THE IMMUNITY REACTIONS OF THE SERUM

IN

GONOCOCCAL INFECTIONS.

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by

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I. ## INTRODUCTION.

The first published observation of clinical value on
this subject was as long ago as 1906, when Mueller and
Oppenheim(1), followed by Bruck(2), described successful
demonstrations of the fixation of complement in gonococcal
infections. Since this date a number of workers have
made contributions which have placed the study of the serum
in these infections on a sound basis, yet there exists
uncertainty on several important points, and in Britian
and to the greater extent in Australia, this field of work
has been little exploited.

The laboratory test which forms the bulk of the present
enquiry is that which is the most important of the immunity
reactions in gonococcal infections, the Complement Fixation
test, referred to hereafter as the C.F. test.

Its clinical recognition has been rather slow. This
has been due in part to the uncertain results often obtained,
owing to the difficulties of a delicate and relatively
unstandardised technique. Perhaps, too, even the Medical
Profession may not have realised that gonorrhœa is not always
merely a very localised infection, but frequently presents
features of what is a systemic invasion. For a serum test to
be of value it is first necessary that the sucessful overthrow

of the infection in question should evoke the general
immunity mechanism of the body.   This aspect of the lesser
but scarcely less serious of the Venereal Diseases has
perhaps not received as wide a recognition as might be.

II.   ## GENERAL CONSIDERATION OF THE IMMUNITY REACTIONS.

   (I). AGGLUTINATION.

       As is well known, the blood serum of an animal artificially
immunised to the gonococcus agglutinates the organism in high
titre.   The conditions in the human infected subject are
not parallel, however; as the usually low antibody content
of the serum, and the difficulty of maintaining agglutin-
able cultures of many strains render this test clinically
inapplicable.

       It is the best means of estimating the immune response
of inoculated animals, but as the technique follows the
customary lines no further mention is needed here.

   (2). PRECIPITATION.

       This reaction also is readily demonstrated in animal
experiments.   Its ease of technique commends it also for
clinical purposes, but its lack of sensitiveness limits
its sphere of usefulness.

       In order to elicit this reaction a more concentrated
antigen is necessary than for C.F.(3), so that the serum
used in the test is not unduly diluted in the presence
of the bacterial antigen.   The antigen employed must be
be a clear solution, made by extracting, dissolving or
autolysing gonococci(vide infra).   The fairly wide employment
of the various flocculation tests in the diagnosis of
Syphilis as confirmation of the Wassermann test might
arouse the hope that a precipitin test might be of clinical

value in gonococcal infections also.   But the cases are
not parallel.   In the former the infecting agent induces
a very strong serum response, and the precipitate obtained
in the test is derived not chiefly from the serum but in
the main from the lipoids of the antigen(4).   In the later
the serum response is relatively feeble, and the precipitate
being derived from the serum proteins(5) is of necessity small
in amount.

A precipitin reaction can be elicited from the serum of
a patient where the C.F. reaction is well marked, but the
latter test will reveal the presence of antibodies where
the former fails to respond.

The method which is most sensitive is that of the
contact ring.   The antigen is floated on the surface of
the serum in a small test tube, and after one hour's
incubation at 37°C, a white ring at the line of contact
indicates the formation of a precipitate.

A series of cases where the serum responded strongly
to the C.F. test was examined by this method, and slightly
less than 80% agreement was obtained.   As this excludes
those cases reacting only feebly to C.F. the method was
not extensively used in the present enquiry.   Herrold(6)
obtained the precipitin reaction in 86% of cases where
the C.F. test was strongly positive, but the limitations
shown by this result alone are sufficient to impugn the
sensitiveness of the test.

A specimen series is appended.

SERUM	PRECIPITIN		C.F.
	After I hour 37 C	After I hour 37 C & I8 hrs room temp.	
I	−	−	−
2	+	+	+ + +
3	−	−	−
4	?	?	±
5	−	slight	+ +
6	−	?	+
7	−	−	±
8	−	−	−
Neg.Control	−	−	−
Pos.Control	+	+	+ + +

While working on this at the close of 1920 I commenced a series of observations on a precipitin test designed to demonstrate the presence of a gonococcal antigen in discharges or urine deposits by testing for a precipitate in the presence of a polyvalent antigonococcal serum. This can be successfully done and presents a close agreement with the results of bacteriological examination.

Since initiating this work I have read the article of Robinson and Meader (7), and as my own independent observations coincide largely with those made fix by these workers they will be presented here, though strictly speaking such a test does not fall within the category of serum reactions.

PRECIPITIN TEST TO DETECT GONOCOCCI OR THEIR BROKEN DOWN PRODUCTS IN DISCHARGES etc.

The clinical material used is the urethral, or prostate or cervical discharge. This is collected directly into a centrifuge tube with a pipette or on a small swab, which is placed in the tube and treated with a small quantity of distilled water or saline, I to 2 c.cs. This may be treated with decinormal N a O H, and subsequently neutralised with N/IO H Cl, but better results are obtained by autolysing

in water or saline alone. The mixture is allowed to stand overnight and incubated at 37°C for 2-3 hours, or else, as Robinson and Meader advise, heated at 37°C for 6 hours.

By spinning the fluid, which may be increased in volume if required, a clear or nearly clear product is obtained, which contains the antigen (precipitogen) if such be present.

The above authors find no special advantage in using a polyvalent antiserum for this test, but the present results are all based on the use of a polyvalent serum.

I n carrying out the test an arbitrary quantity (0 2 to 0 5 c.c.) of antigonococcal serum of high titre is placed in a small test-tube of I cm or less in diameter, and on this is deposited by means of a capillary pipette an equal quantity of the prepared extract, which will float on the surface of the serum.   Extract and serum controls are employed, also with advantage, a control series using normal s serum.

SCHEME OF TEST.

Row of Tubes	Antiserum (Horse)	Normal Serum (Horse)	Extract	Saline
I	0 3 c.c.	—	0 3	—
2	—	0 3	0 3	—
3	—	—	0 3	0 3
4	0 3	—	—	0 3
5	—	0 3	—	0 3

Tubes 4 and 5 suffice of course for the whole series.

These are incubated at 37 C I hour, by preference on a water bath, and allowed to stand at room temperature $\frac{1}{2}$ to I hour.   A positive reaction is shown by the presence

of an opaque ring at the zone of contact, care being t aken not to agitate the rack of tubes.

In general it was found that the results coincided with the clinical and bacteriological findings, but it would be hard to say if any reliable information is often given which cannot be obtained otherwise.

When a definite ring is seen and all controls are clear the reaction is probably quite specific.

It will be seen however that the class of case to which it is applicable is limited, and the C.F. test, for instance, will yield results where no local material for examination can be obtained.   As precipitation requires an optimum concentration of the antigen a further limit ation is placed on the method.

In several cases a successful demonstration of antigen in discharges was made by complement fixation, but the are so many necessary preliminaries and safegaurds in such tests/that the pra ctical value seemed to be very slight.

In several cases urine deposits were subjected to a precipitin test but only limited success was obtained, two positive tests being found in eleven cases which were known to present a demonstrable gonococcal infection.

This table sets out a Summary of precipitin tests carried out on urethral prostatic and cervical discharges.

Nature of case	Smear Examination Positive	Negative	Precipitin test. Positive	Negative
Clinically Gonococcal	38	16	40	14
Clinically Non Gonococcal		II		II

All doubtful precipitin tests were recorded here as negative.

(3).   OPSONIC INDEX.

The determination of the Opsonic Index has been carried out parallel with C.F. tests in a small series of cases, but the results did not warrant continuance of this work.   Even young gonococcal cultures often contain cocci which stain poorly, and may look somewhat swollen.   As these are probably more easily disintegrated than more vigorous individuals this may be an explanation of the divergence which is noted even in the results of examination of normal sera.

The opsonic index in a number of chronic cases which yielded a partially positive C.F. test was below that of normal controls.   An increase of the antibody content of the blood by natural cure, or especially by specific serum or vaccine therapy as gauged by clinical improvement and C.F. tests, was accompanied by a rise in the opsonic index.

However the variable factors are many, and less readily controled than those in haemolytic experiments, so for this reason in no extensive series of cases was the method employed.

(4).   EPIPHANIN REACTION.

This reaction is one method of demonstrating the physical changes which take place when antigen and the supposed antibody are brought together in vitro.   Devised by Weichardt in 1909, and later modified by Seifert it was used as a control for Wassermann tests by Keidel and Hurwitz (8), and confirmed as regards accuracy by Angerer and Stotter (9).

Experience with the serum of Syphilitic patients indicates that the reaction, probably like Noguchi's C.F. test with a spirochaetal antigen (IO), is a true indicator of the presence of specific antibodies, and similar demonstration has also been made in typhoid, diphtheria and tuberculosis.

It has been shown that the conjunction of antigen and antibody causes changes in diffusion rate, owing to alterations in surface tension. In this test/of freshly prepared $Ba SO_4$ a system is used, whose end point to phenolphthalein is shifted towards the side of alkalinity by the presence of colloidal solutions, such as serum alone or antigen alone in high dilutions. The interaction of antigen and antibody in serum alter this relation and shift the reaction of the system in the direction of acidity, i.e. of further concentration of H ions.

Thus, a fresh saturated solution of Ba O H is made exactly equivalent to a normal solution of $H_2 SO_4$. To equal quantities of these are added dilutions of antigen and serum, and finally the indicator, consisting of I% alcoholic solution of phenolphthalein, together with I% of a IO% solution of $S_r Cl_2$, used as a catalyst.

SCHEME OF TEST.

	Antigen dilution	Serum dilution	H_2SO_4 N/I	Ba OH Soln.	Indicator
System control	nil	nil	Ic.c.	Ic.c.	O.Ic.c.
Antigen Control	O.Iac	nil	Icc.	Icc.	O.Icc.
Serum Control	nil	O.Icc.	Iac.	Icc.	O.Icc.
Test	O.Icc.	O.Icc.	Icc.	Icc.	O.Icc.

Several falling dilutions of serum are used, the dilution

of antigen being determined empirically.

A positive result shows the tubes containing the re-
acting serum and antigen lighter pink than the serum and
antigen controls, and a quantitative estimate may be made
by citrating with a very dilute H_2SO_4 solution.

Applying this to the serum of an animal immunised to
the gonococcus, and employing a gonococcal extract as an-
tigen I have been able to demonstrate in this way that
specific antibodies exist in the serum. Apparently this
is a delicate reaction, but as it depends on alterations
in surface tension between the suspended particles of a
nascent insoluble inorganic salt, errors easily creep in.

Exact measurements must be made, using a pipette
mounted inside another glass tube which catches any excess
overflow from the inner measuring pipette, thus ensuring
delivery of the precise quantity required.

When applied clinically this test has not yielded in
my hands any useful results. The experimental error
is apt to be high, and only those sera with a high content
of immune body react demonstrably.

Employing a similar though less sensitive test, the
Meiostagmin reaction, in the diagnosis of Influenza and
Cancer respectively, Edington (II) and Bernstein and Simons(I2)
report a failure to obtain results of clinical value.

(5).   COMPLEMENT FIXATION.

Following upon the pioneer work of Muller Oppenheim
and Bruck the results of othe r observers were somewhat in-
consistent.   Teague and Torrey in 1907 (I3) demonstrated
agglutinins and precipitins in the serum of animals im-
munised against the gonococcus, and by these means and further
work on C.F. in immunised rabbits, Torrey(I4) (I5) (I6) (I7)
showed that the gonococcus belonged to a heterogeneous group.
Schwartz and McNeil (I8) placed the C.F. test on a sound
basis in gonococcal infections and made it of clinical value
fxx by proving the necessity of a polyvalent antigen.

Since their work a number of other investigators have
published slight variants of technique, and confirmation has
not been lacking as to the value of the procedure.   Owers(I9)
Thompson (I9)? Kolmer (20), Schmidt (2I), Jones and Simons(22)
Warden (23), Lees (24) (25), Priestly (26), Dixon and Priest-
ly (27), Magner (28), Smith and Wilson (29), Thomas and Ivy(30)
Irons (3I), Harrison (32), Keyes (33), Herrold (34),Lailey
and Cruikshank (35) and Kelly (36), have all borne testimony
to the value of this laboratory test in clinical medicine.
Nevertheless the percentages of definite reactions in the pre-
sence of known gonococcal infections vary with different
workers, and the Medical Research Committee in Britain in their
report on the laboratory diagnosis of gonococcal infections
(loc. cit.) give four sample techniques and while advising
standardisation are not able to recommend any given tech-
nique as the best.

In appraoching the present enquiry the initial method
employed of testing various technical methods was that of
immunising several rabbits with single strains of gonococ-
cus, and testing their serum against the appropriate strain.
Rabbits readily tolerate intraperitoneal infections of liv-
ing gonococci, which were grown on slopes of Thomson's
serum glucose agar.   In this way in particular different
antigens were tested.

III. <u>TECHNIQUE OF C.F. TEST IN GONOCOCCAL INFECTIONS.</u>

Several points need to be received, for a technique which yields satisfactory results with the Wassermann Test may fail to satisfy the requirements of a much more delicate reaction.

(a). <u>CORPUSCLE EMULSION.</u>

Both human and sheep corpuscles have been used, chiefly the latter.   Results obtained with the former system are mentioned below.

The emulsion employed was usually 5%.   It is of the utmost importance that a regular and standard technique be employed.   It the centrifuge be spun for a longer or shorter time than usual, the bulk of the corpuscles will vary, altering the strength of the suspension.   If a routine be adopted consistent results are obtained, even without the refinement of using a specific gravity method, as advocated by Lewis (37).   Calculation may also be made from the whole blood, reckoning the normal percentage of the blood volume occupied by the corpuscles, and this method gives excellent results.

(b). <u>AMBOCEPTOR.</u>

A series of tests was made to ascertain the relative value of the anti-sheep and anti-human systems, the amboceptors used being obtained by intraperitoneal injection of a rabbit, using five increasing doses of corpuscles. The anti-human system is slightly more sensitive, as it

eliminates the error caused by presence of anti-sheep ambo-
ceptor in human sera.   A recent series illustrates this.

Serum	Human System Incubation I hr.	Sheep System	
		Incubation I hr. at 37	Incubation I8 hrs at 4 C
I	—incomplete	—	—
2	+	—	±
3	+ + +	+ +	+ + +
4	—	—	—
5	±	—	— incomplete

Here the results accord with and may even improve upon the
more sensitive method of long incubation in the cold.   The
drawback to the human haemolytic system is that the ambo-
ceptors thus prepared are hard to make of high titre.   Anti-
sheep haemolysin of high titre is readily obtained and for
this reason most of my tests have been made with it.
Some workers vary the amount of amboceptor used, keeping
the complement constant.   This is well adapted for a "one
tube" method, but as complement is much more instable than
the haemolysin it is better to keep the latter fixed and
vary the former.   That it is more accurate to titrate
complement than amboceptor alone in the daily routine was
shown by Ottenberg (38) in an experimental study.

The method of sensitising used has usually followed
that recommended in No. 4 method of the Wassermann test of
the Medical Research Committee (39) using full sensitis-
ation with 5 M.H.D., allowing incubation for 30 minutes
after mixing the corpuscles with the haemolytic serum.

(c).   **COMPLEMENT.**

Guinea pig serum was almost exclusively used for this
purpose.   In C.F. tests for Syphilis and Tuberculosis
Gradwohl and also Goldenberg and Field (40) have used the
complement in the patient's own serum.   I have tried
this method, but when all due safegaurds of titration of
of the complementary strength are adopted it offers no ad-
vantages.   The negative reactions are probably to be trusted
more than the positives, especially if these latter are
poorly marked.

Complement is best to be pooled, owing to differences
in the relative powers to lyse sensitised corpuscles and to
be fixed by the Antigen-antibody complex.

The method of titration is of the greatest importance.
The usual routine is to incubate sensitised corpuscles with
equal quantities of complement diluted $1/_{10}$, $1/_{20}$, $1/_{30}$ up
to say $1/_{120}$.   As complements vary greatly in activity
the minimum haemolytic dose (M.H.D.) will vary from time
to time.   Now it will be noted that the quantities of
complement used in this trial increase by irregular increments
Thus greater exactness may be attained if the M.H.D. fall
within the higher dilutions than if within the other end of
the scale.   This may be overcome by interpolating dilutions
in order that the series may more nearly approximate to an
arithmetical progression.   For a successful titration it is
desirable to find the dilutions immediately above (i.e. weaker
than) the M.H.D. showing some degree of haemolysis: an abrupt

transition from no change to complete lysis leaves room for doubt.

Thomson (19) advises titration of complement with antigen, and using 2½, 3 and 3½ times the M.H.D. thus found.   The amount of complement absorbed by the antigen alone must be known, but to include this in the M.H.D. invites some lack of uniformity in the quantitative re-sults obtained from week to week, as each multiplication also multiplies the amount absorbed by the antigen alone. This error is slight with a good antigen, but is variable. The routine which I have found most satisfactory is to put up two sets of tubes for preliminary titration, each con-taining graduated amounts of complement, and one contain-ing the previously determined dose of antigen.   These are incubated I hour at 37° C, and the results after adding the other components of the haemolytic system give us the mini-mum haemolytic dose of the complement and also tell how much complement is absorbed by the antigen.

One's experience comes into play here in assessing this evidence and selecting the dilutions which will be used in the test.

A tube containing the positive control serum may be included with advantage, to ensure the activity of the antigen.

Three quantities of complement should be used in the test, in order to measure the strength of the reaction. The smallest amount used may be 2 M.H.D. plus the allowance

made for complement absorbed by the antigen, or $2\frac{1}{2}$ M.H.D. with the same addition if long fixation on ice be used. The ratios between the successive quantities may be $2\frac{1}{2}:3:3\frac{1}{2}$, following Thomson, or else $3:4:5$. In practice the minimum amount of complement used is usually in the neighbourhood of 3 M.H.D. for long fixation (18 hours).

In the Wassermann test large amounts of complement are deviated by strongly reacting sera, and the antigens are but little anti-complementary, so large increments may be used, e.g. 2, 4 and 7 doses. But in this test the reaction is feebler, and its effective and yet safe range more restricted. It should be noted also that if any one given serum fix twice the quantity of complement fixed by another serum, it does not follow that its content of antibody is double that of the other. As it is probable that complement is fixed or deviated according to the laws governing absorption, the quantity of complement bound by increasing concentrations of antibody (in combination with antigen) may not follow a simple linear relation, but rather the general formula of adsorption. (If A = amount adsorbed from a solution, the amount adsorbed from a solution of double concentration is not 2A but A x $2^{1/n}$, where n is a constant).

The practical outcome of this consideration is that the varying quantities of complement used in the test should increase by smaller increments than in the corresponding test for Syphilis, and also that the exact quantitative value of results of C.F. experiments must not be unduly strained in

clinical interpretations.

A further reason for caution in the latter respect is that variations in complement may cause varying quantitative results in the same sera at different times, as proved by Harrison (41) by weekly tests of frozen sera.

(d).   SERUM.

The quantity of serum to be used is important.   One method of carrying out the test is to employ diminishing quantities of serum with all the other factors constant. This gives good results, but those obtained by varying the amount of complement are more satisfactory.

In an anti-sheep system the presence of anti-sheep amboceptor in the sera tested introduces a possible error. This may be avoided by using an anti-human system, or by so diluting the serum as to render the effect negligible, in the latter case taking care to reduce somewhat the quantities of the complement used, so as not to miss faint reactions.   A dilution of 1/5 serum will give good results but for long fixation on ice with small doses of complement there is a risk of this serum dilution being sufficient anticomplement- ary to delay or inhibit lysis in the controls.   It is safer to use serum $I/_{10}$ or $I/_{20}$ as advised by Thomson.

In carrying out a series of tests with varying quantit- ies of serum I have found that the greater concentrations of positive sera do not always give the stronger reactions. This may be xxtxxxx due to natural amboceptors to the oc- casional occurence of the phenomenon of "zones of inhibition"

such as happen in agglutination work.

The following table illustrates this in the case of two positively reacting sera.   All reagents are of such dilutions that equal quantities of each are used.

Serum		Dilution	M.H.D. complement absorbed in presence of antigen
I.	I.	1/5	3
	I.	1/10	4
	I.	1/20	3
	2.	1/5	3.5
	2.	1/10	4.5
	2.	1/20	4.5

This anomaly seems to be rare in clinical diagnostic work but it is an advantage to include two different dilutions of serum with varying doses of complement in the test.

The method of inactivation should be always the same, 15 minutes at $55°C$ being sufficient.   It is probably best to make the period as short as is compatible with thorough destruction of the native complement.   An extra degree of sensitiveness is introduced by inactivating after dilution of the serum.   This modification has the support of some recent work on the Wassermann test (42).

Parallel tests were carried out over more than 60 clinical cases, and showed sufficient improvement in the results in the weaker reactions to warrant its recommendation.   The most striking instances are appended.

Sera	A			B		
	2½ M.H.D.	3	3½	2½ M.H.D.	3	3½
37	sl	p	nc	nc	c	c
58	n	n	n	tr	sl	p
60	sl	sl	p	nc	c	c

A   inactivation after dilution

B   "   before   "

C   complete haemolysis

nc   nearly complete haemolysis

p   partial   "

sl   slight

tr   trace

n   none

A few of the sera used in this work were collected in a Wright's capsule by pricking the finger or ear, but the majority by puncture of a superficial vein. Care was taken to secure clear samples, as haemolysed sera are not infrequently anticomplementary.

(e).   METHOD OF FIXATION.

The method of incubation for one hour on a waterbath or in a thermostat at 37°C gives farily good results, and no strongly reacting serum will thereby fail to give a definite reading. Much more accurate and convincing results are obtained by long fixation at ice chest temperature.

Numbers of observers (19)? (26), (34), (42), (43), (44), (45), (46), have praised this refinement in C.F. work, and my own finding has been that it is a great gain in sensitiveness and that without it the weaker reactions are often missed, while doubtful results with the shorter method become less equivocal and usually definite by this means.

Following the adsorption laws the rate of complement deviation proceeds more rapidly at the higher temperature, but the slower rate near zero is more than compensated by the greater completeness of the process.

With the safegaurds mentioned above even a long period on the ice will not imperil the results. Sometimes lysis is a little delayed, as the rate of deterioration of complement is uncertain. False positives are not obtained if adequate controls are used. On three ocassions the whole series was repeated as the results were not clear cut; these accidents appeared to be due to an error in the balance of the haemolytic system, a source of trouble which increase in experience largely eliminates.

A series of parallel observations was carried out on over 150 sera. Of these a definitely negative or positive reading was unaltered in about 60%. In 31% a definite though in some cases weakly positive reading was rendered perceptibly stronger and in 9% readings which would have to be given as negative or incomplete negative by incubator fixation were transformed to partial or weakly positive results by ice chest fixation. As these weak serum responses are often of importance (vide infra) the gain is considerable.

The racks may be left on ice for 5 to 6 hours, but this method is inconvenient, the time for adding the corpuscles being often at an unsuitably late hour. The most satisfactory period is 17 to 18 hours at ice chest temperature about 4 C.

(f). **QUANTITIES OF REAGENTS**.

There is no special advantage in using large quantities (e.g. 1c.c.) of each ingredient. With careful measurement, using capillary or narrow bore pipettes 1/10 quantities i.e. 0.1c.c. of each can be used with excellent results. The readings can be more easily made with 0.2c.c. or 0.25 of each, bringing the total contents of the tube to about 1c.c.

Latterly I have employed a drop method with every satisfaction, graduating a dropping pipette to deliver 30 drops per c.c.

This obviates error in measuring small quantities and saves time and fatigue. For instance if the three doses of complement used are in the proportion of 2½, 3 and 3½, one dilution is made which contains 2½ M.H.D. in 1/6 c.c. The requisite amounts are then given by 5, 6 and 7 drops.

(g). **ANTIGEN**.

This is the most important of all ingredients of the test; its full discussion is reserved for a separate section. In one method, that of Ower (19), the antigen is

used in varying dilutions.   There seems to be little advantage
in this.

The variation in the behaviour of different anti-
gens is great, but provided the preparation employed binds
complement in the presence of a known positive serum in a
quantity well below its anticomplementary dose an optimum
antigenic dose can easily be found.   This should not be great-
er than I/3 the anticomplementary dose.   If the preparation
in question be quite satisfactory the quantity used in the
test may well remain constant.

Calculation of the actual amount of gonococcal substance
present (i.e. by counting or weighing) may afford some clue
as to the possible effective range of an antigen, but as in
any case a preliminary titration is essential with any new
antigen the step is an unnecessary one.   I have found it most
satisfactory to make up antigen in concentrated form and to
ascertain the correct dilution for use by experiment, without
reference to other measurements.

(h).   <u>READING OF RESULTS</u>.

With ice chest fixation it is hard to assign a definite
period of incubation after adding the corpuscle emulsion.   A
A large number of cold tubes assume the incubation tempera-
ture slowly, but this is not a disadvantage, as the lysis
of the controls can be watched more deliberately.   So soon
as these are lysed a reading is taken, for the reaction is

specific, and with proper technique inhibition of lysis must be due to specific causes.   Another reading is taken after a further period of 15 minutes incubation.

Prolonged incubation will often cause lysis in the case of the feebler reactions.   This is merely a question of the accuracy with which the end-point of titration of complement is determined.   It is better however, to have the complement on the "sharp" side than to risk failure of lysis in controls.

For negative controls I have always used fresh sera, animal or human.   Negative sera kept on ice for a week are apt to become anti-complementary, especially if they are infected, thus vitiating the results.

For positive controls sera may be kept on ice from the preceeding week's tests, but a simple and very satisfactory plan is to use an appropriate dilution of a polyvalent antigonococcal serum.

(i).   SUMMARY OF TECHNIQUE.

For exact work a most accurately controlled haemolytic system must be used, either anti-human of anti-sheep yielding good results.

Inactivation of serum is recommended to be carried out after dilution for 15 minutes at 55°C.   A multiple tube method is essential the variable factor being the complement. The doses of complement used should increase by small increments so as to pick up slight changes in the antibody content of the serum for test.   The serum may with advantage

be used in two different dilutions, a strength of $1/_{10}$ or $1/_{20}$ absorbing very little ~~xxxxxxx~~ complement and containing little natural amboceptor, if this be present.

The use of small quantities (0.1c.c.) of each component is compatible with accuracy, and there is no need to use amounts greater than 0.2 or 0.25 c.c.

Fixation for a long period (17 to 18 hours) on ice is strongly recommended as a routine procedure.

The antigen should be titrated with complement and with positively reacting serum as a preliminary.

The antigens found most satisfactory in this investigation have been

     1.   Polyvalent emulsion of gonococci in carbol-saline.

     2.   Lipoid-free suspension or extract.

     3.   Filtered autolysate (McNeil).

     4.   Neutralised solution in alkali (Thomson).

The question of these and other antigens will now be discussed in detail.

IV. ANTIGENS IN THE C.F. TEST.

The question of the antigen is a vital one, and has
been approached from several standpoints.

I.   Rabbits were immunised with single strains of
gonococcus by repeated intraperitoneal injections
of the living organism.   Though at first they lose weight
they tolerate increasing doses very well.   Their serum
was then used to investigate the antigenic properties of
the various preparations made from the homologous strain.

2.   Antigens were made from a number of strains (not
less than I5) and tested against the polyvalent anti-
gonococcal serum of Parke Davis & Co., and also of the
Commonwealth Serum Laboratories.

3.   Similar tests were made with 5 strains using the
antiserum prepared from these at the Commonwealth
Laboratories (for the purpose of a special investigation
into serum treatment).

4.   Practical trial and comparisons were made in the
weekly routine tests carried out on the patients' sera.
In this way stability and general reliability were in-
vestigated.

In all cases young sub-cultures were used (24 to 48
hours) grown upon Thomson's Glucose agar with the addition
of human or horse serum (the latter being quite satis-
factory especially after several generations).   In order
to secure uniformity this culture medium only was used
for preparing antigens, as there was not opportunity for

any detailed enquiry into the bearing of the medium upon the antigen.   The citrated blood agar of the Commonwealth Serum Laboratories has been used for keeping subcultures with uniform success.

(A).   NEED FOR POLYVALENCY.

This is well known, and though definite knowledge as to the grouping of the gonococcus in Australia is wanting it is certain that the organism belongs to a family of several strains.

The amount of complement absorbed by the serum of a patient infected with the gonococcus varies very considerably with individual strains.   A preparation made from even 5 or 6 random strains will not always elicit a positive reaction where 12 or 15 strains would do so.

By way of illustration the results with 3 different sera are given.

		Antigen 6 strains			Antigen 16 strains.		
Haemolysis with		2½	3	3½	2½	3	3½M.H.D.
Serum	1	tr.	sl.	n.c.	n.	tr.	sl.
"	2.	sl.	p	n.c.	n.	tr.	p
"	3	n.	tr.	sl.	n.	n.	n.

It is advisable to employ at least 12 to 15 strains and to keep regularly subcultured such strains as are found by experience to yield a good antigen.

In the work undertaken on antigonococcal serum (v.i.) in 20% of cases a feeble doubtful or (rarely) negative

response was obtained with 5 strains and positive with
15.

(B.) AGE OF CULTURES.

This does not appear to be an important factor, though
there are so many variables to be taken into account that
it is hard to reach a definite conclusion. However, good
antigens have been made from strains subcultured many months,
and in working with the 5 strains mentioned above no fall-
ing off was observed in antigens made in the same way on
different dates.

(C). AGE OF THE ANTIGEN.

A good antigen should be stable for several months,
but all seem to fall off in potency even at low temperature.
Emulsions and the various forms of extract will keep for
3 or 4 months as a rule, but require periodic testing to
find their antigenic and anticomplementary doses. The
latter often increases with age, and the former decreases,
thus reducing the effective range of the preparation.

For instance after intervals of 3 and 6 months one
antigen (made after Thomson's method) showed a decrease
in antigen power of 40% and 100% and an increase in intrinsic
affinity for complement of 25% and 70% respectively.

It is doubtful if simple emulsions are really less stable
than the various extracts. In both when deterioration oc-

occurs the fall in antigenic power proceeds rapidly.
This question will be further discussed.

(D). <u>REACTION OF THE ANTIGEN</u>.

As certain antigens are prepared by solution in alkali
it is of interest to enquire how exactly neutralisation
should be carried out.   Also the question arises whether
the gonococcal vaccines suspended in Na H_2 PO_4, 0.6% (to
prevent autolysis, Thomson (19), might be employed.

It is better to neutralise such a suspension if it
be used as an antigen, and an alkaline solution (in $N/10$
Na OH or antiformin) should be neutralised to litmus.

The effect of a concentration of H or OH ions is to
increase the anti-complementary properties of the antigen.
Probably this is due to the action of acids or alkalies
on the complement.   It has been suggested that this is
caused by the splitting of the "middle piece" of complement,
which is supposed to unite with the sensitised corpuscles,
from the "end piece" which is left in solution (Browning and
MacKenzie (47)), or alternatively that the active prin-
ciple of complement is inactivated but not "split" (Noguchi
and Bronfenbrenner (48)).

Experiments were made to test the effect of reaction.
Alkali, (Na OH) and Acid (HCl) were added to a fresh gonococc-
cal emulsion in saline in small amounts to make concentrations
of $1/3000$ $1/4000$ $1/5000$ and so on, the actual concentration

in the test-tube being I/9000, I/10,000 etc. These were used in tests to determine the amount of complement inhibited. It was found that the higher concentrations caused chemical lysis of the corpuscles, but those below this point caused definite interference with the normal lytic power of the fresh guinea-pig serum. That is, a quantity of antigen found by trial to be well below the amount necessary to inhibit a given dose of complement was able to present lysis when acid or alkali was added in a concentration less than that sufficient to cause chemical destruction of the corpuscles. Neutrality in an antigen is thus desirable.

(E).   TEMPERATURE USED IN PREPARATION.

This has a decided influence. The C.F. test involves the interaction of colloidal solutions, whose balance is easily disturbed. For instance the denaturing of protein in complement by shaking causes inactivation (49). So undue alteration of protein in antigen is not desirable, not only on account of possible chemical changes, but also of the alteration in surface tension.

Heat is frequently used to extract the bacterial substance, but whether it effects this by hastening a true autolysis by enzyme action or by favouring a mechanical breaking up of the cell the benefit is not an unmixed one.

The following table shows the effect of heat upon portions of the same emulsion of gonococci.

Emulsion heated to	Minimal antigenic dose
55°C I hour	0.08 c.c.
70°C I hour	0.I c.c.
80°C I hour	0.I3 c.c.
Emulsion unheated	0.06 c.c.

In this instance the more rapid disintegration of the cells is accompanied by changes in the protein which tend to lower their antigenic potency.   The actual deterioration by heat for practical purposes may of course be lessened in effect if the preparation in question have a wide range over which it may be used.   In practice heating to 55°C for I hour gives more assurance of sterility and diminishes effectiveness but slightly.

(F).   METHODS OF PREPARATION.

A priori one would expect that a true colloidal solution would give better results than a more or less coarse suspension, on account of the greater surface available for reaction.   Against this must be placed the damage likely to happen to complex bacterial substance when subjected to more or less violent disruptive procedures.

The various methods may thus be summarised.

(a)   Suspensions (the so-called "emulsions") of bacteria

(b)   Autolysates

(c)   Solutions of altered protein e.g. in alkali-
            meta protein

(d)   Extracts   (I)   the lipoid fraction

                      (2)   the lipoid-free residue.

(a)   SUSPENSIONS.

An ordinary suspension of gonococci in saline with the addition of 0.5% carbolic acid serves excellently as an antigen.   It is capricious in its keeping qualities, and its anti-complementary properties usually increase with age.   This occurs with other preparations also, but it may be remarked that if a number of strains of gonococci are kept subcultured in a laboratory it is simple to use a fresh suspension each month.   The only objection is the trouble entailed in an accurate titration of the new antigen, but in any case this is necessary periodically, as the stability of no bacterial antigen should be assumed without proof.

The occurence of autolysis has been advanced as an objection to suspensions of cocci.   This does occur, but after keeping suspensions of gonococci in the ice chest for over 3 months in 0.9% saline, and also in strong (9%) saline, it was found possible to demonstrate antigenic properties in the supernatant fluid after spinning in a centrifuge.

This list shows the comparative degrees of haemolysis in the three tubes (containing increasing doses of complement) as obtained with various antigens and a positive serum.

Antigen	Lysis in Tube I	2	3
Fresh Emulsion	n	n	n
Emulsion 6/12 old	n	tr	sl
Supernatant fluid from above	n	p	n.c.
Solution of cocci 6/12 old	n	sl	p

It would appear therefore that autolysis per se is not the cause of deterioration: this would seem to be rather the instability of the complex molecular structure of the organism.

The antigens considered in this table were not really as nearly equal in power as this phase of their activity would indicate. The older preparations absorbed more ~~xxxxxxx~~ complement than the fresh, and the latter was active in much lower dilutions than the former. The table merely brings out the possession of antigenic power after a number of months, and the transfer of such power from the actual bodies of the cocci to the fluid in which they were suspended.

(b). AUTOLYSATES.

Some writers refer to antigen containing the "endotoxins" This term is of doubtful propriety, as the toxic and antigenic fractions are not of necessity identical.

Included in the term "autolysates " are all those preparations made by breaking down the bacteria whether by a true autolytic process or by a disintegration by heat or by

freezing.

After the contents of the bacterial cell are dispersed the resulting solution or emulsion is filtered or centrifuged, any deposit being rejected.   The product as used is thus extracted from the whole bacteria.

This may be accomplished in various ways.   McNeil [50] advises heating in distilled water for 2 hours at 55°C to disintegrate the gonococci, then centrifugalising and passing through a Berkefeldt filter.   This product is a good one, but always of equal value.   Apparently all strains of gonococci do not disintegrate with equal ease.   At times the organisms require a longer period in order to free their cell contents.   Though but slightly anti-complementary this antigen does not seem to be uniformly potent.   As a rule it is stable and keeps well for a number of months.

The method of Blake [51] is to use successive freezing and thawing to produce lysis of the bacteria; Olitsky and Bernstein [52] autolyse I hour at 60°C and 24 hours at 37°C. These are referred to later.

An experiment to find the influence of time of incubation at 37° of a suspension on antigenic power is tabled here.

	Antigenic dose	Units of complement absorbed
Fresh <u>unheated</u> emulsion	0.03	. I.5
Supernatant fluid from same after <u>24 hours</u> at 37°C	0.05	0.9
ditto <u>after 7 days</u> at 37°C	0.045	0.9

The influence of longer incubation than 24 hours seems to be very slight. In this case the suspensions were well shaken before incubation.

A method which has given a very good antigen is as follows. Heat a suspension 2 hours at $55°$ C on a water bath. The deposit contains bodies resembling gonococci which still possess normal staining capacity. Without filtering the whole is placed on ice and daily shaken for 7 days. It is then centrifuged and the supernatant fluid pipetted off and filtered through paper pulp and preserved with 0.5% carbolic.

(c).   SOLUTIONS OF GONOCOCCI.

As the gonococcus, like most Gram-negative bacteria is readily soluble for the most part in alkali, a neutralised solution of its metaproteins and lipoids obtained in this way has been employed. Merkwijew [53] and Thomson [19] have advised respectively a solution in antiformin and decinormal hydrate of soda or potash. It seems unnecessary to use antiformin when the weaker preparation effects satisfactory solution. The cocci do not dissolve immediately but do so after incubation for a couple of hours. The neutralised solution is almost clear, and a small deposit of the alkali-insoluble fraction falls, but the filtrate gives a good antigen.

I have found this a satisfactory preparation on the whole usually of low anti-complementary properties and highly antigenic, if a little less so than the original emulsion.

It does not show stability superior to other antigens,

but its potency dwindles after a couple of months.

(d)   FRACTIONAL EXTRACTS.

    (I) The lipoid fraction.

Warden (54), (55) in 1915 recommended the use of lipoid-
al antigens in this test, and has since been a strong ad-
vocate of the claims of the lipoids to an important role in
immunity.   Some contributions to this general question will
be discussed later.

As the defensive mechanism of the body against the gone-
coccus seems to be, in the end, bacteriolysis, it is not im-
possible that an antigen consisting of part of the organism
alone will react with the antibodies evoked by the invasion.

Extracts of gonococci made with alcohol, ether (singly
or together) or with acetone, will all display antigenic
power as judged by the C.F. test.   (It is understood that
where by "antigenic capacity" is implied the power to evoke
an antibody response in an animal this is specially stated.
The power to act as an antigen in a reaction in vitro may be
identical with this, but such is not assumed to be the case).

In preparing such extracts the bacterial mass was shak-
en thoroughly for $\frac{1}{2}$ hour with the solvent and allowed to
stand overnight.   This was repeated several times, after
spinning and saving each supernatant.   The lipoids were
evaporated free of their solvent and taken up in ether.
By re-evaporation and solution in alcohol and filtration
after centrifugalisation the product must contain (if any)

too small an amount of protein to possess antigenic power. It was emulsified in saline for use.

It was found that it could fix complement with the serum containing antibodies for the gonococcus, but was inferior to other preparations of the whole bacteria.   Warden claims a special sensitiveness for a lipoid antigen.   Perhaps the fact of its final solvent being alcohol may ensure some degree of stability, but I have not found it preferable to others.

(2) The lipoid-free residue.

The residue obtained after the above treatment shows much superior qualities.   Several workers have used with success a lipoid-free antigen, e.g. Park & Williams (56), Priestly (26), Smith and Wilson (29).   These workers have extracted with alcohol and ether.   I have used with very satisfactory results acetone as the extracting agent, and recently notice that Douglas and Fleming (57) have published some observations on the high antigen capacity (as judged by the results of vaccines and of immunisation experiments) of acetone extracted bacteria.

After extraction they may be used as a suspension, or disintegrated so as to gorm a true emulsion or colloidal solution.   The process of removing the lipoids denatures the protein to some extent, so that true solution of the original bacterial protein is probably not attained.   Park and Williams heat the defatted organisms to $80^{\circ}C$ to extract their substance.   This produces a good antigen: perhaps

the somewhat untoward results of the high temperature are counter-balanced by the greater degree of disintegration effected.   It seems unlikely that true autolysis should occur after subjecting the bacteria to the action of a protein precipitating agent, but solubility of protein is after all relative, as Schmidt (58) has pointed out recently in his studies on denatured protein in immunological experiments.   He has shown that protein dry or in solution subjected to heat can still produce antibodies in animals in spite of denaturation.

I have found the lipoid-free antigen compares very well with all other preparations as regards potency and low anti-complementary value, and is better than the majority as regards stability.

Most of the above observations on antigens must perforce be relative.   It is possible to make many experiments under exact conditions and yet not obtain so much information as by one's general experience.   The consistency of the results obtained in the weekly routine tests on patients' serum, the presence or absence of the various annoying obstacles so abundant in C.F. tests, the ease or difficulty in the reading of the tests; these things are of the greatest weight in assessing the value of such an important constituent as an antigen.

But in addition some comparisons of various preparations studied under identical conditions are of interest.

Luxuriant 48 hour growths of 5 strains of gonococci

were carefully washed off with a minimal amount of normal saline. The resultant thick suspension was subdivided, and different antigens prepared from each aliquot part: these were then tested alone to ascertain their anti-complementary dose, and also with a dilution of $I/_{IO}$ of the horse anti-serum valent for these strains only to find the antigenic dose. Even these determinations are only relative, as different complements alter the absolute results, but they serve well to compare the different prepatations.

The following were the methods employed in preparing the antigens tested.

I. Fresh saline suspension was heated I hour at 55° C.

2. A suspension was autolysed 2 hours in aq. dest. at 55° C centrifuged, filtered and made isotonic

3. A solution was made (with the aid of incubation I hour at 37° C) in $N/_{IO}$ Na OH and neutralised with $N/_{IO}$ H cl.

4. The supernatant fluid was obtained from a suspension autolysed on ice for 2 weeks with frequent shaking.

5. Gonococci were boiled in a 5% solution of antiformin in distilled water till dissolved, and the solution was neutralised with normal HCl. Several volumes of 95% alcohol were added and the deposit separated by the centrifuge. The supernatant fluid was used-- Method of Krumweide and Noble (59).

6. After autolysing a suspension in water at 60°C
   I hour and 24 hours at 37°C and then centri-
   fuging, the supernatant fluid was made iso-
   tonic-- Method of Olitsky and Bernstein (52).

7. Gonococci were dried at room temperature and ex-
   tracted for 3 days at room temperature with suc-
   cessive quantities of alcohol and then ether.
   To this extract was added excess of acetone,
   and the residue after filtration taken up with
   equal parts of methyl alcohol and ether, allow-
   ing this to evaporate to small bulk at room
   temperature.   It was emulsified in saline
   for use.

8. Dried gonococci were ground with a glass rod, ex-
   tracted 3 days at room temperature with suc-
   cessive quantities of acetone: the residue
   was dried in the incubator, finally washed
   with acetone, re-dried and ground up with
   saline and shaken 30 minutes.   This suspen-
   sion was heated I hour at 56°C and stood on
   ice for several days, being shaken at intervals
   After spinning the supernatant fluid was used.

Antigen No.	Minimal dilution fixing 3 M.H.D. complement with positive serum I/10.	Minimal dilution binding 3 M.H.D. complement alone
I	I/I8o	I/I0
2	I/I00	I/I0
3	I/90	I/I0
4	I/45	I/7
5	I/40	I/4
6	I/75	I/I0
7	I/40	I/I0
8	I/I8o	I/2

In this table it will be seen that under the conditions of this experiment the acetone extracted residue was as potent as the original suspension, while it was much less anti-complementary. The two autolysed preparations and the neutral solution in alkali were next in order of merit.

These results were given by readings taken when all controls were haemolysed, and after I5 minutes further incubation On prolonging the period of incubation still more it was found that those antigens which gave the most permanent binding of complement with the anti-serum were placed in the same order as that given above.

To summarise: it is found that a simple suspension of gonococci in carbolised saline constitutes an efficient antigen

By removing the acetone soluble portion of the cocci the anti-
complementary properties are reduced and the antigenic power
at least not decreased: the keeping qualities appear to be
increased thereby, as the lipoid free antigen after some
months of keeping does not display the same degree of in-
dependent affinity for complement as the suspension.

    With regard to all watery solutions or suspensions it
should be noted that infection usually takes place unless
0.5% carbolic acid be added: This is better than a capillary
drop of lysol recommended by some writers.   A non-sterile
antigen, like a contaminated sample of serum absorbs re-
latively large amounts of complement and therefore cannot
be used.

V.   CLINICAL APPLICATIONS.

A study has been made of the clinical aspects of the C.F. test in gonorrhoea, chiefly in the male, since objective information, though often difficult to obtain, is at least less unreliable than in the female.

The points for elucidation were the value of the test in diagnosis and prognosis, its place in the standard of cure, and its relation to the bacteriological tests.   Involved in these considerations is the interpretation of the test.

From August 1920 to December 1921, 410 patients have been studied, and over 300 of these have been under observation for periods of several months.   In the remainder only one serum examination was made: this was due to several causes, firstly, in a number of these cases a single test was made to ensure that a positive C.F. response was being obtained in the presence of frank complications; secondly, in a few a single test was done to give additional evidence of non-infectivity in patients returning long after their cure had been pronounced (for instance when they contemplated marriage); and thirdly, a certain proportion of out-patients in a venereal clinic belong to a floating population and vanish.

Most of the patients were seen at Sydney Hospital Clinic, a few at Royal Prince Alfred Hospital and a number at the Coast Hospital.   As far as possible those patients were selected for test whose clinical condition was most these

thoroughly examined, i.e. where rectal examination, sounds, or urethroscope were employed.

In 75% of the cases several serum tests were made at varying intervals and in those where doubt existed the test was repeated within one or two weeks. In all cases the same routine was observed. A clinical history was taken and kept, and the sera were tested without reference to these data. The results of the tests were compared with the history and past records of tests and where it appeared to be indicated for any reason the test was repeated de novo. In this way it was sought to discover possible errors of technique and arrive at a correct estimate of the meaning of the Serological findings.

Selecting those tests whose accuracy could be checked by clinical observations the results work out as follows.

Strongly Positive sera	88 -- 11%
Positive sera (++ to +)	190 -- 23%
Feebly positive sera, including the class usually called "doubtful"	226 -- 28%
Negative sera	308 -- 38%

Sera described as strongly positive here are those which will completely inhibit lysis in all three tubes in the technique described above. The second class includes those which cause complete inhibition in at least one and less than three tubes. The third class includes all cases where there was definitely demonstrable deviation of complement, up to complete inhibition in the first tube.

It will be observed that it is of great importance
to use a sensitive technique when it is realised that only
in some II% of cases was the reaction very strongly marked.
In fact the serumr rarely shows the same affinity for
complement (in the presence of the appropriate antigen)
as in the Syphilis reaction, except after the artificial
exaltation of the immune response by vaccines or antiserum.

In the above cases the concordance with physical signs
and with bacteriological tests was greatest where the C.F.
response was most intense, thus:--

Degree of Reaction	Concordance with other methods at time of test.
Strong	92%
Moderate	90%
Feeble	82%

In all cases which could be followed up the positive
C.F. finding was confirmed by the subsequent investigation
or history of the patient.   There was only one case where
a marked response was given by the serum which was proved to
be quite inaccurate.   There was no suspicion of any gonorr-
hoeal infection in this patient, who was suffering from
Acne Vulgaris, and a re-test of the serum a week later
failed to elicit any response whatever.   Here it seems like-
ly that the serum was confused with another or else a gross
error in technique committed.

Of the 308 negative tests tabled above there were 22
test
cases where the result of the first/proved inaccurate as

judged by the clinical and other findings.   Putting aside technical error it is found that subsequently a positive test will be yielded by the serum in such cases, and I have not found a case where the serum test remains constantly negative in the presence of an undoubted infection.   Even though the response may be feeble it may always be elicited at some phase of the infection.

In the remainder of these cases the clinical bacteriological and serologocal findings tallied.

The blood of 26 persons who were apparently free from gonococcal infection was examined: in every case there was no binding of complement observed with a gonoccocal antigen.

The sera of 18 patients under treatment for Syphilis was tested: in these the Wassermann test was positive but no gonococcal C.F. reaction was obtained.

The gonoccocal serum test is specific, and for all practical purposes a positive test means an active, latent or recently existing gonoccocal lesion.   The question of specificity will be reverted to later.

Where complications arose, metastatic or otherwise, in the course of a gonorrhoeal infection there was a high proportion of positive tests, shown in the following table. Only cases are included here where there was satisfactory evidence that the particular lesion existed at the time of testing, and multiple tests on the same patient are excluded from these figures.

Complication	C.F.Positive	C.F.Negative	Total
Prostatitis	147	13	160
Epididymitis and Orchitis	32	1	33
Arthritis	19	2	21
Folliculitis and Urethral infiltration	14	0	14
Vesiculitis	22	3	25
Proctitis	2	0	2
Salpingitis	7	1	8

It will be seen that in the presence of an undoubted complication the serum almost invariably responds to the C.F. test.

The question of how early in the disease the reaction may be demonstrated has not been investigated to any extent in this enquiry. It is not of great importance, for the diagnosis is more simply made by other means. Where the area of infection is strictly delimited e.g. to the anterior urethra it is agreed that the serum does not usually respond. Of 10 such cases tested only 1 gave a response; and that feeble. Even as a test of sensitiveness of the technique employed it is fallacious to inquire how early the reaction may be elicited, as it is acknowledged as a matter of extreme difficulty to say when the infection (in the male) invades the posterior urethra.

In a few cases I have obtained a definite positive result within the first 14 days. As a rule it is only after

this time that the serum contains demonstrable antibodies, but as was just pointed out, such cases may be instances of early spread to contiguous structures.

Chronic posterior urethritis generally yields a reaction, though most often cases showing this infection fall also into the category of prostatitis.

In cervicitis in the female the action may usually be demonstrated without difficulty,

With regard to early tests it is of interest to notice that a C.F. test may shed some light on the genesis of an attack in a patient who has previously suffered from the disease.   If the attack is a recurrence of a previously latent infection a Positive test is usually found at a very early stage, so that a definite serum response within the first week would at least support the suspicion of a relapse.

DIAGNOSTIC VALUE OF THE C. F. TEST.

This test was applied in 204 cases where a diagnosis of clinical cure had been made. In all these cases the standard of cure imposed was more rigid than that required by the Regulations under the New South Wales Venereal Diseases Act (60). For a period of 2 months or more there was either no discharge or else merely a slight moisture occasionally in the mornings which on examination revealed nothing inconsistent with the normal prostatic secretion; no gonoccocci or pus cells had been found in the centrifuged morning urine or in the prostatic fluid expressed by massage, there were no threads or flakes in the urine, and in 38 cases an examination with the urethroscope was made.

Of these 204 cases 101 showed a positive C.F. test and 103 a negative: in the latter case confirmed by subsequent re-test in the majority of cases.

Of the 101 positives 44 subsided to a negative after the lapse of variable times; in 17 cases the test remained positive and the patient was afterwards proved clinically or bacteriologically to be still infective: and in the remaining 46 cases no further information was obtained, owing to the patients not being seen again at the Clinic.

It will thus be seen that in at least 8% (and probably about 15%) of patients submitted to the above test of cure the C.F. test proved more accurate than other methods. Now it is not contended that this standard of cure is as exacting as is desirable in all cases: as Clarkson (61)

remarked recently no standard can be too high. In fact where the fullest enquiry was instituted (e.g. with urethroscope, sounds and searching physical examination) the clinician could hardly be corrected at all by a serological test, but it must be pointed out that the final test imposed on most patients, especially in an over worked Hospital Clinic is at best not more thorough than that detailed above. A serum test in a clinic wastes no time and serves the very useful purpose of directing attention to those cases worthy of closer scrutiny.

The cases where help may be afforded by a serum test fall in the following categories: cases of apparent cure, where by no means can a gonococcal infection be demonstrated, cases where there is still an occasional watery morning discharge in which gonococci cannot be found, cases where there is a mixed infection and the question arises whether the original gonococcal infection has died out, cases where an affection such as synovitis or arthritis is suspected as possibly due to gonorrhoea though no signs of this are apparent, and early cases (in the first few days) where there is some suspicion of a recurrence from a previous attack.

The question now arises as to how long after cure a positive C.F. test may persist. It has been found by most observers that the serum response usually persists for 3 months after the termination of the infection, but may be demonstrable for 6 months. The administration of vaccines

may prolong the period a little but apparently not a great
deal.   It is agreed also that an acute complication or a
long continued chronic lesion due to the gonococcus will
prolong the period over which a positive C.F. test is given
by the serum.

It has been my experience that a definitely positive
reaction subsides gradually in the intensity, more rapid-
ly at first, and later more slowly, at last becoming neg-
ative.   This falling response invests the final negative
tests with much greater significance than they might have
otherwise.   A persistent positive test, on the other
hand, after the cessation of obvious signs or symptoms is
of evil omen.   If a patient's serum continue to yield a
positive response for months he should be regarded with
suspicion.   It is not necessary that such test should be
strongly positive.   As will be shown later, even a feebly
positive test, if persistent, may denote the presence of
the gonococcus.   It may on the other hand denote a slow
subsidence of the antibody content of the blood, but such
a finding is a valuable hint to the clinician to investigate
the patient's condition more carefully.

In 82 cases observations were made as to the time taken
for the blood reaction to become negative.   Obviously it
is almost impossible to state with any certainty the time at
which the infection disappeared.   This coupled with the
lack of regularity in the attendances of patients who are
apparently cured makes figures merely approximate.   However

the error is probably common to all cases, and should not vitiate the results to any great extent.

Of these cases 51 were those of complicated gonorrhoea, the lesions being subacute or chronic, and the duration was estimated as nearly as might be. It was found that on the average the C. F. test became negative in 3.3 months, the lesions averaging 4.9 months in duration, reckoning the time from the first intimation of a complication to the date on which no further evidence of such inflammatory disturbance was found.

In 13 cases where there was a single acute complication such as orchitis the test became negative in 3 months.

In 18 cases where these complications could be excluded the average time was 1.8 months.

The intensity of the serum reaction is greater in acute complicating lesions than chronic as a rule, which explains why the times for both types obtained are nearly the same. In some cases classed in the chronic group, where the lesion had not lasted long and yet the blood reaction persisted for a relatively long time, there are two possible explanations. Either the subacute complication in question was relatively acute or else there was a fallacy in the estimation of clinical cure. The results in the 51 chronic cases are presented here in graph form. It will be seen that the longer a lesion persists the longer will the C.F. reaction be demonstrable in the blood. The graph is obtained by averaging each period of a month, and the part enclosed by the circle includes over 70% of the total number of cases.

Duration of Chronic Lesion in Months.

75% of
cases.
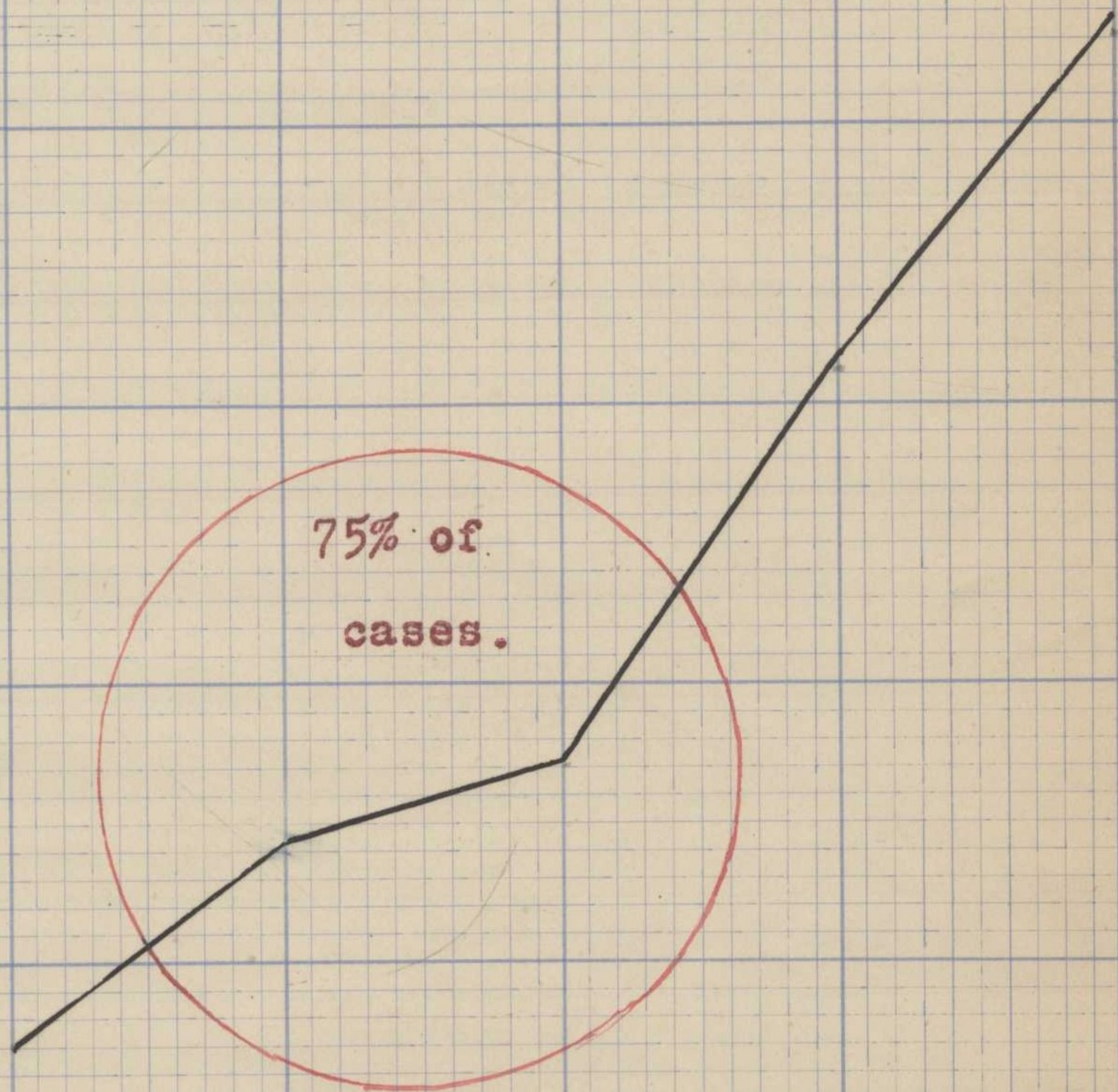
Time in months taken for C. F. Reaction to fall
from Positive to Negative.

Certainly sometime elapses before a serum examination reveals the fact of cure -- but does not the same apply to clinical methods also?   It would seem to be a wise precaution not to sanction the marriage of an individual whose serum gives a positive reaction.

Another question raised in considering the diagnostic value of this test is its relation to other bacteriological methods.   Ordinary urethral smears are admittedly of little use in chronic cases, where the organisms of secondary infections appear and the gonococcus, lurking in strongholds beneath the surface epithelial cells, is hard to find.   Films from the prostatic or vesicular contents are much more likely to contain gonococci, and these organisms may also be demonstrated, though less frequently, in the purulent threads in the first morning urine, by direct examination or by culture.   There has been no opportunity for carrying out culture as a routine method in this research, but some comparison may be made between other bacteriological methods in use at a clinic and the C.F. test.

From a series of 159 cases of chronic complicated gonorrhoea in men the following figures were compiled. They only refer to cases where microscopical examination at the time or subsequently revealed the gonococcus. Therefore they only institute comparison in cases of existing infection on the results of a single examination

	Gonococci found in			No Gonococci found in		
	U.D.	P.M.	M.U.	U.D.	P.M.	M.U.
C.F. Positive	9	54	17	7	22	38
C.F. Negative	2	6	0	2	I	I

U. D. -- smear from urethral discharge.

P. M. --　　"　　after prostatic massage.

M. U. -- film from centrifuged first morning urine.

In many of these cases the condition was latent, and there was practically no demonstrable discharge, hence the number of examinations of simple smears is small.

These results may be summarised as follows.　As the result of one examination, and considered in the light of the later history of the patient,

Microscopic examination	Positive }	
C.F. Test	Positive }	50%
Microscopic Examination	Negative }	
C.F. Test	Negative }	2.5%
Microscopic Examination	Positive }	
C.F. Test	Negative }	5%
Microscopic Examination	Negative }	
C.F. Test	Positive }	42.5%

It is not suggested that any great importance would be attached to single negative examinations for the gonococcus, but the use of a serum test might obviate a certain proportion of the weighty labour of repeated routine examinations of smears.　The examination of urine deposits in particular notably furnishes less positive results than that of a prostatic smear.　It may be remarked also that although

the presence of pus cells in the latter usually denote prostatic infection this need not be still gonococcal.

The relation of the serum examination to clinical cure has already been touched upon.

THE INTERPRETATION OF A NEGATIVE REACTION.

This may be due to a number of causes, viz.,

(I) a failure of technique, owing in general to lack of sensitiveness in the method employed, and often in particular to an imperfect antigen,

(2) a delimited area of disease in a mild infection,

(3) failure of the patient to elaborate antibodies due to low power of general resistance,

(4) a temporary negative phase e.g. after vaccines or as the immediate result of sudden exacerbation or metastasis as noted by Irons (3I), or due to imperfectly understood causes such as perhaps, an intermittency in the stimulus to or response in antibody production,

(5) possibly the establishing of a local tolerance by a chronic infection by an organism of low antigenetic power (to the individual patient) with a consequently slight or inappreciable immune response,

(6) an infection by organisms other than the gonococcus, or

(7) Cure.

The first cause can largely be eliminated by care and experience.   Where the case under investigation is one of apparent clinical cure repeated negative tests are of considerable value in estimating the genuineness of the"cure".

One negative is not sufficient, apart from the chance of technical error, as it may be due to the other causes suggested above.   It is in these cases that some form of

provocative test is useful, of the mild order such as sounds or prostatic massage, or of the severer type such as injection of vaccine or instillation of silver nitrate, if these latter be thought justifiable. A single dose of vaccine would hardly produce a reaction if at all in the blood of a normal person: in the subject of a latent gonorrhoea the response is well marked.

Bearing these points in mind a negative test must be regarded as distinctly helpful, particularly if it be preceded by a gradually falling reaction.

The C. F. test in gonorrhoea has this in common with all such laboratory tests. Apart from its inherent limitations it must be interpreted in the light of an understanding of the phenomena of immunity, and must be regarded as an additional link in the chain of evidence and not as an easy solution of the difficulties of the clinician.

PROGNOSTIC VALUE OF THE TEST.

It has been remarked by Dixon and Priestley (loc. cit.) and others that patients do not do well till the reaction is strongly positive. Certainly in the presence of a frank gonococcal infection a strongly positive C.F. reaction is a favourable sign. The subsequent gradual subsidence of the reaction to a negative in such a case is of happy import also, as stated. But the conjunction of a clinically severe or intractably chronic gonococcal infection with a feeble serum response in not of good omen. There is a type of case where a chronic gonorrhoea (e.g. a prostatitis) is accompanied by an invariably weak C.F. serum test. This is the type which drags on month after month, and may persist for years, never apparently severely affected, but never quite well.

I have observed 43 cases of chronic gonorrhoea, mostly cases of prostatitis, where the result of the serum test was classed as feebly, partially or doubtfully positive, or even as incompletely negative on repeated examinations. Nearly all these have been watched at intervals of months, during which time they were merely receiving a routine clinic treatment. On the average 3 tests were made at intervals on each patient, and in a number of cases as many as 5.

It is because of this that one cannot say that any individual is free from infection who is apparently cured and who still continues to have a feebly positive report returned on each examination of his serum.

Recently Thomsen and Vollmond (62) have described types of gonococci corresponding in some degree with the various clinical types of infection.   It may be that in the above cases the infective agent is of active or potential virulence to such patients but is yet to them of low antigenetic capacity.   In such cases the indication is to raise the general and specific resistance, and vaccine therapy should be of value here.

An instructive illustrative case is that of case 248, who on the occasion of the first C.F. test had been under treatment for 8 months for gonorrhoea complicated by chronic prostatitis.   On 7/7/2I he had no discharge for 6 weeks, no gonococci were found in prostatic secretion or morning urine deposit.   The C.F. test was "incompletely negative". On 28/7/2I the test was feebly positive;   one month and two months later the result was the same, there being no gonococci found on search as before.   Thus for 4 months he presented no sign of infection, and the C.F. test yielded a persistent feeble response.   On 5/I0/2I without further exposure to infection he was suddenly attacked with an acute gonococcal polyarthritis.   The C.F. test then became strongly positive.   This patient was treated with anti-gonococcal serum and made a good, and so far complete, recovery.

Brief accounts of several other cases are appended here to illustrate various points referred to above.
Case 5I. Gonorrhoea contracted 4/2/20: posterior urethritis and (6 months after) stricture.   Treated by dilatation: 4

4 months after appeared free from infection: no discharge
3 months: urine clear: prostatic film normal. C.F. re-
mained strongly positive.   Three months later occasional
return of slight muco-purulent discharge, with a few flakes
and small purulent threads in the urine.   Urethroscope
showed active inflammatory changes in an infiltrated area
of the urethra.

Case I4I. Chronic latent gonorrhoea for several years,
with recrudescences excited by alcohol.   These were only
of the nature of a watery "gleet".   It was questioned
whether this patient were still really suffering from
gonorrhoea or from a residual mixed infection.   A pos-
itive C.F. test was afterwards confirmed by more rigorous
bacteriological examination.

Case 86. (Showing fall from positive to negative). Anter-
ior and Posterior urethritis: urethral abscess 3/I2 previous-
ly.   C.F. strongly positive: no gonococci found in pros-
tatic film.

I/I2 later: Still no discharge, urethra normal, C.F. positive
3/I2 later: Quite well, nil found in prostatic or urine de-
      posit films: C.F. feebly positive.
2/I2 later: Few mucous threads in urine no gonococci found
      in film or culture.   C.F. negative.
2/I2 later: Quite well: C.F. Negative.

Case I44. Patient treated for frequency of micturition and
pain: pus in urine: prostate large and tender: no gonococci

found in prostatic secretion or urine deposit: no urethral
discharge. He positively denied not only the possibility
of infection but even any history of urethral discharge.
C.F. Positive. A week later he admitted infection some
months previously.

Case 344. Husband two undoubted attacks of gonorrhoea in 6/12:
cause not known. Wife on examination has slight endo-
cervicitis of indeterminate nature: C.F. of latter's serum
definitely positive.

RESULT OF TREATMENT ON THE TEST.

It is doubtful ~~per se~~ if treatment per se has any effect
except in so far as it advances the cure of the patient. As
the patient's resistance rises his C.F. reaction becomes
strongly positive and then begins to become less intense (in
the event of successful treatment) as the infection dies out.
Ordinary vaccines increase the intensity of the reaction
slightly when given in large doses: detoxicated vaccines
cause a large rise in the hypothetical complement-fixing
bodies, though the connections between this response and
clinical improvement is still doubtful.

The administration of anti-gonococcal serum also causes
a strongly positive reaction, and the application of the C.F.
test to the control of serum therapy will now be discussed.
This forms the basis of an enquiry which has been started in
order to ascertain the exact status of an antiserum in the

treatment of gonorrhoeal complications, and which is
still proceeding.

treatment of gonorrhoeal complications, and which is
still proceeding.

VI. APPLICATION OF C. F. TEST TO SERUM THERAPY.

As it has been established that the gonococcus falls into a number of serologically distinct groups, as evidenced by the work of Torrey and of Thomsen and Vollmond (loc. cit.) and recently of Hermanies (63), it is presumably necessary to represent all possible types in the strains used for preparing an antiserum.   That this is necessary is supported by the opinion of Flexner who maintains that prevalent strains should be used in the making of an efficient anti-meningococcal serum.   Calderola (64) bases a similar opinion on a recent serological study of the meningo-coccus and its near relatives.

The classification of gonococcal types cannot be regarded as settled definitely, and the Australian strains have never been typed.   Therefore, thanks to the interest and help of Dr. W. J. Penfold a special serum was produced at the Commonwealth Serum Laboratories in Melbourne, using 5 strains only to immunise a horse.   The animal employed for this purpose had not been used previously for the production of anti-meningococcal or -gonococcal sera.   The strains were freshly isolated in Melbourne from cases of active gonorrhoea.

This serum is of high agglutinative titre for all strains and also shows a high content of immune bodies by the C. F. test.   The 5 strains have been kept continuously subcultured both in Melbourne and in the Sydney University Pathological Department, so as to ensure a fresh and active antigen for

the necessary tests.

The patients selected for treatment have been suffering from acute or subacute complications of gonorrhoea. Prior to treatment a serum examination has been carried out in each case, a C.F. test being performed (a) with the special 5 strain antigen (b) with antigens prepared from at least IO distinct local strains.   In this way it was ascertained which patients fell within the category of those infected by a strain serologically homologous with the strains represented in the antiserum.

_Up to the present 30 patients have been tested for this particular purpose and serum treatment has been instituted. All of these have been suffering from acute (or in 2 cases subacute) complications of gonorrhoea and their serum in each case has given a positive C.F. response to a polyvalent antigen.   In 8 of these the response to the antigen made from the selected 5 strains only has been absent or feeble. Where the response ~~has been definite~~ has been definite with the truly polyvalent antigen and absent, indeterminate or feeble with the limited antigen it has been deemed correct to regard the patient as infected by a strain serologically distinct from the chosen group.

The other 22 patients have yielded a strong serum response to the 5 strains, in 4 cases a greater amount of complement being bound with these than with the larger number.

The question of the value of anti-gonococcal serum in treatment will not be dealt with at length here.   But on the whole the results have been encouraging, and while it

cannot be claimed that serum treatment will cure, in the sense of clearing up the cause of the complication (e.g. the urethral infection) it undoubtedly has a well marked ameliorative influence on the complicating lesion.   Thus the pain and local inflammation of salpingitis, orchitis, epididymitis or arthritis rapidly subside in most cases, and especially in the last named condition is the patient's recovery accelerated.   In all such cases allowance must be made for the improvement caused by rest alone - a difficult factor to estimate.   In several cases of acute prostatis the symptoms rapidly subsided, and two patients with acute retention of urine did not need a catheter after the first dose of serum.

But it is with the serological aspects that the present enquiry is most concerned.   One naturally demands the effect of the administration of antigonococcal serum on the serum response of a normal individual.

In three cases the special 5 strain serum was injected subcutaneously into persons who had no history of a gonococcal infection and who showed no signs on examination.   Previous blood examination failed to elicit a C.F. response to a gonococcal antigen in each case.

It was found that IO c.c. of this serum injected subcutaneously into an adult male or female produced a definite C.F. response which was easily demonstrable as a moderately strong reaction 7 days after and as a definite though weak reaction I4 days after.

In a child of II years of age 5 c.c. of serum produced a

definite reaction within 7 days.

The writer produced a demonstrable though feeble re-
action in his blood serum by taking 30 c.c. of serum by
mouth fasting.   This was detected as a feeble positive
test within 48 hours and began to subside after 5 days, in
10 days failing to respond to the ordinary technique.

Hewlett and Sternberg (65) and Aviragnet and
others (66) have failed to confer immunity/tetanus and
diphtheria by the administration of antitoxin by mouth,
but it is not contended that the transient appearance of
complement-fixing bodies in the blood means the coincidence
of immunity.

The effect of the serum when administered to patients
suffering from gonococcal infections was to enhance great-
ly the intensity of the C.F. test given by their serum.
After a total of 30 c.c. or over this was well marked, and
after 75 c.c. had been given in 4 doses it was found that
the blood reaction resembled the positive Wassermann test
as regards intensity.

This high grade of intensity in the reaction does not
persist for long apparently, and begins to fall within 14
days: as yet it is too soon to say when it disappears.

The result in the case of those whose infecting agent
did not fall   in the 5 strain category was also a definite
increase in the strength of the reaction, but hardly to the
same degree as in the others, so it seemed.

The clinical results in both groups are given in the

appended table.   It will be seen that the degree of im-
provement in the first group is much greater than in the
second.   The relapse of two cases in the second is of
great interest: although the number in this group is small
as yet the findings point to the necessity for an antiserum
to be serologically specific.   Although all antigonococcal
sera used for treatment are prepared from many strains
there has been lacking clinical and serological proof that
typing of the immunising strains is necessary to ensure the
true polyvalency of the serum.   The difference in the re-
sults of treatment in the two groups may best be summaris-
ed thus.   There was improvement due to rest and nursing in
most cases of both groups (the great majority being in-
patients of Hospitals): there was also a noticeably great-
er improvement and freedom from setbacks in the process of
recovery in the first group of cases.   In several cases in
the first group there was an undoubted rapid and marked change
for the better clearly following the serum: in the second
group no such clear-cut change was noted.

     This investigation is still proceeding, and it is hoped to
add to these results; in particular to extend the number of
Group II cases/a control.
         as

     Even up to the present the value of a serological investig-
ation in this branch of therapeutics is indicated.

GROUP I.

Lesion.	C.F.5 strain.	C.F.IO strain.	Serum.	Result.
Subacute Prostatitis	+ +	+ +	20 c.c.	Less pain and dysuria
Subacute prostatitis & arthritis	+ +	+ +	30 c.c.	Rapid clearing joint pains: prostate unchanged.
Cervicitis	+	+	20 c.c.	Definite lessening of discharge & inflammation
Cervicitis catarrhal Salpingitis	+++	++	30 c.c.	Definite change from profuse purulent to slight mucoid discharge: no pain
Arthritis ? Endocarditis	+++	+++	50 c.c.	Rapid lessening of pain & swelling: improved general condition
Tenosynovitis	++	++	75 c.c.	Doubtful improvement
Arthritis	+++	+++	75 c.c.	Rapid improvement walking in 14 days
Acute Prostatitis	+ +	+ +	75 c.c.	Rapid decrease in size & tenderness of prostate: no dysuria.
Orchitis	+++	+++	20 c.c.	Pain & swelling quickly subsided.
Arthritis & Cervicitis	+	+	40 c.c.	Improvement: resolution of joint pains.
Epididymitis	+++	+++	30 c.c.	Rapid cessation of pain and swelling
Prostatitis subacute	+ +	+++	20 c.c.	Retention of urine relieved at once: prostatic swelling subsided
Epididymitis	+ +	+ +	20 c.c.	Rapid subsidence of swelling.

Group I. cont.

Lesion	C.F.5 strain	C.F.10 strain	Serum	Result
Prostatitis Acute	+ + +	+ + +	50 c.c.	Almost complete re-solution in 14 days
Prostatitis Acute	+ +	+ +	50 c.c.	ditto
Arthritis	+ +	+ +	50 c.c.	Swelling joints gone: periarticular thick-ening much less: walk-ing well 14 days
Epididymo-orchitis	+ + +	+ + +	20 c.c.	Rapid cessation of pain and swelling
Orchitis	+ + +	+ +	20 c.c.	Practically subsided 14 days: no discharge
Orchitis	+ + +	+ + +	20 c.c.	Pain relieved at once: swelling quickly sub-sided
Epididymo-orchitis	+ +	+ +	20 c.c.	Some improvement swell-ing persists without pain 14 days after
Epididymitis	+ +	+ +	20 c.c.	Rapid relief pain & swelling
Epididymo-orchitis	+ + +	+ +	20 c.c.	ditto

GROUP II.

Lesion	C.F.5 strain	C.F.IO strain	Serum	Result
Epididymitis	±	++	20c.c.	No obvious effect: after 3 weeks resolution incomplete
Prostatitis subacute	±	+	25 c.c.	Unchanged
Cervicitis & Salpingitis	∓	++	40 c.c.	Temporary improvement: rapid relapse in tubal swelling
Arthritis	±	+++	50 c.c.	Definite but not rapid improvement
Subacute Prostatitis & Arthritis	±	++	30 c.c.	More comfort on walking: prostate unchanged
Epididymitis	∓	++	30 c.c.	Temporary improvement relapse after 5 days
Arthritis	?∓	+	50 c.c.	Improvement in joint: but relapse in other joints after IO days
Epididymitis	±	++	20 c.c.	Gradual improvement: thickening resolving slowly

VII. <u>SOME ASPECTS OF THE GENERAL IMMUNITY RELATIONS</u>

<u>OF THE GONOCOCCUS</u>.

I. <u>Group Relations</u>.

The close relationship of the Meningococcus, Micro-coccus Catarrhalis and Gonococcus is well known, and it is obvious thatxxx the question of Specificity of the C.F. test in gonorrhoea may arise.   The serological dis-tinctions between various individuals of each family in the group are readily observed, but there is a certain group relation more or less common to all.   Thus, if an animal be highly immunised to one member of the group a C. F. reaction may be elicited with antigens composed of the other members.

Antimeningococcal serum gives a strong reaction with a gonococcal antigen for instance.   This serum is sometimes prepared for the market from animals used previously for gonococcal serum, but rabbits injected with the meningo-coccus also show a reaction to the gonococcus.   To ascer-tain if this held good for the other well known related organism, the M. Catarrhalis, I have given a rabbit repeat-ed intra-peritoneal injections of this organism.   After 5 injections of increasing strength the serum yielded a marked C. F. reaction with a Catarrhalis antigen (suspen-sion in normal saline).   A well marked though less strong x reaction was also obtained with a gonococcal suspension as antigen.

Conversely a potent anti-gonococcal serum will deviate

complement in the presence of either the M. Meningitidis
or M. Catarrhalis.

The reaction described in the animal injected with
M. Catarrhalis is due to "group" subatances.   That this
is so was proved in this case by agglutination tests.   It
was found on test that the gonococcus absorbs only the
gonococcal agglutinins from an anticatarrhal serum, where-
as the catarrhalis absorbs all agglutinins.

In the case of the meningococcus there is but slight
practical bearing on the diagnosis of gonorrhoea.   Some
observers as noted by the Medical Research Committee (19)
and subsequently Bell and Harmer (67) have demonstrated
deflection of complement by a gonococcal antigen in the
serum of patients suffering from meningitis, but others
have failed to do so.   But there is no serological pro-
blem in diagnosis presented here, as the diseases in ques-
tion are quite distinct.

It may be remarked that there has not been complete uni-
formity in the results obtained by different workers in ex-
amining the cross relationship of these two organisms in
immunised animals.   Though Wollstein (68) like most/workers
later
demonstrated C. F. with anti-miningococcal serum and gono-
coccal antigen and vice versa, Vannod (69) did not confirm
this.   The latter did not use a suspension of cocci for his
antigen but an extract prepared almost indentically as
McNeil (loc. cit.) described his antigen for use in the
clinical test.   McNeil also noted that this preparation will

not react with a polyvalent anti-meningococcal serum, and Arkwright (70) observed that extracts of gono- or meningo- cocci were less satisfactory as antigens than plain suspen- sions when doing similar work.   McNeil claims greater speci- ficity for his antigen on this account, but it may not be al- together due to this, as the antigenic potency of the organ- isms is lowered somewhat in the course of extraction.

I have endeavoured to prepare an antigen which would be in this sense more truly specific than the whole bacteria from which it was derived, but without success.

With regard to the M. Catarrhalis it is possible that a different question may arise, viz., as to whether it could cause an infection which might cloud the diagnostic issues.

Thomas Ivy and B irdsall (7I) (72) have reported the demonstration of a C. F. reaction in a few cases, using M. Catarrhalis as an antigen.   This hardly detracts from the value of the test .   If one could find a positive C. F. test for the Catarrhalis organism in a patient suffering from a respiratory infection and at the same time obtain from the same serum a positive C.F. test for the gonococcus a new element of doubt would enter.   I am not able to bring for- ward any such proof.   Probably it would be necessary to employ many strains of Catarrhalis and examine the serum of many people in whose sputum the organism is found.   It is not certain to what degree this organism is patogenic, and proof would be required that the infections where it is found evoke an antibody response.   In the animal experiment des-

described above a gonococcal fixation test was only demonstrated when the animal showed ~~xxxxxxxxx~~ a high grade of immune response to the M. Catarrhalis.

Some writers (Thomas, Ivy and Birdsall (7I) and Gurd (73)) declare that this coccus is found in inflammations of the genital mucous membranes: whether this finding corresponds to that of Gougerott (74) who states that the gonococcus can exist saprophytically in its usual habitat without demonstrable lesion, might be considered.

But this may be laid down: if the M. Catarrhalis can cause confusion in the serological diagnosis of gonorrhoea it could only do so if the antibody response evoked by it in the patient's body fluids were a strong one.   This seems very unlikely.   In practice no case has been met with where the factors just discussed have caused confusion so far as could be ascertained.

2. **THE HUMAN BODY'S METHOD OF DEFENCE AGAINST THE GONOCOCCUS.**

Certain suggestive points have been raised in the course of this research.

(a) **The rôle of the lipoids in immunity.**

Only the fringe of such a subject can be touched here. Warden (54)(55)(75) has reported good results from the use of a lipoidal antigen in the gonococcal C. F. test, and Kelly (36) has recently employed the same preparation with satisfaction. McNeil (loc. cit.) has not found such antigen to be specific.

Meyer (76) produced C. F. in the serum of rabbits injected with the acetone-insoluble lipoids of taeniae, and though Thiele and Embleton (77) found that the lipoids were non-specific and hence concluded they could not act as antigens, they confirmed Meyer's finding. The same Authors (loc. cit.) found that the removal of lipoids did not hinder precipitation or C. F. in immunological experiments, a conclusion confirmed by Vannod (78) who produced agglutination in rabbits by injections of nuclel protein obtained from gonococci; but they finally concluded that purified phosphatids cannot act as antigens in Bordet-Gengou reaction. These writers in later work (79) found no antibody formation to the fats or phosphatids of higher animal tissues, but produced precipitins from the phosphatids of the tubercle bacillus.

Bechtold (80) remarks that it is possible that lipoids

cannot be prepared pure, and that they may be adsorbed by colloids whose character they may simulate. He says further that it has not been possible (1919) to immunise with chemically known lipoids.

Warden and Connell (81) however, claim to have immunised animals against anthrax with an artificially synthesised fatty antigen, identical in composition with the anthrax fats, previously found by analysis.

It has been established by Wilson (82) and White (83) that pure lipoids cannot cause anaphylaxis, a phenomenon which appears to depend on protein substances.

Other evidence on the subject is rather conflicting. Richie and Miller (84) obtained no result from injection of lipoids of sheep serum and ox corpuscles; Wang (85) found no antigen in lipoids of egg white or horse serum, but demonstrated antigenetic power in those of dry blood, as evinced by the finding of a C. F. reaction in the serum of inoculated animals. Fink (86) regards proteins alone as the essentials in an antigen. Warden (55) produced a haemolysin from the lipoids of red corpuscles, and also obtained positive C. F. tests in typhoid with a fatty antigen.

Thus, although it seems doubtful if lipoids extracted from the higher animal tissues have antigenic power, there is sufficient evidence to/a corresponding further enquiry warrant into the behaviour of bacterial lipoids.

As pointed out above I have confirmed the statements of Warden and others that the fraction of gonococcal substance

extracted by lipoid solvents can act as an antigen/in the C.F. test.   Accordingly I made some further investigation into the relative position of this fraction and the remainder (protein fraction) of the gonococcus as antibody producers.

The method of ~~xxx~~ McLean and Thomas (87) for extracting fats from yeast was used as a basis.

Profuse 36 hour growths of a single strain of gonococcus were washed off slopes and flasks of Thomson's medium with the minimum amount of water.   The pasty mass was dried in a Petri dish 6 hours at 37° C and 48 hours at room temperature.   The dried residue was extracted with absolute alcohol, ground up with a glass rod, and agitated in a mechanical shaker to ensure fine division.   The residue after centrifugalisation was further extracted with equal volumes of absolute alcohol and ether.   This was repeated with the same solvent, and again with ether, the remainder being then dried at room temperature.   Final extraction with alcohol and ether was made under negative pressure.   The solvents were added together, centrifuged and filtered twice and evaporated under negative pressure.   The lipoidal residue was redissolved and evaporated, and finally dissolved for use in absolute alcohol.

Emulsions of both fractions were tested with Osmic Acid to reveal unsaturated fatty acid ~~fxxxpxxtxix~~ compounds, and by the biuret reaction for protein.   The defatted residue caused no change of colour in the Osmic Acid, which was readily reduced by the alcoholic extract, but this latter

gave no biuret test.

Rabbits were inoculated intra-peritoneally with each of these products alone at weekly intervals: one rabbit was also treated with a mixture of both fractions, and one with the whole gonococcus of the same strain.

The quantities used corresponded in each case to two profuse slopes of gonococci for the initial dose, weekly increases of first 50% and then 100% being made.

The serum of each animal was tested for deviation of complement in the presence of each antigen, i.e. the whole bacterial substance, lipoidal and non-lipoidal fractions. The antigenic power of each preparation in C. F. Tests was confirmed by preliminary titration on anti-gonococcal serum, and the dilution used was not greater than one third of the ascertained anti-complementary dose.

After the fifth injection the most definite results were obtained: these are given below. The result obtained from the animal treated with both extracts followed that of the rabbit immunised to the whole gonococcus. It is not further considered here.

The technique of Thomson was followed in the tests, and two dilutions of each serum were tested.

The table shows the results expressed in haemolytic symbols.

The Sera called A. B and C are those respectively of the animals injected with lipoids, non lipoids and whole gonococci. Tubes I, 2 and 3 contain respectively $2\frac{1}{2}$, 3 and $3\frac{1}{2}$ M.H.D. complement.

Serum	No. of Tube	Lipoid Antigen	Lipoid-free Antigen	Suspension of Gonococci
A	I	n	c	n
	2	p	c	n
	3	c	c	tr
B	I	c	n	n
	2	c	tr	n
	3	c	n.c.	n
C	I	n	n	n
	2	tr .	n	n
	3	sl	sl	n

Thus the lipoid inoculations evoked a C. F. response to the whole bacterial substance and slightly to the lipoid fraction, but none to the lipoid-free portion.

The non-lipoidal fraction caused a serum reaction with its own antigen and with the whole gonococci, but none to a lipoidal antigen.

Both antigens react with the immune serum produced by injections of the original bacteria.

Thiele and Embleton in their work referred to above obtained similar results with tubercle, but found that tubercle phosphatids evoked a response not only with the phosphatid and with the whole tubercle antigens but with the fat-free also. In the present instance the failure to elicit a C. F. reaction with gonococcal protein alone in the serum of an animal "immunised" with gonococcal lipoids would seem at least to

indicate that little or no protein was present in the al-
coholic extract.   The degree of "immunisation" may not
have been so great, or perhaps the case may not even be
paralell with that of tubercle, an organism richer in fatty
substances, and with certain affinities to the higher bac-
terial forms.

Tests were also made with the sera A and B against an-
tigens consisting of suspensions of M. Catarrhalis and
Meningitidis.   Serum A showed a slight reaction with both:
the reaction of serum B was doubtful in each case, and in an
ordinary test could not be classed as positive.   This would
indicate that the lipoids are the less truly specific por-
tion of this organism.

The conclusions drawn from these experiments are:--

(I)   that the gonococcus calls forth a so-called im-
munity response in the tissue fluids which can be recog-
nised by a C. F. test, using either protein or lipoidal
portion of the organism as antigen.

(2)   that the presence of either of these fractions in the
body fluids evokes a response which can also be recognised
by C. F. tests.

There is no evidence that the body can directly combat
a toxin produced by the gonococcus; but as there seems to be
humoral (and probably cellular) antagonism to either of the
chief constituents of the gonococcal cell these findings are
consistent with the occurrence of bacteriolysis in the human
body invaded by this microbe.   It is not suggested that ex-
perimental results are synonymous with true immunity, but

they at least offer a basis for speculation.

The position of lipoids in immunity, though perhaps exaggerated by some, cannot be entirely ignored. There is always the possibility of a protein-lipoid complex being involved, and the recent ultra-microscopic work of Bechtold, Salen, Hattory and others (88) on the mechanism of haemolysis is extremely suggestive.

Before passing from the subject of lipoids reference may be made to the claim made by Stuber, quoted by Krumweide and Noble (89), that agglutinins could be extracted by lipoid solvents. The latter workers could obtain no confirmation of his results, any agglutination obtained being in their opinion non specific.

I have tried to extract immune bodies from a potent anti-gonococcal serum with absolute alcohol and ether, but without success. Repeated extraction (with periodic shaking) was made with each solvent, and the extract evaporated to dryness, taken up with a little alcohol and emulsified in saline. The anti-complementary dose of this extract was found, but no binding of complement was observed with one third or even one half this amount in the presence of a good polyvalent gonococcal antigen.

On adding the extract to normal horse serum a negative result was also obtained.

(b)   **The passive immunity conferred by administration**
of antigonococcal Serum.

It has been remarked that an anti-gonococcal serum capable
of producing clinical results contained a high concentration
of immune bodies (in a dilution of 1/80 or 1/100 it will
fix complement with its appropriate antigen): also that
the serum of patients treated with it displays a much en-
hanced intensity of C. F. reaction.   In treating patients
thus a febrile disturbance was nearly always noted after
injecting the serum, which lasted several days.   This frequent-
ly seemed to be part of the reaction to a foreign protein,
being accompanied by urticaria, patchy subcutaneous oedema
and joint pains.   It occurred irrespective of whether the
patient's previous course was afebrile or not.   But a
temperature rise was not always associated with signs of
serum sickness, and in several cases an irregular tempera-
ture was xxxx the only sign of disturbance after large doses
of serum.

Examination of the blood at this time often revealed an
anti-complementary state of the serum, which was at first re-
garded as accidental.   But it was observed in a number of
cases that a serum which had previously been of average be-
haviour in regard to its affinity for complement required more
than ordinary dilution to ensure lysis in serum controls.
Such serum was clear, and free from haemoglobin or infection,
and gave a very strong response to the C. F. test.

The possibility was considered of the presence of small

quantities of gonococcal antigen in the blood stream causing
this condition.   It this were so it might be possible for the
serum in certain concentrations to bind complement alone,
and in appropriate dilutions to do so when reinforced by
either antigen or antibody.

Whether or no this be the explanation of this observ-
ation the sera of a number of patients contained demonstrable
antigen, as snown by the following test.   Besides the in-
gredients shown in the table each tube contained 3 M.H.D.
complement and fully sensitised sheep's corpuscles.

Serum for test	Anti-gonococcal Serum	Normal Horse Serum	Result
0.0I	0.0I	--	n
0.005	0.0I	--	tn
0.0I	0.005	--	tr
0.005	0.005	--	sl
0.0I	--	0.02	c
0.005	--	0.02	c
0.02	--	--	c
--	0.02	--	c
--	--	0.02	c

In other tests the amount of complement absorbed by each ser-
um was ascertained and care was taken then not to use in the
test a quantity greater than one third of this.

This finding has been confirmed in seven sera: other
sera of patients suffering from a complication of gonorrhoea
but not receiving serum gave no reaction.   These latter sera

have been used as the positive serum in the above tests as
well as the original anti-gonococcal serum: in all the re-
sult was the same.

The interpretation of this phenomenon would appear to be
that the sudden access of homologous immune bodies to the
site of infection causes an enhanced bacteriolysis, thereby
setting free disintegrated gonococcal substance.   There is no
evidence that this is actually neutralised, but it is possible
that when bacterial cells are broken down complete elimination
or molecular disintegration is eventually effected by the tis-
sues, a process whose completion might be delayed if the at-
tack were massive, liberating a quantity of bacterial protein.

Torrey (I5), (I6), (I7), produced some evidence in fav-
our of the power of immune bodies in gonococcal sera to ef-
fect bacteriolysis, though he proved that this phenomenon did
not always run parallel with agglutination or C. F.

Summing up: The therapeutic effect of an antigonococcal
serum seems to depend upon its power to bring about lysis of
the gonococcus in situ.   (It should be noted that patients
in whose sera antigen was demonstrated showed definite clinical
improvement).   This, taken with the ability of an animal
to display antagonism (in the serological sense) to isolat-
ed fractions of the gonococcus, leads us to believe that this
organism may initiate certain changes in the body fluids which
eventually lead to its destruction, whether such changes be
the formation of distinct antibodies or some alteration in the
physico-chemical state of the serum.   The question of

phagocytosis has not been touched upon in these considerations, but it is probably a factor in the successful combatting of the infection. Even if the humoral changes which are evidenc-ed by the immunity reactions of the serum merely facilitate phagocytosis the end result is apparently the same.

Finally reference may be made to the bearing of the C.F. reaction on the immunity of the patient. While it cannot be said that the actual immunity of an individual and the "immune response" of his serum in vitro are synonymous there are certain facts which suggest that nevertheless the re-sponse to the C. F. test is a certain measure of a patient's resistance.

The reaction is usually strongest in those who show the greatest clinically demonstrable resistance to their infection whose It is frequently ill-marked in those/efforts to dislodge their invader are feeble or unsuccessful, and an actual spread of infection in such patients (of the nature of an acute com-plication), accompanied by a vigorous response to a serum test test, is not infrequently the beginning of more successful ef-forts to cope with the disease. That the reaction disappears a few months after the dying out of the infection does not mean anything as regards the patient's immunity, which pro-bably stands then just where it did before the attack. The subsidence of the reaction apparently means that the antibodies are eliminated, and fresh formation ceases with the rout of the offending organism.

S U M M A R Y.

I.   Of the serum immunity reactions in gonococcal infect-
ions Complementary Fixation is the most suitable for
practical purposes of diagnosis.

2.   The C.F. test is of considerable value in the diagno-
sis of these infections, especially when obscure, and
is a very useful aid in prognosis and in confirmation of
cure.

3.   Where other reliable microscopic and clinical evidence
was available this test showed accurate agreement with
the diagnosis made by such means.   A positive test means
an active, latent or recently existing infection by the
gonococcus.   A negative test should be confirmed by re-
test preferably after general or local provocation.

4.   Summary of technique -- see above page. 25.

5.   The duration of a positive reaction is usually less than
than 2 months after cure in the absence of complications,
but usually persists 3 months or more in complicated or
chronic cases, the length of time depending upon the dur-
ation or severity of the infection.

6.   A feeble reaction in a patient not presenting signs of
infection does not of necessity mean that cure is being
established.   If this be truly so the reaction will
gradually disappear, but a persistent feeble reaction

may betoken a dormant infection.

7.   The C.F. reaction has been used to determine the suit-ability of patients for treatment with an anti-gonococcal serum prepared from a limited number of strains.   Evidence of the need for typing gonococci in order to secure efficient polyvalency of an anti-serum has thus been adduced.

8.   Work has been done to show that both lipoid and lipoid-free portion of the gonococcus can act as antigen, not only detecting the presence of a gonococcal infection, but also independently evoking an antibody response recognisable by C.F. when inoculated into animals.

9.   Gonococcal Antigen has been demonstrated to be present in the serum of patients successfully combatting an acute com-plication of gonorrhoea immediately after the administration of efficient doses of a potent anti-serum.

10.   From the last conclusions (8 and 9) it is inferred that the body attacks the gonococcus by a process of bacterio-lysis, and whether by phagocytosis or not, the act of re-sistance is rather the breaking up of the bacterial cell than any neutralisation of its toxins, the latter being as yet unproved to take place.

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References to Literature.

1. MUELLER & OPPENHEIM; Wien. klin. Wochenschr. 1906, XIX, 894.

2. BRUCK; Deutsche med. Wochenschr, 1906, XXXII, 1368.

3. DEAN; Lancet, 1917, I, 45.

4. SCHEER; Muenchen med. Wochenschr. Jan. 4 1921.

5. WELSH & CHAPMAN; Proc. Roy. Soc. N. S. W., XLIV .

6. HERROLD; J. Am. Med. Assoc. LXXVI, No. 4, Jan. 22, 1921.

7. ROBINSON & MEADER; J. Urol. 1921, IV, No. 6., 551.

8. KEIDEL & HURWITZ; J. Am. Med. Assoc. Oct. 1912.

9. ANGERER & STOTTER; Muench. Med. Wochenschr. LIX, 2035.

10. NOGUCHI; Jour. Am. Med. Assoc. 1912, LVIII, 1163.

11. EDINGTON; Lancet 1920, II, 340%

12. BERNSTEIN & SIMONS; Amer. J. Med. Sc. CXLII, 862.

13. TEAGUE & TORREY; J. Med. Research. XVI.

14. TORREY; J. Med. Research Vol. XVII.

15. TORREY; ditto vol. XVIII.

16. TORREY; ditto vol. XIX.

17. TORREY; ditto vol. XXII

18. SCHWARTZ & McNEIL; Am. J. Med. Sc. 1911, 693.

19. MEDICAL RESEARCH COMMITTEE; Special Report Series, No.19.

20. KOLMER; "Practical Text-book of Infection, Immunity & Specific Therapy", 1917.

21. SCHMIDT; J. Am. Med. Assoc. 1912, LVIII, 1307.

22. JONES & SIMONS; quoted J. Am. Med. Assoc., vol. LXII.

23. WARDEN; J. Am. Med. Assoc., Dec. 11, 1915.

24. LEES; Lancet, June 28, 1919.

25. LEES; Edin. Med. J., 191 9, XXIII.

26. PRIESTLEY; Lancet, 1919, I, 787.

27. DIXON & PRIESTLY; Lancet 1919, II,964.

28. MAGNER; Lancet, July 17, 1920.

29. SMITH & WILSON; J. Immunol. V, No. 6.

30. THOMAS & IVY; Arch. Int. Med, 1914, XIII, 143.

31. IRONS; Proc. Internat. Cong. Med, Lond, 191 3.

32. HARRISON; J. R. A. M. C., XXII, I, 125.

33. KEYES; J. A. M. A., LXXV, Nov. 13 1920.

34 HERROLD; J. Am. Med. Assoc., LXXVI, Jan. I, 1921.

35. LAILEY & CRUIKSHANK; Surg. Gyn. & Obst. XXXIII, No.4,414.

36. KELLY; N. W. Med.? 1919, XVIII, 187; quoted Abst. Bact.
               vol.IV, No. 5.

37. LEWIS; Lancet, XCVIII, Jan. 3, 1920.

38. OTTENBERG; J. Immunol., 1916, II, 39.

39. MEDICAL RESEARCH COMMITTEE; Special Report Series NO.14.

40. GOLDENBERG & FIELD; Comptes Rendus Soc. Biol., Nov. 6,
               1920.

41. HARRISON; Medical Annual I 920.

42. REPORTS ON PUBLIC HEALTH & MEDICAL SUBJECTS, Ministry of
               Health, No. I, 1920.

43. OWEN & MARTIN; J. Lab. Clin. Med., Jan. 1920.

44. BURDICK; Arch. Derm. Syph., Aug. 1920.

45. RHAMY; J. Indiana. State Med. Assoc., Aug. 15, 1919.

46. FAIRLEY & SULLIVAN; J. R. A. M. C.? 1919, 33.

47. BROWNING & McKENZIE; "Recent Methods in the Diagnosis
               & Treatment of Syphilis" 1911, p. 92.

48. NOGUCHI & BRONFENBRENNER; J. Exp. Med.; 1912, XV.

49. SCHMIDT; Lister Inst. Coll. Papers, No.II, Part I.

50. McNEIL; Coll. Studies Res. Lab. Dept. Pub. Health,
               N. Y. vol. VI, 1911.

51. BLAKE; J. Exp. Med., 1917, XXVI, 27.

52. OLITSKY & BERNSTEIN; J. Inf. Dis. 1916, XIX, 253.

53. MERKIVIJEW; Zeit. f. Immun. Ref., 1910, 864.

54. WARDEN; J. Am. Med. Assoc., LXV, Dec. II 1915.

55. WARDEN; J. Infect. Dis., 1918, XXII, 133.

56. PARK & WILLIAMS; "Pathogenic Bacteria" 1920.

57. DOUGLAS & FLEMING; Br. J. Exp. Path. II No. 3 June 1921.

58. SCHMIDT; J. Immunol., 1921, vol. XXVIII.

59. KRUMWEIDE & NOBLE; J. Immunol, 1918, p. I.

60. VENEREAL DISEASES ACT, AND REGULATIONS N. S. W. 1918.

61. CLARKSON; B. M. J. Sept. 24, 1921, 486.

62. THOMSEN & VOLLMOND; Hosp. Tid. Nov. 1920. quoted B. M. J
                        Feb. 12, 1921.

63. HERMANIES; J. Inf. Dis. 1921, XXVIII, 133.

64. CALDAROLA; Ann. d'Ig. XXX 1920, quoted Abs. Path. Bact,
                        vol. IV, No. 5.

65. HEWLETT; Manual of Bacteriology, 7th Ed. 1921, p. 342.

66. AVIRAGNET; & others; Bull. Soc. Med. Hop. quoted B.M.J.
                        Oct. I, 1921.

67. BELL & HARMER; Lancet 1918, 43.

68. WOLLSTEIN; J. Exp. Med., IX? 1907.

69. VANNOD; Cent. f. Bakt, orig., XLIV.

70. ARKWRIGHT; J. Hyg. 1909, IX, 104.

71. THOMAS, IVY & BIRDSALL; Arch. Int. Med. 1915, XV.

72. THOMAS, IVY & BIRDSALL; Surg. Gyn. Obst. XIX, II, 190.

73. Gurd; J. Med. Res., XVIII.

74. GOUGEROT; Internal. J. Pub. Health, Sept. 1920, quoted
                        B. M. J. 3/11/20.

75. WARDEN; J. Lab. Clin. Med. 1916.

76. MEYER; Zeit. f. Immun., Orig., 1910, VII, 732.

77. THIELE; Zeit. f. Immun., Orig, 1913, XVI, 160.

78. VANNOD; Deutsch. Med. Wochenschr. XXXII, 1984.

79. THIELE & EMBLETON; J. Path. & Bact., 1915, XIX, No.3.

80. BECHTOLD; "Colloids in Biology & Medicine" 1919.

81. WARDEN & CONNELL; J. Inf. Dis. XXV, No. 5.

82. WILSON; J. Path. Bact. 1913, XVIII.

83. WHITE; J. Med. Research; 1914, XXX.

84. RITCHIE & MILLER; J. Path. Bact., 1913, XVII, 429.

85. WANG; J. Path. Bact. XXII, 3 and 4.

86. FINK; J. Inf. Dis. 1919, XXV, No. 2.

87. McLEAN & THOMAS; Biochem. J. 1920. XIV No. 3 and 4.

88. BECHTOLD; Sc. Am. Month., quoting from Salen; Bioch.
                        Zts. 1920, & Hattory; ditto 1921.

89. KRUMWEIDE & NOBLE; J. Immunol. 1921, VI No. 3.

ADDITIONAL   SUMMARY   TO   THESIS

FOR   M.D.   DEGREE

BY

ALLAN   SEYMOUR   WALKER   M.B.;Ch.M.

(Shewing original contributions

to the subject.)

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ADDITIONAL SUMMARY.

The work which forms the basis of this paper is in part confirmatory of conclusions arrived at by workers in the same field, and in part new, in the sense of breaking fresh ground.

The confirmatory work has been chiefly concerning the concordance of the clinical and bacteriological evidence of gonococcal infections with the results of serological tests. In this connection certain additional contributions have been made.

As the result of detailed investigation of the various methods of carrying out the Complement Fixation Test an attempt has been made to consolidate the technique of this test by adopting the following principles.

1. Multiple tube method, with rising doses of complement.

2. Use of a relatively dilute serum for test.

3. Inactivation of serum after dilution.

4. Long fixation in ice-box.

5. Titration of complement in the presence of antigen, with certain reservations in calculating the unit dose of complement on this basis (p. 17)

6. Necessity of recognising the importance of feeble reactions.

Contribution has also been made to the subject of Antigens and the relative claims of the different preparations have been examined; indication has thus been given as to the most reliable of these.

Amongst others recommended is (for the first time) an acetone-extracted antigen: the value of this is confirmed by the more recent observations of Douglas and Fleming on other bacteria.

Exact information has also been gathered correlating the C.F. Test with other methods of investigation, and establishing its value in the diagnosis of obscure and latent forms of Gonorrhoea, and in the estimation of cure.

Prognostic Value of C.F. Test.

While the favourable omen of a strongly positive C.F. Test has been more than once noted, evidence has been here brought forward as to the significance of a persistently feeble response as a sign of chronic infection, poorly combatted and difficult to treat, unless special means be taken to raise the general and local resistance.

Precipitin Test.

Work has been done on a new test for recognising specific gonococcal precipitins in a discharge (see p. 5). These observations were made prior to the publication of this test by Robinson and Meader in 1921, and are in the main consonant with the findings of these workers.

Lipoids in Immunity.

The capacity of the lipoids of the Gonococcus to act as an antigen in the C.F. test has been confirmed.

New ground has been broken here in demonstrating that the gonococcal lipoids can evoke some response in the body fluids(by injection) which can be recognised by the C.F. test. Parllel observations in the case of Tubercle are cited in the text.

Serum Treatment of the complications of Gonorrhoea.

In using a Five-Strain Serum for the treatment of complications, besides confirmng the value of this form of therapy, the following new facts have been elicited.

It has been possible to distinguish between the clinical results in cases infected by homologous and heterologous strains to these five. Though the need for the use of a number of strains in producing an efficient anti-serum has previously been assumed, this is the first direct evidence of the definite necessity for typing the G onococcus for this purpose.

It has also been shewn that nearly 80% of cases treated fell within this group of five, which is important in view of the claim of Torrey to have demonstrated 14 strains.

Additional light has also been shed on the modus operandi of such an anti-serum, confirming the view that an active bacteriolytic process is initiated or encouraged. It has been demonstrated that gonococcal antigen appears in the blood of patients shortly after the injection of the serum, indicating a breaking down of the bacterial invader.

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