THE PRECIPITIN REACTION IN THE DETERMINATION OF THE INFECTIVITY OF GONORRHEAL DISCHARGES

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After the subsidence of the acute symptoms of gonorrheal infection, a condition of chronic inflammation commonly results. This may persist over a long period of time. The demonstration of gonococci by either the culture or the smear method may be difficult or impossible. The complement-fixation test of the blood of treated or untreated patients may be negative. Such negative findings do not necessarily exclude continued infectiousness. There is need, therefore, of a method which could be easily carried out and which would be a better index as to the persistence of infectiousness than are the methods now available.

The work of Robinson and Meader,[1] who reported encouraging results with the application of the precipitin reaction to discharges of gonorrheal origin, seemed to offer a step in this direction. They state that extracts of discharges from gonorrheal inflammations give precipitin reactions with antigonococcus serum, even when examinations of smears of such discharges fail to reveal gonococci. We are indebted to them for an actual demonstration of their original methods which we used as follows:

Production of Immune or Precipitin Serum.—Rabbits whose serum diluted 1:2 did not give ring reactions with the control antigen (see below) were used for inoculation. They received intravenous injections of 1 c c of a suspension containing 500 million per c c of "live" gonococci for the first injection and increasingly larger doses 3 to 5 days apart; later, they were injected at weekly intervals to keep up the antibody content. Trial bleedings, tested against the specific control antigen (see below) showed that the animals varied in their ability to produce precipitins; about 10 injections were necessary before the immune serum could be used. The animals were bled whenever a new supply of serum was needed. Robinson and Meader used the Brady strains 1, 2 and 3 for immunization; we used the 10 Torrey strains.

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[1] Jour. Urol., 1920, 4, p. 551.

We are indebted to Robinson and Meader for a sample of the immune serum which they were using. This gave us the means of determining that the serums we prepared were at least equally as potent as theirs as determined with our gonococcus antigens. With antigens prepared from the Torrey strains both their serum and ours always gave clearly visible reactions when diluted to 1 : 80. Dilutions to 1 : 160 gave faint reactions which appeared irregularly in successive tests.

Antigen for Control Tests.—The 48-hour growth of the gonococcus on North medium was scraped with a platinum loop into 5 c c of salt solution, autolyzed from 3 to 7 days, centrifugalized and the clear supernatant fluid used. The term antigen, unless otherwise specified, is used throughout to signify this specific gonococcus autolysate or precipitinogen.

We avoided the use of blood or serum mediums in the preparation of the gonococcus antigen in order first, to exclude a possible effect on the cultures; second, to avoid the possible transfer of serum or blood to the suspension to be used in the preparation of the antigen. Robinson and Meader used blood agar. The question of the possible influence of the medium on the antigen is noted later.

Material from the Cases to be Tested.—Secretion from the vagina or cervix was obtained on a cotton swab. This was rubbed in 2 c c of salt solution and the mixture incubated over night. In some instances, which are noted, we used salt solution containing 0.3% phenol (carbolic acid). The purpose of incubation was to autolyze the gonococci if they were present. On the following morning, the mixture was centrifugalized. This clear supernatant fluid might or might not contain gonococcus precipitinogen, as the term antigen or even extract is not really applicable, unless one may be justified in using this term for a reagent to be tested. Because of the difficulty of finding a suitable term, "discharge extract" is used throughout to signify the clarified test material. This term is also used for specimens not of gonorrheal origin.

The Reaction to Determine the Presence of Gonococcus Substance.—Two-tenths c c of the "discharge extract" was layered or floated over 0.2 c c of a 1 : 2 dilution of immune serum. A control of the "discharge extract" with a 1 : 2 dilution of normal rabbit serum was also made. The specific antigen as well as salt solution was employed with a 1 : 2 dilution of both immune and normal serums to serve as further controls.

All were placed in the water bath for one hour at 40 C. and then in the icebox for one hour. Observations were then made. The tests were returned to the refrigerator and observed again the following morning. The development of a contact ring of varying opacity and thickness at the line of contact between serum and superimposed clear fluid was recorded as a positive precipitin reaction.

In applying these methods we found that in some instances a ring reaction was not apparent until the tests had been kept in the icebox over night. In other instances, the ring observed after one hour's incubation and one hour in the refrigerator had disappeared when the test was again observed on the following morning. With few exceptions, the disappearance of the ring was accompanied by the development of a precipitate, the few exceptions being almost entirely with normal serum. In other instances a ring reaction was never observed,

TABLE 1

DISCHARGES, GONORRHEAL, SMEARS POSITIVE

Number of Specimens	Precipitin Reaction			
	Normal Serum		Immune Serum	
	Percentage Positive	Percentage Negative	Percentage Positive	Percentage Negative
92	21.7	78.3	82.6	17.4

The specimens from male cases (52) were extracted in salt solution containing 0.3% phenol. The proportion of nonspecific reactions with these and with normal serum was practically the same as with the specimens from females when no phenol was added.

but a precipitate was visible after holding the tests over night. For these reasons the later tests were observed only after refrigeration over night. All other factors excluded, there was another reason for this procedure: Only after this longer period was the percentage of positive reactions with the immune serum sufficiently high from the practical standpoint. Further discussion of this point is given later. The following tables include both ring and precipitate reaction readings as positive, although precipitate readings were not taken at the very beginning of our study.

The first step in our investigations was to apply the test to the discharges of male and female patients; 92 were tested. The smears from these discharges were all positive.

The results in this series of tests (table 1) were disappointing. In spite of the fact that all of the smears were positive and that the majority showed abundant gonococci, only 82.6% gave positive reac-

tions with the specific serum; 17.4% gave negative reactions. With normal serum, 78.3% gave no reaction but 21.7% gave reactions. These reactions were unexpected and disconcerting. If the reactions obtained with normal serum (21.7%) were deducted from those obtained with immune serum (82.6%), the reactions presumably specific would be about 60%.

The results with the "smear negative" patients, the type in which a successful application of the method would be more important, are likewise disappointing. As seen in table 2, the positive results with the immune serum fall to 61%, and the positive reactions with the normal serum rise to 51%. This leaves us with only about 10% of positive reactions which might presumably be considered as specific in character.

Even the reactions which appear superficially to be specific in character and the percentage of which seems to correlate with the smear findings cannot be accepted as such without further analysis.

TABLE 2

DISCHARGES, CLINICALLY GONORRHEAL, SMEARS NEGATIVE

Number of Specimens	Precipitin Reaction			
	Normal Serum		Immune Serum	
	Percentage Positive	Percentage Negative	Percentage Positive	Percentage Negative
49	51	49	61	39

One observation shows this clearly, namely, that of several normal rabbit serums one or more may give a positive reaction, the others not. In other words, the results of the tests are dependent to a considerable extent on the sample of normal rabbit serum used as a control.

Evidently even if specific reactions are present, the results are obscured by a nonspecific factor. The following tests were made in the attempt to determine what this factor was and whether it could be eliminated: Vaginal secretions were obtained from supposedly nongonorrheal children; at the same time, nasal secretions were collected from the same children for further controls. Nasopharyngeal secretions from adults were also obtained. A miscellaneous group of specimens was also tested; one pleuritic fluid; 6 sputums; 6 samples of pus, 2 each from tuberculous, streptococcal and staphylococcal abscesses. Other specimens not of human origin were also included; they were the washings from the peritoneum of one normal and 5 inoculated mice.

The results with these specimens of nongonorrheal origin are contrasted in table 3 with the total number of specimens obtained from presumably gonorrheal sources. The striking point in this table is the high percentage of positive reactions with nongonorrheal material. These reactions occurred with both the normal and the immune serum. If the former are deducted from the latter, it gives the untenable conclusion that from 5 to 20% of these specimens were gonorrheal. This shows at once that the subtraction method is not justified as a criterion of the significance of the reactions. The nonspecific reactions obtained with nongonorrheal material indicate that the method as employed did not yield reliable results.

TABLE 3

COMPARISON OF REACTIONS WITH MATERIAL FROM GONORRHEAL AND NONGONORRHEAL SOURCES

Extracts of Secretions	Number of Specimens	Precipitin Reaction			
		Normal Serum		Immune Serum	
		Percentage Positive	Percentage Negative	Percentage Positive	Percentage Negative
Suspected gonorrheal	174*	24.3	75.7	69.2	30.8
Nongonorrheal vaginal	17	94.1	5.9	100.0	0
Nose and throat	21	66.7	33.3	81.0	19.0
Miscellaneous	19	73.7	26.3	94.7	5.3

* Includes cases on which no smear examinations were made.

The nonspecific reactions with material of other than gonorrheal origin were shown to Robinson and Meader. They had not observed such a percentage of nonspecific reactions and suggested that this factor might be eliminated by greater dilution of the serum or the extract, or of both.

The results of tests made with a higher dilution (1:4) of serum and diluted "discharge extracts" are given in table 4. Although the tests with specimens from gonorrhea are at first glance favorable, the results with the other specimens indicate how little reliance can be placed on the dilution method. The results with the two nasal specimens are especially noteworthy.

In all the preceding work no attempt was made to have the mixture of secretion and of salt solution uniform in turbidity prior to centrifuging. The question arose whether the contradictory results obtained might not be influenced by this factor of irregularity. A turbidity control was prepared which consisted of a suspension of gonococci containing 2,000 million organisms per c c. The control gonococcus

antigen originally of the same turbidity reacted in a dilution of 1:5 with a 1:5 and with a 1:10 dilution of the immune serum, not in higher serum dilutions. The standardized specimens were tested, therefore, in these dilutions, as it was unbelievable that the gonorrheal specimens could contain more gonorrheal substance than was contained in the suspension from which the gonococcus antigen was made. Two immune serums and 2 normal serums were used.

TABLE 4

REACTIONS WITH NORMAL AND IMMUNE SERUM DILUTED 1:4 AND GONORRHEAL AND NONGONORRHEAL MATERIAL

Specimen from	Normal Serum		Immune Serum	
	Extract Dilution	Result*	Extract Dilution	Result*
Nose	1-8	+	1-8	+
Nose	1-2	—	1-8	+
Vagina, nongonorrheal child	1-64	+	1-16	—
Cervix, gonorrheal smear positive	1-8	+	1-32	+
Cervix, gonorrheal smear positive	1-8	+	1-32	+
Cervix, gonorrheal smear negative	1-8	+	1-32	+
Cervix, gonorrheal smear negative	1-8	+	1-32	+

* Highest dilution of extract in which a positive result was obtained or lowest dilution in which a negative result was obtained is recorded.

Symbols used in this and the following tables: ++ = marked reaction; + = moderate reaction; ± = slight reaction; ± = very slight reaction; — = no reaction.

TABLE 5

REACTIONS WITH EXTRACTS STANDARDIZED BY TURBIDITY *

Specimens from	Normal Serum		Immune Serum	
	Rabbit a	Rabbit b	Rabbit c	Rabbit d
Tuberculous abscess, pus	+	+	+	+
Tuberculous abscess, pus	+	—	+	—
Urethra, male, gonorrhea†	—	—	(1:5) +	—
Urethra, male, gonorrhea†	—	—	+	+
Cervix, chronic, gonorrhea†	—	—	+	+
Cervix, chronic, gonorrhea†	—	—	+	+
Cervix, chronic, gonorrhea†	—	—	—	—
Cervix, chronic, gonorrhea‡	+	+	+	+
Cervix, subacute, gonorrhea‡	+	+	+	+
Vaginal, normal‡	+	+	+	+

* Results refer to a dilution of serum of 1:10, except where otherwise noted, and a dilution of extract of 1:5.

† Smear positive.

‡ Smear negative.

The comparison between the results obtained with the gonorrheal cases showing negative smears and those showing positive smears, and of both these types of gonorrheal cases with the nongonorrheal cases, demonstrated that the standardization by opacity was of no value in excluding the nonspecific factor.

The next point studied was whether the nonspecific reaction was due to bacterial substance other than that of the gonococcus.

Suspensions of various bacteria were made in distilled water and the number of bacteria in the suspensions estimated by comparison of the turbidity of each with counted vaccines of the same bacterium. After a few drops of chloroform had been added to prevent multiplication of bacteria, these suspensions were placed in the incubator for 48 hours to autolyze. A concentrated salt phenol solution was then added to bring the salt content to 0.9%, and the phenol content to 0.3%. The equivalent content per c c after this dilution is given in table 6. The suspensions were then centrifuged and the clear supernatant fluid used for test. The results with 3 normal and 2 immune (gonococcus) serums are also shown in table 6.

TABLE 6

REACTIONS WITH GONOCOCCUS AND OTHER BACTERIAL ANTIGENS

Bacterial Antigens	Immune Serum								Normal Serum							
	Rabbit 344				Rabbit 354				Rabbit 321		Rabbit 369		Rabbit 371			
	1:5		1:10		1:5		1:10		1:5		1:10		1:5		1:10	
	R*	P†	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Torrey, 2,000 million	—	+	—	++	—	++	+	++	—	—	—	—	—	—	—	±	—	±
Brady, 2,000 million	—	+	—	±	—	±	—	+	—	—	—	—	—	—	—	±	—	±
Staphylococcus, 2,000 million	—	±	+	—	—	±	—	+	—	+	+	±	—	±	+	±	—	±
Streptococcus, 300 million	—	±	—	±	—	—	—	±	—	—	—	—	—	±	—	±	—	—
Pertussis,‡ 1,000 million	—	±	—	±	—	±	—	±	—	—	—	—	—	±	+	—	—	±
Pneumococcus, 100 million	—	±	—	+	—	—	±	—	—	—	—	—	—	—	—	±	—	±
Colon, 500 million	—	—	—	±	—	—	—	±	—	±	—	±	—	—	—	—	±	—
Catarrhalis,‡ 2,000 million	—	—	—	±	—	—	—	±	—	—	—	±	—	—	—	±	—	±
0.9% salt solution with 0.3% phenol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Ring.
† Precipitate.
‡ Grown or "chocolate" agar. Formula: Glycerol veal agar, neutral to phenolsulphonephthalein before autoclaving. About 5% normal citrated horse blood added while veal agar is about 85 C.

A considerable number of nonspecific reactions were obtained. They were relatively more frequent with the two immune serums. The reactions obtained with normal serums varied considerably. The staphylococcus gave the greatest number of nonspecific reactions. The amount of reaction obtained with the different antigens is recorded in this

table in quantitative terms. The degree of reaction obtained with the heterologous antigens is approximately equal to that obtained in the majority of tests with "discharge extracts." The lack of difference between the reactions obtained with the gonococcus serum with the gonococcus antigens and the reactions with the heterologous antigens is probably due to the fact that the time element was not considered. These tests were made not to determine the optimum conditions under which specific differences could be elicited with bacterial antigens but to determine to what degree nonspecific reactions would develop with the method as adopted for testing the "discharge extracts."

As noted, practical considerations led us to adopt the method of observing reactions only after the tests had been refrigerated over night. The results with the bacterial antigens would seem to indicate that the nonspecific factor was exaggerated by this method.

The overnight method was continued, however, because although the total number of reactions between normal serum and gonorrheal material or between immune serum and nongonorrheal material was increased, this increase was accompanied by a proportionate increase (doubled) in the positive results with the immune serums, and gonorrheal extracts. It seemed, therefore, better to use the method which would give the highest level of positive reactions between immune serum and gonorrheal material and then try to find a means of eliminating the nonspecific factor.

In connection with the results obtained with bacterial antigens it is of interest that extracts of the Brady strains, of relatively recent isolation, were somewhat less sensitive to precipitin action than were the Torrey strains which had been in cultivation for a long time. This naturally raised the question whether the gonococci obtained directly from man might not yield an autolysate which would be still more resistant to precipitin action. Possibly this might be a factor in the failure to obtain with a vaginal discharge, containing numerous gonococci, a reaction which in rapidity and quantity was decidedly greater than with nonspecific material.

The question then arose whether the nonspecific factor might not be limited by heating the extracts. That the reaction between precipitin and its specific precipitinogen (bacterial) is not influenced by heating the precipitinogen is generally accepted. We found no difference in precipitability with the gonococcic antigen whether heated or not heated, when tested with serum dilutions up to the point of extinction of reaction.

Heating of the heterologous bacterial antigens did not reduce appreciably the degree of nonspecific reactions (table 7).

The results obtained with heated and unheated "discharge extracts" are given in table 8. Included in this is the use of both distilled water and salt solution as extractives. Where the former was used the extracts were made isotonic before use by addition of an appropriate amount of concentrated salt solution. The suspensions were all of a uniform capacity before subsequent treatment.

As seen from table 8, there was no constancy in the results before and after heating the extracts, whether the original material showed gonococci in smears or not.

TABLE 7

EFFECT OF HEAT ON REACTIONS WITH BACTERIAL ANTIGENS

Bacterial Antigens	Immune Serum								Normal Serum			
	Rabbit 344				Rabbit 354				Rabbit 316			
	1:5		1:10		1:5		1:10		1:5		1:10	
	N*	H†	N	H	N	H	N	H	N	H	N	H
Torrey, 2,000 million	++	++	+	+	++	++	+	+	—	—	—	—
Brady, 2,000 million	+	+	+	0	+	+	+	0	—	—	—	0
Streptococcus, 300 million	—	—	±	±	—	—	±	±	—	—	±	—
Pertussis, 1,000 million	±	±	—	—	—	—	±	±	—	—	±	—
Colon, 500 million	±	±	±	±	—	—	±	±	—	—	—	—
Pneumococcus, 100 million	—	—	±	±	—	—	±	±	—	—	—	—
Catarrhalis, 2,000 million	±	±	—	—	—	—	—	—	—	—	—	—
0.9% salt solution with 0.3% phenol	—	—	—	—	—	—	—	—	—	—	—	—

* Not heated. † Heated to 100 C. for 10 minutes. 0, No test, insufficient material.

Three factors—dilution of the extract, dilution of the serum and heating of the extract—were of no aid in excluding the nonspecific factor.

The persistence of reactions after the bacterial antigens or the "discharge extracts" had been heated to 100 C. for 10 minutes, seemingly answers the possible criticism that a precipitate due to bacterial growth during the time of extraction and test, might have simulated positive reactions, especially those which were obviously nonspecific.

Naturally, if the reactions were due to bacterial growth a reaction should always appear with both the immune and with the normal serum when both of the salt serum controls showed no evident reaction, thus

demonstrating the absence of bacterial growth originating from the serums. It is difficult to understand how a ring reaction, developing after one hour's incubation, or how a later ring reaction or sediment after 24 hours could be due to bacterial growth when the remaining fluid was nearly always clear. The appearance of reactions with extracts of pus aspirated from unopened cold abscesses is significant. It should also be noted (table 1) that "discharge extracts," containing 0.3% phenol, gave reactions.

TABLE 8

EFFECT OF HEAT ON REACTIONS WITH "DISCHARGE EXTRACTS"

Specimens from	Smear	Extracted With	Extract Diluted	Serum Diluted	Normal Serum Plus Extract		Immune Serum Plus Extract	
					Extract Not Heated	Extract Heated to 100 C. for 10 Min.	Extract Not Heated	Extract Heated to 100 C. for 10 Min.
Cervix, gonorrhea	..	Salt solution	—	1:5	+	—	+	±
		Distilled water	—	1:10	—	±	—	±
Cervix, gonorrhea	—	Salt solution	—	1:5	±	+	±	+
		Distilled water	—	1:10	—	+	—	+
Urethra, male, gonorrhea	—	Salt solution	—	1:5	±	±	±	—
		Distilled water	—	1:10	—	—	—	+
Urethra, male, gonorrhea	..	Salt solution	—	1:5	±	—	—	—
		Distilled water	—	1:10	—	—	±	±
Vagina, non-gonorrheal	—	Salt solution	—	1:5	—	—	±	—
		Distilled water	—	1:10	—	—	—	—
Cervix, gonorrhea	..	Distilled water	1:5	1:5	±	—	—	±
		Distilled water	1:5	1:10	—	—	±	—
Cervix, gonorrhea	—	Distilled water	1:5	1:5	—	—	+	—
		Distilled water	1:5	1:10	—	+	±	—
Cervix, gonorrhea	—	Distilled water	1:5	1:5	+	—	+	—
		Distilled water	1:5	1:10	+	—	±	—
Urethra, male, gonorrhea	..	Distilled water	1:5	1:5	—	±	—	±
		Distilled water	1:5	1:10	—	—	—	—
Urethra, male, gonorrhea	—	Distilled water	1:5	1:5	—	±	—	±
		Distilled water	1:5	1:10	—	±	±	±
Vagina, non-gonorrheal	—	Distilled water	1:5	1:5	+	—	+	—
		Distilled water	1:5	1:10	+	—	+	—

Distilled water extracts made isotonic with salt before use.

For the direct demonstration that bacterial growth was not a factor in the reactions and especially in the reactions with nonspecific material, extracts clarified by prolonged centrifuging were tested with immune and with normal serum and then tested culturally. The materials extracted were obtained from tuberculous abscess, 3 specimens; urethral discharge (male), 4 specimens; and nasal discharge, 2 specimens.

The reactions with the immune and with the normal serums were the same as those previously obtained with similar material. After the tests were completed the contents of each tube were added to melted serum agar and the mixture poured into a petri dish. The majority of the tests yielded no growth. Some showed the development of from 1 to 5 colonies, obviously an insufficient bacterial content to simulate a reaction.

Robinson and Meader have recently attempted to eliminate the nonspecific factor by absorbing gonococcus immune serum with a mixed antigen of Sarcina lutea and Staphylococcus aureus. They have found that these two organisms gave nonspecific reactions.

We have absorbed three samples of our gonococcus immune serum with suspensions of gonococci, meningococci and staphylococci, respectively. When the antigonococcus serum was absorbed by the heterologous types, the precipitin content for the gonococcus was reduced either very slightly or not at all, whereas the precipitin content for the absorbing type was completely or nearly completely removed. When the serum was absorbed by the gonococcus to the point where the precipitins for this organism were removed, the degree of reaction with antigens of the staphylococcus and of the meningococcus was reduced either very slightly—or not at all. This indicates that the reactions obtained with these antigens had no relation to the specific immunization by the gonococcus.

The absorbed serums were also tested with extracts of material from (1) urethra, gonorrheal, smear positive, male; (2) cervix, gonorrheal, smear positive; (3) cervix, gonorrheal, smear negative; (4) vagina, nongonorrheal; (5) tuberculous abscess.

With only one of the extracts, and that from a gonorrheal case (3), did the absorption of the gonococcus immune serum by the staphylococcus or by the meningococcus, result in a diminution of the reaction. As stated, the serums thus absorbed no longer reacted with the staphylococcus or the meningococcus antigen, or at most reacted only slightly.

With the immune serum absorbed by the gonococcus, only one specimen, that from case 3, failed to give a reaction. The extracts from the other two gonorrheal cases still gave undiminished reactions. We have, therefore, the striking result that a gonococcus serum absorbed to the point where it no longer reacts with a specific gonococcus antigen still reacts with the "discharge extracts" from two gonorrheal cases showing positive smears.

These results indicate that some reacting substance in the extracts, other than of bacterial origin, is most frequently responsible for the nonspecific reactions.

Some observations on the reactions obtained with antigens prepared in different ways are suggestive in this connection. Robinson and Meader prepared their antigens by growing the gonococcus on blood agar. They obtained reactions with immune serums in extraordinary high dilutions. On the other hand, with gonococcus antigens prepared from North medium cultures, we obtained reactions with our immune serums and even with their immune serum in dilutions of only 1:80 or slightly higher. These observations indicated that the blood in the medium might influence the results. We prepared two antigens, therefore, with the same medium, using rabbit blood in one case and not in the other. With the medium containing no blood, the resultant antigens reacted slightly with one [2] immune serum as high as 1:100, with 3 others diluted 1:50. With the medium containing blood, the resultant antigens reacted when these 4 serums were diluted 1:500 to 1:1,000 and with 2 of these serums even in dilutions of 1:2,000. One of these rabbits was immunized with vaccine grown on blood medium, the other with vaccine grown on medium without blood. Diluted normal rabbit serum likewise gave a reaction when added instead of the gonococcus antigens to the gonococcus immune serums used in the foregoing tests. Normal rabbit serum diluted 1:10 gave a reaction with one immune serum obtained from Robinson and Meader diluted 1:250.

These observations indicated that the addition of blood to the medium might be a factor in the reaction. The importance of this factor varied apparently with the serums of the individual immune rabbits.

Because of the variation noted with the gonococcus antigens, the experiment was repeated with the serums from 4 rabbits immunized with a vaccine grown on blood-free medium and from one rabbit immunized with vaccine grown on blood mediums. The results with the serums from all 5 rabbits have shown an average lower reaction (1:40) with blood-medium grown antigens as compared with the reaction (1:60) with antigens grown on blood-free medium, these results differing very much from those obtained in the experiment outlined in the foregoing.

As has been previously noted, the serums from normal rabbits vary considerably as regards reactions with bacterial antigens, including gonococcal, or with "discharge extracts." The two series of experi-

[2] Sample of serum used by Robinson and Meader in most of their tests.

ments with animals immunized by the injection of gonococci show, a still more marked variation. The fact that certain rabbits after immunization give materially higher reactions with blood grown antigens than with antigens prepared with blood-free mediums indicates that the nonspecific reactions obtained with "discharge extracts" are due to some substance present in the extracts which give such reactions.

The fact that the sample of serum received from Robinson and Meader, which they employed for most of their tests, gave very high reactions with the blood-grown antigen seems significant in view of their high incidence of positive results with "discharge extracts."

The heating of blood-grown antigens reduced the reactivity of these antigens slightly, if at all. This was true even when the blood-grown antigen reacted with the immune serum in dilutions as high as 1 : 1,000.

SUMMARY

Although reactions are obtained when extracts of discharges from gonorrheal patients are added to the serum of a rabbit immunized with gonococci, similar reactions are frequently encountered with the serums of normal rabbits. Likewise, extracts of material from the genital organs, known not to be infected with the gonococcus, as well as extracts of exudates due to infection by other organisms, give reactions with both antigonococcus serum and normal serum.

Attempts to exclude the nonspecific factor and thus obtain a specific reaction have failed. Dilution of serum of extract or of both, has not served to differentiate between a specific and a nonspecific reaction. Likewise, absorption of the antigonococcus serum by heterologous bacteria has failed to eliminate the nonspecific factor.

This nonspecific factor has obscured any reaction which might have occurred between the gonococcus serum and any gonococcus precipitinogen which might have been present in the known gonorrheal specimen.

Comparison between the probable amount of gonococcus substance present in the specimens as indicated by smear examination with the number of organisms necessary in a suspension to give a satisfactory precipitating antigen, raises a strong presumption that a specific precipitate development infrequently if at all.

When the gonococcus precipitins were absorbed from the serum, there was no uniform diminution in the reaction obtained with extracts from gonorrheal cases.

The precipitin reaction as recommended by Robinson and Meader is not applicable for the determination of the presence of the gonococcus in discharges from the cervix, urethra, etc.