THE SEROLOGICAL CLASSIFICATION OF GONOCOCCI BY COMPARATIVE AGGLUTINATION

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Received for publication Jan. 11, 1937

It is only recently that serological methods have attained practical significance in the diagnosis of gonococcus types. Torrey (1907) and Teague and Torrey (1907) demonstrated differences of type in different strains of gonococci by agglutination as well as by complement fixation and, in addition, by precipitation in rabbit immune sera. They examined 10 strains, of which 6 could be classified into 3 serological groups. In a later investigation, Torrey and Buckell, (1922) by serological examination, endeavored to obtain data concerning the frequency of the individual types in a large number (77) of strains, some of which were twelve years old. Since they encountered great difficulties in the production of suitable diagnostic sera, they obtained unsatisfactory results both by agglutination and absorption. They assumed that the gonococcus antigen is very unstable as they did not find any sharp differences of types in the 3 serological groups (regular, intermediate, irregular) which they finally established. They found several strains whose sera had a very great range of reaction so that the majority of the different strains of gonococci examined were able to absorb several of these 9 sera for the homologous strains. On the other hand, it was not found that all the strains which furnished the overlapping and apparently very good sera, were identical. They were by no means able to absorb all the antisera of those strains which, on their part, had absorbed these “sera with wide antigenic valency.”

These authors, therefore, could not combine all the strains which absorbed exactly the same sera, into one group as the
groups would overlap with each other. They finally assigned 39 strains which had absorbed the majority of the 9 sera to the "regular" group, 18 strains which had only partially or totally absorbed a few of the nine sera to the "intermediate" group and 19 strains which had absorbed none of the sera to the "irregular" group. They also observed that with cultivation for a longer period of time an irregular type changed into a regular type. They stated, therefore, that, despite the unstable nature of the gonococcus antigen, there is a general tendency to revert to the regular type. Consequently, they believe that it is not logically permissible to speak of "types" per se.

The investigations of several other authors have not led to concordant answers to this question. Direct examination of patients' sera by the commonly used antigens of "wide valency" could not, for understandable reasons, lead to definite conclusions (experiments of M. Stern).

Tulloch (1922) examined only freshly isolated strains from acute and subacute gonorrheal urethritis of males for their types, using only one high titer rabbit immune serum and its homologous strain. With this antiserum he selected those strains which, by absorption, decreased the titer of the immune serum for its homologous strain to \( \frac{1}{3} \) or \( \frac{1}{4} \) of its original value. These strains were assigned to type I. He found 43 strains which decreased the titer to less than \( \frac{1}{4} \) and 29 "closely related" strains which decreased the titer to less than \( \frac{1}{2} \) of its original value. The remaining 28 strains did not influence the titer of the type I serum at all or only lowered it to \( \frac{1}{2} \). At the end of both series, the remaining strains were further classified by absorption, using antisera some of which were obtained from old and some from freshly isolated heterologous strains. Twenty strains could be identified by means of 4 sera, 3 of which certainly belonged to completely different serological groups. Eight strains remained uncertain. Nevertheless, Tulloch classified his type I strains according to the height of titer of agglutination.

Atkin (1925) has objected not only to the investigations of Torrey and Buckell but, in general, to the entire serological classification. This author had already applied cultural methods of differentiation on special media (alkaline pea-broth agar in a
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Semi-solid state) to meningococci with particular reference to colony morphology. He made a comparison between Gordon's type II meningococcus and a strain of the same author's type I, which had been cultivated for a long period of time, and showed that such a degenerated type I could not be differentiated from a type II on the basis of colony morphology. When incubated for a long period of time on this culture medium, the shape of the freshly isolated and undegenerated type I could be easily distinguished because it formed papillae and exhibited a characteristic form of colony with a halo while type II had neither papillae nor halo.

Atkin found quite analogous varieties of gonococci and has established a parallelism of sharp serological differences between the antisera of both of these colony types. He was able to show, in a comparative study of a great number of chronic and of some acute cases of gonorrhea, that gonococci which had been isolated from chronic cases were acted upon by a type II serum obtained from a 2-year-old cervix strain which exhibited all the characteristics of a degenerated type I or were influenced by the sera of both types. Gonococci which had been isolated from acute cases were agglutinated either by the serum of a strain which formed papilla-bearing colonies (type I) or remained altogether unaffected by either serum.

However, these investigations did not clarify the question of the importance and frequency of the single types of gonococci.

Another method, namely, the cuti-reaction, has played an equally important rôle in the indirect demonstration of gonorrhea by immunological procedures. Because of the sensitivity of healthy individuals, however, this reaction was repeatedly rejected. This sensitivity was a consequence of the use of suspensions of gonococcus cultures or its soluble extracts as test antigens.

In a preceding paper (Casper, 1930) we have demonstrated that in a great number of cases a protein-free derivative which had been obtained from a gonococcus strain designated by us as type I, gave a strictly specific reaction, i.e., normal persons were not at all affected, while gonorrhreal individuals, as a rule, showed characteristic skin reactions.

As was emphasized in that paper, this antigen was obtained
from a strictly type-specific gonococcus strain which was not
agglutinated by a high-titer heterologous antiserum. The antigen
was examined by precipitation with the antisera of both types
and was demonstrated to be strictly type-specific. Conversely,
the protein-free antigen obtained from the more infrequent type
II was also found to be strictly type-specific.

The purpose of the present paper is to report the results of the
fundamental diagnostic agglutination tests which preceded the
production of these type-specific antigens (Casper, 1937). In
these agglutination tests for the recognition of the different types
of gonococci we started with the assumption, in accordance with
earlier experiments on pneumococci, that strains from fresh cases
should be most suitable for the demonstration of type charac-
teristics. Tulloch also followed this principle and laid stress
on the fact that he limited himself to only acute and subacute
cases of gonorrhea. We have gone even further and have ex-
amined, in general, only quite recent, untreated cases. In addition,
we attempted, above all, to demonstrate the existence of 2 types
which were completely different from each other and which were
not overlapping in agglutination. This requirement appeared
to be necessary because, in the case of pneumococci Avery and
Heidelberger found that carbohydrates of a specific chemical
structure represent the type-determining factor and that auto-
lyzed type-specific cultures react with the antisera of heterologous
types. In the same way a partially degenerated culture might
show an overlapping reaction. In such cases, therefore, these
types could be differentiated from each other only by an absorp-
tion test.

But, in the interpretation of an absorption test, the question
always arises as to whether the overlapping reaction was caused
by the common portion of the antigen or by the relationship of the
type-specific factors. The solution of all these questions is
simplified if, as in our experiments, the diagnostic sera are pre-
pared with 2 completely different types which have no sort of
relationship between the type-specific factors.

Culture media: we have taken into consideration still another
difficulty, namely, that cultures on artificial media frequently
tend to agglutinate spontaneously in saline or in normal rabbit serum. In our experiments, we first used 5 per cent horse blood agar and later the blood-water agar (Casper, 1929) on which gonococci showed an optimum growth and did not degenerate so quickly. But even when the best possible culture media are selected the tendency towards spontaneous agglutination cannot always be excluded. Therefore, we never use such strains for the production of antisera and have considered them separately in establishing the frequency of the types.

Preparation of serum: in the preparation of our first batch of sera we followed the precautionary measures advised by Tulloch namely, the use of killed vaccines in order to avoid autolysis and the production at one time of all the antigen necessary for the entire process of immunization. Although Torrey and Buckell consider the temperature of 60°C. used by Tulloch as too high and deleterious to the antigen, we obtained good results with this method. But we also obtained good sera with living cultures which had been freshly prepared for each injection if the strains used had not been isolated too long previously. We obtained a good serum from a 1-year-old laboratory culture of our strain 1 (type I). Other strains of this age, however, were not suitable because they overlapped too much.

A partly living, partly dead vaccine was injected intravenously every 4 to 6 days in increasing doses of $\frac{1}{4}, \frac{1}{2}, 1$ to 16 loops. After the injection of 8 loops, a trial bleeding was made and agglutination tests performed. After the injection of 16 loops, the animals were exsanguinated and phenol added to the serum.

To find suitable types we proceeded in the following manner: rabbits were first immunized with 3 freshly isolated strains. The sera of these 3 strains proved to be identical in the absorption and had a titer of 1:800 to 1:1600. A number of freshly isolated strains were then examined for agglutination in 1:25 to 1:1600 dilutions of these 3 sera. The sixth and seventh strains examined did not react at all with these sera, not even in the strongest concentration (1:25). A high-titer antiserum was prepared with one of these strains. This antiserum strongly agglutinated the second strain which also did not react with type I serum. The
<table>
<thead>
<tr>
<th>STRAIN AND SERUM NUMBER</th>
<th>PERIOD</th>
<th>GROUP</th>
<th>TYPE</th>
<th>PREPARATION OF THE ANTIGEN</th>
<th>AGE OF CULTURE AT SPAN OF IMMUNIZATION</th>
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<tbody>
<tr>
<td>1</td>
<td>First</td>
<td>1a</td>
<td>I</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>38 days</td>
</tr>
<tr>
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<td>1a</td>
<td>I</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>38 days</td>
</tr>
<tr>
<td>3</td>
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<td>1a</td>
<td>I</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>38 days</td>
</tr>
<tr>
<td>10</td>
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<td>I</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>86 days</td>
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<tr>
<td>41</td>
<td>First</td>
<td>1a</td>
<td>I</td>
<td>Organisms killed at 60°C., prepared freshly for each injection</td>
<td>7 days</td>
</tr>
<tr>
<td>41</td>
<td>First</td>
<td>1a</td>
<td>I</td>
<td>Living</td>
<td>7 days</td>
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<tr>
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<td>Second</td>
<td>1b</td>
<td>I</td>
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<td>39 days</td>
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<tr>
<td>83</td>
<td>Second</td>
<td>1b</td>
<td>I</td>
<td>Living</td>
<td>35 days</td>
</tr>
<tr>
<td>76</td>
<td>Second</td>
<td>1e</td>
<td>I</td>
<td>Organisms killed at 60°C., preserved with phenol</td>
<td>8 days</td>
</tr>
<tr>
<td>75</td>
<td>Second</td>
<td>1b</td>
<td>I</td>
<td>Organisms killed at 60°C., preserved with phenol</td>
<td>8 days</td>
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<td>Third</td>
<td>1a</td>
<td>I</td>
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<td>20 days</td>
</tr>
<tr>
<td>7</td>
<td>First</td>
<td>2a</td>
<td>II</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>34 days</td>
</tr>
<tr>
<td>25</td>
<td>First</td>
<td>2a</td>
<td>II</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>26 days</td>
</tr>
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<td>Second</td>
<td>2a</td>
<td>II</td>
<td>Living</td>
<td>30 days</td>
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<td>57</td>
<td>Second</td>
<td>2b</td>
<td>II</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>10 days</td>
</tr>
<tr>
<td>72</td>
<td>Second</td>
<td>2d</td>
<td>II</td>
<td>Organisms killed at 60°C., prepared freshly for each injection</td>
<td>8 days</td>
</tr>
<tr>
<td>116</td>
<td>Third</td>
<td>2b</td>
<td>II</td>
<td>Living</td>
<td>10 days</td>
</tr>
<tr>
<td>81</td>
<td>Second</td>
<td>Irregular</td>
<td>I + II</td>
<td>Living</td>
<td>3 months</td>
</tr>
<tr>
<td>51</td>
<td>Second</td>
<td>3</td>
<td>Heterologous</td>
<td>Organisms killed at 65°C., preserved with phenol</td>
<td>4 days</td>
</tr>
<tr>
<td>41a</td>
<td></td>
<td></td>
<td>I</td>
<td>Organisms killed at 100°C., prepared freshly for each injection</td>
<td>$\frac{1}{2}$ year</td>
</tr>
<tr>
<td>41a</td>
<td></td>
<td></td>
<td>I</td>
<td>Living</td>
<td>$\frac{1}{2}$ year</td>
</tr>
<tr>
<td>1a</td>
<td></td>
<td></td>
<td>I</td>
<td>Organisms killed at 60°C., prepared freshly for each injection</td>
<td>2 years</td>
</tr>
<tr>
<td>25a</td>
<td></td>
<td></td>
<td>II</td>
<td>Organisms killed at 60°C., prepared freshly for each injection</td>
<td>1$\frac{1}{2}$ years</td>
</tr>
</tbody>
</table>
type I strains (1 to 3), on the other hand, remained completely (1:25) uninfluenced. From this it was concluded that the type-specific factors of both types were completely different from one another. In a manner similar to that used with pneumococci, therefore, it seemed possible to make a classification of types by means of simple agglutination without the use of the absorption test.

Representative results are shown in table 2. We regarded as definitely type-specific only those strains which were agglutinated

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TITERS OF COMPARATIVE AGGLUTINATION IN TYPE I SERA</th>
<th>TITERS OF COMPARATIVE AGGLUTINATION IN TYPE II SERA</th>
<th>CO-AGGLUTINATION</th>
<th>RATIO OF TITERS TYPE I:TYPE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>800 to 1600+</td>
<td>25— to 50+</td>
<td>None or slight</td>
<td>16:1</td>
</tr>
<tr>
<td>b</td>
<td>400+</td>
<td>25— to 50+</td>
<td></td>
<td>8:1</td>
</tr>
<tr>
<td>c</td>
<td>800 to 1600+</td>
<td>100+</td>
<td>Relatively strong</td>
<td>8:1</td>
</tr>
<tr>
<td>d</td>
<td>1600+</td>
<td>200+</td>
<td></td>
<td>4:1</td>
</tr>
<tr>
<td>e</td>
<td>400 to 800+</td>
<td>100 to 200+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>25— to 50+</td>
<td>800 to 1600+</td>
<td>None or slight</td>
<td>1:16</td>
</tr>
<tr>
<td>b</td>
<td>25— to 50+</td>
<td>400+</td>
<td></td>
<td>1:8</td>
</tr>
<tr>
<td>c</td>
<td>200+</td>
<td>1600+</td>
<td>Relatively strong</td>
<td>1:8</td>
</tr>
<tr>
<td>d</td>
<td>100 to 200+</td>
<td>400 to 800+</td>
<td></td>
<td>1:4</td>
</tr>
<tr>
<td>3</td>
<td>25—</td>
<td>25—</td>
<td>None</td>
<td>0:0</td>
</tr>
</tbody>
</table>

by 1:400 to 1:1600 dilutions of the corresponding serum but which were not influenced by heterologous serum in dilutions greater than 1:50. If co-agglutination took place up to 1:100 or to 1:200 but a higher titer was reached in the antiserum of the heterologous type we have likewise considered this strain as definitely classified. Differential diagnosis by comparative agglutination can still be made if a titer of 1:400 to 1:800 is followed by a co-agglutination in 1:100 to 1:200 heterologous antiserum. (Strains with the relatively high co-agglutination-agglutination titer ratio 1:4.)
Controls were done in all cases consisting of: (1) normal rabbit serum in various dilutions and (2) one control in saline. We regarded as uncertain all reactions in which a strain was spontaneously agglutinated in saline (even to a slight degree) or influenced by normal rabbit serum. These we have gathered into a special group which will not be taken into consideration in the calculation of the frequency of the types. According to our preliminary tests with strains from chronic gonorrhea it seems that these strains, particularly, tend to agglutinate spontaneously to a lesser or greater degree.

In addition to both of these principal types, we distinguished those types which are not influenced by either serum and which, therefore, probably belong to another type (shown under the heading group 3 in table 2).

Furthermore, strains which were sensitive to the same degree in both sera and strains which were only weakly sensitive in both sera (never more than 1:200) have been found. In the latter case a difference in titer in one serum of 1:200 and 1:50 in the other serum seemed to be insufficient for diagnosis without the aid of the absorption test (cf. table 4).

Since most of the strains could be typed by comparative agglutination, we have used the absorption test in only a small portion. In the absorption test, both sera were simultaneously absorbed for the respective test strain. The difficulties which resulted from this, especially in those cases in which the strains were degenerated as a consequence of long cultivation, will be later discussed in detail.

The confirmation of the diagnosis by preparation of an antiserum from the examined strain was relatively seldom necessary. However, sera were prepared with 18 strains. Moreover, purified protein-free carbohydrate fractions (Casper, 1937) were also prepared from 6 strains and the type-specificity determined by examination with both antisera. Conversely, some of the antisera were established as type-specific by precipitation tests with both carbohydrate fractions.

In the following we report on the results of our investigations which extended over a period of several years (1927–1933).
TABLE 3
Comparison of the type I and type II strains of the first period (1937 to 1930) which had clear controls by means of crossagglutination tests in type I sera (on the left) and type II sera (on the right)
The test material consisted of living gonococci suspended in saline

| Strain Nr | 1* | 2* | 3* | 10* | 14 | 15 | 16 | 17 | 19 | 20 | 26 | 28 | 36 | 11 | 23* | 44 | 46 | 6 | 7 | 25 | 46 | 6 | 7 | 25 |
|-----------|----|----|----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|
| 1:1,600   | ±  | ±  | ±  | ±   | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  | ±  |
| 1: 800    | +  | +  | +  | +   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 1: 400    | +  | +  | +  | +   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 1: 200    | +  | +  | +  | +   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 1: 100    | +  | +  | +  | +   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 1:  50    | +  | +  | +  | +   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

Controls: homogeneous in saline; negative in 1:50 to 1:400 normal rabbit serum

Twenty-one strains could be classified without supplementary tests.

* Seven strains were conclusively classified by production of an antiserum.

† Five strains were conclusively classified by absorption test.
reason for this was that we scrupulously selected only acute untreated cases of gonorrhea as the source of our strains.

Table 3 shows the results obtained with 21 gonococcus strains with good controls. These could be immediately classified on the basis of differences in titer when they were tested simultaneously with type I and type II sera. This table also shows that 11 of the 21 strains remained perfectly homogeneous even when treated with the strongest concentration (1:50) of type II serum while they reacted to the maximum titer or at least to a 1:400 dilution of type I serum.

Three other strains (19, 26, 36) gave a + reaction with the heterologous type II serum but in no dilutions higher than 1:50; a fourth strain (41) also gave a + reaction with a 1:50 dilution. Strain 41, however, reacted with a 1:1600 dilution of type I serum. All cases in which the titer is increased more than eight fold belong to our group 1a.

Strain 11 which reacted ± with a 1:400 and + with a 1:200 dilution of type I serum and which did not react with type II serum should be classified with our group 1b.

Only with strains 42, 43, and 44 is the titer difference lower and co-agglutination somewhat higher. Strain 43, which has a four times higher titer (+ reaction in 1:400 type I serum and + and ± reaction with 1:50 and 1:100, respectively, type II serum) belongs to group 1e. Strain 42, which reacts four times more strongly with type I serum (1:800 ± and 1:400 +) than with type II serum (1:200 ± and 1:100 +) also belongs to this group. Strain 44 which gives a + reaction with 1:200 type II serum and a ± reaction with 1:1600 type I serum belongs to group 1d.

The last 3 strains examined (6, 7, 25) belong, undeniably, to type II. They all reacted with 1:800 type II serum while they showed a perfectly negative reaction with 1:50 type I serum.

The reading of table 4, which presents the results of the investigations of the second period, is somewhat more difficult because the results are not so clear cut. In it are found those type I and type II strains which can be classified only by absorption as well as those strains which, according to the absorption test, are different from both types. Along with the absorption
### TABLE 4

Comparison of the type I and type II strains of the second period (May 1930 to May 1931) which had clear controls by means of cross agglutination tests in type I sera (left) and type II sera (right)

Different kinds of antigen suspensions, heated suspensions of some of the strains were examined

<table>
<thead>
<tr>
<th>Strain Nr</th>
<th>Type I serum 41</th>
<th>Type II serum 41f</th>
<th>Type II serum 41n</th>
<th>Type II serum 25</th>
<th>Type II serum 57</th>
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<tr>
<td>1: 1,600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>1: 800</td>
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<td>1: 400</td>
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<td>1: 100</td>
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<td>1: 50</td>
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<td>1: 25</td>
<td></td>
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<tr>
<td>Saline control</td>
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<td>0 0 0 0 0 0 0 0 0 0</td>
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<td>Normal serum</td>
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<tr>
<td>1: 25</td>
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<tr>
<td>1: 100</td>
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</tbody>
</table>

**Key to symbols:**
- *Absorption tests were made with these strains. This symbol also indicates the type serum with which absorption was successful.
- † Strain 51 was classified as a heterologous type.
- ‡ According to absorption test, these strains (52, 62) were classified as belonging neither to type I nor to type II.
test the indirect method of identification was used, namely:

examination of the agglutinins and precipitins produced by the

immunization of rabbits with the strains in question.

Moreover, the diagnostic sera I as well as II, have been changed several times.

In the preparation of the antigen we have employed all possible variants.

Of the 3 strains (47, 48, 49) which were heated to 100°C.,

only strain 49, which reacted four times more strongly in type I

serum than in type II serum, could be identified as of type I by

the difference in titer.

Strains 47 and 48 could be identified as type II only with the

aid of the absorption test. It is remarkable that strain 47

reacted twice as strongly in a high titer II serum (25) and that

strain 48 gave an equally low reaction (1:100) in both sera.

According to our scheme, these two strains had to be assigned to

groups 5d and 6b, respectively (table 5).

Whether it can be concluded from this that the type II antigen

is particularly sensitive to heat remains uncertain. The fact

that, in testing the thermostability of the isolated type-specific

carbohydrate fractions, precipitation with the undiluted type II

substance decreased markedly after heating while the substance

prepared from a type I strain withstood heating, speaks in favor

of this conclusion.

Two (65, 67) of the 7 strains, which were tested with the un-

filtered I serum 41, reacted strongly with I serum and \( \frac{1}{2} \) to \( \frac{1}{4} \)

as strongly with type II serum (groups 1b and 1c, respectively).

Strain 57 is a distinct type II as it does not react at all with I

serum and gives a + reaction in 1:400 II serum. Strain 56

reacted ± in 1:200 I serum and + in 1:400, ± in 1:800 and

± 1:1600 II serum.

In the determination of the end titer of the reactions carried

out in several concentrations, we decided to count only the first

± following a + reaction for the comparison of the titer with

that in the heterologous serum. Experience had shown that

when these weak reactions were repeated variations in the end

titer were frequent.
SEROLOGICAL CLASSIFICATION OF GONOCOCCI 365

Strains 51, 52 and 62 in the table occupy a special position since these 3 strains belong neither to type I nor to type II. Strain 51, particularly, showed no reaction (- in 1:25) in either type I or type II serum. The 2 strains, 52 and 62, reacted in 1:100 and 1:400 I serum and 1:100 and 1:200 II serum and, therefore, are approximately the same in both sera. In the absorption test, however, strain 52 was unable to remove the specific agglutinins for either of these 2 types while strain 62, as a special type, removed the agglutinins from both sera. Of the 6 strains which were tested by the filtered serum 41f, 3 were heated to 56°C. and 3 were tested while living. Of the former, strains 76 and 78 proved to be type I by comparative agglutination and belonged, respectively, to the groups 1e and 1c. The classification of strains 75 and 76 was confirmed by the production of an immune serum and the testing of its agglutinins. As further confirmation absorption tests were repeated with the immune serum. Strain 75 had been previously identified as a type I by the absorption test. The high co-agglutination of strain 75 and the relatively high co-agglutination of strains 76 and 78 warrants the conclusion that the gonococcus antigen which gives an overlapping reaction is not destroyed by heating to 56°C.

Of the 3 unheated cultures which were tested with serum 41f, strains 82 and 83 gave a + reaction with 1:400, and strain 84 a + reaction with 1:50 I serum. The reactions were reversed in type II serum, strain 84 giving a + reaction with 1:800 and strains 82 and 83 negative reactions with 1:50 so that diagnosis can be clearly made. The classification was confirmed by the preparation of immune sera and the examination of their agglutinins.

The last strain examined (72) showed itself to be a type II as it agglutinated to the end point (1:1600) in type II serum (57) while the I serum (1a) with its overlapping valency agglutinated it only to 1:200, i.e., was ½ as strong. In this case also the diagnosis was confirmed by the preparation of an immune serum. Of the 17 strains tested, 7 were clearly type I and 3 clearly type II. Two other strains were proven to be type II by the use of the absorption test. The percentage of type I is, therefore, much
smaller in this series than in the first series. To these must be added the 3 strains classified as heterologous and the irregular strains excluded from this table. It seems possible, therefore, that an epidemiologically significant change in the types is taking place.

In consideration of the importance of this problem, we decided, in 1933, to resume these experiments and give the results obtained with 15 strains with good controls. In these experiments, we used the diagnostic sera 82 (type I) and 84 (type II). These sera were prepared from the type-specifically reacting strains just mentioned shortly after their isolation.

In this period again a definite preponderance of type I can be shown. Nine strains reacted strongly with type I serum and only weakly with type II serum. Of these, 2 reacted up to 1:100 in type II serum and, therefore, belong to group 1e.

Three strains reacted with a 1:400 to 1:800 dilution of type II serum and much less strongly with type I serum (1:25, 1:50). They, therefore, belong to groups 2c and 2d respectively. Three strains only reacted with 1:25 dilution of type I and type II serum. They, therefore, are heterologous types of group 3.

Eight strains of this period, belonging to the "irregular" or "degenerated" strains will be discussed later.

Absorption tests were not performed during this period. At this time, again, the majority of the strains could be easily classified by "comparative agglutination." As in the first period, type I was predominant and the absorption test was unnecessary.

If we divide the material thus far considered as to the frequency of types I and II and the heterologous types which can be identified by comparative agglutination, we find, in the total of 53 strains:

<table>
<thead>
<tr>
<th></th>
<th>TYPE I</th>
<th>TYPE II</th>
<th>HETEROLOGOUS</th>
<th>RELATED TYPES I AND II BY ABSORPTION</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>First period</td>
<td>18</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Second period</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Third period</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>53</td>
</tr>
<tr>
<td>In per cent</td>
<td>66%</td>
<td>22.6%</td>
<td>9.4%</td>
<td>1.8%</td>
<td></td>
</tr>
</tbody>
</table>
If we make our judgment concerning the frequency of the types only on the basis of cases which are quite clear diagnostically, we find a great preponderance of type I in the first as well as in the third periods. Totalling the cases of all 3 periods, we find type I present in 66 per cent, a figure which approaches the result obtained by Tulloch (72 per cent). In the first period the percentage is much greater (85 to 86 per cent); in the second period type II is relatively more frequent (37.5 per cent) and type I more rare (50 per cent). In this and in the third periods, there occur 5 strains which are classified as belonging to "neither type I nor type II." Two of these strains were thus classified by absorption and 2 by the complete absence of any effect by either serum. In addition, 1 strain (62) showed the striking characteristic that it could remove, to a high degree, the agglutinins for both types. We shall return to a detailed discussion of this—as yet not clearly explained—group of strains which seemed to play an important rôle in chronic gonorrhea.

The irregular strains

These figures do not correspond perfectly with actual conditions. They must be corrected by considering the results of irregular and degenerated strains.

As "irregular strains" we understand those with which we did not obtain a clear result by comparison of the titer in both type sera. With some of these strains, neither serum was effective in dilutions higher than 1:200. In these cases, the objection might be raised that we are dealing only with co-agglutination, whereas, if a heterologous serum had been used a higher titer might have been reached. With other of these strains, on the other hand, both sera were effective in dilutions much higher than 1:200. In all of these cases, the absorption test should have been used. But, just in these cases, the absorption test was unsuccessful. In order to achieve a uniform understanding with later investigators, we therefore tried to form a plan, for the classification, according to the height of the titer in the sera of both types, of those strains whose types could not be determined by comparative agglutination.
The resume shows the frequency and the classification into the
different groups for all 3 periods.
In 8 cases we obtained a higher titer in type I serum than in
type II serum; in 5 cases the titer was higher in type II serum than
in type I serum and in 8 cases the titer was exactly the same in
both sera. Strong reactions (above 1:200) occurred eleven times
among these and below 1:200 ten times. Of the 11 cases with a
high titer, 2 showed a four times stronger agglutination (up to

| TABLE 5 |
| Plan for the classification of the irregular strains which had clear controls but which could not be typed by means of comparative agglutination |

<table>
<thead>
<tr>
<th>NUMBER OF IRREGULAR STRAINS</th>
<th>GROUP 4a</th>
<th>GROUP 4b</th>
<th>GROUP 4c</th>
<th>GROUP 5a</th>
<th>GROUP 5c</th>
<th>GROUP 5d</th>
<th>GROUP 6a</th>
<th>GROUP 6b</th>
</tr>
</thead>
<tbody>
<tr>
<td>First period reaction in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum type I</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>1</td>
<td>None</td>
<td>None</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Serum type II</td>
<td>1:3200±</td>
<td>1:1000</td>
<td>1:300</td>
<td>1:100±</td>
<td>1:300</td>
<td>1:100±</td>
<td>1:300</td>
<td>1:100±</td>
</tr>
<tr>
<td>Serum type III</td>
<td>1:200</td>
<td>1:1000</td>
<td>1:200</td>
<td>1:400</td>
<td>1:400</td>
<td>1:200</td>
<td>1:200</td>
<td>1:200</td>
</tr>
<tr>
<td>Second period reaction in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum type IV</td>
<td>1:200</td>
<td>1:1000</td>
<td>1:200</td>
<td>1:400</td>
<td>1:400</td>
<td>1:200</td>
<td>1:200</td>
<td>1:200</td>
</tr>
<tr>
<td>Serum type V</td>
<td>1:200</td>
<td>1:1000</td>
<td>1:200</td>
<td>1:400</td>
<td>1:400</td>
<td>1:200</td>
<td>1:200</td>
<td>1:200</td>
</tr>
<tr>
<td>Third period, reaction in</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum type I</td>
<td>None</td>
<td>None</td>
<td>1</td>
<td>None</td>
<td>1</td>
<td>None</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Serum type II</td>
<td>1:25</td>
<td>1:100</td>
<td>1:200</td>
<td>1:400</td>
<td>1:400</td>
<td>1:200</td>
<td>1:200</td>
<td>1:200</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Type I &gt; Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II &gt; Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I = Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1:3200) in type I serum than in type II serum. However, we
did not feel justified in explaining the high agglutination (1:800)
in type II serum as co-agglutination. Furthermore, the great
number of strains which react exactly alike in both sera is striking;
among these were strains (31, 86 and 119) which had a titer of
1:400 to 1:800 in both sera and 3 to 4 strains (55, 94, 104, 53)
with a lesser titer (1:100 to 1:200) in both sera. As a whole,
type I also predominates in these 21 cases.
SEROLOGICAL CLASSIFICATION OF GONOCOCCI

Since in a few random tests absorption did not succeed and shed no light on the problem, it was not performed in these cases.

THE DEGENERATED STRAINS

In the group of degenerated strains we assembled all those which did not show perfectly homogeneous controls in saline or in normal rabbit serum. As was previously mentioned, we came to this conclusion by the experience that a reaction in saline or normal rabbit serum must be regarded as a sign of degeneration; and the results showed that such strains also acted very irregularly with immune sera. Such strains are probably also more sensitive in their reactions with the different immune sera which, in different animals, do not always contain equal quantities of normal agglutinins.

Altogether we found 35 of these degenerated strains. In the first period, 8 strains were found to be degenerate.

In a few cases, (strain 18), in which the reaction in type II serum is negative while it reaches 1:1600 in type I serum and \( \pm \) in 1:50 normal serum, perhaps the repetition of the test and titration of the normal serum would have shown that this strain was only transitorily influenced by normal serum. Another strain (27) too, which shows a homogeneous control in normal serum and only a slight \( \pm \) reaction in saline, can be considered to be a type I only if one assumes selective influence by type I serum. However, we have recorded these strains here in order to show the different grades of degeneration.

Table 6 combines the degenerate strains of the second and third periods. In it, however, are presented only those strains which showed differences in titer and which were examined by the absorption test or those similar to strain 77 which gave a \( \pm \) reaction in normal serum and the same reaction in the sera of both types and, therefore, are to be considered as most probably of heterologous type.

The strains examined by absorption (50, 61, 59, 73) are marked by a star. It was found that strain 61 absorbed the sera of both types to a large extent.

Strains 50 and 73 showed clear controls during absorption and
exhausted the serum for their own antigen, an indication that at the time of the absorption test they had recovered from their

### Table 6

**Degenerated strains of the second and third periods**

<table>
<thead>
<tr>
<th>Strain Nr.</th>
<th>SECOND PERIOD, ANTIGEN RELATED TO</th>
<th>THIRD PERIOD, ANTIGEN RELATED TO</th>
<th>Immune sera type I</th>
<th>Immune sera type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 3200</td>
<td>41 69 77 50 73 61 59</td>
<td>100°C. 56 100 56 100°C.</td>
<td>115 101 25 87 25 84</td>
<td>54</td>
</tr>
<tr>
<td>1: 1600</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 800</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 400</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 200</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 50</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 25</td>
<td>0 0 - 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td>0 0 - 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Control: Normal serum</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 400</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 200</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 50</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>1: 25</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td>0 0 ± 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

Key to symbols:

- Absorption test showed that: Strains 50 and 73 were "neither type I nor type II" = heterologous. Strain 61 was "type I and type II." The absorption test was unsuccessful with strain 59.

† 9 strains which, similar to strain 59, were agglutinated to the end titer by the sera of both types must be added to the second period. Six of these were agglutinated by 1:400 normal serum and 3 by 1:100 normal serum. Living suspensions of the latter were used as antigen. Nine strains which reacted like strain 59 must be added to the third period.

. Not done.

degeneration. However, they were unable to influence the I and II agglutinins; they therefore had to be assigned to the heterologous types, with this result considered as not final.
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Strain 59 could not even remove its own agglutinins, as one would expect from the experimental conditions applied. Strain 61 absorbed the specific agglutinins from the sera of both types to a high degree so that it is related to both of them.

In the second period, strain 46 could be assigned to type I and strain 69 to type II on the basis of titer difference alone. In the third period, similarly, strains 115 and 101 could be classified as type II.

Of the remaining strains not specified in this table, 9, which reacted like strain 59, must be added for the second period. Of these, 6 strains were agglutinated in dilutions of normal serum up to 1:400 and 3 up to 1:100. Nine strains of the third period agglutinated up to the end titer in both immune sera as well as in normal serum.

In 23 of these 35 cases no indirect methods were applied. In 1 case the absorption test was unsuccessful and in the other 11 a diagnosis could be made only with reservations.

If we now take into account, in the classification of types, the results obtained in the examination of the irregular and degenerated strains, we get the following picture:

<table>
<thead>
<tr>
<th></th>
<th>53 Strains, Greatest Number of Diagnosed Types, Strains with Good Controls</th>
<th>74 Strains, with Addition of the 21 Irregular Strains</th>
<th>100 Strains, Smallest Number with Addition of the 35 Degenerated Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>35 per cent</td>
<td>47.3 per cent</td>
<td>4 per cent</td>
</tr>
<tr>
<td>Type II</td>
<td>12 per cent</td>
<td>18.2 per cent</td>
<td>3 per cent</td>
</tr>
<tr>
<td>Heterologous</td>
<td>5 per cent</td>
<td>6.7 per cent</td>
<td>3 per cent</td>
</tr>
<tr>
<td>Type I and II</td>
<td>1 per cent</td>
<td>1.3 per cent</td>
<td>1 per cent</td>
</tr>
<tr>
<td>Not classified</td>
<td>0 per cent</td>
<td>28.3 per cent</td>
<td>24 per cent</td>
</tr>
<tr>
<td>Altogether a diagnosis was made in per cent</td>
<td>100 per cent</td>
<td>71.5 per cent</td>
<td>60.6 per cent</td>
</tr>
</tbody>
</table>

From this summary it is obvious that the number of type I strains, after addition of the degenerated and undiagnosed irregular strains omitted in the first summary, is relatively much smaller but, nevertheless, constitutes almost half of all the strains. Type II is the most frequent of the other types.
Altogether we find 71.1 per cent of the cases diagnosed. In this number about 10 per cent (11) of the degenerated strains, in which the diagnosis was made with reservations, are not included.

We consider this a favorable result for the evaluation of our methods; for, as we mentioned above, no definite conclusions as to the degenerated strains and, as will be shown later, as to most of the cases, can be drawn from the use of the absorption test. Therefore, according to our experiences with strain 59, it cannot be expected that the results of absorption tests with the 21 irregular strains would have essentially decreased the percentage of type I.

Even 47.3 per cent would mean a great spread of the strains which we have designated as type I. Of course, it seems important to us that 16.2 per cent belong to type II. 6.7 per cent of the cases, we designate as heterologous, are also epidemiologically important. The percentage, like that of type II, varied in the different periods.

On the other hand, the type designated by us as “related to types I and II” seems to have a lesser significance in acute cases of gonorrhea, although among the irregular strains there were 3 which reacted equally high in both sera and 5 which reacted to a lesser degree in both sera and which, perhaps, should be assigned to this type. This type seems to be of rather great significance in the strains isolated from cases of chronic gonorrhea.

THE STRAINS FROM CHRONIC GONORRHEA

We have examined only a few strains from chronic gonorrhea (6 in the first period and 10 in the second); all these strains had a certain tendency towards agglutination in normal serum; 2 strains were also agglutinated in saline. The majority of them, therefore, have been classified with the degenerated strains.

It was found that all these strains were highly affected by the sera of both types. This could also be proven by the absorption test which was performed with 5 of these strains (34, 37, 38, predominant strain (Tulloch) and 81 which showed clear con-
controls.) They all absorbed the I and II agglutinins to the same extent.

In regard to the frequency of occurrence, this behavior is characteristic of the chronic strains. It is true that this behavior is in contrast to that of pneumococci. No parallel can be drawn with this organism, even when it has lost its specificity. However, the possibility of the relationship of 2 type-specific substances, different from each other, to a third, should not be regarded as outside the realm of feasibility. It must be mentioned that Griffith made a similar observation with streptococci. He

**TABLE 7**

_Crossagglutination tests in their reciprocal sera, of 4 strains of gonococci (82, 83 (type I), 84 (type II) and 81)_

<table>
<thead>
<tr>
<th>Gonococcus strain</th>
<th>Serum 81</th>
<th>Serum 82</th>
<th>Serum 83</th>
<th>Serum 84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:50</td>
<td>100</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>81</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>82</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>83</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>84</td>
<td>(+)</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

found that one of his types could absorb the agglutinins of 2 heterologous type sera which were strictly different from each other. We prepared an immune serum with strain 81 and examined, with this serum, two I strains (82, 83) and one II strain (84).

As is seen from table 7, serum 81 influenced all 4 strains up to the end titer. Strains 82, 83 and 84, however, proved to be strictly type-specific when examined with the homologous sera (82, 83 (type II)). From this, it seems that there exists a type “I + II.”
Strain 81 was also used several times for absorption tests. With only one exception, it absorbed, to the same degree, the agglutinins for most of the type I and type II test strains from their respective sera.

This strain, therefore, offers an analogy to the observations made by Griffith on streptococci. Furthermore, the relationship between types I and III meningococci should be remembered. In his cases, Griffith assumed "phases" similar to those of the paratyphoid bacilli. Here we might refer to an earlier paper by Casper (1928) who, in trying to prepare the carbohydrates of paratyphoid B and Breslau bacilli (in which we worked with purely specific and non-specific phases) had found that the carbohydrates obtained from all four variants were apparently identical. They were all heavily precipitated by specific Breslau serum while they were not at all or only very little influenced by non-specific Breslau serum or specific Schottmueller serum. From this, it follows that the "serological factor" is contained in all these phases of the paratyphoid bacillus but "is masked" in the production of antibodies, in the rabbit, by the specific Schottmueller and the non-specific Breslau bacillus. Another serological factor must, therefore, be dominant in these 2 phases. The conception is derived, therefore, that during a change of phase the species-specific carbohydrate can become dominant for the non-specific type of Schottmueller, while the same carbohydrate, which represents the dominant haptene of the specific Breslau type, is masked in the specific type of Schottmueller.

If one assumes, therefore, that in the cultivation of gonococci a change of phase eradicated the serological differences between the types, in a manner similar to that of the paratyphoid bacilli, the explanation of the following is made clear: (1) the relationship between strain 81 and types I and II, and (2) the frequency of the appearance of such mixed phases, (not only in chronic cases of gonorrhea but also in some acute cases) in which equally high agglutination titers were obtained in both type-specific sera, an occurrence which is not infrequent with paratyphoid bacilli.

At that time we thought of this possibility and examined different sera of type I and II gonococci, prepared partly with living
and partly with heated cultures. This was done by means of slide agglutination using several strains of each type in order to demonstrate the combination of the different type-specific strains from two different phases.

The result was not quite uniform. However, a dominant overlapping of the I serum, 82, which influenced a large number of single colonies of almost all type II strains, was always observed. The II serum, 57, on the other hand, acted upon a large number of I colonies. This experiment, which suggests further illuminating observations in this direction, will be discussed in a separate paper.¹

As was mentioned at the start, we separated the chronic strains, which showed only a relatively overlapping reaction in normal serum, from those obtained from cases of acute gonorrhea. We also tried to explain the overlapping reaction in both type-specific sera as a sign of degeneration.

On this fact is based the conception that normal agglutinins are increased during immunization. Absorption tests have shown, however, that the chronic strains were able to remove the specific agglutinins for the homologous strain from sera of both types. Now, however, our homologous test strains, in fixation experiments, proved to be strictly specific on every occasion, so that it is doubtful whether we can assume that they are acted upon by normal agglutinins. Perhaps one might rather assume that the chronic strains contain both phases and that they are thus also able to remove the agglutinins for the heterologous type.

THE "PREDOMINANT" STRAIN OF TULLOCH

For the purpose of comparison, we included this strain here although it had been cultivated for a long time on artificial media. It was kindly placed at our disposal by Dr. A. Cohn (1925). He found it to be equivalent in the complement fixation test to the strain he formerly used.

It seems noteworthy that the predominant strain of Tulloch showed a close relationship to both our types, not only in agglutination but also in absorption. It absorbed the specific agglu-

¹ The Cultural and Serological Behavior of Degenerated Gonococci.
tinins of both the I and II strains and, therefore, proved to be a 
"type I and II."

It is interesting now to recall how Tulloch selected the type 
which he later designated as "predominant."

He examined 13 strains in his preliminary experiment. Six of 
these were used for the preparation of sera. These sera proved 
to be little different when tested by all 13 strains. By the ab-
sorption test, however, 2 types were found, one of which, the 
so-called type I, was later used for the examination of all of his 
100 cases.

Type II and the other heterologous types, however, were not 
tested at the same time but were only later identified by their 
corresponding sera. By this method of examination, the differ-
ences of titer which he would have obtained by a simultaneous 
test with a heterologous serum could not become manifest. Cer-
tain differences in titer were present in the 13 strains. For, 
several I strains reacted more strongly and others less strongly 
with type II serum. Likewise, one I serum exerted a stronger 
influence on the II strains than did the others. The strain which 
yielded the most overlapping serum was chosen by Tulloch for 
his absorption experiments. This strain, therefore, would corre-
spond to our strain 81, i.e., a strain which, until the problem of 
phase has been clarified, we wish to designate provisionally as 
"I and II."

DISCUSSION

In the course of several years we performed serological tests on 
109 strains of gonococci isolated from cases of acute gonorrhea 
and 16 strains isolated from cases of chronic gonorrhea. All 
strains were simultaneously subjected to comparative agglutina-
tion tests with 2 type-specific sera. A large proportion of the 
strains showed sharp differences in titer, i.e., they were strongly 
agglutinated by the serum of one type while the serum of the 
other type agglutinated them weakly or not at all. The types 
found were called type I and type II, respectively.

An additional small group of strains impressed us as of heter-
ologous type. These strains were not agglutinated by either of
the type-specific sera, or, if agglutination did occur, the titer never rose over 1:50. From this we must draw the conclusion that there is another type which, however, is seldom found.

These investigations were originally undertaken with the purpose of finding several different type-specific strains of gonococci, isolating the type-specific portion of their antigen and examining their carbohydrate content. We were able to demonstrate that each of our 2 types contained different polysaccharide-like substances to which, as is the case with pneumococci, the specific reaction had to be ascribed.

With the finding of these 2 characteristic types, difficulties arose in our investigations. The diagnostic sera had to be prepared by the immunization of rabbits with freshly isolated strains. All of these sera did not prove to be equally selective when tested with fresh gonococcus cultures. We encountered great obstacles, especially in the second period of our investigations, because of overlapping and weaker reactions. In this period, old instead of freshly isolated strains were used for the preparation of antisera. Thus it was shown that old laboratory strains suffer a change in their antigenic properties. A series of experiments with one group of freshly isolated strains demonstrated, however, that the structure of the whole bacterial antigen is more complicated than appeared to be the case after the first period of experimentation. In order to show the difficulties of the serological differentiation of types, therefore, we have considered each strain as an individual, and have shown its reaction according to various methods of examination.

In the second period we used the absorption test more often for the purpose of classification. In only a few of the cases, however, in which comparative agglutination did not succeed in classifying our strains was any conclusion reached by the use of the absorption test.

The irregular and degenerated strains make up the major portion of the unclassifiable strains. The strains obtained from cases of chronic gonorrhea belong to the degenerated strains. While gonococci of both types I and II (similar to pneumococcus I and II) were strictly specific in agglutination, as were their carbo-
hydrates in precipitation, the cultures obtained from cases of chronic gonorrhea were able to absorb the sera of both types. In only one case of acute gonorrhea with good controls did we find that the specific agglutinins of both sera were absorbed to any great degree (strain 62). It seems, therefore, that these strains with antigenic valencies which overlap both type I and type II only seldom occur in acute gonorrhea while they appear more often in chronic gonorrhea and disrupt the classification. One might wish to classify a separate, “type I + type II.” It is uncertain as to whether one must assume that there is a degenerated or special type which is particularly frequent in chronic gonorrhea or which might even be the etiological agent in the chronic disease.

Our bivalent strain (81) according to our experimental results, apparently corresponds to Tulloch’s “predominant strain.” We cannot judge whether the appearance of degeneration, with which we are dealing here, can be ascribed to the age of the culture and to its continued transplantations on artificial media. We do consider the possibility that a change of phase occurred and refer to similar observations by Griffith on streptococci.

In any event, the results of our investigations show that one must reckon with the appearance of degeneration even with some freshly isolated strains of gonococci and that, in the choice of test strains for the production of immune sera this must be considered.

Under the given conditions, therefore, the technique is not ideal since degeneration, even at the time of isolation cannot be avoided. With further improvement of culture media a greater number of strains will probably be classifiable by means of comparative agglutination.

Despite this, in 71 per cent of the cases we were able to determine the type of the strain by comparative agglutination with our type-specific immune sera. Type I was preponderant among our strains. According to our examinations, however, our type I is not identical with the “predominant” strain which Tulloch found in the majority (72 per cent) of cases.
CONCLUSIONS

(1) By means of comparative agglutination two different types of gonococci (type I and type II) were found. With the aid of the immune sera prepared with them, we endeavoured to classify a large number of gonococcus strains into either of these types.

(2) The difficulties arising from this procedure are described for each individual strain.

(3) The reproduction of type-specific carbohydrate is indispensable for the solution of the problem of classification.

REFERENCES


