A MODIFIED LOEFFLER'S BLOOD SERUM MEDIUM USEFUL IN THE ROUTINE HEALTH DEPARTMENT EXAMINATION FOR DIPHTHERIA AND STREPTOCOCCUS INFECTIONS

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INTRODUCTION

The shortcomings of Loeffler blood serum for the laboratory diagnosis of diphtheria were brought home to one of us (Medalia) while chief of the laboratory and infectious disease service at a divisional camp and base hospital during the War, when an outbreak of diphtheria occurred. The contacts, sometimes as many as 500 to 750 men (two or three companies), had to be cultured. Since the diagnosis was based upon microscopic examination, the amount of labor entailed is obvious. In addition to the actual labor required in the microscopic examination of so many cultures, there was the difficulty of securing agreement between fairly well trained technicians as to the positive and negative smears. The discrepancy between the findings of the laboratory workers was also met with in the release cultures, mainly because of the scarcity of diphtheria organisms in such cultures.

On the other hand, in the cultivation for typhoid carriers of food handlers, or in cases of epidemic meningitis, where similar routine examination of contacts had to be done, the work, because of special differential media aiding the identification of these two organisms with the naked eye by their characteristic growth on the transparent media, was fairly simple. It, naturally, suggested itself that if a special medium could be found, preferably a transparent medium, which would permit the identification of
C. diphtheriae by the appearance of the colonies on plates, as is true in typhoid and meningitis, or if the selective character of the medium would grow C. diphtheriae more luxuriantly or to the exclusion of the other throat organisms, the work in such emergencies would be greatly minimized and possibly better results obtained.

The search for such a special medium was started by one of us (Medalia) in 1920. This was carried on in a research laboratory of the Department of Biology and Public Health of the Massachusetts Institute of Technology. It was at once discovered that in order to duplicate a culture medium, the first prerequisite was the possibility of titrating the culture medium by means of the pH. This resulted in the study of the colorimetric method of titration and the development of the method published by Medalia (1920 and 1922).

The actual development of the special culture medium here reported was accomplished at the Bacteriological Laboratory of the Health Department of the City of Boston, where the facilities for testing such a special medium on a large scale in the routine examination of diphtheria cultures were available. The medium was also tested in a controlled study of cultures obtained at the South Department of the Boston City Hospital and in another controlled study of cultures obtained at the Haynes Memorial Hospital for Contagious Diseases.

This report therefore, deals with three distinct studies: on the one hand, with 2297 cultures obtained by placing the special medium in the same diphtheria outfit which is used by the physicians, with a request to the physicians to plant both media simultaneously. Over this study we had no complete control. We could not positively tell that all such cultures were planted as we requested. On the other hand, the other two studies deal with definitely controlled cultures, which were taken at the South Department of the Boston City Hospital, from newly arrived cases, as well as those cultured for release and others, and at the Haynes Memorial Hospital for Contagious Diseases. The swabs obtained from the nose and throat were placed in a tube containing 2 cc. of nutrient broth. These swabs
were washed in the broth and discarded; fresh sterile swabs were then used for making comparative cultures on the *routine* Loeffler's and the *modified* Loeffler's media. These cultures were incubated under identical conditions, were smeared and stained in exactly the same way, and examined by three and sometimes by four different laboratory workers. The results obtained in these last two studies, we feel, are of definite value.

**SPECIAL MEDIA AS FOUND IN THE LITERATURE**

The disadvantages of Loeffler's coagulated blood serum medium have been recognized by many laboratory workers ever since Loeffler's classical work on the subject in 1884.

A number of special media, as found in the literature, have been recommended by these workers in an attempt to overcome some of the disadvantages of Loeffler's medium. The more important of these will be briefly discussed here.

Rankin (1911) in an attempt to overcome the necessity for microscopic examination, recommended the use of a medium consisting of three parts of sheep serum, one part bouillon, 1 per cent glucose, 2 per cent potassium sulpho-cyanide 50 per cent solution, and 1 per cent of a 1 per cent solution of neutral red. This is distributed in sterile tubes and coagulated in a slanted position at a temperature high enough to render it sterile.

It is recommended that throat cultures made on this medium be kept in the incubator over night, and if no colonies with red centers are present, that the cultures be returned to the incubator for another twenty-four hours and re-examined. If any colonies with red centers are found they are examined under the microscope to make sure they are *C. diphtheriae*. If no such colonies are present, the cultures are discarded as negative.

The disadvantages of such a method for routine practice are obvious. The element of time alone, would practically eliminate it for purposes of routine work. While some workers believed Rankin's medium to have merit (Coplans, 1911), others (Hanau, 1914) have found it less valuable than Loeffler's medium.

We checked up the medium and found it less selective than Loeffler's. There was no evident inhibition of growth of other
organisms, e.g. the staphylococci, *C. Hoffmanni*, *Streptococcus hemolyticus*, and *Streptococcus viridans*. There was also no characteristic differentiation between the other organisms and *C. diphtheriae* on such plates; i.e., when mixed cultures were planted, it was found impossible to distinguish *C. diphtheriae* from the other organisms; nor was there any apparent inhibition of growth of the organisms that were not *C. diphtheriae*. The chief drawback of this culture medium, however, is the difficulty of its preparation as compared with that of Loeffler's; and, in a comparative study of the two media the former was found not much, if at all, superior to Loeffler's. Such a comparative test was not carried out by Rankin himself. This was done, however, by Hanau (1914), who found the results on Loeffler's medium to be similar to those on Rankin's medium, and in one case he found that *C. diphtheriae* grew on Loeffler's and not at all on Rankin's medium.

Conradi and Troch (1912) made use of a culture medium containing calcium tellurite. No special advantage of this medium over Loeffler's has been found by Hanau (1914) and others sufficient to make it worth while to discard Loeffler's. The same can be said of other special media advanced by their respective proponents, such as the one recommended by Smith (1914) and recently by Douglas (1922).

Practically all of the special media reported in the literature have not been sufficiently checked and tested before they were published. Douglas (1922), for instance, bases his conclusions as to the value of his culture medium on a study of twenty-nine cases (29 cultures) as compared with Loeffler's, where he found nineteen positives on his medium and sixteen on Loeffler's; while Smith (1914) tested his medium on fifty suspected cases of diphtheria against Loeffler's medium and also on Conradi and Troch's medium with the following results: his own medium, ten positives; Loeffler's, eight; while Conradi and Troch's had only six positives. To draw conclusions as to the value of a special culture medium for diphtheria from so few test cultures, is, we feel, not justified.
A number of other special media for *C. diphtheriae* are found in the literature, but as they have not stood the test of time they are not referred to here.

Belding and Fogel (1929) reported, a comparative study by two laboratories of 500 cultures on 77 convalescent diphtheria patients for release. The finding of 43 per cent disagreements as reported by these authors, between their two laboratories is a grave indictment of our means of diagnosis and release of diphtheria cases if that study may be taken as representative of the findings in other laboratories. Belding and Fogel ascribe their main sources of error to the scarcity of typical diphtheria organisms and the technical difficulties in taking perfect cultures. According to these authors, the experience of the workers shows a difference in the percentage of disagreements from 10.5 per cent for laboratory directors, to 21 and 30 per cent for experienced and inexperienced technicians respectively.

We feel, therefore, that a culture medium which will grow the *C. diphtheriae* more readily and to the exclusion of the other throat organisms, more especially the staphylococci, as compared with the ordinary Loeffler's medium would eliminate the greatest source of error in diagnosis and release cultures. This, in turn, will help overcome, to a great extent, the endemic prevalence of diphtheria brought about by overlooking cases of diphtheria and by releasing a number of convalescents too soon. Such a culture medium would also tend to avoid the bitter experience met with occasionally, where a child comes home from the contagious hospital supposedly recovered from diphtheria, having been released following three or more negative cultures as required, and a few days later another child in the same household comes down with diphtheria.

The scarcity of growth of *C. diphtheriae* on the ordinary Loeffler's medium and the overgrowth of the cultures by other bacteria is a recognized factor responsible for errors in diagnosis. This scarcity of growth is also referred to by Belding and Fogel as one of the factors responsible for the 43 per cent error found by them. These features are what we hope to overcome with the
culture medium here reported. Such advantages, together with the simplicity of preparation of this culture medium, since it is only a modification of the routine Loeffler's medium, should make it the culture medium for use as a routine in laboratory diagnoses and release cultures of diphtheria.

PERSONAL WORK

The routine diphtheria culturing for diagnosis in the City of Boston has been done at this laboratory with Loeffler's blood serum, prepared from pig serum three parts, beef heart infusion one part, to which are added 1 per cent peptone, 1 per cent glucose, and 5 per cent glycerol. The broth is titrated to 0.8 per cent acid.

Since 1927, the pH colorimetric method (Medalia, 1920 and 1922) has been used in this laboratory and the broth standardized to pH 7.2, in place of the titration method.

The serum broth mixture is poured in sterile tubes, slanted and coagulated and sterilized at the same time in the autoclave by heating it at 15 pounds pressure for one hour.

Early in the search for a transparent blood serum culture medium (February, 1919), it was found by one of us (Medalia) that, when Loeffler's blood serum mixture was adjusted to 4.2 per cent alkaline, slanted in tubes, coagulated for three hours at 70° to 80°C, and sterilized in an Arnold sterilizer on three successive days at a temperature between 80° to 85°C., the medium would coagulate and remain clear and transparent, like agar. Attempts at obtaining such a transparent blood serum medium were continued recently by us at the Health Department Laboratory. After many trials, studying different media prepared from beef serum as compared with pig serum with the addition of different amounts of NaOH, the following medium gave the best satisfaction as to transparency and growth:
Pig serum (pH 6.8) 3 parts; beef heart infusion\(^1\) broth (pH 7.2) 1 part; to which are added 4.6 per cent N/1 NaOH, 1 per cent peptone, and 1 per cent glucose. No salt

This mixture gave a reaction of pH 7.6. It was poured into Petri dishes and autoclaved for one hour at 15 pounds pressure, resulting in a solid, clear, transparent medium.

Attempts were made to identify the \textit{C. diphtheriae} colonies by their naked eye appearance from a number of other organisms, such as \textit{C. Hoffmanni}, \textit{Staphylococcus aureus}, the pneumococcus, and \textit{Streptococcus viridans}. It was found, after a long series of cultures, studying the above bacteria on portions of the same plate, that while the streptococci and pneumococci could be identified and distinguished from \textit{C. diphtheriae} because of their characteristic size, the same was not true of the Hoffmann bacillus, or the various staphylococci. Even attempts to identify the colonies of \textit{C. diphtheriae} under the microscope with low power lens were found unsuccessful. At times, we felt that the granular appearance of the colonies would make it possible to do so, yet other organisms at various stages of their growth were found practically similar in appearance, and we were forced to conclude that the diagnosis of the presence or absence of \textit{C. diphtheriae} by the appearance of the colonies in a transparent medium from throat cultures, was not feaseable.

Attempts were then made to identify \textit{C. diphtheriae} by making use of a double colored indicator which was found by one of us (Medalia, 1920) to stand sterilization without changing. As early as 1888 Roux and Yersin (1888) found that alkaline broth was made acid by the growth of \textit{C. diphtheriae}, becoming alkaline again after a few days' growth. The acid production by \textit{C. diphtheriae} in an alkaline culture medium of a serum broth mixture containing glucose, with the addition of a double colored indicator, was, therefore, thought by us to be an ideal method of differentiating this organism from other bacteria. Various attempts were made with the transparent culture medium to

\(^1\)Beef heart infusion is prepared from minced beef heart 500 grams water, 1000 cc. kept in ice chest over night, pressed in meat press, made up to 1000 cc. and used for the meat infusion broth.
which were added, before autoclaving, the following indicators of the Clark and Lubs series: brom cresol purple, brom thymol blue, and thymol blue. These were tested in different strengths ranging from 0.05 per cent of a 0.2 per cent alcoholic solution to 0.1 per cent of the same alcoholic solution. The best medium for this study was found to be a serum mixture made with one part beef extract broth, 1 per cent glucose, and three parts pig serum, to which was added 4.5 per cent of N/1 NaOH; reaction pH 7.6; to this was added 0.05 per cent of a 0.2 per cent brom thymol blue alcoholic solution. This was poured in Petri dishes and tubes, coagulated, and sterilized in the autoclave at 15 pounds pressure for one hour. On this medium, too, the C. diphtheriae colonies were not any easier to be recognized when grown in mixed cultures with diphtheroids and staphylococci. The attempts to make use of the special culture medium, with or without indicators, for the diagnosis of C. diphtheriae by cultural characteristics, without the use of a microscope, was, therefore, abandoned.

In this study the following facts were brought to light:

1. The pig serum, used to prepare the routine Loeffler's medium for the laboratory and which was used in this study, was found to vary from pH 6.4 to 7.0.

2. When the serum was a week old, containing 0.5 per cent chloroform for preservation, and kept in the ice chest, it was found to be at pH 6.4; the addition of 4.5 per cent N/1 NaOH brought the mixture of serum and broth to pH 7.2 (the broth alone was originally pH 7.2). This mixture, when autoclaved and coagulated, instead of being transparent, was opaque.

3. On the other hand, when fresh serum (pH 6.8) was used, the addition of 4.5 per cent N/1 NaOH to the serum broth mixture would bring the mixture up to pH 7.6, which when poured in plates and autoclaved, gave a transparent solid medium.

4. The serum which showed an initial pH of 7.0 instead of pH 6.8 when mixed with broth of pH 7.2, to which mixture was added 4.5 per cent N/1 NaOH, showed a final pH of 7.8 for the mixture. This, when poured into plates and tubes, gave a clear transparent medium, but one which did not coagulate sufficiently for practical use.
It is evident from the facts just described that serum of an unknown pH can not be depended upon to yield the same reaction in the serum broth mixture for Loeffler's medium.

In this study, while using the routine laboratory blood serum medium as a control for the new media studied, the impression gained was that the medium to which 4.5 per cent N/1 NaOH was added and which gave a pH of 7.6 has yielded a much better growth of *C. diphtheriae* than the control, laboratory routine Loeffler's medium. The serum of the latter was not titrated, but was added in the usual proportion, three to one of broth, the broth having been titrated to pH 7.2.

In order to test this "impression," a special study was undertaken for the purpose of obtaining definite data in a large series of cultures, using the "special" medium simultaneously with the routine Loeffler's medium. Before undertaking this study, however, it was thought desirable to test different serum broth media with a view to deciding definitely upon a mixture which would yield the best results.

Six serum-broth media mixtures were prepared:

1. Beef heart infusion broth pH 7.2, 1 part; glucose, 1 per cent; glycerol, 5 per cent; pig serum pH 6.8, 3 parts.
2. Beef *extract* broth, 1 part, in place of the beef infusion broth; glucose, 1 per cent; glycerol, 5 per cent; pig serum pH 6.8, 3 parts.
3. Beef extract broth, 1 part; glucose, 1 per cent; no glycerol; 3 parts of same serum.
4. Beef infusion broth; pH 7.2, 1 part; glucose, 1 per cent; no glycerol; 3 parts of same serum.
5. Beef infusion broth, pH 7.2, 1 part; glucose, 1 per cent; glycerol, 5 per cent; 3 parts of same serum to which was added 4.5 per cent N/1 NaOH, final mixture pH 7.6.
6. Same as no. 5, only substituting beef extract broth for the beef infusion broth.

The composition of the above mixtures was decided upon in order to test whether the heart infusion broth has any advantage over the beef extract broth, the latter being so much easier to prepare; also, to decide whether the glycerol, which has been
used in the routine media, is necessary for its keeping qualities. The addition of the 4.5 per cent N/1 NaOH was made to both the mixture containing infusion broth and the one containing beef extract broth. This was done to decide definitely whether our "impression," that the addition of the 4.5 per cent N/1 NaOH, does make a difference in the growing of the C. diphtheriae.

Following a comparative study of cultures made on the six media by planting a mixture of laboratory bacteria (Staphylococcus aureus, Staph. albus, Streptococcus, Pneumococcus, with C. diphtheriae), it was found that the media containing the NaOH (pH 7.6) with or without the glycerol, were far superior to any of the others.

It was also found that the beef extract broth serum mixture containing the NaOH was in all respects similar to the one made with the beef infusion broth. Consequently, the medium decided upon was that described under no. 6, namely; beef extract broth, pH 7.2, 1 part, containing 1 per cent glucose, 5 per cent glycerol, pig serum pH 6.8, 3 parts; to which was added 4.5 per cent N/1 NaOH, the final pH of the mixture being 7.6. We decided upon the use of the pig serum because the beef and horse serum were found unsatisfactory for the preparation of modified Loeffler's because they yielded too soft a medium for routine use. The pig serum we found could easily be obtained fresh from the nearest abattoir by giving one days' notice. It is also more economical as compared with beef or horse serum.

The advantage of this last medium over all the others is that it grows C. diphtheriae very luxuriantly and almost in a selective way, while the other media, not containing 4.5 per cent N/1 NaOH, were overgrown by staphylococci, showing only a rare organism of C. diphtheriae. On the modified Loeffler's containing the NaOH, C. diphtheriae were very abundant and very easy to detect in stained smears. The glycerol was retained because it prevents the drying of the media when kept for a long time, as occasionally happens in the 73 different Board of Health culture stations and Health Units. This mixture, when put in a

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2 The stain used in our laboratory is Laybourn's Modification of Albert's Stain.
LOEFFLER'S BLOOD SERUM MEDIUM

Tube and coagulated, gave an opalescent, semi-transparent medium, which readily withstood the rough use of swab planting, in some respects even better than the routine Loeffler's medium.

That being done, we were ready to undertake the study of testing this medium in the actual culturing of patients in the routine diagnoses or release cultures of the Health Department Laboratory. For that purpose, we placed two culture tubes, of the "special" and "routine" media, together with the swab, in the diphtheria outfits, with a request to the physician to plant both culture tubes at the same time.

This study comprises 2297 routine cultures made by the physicians, or by the medical inspectors and district nurses for diagnosis of suspected cases and of contacts as well as for release cases.

The medical inspectors and nurses on the districts have been taking the release cultures in this study as they usually do and planting them on both culture media. Better control over the work was, of course, possible when the medical inspectors and nurses did the culturing rather than when the private practitioners did it. However, in order to verify these results a comparative

### Table 1

<table>
<thead>
<tr>
<th>Routine Loeffler's Serum, 2297 Cultures</th>
<th>Modified Loeffler's Serum, 2297 Cultures</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>220</td>
<td>2006</td>
</tr>
<tr>
<td>9.6%</td>
<td>87.4%</td>
</tr>
</tbody>
</table>

Of the above number of positive cultures 29, or 1.3 per cent, were found to be practically pure cultures of *C. diphtheriae* on the modified Loeffler's, while only a rare or a few organisms developed on the routine Loeffler's. This was particularly noted in cultures taken for release.

The greater number of "no growth" or "scanty growth" cultures on the modified Loeffler's may be attributed to the fact that frequently physicians failed to plant both sera, using only the accustomed Loeffler's tube. Contaminated cultures were rare, and then occurring in both cultures.

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study under absolute control was undertaken, which will be described later.

Table 1 represents a study of the 2297 cultures sent to the Health Department Laboratory by the private practitioners, medical inspectors and nurses. Out of this number there were 220 “positives,” or 9.6 per cent, on the routine Loeffler’s and 306, or 13.3 per cent, “positives” on the modified Loeffler’s, which is higher by approximately 33 per cent. The more important fact, however, which is not brought out by merely studying the figures of the table, is that the modified Loeffler’s, because of the more luxuriant growth of *C. diphtheriae* and the ease of detecting the organism microscopically, enabled the diagnosis to be made in a very short time, while it took a considerably longer time and was much more difficult to reach a conclusion when examination was made of the routine Loeffler’s. In fact, out of the total number of positive cultures, there were on the modified Loeffler’s 29, or 1.3 per cent, which were practically pure cultures of *C. diphtheriae*, while only an occasional or rare organism was found on the routine Loeffler’s. This was particularly true in the cultures taken for release.

The number of “suspicious” cultures was about the same on both media. The greater number of “no growth” or “scanty growth” on the modified Loeffler’s may be accounted for by the fact that the physicians failed to plant both media, using only the routine media to which they were accustomed, and which had the label, while the modified Loeffler’s did not have such a label.

There rarely was a case where the *C. diphtheriae* was positive on the routine Loeffler’s and negative on the modified medium. Another important finding in this study of the 2297 cultures was the ease with which the streptococcus grows on this medium. Since the finding of streptococci has to be reported by the laboratory to the physicians, a careful record was kept of such findings parallel with the finding of *C. diphtheriae*. The results were astonishing, namely: only 11, or 0.5 per cent, of the total number showed the presence of streptococci on the routine Loeffler’s, while 133, or 6.2 per cent, showed the presence of
streptococci as a predominating organism on the modified Loeffler's.

As already referred to, the results of the study just described, dealing with comparative cultures obtained at the Laboratory in a routine way, were not considered by us to be entirely satisfactory because of our inability to control the culturing. There might, for instance, have been cases where some practitioners, in

<table>
<thead>
<tr>
<th>ROUTINE Loeffler'S Serum, 2297 cultures—Streptococcus</th>
<th>MODIFIED Loeffler'S Serum, 2297 cultures—Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>133</td>
</tr>
<tr>
<td>0.5%</td>
<td>6.2%</td>
</tr>
</tbody>
</table>

It will be noted that modified Loeffler's permits the growth of a much larger percentage of streptococcus cultures than the routine Loeffler's.

**TABLE 3**
Comparison of 494 diphtheria cultures planted simultaneously on two media in the laboratory

<table>
<thead>
<tr>
<th>ROUTINE Loeffler'S Media, 494 Cultures</th>
<th>MODIFIED Loeffler'S Media, 494 Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>20</td>
<td>457</td>
</tr>
<tr>
<td>4.04%</td>
<td>92.5%</td>
</tr>
</tbody>
</table>

This study was controlled at every step.

It will be noted that the modified Loeffler's media grew over four times as many "positives" as the routine Loeffler's. The growth was far more abundant and in some instances almost a pure culture of *C. diphtheriae*.

spite of the request to plant the two culture media alike, planted one with the swab obtained from the throat and the other with one obtained from the nose. Although we have tried always to check up in those cases, when a positive was found on the modified Loeffler's and a negative on the routine Loeffler's, still we were not satisfied that this could be considered an absolutely controlled piece of work. We, therefore, undertook the following study.
The cultures were made at the South Department of the Boston City Hospital. The swabs, one from the throat and one from the nose, were placed in a tube containing 2 cc. of nutrient broth. These swabs, when brought to the Laboratory in the broth, were carefully washed in this broth and removed from it and discarded. A fresh sterile swab was used to plant each of the routine and modified media, properly labeled and incubated over night. They were then smeared, stained, and examined microscopically by at least three different Laboratory workers, and sometimes four. Table 3 gives the result of this study.

It will be seen that this study was controlled at every step, namely, the broth was used merely to wash the nose and throat swabs; fresh sterile swabs were used to plant from the broth onto the culture media, which were studied. The media were incubated under exactly the same conditions, stained and examined in a similar way.

A second controlled, comparative study was also made by using, not only the routine Loeffler's medium of the Board of Health Laboratory alongside the new medium, but also by using

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**Table 4**

Comparison of 189 diphtheria cultures planted simultaneously on three media in the laboratory

<table>
<thead>
<tr>
<th></th>
<th>Laboratory Loeffler's Media, 199 Cultures</th>
<th>Hospital Loeffler's Media, 198 Cultures</th>
<th>Modified Loeffler's Media, 189 Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>Negative</td>
<td>179</td>
<td>165</td>
<td>149</td>
</tr>
<tr>
<td>Suspicious</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>No growth or contaminated</td>
<td>0.5%</td>
<td>11.0%</td>
<td>18.1%</td>
</tr>
</tbody>
</table>

This is another controlled study on three different media.

The modified Loeffler's again showed over four times as many “positives” as the routine Loeffler's.

The Hospital media to which is added NaOH until it reaches a definite pink to phenolphthalein made a better showing but is not up to the modified Loeffler's in character of growth nor in number of “positives”.

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the hospital Loeffler's serum. Table 4 gives the result of this study, which was carried out in exactly the same manner described for the one reported in table 3.

A critical examination of table 3, which represents the controlled study on the two media, shows that there were 494 cultures, of which 20, or 4 per cent, were found positive on the routine medium, as against 85, or 17.2 per cent, positives on the modified Loeffler's. The "suspicious" cultures in this study were 9, or 1.8 per cent, on the routine, and 19, or 3.8 per cent, on the modified Loeffler's media.

The streptococcus findings were not included in this study. It was limited to diphtheria only. The positives on the modified Loeffler's, according to our findings, are over four times those found on the routine Loeffler's, the significance of which speaks for itself.

Table 4, which represents another controlled study on three different media instead of two, shows again the value of the modified Loeffler's, medium for the growth of the C. diphtheriae. Here we find 7 positives, or 3.7 per cent, in a total of 189 cultures, on the routine media, while the modified Loeffler's shows 34, or 18 per cent, positives, which is again 4 ½ times as many positives as on the routine. The "suspicious" findings here too were twice as many as on the routine media. The hospital media made a better showing in this study than the routine: 20 positives, or 11 per cent, out of the total 189, as against 3.7 per cent on the routine, and 18.1 per cent on the modified media. Upon investigation we found that the hospital media contained NaOH, which was added by testing the regular pig serum-broth mixture to a drop of phenolphthalein on a porcelain plate. Enough normal NaOH was added so that the indicator turned a definite pink. In case too much NaOH had been added, HCl was used until the desired pink was obtained.

The method of titration just described, as followed in the preparation of the hospital medium, is unreliable because this method does not yield the same pH for the media prepared at different times. However, even at that, the results of growing C. diphtheriae were better than without the addition of the
NaOH. The use of the pH method in the preparation of any media is now recognized as the standard reliable way of obtaining a culture medium which can be duplicated at all times.

Another study was carried out with cultures obtained at the Haynes Memorial Hospital for Contagious Diseases by a student of Boston University, Elizabeth Goodman, to whom we are indebted for this work.

The swabs were placed in 2 cc. nutrient broth. The secretions were washed off them and the original swabs discarded. Fresh sterile swabs were used to make cultures on the routine Loeffler's and the modified Loeffler's. They were incubated, stained, and examined in identically the same way. The work as a whole was

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<thead>
<tr>
<th>ROUTINE LOEFFLER'S MEDIA, 176 CULTURES</th>
<th>MODIFIED LOEFFLER'S MEDIA, 176 CULTURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>167</td>
</tr>
<tr>
<td>2.2%</td>
<td>95%</td>
</tr>
</tbody>
</table>

This is another controlled study carried out in a different hospital by another worker.

It will be noted that here too the modified Loeffler's showed four times as many "positives" as the routine Loeffler's.

done in a similar manner to that employed in the studies referred to in tables 3 and 4.

Table 5 gives the results of this study. There were 176 cultures planted simultaneously on the two media. There were four positives, or 2.2 per cent, on the routine Loeffler's medium, while there were 15 positives, or 8.5 per cent, on the modified Loeffler's. In this study, as in the other two controlled studies, the modified Loeffler's showed more than four times as many positives as the routine Loeffler's. Here, too, the striking factor, however, was not merely the greater number of positives in the modified Loeffler's, but the greater abundance of growth as compared with that on the routine Loeffler's, which means
greater ease of diagnosis, in the case of the use of modified Loeffler's.

In the light of our findings, showing the great sensitiveness to changes in pH of culture media of the bacteria here considered, C. diphtheriae and particularly the streptococci, we feel that the methods used in some laboratories, of determining the pH by merely adding a few drops of phenol red to a solution and guessing the pH by the change of color produced in the solution is unreliable. We found brom thymol blue to be the best and most reliable indicator and the use of the method with the color standards as described by Medalia (1920 and 1922) almost as easy as the indiscriminate use of a few drops of phenol red.

The use of brom thymol blue with the color standards and the "comparator" block permits the definite determination of the pH within 0.1 of a pH; but what is more important, the method permits the use of additional tubes to offset the color of the culture medium and also to determine whether the particular indicator can be used at all.

We found, for instance, that phenol red does not permit the matching of color in the preparation of the Loeffler's or the modified Loeffler's media, possibly because of the "protein" or "salt" error in the serum. This difficulty is overcome by the use of brom thymol blue. The importance of definitely determining the pH in culture media is brought home by this study since, in such a highly buffered medium as we have used, the difference of 0.4 pH between the two media has made such a marked change in the growing of C. diphtheriae, yielding four times as many positives and growing it almost in a selective way, while the streptococci grew more than twelve times as often as on the routine media.

SUMMARY

A blood serum medium has been described, which is almost a selective culture medium for C. diphtheriae. It is easy to prepare, since it is only Loeffler's medium, modified by the addition of 4.5 per cent N/1 NaOH yielding a final pH of 7.6 using brom thymol blue as an indicator.
The possibility of checking the reaction in the final mixture by the pH "colorimetric" method (Medalia, 1920 and 1922) makes this culture medium easy to duplicate and yields uniform results.

In definitely controlled studies (tables 3, 4, and 5), the positive findings on the modified Loeffler's medium are more than four times as many as on the routine medium. The ease with which this modified Loeffler's grows C. diphtheriae, almost in a selective way, permits the examination to be made in a much shorter time and the positives are more readily discernible.

From the standpoint of the Health Department Laboratory examination, this culture medium has also been of great value because of the ease with which it grows the streptococcus; in a study of 2297 cultures (table 2), where on the routine Loeffler's medium only 0.5 per cent streptococci were found predominating, on the modified Loeffler's, 6.2 per cent were found.

The modified Loeffler's culture medium here described, is therefore, we feel a very valuable one for the routine examination of diphtheria and streptococcus organisms, and we especially recommend it to Health Department Laboratories for this purpose.

In concluding, we wish to express our appreciation and thanks to Dr. Francis X. Mahoney, Health Commissioner, City of Boston, to Professor Hans Zinsser of Harvard, Dr. D. L. Belding of Boston University, Dr. Edwin H. Place of the Contagious Department of the Boston City Hospital, Dr. Conrad Wesselhoeft of the Haynes Memorial Hospital for Contagious Diseases, and J. Etta Mullen, Bacteriologist, Health Department, through whose kindly advice and sympathetic cooperation this study was made possible.

REFERENCES

Belding and Fogel 1929 New England Journal of Medicine, 17, 200, 876.
Coplans, M. 1911 Jour. Hyg., 11, 274.
Swabs were taken from nose and throat washed in 2 cc. of bouillon and cultured simultaneously with fresh sterile swabs on routine Loeffler's media and modified Loeffler's media.

Fig. 1. Microphotograph of the growth on the routine Loeffler's media showing occasional *C. diphtheriae* with a large number of other organisms both staphylococci and bacilli.

Fig. 2. Microphotograph of the same culture as figure 1 grown on the modified Loeffler's media. This proved to be almost a pure growth of *C. diphtheriae*. 

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**PLATE 1**

Swabs were taken from nose and throat washed in 2 cc. of bouillon and cultured simultaneously with fresh sterile swabs on routine Loeffler's media and modified Loeffler's media.

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FIG. 1

(L. S. Medalia, et al.: Modified Loeffler's blood serum medium.)
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