المعهد: المصلى واللقاح - العباسيه.
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المواد المؤثرة على نشاط الفيبرينوليسين المفرز بواسطة الكلوستيرديم شوفييادم كلوسيريد دم سبتكم

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تم اختبار عشة عترات من كل من ميكروب الكلوستيرديم شوفييادم والكلوسيريد دم سبتكم لمعرفة مدى افراز هذه العтратات للفيبرينوليسين.

وقد وجد أن 40% من عرات الكلوستيرديم شوفييادم والكلوسيريد دم سبتكم تقوم بعمل تحلل كامل لبلازما الارانب بينما بلازما الأرانب تتحلل تحلل كامل بواسطة 50% من عرات الكلوستيرديم شوفييادم و20% من عرات الكلوستيرديم سبتكم. أيضاً ثبت بالبحث أن 10% فقط من عرات الكلوستيرديم سبتكم والكلوسيريد دم شوفييادم تقضيم بتحلل بلازما الأرانب الهندية.

وقد لوحظ أن الوسط الغذائي للحم الطيبي لا يفرز أي كمية من الفيبرينوليسين الخاص بميكربي الكلوستيرديم شوفييادم السبتكم.

وجد أن أعلى معدل لافراز الفيبرينوليسين الخاص بميكربي الكلوستيرديم سبتكم يكون في خلال من 48-72 ساعة بينمما الفيبرينوليسين الخاص بميكربي الكلوستيرديم وشوفييادم يصل إلى أعلى معدل له في خلال 48 ساعة.

وجد أيضاً أن درجة الاستهيد روغيني المناسبة لإجراء اختبار الفيبرينوليسين تكون بين 8 - 8.6 رئ.
FACTORS AFFECTING FIBRINOLYTIC ACTION OF
CL. CHAUVOEI AND CL. SEPTICUM
(With 5 Tables)

By
IKBAL FARRAG and A.Z. HUSSEIN
(Received at 1/5/1983)

SUMMARY

Ten strains of each of CL.chauvoei and CL.septicum were tested for their fibrinolytic activities. 40% of CL.chauvoei and CL.septicum strains produced complete lysis of sheep plasma, while rabbit plasma was completely lysed by 50% of CL.chauvoei strains and 20% of CL.septicum strains. Only 10% of both CL.septicum strains lysed guinea-pigs plasma. Cooked meat broth did not produce any fibrinolytic activity with either CL.chauvoei or CL.septicum.

The optimum production of fibrinolysin of CL.chauvoei was at 24 hours while those of CL.septicum at 48 - 72 hour.

The most suitable pH for the fibrinolysin test was between 5.8 - 6.8.

INTRODUCTION

Many species of bacteria produce enzymes or toxin which tend to breakdown coagulated plasma and therefore influence the course of infection to a significant degree.

The very rapid spread of gas-gangrene infections in man and animals suggests that fibrinolysis is involved. REED et al. (1941) proved that the 4 species principally concerned in gas gangrene, i.e. CL.perfringens, CL.novyi, CL.septicum and CL.sordelli produce active fibrinolysin. They found that more than half of the CL.chauvoei strains were inactive on human, rabbit, guinea-pigs and sheep plasma. The nature of this enzymes has not been investigated.

In Egypt, blackleg is the most prevalent clostridial disease, and in cases of gas-gangrene, CL.septicum is the more important organism associated with this disease.

Accordingly a series of preliminary trials had been carried with 10 strains of each of CL.chauvoei and CL.septicum to study their fibrinolytic activities and factors affecting it.

MATERIALS and METHODS

1. Strains:

Ten strains of each of laboratory stock strains of CL.chauvoei and CL.septicum were used. All the strains have been passaged through guinea-pigs and pure cultures were obtained.
Il. Media

1. Robertson media with liver particle (SMITH and HOLDMAN 1968).
2. Robertson media with meat particle.
3. Papain digest liver (MURATTA et al. 1956).
4. Thioglycolate fluid medium (patent preparation, Oxoid).

Fibrinolytic procedures

The test procedure was that used by TILLET and GARNER (1933) and slightly modified by BOISVERT (1940) was conducted.

Fresh oxalated plasma was obtained from rabbit, guinea-pigs and sheep. To 0.2 ml of oxalated plasma, 0.8 ml of sterile saline solution and 0.5 ml of serial doubling dilutions of culture filtrate was added and mixed. To this mixture 0.25 ml of a 0.25% solution of CaCl was added and placed in a water bath at 37°C. The time at which solid coagulation occurred was noted. The time at which complete dissolution of the clot (fibrinolysis) occurs was noted for 4 hours and then for 24 hours. Controls containing 0.5 ml of sterile media were tested in the same manner.

Studies of factors affecting fibrinolytic activity of C. chauvoei and C. septicum

The strains were compared for their ability to produce fibrinolysin. The best strains were chosen and tested on different culture media.

To test the effect of different pH, phosphate buffer of pH ranges 5-8 were used in comparison with normal saline.

RESULTS

1- Fibrinolytic activity of different strains of C. chauvoei and C. septicum

Ten strains of each of C. chauvoei and C. septicum were isolated in Robertson media containing liver particles. The test was applied on 24 hours old culture.

Most of the cultures which have fibrinolytic activities broken the plasma in 24 hours or less but not before 4 hours.

A few of the cultures from each fibrinolytic species prevented the clotting of plasma.

Table 1 and 2 show the fibrinolytic activities of C. chauvoei and C. septicum strains.

From table 1 it is evident that 40% of C. chauvoei and C. septicum strains produced complete lysis of sheep plasma, while rabbit plasma was completely lysed by 50% of C. chauvoei strains and 20% of C. septicum strains. Guinea-pig plasma was less sensitive to both organisms and only 10% of the strains of each was completely lysed.

The cultures which produced complete solution of the clot of a certain plasma were serially doubly diluted and retested. Table 2 illustrated the results.

According to the results of table 2, strains No. 5 and 6 of C. chauvoei were chosen for further studies as they produced lysis for different types of plasma. C. septicum strains No. 3, 4, 10 were chosen.

2- Effect of different media on the fibrinolytic activities of C. chauvoei and C. septicum

Each of the selected strains was inoculated on Robertson medium containing meat particles and other containin liver particles, fluid thioglycolate medium and liver digest medium.

FIBRINOLYTIC ACTION OF CL.chauvoei and CL.septicum

The cultures were incubated for 24 hours before being tested for their fibrinolytic activities against the three plasma.

Robertson medium with meat particles did not produce any fibrinolytic effect with any strain against any plasma.

Results of other media are illustrated in tables 3.

Table 3 shows that the fibrinolytic activities of the cultures of the same strain were irregular, it may produce lysis to a certain plasma in high dilution and on other media it may not produce any lysis and vice versa.

CL.chauvoei strains cultivated on thioglycolate medium were active only against sheep plasma in concentrated cultures, while CL.septicum cultures on the same media produced lysis to all types of plasma with a varying degree.

3- Effect of incubation times

CL.chauvoei No. (6) and CL.septicum (10) were inoculated on liver digest medium, as these strains produced lysis of all the 3 plasma on this medium. They were incubated for 72 hours. Every 24 hours one ml of cultures was siphoned and tested for their fibrinolytic activities.

The results are shown in table 4.

From table 4, it is evident that CL.chauvoei produced the maximum fibrinolytic activity at 24 hours then declined gradually. The fibrinolytic activity of CL.septicum strain increased slightly at 48 and 72 hours against the rabbit plasma, while remained at the same level against the sheep plasma with slight drop against guinea-pig plasma.

4- Effect of diluents

Twenty four hours cultures of CL.chauvoei (Na6) and CL.septicum (No. 10) were tested for their fibrinolytic activities against rabbit plasma using normal saline and phosphate buffer with pH ranges of 5.8 - 8 as diluents.

The results are illustrated in table 5.

The result in table 5 shows that normal saline and phosphate buffer at pH 5.8 and 6.6 when used as diluent for fibrinolytic activity gave the same effect, when phosphate buffer at pH 7.2 and 8.0 were used, the plasma was not clotted but precipitated down like flocules leaving upper clear supernatent in all dilutions and also in the controls.

DISCUSSION

The findings presented in this communication demonstrate the capacity of both cultures of CL.chauvoei and CL.septicum to liquify the clotted fibrin of normal sheep, rabbit and guinea-pig plasma. In the case of sheep plasma both organisms produced the same activity, while CL.chauvoei was more active against rabbit plasma. Small percent of strains of both organism (10%) produced complete lysis of guinea-pig plasma clots.

REED et al. (1941) working on human, sheep, rabbit and guinea-pig plasma reported that more than half of the cultures of CL.chauvoei were inactive in all four plasma, while 10% of CL.septicum strains produced active fibrinolysin. A few cultures from each fibrinolytic strains prevented clotting of plasma, this anticoagulating factor was exhibited not always by the same strains or the same plasma. It may be exhibited by a certain strain in one media.
and did not exist with another media. This condition was observed by REED et al. (1941) with several cultures of clostridium. They found that C.liperfringens in chopped meat medium eight cultures and in Brewers broth with 0.2% glucose six cultures out of 33 tested prevented CaCl from clotting rabbit plasma. They found that the same proportion of cultures of C.novyi, C.septicum, C.laspergillus and C.histolyticum exhibited the anticoagulating. A similar anticoagulating factors has been observed by DENNIS and ADHAM (1937), TILLETT (1937) and CHRISTENSEN (1940) in certain cultures of haemolytic streptococcus. TILLETT (1937) points out that the critical pH of the clotting plasma is 5.0 to 5.5 and he suggested that if the culture is sufficiently acid to bring the plasma-culture mixture to a more acid reaction, clotting will be prevented. However, in 24 hours culture of C.lachauvoei or C.septicum the reaction never exceeds pH 6.0 so, we consider this factor as a variable one and independent of pH.

In our hands neither C.septicum or C.lachauvoei produced any fibrinolysis when meat particles were used in the medium, while when liver particles used it gave good result. The fibrinolytic activity was variable with the 3 types of media used, so we cannot prefer one on the other. REED et al. (1943) found that cultures in Brewers medium or pepton thioglycolate medium generally gave reactions the same as parallel cultures in cooked meat.

The optimal incubation time for the fibrinolytic activity was 24 hours for C.septicum. Most authors used overnight cultures (18 hours).

When phosphate buffer was used as diluent, in comparison with saline, it was found that between pH 5.8-6.8, the pH of the test mixture has no effect on either clotting time or lysis time of the clot when found, but at pH 7.2 and 8 the plasma was not clotted and precipitated down like floccules. This result differ from that reported by CHRISTENSEN (1940) on streptococcus fibrinolysin that it was quiet stable between pH 5.0-9.0.

REFERENCES


**Table 1:** Fibrinolytic activities of undiluted culture filtrates of *Cl.chauvoei* and *Cl.septicum*

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Cultures tested</th>
<th>Plasma</th>
<th>No. of culture producing solution</th>
<th>Complete</th>
<th>Partial</th>
<th>No. lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cl.chauvoei</em></td>
<td>10</td>
<td>Sheep</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Rabbit</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>G-pig</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>Cl.septicum</em></td>
<td>10</td>
<td>Sheep</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Rabbit</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>G-pig</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 2:** Fibrinolytic activities of different filtrate dilutions of *Cl.chauvoei* and *Cl.septicum*

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Filtrate dilution producing solution of plasma of</th>
<th>Strain No.</th>
<th>Filtrate dilution producing solution of plasma of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep</td>
<td>Rabbit</td>
<td>Guinea-pigs</td>
</tr>
<tr>
<td><em>Cl.ch.2</em></td>
<td>-</td>
<td>1/2(p)</td>
<td>-</td>
</tr>
<tr>
<td><em>Cl.ch.4</em></td>
<td>-</td>
<td>1/4(p)</td>
<td>-</td>
</tr>
<tr>
<td><em>Cl.ch.5</em></td>
<td>undil</td>
<td>1/4</td>
<td>1/2</td>
</tr>
<tr>
<td><em>Cl.ch.6</em></td>
<td>1/2(p)</td>
<td>1/4</td>
<td>-</td>
</tr>
<tr>
<td><em>Cl.ch.7</em></td>
<td>-</td>
<td>1/4(p)</td>
<td>-</td>
</tr>
<tr>
<td><em>Cl.ch.9</em></td>
<td>undil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Cl.ch.10</em></td>
<td>1/2(p)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Nolysis  
(p) = Partial lysis

**Table 3:** The fibrinolytic activities of *Cl.chauvoei* and *Cl.septicum* strains cultivated on different media

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>C.L.M.</th>
<th>P.L.D.</th>
<th>Thioglycolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cl.ch. (5)</em></td>
<td>undil.</td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td><em>Cl.ch.6</em></td>
<td>1/2(p)</td>
<td>-</td>
<td>1/4</td>
</tr>
<tr>
<td><em>Cl.sept.3</em></td>
<td>-</td>
<td>1/32(p)</td>
<td>1/2(p)</td>
</tr>
<tr>
<td><em>Cl.sept.4</em></td>
<td>1/16(p)</td>
<td>-</td>
<td>1/2</td>
</tr>
<tr>
<td><em>Cl.sept.10</em></td>
<td>1/2</td>
<td>1/16</td>
<td>-</td>
</tr>
</tbody>
</table>

C.L.M. = cooked liver medium  
Undil. = undiluted  
P.L.D. = Papain liver digest medium  
Sh. P. = Sheep plasma  
Rb. = Rabbit plasma  
G.P.P. = Guinea-pig plasma  
(p) = partial

### Table (4): Effect of incubation time on the fibrinolytic activities of *C. l.chauvoei* and *Cl. septicum* cultures

<table>
<thead>
<tr>
<th>Strains</th>
<th>Activities of cultures incubated for</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. l.chauvoei (6)</em></td>
<td>1/4</td>
<td>1/2(p)</td>
<td>1/2</td>
<td>1/2(p)</td>
</tr>
<tr>
<td><em>C. l.septicum (10)</em></td>
<td>1/2</td>
<td>1/2</td>
<td>1/2(p)</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Key as table 3.

### Table (5): Effect of different ranges of pH on the fibrinolytic activities of *C. l.chauvoei* and *Cl. septicum* cultures

<table>
<thead>
<tr>
<th>Diluents</th>
<th>Dilution of <em>C. l.chauvoei</em> culture producing lysis</th>
<th>Dilution of <em>Cl. septicum</em> culture producing lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline pH 6.8</td>
<td>1/4</td>
<td>1/2</td>
</tr>
<tr>
<td>Ph. buf. pH 5.8</td>
<td>1/4</td>
<td>1/2 (p)</td>
</tr>
<tr>
<td>Ph. buf. pH 6.6</td>
<td>1/4</td>
<td>1/2 (p)</td>
</tr>
<tr>
<td>Ph. buf. pH 7.2</td>
<td>precipitate</td>
<td>precipitate</td>
</tr>
<tr>
<td>Ph. buf. pH 8.0</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*Ph. buf.* = Phosphate buffer