معهد : المصل واللقاح - العباسية.
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العوامل المؤثرة على نشاط الفيبرينوليسين المفرز بواسطة الكلوستريد يم شوفياى وكلوستريد يم سبتكم

اقبال فراج ، عبد السلام حسين

تم اختبار عشرة عترات من كل من ميكروبي الكلوستريد يم شوفياى والكلوستريد يم سبتكم لمعرفة مدى افراز هذه العترات للفيبرينوليسين. ولقد وجد أن ٤٠٪ من عترات الكلوستريد يم شوفياى والكلوستريد يم سبتكم تقوم بعمل تحلل كامل لبلازما الاغنام بينما بلازما الأرانب تتحلل تحلل كاملا بواسطة ٥٠٪ من عترات الكلوستريد يم شوفياى و ٢٠٪ من عترات الكلوستريد يم سبتكم. أيضا ثبت بالبحث أن ١٠٪ فقط من عترات الكلوستريد يم سبتكم والكلوستريد يم شوفياى تقوم بتحلل بلازما الأرانب الهندية.

ولقد لوحظ أن الوسط الغذائي للحم المطبوخ لا يفرز أى كمية من الفيبرينوليسين الخاص بميكروبي الكلوستريد يم شوفياى والسبتكم. وجد أن أعلى معدل لافراز الفيبرينوليسين الخاص بميكروب الكلوستريد يم سبتكم يكون في خلال من ٤٨-٧٢ ساعة بينما الفيبرينوليسين الخاص بميكروب الكلوستريد يم شوفياى يصل الي أعلى معدل له في خلال ٢٤ ساعة.

وجد أيضا أن درجة الاس الهيدروجيني المناسبة لاجراء اختبار الفيبرينوليسين تكون بين ٨ره - ٨ر٦.

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FACTORS AFFECTING FIBRINOLYTIC ACTION OF CL. CHAUVOEI AND CL. SEPTICUM

(With 5 Tables)

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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 produced 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 infleunce 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

I. 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 obtainded.

II. 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 procedure:

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 filterate 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 occured 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 Cl.chauvoei and Clsepticum:

The strains were compared for their ability to produe 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 strins of Cl.chauvoei and Cl.septicum:

Ten strains of each of Cl.chauvoei and Cl.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 cloting of plasma.

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

From table 1 it is eviedent that 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. 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 Cl.chauvoei were chosen for further studies as they produced lysis for different types of plasma. Cl.septicum strains No. 3, 4, 10 were chosen.

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

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

The cultures were incubated for 24 hours before bein 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 time:

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 diluent:

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 dilutent for fibrinolyftic 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 floccules leaving upper clear supernatent in all dilutions and also 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-pigs 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-pigs plasma clots.

REED et al. (1941) working on human, sheep, rabbit and guinea-pigs 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 anticloting 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 Cl.perfringens 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 Cl.novyi, Cl.septicum, Cl.sporogenes and Cl.histolyticm exhibited the anticloting. A similar anticloting factors has been observed by DENNIS and ADHAM (1937), TILLET (1937) and CHRISTENSEN (1940) in certain cultures of haemolytic streptococcus. TILLET (1937) points out that the critical pH of the clotting plasma is 5.0 to 5.5 and he sugested 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 Cl.chauvoei or Cl.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 Cl.septicumn or Cl.chauvoei produced any fibrinolysin when meat particles were used in the medium, while when liver particles used it gave good result. The fibrinolytic activities 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 Cl.septicum. Most authros 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 cloting 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 streptococcous fibrinolysin that it was quiet stable between pH 5.0-9.0.

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FIBRINOLYTIC ACTION OF CL.CHAUVOEI and CL.SEPTICUM

Table (1): Fibrinolytic activities of undiluted culture filtrates of Cl.chauvoe and Cl.septicum

Species	No. of Cultures tested	Plasma	No. of culture producing solution of clot		
			Complete	Partial	No. lysis
Cl.chauvoei	10	Sheep	4	2	4
	10	Rabbit	5	4	1
	10	G.pig	1	5	4
Cl.septicum	10	sheep	4	4	2
	10	Rabbit	2	4	4
	10	G.pig	1	2	7

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

Strain No.	Filtrate dilution producing solution of plasma of			Strain No.	Filtrate dilution producing solution of plasma of		
	Sheep	Rabbit	Guinea-pigs		Sheep	Rabbit	Guinea-pig
Cl.ch.2	-	1/2(p)	-	Cl.sept.1	-	1/16	-
Cl.ch.4	-	1/4(p)	-	Cl.sept.2	1/2(p)	-	-
Cl.ch.5	undiluted	1/4	1/2	Cl.sept.3	-	1/32(p)	-
Cl.ch.6	1/2(p)	1/4	-	Cl.sept.4	1/16(p)	-	-
Cl.ch.7	-	1/4(p)	-	Cl.sept.9	undiluted	-	-
Cl.ch.9	undiluted	-	-	Cl.sept.10	1/2	-	-
Cl.ch.10	1/2(p)	-	-				

- : = Nolysis (p) = Partial lysis

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

Strain	Fibrinolytic activites of cultures on								
	C.L.M.			P.L.D.			Thioglycolate		
	Sh.p	G.P.P.	Rb.P.	Sh.P	G.P.P.	Rb.P.	Sh.P.	G.P.P.	Rb.P
Cl.ch. (5)	undil.	1/2	1/4	1/8(P)	undil.	-	1/2(p)	-	-
Cl.ch(6)	1/2(p)	-	1/4	1/4	1/2	1/4	undil.(P)	-	-
Cl.sep.(3)	-	-	1/32(P)	1/2(p)	1/2(p)	-	undil.	1/2(p)	1/2(p)
Cl.sep.(4)	1/16(p)	-	-	1/2	1/8	-	undil.	1/2(p)	1/4
Cl.ep.(10)	1/2	1/16	-	1/2	1/2(p)	1/2	1/2	1/4(p)	1/2

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 fibrionlytic activities of Cl.chauvoei and Cl.septicum cultures

Strains	Activities of cultures incubated for								
	24 hours			48 hours			72 hours		
	Sh.P.	Rb.P.	G.P.P.	Sh.P.	Rb.P	G.P.P.	Sh.P.	Rb.p.	G.P.P.
Cl.ch.(6)	1/4	1/2(p)	1/2	1/2(p)	(1/2(p)	1/2(p)	-	-	undil.
Cl.sep(10)	1/2	1/2	1/2(p)	1/2	1/4	undil.	1/2	1/4	undil.

Key as table 3.

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

Diluents	Dilution of Cl.chauvoei culture producing lysis	Dilution of Cl.septicum culture producing lysis
Saline pH (6.8)	1/4	1/2
Ph.buf.pH (5.8)	1/4	1/2 (p)
Ph.buf.pH (6.6)	1/4	1/2 (p)
Ph.buf.pH (7.2)	precipitate	precipitate
Ph.buf.pH (8.0)	"	"

Ph. buf. = Phosphate buffer