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BACTERIA AND FIBRINOLYTIC ACTIVITY IN "DRY SOCKET"

by
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INTRODUCTION

It has been shown that the pathogenesis of »dry socket« or *alveolitis sicca dolorosa* (ASD) may be explained by an increased fibrinolytic activity in or around the dental socket leading to partial or complete lysis and destruction of the blood clot (*Birn*, 1970). This activity could be due to enzymes produced by bacteria invading the extraction wound, or tissue kinases liberated during the inflammation (*Birn*, 1970).

A wide variety of bacteria is known to possess fibrinolytic activity (*Weiss*, 1937—38; *Tillett*, 1938; *Reed at al.*, 1943; *Dixon*, 1945; *Lewis et al.*, 1949; *Lewis & Ferguson*, 1950; *Lewis & Ferguson*, 1951; *Clifton*, 1953; *Adamis*, 1961; *Kopper*, 1962; *Yaromyuk & Domaradskii*, 1963). Some of these bacteria show fibrinolytic activity only in the presence of human proactivator, while others also act in the presence of animal sera, especially dog serum.

One of the most widely accepted theories concerning the aetiology of ASD is infection of the extraction wound with bacteria from the normal oral flora (*Schroff & Bartels*, 1929; *Belding & Belding*, 1934; *Grandstaff*, 1935; *Archer*, 1939; *Sinclair*, 1943; *Russel*, 1944; *Faillo*, 1948; *Harnisch*, 1949; *Branzi & Kovacs*, 1953; *Hahn*, 1962; *Rud et al.*, 1963; *Hahn & Lange*,

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1966; *Schilli et al.*, 1967). In accordance herewith, bacteria isolated from ASD are normal inhabitants of the oral cavity (*Schroff & Bartels*, 1929; *Belding & Belding*, 1934; *Grandstaff*, 1935; *Archer*, 1939; *Lehnert*, 1953; *Waite*, 1957; *Scharf & Lange*, 1967).

Table I.

Fibrinolytic activity of microorganisms found in the oral cavity and in ASD

Organism	Found in the oral cavity	Isolated from ASD	fibrinolytic
Pseudomonas	tr.*)	yes	(+)**)
Escherichia	tr.	yes	0***)
Klebsiella	tr.	yes	(+)
Haemophilus	tr.	yes	0
Bacteroides	yes		+
Fusobacterium	yes	yes	
Staphylococcus aureus	yes	yes	++
Other staphylococci	yes	yes	(+)
Neisseria meningitidis	no	yes	
Other neisseria	yes	yes	0
Veillonella	yes		
Diplococcus pneumoniae	tr.	yes	0
β-haemolytic streptococcus	tr.	yes	+++
α-γ-haemolytic streptococci	yes	yes	(+)
Anaerobic streptococci	yes	yes	0
Corynebacterium	yes		
Actinomyces	yes	yes	
Borrelia	yes		
Treponema	yes	yes	

Some of the microorganisms isolated from ASD possess fibrinolytic activity, but only β-haemolytic streptococci show this activity to any greater extent. This is shown in Table I, which is compiled on the basis of the aforementioned reports. There is some doubt about the exact nature of the fibrinolytic activity of some of the microorganisms listed in Table I. In many instances the activity is believed to be the result of an unspecific proteolytic activity.

*) tr. = transient

**) The number of plus-signs indicate the degree of fibrinolytic activity. Brackets are used when reports on the fibrinolytic activity are controversial.

***) 0 = no activity.

The aim of this study was to investigate the possibility that a fibrinolytic activity of bacterial origin could account for the highly increased activity in ASD and thus contribute to the aetiology of this disease.

MATERIAL AND METHODS

ASD was diagnosed by partial or total loss of the blood clot after extraction, severe, often irradiating pain, and putrid odour. In 10 patients suffering from ASD, bacteriological specimens were taken from the alveolus with a platinum loop after thorough swabbing and drying of the surrounding mucosa with cotton rolls. In addition, a sample of the liquid content of the alveolus was obtained by inserting a gauze strip into the alveolus until it was saturated. After placing the gauze strip in 0.75 ml sodium diethylbarbiturate buffer (pH = 7.8, ionic strength = 0.15) the resulting solution was tested on fibrin plates with and without an admixture of 5 per cent human plasma to ensure that a fibrinolytic activity was present in all the cases of ASD used for bacteriological examination.

The bacteriological specimens were transferred to test tubes containing a special tryptone yeast extract medium described by *Jensen et al.* (1968) and to the same medium with an admixture of 20 per cent fresh frozen human plasma*). In addition, they were cultured on blood agar plates and on the same plates with an admixture of 2 per cent fresh frozen human plasma. In all cases the liquid cultures and plates were incubated aerobically as well as in anaerobic jars in H_2 with 5 per cent CO_2**).

After incubation for 2—3 days the liquid cultures were tested for fibrinolytic activity on fibrin plates according to the technique described by *Birn* (1970) and on fibrin plates with an admixture of 5 per cent human plasma. From the blood agar plates representative colonies were transferred to fibrin plates with and without human plasma and tested for fibrinolytic activity.

One part of the liquid culture was disintegrated by ultrasound and tested on fibrin plates, while the other part was further incubated for one week, after which it was tested.

If no fibrinolytic activity was found, no attempt was made to classify the bacteria.

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From another series of 10 patients suffering from ASD, alveolar liquid was obtained as described above. The gauze strip was placed in 0.75 ml sodium diethylbarbiturate buffer (pH = 7.8, ionic strength = 0.15). The resulting solution was centrifuged at 1,000 × *g*. The supernatant was removed and tested for fibrinolytic activity on fibrin plates. The sediment was resuspended in 1.5 ml buffer and one half of this volume withdrawn to be tested for fibrinolytic activity. The rest of the sediment was washed with 10 ml buffer, centrifuged, and tested for fibrinolytic activity.

Bone biopsies of the alveolar wall were obtained from five patients suffering from ASD. When storage of the specimens were necessary, this was done at −20°C. The biopsy specimens were cut into two fragments. One fragment was washed and gently brushed in saline to remove blood and decomposed remnants of the blood clot. The other fragment was left untreated. Both fragments were placed on fibrin plates and tested for fibrinolytic activity. The fibrinolytic activity was only estimated, as it was impossible to express the activity in exact figures when using this method.

RESULTS

The fibrinolytic activity in the alveolus of patients used in the bacteriological study is shown in Fig. 1. In spite of the high activity encountered in all cases it was not possible to demonstrate fibrinolytic activity in any of the bacteriological cultures, whether tested on pure fibrin plates or on fibrin plates with an admixture of human plasma. Only in one case of contamination with an aerobic Gram-positive spore-forming rod, not normally found in the oral cavity, fibrinolytic activity was recorded. β-haemolytic streptococci and Staphylococcus aureus were not found in any of the cultures.

The separation into sediment and supernatant of the liquid content of the alveolus in ASD showed that the highest fibrinolytic activity was encountered in the supernatant (Fig. 2). In the unwashed sediment the fibrinolytic activity was rather low, and in the washed sediment it constituted only about 13 per cent of the activity in the supernatant (Fig. 2).

Biopsy specimens from the alveolar bone in ASD tested for fibrinolytic activity showed no activity in the uncleaned fragments. A rather pronounced activity, however, was encountered in the cleaned fragments (Fig. 3).

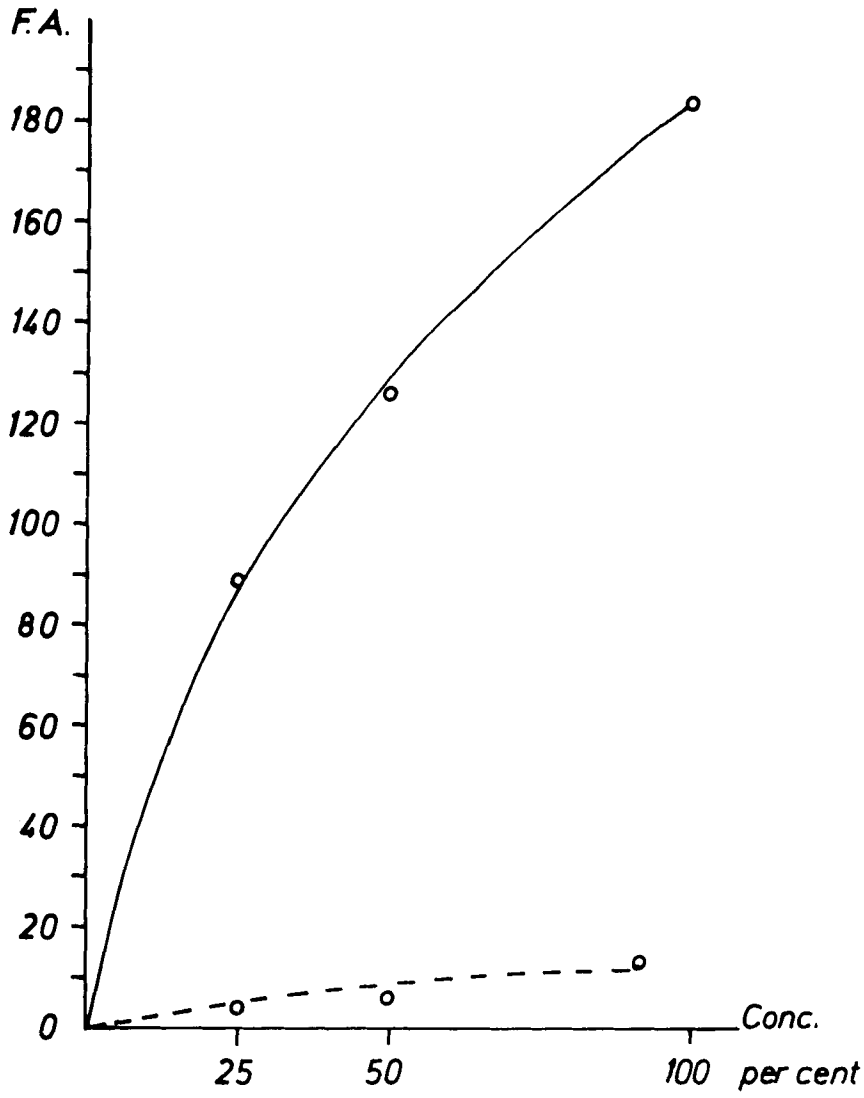

Fig. 1. Fibrinolytic activity in the liquid content of the alveolus in ASD (N = 10) tested on pure fibrin plates (unbroken line) and on fibrin plates with an admixture of human plasma (broken line). F. A. = fibrinolytic activity.

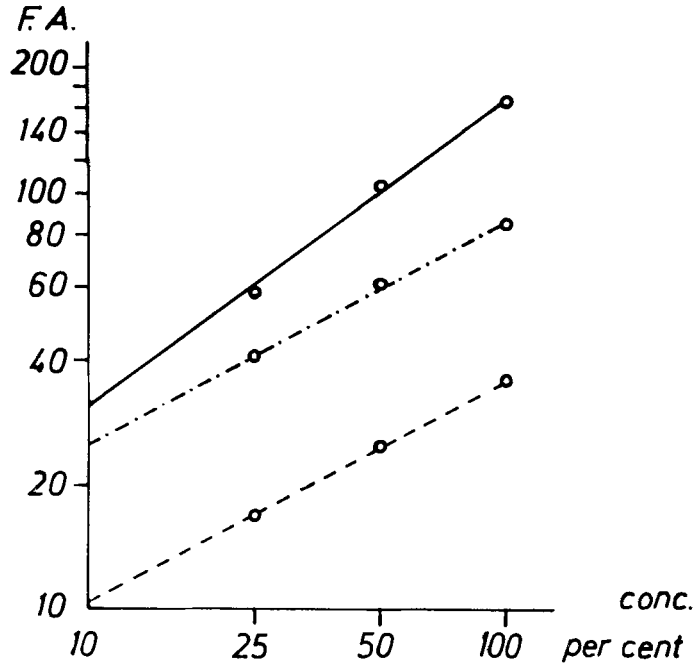

Fig. 2. Fibrinolytic activity in centrifuged liquid content of the alveolus in ASD (N = 10). Unbroken line: supernatant. Dot-and-dash line: sediment. Broken line: washed sediment. Values logaritmic plotted. F.A. = fibrinolytic activity.

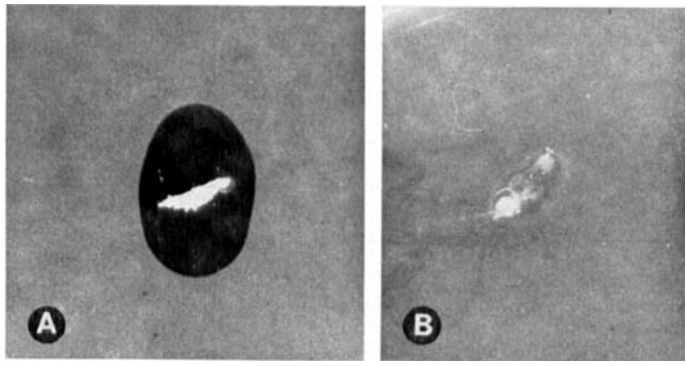

Fig. 3. Fibrinolytic activity of biopsy specimens from the alveolar wall in ASD tested on fibrin plates. A = cleaned fragment. B = uncleaned fragment.

DISCUSSION

Most of the fibrinolytic bacteria isolated from the oral cavity are only present as transients and in small numbers (Table I). Staphylococcus aureus is constantly found in the oral cavity in about half of the subjects, but usually in very small numbers (*Handelman & Mills*, 1965; *Knighton*, 1965), and the fibrinolytic activity of these bacteria is slow acting and differs in many respects from the rapid fibrindissolving effect of the haemolytic streptococci (*Tillett*, 1938; *Cliffton & Cannamela*, 1953). Besides, it has been shown that the fibrinolytic activity is proportional to the pathogenecity of the bacteria (*Weiss*, 1937—38; *Zinsser & Williams*, 1949). Thus it is uncertain whether the relatively apathogenic oral microflora is fibrinolytic, the more so as it is a matter of discussion whether the activity recorded in many of the bacteria listed in Table I is real fibrinolysis or some unspecific proteolytic activity (*Tillett*, 1938; *Lewis et al.*, 1949). In this connection it may be worth mentioning that *Söder* (1967) has shown dental plaque material to possess a weak proteolytic activity.

In the present investigation the microorganisms isolated from ASD exhibited neither extracellular nor intracellular fibrinolytic activity, even in the presence of human proactivator. β-haemolytic streptococci were not found in the cultures from ASD. *Brightman* (1957) has, however, tested the fibrinolytic activity of β-haemolytic streptococci, isolated from the mouth, and found it to be very weak. In no cases was it possible to correlate the occurrence of these bacteria with the development of ASD. *Brightman* concludes that there is no evidence that β-haemolytic streptococci are a common aetiological factor in ASD.

The fibrinolytic activity of the liquid content of the alveolus in ASD after centrifugation was mainly restricted to the supernatant (Fig. 2). The sediment with the microorganisms showed only a very weak activity, indicating that the fibrinolytic activity in the alveolus of ASD is derived from other sources than the microorganisms.

This concept is supported by the results obtained with bone fragments from ASD (Fig. 3). The cleaned bone fragments showed a very high fibrinolytic activity, even though the coating composed of bacteria and detritus had been removed. In uncleaned bone fragments no activity was recorded. The explanation of this unexpected fact is undoubtedly that the bone fragments were contaminated with fresh blood during the biopsy procedure. It is well known that human blood contains antiplasmines which inactivate the fibrinolytic system (*Norman & Hill*, 1958). This theory is further supported by the experiments dealing with the fibrinolytic activity of the liquid content

of the alveolus in ASD, where addition of fresh frozen human plasma to the fibrin plates (Fig. 1) led to a similar reduction.

In conclusion, lack of fibrinolytic activity in bacteria isolated from ASD indicates that bacteria are not responsible for the fibrinolytic activity recorded in this disease. The oral microflora, according to our present knowledge, has no or insignificant fibrinolytic activity. Even if a more detailed study of the oral microflora should reveal fibrinolytic microorganisms, these could hardly be of any significance in the production of the high activity recorded in ASD, and, as shown in the present study, their presence would certainly not explain the development of ASD in all cases.

On the other hand, this investigation has shown that in cases of ASD a high fibrinolytic activity of another origin is constantly present. The experiments also indicate that this activity is derived from the alveolar bone.

SUMMARY

This investigation dealt with the fibrinolytic activity of bacteria isolated from »dry socket« (alveolitis sicca dolorosa, ASD) and showed

1. that no fibrinolytic activity could be recorded in bacteria isolated from ASD,

2. that separation of the liquid content of the alveolus in ASD by centrifugation yielded a high activity in the supernatant and only very little activity in the sediment with the bacteria,

3. that biopsy specimens from the alveolar wall in ASD exhibited, upon thorough washing and cleaning to remove detritus and bacteria from the surface, a pronounced fibrinolytic activity.

It is concluded that the fibrinolytic activity in ASD is hardly derived from bacteria, but, more likely, is liberated from the alveolar bone.

RÉSUMÉ

BACTÉRIES ET ACTIVITÉ FIBRINOLYTIQUE DANS L'ALVÉOLITE SÉCHE

Cette étude a porté sur l'activité fibrinolytique des bactéries isolées à partir de cas d'alvéolite sèche douloureuse (ASD). Les faits suivants ont été mis en évidence:

1. Aucune activité fibrinolytique ne pouvait être observée dans les bactéries isolées à partir d'ASD.

2. La séparation par centrifugation du contenu liquide de l'alvéole dans l'ASD permet d'obtenir une forte activité dans le surnageant et une activité très faible dans le sédiment contenant les bactéries.

3. Après lavage et nettoyage soigneux pour enlever de la surface les débris et les bactéries, les biopsies provenant de la paroi alvéolaire dans l'ASD présentaient une activité fibrinolytique marquée.

En conclusion, il est peu probable que l'activité fibrinolytique dans l'ASD soit due aux bactéries, mais il est plus vraisemblable que cette activité provienne de l'os alvéolaire.

ZUSAMMENFASSUNG

BAKTERIEN UND FIBRINOLYSE BEI »DRY SOCKET«

Die Untersuchung bezog sich auf die fibrinolytische Aktivität bei von »dry socket« (alveolitis sicca dolorosa, ASD) isolierten Bakterien und ergab,

1. dass bei von ASD isolierten Bakterien keine fibrinolytische Aktivität nachzuweisen var,

2. dass ein Zentrifugieren von dem fliessenden Inhalt der Alveole in Fällen der ASD eine hohe fibrinolytische Aktivität der Supernatante, dagegen nur ganz geringe Aktivität des bakterienhaltigen Bodensatzes ergab,

3. dass Biopsien des Alveolenknochens in Fällen der ASD nach sorgfältiger Spülung und Reinigung, um Detritus und Bakterien von der Oberfläche zu beseitigen, eine ausgesprochen fibrinolytische Aktivität ergaben.

Es wird die Schlussfolgerung gezogen, dass die fibrinolytische Aktivität in Verbindung mit der ASD kaum von den Bakterien herrührt, sondern sich eher von dem Alveolenknochen freimacht.

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