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FIBRINOLYTIC ACTIVITY OF ALVEOLAR BONE IN »DRY SOCKET«

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The present study dealt with the fibrinolytic activity of alveolar bone in *alveolitis sicca dolorosa* (ASD) or »dry socket«. It was shown that 1) alveolar bone in ASD contains stable tissue activators but no labile activators, and 2) alveolar bone in ASD contains free plasmin.

These findings were compared with previous investigations of the fibrinolytic activity in normal alveolar bone, and it was concluded that liberation of the tissue activators has taken place in ASD. The importance of this conclusion for the etiology and pathogenesis of ASD were discussed.

One of the most outstanding clinical characteristics of *alveolitis sicca dolorosa* (ASD) or »dry socket« is the liquefaction of the blood clot in the extraction wound. This may be explained from the fact that such alveoli exhibit a high fibrinolytic activity (*Birn*, 1970a). Fibrinolytic activity is known to be generated by chemical agents (i.e. chloroform, urethane and salicylic acid), and bacteria (i.e. β-haemolytic streptococci, staphylococci and coli bacilli) and activators in different tissues and body fluids (i.e. the uterus, prostate, urine and saliva) by a direct or indirect activation of plasminogen in human blood (*Konttinen*, 1968). The normal oral microflora does not possess any fibrinolytic activity (*Birn*, 1970b) and the fibrinolytic activity of saliva is in no way strong enough to account for the high activity in ASD (*Birn*, 1970a). On the other hand, alveolar bone contains tissue activators of plasminogen, which may be released during inflammation and thus give rise to the high fibrinolytic activity in ASD (*Birn*, 1971).

The aim of this study was to investigate if such liberation of tissue activators

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of alveolar bone in ASD and succeeding activation of the fibrinolytic system actually takes place in ASD.

MATERIAL AND METHODS

Bone biopsies devoided of periosteum were obtained from 10 patients suffering from ASD. The diagnosis of ASD was made according to the recognized criteria: partial or complete loss of blood clot denuding the alveolar bone, exagerating pain and putrid odour or taste. The age of patients ranged from 26 to 56 years, the average being 39 years. All cases of ASD occurred in the region of the lower third molars following surgical removal of the teeth. Pain was initially encountered from one to five days postoperatively, the average being 3 days. The duration of pain ranged from 8 to 17 days, the average being 12 days. The biopsies were taken at a time where the course of the disease on the basis of patients information about pain intensity and the appearance of the empty alveolus was considered to be at its maximum. A mucoperiosteal flap was raised and the buccal part of the alveolar wall was removed with chisel or bone rongeur.

According to a method previously described in detail by *Birn* (1971) and modified from a technique developed by *Albrechtsen* (1958), the bone biopsies were grounded in a morter and the activators were extracted with saline and a 2M solution of potassium thiocyanate (KSCN) (Table I). The extracts were tested for fibrinolytic activity on fibrin plates using a slight modification (*Birn*, 1970a) of the technique described by *Astrup* and *Müllertz* (1952). The fibrinolytic activity was measured as the product in mm^2 of two perpendicular diameters of the lysed zone around the testdrops, which constitute three concentrations of each sample (100, 50 and 25 per cent) and three drops of each concentration. Diethylbarbiturate buffer (pH = 7.8, ionic strength = 0.15) was used for all dilutions.

In both saline and KSCN extracts of all biopsies (Table I) the fibrinolytic activity was tested after adding epsilon amino caproic acid* (EACA) dissolved in diethylbarbiturate buffer in concentrations of 10^{-5}, 10^{-4} and 10^{-3}M. Furthermore, the extracts (Table I) were tested for plasmin activity on heated fibrin plates according to the technique described by *Lassen* (1953). The method used is identical to the one previously described (*Birn*, 1970a), and the absence of fibrinolytic activity when urokinase was placed on the heated fibrin plates has shown that all plasminogen present in the plates has been

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Table I.

Distribution according to patients of different tests for fibrinolytic and histological examination used in the investigation

Pt. no.	Saline extract		KSCN extract		Plasmin activity test	Liquid content of alveolus	Acidity and heat stability test	Histological examination
	Pure	EACA added	Pure	EACA added				
2936 D	+	+	+	+	+	+		
3068 D	+	+	+	+	+	+		+
4286 D	+	+	+	+	+	+		
4384 D	+	+	+	+	+	+		
4983 D	+	+	+	+	+	+	+	
5198 D	+	+	+	+	+	+		+
5320 D	+	+	+	+	+	+		+
5709 D	+	+	+	+	+			
6812 D	+	+	+	+	+	+	+	
7110 D	+	+	+	+	+	+		

destroyed. Besides, it was shown that drops of a 2M solution of KSCN did not cause any lytic zone on heated or unheated plates.

In 9 patients (Table I) the liquid content of the alveolus was sampled by placing a gauze strip in the alveolus until saturated. The material thus obtained was immersed in 0.75 ml diethylbarbiturate buffer and tested for fibrinolytic activity on fibrin plates as mentioned above.

In two bone biopsies (Table I) the amount of saline and KSCN extracts were sufficient for acidity and heat stability tests. One fraction of the extract was adjusted to pH 3 by means of 0.1 N HCl and then stabilized by a phosphate buffer solution. After incubation at 37°C in water bath for 30 min. the fraction was readjusted to neutral pH with solid $NaHCO_3$ and tested for fibrinolytic activity on fibrin plates. The other fraction was diluted with diethylbarbiturate buffer to the same volume as the fraction mentioned above. After incubation for 30 min. at 37°C in water bath this fraction was tested for fibrinolytic activity on fibrin plates, too. On the basis of an investigation by *Albrechtsen* (1958) it was concluded that this procedure ensured optimum possibility for differentiation between labile and stable activators.

Three biopsies (Table I) were divided in two fragments before the extraction procedure, and the one fragment was examined histologically after fixation in 10 per cent formalin, decalcification in EDTA and staining with haematoxylin and eosin.

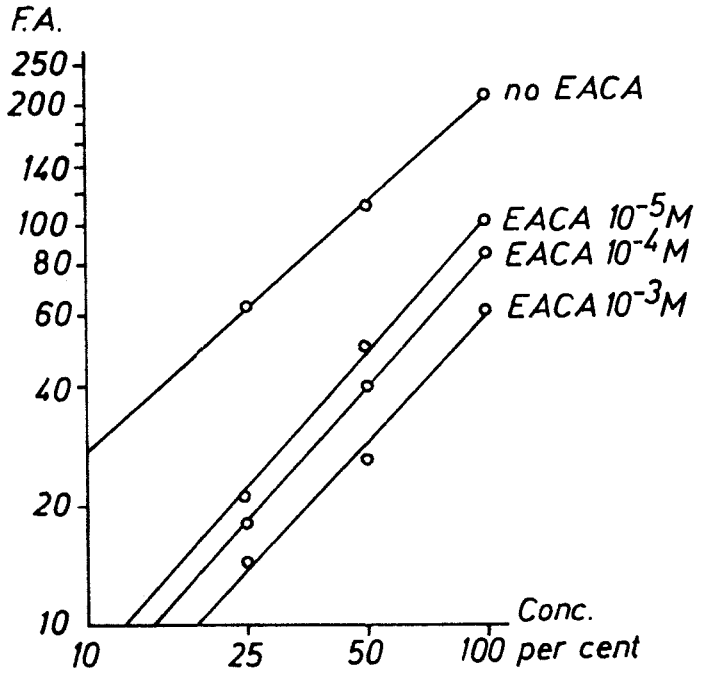

Fig. 1. Mean inhibitory effect of different concentrations of EACA on 10 samples of KSCN extracts of alveolar bone from ASD. F.A. = fibrinolytic activity expressed in mm². The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

RESULTS

Saline extracts showed only a very weak fibrinolytic activity, the average activity of undiluted extracts without addition of EACA being 4 mm². All other tests for fibrinolytic activity of saline extracts showed no activity at all.

The fibrinolytic activity of KSCN extracts with and without addition of EACA is shown in Fig. 1. The activity was high and to a great extend inhibited by EACA, although complete inhibition was not seen.

The activity of KSCN extracts on heated fibrin plates was low, but clearly demonstrated in all cases (Fig. 2).

The stability of KSCN extracts to acidity and heat is shown in Fig. 3. The saline extracts did not show any activity at all, whereas KSCN extracts possessed an activity, which was unaffected by a change in pH at 37°C.

The fibrinolytic activity of the liquid content of the alveolus was pronounced (Fig. 4).

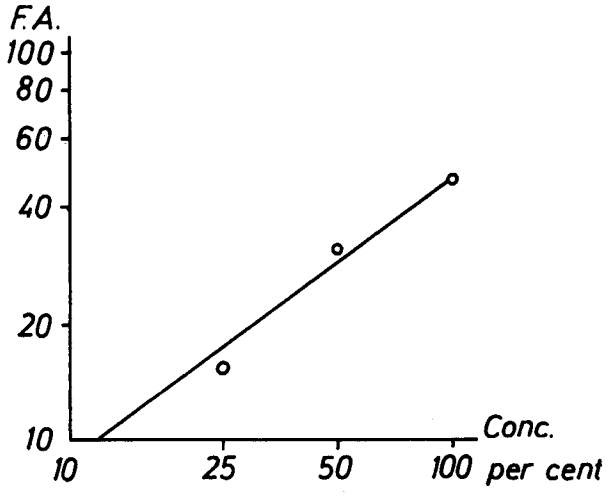

Fig. 2. Mean fibrinolytic activity of 10 extracts from alveolar bone in ASD on heated fibrin plates. F.A. = fibrinolytic activity expressed in mm². The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

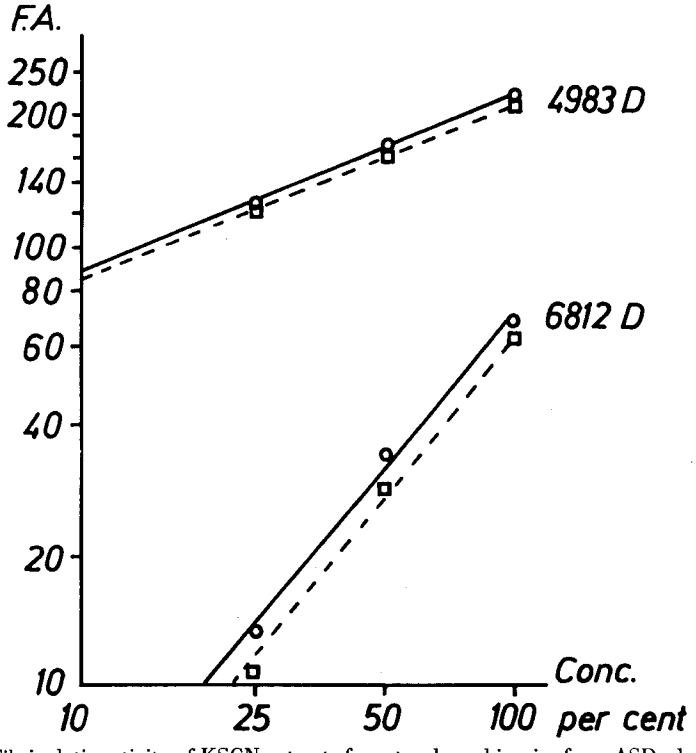

Fig. 3. Fibrinolytic activity of KSCN extracts from two bone biopsies from ASD when heated to 37°C at neutral pH (unbroken lines) and when heated to 37°C at pH 3 (broken lines). F.A. = fibrinolytic activity expressed in mm². The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

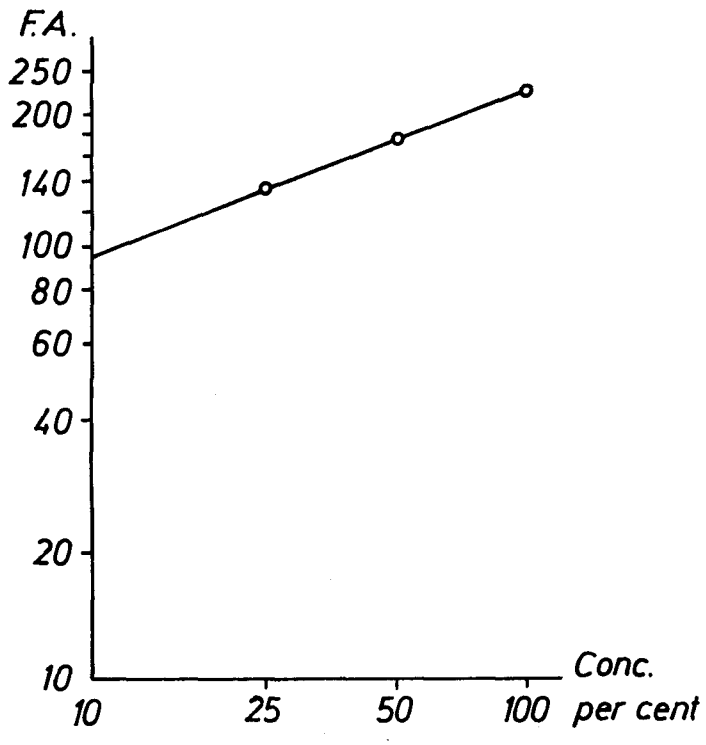

Fig. 4. Mean fibrinolytic activity of 9 samples of the liquid content of the alveolus in ASD. F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the samples in per cent (obtained by serial dilutions). All values are plotted logarithmically.

In 3 biopsies the microscopy showed marrow spaces of the loose connective tissue type with pronounced inflammation (Fig. 5).

DISCUSSION

In the present study the distribution of the patients according to age and sex and the course of the disease in each case are in good accordance with the recognized pattern for ASD (*Krogh*, 1937; *Lehner*, 1958; *MacGregor*, 1968).

The high fibrinolytic activity of the liquid content of the alveolus as shown in Fig. 4 is in good accordance with the highest activity recorded in a previous investigation on the fibrinolytic activity in ASD (*Birn*, 1970a) and indicates that the bone biopsies actually have been obtained at a peak in the course of the disease.

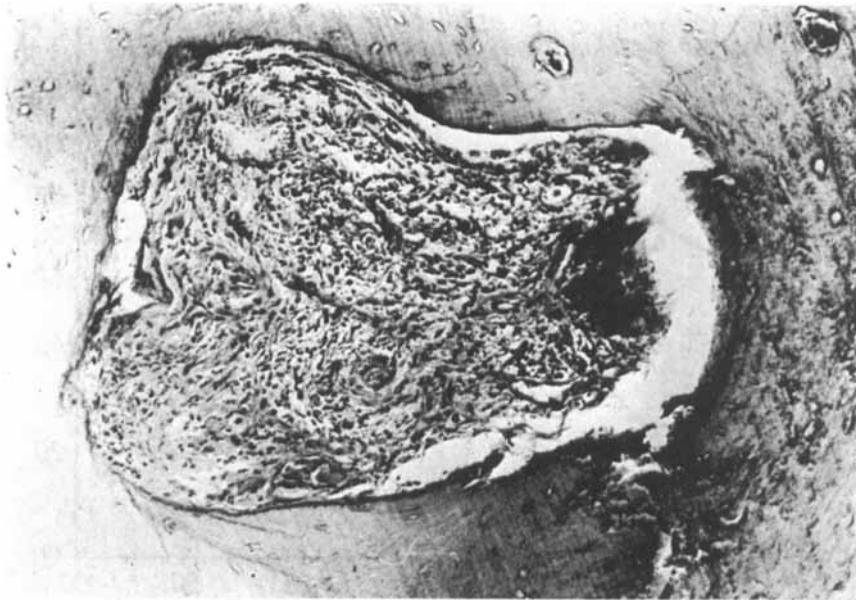

Fig. 5. Typical appearance of bone marrow in ASD. Note the pronounced inflammation of the loose connective tissue.

It is known that EACA in concentrations from 10^{-4}M to 10^{-3}M is a powerfull inhibitor of activator activity, whereas the plasmin activity remains almost unaffected (*Ablondi et al.*, 1959; *Alkjaersig et al.*, 1959; *Egeblad*, 1967). Fig. 1 shows that the fibrinolytic activity of the KSCN extracts to a great extent was inhibited by EACA in the aforementioned concentrations. This indicates that the fibrinolytic activity in the KSCN extracts is mainly due to activators of plasminogen. The KSCN extracts has been found to contain a tissue activator, which is stable to acidity and heat (*Albrechtsen*, 1958). Fig. 3 shows that the fibrinolytic activity of the KSCN extracts from alveolar bone in ASD was unaffected when changing the pH from neutral to 3 at 37°C, and this result thus confirms that the tissue activator in KSCN extracts of alveolar bone from ASD is of the stable type. In contrast to this finding no labile activator, which is to be contained in the saline extracts (*Albrechtsen*, 1958), could be demonstrated in the alveolar bone from ASD. *Lassen* (1953) has shown that heating of fibrin plates destroyes the plasminogen present, and the urokinase tests confirmed that all plasminogen has been destroyed in the method used in the present study. As fibrinolytic activity could be demonstrated on heated fibrin plates it thus may be concluded that the extracts of alveolar bone from ASD contains free plasmin. To summarize,

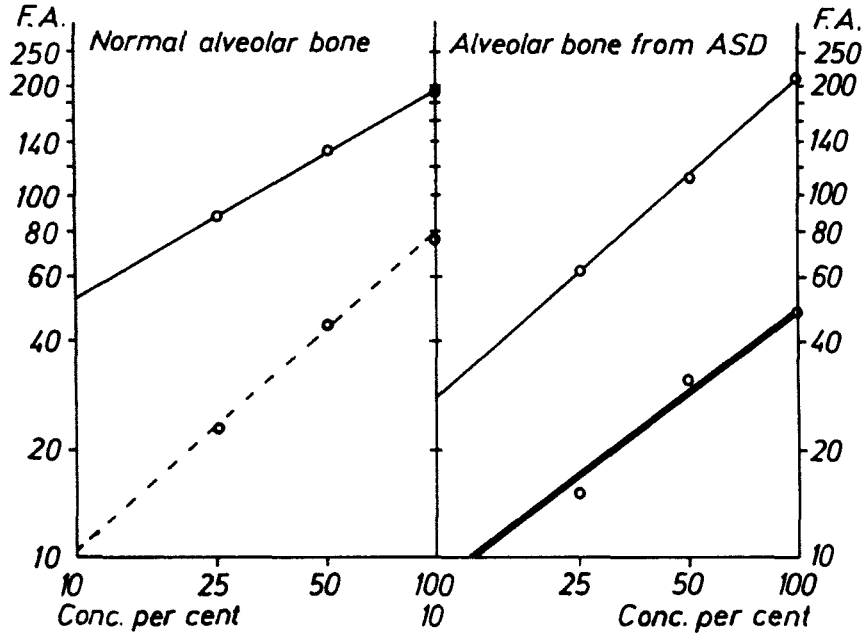

Fig. 6. A comparison of the fibrinolytic activity in normal alveolar bone (from *Birn*, 1971) and in alveolar bone from ASD. Heavy unbroken line = plasmin activity. Unbroken line = stable activator activity. Broken line = labile activator activity. F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the different extracts in per cent (obtained by serial dilution). All values are plotted logarithmically.

the extracts of alveolar bone in ASD contains stable tissue activator and free plasmin, but no labile activator.

Normal alveolar bone contains tissue activators of both the stable and labile type, but no plasmin (*Birn*, 1971). It is believed that the labile activator, when released, disappear from the area, possibly by entering the blood stream (*Albrechtsen*, 1958), whereas the stable activator remains locally and is responsible for a local fibrinolytic activity by transforming plasminogen from blood to plasmin (*Astrup*, 1956).

When comparing the results of the present study with those of the previous investigation (*Birn*, 1971), it may thus be concluded that a release of tissue activators of the alveolar bone and subsequent activation of plasminogen from the blood or bloodclot has taken place in ASD (Fig. 6).

The presence of free plasmin in the extracts of bone biopsies in ASD should not suggest that plasmin is liberated or generated in the living bone tissue itself. It is more likely that the plasmin present in the extracts originates

from contamination of the biopsy with the content of the alveolus or maybe from the necrotic parts of the alveolar bone. Thus, the presence of plasmin in the extracts of alveolar bone in ASD indicates that liberated stable tissue activators diffuse towards the alveolus giving rize to plasmin formation in areas beyond the extent of the inhibiting properties of the living organism.

The reason for this liberation of tissue activators of alveolar bone is probably to be searched for in the pronounced inflammation of the marrow spaces in ASD (Fig. 5). It has been shown that inflammation is able to cause release of tissue activators by injury to the cells (*Ungar*, 1952; *Astrup*, 1956).

This investigation and earlier studies (*Birn* 1970a, b; *Birn* 1971) thus indicate that the etiology of ASD may be either trauma excerted during removal of the tooth, infection of the extraction wound or both. The patogenesis is inflammation of the marrow spaces, which causes liberation of the tissue activators transforming plasminogen to plasmin, which in turn dissolves the fibrin and thus produces lysis of the blood clot.

Furthermore, it should be mentioned that fibrinolysis liberates bradykinin, a nonapeptide. This biologically active substance is strongly pain producing (*Frey et al.*, 1968) and may thus explain the pronounced pain in ASD. Studies are in progress to substantiate this hypothesis.

REFERENCES

Ablondi, F. B., J. J. Hagan, M. Philips & E. C. de Renzo, 1959: Inhibition of plasmin, trypsin and the streptokinase-activated fibrinolytic system by ε-aminocaproic acid. Arch. Biochem. *82:* 153—160.

Albrechtsen, O. K., 1958: The fibrinolytic agents in saline extracts of human tissues. Scand. J. clin. lab. Invest. *10:* 91—96.

Alkjaersig, N., A. P. Fletcher & S. Sherry, 1959: ε-aminocaproic acid: an inhibitor of the plasminogen activation. J. biol. Chem. *234:* 832—837.

Astrup, T., 1956: Fibrinolysis in the organism. Blood *11:* 781—806.

Astrup, T. & S. Müllertz, 1952: The fibrin plate method for estimating fibrinolytic activity. Arch. Biochem. *40:* 346—351.

Birn, H., 1970a: Fibrinolytic activity in »dry socket«. Acta odont. scand. *28:* 37—58.

—»— 1970b: Bacteria and fibrinolytic activity in »dry socket«. Acta odont. scand. *28:* 773—783.

—»— 1971: Fibrinolytic activity of normal alveolar bone. Acta odont. scand. *29:* 141—153.

Egeblad, K., 1967: Inhibitors of fibrinolysis. Scand. J. clin. lab. Invest. *20:* suppl. 98.

Frey, E. K., H. Kraut & E. Werle, 1968: Das Kallikrein-Kinin-System und seine Inhibitoren. Ferdinand Enke Verlag, Stuttgart, pp. 182—184 and 220—228.

Kottinen, Y. P., 1968: Fibrinolysis: chemistry, physiology, pathology and clinics. Star Pharmaceutical Manufacturers, Tampere, Finland, pp. 84—137.

Krogh, H. W., 1937: Incidence of dry socket. J. Amer. dent. Ass. *24:* 1829—1836.

Lassen, M., 1953: Heat denaturation of plasminogen in the fibrin plate method. Acta physiol. scand. *27:* 371—376.

Lehner, T., 1958: Analysis of one hundred cases of dry socket. Dent. Pract. dent. Rec. *8:* 275—279.

MacGregor, A. J., 1968: Aetiology of dry socket: a clinical investigation. Brit. J. oral Surg. *6:* 49—58.

Ungar, G., 1952: Inflammation and its control. A biochemical approach. Lancet *263:* 742—746.

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