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FIBRINOLYTIC ACTIVITY OF NORMAL ALVEOLAR BONE

by
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INTRODUCTION

Several tissues and organs possess fibrinolytic activity (*Permin*, 1949; *Albrechtsen*, 1959). The activity is due to activators of plasminogen which are of two different kinds. The first type is easily extracted by saline; this activator is labile, i.e. it looses its activity by heating at a low pH. The second type of activator is firmly attached to the structural cell proteins and can only be extracted by such solvents as potassium thiocyanate (KSCN). This latter type of activator is stable and is not destroyed by heat and acidity. The function of the two types of tissue activator is schematically outlined in Fig. 1. It has been assumed that the labile activator under certain conditions (i.e. trauma, burns and stress situations) is easily liberated from the tissues and finds its way into the bloodstream, thus activating the plasminogen in the plasma. In this way, the labile activator is immediately available to meet the varied needs of the organism as a whole (*Albrechtsen*, 1958). On the other hand, it is believed that the stable activator remains locally and is responsible for the local fibrinolytic activity of the tissue in question, without influencing the general fibrinolytic activity of the organism (*Astrup*, 1956).

The fibrinolytic activity of human tissues shows wide variations. Some organs such as the adrenal, prostate, uterus, lymph nodes, thyroid and ovary

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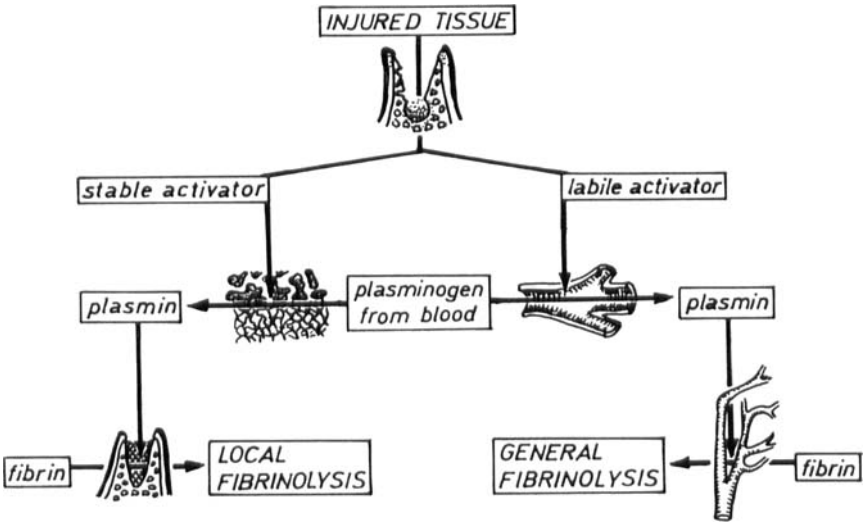

Fig. 1. Schematic outline of the function of tissue activators in fibrinolysis. See text for further explanation.

show a high fibrinolytic activity. A moderate fibrinolytic activity has been found in the pituitary body, kidney, skeletal muscles, heart, brain and testis, whereas no or only slight activity has been noted in samples from the spleen and liver (*Albrechtsen*, 1957). The fibrinolytic activity of bone has been sparsely investigated. *Roberts* and *Astrup* (1957) in a study of tissue activators in monkeys found no fibrinolytic activity in bone, whereas the periosteum in both monkeys and humans showed a very high activity. In a report on the fibrinolytic activity of human bone marrow *Björkman* and *Nilsson* (1961) have shown that red bone marrow possesses a high fibrinolytic activity in contrast to yellow bone marrow which is nearly afibrinolytic. The tissue activator in red bone marrow is exclusively of the labile type. The fibrinolytic activity of haematopoetic bone marrow has been shown to be connected with the eosinophilic granulocytes (*Barnhart* and *Riddle*, 1963).

The oral mucosa like the gingival fluid from the crevice of clinically healthy gingiva possesses fibrinolytic activity, probably originating from the connective tissue (*Gustafsson* and *Nilsson*, 1961; *Björlin* and *Nilsson*, 1968). Otherwise the fibrinolytic activity of human oral tissues has not been clarified.

The aim of this study was to investigate the content of tissue activators in normal alveolar bone in the posterior and anterior regions of the jaws and to characterize this activator if present. The incentive to carry out this

investigation was the finding that a high fibrinolytic activity is present in the alveolus of »dry socket« and that this activity might be derived from the alveolar bone (*Birn*, 1970 a and b).

MATERIAL AND METHODS

Bone biopsies devoided of periosteum were obtained during minor surgical procedures from 27 patients consisting of 14 males and 13 females, age ranging from 17 to 73 years, the average being 33 years. All patients were treated at the Department of Oral Surgery, Royal Dental College, Aarhus. In 20 patients biopsies were taken from the alveolar bone during operative removal of impacted lower third molars without signs of pericoronitis. In 7 patients biopsies were taken from the alveolar bone in the anterior regions of the upper and lower jaws during different operative procedures in clinically noninfected areas. All biopsies used for fibrinolytic activity estimation were stored untreated in specimen tubes at −20°C if not processed immediately.

Extraction of activator from all biopsies was performed by a technique modified from the one described by *Albrechtsen* (1958) using saline and a 2M solution of KSCN. The bone fragments were ground in a mortar with 20 ml saline per one gram of tissue and shaken for one hour. After centrifugation at 1000 × *g* the supernatant was removed and tested for fibrinolytic activity on fibrin plates using a slight modification of the technique described by *Astrup* and *Müllertz* (1952) and mentioned in a previous report (*Birn*, 1970a). The fibrinolytic activity, according to this technique, is measured as the product in mm^2 of two perpendicular diameters of the lyzed zone around the test drops, which constitute three concentrations of each sample (100, 50 and 25 per cent) and three drops of each concentration. All dilutions were made with diethylbarbiturate buffer (pH = 7.8, ionic strength = 0.15). The sediment remaining after centrifugation and containing the crushed tissue was suspended in 20 ml of 2M KSCN and once again shaken for one hour. After centrifugation at 1000 × *g* the supernatant was removed for fibrinolytic activity measurements by the fibrin plate method.

In both saline and KSCN extracts from 10 of 20 bone biopsies from the lower third molar region, the stability to heat and acidity was tested. One fraction of the extracts 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 re-adjusted to neutral pH with solid $NaHCO_3$ and placed on fibrin plates for evaluation of the fibrinolytic activity. 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 it was tested on fibrin plates, too.

In the 10 remaining samples of saline and KSCN extracts from the 20 alveolar bone biopsies from the lower third molar region the fibrinolytic activity was tested by adding epsilon aminocaproic acid (EACA)* dissolved in diethylbarbiturate buffer in concentrations of 10^{-5}, 10^{-4} and 10^{-3} M. Furthermore, tests were made for a possible plasmin activity on heated fibrin plates according to the technique described by *Lassen* (1953).

Eleven bone biopsies which were of sufficient size to allow partition and yet leaving enough material for the fibrinolytic activity measurements were divided into two fragments, one of which was used for fibrinolytic activity tests, while the other was examined histologically. The latter was fixed in formalin, decalcified in EDTA and stained by haematoxylin and eosin.

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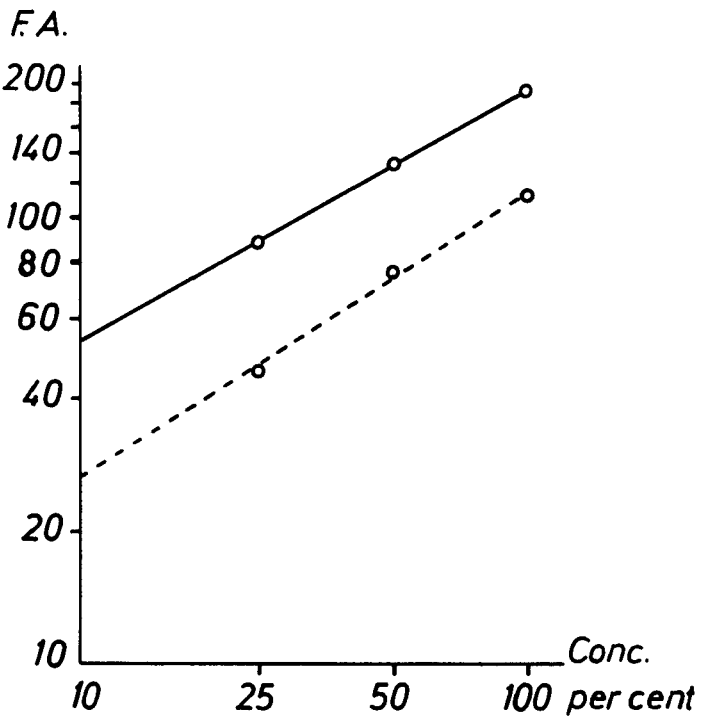

Fig. 2. Mean fibrinolytic activity of KSCN (unbroken line) and saline (broken line) extracts from 27 alveolar bone biopsies. F.A. = fibrinolytic activity expressed in mm². The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

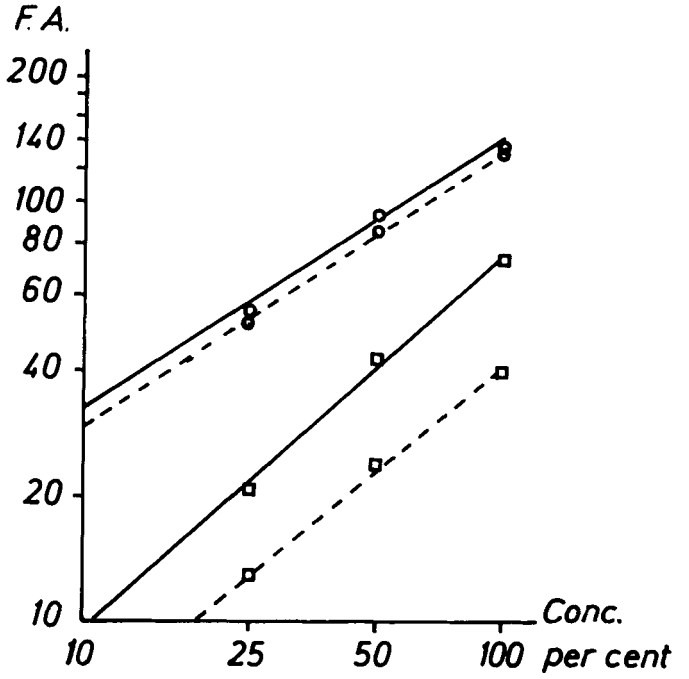

Fig. 3. Mean fibrinolytic activity of 10 KSCN extracts (circles) and 10 saline extracts (squares) when heated to 37°C at neutral pH (unbroken lines) and when heated to 37°C at pH 3 (broken lines). F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

RESULTS

KSCN extracts of all biopsies demonstrated a high fibrinolytic activity as shown in Fig. 2. The variations in activity of different biopsies extracted with KSCN were within 50 mm^2 from the mean values (unbroken line). Extractions with saline (broken line) showed considerably less activity than the KSCN extracts (Fig. 2). Moreover, the fibrinolytic activity of the saline extracts showed large variations from one patient to another (from 25 to 324 mm^2).

The results of the combined heat and pH stability test on the tissue extracts are demonstrated in Fig. 3. It is clearly shown that the fibrinolytic activity of the KSCN extracts was unaffected by changing the pH from neutral to 3 at 37°C (circles). On the other hand, the fibrinolytic activity of saline extracts had decreased to about 50 per cent at pH 3 and 37°C (squares).

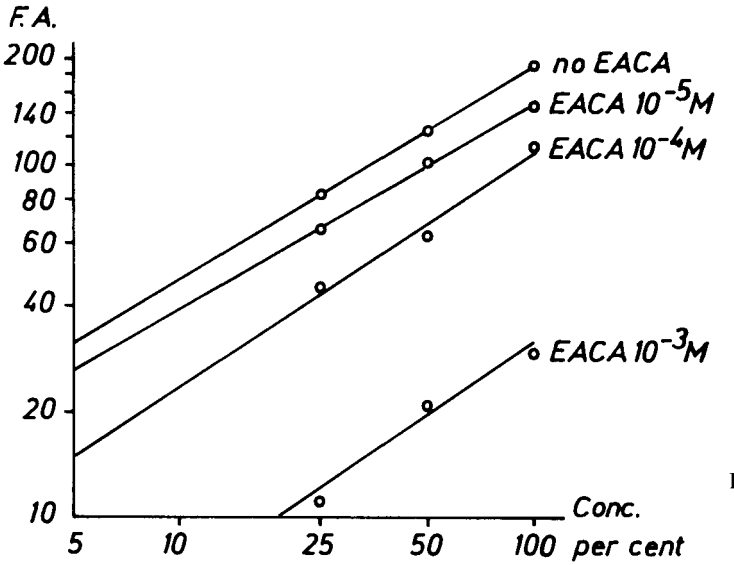
F.A.
200
140
100
80
60
40
20
10
no EACA
EACA 10⁻⁵M
EACA 10⁻⁴M
EACA 10⁻³M
Conc.
5
10
25
50
100 per cent

Fig. 4.

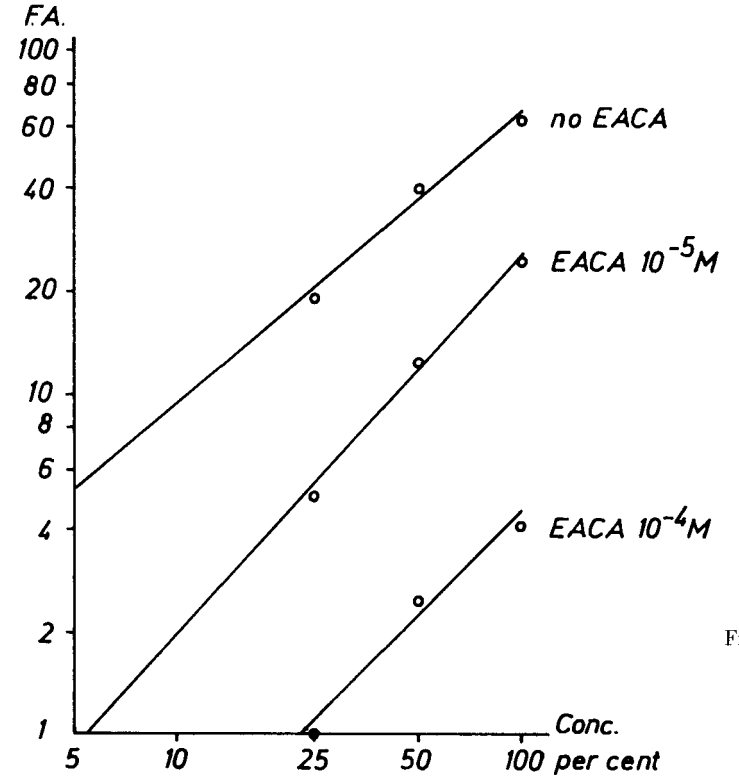
F.A.
100
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Fig. 5.

Fig. 6.

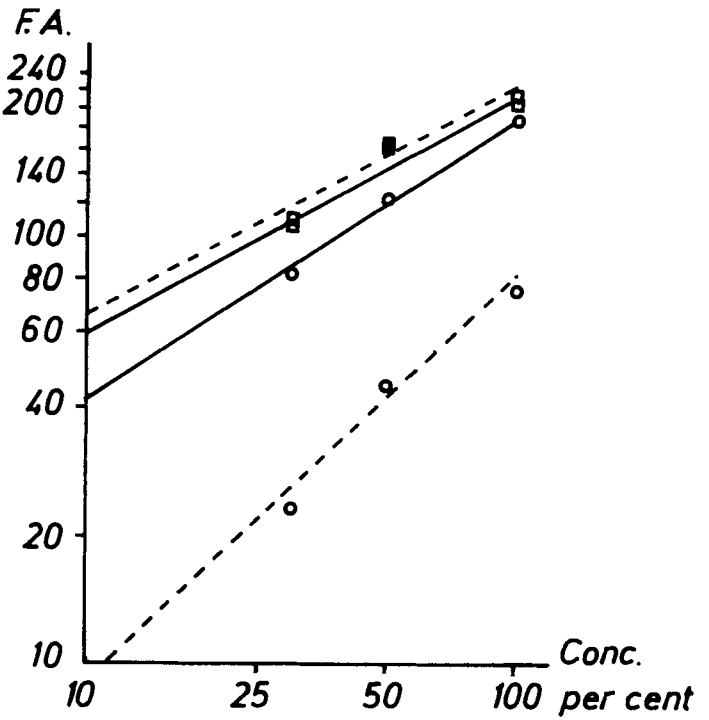

Fig. 4. Mean inhibitory effect of different concentrations of EACA on 10 samples of KSCN extracts. F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

Fig. 5. Mean inhibitory effect of different concentrations of EACA on 10 samples of saline extracts. F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

Fig. 6. Mean fibrinolytic activity of KSCN extracts (unbroken lines) and saline extracts (broken lines) from 20 bone biopsies from the lower third molar region (circles) and from 7 bone biopsies from the anterior regions (squares). F.A. = fibrinolytic activity expressed in mm^2. The abscissa expresses the concentration of the extract in per cent (obtained by serial dilutions). All values are plotted logarithmically.

The inhibitory effect of different concentrations of EACA on KSCN extracts of the bone biopsies is shown in Fig. 4. The decrease in fibrinolytic activity at low concentrations of EACA was obvious. When EACA was added in concentrations of 10^{-3}M the activity was less than five per cent of the original fibrinolytic activity. The effect of EACA on saline extracts is illustrated in Fig. 5. Inhibition of fibrinolytic activity was very pronounced. Addition of

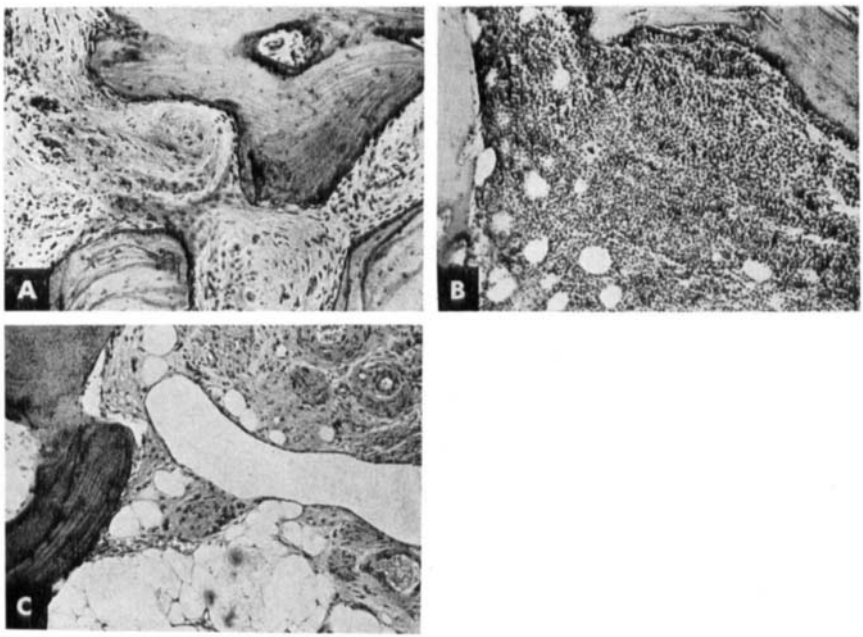

Fig. 7. Types of bone marrow found in alveolar bone biopsies used for examination of fibrinolytic activity. A: loose connective tissue. B: haematopoetic bone marrow. C: same as in (A) with fat cells.

EACA in concentrations of 10^{-3}M completely inhibited the fibrinolytic activity and could therefore not be shown on the diagram.

When the saline and KSCN extracts on heated fibrin plates was tested, no fibrinolytic activity was recorded.

The fibrinolytic activity of bone biopsies from the lower third molar region (circles) and from the anterior regions (squares) is shown in Fig. 6. The KSCN extracts from the two regions showed nearly the same fibrinolytic activity (unbroken lines), whereas the activity of the saline extracts (broken lines) was considerably higher in bone biopsies from the anterior regions than in biopsies from the lower third molar region.

The microscopic appearance of the bone biopsies is shown in Fig. 7. In most cases, the bone marrow was composed of loose connective tissue (A). In some biopsies, areas of red bone marrow was found (B), and occasionally fat cells were present in the marrow (C). There were no striking differences between biopsies from the lower third molar regions and the anterior regions with regard to composition and cell content of the marrow spaces. A slight round cell infiltration in some of the marrow spaces indicating mild inflammation was encountered in a few biopsies from both regions.

DISCUSSION

The present study has shown that it is possible to extract fibrinolytically active material from normal alveolar bone (Fig. 2). However, no fibrinolytic activity is recorded on heated fibrin plates. This indicates that the fibrinolytic activity in both saline and KSCN extracts is due not to plasmin but to some activator (*Lassen*, 1953). This is confirmed by the inhibition of the fibrinolytic activity when adding low concentrations of EACA (Figs. 4 and 5). EACA is known as a powerful inhibitor of activator activity when used in concentrations between 10^{-4} and 10^{-3}M, whereas the plasmin activity remains almost unaffected (*Ablondi et al.*, 1959; *Alkjaersig et al.*, 1959; *Egeblad*, 1967). The stronger inhibition by EACA of the saline extracts in comparison with the KSCN extracts is probably due to the weaker primary activity of saline extracts and not to differences in the origin of fibrinolysis.

As the fibrin plates used were made from bovine fibrinogen which does not contain proactivator, no kinase activity would be able to produce lysis of the plates (*Müllertz*, 1955). It can thus be concluded that the fibrinolytic activity of normal alveolar bone is due to tissue activators.

The fibrinolytic activity of the KSCN extracts is unchanged when heated to 37°C at pH 3, whereas the activity of the saline extracts decreases when handled in the same way (Fig. 3). This indicates that the KSCN extracts contain a tissue activator of the stable type, and the saline extracts an activator of the labile type, although it cannot be excluded that some stable activator is present in the latter extract, too (*Albrechtsen*, 1958).

The amount of stable activator in the extracts of normal alveolar bone is high. On the other hand, the labile activator avtivity as a whole is low, but shows large variations from one biopsy to another (Fig. 2). The high activator activity of bone as found in the present investigation is in contrast to the results of *Roberts* and *Astrup* (1957), who found no activator activity in bone from monkeys. On the other hand, *Björkman* and *Nilsson* (1961) showed that human red bone marrow has a high content of labile activator but no stable activator. It is possible that the discrepancies between the aforementioned reports as well as the diverging results obtained in the present study should be related to the composition and cell content of the bone marrow examined in the different studies. In the present investigation the bone marrow most often was composed of loose connective tissue (Fig. 7A). The occasional finding of red bone marrow (Fig. 7B) may explain, why the labile activator content as a whole is low in the present material and shows wide variations from one biopsy to another. The difference in the labile activator content between the anterior regions and the lower third molar region is

most likely due to contingencies as no striking differences are known to be present between the two regions with regard to the occurence of red bone marrow. The stable activator may be related to the loose connective tissue type of bone marrow found in the biopsies. The composition of this tissue as well as the composition of the periodontal membrane, which most often was included in the biopsies, resembles the deeper layers of the periosteum, which has been shown to be fibrinolytically active to a great extent (*Roberts* and *Astrup*, 1957).

As no plasmin activity was recorded in the extracts, no activation of plasminogen has occured previous to placing the test material on fibrin plates. This means that the mild inflammation seen in some of the biopsies has not been sufficient to liberate the tissue activators to any significant extent and thus does not influence the results obtained in the present study. The same conclusions may be drawn with regard to any possible liberation of activators during the biopsy procedure. A prerequisite of these conclusions is that plasminogen actually has been at hand at the moments where the possibility for liberation of activators is conceivable. It is likely that the normal blood supply to the alveolar bone before the biopsy-taking and later on the blood adhering to the biopsy could be the sources of plasminogen.

In a previous report (*Birn*, 1970a) it was shown that the liquid content of the alveolus in patients suffering from alveolitis sicca dolorosa (ASD) or »dry socket« exhibits a high fibrinolytic activity, which may explain the liquefaction of the blood clot in this disease. The fibrinolytic activity in ASD is most likely of local origin and restricted to the area of pathological changes. It may be derived either from the tissues surrounding the alveolus or from bacteria infecting the extraction wound. In a report on the fibrinolytic activity of bacteria isolated from ASD it was found that the microorganisms do not possess any fibrinolytic activity (*Birn*, 1970b). On the other hand, it was shown that alveolar bone from ASD exhibits fibrinolytic activity. In the present study it has been shown that normal alveolar bone contains activators of plasminogen to a great extent. As mentioned in the introduction and shown in Fig. 1 the stable type of these activators may be released during inflammation of the tissue and cause localized fibrinolysis. The fibrinolytic activity of the alveolar bone in ASD may be explained on this basis and it seems reasonable to believe that the fibrinolytic activity in ASD is derived mainly from the alveolar bone and released during inflammation of the tissue. It is possible, though, that the gingival and mucosal tissues play a role in the production of fibrinolysis (*Björlin* and *Nilsson*, 1968; *Gustafsson* and *Nilsson*, 1961). Inflammation can be caused by trauma or infection,

and these etiological factors are to-day considered to be the most likely factors in the development of ASD (*Birn*, 1970a).

It is well known that ASD develops more often in the region of the lower molars than in the anterior regions (*Krogh*, 1934; *Lehner*, 1958; *MacGregor*, 1968). However, the stable activator content in these two regions is nearly the same (Fig. 6). Therefore, supposing ASD is the result of local fibrinolysis, the prevalence of this disease in the lower molar region cannot be explained by differences in the stable activator content of the alveolar bone, but must be related to differences in the degree of liberation of these activators in the two regions, i.e. the severity and frequency of inflammation in connection with removal of the teeth.

SUMMARY

This investigation dealt with the content of tissue activator of plasminogen in normal alveolar bone from the posterior and anterior regions of the jaws. It showed

1) that the content of stable tissue activator in normal alveolar bone is high,
2) that the amount of stable tissue activator is nearly the same in alveolar bone from the lower third molar region and the anterior regions of the jaws, and
3) that the content of labile activator in normal alveolar bone as a whole is low but shows wide variations from one biopsy to another, independent of its regional origin.

The relationship between the type of activator and the type of bone marrow found in alveolar bone biopsies is discussed as is the significance of the presence of stable tissue activators in normal alveolar bone for the development of »dry socket«.

RÉSUMÉ

ACTIVITÉ FIBRINOLYTIQUE DE L'OS ALVÉOLAIRE NORMAL

Cette étude concerne la teneur en activateur tissulaire du plasminogène dans l'os alvéolaire normal des régions antérieures et postérieures des maxillaires. On a constaté:

1) que la teneur en activateur tissulaire stable dans l'os alvéolaire normal est élevée,

2) que la quantité d'activateur tissulaire stable est à peu près la même dans l'os alvéolaire de la région de la dent de sagesse inférieure et des régions antérieures des maxillaires, et
3) que la teneur en activateur labile dans l'os alvéolaire normal est dans l'ensemble peu élevée, mais qu 'elle présente de larges variations d'une biopsie à l'autre, indépendamment de la région dont celles-ci proviennent.

La relation entre le type de l'activateur et le type de moelle osseuse trouvés dans les biopsies de l'os alvéolaire fait l'objet d'une discussion, de même que la signification de la présence d'activateurs tissulaires stables dans l'os alvéolaire normal pour le développement des alvéolites séches.

ZUSAMMENFASSUNG

FIBRINOLYTISCHE AKTIVITÄT DES NORMALEN ALVEOLARKNOCHENS

Der Zweck dieser Untersuchung war Erforschung des Gehalts an Gewebeaktivatoren des Plasminogens im normalen Alveolarknochen aus verschiedenen Gebiete der Kiefer. Die Untersuchung ergab:

1) dass der Gehalt an stabilen Gewebeaktivatoren im normalen Alveolarknochen ziemlich hoch war,
2) dass die Menge stabiler Gewebeaktivatoren im normalen Alveolarknochen aus der Weisheitszahngegend des Unterkiefers fast dieselbe war wie im Alveolarknochen aus der Vordergegend, und
3) dass der Gehalt an labilen Gewebeaktivatoren im normalen Alveolarknochen niedrig war und von einem Gewebeausschnitt zum anderen anders war, unabhängig davon, woher sie stammten.

Die Verbindung zwischen dem gefundenen Typ Gewebeaktivator und dem Aussehen des Knochenmarks wird erörtert. Ausserdem wird die Bedeutung des Vorhandenseins stabiler Gewebeaktivatoren für die Entwicklung von »dry socket« (alveolitis sicca dolorosa (ASD)) erwähnt.

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