A computerized method for quantitative estimation of the epithelium-connective tissue interface applied to the gingiva of various age groups

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A computerized method has been developed for the quantitative estimation of the contact area between the epithelium and connective tissue of oral mucosa. Magnified (× 200) paper replicas are produced from histologic sections (10 microns) and are placed in a fixed system of co-ordinates. By recording the co-ordinates to points placed at intervals along the basement membranes the course of these structures is described. The resulting curves are analysed by a computer, programmed to calculate the area of the epithelium-connective tissue interface on the basis of triangulation. The study utilized thirty gingival specimens in which the contact area between the epithelium and connective tissue had been quantitated in an earlier study using a trapezoid method. The results of the two different methods were moderately close. The results of the computerized method confirmed the previous finding of an increased contact area between the epithelium and connective tissue from prepuberty to 20—25 years of age. It is concluded that the computerized triangulation method, developed in the present study, is a more satisfactory method of measuring the area of the epithelium-connective tissue interface than the trapezoid method.

Key-words: Gingiva; age factors; epithelium connective tissue

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The study of biological structures frequently necessitates the subjective evaluations of tissue changes. Such evaluations do not lend themselves to ready comparisons with those of other investigators and may lead to inaccurate conclusions. One way to eliminate subjective errors in the description of tissue changes is to develop accurate quantitative methods based on objective measurements.

In recent studies of human gingiva we have introduced a method for the quantitative estimation of the epithelium-connective tissue interface based on adding trapezoid areas (*Löe & Karring*, 1969; *Karring & Löe*, 1971; *Löe & Karring*, 1971). It was apparent, however, that this method suffered from various shortcomings. 1) The measurements were performed manually which made the method extensively time consuming. 2) The method was not optimal because the mathematical approximation of the measured surface using trapezoids was poor and a methodical error could be introduced into the results by different configurations of the

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epithelium-connective tissue interface. 3) Finally, the reliability of the method was not tested.

The purpose of the present investigation was to develop an improved computerized method for the quantitative assessment of the epithelium-connective tissue interface and to test the reliability of the data previously published (*Löe & Karring*, 1971).

MATERIAL

This study utilized thirty gingival biopsies of 20 male and 10 female caucasians. Ten were 7—13 years of age, ten 20—25 and other ten 65—77 years of age. These biopsies were the same as used in a previous study on the morphology of the epithelium-connective tissue interface as related to age and sex (*Löe & Karring*, 1971). The biopsies were cut serially at 10 microns. Reference points had been introduced according to the method previously described (*Karring & Löe*, 1970). Of 50 sections from each biopsy every ninth and tenth were projected at 200 × magnification on to white paper. The outline of the epithelium including the reference points were drawn on the paper. On the first replica a region corresponding to a unit length of the epithelial surface was designated and transferred to the succeeding replicas using the reference points. From two of the biopsies such replicas were produced of fifty consecutive sections.

METHOD

Mathematically the use of triangulation for the approximation of a curved plane, such as represented by the epithelium-connective tissue interface, is superior to that utilizing trapezoids. Triangulation of the epithelium-connective tissue interface was performed by the placement of triangles between pairs of succeeding histologic sections (Fig. 1). The curves formed by the epithelium-connective tissue junction (basement membrane) served as the basis for this procedure. The replicas of the magnified (× 200) histologic sections were placed one after another in a fixed system of co-ordinates so that the reference points coincided. The basement membrane curves were described by placing points at intervals along these curves so that a proper approximation of their course was obtained by the connecting straight lines. The co-ordinates to these points were recorded and fed into a computer programmed to perform the triangulation and the subsequent calculation of the interface area. The essential features of the computer program as developed in cooperation with a statistician, is described in the following.

Computer Program

In the computer the basement membrane curves were placed in parallel planes with intervals equal to the thickness of the sections (× 200). Between the recorded co-ordinates for each curve, points were inserted at intervals of a maximum of 4 mm in order to improve the approximation of the surface. Triangulation of the surface between two adjacent curves (Fig. 1) was started by connecting the first point at one curve with the first point at the other. Gradually a triangulation net was produced by connecting the points at the two curves. As far as possible the individual triangles were constructed using sides which represented the connecting lines between points with the shortest distance between them. When these con-

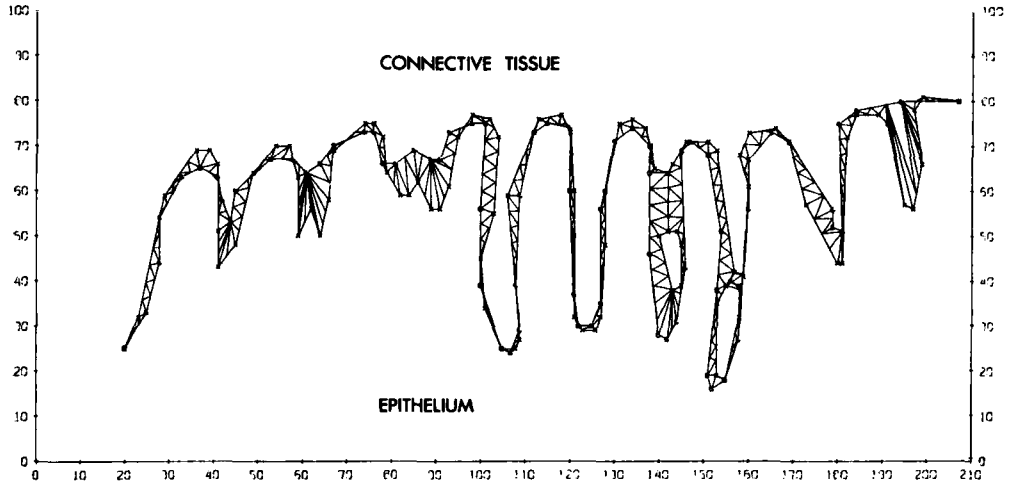

Fig. 1. Drawing produced by the computer. The triangulation performed between the approximated basement membranes of two adjacent sections is designated.

necting lines alone did not complete the triangulation of the surface, the net was supplied with the shortest diagonals (Fig. 2A).

Occasionally recordings of the points with the shortest connections were found to be inconsistent with each other (Fig. 2B). Point A at one curve is the point showing the shortest distance to point B at the other. However, this recording is inconsistent with the pairing of points in the adjacent portion of the curves. The computer was programmed to reveal these areas, and perform the triangulation by progressing stepwise an equal relative length along each curve. In Fig. 2B the triangulation net between the two curves has been produced in this manner.

Following input of the recorded co-ordinates, the computer produced drawings of each pair of succeeding sections with demonstration of the triangulation net (Fig. 1). Comparison of these drawings with the original replicas allowed the recorded co-ordinates and the triangulation to be controlled.

Where connective tissue papillae were cut so that they appeared in the epithelium as isolated areas (islands) special precautions were taken. Simultaneously with the placement of points along the basement membranes, it was recorded whether these islands were related to 1) a connective tissue papilla (Fig. 2C), 2) another island (Fig. 2D) or 3) had no counterpart in the adjacent section (Fig. 2E).

When the connective tissue island was related to a papilla in the succeeding section, a construction line (CL) was drawn between a point at the periphery of the island and the subjacent basement membrane as shown in Fig. 2C. The recording of the co-ordinates to the end-points of this line allowed the island to be included in the measurements of the total area between the two sections. Islands, which could be related to another island or had no counterpart in the adjacent section were handled as separate curve sections. After computation the areas of these curve sections were added to the total surface area. The triangulation of the surface between two islands was performed as described (Fig. 2D). However, if the island

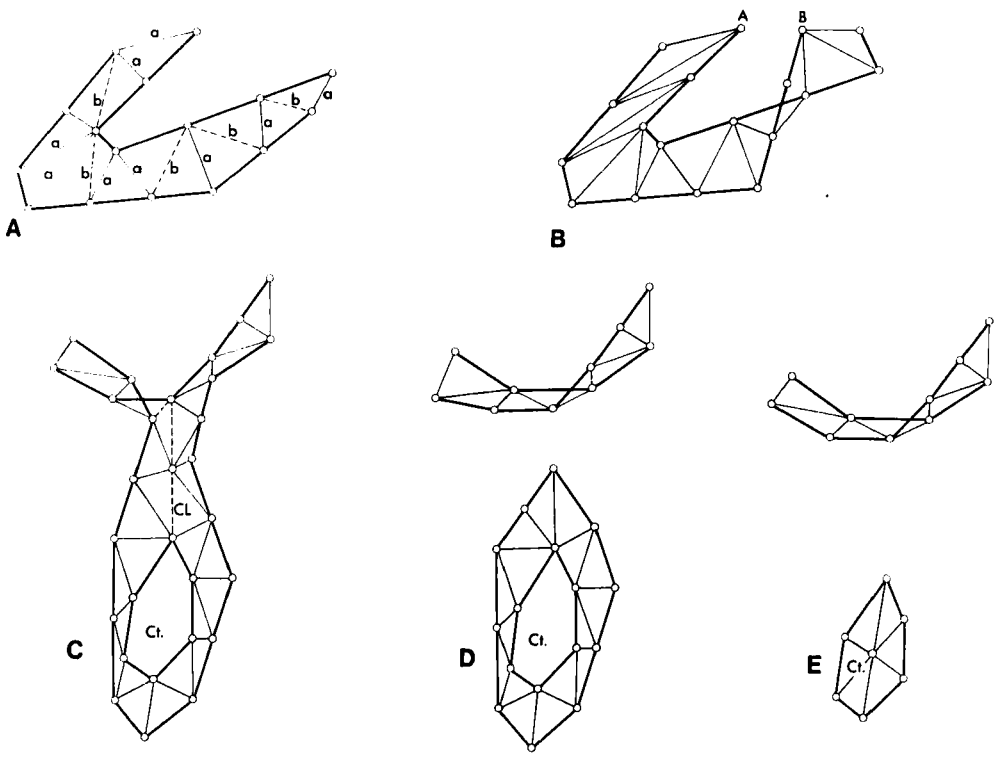

Fig. 2. Schematic drawing showing the fundamental principles in the triangulation of the epithelium-connective tissue interface between two adjacent sections.
A. The individual triangles are constructed using sides which represent either the connecting lines between the points with the shortest distance (a) or the shortest diagonals (b).
B. Example where the triangulation net has been produced by progressing stepwise with an equal relative length along each curve.
C-E. Demonstration of the triangulation net in areas where an isolated connective tissue area (Ct.) in the epithelium is related to C) a connective tissue papilla, D) another isolated connective tissue area or E) has no counterpart in the adjacent section. CL: Construction line.

could be found in only one section, the triangulation net was produced using a point in the other section (Fig. 2E). This point represented the center for the points placed along the periphery of the island.

The area of each triangle was calculated according to the formula of *Heron*. Thus, in Fig. 3, the area of the projected triangle $\acute{A}B_1B_2$ is:

$$\sqrt{S(S-B_1B_2)(S-B_1\acute{A})(S-B_2\acute{A})} \quad \text{where}$$

$$S=\tfrac{1}{2}(B_1B_2+B_1\acute{A}+B_2\acute{A})$$

It follows that the area of AB_1B_2 which represents a triangle in the approximated epithelium-connective tissue interface is:

$$\sqrt{S(S-B_1B_2)(S-B_1\acute{A})(S-B_2\acute{A})+\frac{(A\acute{A}\,B_1B_2)^2}{4}}$$

In addition the computer was programmed to calculate the mean length of the approximated basement membrane curves relative to 1 mm of the epithelial surface.

Treatment of data

The measurements obtained using the triangulation method were compared to those found in the same biopsies with the method formerly developed. Similarly,

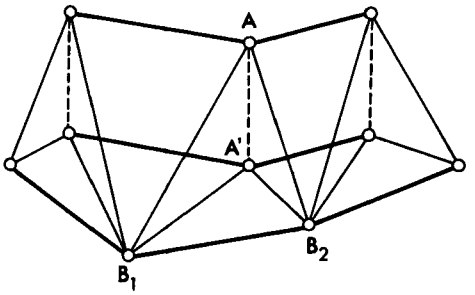

Fig. 3. Schematic drawing showing the relationship between the projected triangles ($\acute{A}B_1B_2$) and those (AB_1B_2) positioned at the approximated epithelium-connective tissue interface.

the method was used for the reexamination of the data related to age (*Löe & Karring* 1971).

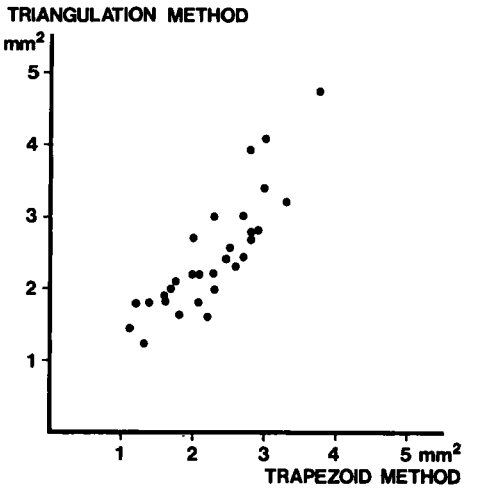

Fig. 4. Correlation between the area of the epithelium-connective tissue interface computated with the triangulation method and the method previously developed (trapezoid method).

RESULTS

The dispersion of the areas measured between adjacent sections was small for both of the specimens in which fifty consecutive sections were used. The measurements between each tenth pair of sections demonstrated a relatively small dispersion. This indicates that just as with the method previously presented (*Löe & Karring*, 1969), measurements between each tenth pair of sections are representative of the whole specimen.

In the thirty gingival specimens, the area of the epithelium-connective tissue interface relative to 0.5 mm² of the epithelial surface varied between 1.24 mm² and 4.74 mm². Between these measurements and those obtained with the method developed previously, there was a low correlation due to a few extreme measurements. The specimens corresponding to the ten most extreme pairs of values were subjected to re-computation with the previous method. For six specimens this reanalysis produced results which deviated from 0 to 17 per cent from those obtained earlier. However, the remaining four specimens, were found to be from 32 to 64 per cent lower than the results of the earlier method. It appeared that these specimens had been measured incorrectly at the first examination, which may have escaped detection because of the complicated technique of computation. Using the results obtained by the reanalysis the correlation between the data of the two different methods of computation was moderately high (Fig. 4). Tested statistically, using a linear regression model, this correlation showed to be highly significant ($T=8.30$; $f=28$; $p << 0.001$).

On the basis of the results obtained in this study the mean area of the epithelium-connective tissue interface for the ten specimens which constituted the age group from 7 to 13 years was found to be 2.113 mm². For the specimens which constituted the groups from 20 to 25 years and from 65 to 77 years, the mean area was 2.726 mm² and 2.634 mm² (Table I). Compared to the assessments in the earlier study these values have increased for the young-

Table I. *Re-computation of the area and length of the basement membrane in the gingiva of various age groups*

Age groups	No. of specimens	Basement membrane	Present study		Previous study (*Löe & Karring*, 1971)	
			$\bar{x}$	S.D.$_k$.	$\bar{x}$	S.D.$_k$
7—13	10	Area	2.113	0.778	2.02	0.51
		Length	2.74	0.60	2.7	0.6
20—25	10	Area	2.726	0.712	2.94	0.90
		Length	4.04	1.22	4.1	1.6
65—77	10	Area	2.634	0.874	2.71	1.01
		Length	3.23	1.16	3.0	1.2

est group whereas for the older groups they have become smaller (Table I). For the latter groups the decreased area is caused mainly by the correction of the inaccurate measurements. As in the earlier study, an analysis of variance showed that the differences between the areas of the epithelium-connective tissue interface in the various age groups are not significant ($0.05 < p < 0.10$) although there is a clear tendency for the area to increase from prepuberty to 20—25 years of age. Between the basement membrane lengths and the area of the epithelium-connective tissue interface there was a fairly high correlation (Fig. 5). However, it was apparent that the correlated data were heterogenous since the specimens in which the epithelium-connective tissue interface was characterized by connective tissue ridges and those in which this was characterized by exclusively connective tissue papillae exhibited two different correlations. At identical lengths of the basement membrane, the area of the epithelium-connective tissue interface was greater in specimens showing papillae than in those showing ridges. Tested statistically, using a linear regression model of the logarithmic basement membrane areas upon the logarithmic basement membrane lengths, this difference showed to be highly significant ($T=7.29$; $f=28$; $p << 0.001$).

For the various age groups the mean length of the basement membrane were

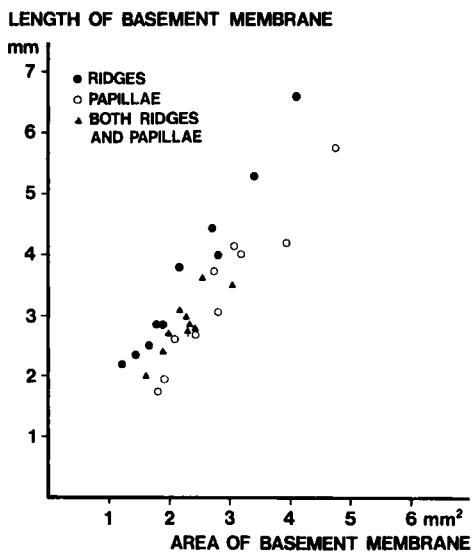

Fig. 5. Correlation between the length and area of the basement membrane. Specimens in which the epithelium-connective tissue interface is characterized by connective tissue papillae and those in which it is characterized by connective tissue ridges exhibit different correlations.

almost identical to those found in the earlier study (*Löe & Karring*, 1971) (Table I). Similarly, an analysis of variance showed that the differences between the basement membrane lengths of the various age groups were significant ($0.01 < p < 0.05$).

DISCUSSION

It may readily be seen on the basis of the mathematics that the method developed for triangulation of the epithelium-connective tissue interface offers the possibility of accurate quantitation. With the method previously presented, the contact area between the epithelium and connective tissue was approximated using trapezoids. It was apparent that this approximation was not optimal and that due to the technique of measuring certain discrepancies between the calculated and the actual area of the approximated surface might occur.

In two previous studies the trapezoid method was used for the quantitative estimation of the epithelium-connective tissue interface area of the human gingiva. In 20 gingival specimens it was found that the epithelium-connective tissue interface varied between 14.81 and 48.55 mm^2 relative to a unit area of the epithelial surface (*Löe & Karring*, 1969). Although these results have been expressed erroneously relative to 5 mm^2 of the epithelial surface instead of 0.5 mm^2 as stated, it is apparent that this range of variation is in agreement with that found in the present study. Also, the observation of an increased area of the epithelium-connective tissue interface with age is in keeping with that found in the earlier study (*Löe & Karring*, 1971). Despite the agreement between the results of the present and previous study, the correlation between the data of the two different methods was only moderately high. This may indicate that although the computations with the trapezoid method are inaccurate, this method can be used under certain conditions for the demonstration of quantitative differences in the epithelium connective tissue interface.

As the original measurements of the lengths of the basement membrane were performed with a curvometer these results express the actual length of this structure in the projected histologic sections. The results produced by the computer represent the lengths of the approximated basement membrane. The concordance between these measurements indicates that the configuration of the basement membranes has been properly approximated.

In the present and earlier investigation (*Löe & Karring*, 1969) a fairly high correlation was found between the length of the basement membrane and the area of the epithelium-connective tissue interface. In both studies it was observed that specimens showing connective tissue ridges and those showing connective tissue papillae exhibited different correlations. Therefore, comparative studies of the area of the epithelium-connective tissue interface on the basis of only the length of the basement membrane may produce inaccurate results. This is also apparent from the present investigation, where the difference between the lengths of the basement membrane of the groups aged 7 to 13 years and 20 to 25 years is definitely greater than that between the corresponding areas of the epithelium-connective tissue interface.

It is concluded that comparative studies on the basis of the trapezoid method are liable to reveal major differences in the

area of the epithelium-connective tissue interface. However, the computerized method based on a triangulation is a more satisfactory method of measuring this area, especially as this method is considerably less time-consuming and easier to check than the trapezoid method.

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