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THE EFFECT OF INFLAMMATION ON THE PERMEABILITY OF HUMAN PALATAL EPITHELIUM TO UREA

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Tissue fluid does not readily escape from normal oral epithelium except at the gingival pocket. At this site the permeability of the epithelium to tissue fluid is increased following mechanical stimulation (*Brill,* 1959) and also in the presence of clinically detectable marginal gingivitis (*Brill & Björn,* 1959). The present investigation attempts to determine whether a similar increase in permeability to tissue fluid occurs in the hard palates of denture wearers, these areas frequently being sites of inflammatory change.

Brill & Björn (1959) used fluorescein sodium as a tracer in their experiments on the gingival pocket. This is somewhat toxic, and it was considered preferable to use urea, a physiologically occurring substance (*Diem* 1962).

MATERIAL AND METHODS

Selection of subjects

19 females and 14 males were selected for the present study. All subjects were edentulous. Their ages ranged from 35 to 78, their mean age being 58 years. 18 subjects had clinically healthy palatal

mucosa. Data concerning these 18 subjects are entered in Table I. 15 subjects had clinically observable inflammatory changes in the palate presumably produced by their dentures. These reactions ranged from slight hyperemia in a restricted part of the area, to heavy reddening of the whole area, in severe cases accompanied with papillomatosis. Data concerning this group are entered in Table II.

Clinical procedure

Each subject was given fifty g of urea dissolved in 180 ml of water. Two drops of an alcoholic solution of oil of peppermint were added to disguise the taste. Although urea occurs naturally in body fluids, it was nevertheless thought, that a raised urea level in these fluids would ensure greater amounts of urea to be available for the subsequent analysis of the fluid that might pass from the subepithelial spaces into the oral cavity.

Moreover, one pill of atropine sulphate 0.5 mg (Pilulae atropini majores, Ph. Dan. 48) was administred to each subject to suppress activity of salivary and mucous glands. This measure was taken to prevent the escaping tissue fluid from being contaminated by urea contained in the glandular secretions.

Two hours later samples of fluid were collected. Before the collection of samples, the mouth was prepared in the following manner: the hard and soft palate and the alveolar ridges were sprayed with tap water, and afterwards carefully dried with cellulose sponges. Any water remaining was dried by blasts of air. Saliva pooling in the mouth during the subsequent sampling procedure was removed by a saliva ejector.

Fluid was recovered from the hard palate by means of a circular piece of unbleached cotton material, thirty-four mm in diameter (Fig. 1). To ensure uniformity of size and form, the pieces were cut out by a punching machine.

Further, to be sure that these patches did not provide a source of urea, several patches not used for sampling were subjected to a separate analysis. They were either (1) taken by means of tweezers directly from the punching machine, or (2) touched by recently washed and dried fingers, or (3) washed and dried and afterwards handled by clean fingers. In no case was urea demonstrated.

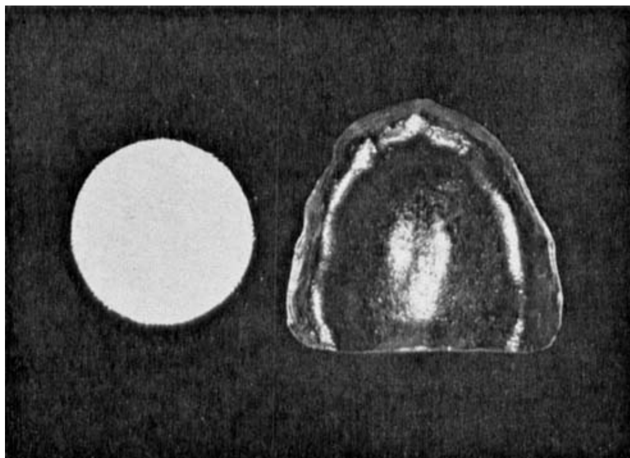

Fig. 1. (Left) Cotton patch used for collecting fluid from the hard palate. (Right) Baseplate used for keeping the patch in contact with the palatal mucosa, as shown in Fig. 2.

In each subject the patch was carefully adapted to the hard palate and the lingual aspect of the anterior part of the alveolar ridge. The patch was kept in this position by means of a plate (Fig. 2) made of clear thermoplastic baseplate material (Fig. 1). This plate was supported by light finger pressure for fifteen minutes, and then removed. The cotton patch was removed by means

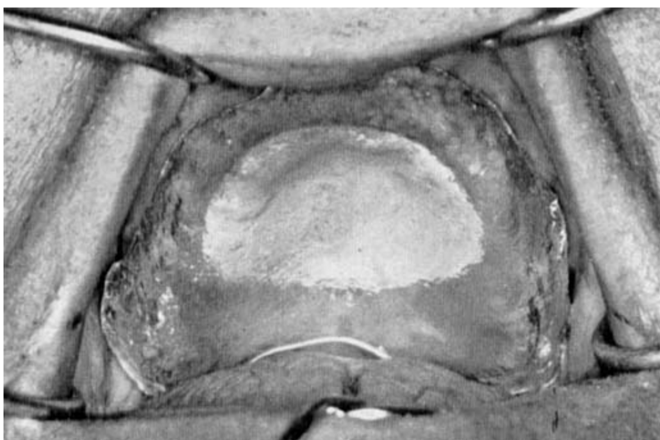

Fig. 2. Patch of cotton kept in contact with palatal mucosa by means of an individual baseplate.

of tweezers and transferred to a stoppered glass vessel, where it remained until the microanalysis for urea was performed.

Finally, a blood sample was taken, and its urea content was determined using the method described by *Friedman* (1953).

Laboratory procedure

Principle of analysis

Urea is decomposed by urease into carbon dioxide and ammonia. The latter reacts with sodium phenolate and hypochlorite (Berthelot's reaction) yielding a blue colour, the absorbance of which is measured in a spectrophotometer at 625 nm.

Applying this principle *Chaney & Marbach* (1962) determined the content of urea in biological fluids, and *Brown et al.* (1957) the content of ammonia in human plasma and rat tissues. For the present investigation the methods described by these authors have been modified by one of us (V.L.).

Technique

The patch is flooded in the glass vessel with 4 ml of distilled water and shaken at intervals for five minutes. 1 ml of the fluid is transferred to each of two test tubes. To tube 1 is added 1 ml from a urease solution consisting of 150 mg urease and 1 g EDTA in 100 ml of distilled water, the pH being 6.5. To tube 2 is added 1 ml of distilled water. Both tubes are left for twenty minutes at room temperature (20° C), whereafter 1 ml of each of the following colour developing reagents are added to both.

(1) 2.5 % solution of sodium phenolate prepared from phenol 2.5 g, sodium hydroxide 1.25 g and distilled water to a final volume of 100 ml.

(2) 0.005 % solution of sodium nitroprusside. A freshly made 1—100 dilution of a 0.5 % stock solution of this reagent is used.

(3) Sodium carbonate 0.05 M.

(4) Sodium hypochlorite *circa* 0.06 N.

After mixing, the tubes are allowed to stand for thirty minutes in darkness and then diluted with 4 ml of distilled water. Finally the optical density (Es) of tube 1 is measured in a spectrophotometer at 625 nm using tube 2 as blank. The ammonia content of the urease solution is determined by adding the reagents (1),

(2), (3), and (4) to 1 ml of the urease solution and 1 ml distilled water, and measuring the optical density (Eu) after thirty minutes in darkness and dilution with 4 ml of distilled water. The blank used, is made from the reagents (1), (2), (3), and (4) diluted with 4 ml of distilled water after thirty minutes in darkness.

Finally the optical density (Est) of 1 ml of a standard solution, containing 2 μg N/ml as $(NH_4)_2SO_4$ (Ammonium sulphate), is measured after addition of the reagents as described above.

The amount of urea-N contained in an experimental patch is calculated from the formula

$$\frac{Es - Eu}{Est} \times 8.$$

RESULTS

In columns five of Tables I & II are entered the urea-N values arising from the microanalyses. Table I comprises data from subjects with clinical signs of inflammation; Table II comprises

Table I

Urea-N passage through an inflamed epithelial barrier of the hard palates in 15 subjects. In column 5 are entered urea-N levels in fluid absorbed from the hard palates. In column 4 are entered blood-urea levels.

Subject	Age	Sex	Urea concentration in blood in mg %	Urea-N content of cotton patch in μg
1	40	♂	100	— 2
2	67	♀	152	1
3	46	♀	92	2
4	54	♀	122	2
5	74	♂	115	2
6	72	♂	112	3
7	70	♀	152	3
8	42	♂	109	3
9	74	♂	108	5
10	61	♀	111	5
11	66	♀	148	6
12	67	♂	126	6
13	47	♀	96	9
14	73	♂	111	17
15	74	♀	136	17

Table II

Urea-N passage through a healthy epithelial barrier of the hard palates in 18 subjects. In column 5 are entered urea-N levels in fluid absorbed from the hard palates. In column 4 are entered blood-urea levels.

Subject	Age	Sex	Urea concentration in blood in mg %	Urea-N content of cotton patch in μg
I	73	♀	148	— 2
II	71	♂	190	— 2
III	47	♂	116	— 1
IV	70	♂	144	— 1
V	49	♂	172	— 1
VI	65	♀	85	— 1
VII	75	♂	112	0
VIII	55	♀	117	0
IX	52	♀	112	0
X	78	♀	108	0
XI	49	♀	98	0
XII	37	♂	103	1
XIII	58	♂	119	1
XIV	52	♀	114	1
XV	40	♀	118	1
XVI	35	♀	132	3
XVII	39	♀	132	4
XVIII	55	♀	115	12

data from subjects in whom these signs were absent. The figures in both tables are arranged according to the amount of urea-N demonstrated in each sample of fluid.

When the two sets of data are compared, a difference is noted. This is brought out more clearly in Fig. 3. It appears that the urea-N values from subjects with healthy palatal mucosa are grouped rather closely around zero with a single extreme positive value. The values of urea-N from subjects with inflamed palatal mucosa are spread over a larger interval but they show a clear tendency to be higher than those of the former group.

The significance of this difference was evaluated in a distribution-free median test, in which the two sets of data were combined. The number of observations that were above, and the number that were below the median of the combined data were determined. The resulting 2×2 contingency table was then subjected to a χ^2 homogeneity test, employing Yates' correction. This

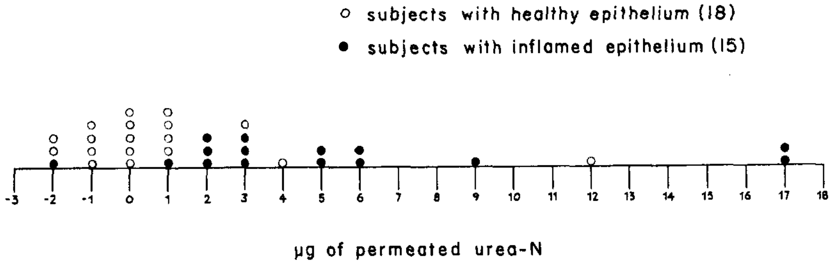

Fig. 3. Diagrammatic presentation of data from columns 5 in Tables I & II, showing the permeability of the palatal epithelium in 33 edentulous subjects; viz. 18 subjects with clinically healthy palatal mucosa and 15 subjects with inflamed palatal mucosa.

test produced a P value of less than 0.001. Thus, ample significance was demonstrated concerning the difference between the original two sets of urea-N values from Tables I & II. Had a one-sided test been used, this significance would have been even greater.

DISCUSSION

Urea-N escaped from the palatal epithelium of all subjects with inflammatory changes, except for subject 1 (Table I). This finding suggests that, when clinically observable inflammation is present, the epithelial membrane is rendered permeable to the extent that tissue fluid escapes into the oral cavity.

However, the tracer substance was also collected from the palatal epithelium of about one third of the subjects classified as having healthy mucous membranes. This may mean that overt stages of inflammation are preceded by subclinical stages in which epithelial permeability is increased; or, the activity of the mucous glands is not suppressed sufficiently by atropine to prevent contamination; or, both may occur simultaneously.

Concerning subject 1 (Table I) the negative value for permeability in spite of palatal inflammation may be correct, or may be due to an accidental error occurring when the diagnosis of inflammation was made.

SUMMARY

33 edentulous human subjects were tested for permeability of the epithelial membrane covering the hard palate. 18 subjects had clinically healthy palatal mucosa, while 15 subjects showed signs of inflammatory changes. Urea administered perorally was used as a tracer substance. By means of circular patches of cotton material (Fig. 1) samples of tissue fluid containing urea could be recovered from the hard palates (Fig. 2) of all but one of the subjects with inflammation. Urea could also be recovered from about one third of the palates classified as healthy. These observations suggest that palatal epithelium is rendered permeable in the presence of inflammation, and that clinically observable inflammation may be preceded by subclinical changes, which are also accompanied by a break-down of the epithelial barrier.

RÉSUMÉ

PASSAGE DE L'AZOTE URÉIQUE À TRAVERS LA MEMBRANE ÉPITHÉLIALE DE LA MUQUEUSE PALATINE CHEZ L'HOMME

La perméabilité du revêtement épithélial de la voûte palatine a été étudiée sur 33 personnes. Chez 18 personnes, la muqueuse palatine apparaissait saine à l'examen clinique, tandis que chez 15 personnes, on trouvait des signes cliniques d'altérations inflammatoires. La substance traçante employée était l'urée administrée par voie buccale. A l'aide de pastilles de toile (fig. 1) mises au contact de la muqueuse (fig. 2), il a été possible, à une exception près, de recueillir un liquide tissulaire contenant de l'urée chez toutes les personnes présentant une inflammation. Il était aussi possible de recueillir de l'urée chez environ un tiers des personnes dont la muqueuse palatine s'était présentée comme saine à l'examen clinique. Les résultats de cette étude semblent montrer que le revêtement épithélial de la muqueuse palatine est en général perméable quand il est possible de constater des signes cliniques d'inflammation, et que, de plus, avant qu'il soit possible de faire le diagnostic clinique de l'inflammation, il peut s'être produit dans la muqueuse des altérations subcliniques rendant également la muqueuse perméable.

ZUSAMMENFASSUNG

DIE DURCHLÄSSIGKEIT DER EPITHELMEMBRAN DER MENSCHLICHEN GAUMENSCHLEIMHAUT

An 33 Personen wurde die Durchlässigkeit der Epithelmembran des harten Gaumens untersucht. Von diesen hatten 18 Personen eine klinisch gesunde Gaumenschleimhaut; 15 Personen wiesen hingegen klinische Zeichen entzündlicher Veränderungen auf. Als Nachweissubstanz kam peroral gegebener Harnstoff zur Anwendung. Unter Zuhilfenahme von Leinenoblaten (Fig. 1), die mit der Schleimhaut in Kontakt gebracht wurden (Fig. 2), war es möglich, bei allen Personen — ausser einer — mit entzündlicher Schleimhaut Harnstoff enthaltende Gewebsflüssigkeit anzusammeln. Dieser Stoff konnte ebenfalls bei einem Drittel der Personen mit klinisch gesunder Schleimhaut angesammelt werden.

Die Ergebnisse der Untersuchungen scheinen zu zeigen, dass die Epithelbekleidung der Gaumenschleimhaut allgemeinhin durchlässig ist, wenn klinische Zeichen einer Entzündung vorliegen, und ferner, dass an der Gaumenschleimhaut subklinische Veränderungen eintreten können, bevor diese sich klinisch nachweisen lassen, und dass die Epithelmembran auch in solchen Fällen durchlässig ist.

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