Studies on the permeability of human oral mucosa

V. The inflow of water through dehydrating oral mucosa

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Kaaber, S. Studies on the permeability of human oral mucosa. V. The inflow of water through dehydrating oral mucosa. *Acta Odont. Scand.* 31, 101—108, 1973.

The inflow of water through human oral mucosa during and after secretion block with scopolamine has been investigated in 9 young adults with healthy mucosae by analysing the changes of weight in water-saturated discs of ash-free filter paper during and after mucosal contact. Buccal and palatal mucosae both showed a continuous uptake of water from the filter paper, proportional in amount to the area of mucosa covered and to the contact period. The inflow ratio for buccal epithelium in relation to palatal epithelium was 2:1. The values in the regression equations for the inflow-time relation corresponded closely to the values for water outflow from analogous slightly dehydrated mucosae, demonstrating a ratio of 1:1 for inflow: outflow of water through oral mucosa.

Key-words: Mouth mucosa; biological transport; water; permeability

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The transport of water through oral mucosa under a vapour pressure gradient between the exposed mucous surface and the ambient oral air has been studied previously. Under these conditions the mucous membranes lose water at a rate which depends on the structure and water content of the epithelium (*Kaaber*, 1973). The effect of an elimination of the vapour pressure gradient over the mucosa on the direction and rate of water transport has not yet been elucidated. The corresponding epidermal function during submersion has been investigated several times, either in subjects immersed in water (*Whitehouse, Hancock & Haldane*, 1932, *Marx* 1935) or in small areas of skin exposed to water for long periods (*Folk & Peary*, 1951; *Buettner*, 1953, 1959; *Peiss, Randall & Hertzmann*, 1956; *Szakall* 1958). Both types of experiments have demonstrated a definite uptake of water into epidermis. It occurs rapidly at first, then at a slower but constant rate for several hours (*Buettner*, 1953, *Szakall*, 1958). This uptake is determined by the osmotic properties of epidermis due to the high concentration of water-soluble substances in the deeper part of the stratum corneum, especially in the stratum lucidum (*Stüpel & Szakall*, 1957; *Szakall*, 1958).

In some areas of the oral cavity, especially the floor of the mouth, the mucous membrane is almost constantly bathed in saliva. The barrier properties of the oral mucosa under these circumstances has not yet been investigated. The aim of the present study has therefore been to study the transport of water through non-keratinized and keratinized oral

Received for publication, November 20, 1972.

mucosa under experimental conditions equivalent to immersing the mucous membrane in water.

MATERIAL AND METHODS

The investigation was performed on 9 young adult persons: 8 men and 1 woman aged 21—25 years with clinically normal oral mucosae. Two mucosal areas on the hard palate and the cheeks, as described in previous papers (*Kaaber*, 1917b, 1971c), were selected for sampling. The water transport was studied by recording the changes in weight of saturated 0.13 mm thick discs of ash-free filter paper* with a diameter of 6.3 or 8.0 mm, after contact with the mucosal surface. 3.00 and 4.80 mg distilled water respectively were applied to the discs with a microsyringe (Hamilton type 7105-N).**

These quantities were calculated from the volume of the discs and a filter paper density of 0.46, to ensure saturation of the disc plus an excess of about 10% to compensate for evaporation loss during transport to and from the mucous membrane. After the transfer of water, the discs were kept in closed polyethylene containers as described elsewhere (*Kaaber*, 1971a). The changes in weight were recorded 30 minutes before and after mucosal contact by means of a microbalance (*Kaaber*, 1971b).

Sampling occurred at 22±2°C and at a vapour pressure of 11±0.5 mm Hg equivalent to a relative air humidity of 50%. Temperature and vapour pressure of the air surrounding the subject were recorded at 15-minute intervals with a psychrometer coupled to an electric thermometer and adjusted as described earlier (Kaaber 1973). Secretion block was produced by submucous injection of 0.5 ml 0.05% scopolamine nitrate 30 minutes before sampling. The sampling areas were carefully cleaned just before sampling with sterile gauze and protected from saliva by cotton rolls and a saliva ejector. The saturated filter paper was kept in close contact with the surface of the mucous membrane under a disc of 0.12 mm thick polyethylene with a diameter of 11.5 mm stuck to a piece of transparent tape of 3—4 cm^2. To avoid stimulation of the glandular tissue of the palatal mucosa, and to reduce evaporation, the original covering was retained as long as possible. After the application of a new covering, the evaporative loss from the mucosal surface was compensated for by applying a saturated filter paper disc to the sampling area for 5 minutes before fresh sampling.

Three sampling series, each of 100—150 minutes' duration, were used, cf. Table I. In the first of these, both types of mucosae were sampled unilaterally at the same time, using a uniform disc size and contact period. In the second, each type of mucosa was sampled bilaterally with different sizes of discs and a uniform contact period. In the third series, sampling was performed bilaterally on both types of mucosae, using contact periods between 2 and 30 minutes; in this series the main sampling period was divided into two periods of equal length (75 minutes), in which two or three samples were taken with each of the shorter periods and one with the 30 minutes' contact period, with sampling out of phase in the two mucosae. All series used blinds at 5- or 10-minute intervals throughout the sampling period to measure the evaporation loss. If the covering became loose or the sampling

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Table I. *Variations in sampling procedure*

Series	Mucosal area	Diameter of sample in mm	Contact periods in minutes	Number of samples per series
I	palatal	6.3	5	16—20
	buccal			16—18
II	palatal	6.3—8.0	5	12—16
	buccal			
III	palatal	6.3	2, 5, 10, 15, 30	20—24
	buccal			20—24

area was obviously contaminated with saliva, the samples were rejected. The statistical analysis employed parameters calculated by conventional methods (*Sokal & Rohlf* 1969). The X_2-test was used to ascertain whether the distribution in each group was normal. A significance level of 1% was employed. All calculations were performed by an electronic computer.

RESULTS

The weight changes in corresponding samples and blinds in series I are shown in Fig. 1. In both buccal and palatal

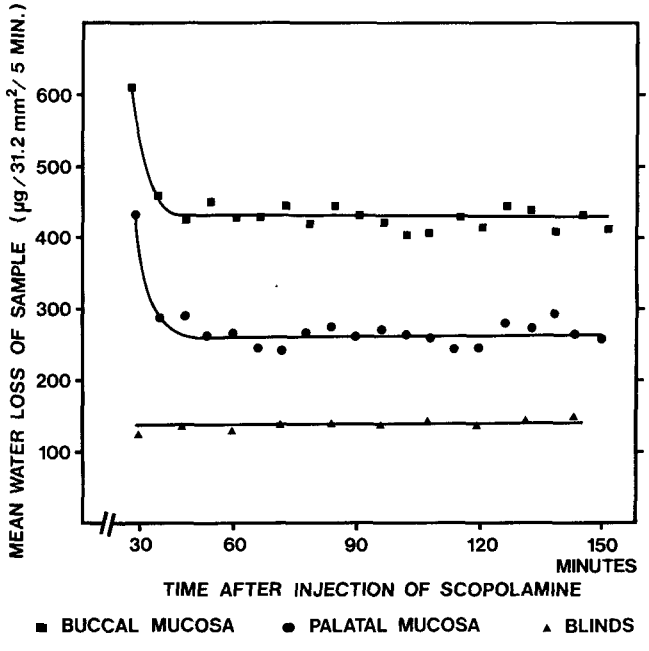

Fig. 1. Mean water loss from water-saturated filter paper samples during consecutive 5-minute contact periods with buccal and palatal mucosa, compared with the evaporative water loss from corresponding blinds.

Table II: *Alteration in weight ratio for two sizes of filter paper discs after 5 minutes' contact with oral mucosa.*

Person and sex	Mucosal area	Number of sample pairs	Dry weight ratio $\bar{x}$	S.D.$_k$	Number of sample pairs	Wet weight ratio $\bar{x}$	S.D.$_k$	Value of t S.D.$_k$
TN ♂	Buccal				12	1.54	0.30	2.43
KK ♂	Buccal				13	1.55	0.41	1.73
BJ ♂	Buccal				13	1.56	0.33	1.92
		18	1.68	0.16				
CJ ♂	Palatal				10	1.55	0.37	2.35
MO ♂	Palatal				14	1.52	0.58	1.58
KS ♂	Palatal				16	1.55	0.33	2.08

Table III: *Individual and interindividual variation in water loss from wet filter paper to human oral mucosa*

Person and sex	Buccal mucosa Number of samples	Inflow of water (mg/cm²/5 min) $\bar{x}$	S.D.$_k$	Palatal mucosa Number of samples	Inflow of water (mg/cm²/5 min) $\bar{x}$	S.D.$_k$
KS ♂	18	0.757	0.131	18	0.387	0.070
	4	0.745	0.114	4	0.379	0.142
TN ♂	14	*0.820*	*0.158*	11	*0.380*	*0.084*
	5	0.817	0.135	6	0.399	0.035
CJ ♂	13	0.859	0.160	11	0.479	0.066
	4	1.045	0.084	4	0.455	0.079
BJ ♂	16	1.051	0.156	18	0.480	0.118
	5	1.065	0.123	3	0.578	0.055
FP ♂	16	1.008	0.120	18	0.384	0.113
	4	1.104	0.163	6	0.568	0.081
OJ ♂	15	*1.165*	*0.108*	11	*0.516*	*0.098*
	3	1.214		2	0.737	
MO ♂	12	*0.901*	*0.140*	13	*0.422*	*0.152*
	2	1.337		2	0.661	
KK ♂	10	*0.884*	*0.109*	11	*0.521*	*0.129*
	5	1.229	0.168	5	0.474	0.152
JC ♀	14	*1.005*	*0.115*	19	*0.371*	*0.110*
	7	1.329	0.356	5	0.611	0.172

Italics denote independent sampling periods in buccal and palatal mucosa.

Table IV. *Mean uptake of water by oral mucosa during (period I) and after suspension of salivary secretion (period II). Values in milligrams per cm² mucosa*

Sampling area	Sampling period in minutes	Uptake of water (Mean Value ± 1 S.E.) Period I 30—105 minutes after injection $\bar{x}$	S.D.$_k$	Period II 105—180 minutes after injection $\bar{x}$	S.D.$_k$	Value of t	Value of F
Buccal mucosa	2	0.696	0.121	0.651	0.122	1.03	1.03
	5	1.084	0.217	1.114	0.208	0.42	1.09
	10	1.549	0.381	1.570	0.434	0.16	1.30
	15	1.931	0.366	2.084	0.443	1.14	1.46
	30	2.731	0.528	2.879	0.706	0.68	1.79
Palatal mucosa	2	0.340	0.135	0.334	0.096	0.23	1.97
	5	0.559	0.130	0.509	0.146	1.01	1.27
	10	0.821	0.162	0.587	0.223	3.61*	1.90
	15	0.932	0.226	0.894	0.350	0.36	2.39
	30	1.372	0.287	1.260	0.355	1.02	1.53

* Significant at the 1% level.

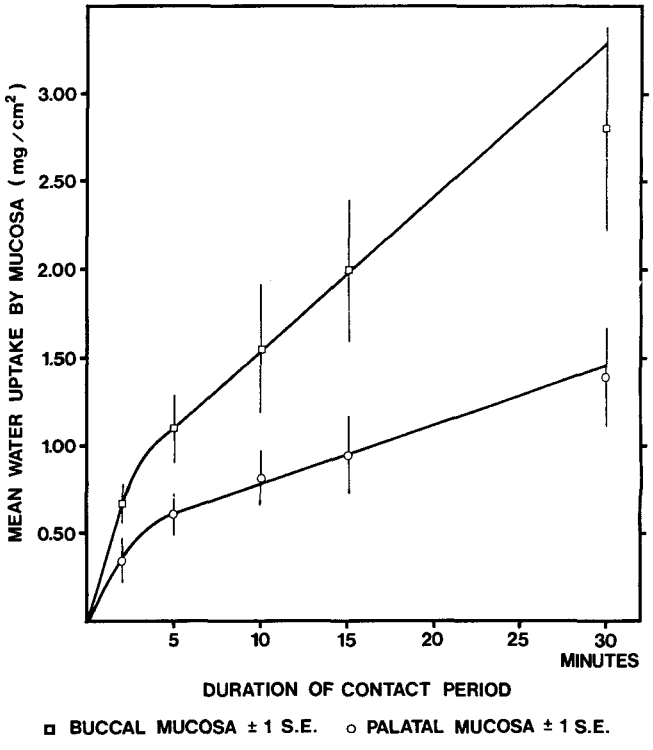

Fig. 2. The relation between time and the uptake of water by human oral mucosa.

Mean values for buccal mucosa obtained during main sampling period. Mean values for palatal mucosa obtained during temporary suspension of salivary secretion. Cf. Table IV.

Table V. *Regression equation values for the inflow of water through oral mucosa during (period I) and after suspension of salivary secretion (period II)*

	Sampling period	Buccal mucosa $\bar{x}$	S.E.M.$_k$	Palatal mucosa $\bar{x}$	S.E.M.$_k$	Ratio buccal/palatal mucosa
Regression line constant (a)	I	0.620	0.072	0.275	0.051	2.3
	II	0.675	0.062	0.395	0.034	1.7
Regression coefficient (b)	I	0.970	0.032	0.385	0.137	2.5
	II	0.846	0.048	0.377	0.090	2.2

mucosae, the water loss from the filter paper samples followed a characteristic course, proceeding from an initially high level to uniform values after only the third sample. After a correction for the evaporative water loss from the blinds, the net water loss from the filter paper in the stable phase was 125 μg/5 minutes to the palatal mucosa and 290 μg/5 minutes to the buccal mucosa. The difference between the two areas was significant, $P < 0.001$. The relation between this water loss and the size of the filter paper discs is shown in Table II. In all series, the dry weight ratio was slightly lower than the corresponding wet weight ratio, but this difference was not significant. The individual and interindividual variation in water uptake for the two mucosal regions is shown in Table III. For buccal mucosa, the range for the material as a whole was 0.591 mg/cm^2/5 minutes. The individual range was 0.435 mg/cm^2/5 minutes. The dispersion of values from the palatal mucosa was less pronounced, with a range of 0.366 mg/cm^2/5 minutes for the whole material, and an individual range of 0.240 mg/cm^2/5 minutes. Table IV gives the mean values for water uptake by the mucosae in relation to the duration of secretion block. In buccal mucosa corresponding values during and after secretion block showed uniform variation, whereas the palatal mucosa showed a tendency to falling mean values and increased variability after secretion block. The water uptake was in both mucosae a distinctly linear function of time, Fig. 2. Table V gives the regression constants *a* and *b* for the relation between water uptake and time and their relation to secretion block. The values of both constants were uniform in the same mucosal area for the two series. The ratio of buccal values to palatal values was between 1.7 and 2.5.

DISCUSSION

The present method was an elaboration of the principles which have proved applicable to quantitative studies of the water loss from oral mucosa (*Kaaber*, 1973). The analysis in Table II showed that the technique employed was suited to corresponding studies of water uptake through the oral mucosal surfaces. The slightly lower wet weight ratio in the different sizes of discs in relation to their dry weight ratio did suggest, however, the presence of systematic errors. An important source of variation was air trapped between filter paper and mucosa,

which prevented water absorption. These pockets were most easily eliminated without water loss of consequence in discs of 6.3 mm in diameter; this diameter was therefore employed in the other experiments. But other factors caused increased variation in this *in vivo* technique. The difficulties of maintaining even contact over longer intervals between filter paper and mucous membrane were thus clearly reflected in a low mean value for water absorption from the cheek in the 30-minute contact period in Fig. 2, just as the large individual variations in Table II showed the difficulties of establishing identical hydrated conditions in the mucosa during repeated sampling.

In spite of a coefficient of variation of 20—25%, the technique permitted a number of observations on the water uptake by hydrated oral mucosa. The continuous inflow of water through the mucous membrane could be ascribed to the prevailing osmotic gradient between water and the mucous membrane and the interstitial fluid. The osmotic activity of the oral epithelial strata is not known, but the gradient between water and plasma is 0.301 osmol/l (*Diem*, 1962), enabling a continuous inflow through the mucosa. The course of this inflow in Fig. 1 with an initially high level and a later steady-state rate compared closely with the corresponding inflow of water in epidermis (*Buettner*, 1953; *Szakall*, 1958). The linear relation between time and intake in the steady-state phase in Fig. 2, and the ratio of 2:1 between buccal and palatal mucosa for the inflow-time relation, which the values in Table V expressed, were closely paralleled in the corresponding outflow of water through the same mucosal areas under a hydrostatic pressure gradient (*Kaaber*, 1973). The mean values in Table V for the regression line constant *a* for buccal mucosa and for palatal mucosa during secretion block, occupied a level between the corresponding *a*-values for hydrated and moderately dehydrated mucosa in the series I and phase IIA in Table IV in *Kaaber* (1973). The present values thus corresponded to an inflow of 5.1—5.9 mg water/cm²/hour for buccal mucosa and 2.3—2.4 mg/cm²/hour for palatal mucosa. Considering their intermediate level, these values clearly manifested a ratio of 1:1 between inflow and outflow of water through the two mucosal areas. These flow values were of the same magnitude as in other types of stratified squamous epithelium. In rabbit cornea the hydrostatically determined outflow of water is about 3 μl/cm²/hour (*Mishima & Maurice*, 1961), just as frogs under water at 22°C show an osmotically determined inflow of water of 4—7 mg/cm²/hour (*Hevesy, Hofer & Krogh*, 1935).

The present investigation indicated that the osmotic conditions in the oral cavity give rise to an inward transport under physiological conditions in areas like the floor of the mouth, where the surface of the mucous membrane is almost constantly bathed in mixed saliva, the osmotic pressure of which is between 50 and 75% of the value for blood (*Willsmore*, 1937; *Köstlin & Rauch*, 1957). Whether a true diffusion of water may occur into the capillary bed during alterations in the osmolyte concentration of saliva, depends on the osmotic properties of the epithelial layers. Their magnitude is at present unknown and should therefore be the object of continued research.

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