Studies on the permeability of human oral mucosa

IV. Regional changes in outflow of water from hydrated and dehydrating oral mucosa

SVEND KAABER

The Department of Prosthetic Dentistry, Royal Dental College, Aarhus, Denmark

Kaaber, S. Studies on the permeability of human oral mucosa. IV. Regional changes in outflow of water from hydrated and dehydrating oral mucosa. *Acta Odont. Scand.* 31, 89—99, 1973.

The discharge of water from hydrated and dehydrating oral mucosa has been investigated in 10 young adults by analysing the change in weight in standardized discs of ash-free filter paper and membrane filter after mucosal contact in a non-ventilated sampling chamber. The rate of water loss was from hydrated buccal mucosa 6.2—7.4 mg/cm²/hour and from hydrated palatal mucosa 3.0—3.6 mg/cm²/hour. Dehydration of the mucosae was achieved by creating a vapour pressure gradient to the ambient external atmosphere after suspension of the salivary secretion with scopolamine. During the first 60 minutes the water discharge showed decreasing values, followed by stabilization. A regression analysis of the relationship between time and water loss demonstrated constant values for the regression coefficients but gradually falling values for the intercept for the regression lines on the axis for the water loss, indicating a declining permeability to water due to the decreasing water content in the mucosa during dehydration. The ratio of water discharge from buccal and palatal mucosa was a constant 2:1. In the palatal mucosa local variations in water discharge were observed, probably on account of regional differences in the hydrostatic pressure. The observations are discussed in relation to ultrastructural barrier components of stratified squamous epithelium.

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

Svend Kaaber, The Department of Prosthetic Dentistry, Royal Dental College, 8000 Aarhus C, Denmark

The oral epithelium performs its protective functions under a highly variable fluid covering. In an area like the floor of the mouth, the mucous membrane is almost constantly bathed in mixed saliva, while the other parts of the mouth are subject to varying conditions ranging from periodic inundation with saliva to strong dehydration. These extremes are especially pronounced in the transition zone between labial epidermis and labial mucosa.

The effect of variations in the saliva covering on the functions of the mucous membranes has not previously been subjected to detailed investigation. Observations on keratinized gingival mucosa (*Schilli*, 1968) and on non-keratinized buccal mucosa (*Kaaber*, 1971c) suggest that the superficial mucosal layers are in equilibrium with the ambient saliva. Under changed conditions like prolonged dehydration, the oral mucosae lose water at a rate inversely proportional to the humidity of the ambient external air, varying with the structure of the epithelium (*Kaaber*, 1971b, 1971c).

Received for publication, November 20, 1972.

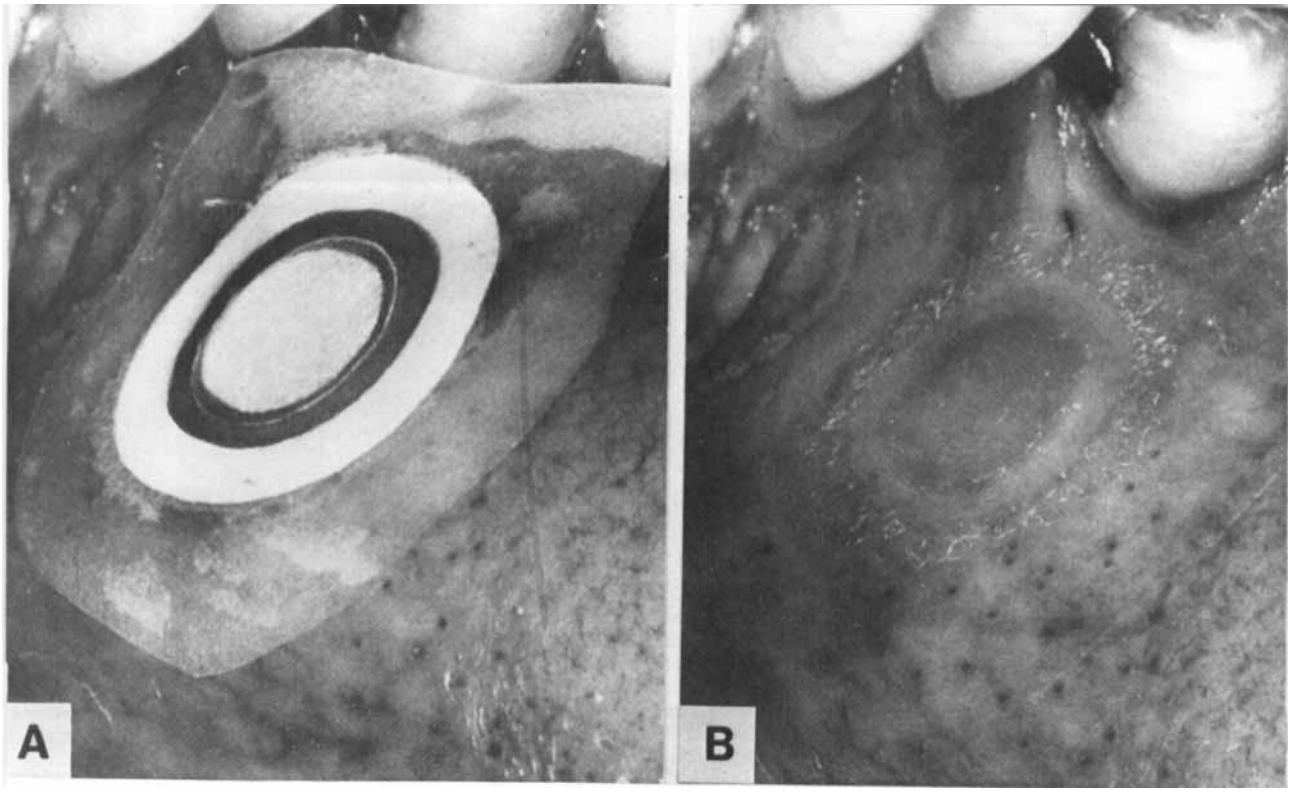

Fig. 1. A: Sampling chamber with a disc of filter paper located on palatal mucosa. B: The same area after removal of the sampling appliances.

The rate of this oral water loss is not yet known, whereas there are numerous studies on the corresponding transport through the epidermis, cf. for example reviews by *Tregear* (1966) and *Lamke & Wedin* (1971). The purpose of the present investigation was thus to study under standardized external conditions the rate of water discharge from a keratinized and a non-keratinized type of oral mucosa and its variability during prolonged dehydration of the mucosal surface.

MATERIAL AND METHODS

The investigations were carried out on 10 persons: 5 men and 5 women aged 22—25 years with clinically normal oral mucosa, except that one woman had moderate bilateral leukoedema of the buccal mucosa. None of the subjects were smokers. Two non-glandular areas of the cheeks and hard palate, the topography of which has been described earlier (*Kaaber*, 1971b, 1971c), were selected as sampling areas with non-keratinized and keratinized mucosa. The water passed from these areas was collected in discs of ash-free filter paper (Frisenette 644—25),* with an underlying disc of porous cellulose ester membrane (Millipore MF filter, type 11301, pore diameter 5 μ).** The discs were kept in close contact with the mucous membrane under a transparent plastic disc with a diameter of 8 mm, stuck to a piece of transparent tape, with a surrounding ring of ash-free filter paper (Fig. 1A). Before sampling, surface saliva was removed from the mucosa by careful wiping with sterile gauze. The sampling area was kept dry by means of cotton rolls in the buccal vestibule and by a saliva ejector. Two series of samples were taken: in series I the samples were taken im-

* A.G. FRISENETTE & SONS, Ebeltoft, Denmark

** MILLIPORE Corp. Bedford, Mass., U.S.A.

Table I. *Sampling procedure*

Series	Phase	Number of samples	Sequence of sampling (contact period in min.) 30	15	10	5	2	1
Without injection of scopolamine	I	5	—	1	1	1	1	1
With injection of scopolamine	IIA	5	—	1	1	1	1	1
	IIB	6	1	1	1	1	1	1
	IIC	5	—	1	1	1	1	1

mediately after cleaning the mucous membrane; in series II the oral cavity was dehydrated by suspending the salivary secretion with a submucous injection of 0.5 ml 0.05 % scopolamine nitrate 30 minutes before sampling. A consecutive sampling scheme with three phases was employed using decreasing length of contact period (Table I). The contact period was in each case regulated with a stop watch. In both series I and II the right and left aspects were sampled simultaneously with different sizes of disc. For the palatal mucosa, discs with a diameter of 6.3 and 7.4 mm were employed, and for the buccal mucosa discs with a diameter of 4.7 and 6.3 mm. For each subject, 10—16 blind samples were taken to register the effect of manipulation.

During sampling, the subject kept the mouth slightly open and breathed through the nose. The samples were taken at a temperature of $22 \pm 2°C$ and a vapour pressure of 11 ± 0.5 mm Hg, equivalent to a relative air humidity of 50 %. The humidity was continuously adjusted with an electric evaporator (Klimalux).* Temperature and vapour pressure were checked at 15-minute intervals with a battery-operated psychrometer (Ellab type B 19), coupled to an electric thermometer (Ellab type TE 3).** The psychrometer was read after an adjustment period of 2 minutes, and the registrations comprised both the air mass at the height of the subject's face and that in the oral cavity above the top of the tongue. After the final samples the surface temperature of the mucous membrane was measured with a thermoprobe, coupled to the electrical thermometer.

The water content of the discs was measured by weighing them in closed polyethylene containers before and after contact. Weight determinations were carried out on a microbalance (Mettler M5) in a weighing room at 70 % relative air humidity, according to a procedure described earlier (*Kaaber*, 1971a, 1971b).

The statistical analysis of the collected data was performed with the aid of an electronic computer using parameters calculated by conventional methods. An X^2-test was used to investigate whether the distribution of values in each group was normal. A significance level of 5 % was employed, and parameter values with significant deviation at a 5 %, 1 % or 0.1 % level are indicated in the tables by one, two or three asterisks respectively.

RESULTS

Fig. 2 shows the changes in vapour pressure and temperature in the oral cavity and ambient air during sampling. While the oral air temperature showed constant

* H. Bødtcher-Hansen A/S, Copenhagen, Denmark

** Ellab A/S, Copenhagen, Denmark

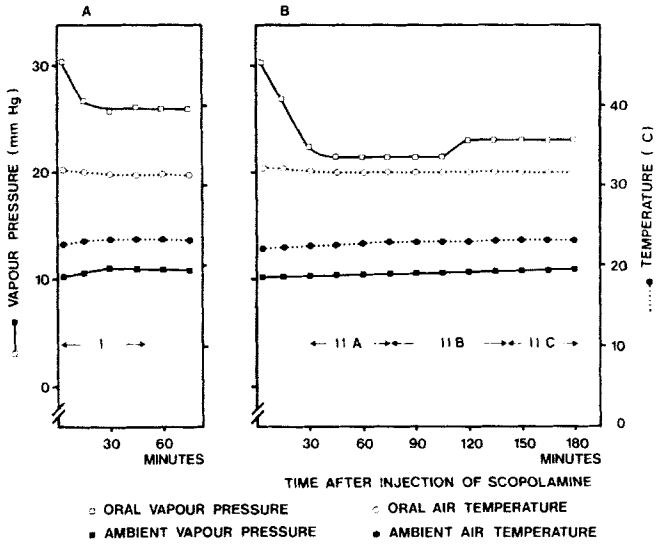

Fig. 2. Changes in vapour pressure and in temperature of the oral cavity and ambient air during sampling without (series I) and with suspended salivary secretion (series II). Area inside arrows denotes duration of sampling period. A: Changes during sampling in series I. B: Changes during sampling in series II.

values in both series, the oral vapour pressure fell during the first 15 minutes in series I from 31 mm Hg to 26 mm Hg, where it remained during the remaining period (Fig. 2A). This change was equivalent to a fall in relative air humidity from 90 to 76 %. In series II, the oral vapour pressure showed falling values for the first 30 minutes, until stabilization occurred around 22 mm Hg, equivalent to a fall in the relative air humidity from 90 to 60 % (Fig. 2B). 120 minutes after scopolamine injection, the vapour pressure showed a slight but distinct rise to 23 mm Hg, equivalent to an increase in the air humidity from 60 to 65 %. The mucosal temperatures were uniform in both series, with a mean value for the buccal mucosa of 35.3 ± 0.5°C and for the palatal mucosa of 34.6 ± 0.4°C.

Table II. *Variations in weight ratios for two different sizes of discs before and after contact with human oral mucosa during dehydration*

Person	Sex	Buccal sample series				Palatal sample series			
		Dry weight ratio		Wet weight ratio		Value of t	Dry weight ratio		Wet weight ratio		Value of t
		$\bar{x}$	S.D.$_k$	$\bar{x}$	S.D.$_k$		$\bar{x}$	S.D.$_k$			
OJ	♂	1.95	0.14	2.06	0.36	1.47	1.25	0.08	1.24	0.18	0.25
KK	—	—		1.98	0.39	0.37	—		1.22	0.20	0.69
TN	—	—		1.82	0.24	2.45*	—		1.41	0.13	3.81***
ON	—	—		2.06	0.30	1.18	—		1.06	0.21	4.14***
CJ	—			1.89	0.40	0.71	—		1.09	0.16	4.43***
BB	♀	1.95	0.14	2.26	0.60	2.57*	1.25	0.08	1.23	0.29	0.34
KT	—	—		1.78	0.32	2.50*	—		1.17	0.19	1.93
JC	—	—		2.10	0.36	1.86	—		1.36	0.25	2.07*
CN	—	—		1.92	0.34	0.42	—		1.36	0.18	2.83**
BS	—	—		1.89	0.24	1.09	—		0.94	0.13	10.33***

Table III. *Water discharge from oral mucosa in persons without (series I) and with (series II) suspended secretions. Values in milligrams per cm² mucosa*

Sampling area	Sampling period in minutes	Mean water content of samples (mg/cm²) Series I $\bar{x}$	S.D.$_k$	Series II A $\bar{x}$	S.D.$_k$	Series II B $\bar{x}$	S.D.$_k$	Series II C $\bar{x}$	S.D.$_k$	Value of t I—IIA	IIA—IIB	IIB—IIC
Buccal mucosa	30					1.698	0.341					
	15	2.319	0.292	1.981	0.412	1.366	0.292	1.176	0.169	3.87***	7.70***	3.43**
	10	1.772	0.197	1.391	0.162	1.173	0.221	1.066	0.175	9.08***	4.91***	2.34**
	5	1.381	0.227	1.044	0.171	0.891	0.142	0.830	0.109	7.24***	4.25***	2.07
	2	0.978	0.165	0.723	0.120	0.604	0.081	0.590	0.082	7.91***	5.03***	0.75
	1	0.741	0.105	0.528	0.089	0.432	0.087	0.415	0.092	9.38***	4.86***	0.85
Palatal mucosa	30					1.084	0.159			I—IIA	I—IIB	I—IIC
	15	1.052	0.129	0.990	0.180	0.832	0.157	0.766	0.142	1.56	5.92***	8.22***
	10	0.799	0.155	0.740	0.168	0.689	0.123	0.673	0.119	1.53	3.35**	3.85***
	5	0.649	0.098	0.502	0.119	0.474	0.072	0.466	0.071	1.77	3.63***	3.96***
	2	0.403	0.068	0.331	0.088	0.283	0.061	0.298	0.061	3.74***	7.66***	6.69***
	1	0.311	0.064	0.200	0.055	0.190	0.038	0.208	0.044	7.93***	10.00***	7.62***

Table II shows the dry weight ratios of the different sizes of discs employed and their wet weight ratios after contact with the mucous membrane. The wet weight values for buccal mucosa showed accordance with the dry weight value in most of the material with minor differences in three persons, indicating proportionality between water absorption and disc size, whereas the values for the palatal mucosa in five persons — 50 % of the material — showed significant differences between the corresponding dry and wet weight ratios.

Table III gives the mean values for the water discharge from the two mucosal areas, expressed as the water content per

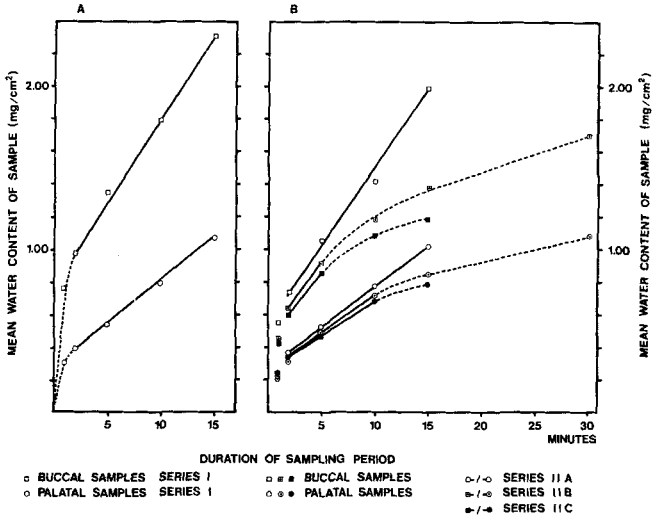

Fig. 3. Water discharge from oral mucosa during sampling without (series I) and with suspended salivary secretion (series II). A: Mean values for water content of samples obtained in series I. B: Corresponding mean values during sampling in series II. Values with a non-linear relation between water content and time are connected by broken lines.

Table IV. *Regression equation values for the water discharge from oral mucosa in persons without (series I) and with (series II) suspended salivary secretion*

	Sampling series	Buccal mucosa $\bar{x}$	Buccal mucosa S.E.M.$_k$	Palatal mucosa $\bar{x}$	Palatal mucosa S.E.M.$_k$	Ratio buccal/palatal mucosa
Regression line constant (a)	I	0.821	0.027	0.301	0.015	2.7
	IIA	0.534	0.028	0.242	0.022	2.2
	IIB+C	0.419	0.017	0.207	0.008	2.0
Regression coefficient (b)	I	0.996	0.056	0.499	0.030	2.0
	IIA	0.933	0.057	0.458	0.080	2.0
	IIB+C	0.883	0.120	0.483	0.025	1.8

cm^2 sample area. In both areas a fall occurred in corresponding values as sampling progressed from series I to series II. except in the last phase (IIC). In series I the water discharge from both mucosal areas was a distinctly linear function of time, apart from the 1-minute values, Fig. 3A. A similar relation was also apparent in the first phase of series II, whilst in the following phases, IIB and IIC, it was manifested only in the 2- and 5-minute values for the buccal mucosa and in the 2-, 5- and 10-minute values for the palatal mucosa Fig. 3B. The other values deviated clearly from linearity with their lower level.

Table IV gives the regression equation values for those values in Fig. 3A and 3B which showed a linear relation between water uptake and time. In both series I and II the regression coefficient b was a uniform value, twice as high in the buccal mucosa as in the palatal mucosa. The intercept of the regression line on the y-axis, the regression line constant *a*, was in both buccal and palatal mucosa systematically lower in series II than in series I. The ratio between the values of the two areas ranged in the single phases between 1.8 and 2.7.

DISCUSSION

The influence of physical factors on the discharge of water from the oral mucosa

The distinct demarcation between the covered and the exposed part of the palatal mucosa in Fig. 1B and the direct correlation between the dry weight of the different sizes of discs employed and their wet weight after contact with buccal mucosa in Table II were two clear indications that the water content of the samples derived from the covered mucosa and was in consequence an expression of the water loss from this area. The sampling appliance thus constituted a non-ventilated chamber filled with absorbent material. This principle has been employed with various modifications in a number of studies of water and fluid transport through epidermis, for example by *Randall & McClure* (1949), *Buettner* (1953, 1959), *Monash & Blank* (1958), *Ohara & Ono* (1963), *Wilson & Moncrief* (1965), and *Shahidullah, Raffle & Frainbell* (1967). An important drawback with this technique in epidermal studies is the uncontrollable rise in vapour pressure in the chamber during absorption and its lowering effect on the rate of discharge from the skin (*Lamke*

1970). In the present material a similar lowering effect was observed towards the end of the phases IIB and IIC in contrast to the linear relation between time and water loss in the preceding phase IIA and series I, Fig. 3A—B. This deviation from linearity coincided with a reduced content of water in the samples compared with the preceding corresponding samples in Table III, which reflected a lower water content in the tissue and a lowering of the vapour pressure on the mucosal surface. Thus the rise in vapour pressure in the sampling chamber due to the outward flow of water would reduce the vapour pressure gradient in the chamber and tend to decrease the loss of water from the mucosa. This mechanism might explain the lowering of the water content in the latter samples in the phases IIB and IIC. Accordingly, the increased water content of the samples in series I and phase IIA reflected a higher water content of the mucosa, which at the beginning of sampling in series I must be considered to have been hydrated and in equilibrium with saliva and the vapour-saturated oral air, cf. Fig. 2. This condition indicated the presence of a uniformly high vapour pressure over the mucous membrane in the sampling chamber. The continuous water discharge from the mucosa and its linear course during these phases demonstrated its independency of the high vapour pressure and indicated the presence of another pressure gradient, sustained by a positive hydrostatic pressure in the two mucosal areas. This factor and its importance for the oral water discharge has previously been demonstrated indirectly on the dehydrated palatal mucosa, Fig. 4 in *Kaaber* (1971b). The present observations indicated a major difference between water loss from non-sweating epidermis and oral mucosa, since the main driving force for transepidermal water loss seems to be the vapour pressure gradient between the stratum corneum surface and the ambient air (Grice, Sattar & Baker 1972). Other epidermal studies have demonstrated the influence of a high water content in stratum corneum on the rate of the transepidermal water loss (*Spruit & Malten*, 1969), which explains the initially high level of the water discharge from hydrated epidermis (*Peiss, Randall & Hertzmann*, 1956). The significantly higher water content of the samples from the hydrated buccal mucosa in series I compared with series IIA in Table III thereby indicated a maximal value for water discharge from the mucosa. For a 60-minute period and with a confidence level of 95 % the values in Table IV corresponded to a discharge of 6.2—7.4 mg water per cm^2 hydrated buccal mucosa. The similar values for the hydrated palatal mucosa were 3.0—3.6 mg water per cm^2 and hour. The exposure of the unprotected mucosal surface to a high vapour pressure gradient created the requisite conditions for a continuous water discharge from the mucosa and a dehydration of it. The consistent level of the *b*-values in Table IV demonstrated a rate-limiting effect from structural factors on the water loss, while the changing *a*-values indicated a declining permeability of the mucosal areas to water during the period. The principal effect from the dehydration was a gradually decreasing water content in the surface layers of the mucosa. These changes were reflected in the reduction of the *a*-values in Table IV, which were most pronounced during the first 30 minutes in phase IIA. Compared with the values in series I the reduction was 35 % and 20 % in buccal and palatal mucosa respectively, whilst the reduction in relation to phase IIA in the

following phases IIB—IIC was 22 % and 15 % for these areas respectively. The values in Table III showed that the water discharge from both mucosal areas was not essentially altered between phases IIB and IIC, i.e. after 120 minutes' dehydration. The tendency to further reduction in Table III found in the 10- and 15-minute values from the buccal mucosa in phase IIC might be ascribed to another factor, the weak increase in the vapour pressure of the oral air about 100 minutes after the injection of scopolamine, cf. Fig. 2. This increase was most likely due to the cessation of the effect of scopolamine, as the duration of the period coincided with earlier observations on the blocking effect of the same dosage (*Östlund & Åkesson*, 1959; *Pohto & Ahtee*, 1966). Furthermore, the range of the *a*-values in Table IV demonstrated the amount of passively held water in the surface layers of the mucosa. The values indicated that about 0.40 mg water was retained per cm^2 hydrated buccal mucosa and about 0.10 mg water per cm^2 hydrated palatal mucosa, constituting a considerable reservoir of solutes in relation to the total oral area.

Regional variations in the water loss from oral mucosa

The values in Table IV indicated a permeability to water which was twice as great in buccal as in palatal mucosa. The higher permeability of non-keratinized buccal mucosa agreed closely with earlier qualitative observations (*Kaaber*, 1971b, 1971c). Compared to the earliest 30-minute values at a relative ambient air humidity of 50 %, see Table IV in *Kaaber* (1971c), the present values in Table III represented a 10 % higher level, probably on account of the more gentle drying of the mucous membrane prior to sampling. Besides the regional variation, the palatal material showed distinct local differences in water discharge. The reason for these differences in Table II was probably regional variations in the hydrostatic pressure in the hard palate, due either to a heterogeneous vascularization of the glandular tissue in the vicinity of the sampling area or to the absence in the palate of a collateral circulation owing to its being supplied from the terminal palatine artery. The distinctly lower water discharge from the anterior horizontal part of the palate compared with the present sampling area (*Kaaber*, unpublished data) seemingly favoured this latter interpretation, although the influence from regional differences in keratinization of the palatal mucosa could not be neglected.

The influence of structural factors on the water permeability in oral mucosa

The regional variation in the present material demonstrated clearly the effect of various structural factors on the water balance in the two different types of oral mucosa. Although the material did not include histological data, earlier cytological observations from the same region in a corresponding material (*Kaaber*, 1971b, 1971c) indicated the presence of a predominant parakeratotic cell surface layer in the palatal sampling area and a non-keratinized surface in the buccal sampling area. The structural differences between palatal and buccal mucosa have recently been thoroughly reviewed (*Silverman*, 1971; *Alvares & Meyer*, 1971; *Chen & Meyer*, 1971). According to these surveys, the general ultrastructural features of keratinized and non-keratinized oral epithelia are well described, while special data, e.g. regarding age-dependent variation and quantitative differences are not

yet available for human mucosa. These gaps and the lack of data from correlated physiological and histological studies complicate an understanding of the functional and structural interrelationship, for which reason the following discussion has to be restricted to a mention of structural observations which are coincident with the present results.

The falling *a*-values in Table IV demonstrated a regional difference in the water-binding capacity of the mucosa which was approximately only half as great in the palatal as in the buccal area. Structural correlates to this observation are the dense packing of tonofilaments (*Meyer & Gerson*, 1964; *Silverman*, 1971) and the narrow intercellular spaces (*Thilander & Bloom*, 1968; *Silverman*, 1971) in palatal surface cell layers compared to corresponding buccal cell layers. Quantitative data on the dry mass content in these layers are not yet available for human mucosa, but analyses of rat mucosa have demonstrated a ratio of 3:1 between palatal and buccal epithelium (*Barrington & Meyer*, 1969). This ratio may be comparable to the present ratio of about 2:1 in Table IV considering the differences between the orthokeratinized rodent and the presumably parakeratinized human palatal epithelium. Similar studies on human ortho- and parakeratinized palatal epithelium would therefore be of interest to elucidate their water-binding properties.

The constant level of the *b*-values in Table IV indicated a consistent rate-limiting effect in the mucosal membrane on water transport. The regional differences thus demonstrated the presence of structural barriers other than those controlling the water-binding properties of the two mucosal areas. Studies of biological transport phenomena have demonstrated two main pathways for water, a transcellular and an intercellular one. The transcellular route, which is of importance in ensuring the osmotic balance between the cell and its environment, has mainly been studied on red blood corpuscles, cf. *Stein* (1967). The intercellular pathway, which is of importance for the active transport of water and solutes, has been demonstrated in water-transporting epithelia such as that of the gall bladder (*Tormey & Diamond*, 1966; *Kaye et al.*, 1966) and the kidney (*Schmidt-Nielsen & Davies*, 1968). According to ultrastructural investigations by *Farquhar & Palade* (1964, 1965) this pathway also seems to exist in stratified squamous epithelia like that of the frog skin. The pathways for water in the highly differentiated human oral epithelia are still unknown. Comparative electron microscopic studies on keratinizing and non-keratinized oral epithelium have, however, demonstrated structural differences in keratinizing palatal epithelium consistent with its lower permeability to water. These differences are present partly as aggregations of dense lamellated material deposited in the superficial intercellular spaces of the granular layer (*Frithiof & Wersäll*, 1965) and partly as an increasing thickness of the plasma membrane of the superficial cell layers (*Frithiof*, 1970). The supposed contribution of the palatal membrane-coated »microgranules» in the prickle cell layer to the thickening of the inner leaflet of the plasma membrane and to the formation of intercellular electron-dense material (*Silverman*, 1970) points to a central role for these granules in the barrier properties of this epithelium. This point of view can be supported by the structural similarity of the intercellular material to synthetic phospholipid preparations (*Frithiof & Wersäll*, 1965) in view of the known role of phospholipid-protein layers as semi-

permeable membranes with water barrier properties in human epidermis (*Crounse*, 1965).

The main result of the present investigation has been the demonstration of two barrier systems which restrict the water loss from human oral mucosa, one controlling the amount of passively held water in the surface layers of the mucosa, the other regulating the discharge rate. The regional differences and the complexity of the factors which may affect the oral water balance clearly demonstrate the need of detailed studies of the relation between structure and function in this area.

REFERENCES

Alvares, O. F. & Meyer, J. 1971. Variable features and regional differences in oral epithelium, in *Current concepts of the histology of oral mucosa*. Ed. by *Squier, C. A. & Meyer, J.* C. C. Thomas, Illinois, pp. 97—113

Barrington, E. P. & Meyer, J. 1969. Regional differences in gradients of dry residue density in oral epithelia of the rat: a quantitative study. Arch. Oral Biol. 14, 1085—1099

Buettner, K. J. K. 1953. Diffusion of water and water vapor through human skin. J. Appl. Physiol. 6, 229—242

Buettner, K. J. K. 1959. Diffusion of liquid water through human skin. J. Appl. Physiol. 14, 261—268

Chen, S. Y. & Meyer, J. 1971. Regional differences in tonofilaments and keratohyaline granules, in *Current concepts of the histology of oral mucosa*. Ed. by *Squier, C. A. & Meyer, J.* C. C. Thomas, Illinois, pp 114—128

Crounse, R. G. 1965. Keratin and the barrier. Arch. Environ. Health 11, 522—528

Eriksson, G. & Lamke, L. O. 1971. Regeneration of human epidermal surface and water barrier function after stripping. Acta dermatovener (Stockholm) 51, 169—178

Farquhar, M. G. & Palade, G. E. 1964. Functional organization of amphibian skin. Proc. Nat. Acad. Sci. 51, 569—577

Farquhar, M. G. & Palade. G. E. 1965. Cell junctions in amphibian skin. J. Cell Biol. 26, 263—291

Frithiof, L. 1970. Ultrastructural changes in the plasma membrane in human oral epithelium. J. Ultrastruct. Res. 32, 1—17

Frithiof, L. & Wersäll, J. 1965. A highly ordered structure in keratinizing human oral epithelium. J. Ultrastruct. Res. 12, 371—379

Grice, K. A., Sattar, H. & Baker, H. 1972. The effect of ambient humidity on transepidermal water loss. J. Invest. Derm. 58, 343—346

Kaaber, S. 1971a. Studies on the permeability of human oral mucosa. I. Gravimetric determination of biological fluids at microgram levels. Acta Odont. Scand. 29, 653—662

Kaaber, S. 1971b. Studies on the permeability of human oral mucosa. II. The permeability of dry palatal mucosa to water, sodium and potassium. Acta Odont. Scand. 29, 663—681

Kaaber, S. 1971c. Studies on the permeability of human oral mucosa. III. The permeability of dry buccal mucosa to water, sodium and potassium. Acta Odont. Scand. 29, 683—698

Kaye, G. I., Wheeler, H. O., Whitlock, R. T. & Lane, N. 1966. Fluid transport in the rabbit gall bladder: a combined physiological and electron microscopic study. J. Cell. Biol. 30, 237—268

Lamke, L. O. 1970. An instrument for estimating evaporation from small skin surfaces. Scand. J. Plast. Reconstr. Surg. 4, 1—7

Lamke, L. O. & Wedin, B. 1971. Water evaporation from normal skin under different environmental conditions. Acta dermatovener (Stockholm) 51, 111—119

Meyer, J. & Gerson, S. J. 1964. A comparison of human palatal and buccal mucosa. Periodontics 2, 284—291

Peiss, C. N., Randall, W. C. & Hertzmann, A. B. 1956. Hydration of the skin and its effect on sweating and evaporative water loss. J. Invest. Derm. 26, 459—470

Pohto, P. & Ahtee, L. 1966. Effect of atropine, methylatropine, methylscopolamine and pilocarpine on salivary secretion and heart rate in man. Ann. Med. Exp. Fenn. 44, 411—414

Randall, W. C. & McClure, W. 1949. Quantitation of the output of individual sweat glands and their response to stimulation. J. Appl. Physiol. 2, 72—80

Schilli, W. 1968. The most superficial zone of the stratum corneum of the gingiva. Oral Surg. 25, 896—900

Schmidt-Nielsen, B. & Davis, L. E. 1968. Fluid transport and tubular intercellular spaces in reptilian kidneys. Science 159, 1105—1108

Shahidullah, M., Raffle, E. J. & Frain-Bell, W. 1967. Insensible water loss in dermatitis. Brit. J. Derm. 79, 589—597

Silverman, S., 1971. Nonkeratinization and keratinization: The extremes of the human range, in *Current concepts of the histology of oral mucosa*. Ed. by *Squier, C. A. & Meyer, J.* C. C. Thomas, Illinois, pp. 80—96

Spruit, D. & Malten, K. E. 1969. Humidity of the air and water vapour loss of the skin. The changing permeability. Dermatologica 138, 418—426

Stein, W. D. 1967. *The movement of molecules across cell membranes.* Academic Press. New York & London.

Tormey, J. McD. & Diamond, J. M. 1966. The ultrastructural route of fluid transport in rabbit gall bladder. J. Gen. Physiol. 50, 2031—2060

Thilander, H. & Bloom, G. D. 1968. Cell contacts in oral epithelia. J. Periodont. Res. 3, 96—110

Tregear, R. T. 1966. *Physical functions of the skin.* London.

Wilson, J. S. & Moncrief, A. 1964. Vapor pressure of normal and burned skin. Ann. Surg. 162, 130—134

Östlund, S. G. & Åkesson, N. Å. 1959. Hämning och stegring af salivkörtlernas verksamhet. Odont. Rev. 10, 147—166