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STUDIES ON THE PERMEABILITY OF HUMAN ORAL MUCOSA

I. GRAVIMETRIC DETERMINATION OF BIOLOGICAL FLUIDS AT MICROGRAM LEVELS

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The variation from the determination of small water quantities in standardised discs of ash-free filter paper in closed polyethylene containers and weight determinations with a microbalance have been studied. Mean weight differences of +7 micrograms or less were obtained for antistatic containers with dry filter paper discs after use of a pre-weighing period of 60 minutes and an inactivity period of 30 minutes after manipulation and closure of the containers. Weight determination of moistened filter paper was carried out using a microsyringe for water transfer. The syringe showed a uniform discrepancy between its scale indications and the corresponding weight determinations, which was attributed to an erroneous graduation level. The evaporative water loss from moistened filter paper at varying air humidity occurred at a constant rate at amounts of 500 micrograms or above. Closed weighing bottles containing 100—1,000 micrograms of water in the filter paper showed a mean loss of 7.5 micrograms after 30 minutes' standing and 20.8 micrograms after 60 minutes'. The mean recovery value after 30 minutes' standing was 99.1 % after correction for the evaporative water loss. The variation caused by direct sampling of water was estimated to 10 micrograms.

Several aspects of the physiology of the oral mucosa have not yet been fully elucidated. Thus only a little information is available on the permeability of healthy oral tissue, whereas the conditions in inflamed tissue have been intensively studied in periodontal mucosa (*Brill*, 1962; *Weinstein & Mandel*, 1964; *Brandtzaeg*, 1966; *Egelberg*, 1967). The limited knowledge of the physiological reactions of oral tissue is mainly due to difficulties of collecting and quantifying the small amounts of material available. This problem has further complicated the investigation of oral inflammatory exudate, which has mainly utilized volumetric methods (*Brandtzaeg & Mann*, 1964; *Sueda, Bang & Cimasoni*, 1969; *Kaslick et al.*, 1968). A volumetric technique using capillary microtubes for sampling is unsuited

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for use on most oral surfaces. Gravimetric determination of the exudate quantities collected in absorbing materials represents an alternative method, which has only been employed to a limited extent (*Matsue*, 1967; *Hara & Löe*, 1969).

The latter principles offer distinct advantages for quantitative studies of oral fluids. The aim of the present study has been to analyse the variation arising from the use of filter paper for the sampling and a highly sensitive microbalance for the determination of small quantities of water, in order to devise a quantitative sampling method with an established precision.

MATERIALS AND METHODS

Circular pieces of ash-free filter paper (Frisenette 644—25)* with a diameter of 6.3 mm were mployed for sampling. For transport and weighing of the discs, cylindrical containers of polyethylene (height 28 mm, diameter 10 mm), closed with stoppers of polytetrafluorethylene (Teflon) were used. The stoppers had an angle of convergence of 3° and were equipped with a collar at the top to facilitate manipulation (Fig. 1). Static electricity on the surface of the container was removed by gently wiping with a piece of linen moistened with a 0.5 % aqueous solution of quaternary ammonium bases (Rodalon®).** All other handling was carried out with clean chromium-plated steel tweezers.

A micro balance (Mettler M5) with a maximum capacity of 20 grams and a reading accuracy of 2 micrograms was used for weight determination. The balance was placed on a vibration-free base in a closed weighing room, the temperature of which was maintained at $22 \pm 2°$ C. The humidity in the room was regulated with a portable humidifier (Klimalux)*** and continually registrated with a hygrometer. Before use, pre-weighing was carried out, until a constant zero level was obtained. The zero level was checked after every weighing and any displacement adjusted immediately. Readings were made 30 seconds after the pan was apparently still. All registrations were checked for gross errors by duplication.

The water content of samples was calculated from the difference in weight between the closed bottle before and after absorption of water by the filter paper disc. The transfer of water to the filter paper was made with a micro-

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

Fig. 1. Weighing bottle of polyethylene with a stopper of polytetrafluorethylene.

syringe. The syringe employed (Hamilton type 7001)* had a capacity of 1 microlitre and was calibrated in units of 10 nanolitres. Its variation in volume determination and fluid yield was investigated by the following technique:

Before filling the chamber, the syringe was carefully pumped through with distilled deionized water (DDW) to eliminate air trapped. It was filled to an excess of c. 20 nanolitres, which were removed with filter paper. The content was then transferred as a single drop to a waterrepellent plastic disc (Styroflex ®), the weight of which was checked in each case. 30 and 60 seconds after discharge, the plastic disc with the droplet was weighed, reading to the nearest 5 micrograms on the balance. The water content of the syringe at discharge was calculated from the formula $(W_{s+30}-W_s) + (W_{s+30}-W_{s+60})$, where W_s represented the dry weight of the unit, and W_{s+30} and W_{s+60} the corresponding wet weight values for the unit 30 and 60 minutes respectively after the discharge of the syringe.

* Micromesure N.V., Den Haag, Holland.

The evaporation of water from samples in free air was investigated with filter paper discs placed on a piece of waterrepellent plastic (Styroflex). After determination of the dry weight of this unit, DDW was applied to the disc. With the balance pan continuously released, the changes in weight in the filter paper were registered at 60 second intervals until constant values were obtained. After repeated control of the zero level, the dry weight value was checked. The final value was used in calculation of the water loss.

The statistical analysis employed parameters calculated by conventional methods. Differences between means were analysed with Student's t-test and differences in variances with Snedecor's F-test. Significance limits at a 5 %, a 1 % and a 0.1 % level are indicated with 1, 2 or 3 asterisks in the tables respectively.

RESULTS

Table I indicates the reproducibility of weight determinations with containers before and after antistatic treatment. After treatment, the weight differences exhibited a uniform mean value and significantly lower values for the standard deviation ($P<0.01$).

Table II gives the corresponding values from weight determinations of antistatic containers related to the frequency in use of the microbalance and the level of humidity of the air. With daily use of the balance, weight determinations obtained at an interval of 60 minutes exhibited only moderate variation during the first hour, compared with the following hour. Least variation was observed at a relative humidity level above 60 %. After a period of inactivity of 24 hours or more for the balance, a significantly greater variation was observed, especially during the first hour and at a low relative

Table I.

Weight determination with polyethylene containers before and after antistatic treatment with 0.5 % Rodalon-solution. Number of containers: 20. Pre-weighing period: 60 minutes. Interval between first and second weight determination: 60 minutes

Time after initial pre-weighing	Weight differences in micrograms Before antistatic treatment		After antistatic treatment		
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	F
1 hour	0	9.75	+4.20	5.00	3.80**
25 hour	—6.65	7.44	+5.45	4.71	2.49**

Table II.

Weight determination with antistatic polyethylene containers. Weight differences related to relative humidity and the extent of time prior to the use of the microbalance. Number of containers in each group 24. No pre-weighing

Inactivity period	Relative humidity (in %)	Weight differences in micrograms					
		First hour		Second hour		Value of t	Value of F
		$\bar{x}$	S.E.	$\bar{x}$	S.E.		
	32—37	+ 6.16	4.26	+6.32	5.07	0.1194	1.42
< 24 h.	40—45	— 0.38	8.01	+4.58	3.74	2.7555**	4.58**
	50—55	+ 5.12	4.77	+0.33	5.63	3.1867**	1.39
	60—65	+ 4.77	3.85	+3.21	3.25	1.7333	1.40
	32—37	+12.48	8.48	+6.20	4.30	3.2538**	3.88**
< 24 h.	40—45	+11.21	7.30	+0.75	5.90	5.4760***	1.53
	50—55	+ 9.71	10.27	+6.16	4.82	1.5367	4.59**
	60—65	+ 5.23	7.25	+2.38	4.15	1.6764	3.05**

humidity. An increase of the weighing period after the first hour to 150 minutes produced no significant changes in the magnitude of the weight differences.

Table III gives the results from weight determinations of the water content of the microsyringe used. In all cases, the actual weight value was less than the corresponding syringe reading, with a systematic difference of —20 micrograms.

Fig. 2 shows the evaporative loss of water from a filter paper disc containing 3000 μg DDW. Evaporation from the surface occurred at a constant rate

Table III.

Weight determination of water quantities in a microsyringe. Number of samples in each group: 20. Microsyringe readings, 250, 500 and 1000 nanoliters respectively, converted into micrograms according to temperature of water

Water content of microsyringe (micrograms)	Water content after weight determination (micrograms)		Mean deviation from expected value	
	$\bar{x}$	S.E.	micrograms	%
249.50	228.50	5.93	—21.00	8.4
499.00	478.00	8.16	—21.00	4.2
998.00	978.00	5.42	—20.00	2.0

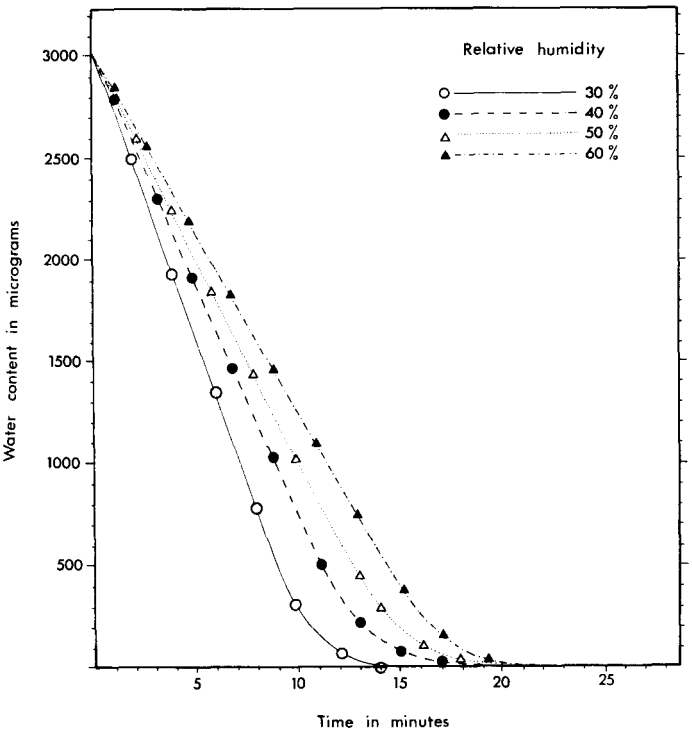

Fig. 2. Rate of evaporation for 3000 μg distilled deionized water absorbed in filter paper discs (diameter 6.3 mm), related to the humidity of the surrounding air. Mean values from five tests at each humidity level.

until the water content had fallen to c. 500 micrograms. After this, the rate fell gradually.

Fig. 3 shows the corresponding values of the percentage loss of water vapour per minute related to the remaining water content in the filter paper. The loss exhibited a hyperbolic curve after a maximal value at amounts of 100—150 micrograms in the filter paper.

Fig. 4 shows displacements in the weight level of closed containers with varying water content in the filter paper disc. 30 minutes after closure a strong increase was noted compared to the weight level 5 minutes after closure. 60 and 90 minutes after closure, the values showed a distinct decline. The mean value for the total loss during the first 30 minutes was −7.50 micrograms, and −13.27 micrograms for the following 30 minute period. The corresponding value for the control material was +3.07 micrograms.

Table IV gives the results of the weight determinations of the same contain-

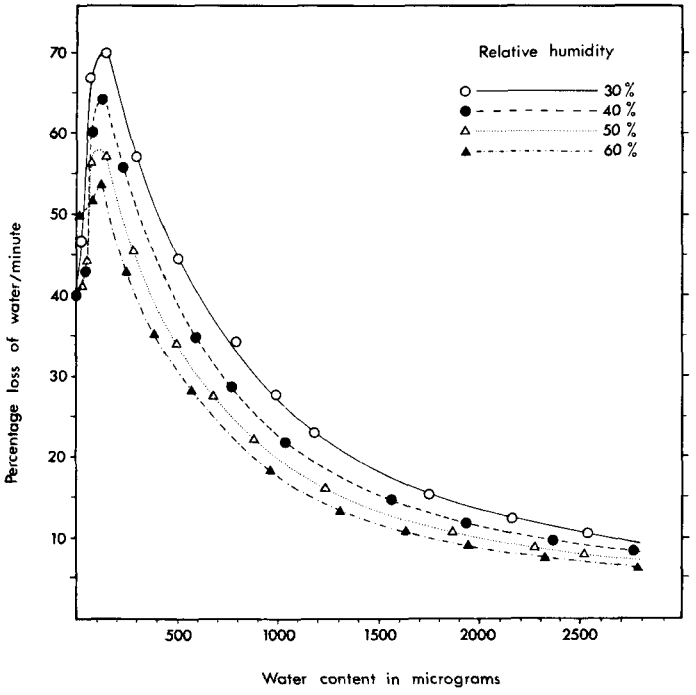

Fig. 3. The percentage evaporative loss from humid filter paper discs (diameter 6.3 mm) related to the humidity of the surrounding air. Mean values from five tests at each humidity level.

Table IV.

Weight determination of microquantities of water in filter paper. Recovery rates 30 minutes after closure of container

Water content of microsyringe (values in micrograms)		Water content after weight determination (values in micrograms)			Recovered weight in %
Read values $\bar{x}$	Corrected values $\bar{x}$	Observed values $\bar{x}$	S.E.	Corrected values $\bar{x}$	
149.70	128.70	114.26	13.50	126.59	98.3
499.00	478.00	445.34	15.24	488.36	102.1
948.00	928.50	836.12	16.72	901.11	97.0

Number of samples in each experiment = 2 × 12. Microsyringe readings 150, 500 and 950 nanoliters respectively, converted into micrograms according to temperature of water, and corrected for reading errors according to Table III. Quantities of absorbed water corrected for loss due to evaporation between water application and closure of container. Corrected values calculated at a relative humidity of air of 50 % and a duration of the evaporating period of 10 sec./128 micrograms, 15 sec./478 micrograms and 20 sec./928 micrograms of water.

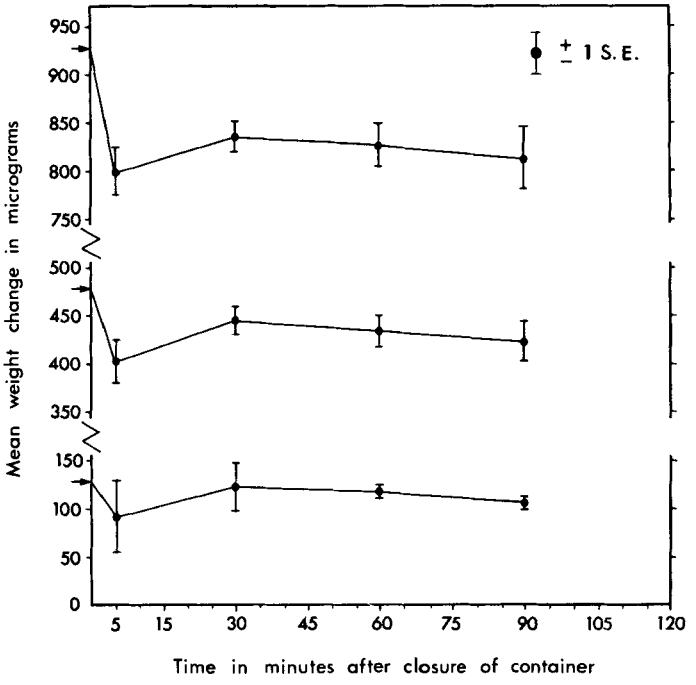

Fig. 4. Weight changes of closed weighing bottles containing filter paper discs with 128.7, 478.0 and 928.5 μg double distilled water added. Mean values from 2×12 samples in each group.

ers 30 minutes after closure. After correction due to errors from the microsyringe and evaporative water loss before closure of the bottle, the weight determinations gave values corresponding to 97–102 % of the applied water.

DISCUSSION

The present method represents a further development of earlier gravimetric sampling methods, both at a milligram level (*Dole, Stall & Schwarz*, 1951; *Thaysen*, 1955) and at a microgram level (*Matsue*, 1967; *Hara & Löe*, 1969). On account of the sensitivity of the analytical balance and with a view to subsequent microanalyses, it was necessary to develop a more suitable type of container than the previously used weighing glasses. The two plastic materials polyethylene and polytetrafluorethylene were preferred on account of their low specific gravity, inability to absorb water, dense structure and

chemical inactivity (*Schifter-Holm*, 1968). Their main disadvantage was a marked tendency to build up an electrically active surface. Electrostatic containers could at a distance of 2—3 mm from the released balance pan displace the zero point position by up to 600 units on the scale. A thin coating of quaternary ammonium bases on the polyethylene provided a stable antistatic surface which did not influence the balance (Table I). A high reproducibility of readings was only obtained after a preweighing period of sufficient length. After an inactivity period of more than 24 hours, a preweighing period of 60 minutes was necessary in order to obtain readings with a discrepancy of less than 6 micrograms (Table II). The balance proved to be less sensitive to variations in humidity at room temperature. The results in Table II indicated a greater precision at humidity values above 60 %. Reproducible determinations could only be achieved on the same day, as experiments employing intervals of 16—24 hours showed weight differences varying from —20 to +20 micrograms.

The necessary manipulation of the weighing bottles and their filter paper discs caused a strong displacement in the weight level immediately after the closure of the bottles, cf. fig. 4. This displacement was of a reversible nature and weight determinations with values which were too low could be avoided by including a period of 30 minutes delay between closure and the subsequent weight determination. The reason for the displacement was not apparent. The closed bottle's resistance to loss of water vapour showed that the most favourable period for a quantitative determination was 30 minutes after closure.

The use of a microsyringe proved to be a source of discrepancies, as the syringe invariably yielded a smaller amount of water than indicated by its scale. The constant magnitude of this discrepancy and its small variation at all volume levels indicated an erroneous graduation level. The variation in the water transfer was, however, in accordance with the available information on the precision of this type of syringe (*Hamilton*, 1967). The rate of evaporation from moist filter paper was another potential source of error on account of the time-consuming manipulation of the containers. The evaporation curve in fig. 4 revealed a loss, which in 10 seconds could remove 10—15 % of a water content of 100—700 micrograms in the filter paper. This discrepancy could be partly eliminated by a correction with values calculated on the basis of the length of the period, and the temperature and relative humidity of the air during evaporation. Determination of water quantities between 100 and 1,000 micrograms (Table IV) was affected by a variation of 13—17 micrograms including a value of 5—8 micrograms caused by use of the microsyringe. The variation from a determination

of fluid content obtained by direct sampling could thus be assumed to be about 10 micrograms.

REFERENCES

Brandtzaeg, P., 1966: Local factors of resistance in the gingival area. J. periodont. Res. *1:* 19—42.

Brandtzaeg, P. & W. V. Mann, 1964: A comparative study of the lysozyme activity of human gingival pocket fluid, serum, and saliva. Acta odont. scand. *22:* 441—455.

Brill, N., 1962: The gingival pocket fluid. Acta odont. scand. 20, suppl. 32.

Dole, V. P., B. G. Stall, & I. L. Schwarz, 1951: Methods for local induction and quantitative analysis of human sweat. Proc. Soc. exp. Biol. & Med. *77:* 412—415.

Egelberg, J., 1967: The topography and permeability of vessels at the dento-gingival junction in dogs. J. periodont. Res., suppl. 1.

Hamilton Co., 1967: Catalog H-67 on precision syringes and related equipment. Whittier, California, U.S.A.

Hara, K. & H. Löe, 1969: Carbohydrate components of the gingival exudate. J. periodont. Res. *4:* 202—207.

Kaslick, R. S., A. I. Chasens, D. Weinstein, & R. Waldman, 1968: Ultramicromethod for the collection of gingival fluid and quantitative analysis of its sodium content. J. Dent. Res. *47:* 1192.

Kaslick, R. S., A. I. Chasens, I. D. Mandel, D. Weinstein, R. Waldman, T. Pluhar, & R. Lazara, 1970: Quantitative analysis of sodium, potassium and calcium in gingival fluid from gingiva in varying degrees of inflammation. J. periodont. *41:* 25—33.

Matsue, I., 1967: Study of subgingival scaling. 3: Quantitative determination of sodium, potassium and calcium in periodontal pocket fluid. J. Jap. stomat. Soc. 34: 452—460. (in Japanese, cit. Kaslick et al. 1970).

Schifter-Holm, B., 1968: Introduktion til plastmaterialer. Copenhagen.

Sueda, T., J. Bang, & G. Ciamasoni, 1969: Collection of gingival fluid for quantitative analysis. J. Dent. Res. *48:* 159.

Thaysen, J. H., 1955: Sekretionsstudier. Copenhagen.

Weinstein, E. & I. D. Mandel, 1964: The fluid of the gingival sulcus. Periodontics *2:* 147—153.

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