Lowering of interstitial fluid pressure in rat submandibular gland: a novel mechanism in saliva secretion

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Berggreen, Ellen, and Helge Wiig. Lowering of interstitial fluid pressure in rat submandibular gland: a novel mechanism in saliva secretion. Am J Physiol Heart Circ Physiol 290: H1-10-H1468, 2006.—The submandibular gland transports fluid at a high rate through the interstitial space during salivation, but the exact level of all forces governing transcapillary fluid transport has not been established. In this study, our aim was to measure the relation between interstitial fluid volume (Vi) and interstitial fluid pressure (Pif) in salivary glands during active secretion and after systemically induced passive changes in gland hydration. We tested whether interstitial fluid could be isolated by tissue centrifugation to enable measurement of interstitial fluid colloid osmotic pressure. During control conditions, Vi averaged 0.23 ml/g wet wt (SD 0.014), with a corresponding mean Pif measured with micropipettes of 3.0 mmHg (SD 1.3). After induction of secretion by pilocarpine, Pif dropped by 3.8 mmHg (SD 1.3) whereas Vi was unchanged. During dehydration and overhydration of up to 20% increase of Vi above control, a linear relation was found between volume and pressure, resulting in a compliance (ΔV/ΔPp) of 0.012 ml/g wet wt−1·mmHg−1. Interstitial fluid was isolated, and interstitial fluid colloid osmotic pressure averaged 10.4 mmHg (SD 1.2), which is 64% of the corresponding level in plasma. We conclude that Pif drops during secretion and, thereby, increases the net transcapillary pressure gradient, a condition that favors fluid filtration and increases the amount of fluid available for secretion. The reduction in the relatively low interstitial compliance of the organ will enhance the effect of the myoepithelial cells on Pif during reduced Vi.

interstitial fluid volume; micropuncture; pilocarpine

IN SALIVARY GLANDS, blood, as well as salivary, flow is under autonomic nervous control, and transcapillary fluid transport and fluid flow through the interstitial space change dramatically after nervous stimuli before secretion of saliva from the epithelial cells. The high rate of fluid transport is reflected in the extremely high total capillary surface area in this organ, up to 500 cm²/g, which is among the highest in the body (6), and it has been estimated that the total fluid flux across the capillaries increases from a resting value of ~3 to 30 ml·min⁻¹·100 g⁻¹ during maximal parasympathetic stimulation (22). During salivation, gland volume, as well as interstitial fluid pressure (Pif), has been reported to decrease (15, 22). The decrease in tissue volume was suggested to reflect expulsion of preformed saliva from the salivary ducts caused by contraction of the myoepithelial cells and/or reduction of cellular and/or interstitial fluid volume (Vi) in the initial phase of secretion after onset of parasympathetic stimulation (15).

The 10-fold increase in fluid flux across the capillaries during activation of parasympathetic nerves to the salivary glands demands dramatic changes in the Starling forces that regulate fluid flow across the capillary walls.

Our aim was to obtain a better quantitative estimate of the factors involved in interstitial fluid and saliva transport, and this led us to study the Starling forces, with our focus on the interstitial side. Transcapillary fluid transport is described by the so-called Starling equation

\[ J_v = C_{FC}(P_c - P_d) - \sigma(COP_p - COP_if) - L \]

where \( J_v \) is net flow across the capillary, \( C_{FC} \) is capillary filtration coefficient, \( P_c \) is capillary hydrostatic pressure, \( P_d \) is interstitial fluid pressure, \( \sigma \) is osmotic reflection coefficient to plasma proteins, \( COP_p \) and \( COP_if \) represent colloid osmotic pressure (COP) in plasma and interstitium, respectively, and \( L \) is lymph flow. For the salivary gland, many of these parameters of the Starling equation have been determined (for review see Ref. 21); recently, we used micropipettes to measure \( P_{if} \) in the salivary gland. \( COP_{if} \) is, however, unknown in this organ, because the interstitial fluid is difficult to access. Recently, we showed in tumors, another tissue where interstitial fluid is not readily available, that interstitial fluid can be isolated by exposure of the tumor tissue to centrifugation at less than ~400 g (25). Exclusion of wicks and microdialysis as potential methods to gain access to salivary gland interstitium because of the more traumatic nature of these techniques led us to test whether a centrifugation method similar to that used in our recent experiment (25) could also be applied to isolation of salivary gland interstitial fluid and, thereby, enable us to measure \( COP_{if} \).

\( P_{if} \), another determinant of transcapillary fluid flux, is influenced by the interstitial compliance (C) of the organ, defined as the change in Vi divided by the corresponding change in Pif, i.e., \( C = \Delta V / \Delta P_{if} \). In a low-compliant organ, a change in vascular volume (Vi) or/and Vi will induce a change in Pif. In a previous study, we observed immediate changes in \( P_{if} \) after changes were induced in vascular perfusion in rat submandibular gland (4). The changes induced in \( V_i \) and the concomitant change in \( P_{if} \) were interpreted to be a consequence of a relatively low compliance in the submandibular gland. Because the compliance may be important for fluid secretion, our aim was to determine Vi and \( P_{if} \) in secreting and nonsecreting glands. In nonsecreting glands, we changed the forces acting across the capillary wall by altering the tissue hydration systemically, whereas we administered pilocarpine, which is known to act via muscarinic receptors in the organ, to induce...
secretion locally in the salivary gland. We were especially interested in the possible mechanistic role of $P_H$ in the initial phase of saliva secretion. Here we demonstrate that a reduction in $P_H$ contributes significantly to the net filtration pressure, leading to salivation, and that samples representative for interstitial fluid can be isolated by centrifugation of the salivary gland. We also propose a novel mechanistic role for the myoepithelial cells in saliva secretion.

**METHODS**

Female Wistar rats (190–240 g body wt, $n = 54$) were anesthetized with pentobarbital sodium at 50 mg/kg body wt ip (50 mg/ml; Mebumal, Svaneapoteket, Bergen, Norway) and maintained with 2–3 mg/kg iv. The animals were killed by an overdose of anesthetic at the end of the experiments. Body temperature was maintained at 37–38°C with a servo-controlled heating pad. All experiments were performed in accordance with recommendations of the Norwegian State Commission for Laboratory Animals and were approved by the local ethical committee.

**Fluid Distribution Volumes and $P_H$**

The rats were studied in a supine position. A femoral vein was catheterized for injection of supplemental anesthesia and a femoral artery for continuous systemic blood pressure [arterial pressure ($P_A$)] recordings with a Gould pressure transducer and recorder. The submandibular gland was isolated and placed in a cup for immobilization just before $P_H$ measurements. In the cup, the salivary gland was flushed with saline at 37°C to keep the surface moist. Fluid was drained from the bottom of the cup.

**Micropuncture Measurements of $P_H$**

Under stereomicroscopic guidance, $P_H$ was measured with sharpened glass micropipettes (2- to 6-µm-diameter tip) filled with Evans blue-dyed 0.5 M NaCl. The micropipette was connected to a servo-controlled pressure transducer. The submandibular gland was isolated and placed in a cup for immobilization just before $P_H$ measurements. In the cup, the salivary gland was flushed with saline at 37°C to keep the surface moist. Fluid was drained from the bottom of the cup.

**Fluid Volume Measurements**

To eliminate interindividual differences, volume was measured in the same animal in the control situation in one submandibular gland and after changes in hydration or after pilocarpine infusion (see below) in the contralateral gland. The selection of right or left submandibular gland for control or experimental condition was alternated. After anesthesia and placement of catheters, both kidney pedicles were ligated via flank incisions, and $^{51}$Cr-EDTA (60–70 µCi) was injected intravenously for measurement of $V_r$. After 90 min of tracer equilibration, control $P_V$ was measured. In rats where hydration changes were induced, a ~100-µl blood sample was withdrawn from the femoral artery into a heparinized syringe; then the vessels to the submandibular gland were ligated, and the control gland was removed.

After an equilibration period following the induced change in hydration or 2–3 min before pilocarpine infusion (see Experimental Protocol), 3–4 µCi of $^{125}$I-human serum albumin (HSA) were injected and allowed to circulate for 5 min. A final 0.5- to 0.7-ml blood sample was obtained from the arterial catheter, and the rat was killed with an overdose of intravenously administered anesthetic. The remaining submandibular gland was removed. Tissue samples were placed in tared, covered vials, weighed, and then counted in a gamma counter (model 1282 Compu gamma, LKB) with window settings of 15–75 keV for $^{125}$I and 290–350 keV for $^{51}$Cr. Counts were corrected for background and spillover.

**Measurement of $V_r$.** Our experimental protocol using each animal as its own control required special attention regarding measurements of plasma volume. Labeled $^{125}$I-HSA will gradually leak from the vascular compartment, and tracer injected at the end of the control period cannot be used to measure $V_r$ at the end of the experimental period. Because $V_r$ is small relative to $V_V$, and may be assumed to be fairly stable under control conditions, we measured $V_V$ (and $V_r$) in a separate series of experiments and used these volumes to estimate $V_r$ in the control situation in the groups of animals subjected to over- or dehydration. Thus we used no intravascular tracer for the control situation in the latter animals and included 10 rats as controls for fluid distribution volume measurements in the submandibular gland (basal group). The mean $V_V$ as percentage of total extracellular fluid volume ($V_V$) in the submandibular gland estimated in the basal group of rats was used for calculation of $V_r$ in the control situation before over- or dehydration.

**Possible Transport of Isotopes to Saliva**

In three animals, a polyethylene catheter was inserted into the submandibular gland duct and ligated before infusion of $^{51}$Cr-EDTA to test whether the tracer was transported across the epithelial layer in the gland and into saliva. The tubes were removed after the animals were killed and placed in vials for counting.

**Experimental Protocol**

**Systemic dehydration (group 1).** Dehydration was induced by peritoneal dialysis using a hypertonic glucose solution in seven rats. After measurements of $P_r$ and sampling of tissue in the control situation, 10–15 ml of 20% glucose in Ringer acetate (Fresenius-Kabi, Oslo, Norway; pH 6.0) that had been preheated to 37°C were instilled via a catheter introduced into the peritoneal cavity from the lumbar region. After an equilibration period of 45 min, the dialyzing fluid was withdrawn and the dialysis was repeated. This procedure was used to remove a net volume of 5–15 ml from each rat. During dialysis, repeated intravenous infusions of 2 ml of preheated 10% HSA in Ringer acetate solution (~6 ml) were given in an attempt to prevent hypotension and induce additional interstitial dehydration. Measurements of $P_H$ were started in the remaining experimental submandibular gland 1 h after withdrawal of the last sample of dialyzing fluid and were finished within the following hour. Then $^{125}$I-HSA was injected, and blood and tissue were sampled for volume determination as described above.

**Systemic overhydration (group 2).** Previous studies of the volume-pressure relation in skin and muscle of rats suggested a linear relation between $V_r$ and $P_H$ at low degrees of overhydration followed by no increase in $P_H$ as the overhydration increases (20, 28). A gradual overhydration was therefore induced by volume loading. After measurements of $P_H$ and sampling of tissue in the control condition, we infused 20, 25, and 35 ml of Ringer acetate solution that had been preheated to 37°C through the femoral vein catheter. The infusions lasted 15, 30, and 60 min, respectively. Measurement of $P_H$ was initiated 90 min after the end of infusion and was completed within 1 h. After pressure measurements, $^{125}$I-HSA was injected for plasma
volume determination, and plasma and tissue were sampled for volume measurements as described above.

Active secretion (group 3). Pilocarpine (1 mg/kg iv dissolved in 0.1 ml saline) was administered to 11 rats as bolus infusions immediately after injection of $^{125}$I-HSA. $P_d$ was measured continuously in the experimental submandibular gland before, during, and after pilocarpine administration. We chose this agent to induce salivation because of its ability to induce maximal secretion even without activation of all the available receptors (3). In three of the rats, saliva produced during stimulation was collected from the oral cavity with micropipettes, placed in vials, and assayed for radioactivity. Submandibular glands collected for fluid volume measurements were dried after gamma counting to determine wet-to-dry weight ratio.

In four of the rats in this group and in one rat not given isotopes, local changes in red blood cell flux were measured (as an index of blood flow in the submandibular gland) during the experimental procedure with a laser-Doppler flowmeter (Periflux model 4001 Master, Perimed, Järfalla, Sweden) equipped with a needle probe (model PF 415:10; 125-μm fiber diameter with 500-μm separation). The laser probe was positioned with a micromanipulator above the area for $P_d$ recordings and rotated to the position at which the largest resting blood flow signal [in arbitrary perfusion units (PU)] was measured. Calibration was performed using a motility standard giving a signal output of 250 PU. Zero blood flow was determined as the value recorded with the probe positioned at the submandibular gland after heart arrest. The flowmeter’s time constant was set at 0.03 s, and upper and lower bandwidths were 20 kHz and 20 Hz, respectively.

Isolation of Interstitial Fluid and Measurements of COP

Centrifugation technique. In this subset of experiments ($n = 12$ rats), our aim was to isolate interstitial fluid from the salivary gland to enable us to estimate $COP_p$ using an approach similar to that described previously for tumors and skin (25). After anesthesia, blood was sampled by cardiac puncture and the rats were killed by an intravenous injection of saturated KCl. Immediately after euthanasia, was sampled by cardiac puncture and the rats were killed by an described previously for tumors and skin (25). After anesthesia, blood was injected and allowed to circulate for 5 min before blood was sampled for isolation of plasma, and the rat was killed. The submandibular glands were removed, transferred to centrifuge tubes, and handled as described above. Fluid isolated from the submandibular gland in these experiments was collected in microcapillaries for exact volume measurements and transferred to vials for gamma counting. Initially, using a procedure described previously, we explored centrifugation rates of 68–955 g (800–3,000 rpm) for 10 min. At $\geq 663$ g (2,500 rpm), massive hemolysis and blurring of the centrifuge occurred; these samples were discarded. At $\leq 663$ g, the isolated fluid was, with a few exceptions, clear; however, in all experiments using a centrifugation time of 10 min, we found an increase in the concentration of interstitial fluid, as evidenced by a $^{51}$Cr-EDTA tissue fluid-to-plasma concentration ratio significantly $>1.0$, suggesting that the tissue fluid became concentrated during the isolation procedure (see below). We then turned to an alternative centrifugation protocol, in which the tissue was subjected to 424 g for 5 min. Because we suspected that swelling of the metabolically active salivary gland cells could increase the concentration of substances dissolved in the extracellular fluid, we avoided all possible delay in the isolation procedure before the centrifugation. Thus $<10$ min elapsed from the time the animal was killed until fluid was isolated.

Fluid that accumulated at the bottom of the tubes after centrifugation was collected in graduated glass micropipette tubes for volume measurement in a humidity chamber. The fluid was removed from the micropipette tubes for COP measurements or diluted in buffer for HPLC. Samples that were visually contaminated with blood (<5% of total) were discarded.

To determine whether the connective tissue sheath (“capsule”) surrounding the submandibular gland represented a barrier to plasma protein, leading to sieving of interstitial fluid and an underestimation of $COP_i$, we added a series of experiments ($n = 3$) in which fluid was isolated after “decapsulation” of the submandibular gland. After anesthesia, one gland was isolated with the capsule intact as described above, whereas in the contralateral gland the thin connective tissue sheath surrounding the gland was removed by careful blunt dissection. Both glands from one animal were centrifuged simultaneously, and the fluid was isolated and handled as described above.

HPLC of Interstitial Fluid

The distribution of macromolecules in fluid isolated by centrifugation from the submandibular gland and in plasma was determined by HPLC using a Superose 12 HR 10/30 exclusion column (Pharmacia-Biotech, Uppsala, Sweden) with an optimal separation range of 10–300 kDa, as described in detail elsewhere (25). The pherograms were compared with relevant plasma protein standards.

COP

$COP_p$ and $COP_p$ were measured in a colloid osmometer designed for submicroliter samples (26) using membranes with a 30-kDa cutoff.

Calculations

Distribution volumes. Distribution volumes were calculated as the plasma equivalent distribution volumes of the tracers [counts given as corrected counts per minute (cpm)] with the assumption that $^{51}$Cr-EDTA will distribute in the extracellular fluid phase and labeled HSA will distribute only in plasma. $V_i$ in a tissue sample was calculated as the 5-min $^{125}$I-HSA distribution volume

$$V_i (ml/g) = \frac{cpm \ 125I-HSA/g \ tissue}{cpm \ 125I-HSA/ml \ terminal \ plasma}$$

(2)

Because $^{125}$I-HSA has been in the animal for only 5 min, extravasation can be assumed to be negligible.

Tissue $V_i$ was calculated as the distribution volume of $^{51}$Cr-EDTA before and after experimental intervention

$$V_i (ml/g) = \frac{cpm \ 51Cr-EDTA/g \ tissue}{cpm \ 51Cr-EDTA/ml \ terminal \ plasma}$$

(3)

$V_i$ was the difference between $V_i$ and plasma volume ($V_p$)

$$V_i (ml/g) = V_i - V_p$$

(4)

All distribution volumes are expressed in terms of wet tissue weight. Interstitial compliance. Interstitial compliance was calculated from the relation between $V_i$ and $P_d$ during changes in hydration. In regular regression analysis, it is assumed that there are errors only in the independent variable; therefore, the coefficient of regression will depend on which parameter is chosen as the independent variable. Because we cannot know which parameter is independent, the regression coefficients are given as the geometric mean obtained when $V_i$ is used as the independent, as well the dependent, parameter in the regression analysis, as described by Brace (5).
RESULTS

Basal Group

The volumes and pressures obtained in the basal group were used for determination of Vv, under control conditions, i.e., no stimulation or secretion or change in hydration. Vv averaged 0.26 ml/g wet wt (SD 0.05) with a corresponding Vx of 0.03 ml/g wet wt (SD 0.02), resulting in an average Vi of 0.23 ml/g wet wt (SD 0.01). The mean Pif in this group was 3.0 mmHg (SD 1.3). Neither 51Cr-EDTA nor 125I-HSA was detected in saliva sampled in canulas in the submandibular gland ducts, showing that no tracer crossed the epithelial layers in the glands.

Volume and Pressure During Systemic Changes in Hydration

Peritoneal dialysis (group 1), which was performed to reduce Vx, reduced mean Vx and Vv to 70% of control values (Table 1). As expected, we observed a significant drop in mean systemic blood pressure averaging 20 mmHg (P < 0.05; Table 1) and a variable increase in heart rate (data not shown), despite infusions of hyperoncotic HSA. The amount of fluid that was removed by dialysis showed large variations between the rats: 8–20.5 ml (median 15 ml). The net volume removed that was removed by dialysis showed large variations between despite infusions of hyperoncotic HSA. The amount of fluid 1) and a variable increase in heart rate (data not shown), systemic blood pressure averaging 20 mmHg (Fig. 1A). The maximal increase in Pif was 4.5 mmHg after an increase in Vi of 0.11 ml/g. There was a linear relation between volume and pressure during overhydration for the first 20% increase of Vi above control as well as for dehydration (Fig. 1B).

Measurements within the volume-pressure area of the curve (Fig. 1B) with a linear relation were used for calculation of interstitial compliance (see METHODS). The change in Vi in this area was 5.59% per mmHg change in Pif, and the compliance was 0.012 ml·g wet wt⁻¹·mmHg⁻¹.

Volume and Pressure During Active Saliva Secretion

Pilocarpine bolus infusions caused immediate responses in systemic pressures. Thus the mean systemic pressure dropped from 99 mmHg (SD 21) in the control condition to 51 mmHg (SD 30) shortly after infusion before it stabilized at an elevated level [121 mmHg (SD 39)] within 40 s after infusions (Fig. 2). The increase in pressure was interpreted as a baroreceptor effect caused by the initial drop in systemic pressure. In the subgroup of rats where blood flow was measured (n = 4), pilocarpine resulted in an increase from 132 PU (SD 25) to a maximal level of 284 PU (SD 25), corresponding to a 97% (SD 2) increase in flow. The blood flow increase was immediate and remained elevated until the submandibular gland was removed for fluid volume measurements. In one rat, the glands were not removed, and measurements were monitored until blood flow and Pif returned to control levels after 15 min.

Pilocarpine infusion also caused immediate changes in Pif. In seven rats, there was a temporary increase in Pif from basal levels of 3.2 mmHg (SD 1.7) to 5.0 mmHg (SD 3.9). In two other rats, no increase was observed. In another two rats, we were not able to measure Pif continuously after pilocarpine infusion. In all rats, Pif started to drop 1–3 min after the start of

Table 1. Hemodynamic parameters and body fluid volumes during control and experimental conditions

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<thead>
<tr>
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<th>Control</th>
<th>Exptl</th>
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<tbody>
<tr>
<td>P_A, mmHg</td>
<td>82.1 (9.9)</td>
<td>62.1 (10.7)†</td>
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<tr>
<td>P_D, mmHg</td>
<td>3.7 (0.6)</td>
<td>-0.4 (0.5)</td>
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<tr>
<td>V_x, ml/g</td>
<td>0.23 (0.06)</td>
<td>0.17 (0.02)†</td>
</tr>
<tr>
<td>V_v, ml/g</td>
<td>0.034 (0.008)</td>
<td>0.024 (0.006)*</td>
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+ 20 ml (n=2)

<table>
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<th></th>
<th>Control</th>
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<tr>
<td>P_A, mmHg</td>
<td>95 (14.1)</td>
<td>87.5 (17.7)</td>
</tr>
<tr>
<td>P_D, mmHg</td>
<td>1.5 (0.7)</td>
<td>4.5 (0.7)</td>
</tr>
<tr>
<td>V_x, ml/g</td>
<td>0.20 (0.003)</td>
<td>0.27 (0.0)</td>
</tr>
<tr>
<td>V_v, ml/g</td>
<td>0.03 (0.0007)</td>
<td>0.026 (0.001)</td>
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+ 25 ml (n=3)

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<tr>
<td>P_A, mmHg</td>
<td>85 (5)</td>
<td>95 (35)</td>
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<tr>
<td>P_D, mmHg</td>
<td>3 (0)</td>
<td>5.5 (0.7)</td>
</tr>
<tr>
<td>V_x, ml/g</td>
<td>0.22 (0.004)</td>
<td>0.29 (0.08)</td>
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<tr>
<td>V_v, ml/g</td>
<td>0.03 (0.002)</td>
<td>0.04 (0.03)</td>
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+ 35 ml (n=2)

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<th>Exptl</th>
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<tr>
<td>P_A, mmHg</td>
<td>100 (0)</td>
<td>90 (5)</td>
</tr>
<tr>
<td>P_D, mmHg</td>
<td>1.7 (0.3)</td>
<td>5.3 (0.6)</td>
</tr>
<tr>
<td>V_x, ml/g</td>
<td>0.22 (0.03)</td>
<td>0.35 (0.09)</td>
</tr>
<tr>
<td>V_v, ml/g</td>
<td>0.032 (0.004)</td>
<td>0.03 (0.001)</td>
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Values are means (SD), n, number of rats. P_A, systemic blood pressure; P_D, interstitial fluid pressure; V_x, extracellular fluid volume; V_v, vascular fluid volume. *P < 0.05; †P < 0.005 vs. control.
infusions (Fig. 2). The maximal decline of $P_{if}$ was observed 3–5 min after the start of infusion and averaged $3.8 \text{ mmHg}$ (SD 1.5) (Fig. 3B). When $P_{if}$ had stabilized at a lower level, usually at 4–6 min, the experimental submandibular gland was removed for fluid volume measurements.

Despite marked changes in $P_{if}$, surprisingly, no significant changes in $V_{i}$ were observed when volumes in submandibular glands in the control condition before pilocarpine infusion were compared with volumes in glands subjected to experimental conditions ($P = 0.261$; Fig. 3A). Furthermore, these volumes did not differ from corresponding volume measurements in the basal group ($P = 0.44$; see above), suggesting that the submandibular gland did not swell while it was in the cup flushed with saline. Although the $V_{i}$ tended to be lower after stimulation, this reduction was not statistically significant ($P = 0.08$; Fig. 3A). In saliva collected from the oral cavity with micropipettes after pilocarpine injections, $^{51}$Cr-EDTA and $^{125}$I-HSA levels were not different from background, suggesting that neither of these substances crossed the epithelial layer in the submandibular glands during salivation.

The wet-to-dry weight ratio was 4.23 (SD 0.17) for the experimental submandibular glands ($n = 5$) and 4.02 (SD 0.16) for the control glands ($P = 0.11$). These data, along with the data for $V_{i}$ (see above), show that salivation did not result in changes in interstitial or cellular hydration at the time of gland collection after pilocarpine infusion. To ensure that saline flushing during $P_{if}$ measurements did not influence the distribution volume in the experimental submandibular gland, we compared the $V_{x}$ values from the contralateral control side (without $P_{if}$ measurements) in this group of animals with those in the basal group (with $P_{if}$ measurements) and found that they were not significantly different ($P = 0.44$).

**Isolation of Interstitial Fluid and COP Measurements**

As described in methods, we measured the recovered centrifugate tracer ($^{51}$Cr-EDTA) that had equilibrated in extracellular fluid after exposure of the tissue to $424 \text{ g}$ for 5 min. At $424 \text{ g}$ for 5 min, the $^{51}$Cr-EDTA fluid-to-plasma concentration ratio was 0.99 (SD 0.07, $n = 6$), which is not significantly different from background. The interstitial fluid volume and pressure were measured by centrifugation after exposure of the tissue to $424 \text{ g}$ for 5 min. The $^{51}$Cr-EDTA fluid-to-plasma concentration ratio was 0.99 (SD 0.07, $n = 6$), which is not significantly different from background.

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different from 1.0, indicating no concentration or dilution of the interstitial fluid. The corresponding ratio for $^{125}$I-HSA was 0.07 (SD 0.04). No other combinations of centrifugation speed and time resulted in a ratio for $^{51}$Cr-EDTA closer to 1.0; therefore, this combination of speed and time was chosen for isolation of interstitial fluid. The isolated fluid was clear and straw colored, and the volume was usually ~2 μl.

COP$_{if}$ averaged 10.4 mmHg (SD 1.2), and COP$_{p}$ was 16.7 mmHg (SD 2.0, n = 6). Thus COP$_{if}$ corresponded to 64% of COP$_{p}$.

The connective tissue sheath surrounding the submandibular gland did not result in any sieving of plasma proteins on centrifugation, as evidenced by an average COP in the centrifugate isolated from decapsulated glands of 11.0 mmHg (SD 0.6, n = 3), which is not different from the corresponding pressure of 11.2 mmHg (SD 0.6, n = 3) in fluid isolated from glands with intact capsule. COP$_{p}$ in these rats averaged 20.0 mmHg (SD 0.6, n = 3).

**HPLC**

An elution pattern from HPLC of interstitial fluid from a submandibular gland is shown in Fig. 4B; the elution pattern of rat plasma is shown for comparison in Fig. 4A. The pattern for interstitial fluid closely resembles that of plasma. There was, however, a lower fraction of high-molecular-weight substances in interstitial fluid, suggesting a higher albumin-to-globulin ratio. There was also a small fraction of low-molecular-weight substances in interstitial fluid that was not found in plasma. The HPLC also showed that the interstitial fluid was not contaminated with saliva, which would be expected to give a large peak in the fractions eluting before albumin, represented by amylase.

**DISCUSSION**

As pointed out by Smaje (21), little attention has been given to the important process of fluid and solute transport from the blood to saliva during salivation. Considering the secretion from the vascular and interstitial compartments, we have presented novel information that provides mechanistic insight into this process. The main novel finding of our study is that the interstitium plays an active role in the induction of active secretion, as evidenced by the reduction in P$_{if}$ after activation of muscarinic receptors, despite no change in submandibular gland fluid volumes. Furthermore, we have shown that interstitial fluid can be isolated from the salivary gland interstitium and, thus, provides a new tool for studies of salivary gland interstitial fluid balance. Finally, we were able to verify our...
pressure will likely equal $P_i$ if, because hydrostatic pressure
measurements showing similar $COP_i$ from intact and decapsulated
dibular gland, but such sieving was ruled out by the experi-
ments. Another potential problem would be sieving of plasma proteins
increase in concentration or dilution of the isolated fluid.
which is not significantly different from 1.0, suggesting no
swelling had occurred. Assuming that such swelling occurred
pressure recorded by micropipettes will give the best esti-
mate another previously unknown parameter in the Starling
equation for this organ. On the basis of previous experience
we found a positive $P_i$ in the salivary gland, in agreement with
the suggestion of a previous study that the salivary gland was
a low-compliant organ and that this phenomenon could influ-
ence transcapillary fluid transport (4). In the control situation,
we found a positive $P_i$ in the submandibular gland, which
has been validated in a number of studies (for references see
Ref. 24). As pointed out previously (4), we cannot rule out the
possibility that the micropipette tip could be placed intracellu-
larly in some recordings. Nevertheless, if some of our record-
ings should reflect intracellular pressure, rather than $P_i$, the
pressure will likely equal $P_i$, because hydrostatic pressure
gradients are unlikely across the compliant plasma membrane
of cells (18). The absence of a gradient between interstitial fluid and parenchymal cells is of particular importance during
peritoneal dialysis, which will result in cellular dehydration of
most organs, including the salivary gland, because of the high
capillary filtration is important when we consider fluid dynam-
ics in the salivary gland, in that it enhances the effect of the
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interstitial fluid and parenchymal cells is of particular importance during
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filtration induced by active secretion. Nevertheless, the development of a
glands. The pherograms showed a higher albumin-to-globulin
ratio for salivary gland interstitial fluid than for plasma, which
could have been the result of sieving of proteins during passage
of fluid through the gland tissue. This observation is, however,
most likely a consequence of the known sieving occurring at the
capillary wall, because a similar increased albumin-to-
ceeduced gradient has been found also in fluid isolated using wick
techniques in skin and muscle interstitial fluid (25, 30). Taken
together, these observations suggest that we have presented a
method to isolate salivary gland interstitial fluid and, thereby,
determine $COP_i$ in this organ. Another implication of our
observations is that the concentration of signaling substances,
e.g., inflammatory mediators, can be measured locally in the
fluid bathing the glandular cells.

$\nu_i$-$P_i$ Curve in Passive Alteration of Salivary
Gland Hydration

The $\nu_i$-$P_i$ relation in large tissues, such as skin and muscle,
is important for whole body fluid distribution, but the same
as cannot be said for this relation in the submandibular gland. Our
reason for determining interstitial compliance in this organ was
the suggestion of a previous study that the salivary gland was
a low-compliant organ and that this phenomenon could influ-
ence transcapillary fluid transport (4). In the control situation,
we found a positive $P_i$ in the salivary gland, in agreement with
previous data from the salivary gland (4) as well as other oral
tissues (11, 13, 14). Finding an average compliance 36% of
that found during corresponding changes in hydration in rat
skin and muscle (20, 28), we were able to verify our original
hypothesis. This means that changes in hydration induced from
the vascular side will be more strongly counteracted in the
salivary gland than in the two latter organs. This effect is,
however, even more pronounced in other organs with a stron-
ger capsule, such as brain (29), rat tail (2), bone marrow (12),
and dental pulp (10). Nevertheless, the development of a
substantial hydrostatic counterpressure during increased trans-
capillary filtration is important when we consider fluid dynam-
ics in the salivary gland, in that it enhances the effect of the
changes in transcapillary filtration induced by active secretion.

$\nu_i$-$P_i$ Relation During Active Secretion

A novel and surprising finding was that activation of mus-
caric receptors inducing salivation led to a significant drop in
$P_i$ without a concomitant drop in $\nu_i$ or total tissue water
content. During systemic changes in hydration, the change in
pressure was a consequence of an altered hydration, requiring
an alternative explanation for the reduction in $P_i$ during sali-
vation. In this context, it is of interest to consider observations
by Lung (17), who reported that subcapsular pressures in
canine submandibular glands dropped during stimulation of
parasympathetic, as well as sympathetic, nerves to the glands.
These observations were interpreted as a receptor-mediated
activation of myoepithelial cells, causing a contraction of the
acinar cells and intercalated ducts that moved the tissue away
from the capsule. The subcapsular pressure dropped, despite
increased submandibular gland blood flow, after stimulation of
parasympathetic nerves. When ductal occlusion was induced
during stimulation, the decline in subcapsular pressure was
enhanced, indicating that the myoepithelial cells can contract
more when they are distended and, even more important,
That the pressure dropped even when no fluid was removed from the organ.

It is possible that the total water content in the experimental salivary glands in our study was reduced immediately after onset of secretion and gradually returned to baseline, as reported in cat submandibular gland (16). However, in salivary glands collected for fluid volume measurements, when \( P_{df} \) had stabilized at a lower level (after 4–6 min), there was no change in total fluid content of the submandibular glands.

Lung (17) measured pressure by introduction of a miniature transducer in the subcapsular space until it abutted the parenchyma and, thereby, separated the capsule from the submandibular gland parenchyma, whereas our pressure measurements were done in the interstitial fluid. Despite differences in methodology and the possibility that the pressure recorded by Lung may partially reflect solid tissue pressure (9), subcapsular pressure recorded by Lung during rest (3.0 mmHg) is close to our \( P_{df} \) measured during similar conditions (3.3 mmHg). The pressure drop recorded by Lung when acetylcholine was given systemically (about -4.2 mmHg) is similar in magnitude to that observed by us after pilocarpine infusions (about -3.8 mmHg), suggesting that there is no pressure gradient between the subcapsular space and interstitial fluid during salivation, and it is reasonable to assume that our measurements are comparable. We have, however, documented for the first time that the pressure drop takes place also in the interstitial space surrounding the secreting acinar cells and without changes in the \( V_i \) and that this effect is important for the formation of saliva.

The observed change in \( P_{df} \) during pilocarpine stimulation is the sum of temporary changes in \( V_i \) and \( V_d \) and possible conformational changes. We propose that the myoepithelial cells have an important role in the lowering of \( P_{df} \) and, thereby, in increasing the filtration pressure gradient leading to saliva formation. When the myoepithelial cells contract as a result of receptor activation, the interstitial tissue that surrounds the acinar cells and intercalated ducts undergoes a change in conformation that may lead to a reduction of hydrostatic tissue pressure. This may be considered to parallel changes in skin, where the integrins play an active role in control of \( P_{df} \). After the \( \beta_1 \)-integrin is blocked, the transcapillary fluid transport is increased by a reduction in \( P_{df} \) followed by edema formation (19). The lowering of pressure (\( P_{df} \)) causes a dramatic increase in the net filtration pressure. The myoepithelial cells may thus have an active and important role in reducing \( P_{df} \) and increasing transcapillary filtration. Also, the myoepithelial cells have an important role in reducing the luminal capacity and increasing the secretory pressure in the ducts and, thereby, accelerating the outflow of saliva (8). Even though we found no increase in \( V_i \) during secretion, the fluid flow through the epithelium may exceed the transport into the interstitium from the capillaries during brief periods of secretion, resulting in reduction in \( V_i \). If this is the case, the reduction of \( P_{df} \) will be enhanced, because we can assume that the contraction of myoepithelial cells, as well as the volume reduction, will lead to a lowering of \( P_{df} \). The latter is a result of a relatively low compliance in the organ as measured in this study. Our explanation is only partly consistent with that offered Smaje (21), who proposed that such a pressure reduction could be a consequence of a reduced \( V_i \).

We may estimate the quantitative importance of a reduction in net transcapillary fluid pressure at about \(-4 \text{ mmHg} \) with respect to transcapillary fluid flow. \( \text{COP}_{if} \) and \( \text{COP}_{p} \) were 10.3 and 16 mmHg, respectively. Because we measured no changes in \( V_i \) during lowered \( P_{df} \) and \( \sigma \) has been shown to remain relatively unchanged during secretion (0.79) (22), we can assume that there was no change in \( \text{COP}_{if} \) when we measured the lowered \( P_{df} \) and collected the salivary glands for fluid volume distribution. Using these assumptions and the Starling equation, we can calculate the transcapillary fluid flow during secretion when applying all known parameters and, furthermore, estimate the contribution of the drop in \( P_{df} \). The composite CFC, determined in the isolated perfused rabbit submandibular gland, is 1.35 ml·min⁻¹·100 g⁻¹·mmHg⁻¹ and does not change in the presence of acetylcholine (7). The \( P_{df} \) in the submandibular gland has been estimated to 31 mmHg during secretion (7). If we calculate the transcapillary fluid flow during secretion with and without a drop in \( P_{df} \), the respective values are 36.9 and 31.8 ml·min⁻¹·100 g⁻¹. Accordingly, the drop in \( P_{df} \) in this situation will increase the transcapillary fluid flow \(-16\% \) without a reduction in \( V_i \). To achieve a drop in \( P_{df} \) of the magnitude we measured after pilocarpine infusion without involvement of the myoepithelial cells, a reduction in \( V_i \) of \(-25\% \) would have been needed, as calculated from our value for interstitial compliance, showing the significant importance of the myoepithelial cell effect for filtration.

In conclusion, we have shown that the salivary gland has a low interstitial compliance that may influence the secretion process. During active secretion, \( P_{df} \) drops, increasing the net filtration pressure and, thereby, contributing to the salivation. This drop in \( P_{df} \) is most likely mediated via the myoepithelial cells, suggesting an active and new role for these cells in salivary secretion.

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