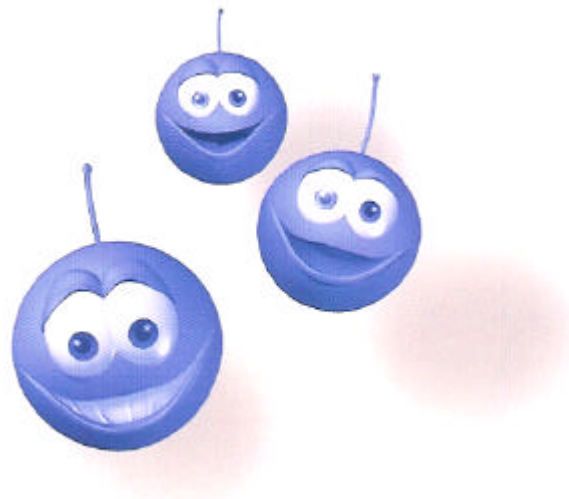


Saliva Diagnostics



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Introduction

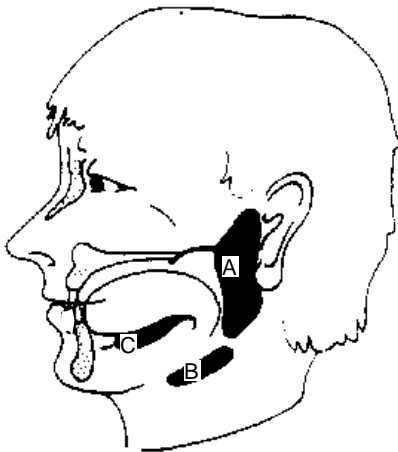
Saliva is used as biological fluid for the detection of different biomarkers such as electrolytes, hormones, drugs and antibodies in human and veterinary medicine. The sample collection process is non-invasive, painless and convenient. Collecting saliva samples is possible several times a day and can be conveniently accomplished under circumstances where it is difficult to get blood samples.

Therefore, saliva is an appropriate specimen in psychology, sports medicine, pharmacology, pediatrics and anti-aging medicine among many other scientific fields. With the possibility to evaluate a mixed saliva sample from multiple collections you may get an actual value of hormones with distinct diurnal fluctuations.

In saliva only the unbound (biologically active) hormones and drugs are measured.

However, because of the small concentrations of unbound hormones and drugs present in saliva, special assays have to be used in order to yield accurate quantitation of analytes.

Morphology of the Salivary Glands



Saliva is produced by three pairs of greater salivary glands besides smaller glands spread over the whole oral cavity. The greater glands are named parotid glands lying cranial of the ears, sublingual glands under the tongue and submandibular glands at the inner part of the lower jaw.

Fig. 1: Site of the salivary glands in humans:
A = Parotid Gland; B = Submandibular Gland; C = Sublingual Gland (Van Dam and Van Loenen, 1978)

The tissue of the salivary glands consists of a system of blind ducts surrounded by webs of capillary vessels and embedded in connective tissue. In the extremity of these ducts the primary saliva is produced by filtering the blood of the capillaries through the membranes of the acinar cells. These membranes prevent certain blood components from passing, and so they have a selective function.

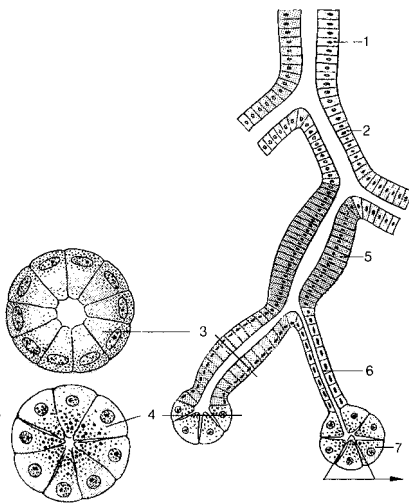


Fig. 2: Schematic micromorphology of the ductal system of the salivary glands:
 1 – 6 = parts of the ductal system
 3 = mucous producing tubules
 7, 8 = endpiece with acinar cells
 (H. Leonhardt, 1980)

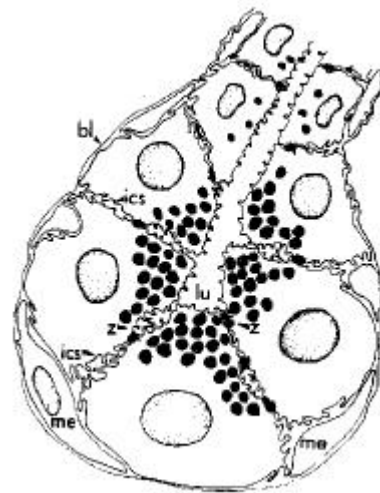


Fig. 3: Micromorphology of an endpiece
 bl = blood capillary
 ics = intercellular space
 z = tight junctions
 me = myoepithelial cells
 (Young and Van Lennep, 1978)

The saliva of humans produced by the various glands differs in the content of enzymes and the viscosity depending on the production of mucous. However, this is not important for the release of small non-polar molecules like that of steroid hormones.

In stained sections of the human mandibular glands can be seen many clusters of mucous producing ductal cells, whereas in the section of parotid glands the endpieces are most prominent.

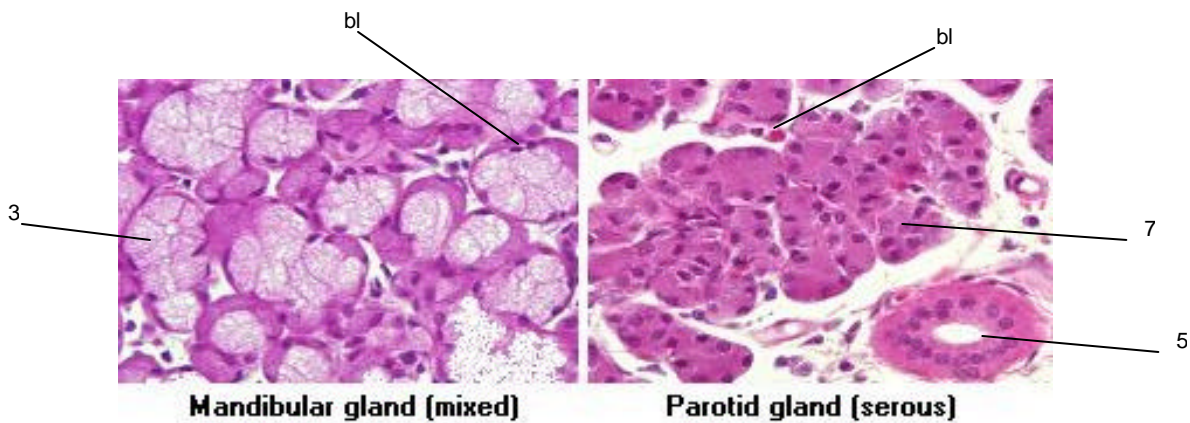


Fig. 4: Hematoxylin stained cross-sections of salivary glands: bl = blood capillary; 3 = mucous producing tubulus; 5 = larger ductus; 7 = endpiece

Physiology of the Salivary Glands

The cell membrane of the acinar cells in the distal end of the salivary glands consists, as in other cells, of a duplicate layer of lipids with a hydrophil end (Phosphorylcholine) at the outer side and a lipophil end (fatty acids) at the inner part. Across this membrane only small lipophil substances can pass freely. Integrated proteins may form channels through this duplicate layer and therefore make it possible for some smaller hydrophil molecules to pass through. Peripheric proteins may serve as receptor molecules for substances like hormones.

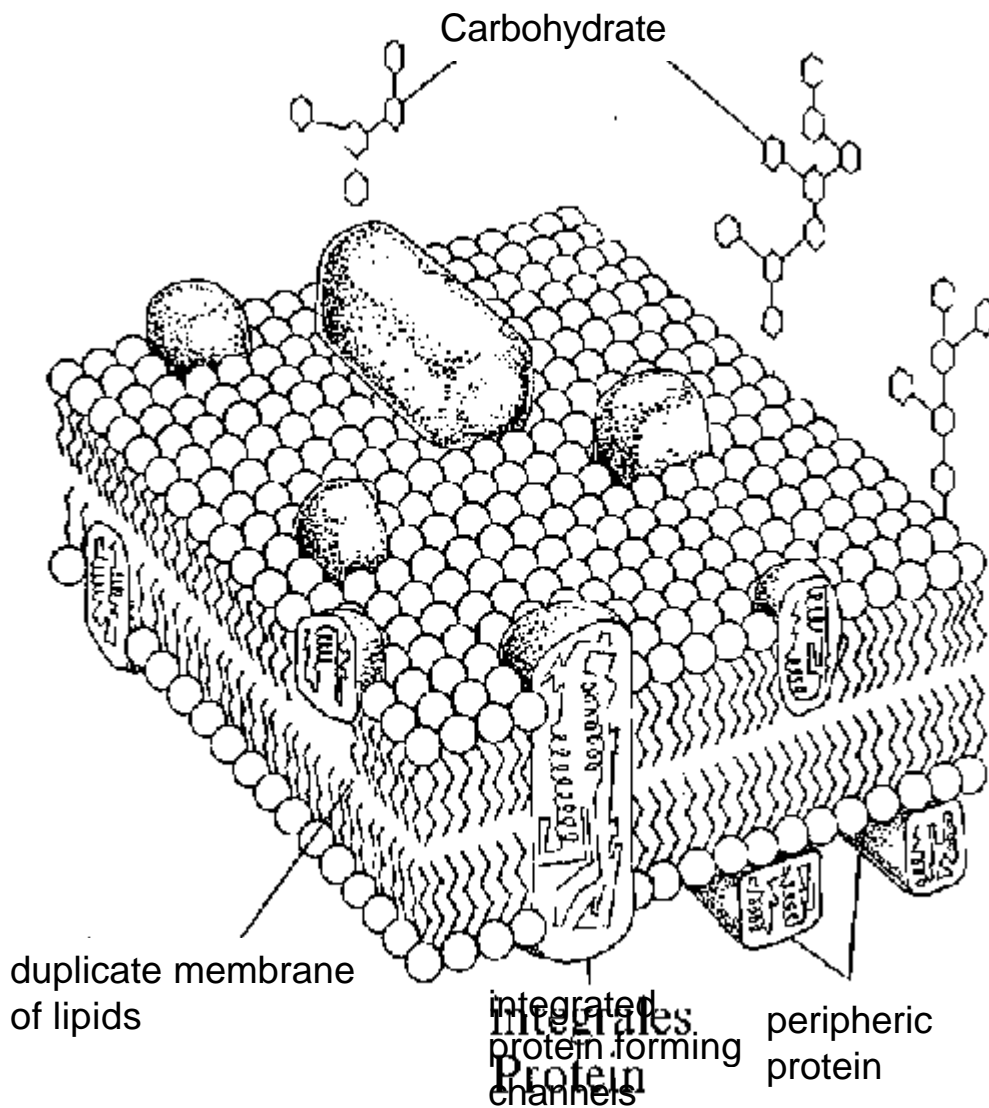


Fig. 5: Structure of the duplicate layer of a cell membrane

More than one mechanism exists for blood components to pass the membrane barrier into the salivary ducts.

1. Passing through the space between the acinar cells. Because of barriers in the intercellular space, called tight junctions, only molecules with a relative small molecular weight ($Mr < 1900$) may pass through ($Mr \text{ H}_2\text{O} = 18$; $Mr \text{ Na} = 23$; $Mr \text{ steroids ca. } 300$; $Mr \text{ albumin} = 66,000$; $Mr \text{ CBG} = 49,500$; $Mr \text{ SHBG} = 115,000$; $Mr \text{ AST} = 93,000$).
2. Filtration through pores of the cell membranes. This transfer is only possible for substances of a $Mr < 400$ (e.g. water, electrolytes)
3. Selected transport across the cell membrane
 - a. Passive diffusion of lipophilic molecules (e.g. steroids)
 - b. Active transport through protein channels (e.g. peptides)
 - c. Pinocytosis: passing into the cell by taking along a part of the cell membrane and forming a vacuole in the cell; on the other side of the cell the vacuole membrane is reintegrated into the cell membrane and the contents of the vacuole are released in the duct of the glands (e.g. larger proteins such as enzymes)
4. Sodium ions are actively pumped into the acinar cells and therefore build up an osmotic gradient so that water and small molecules will flow into the cell. In the ductal cells the sodium in the saliva is exchanged with potassium ions. This process is dependent on the saliva flow rate. So during a great flow the sodium concentration in saliva is relative greater than that of potassium compared to a reduced flow rate.

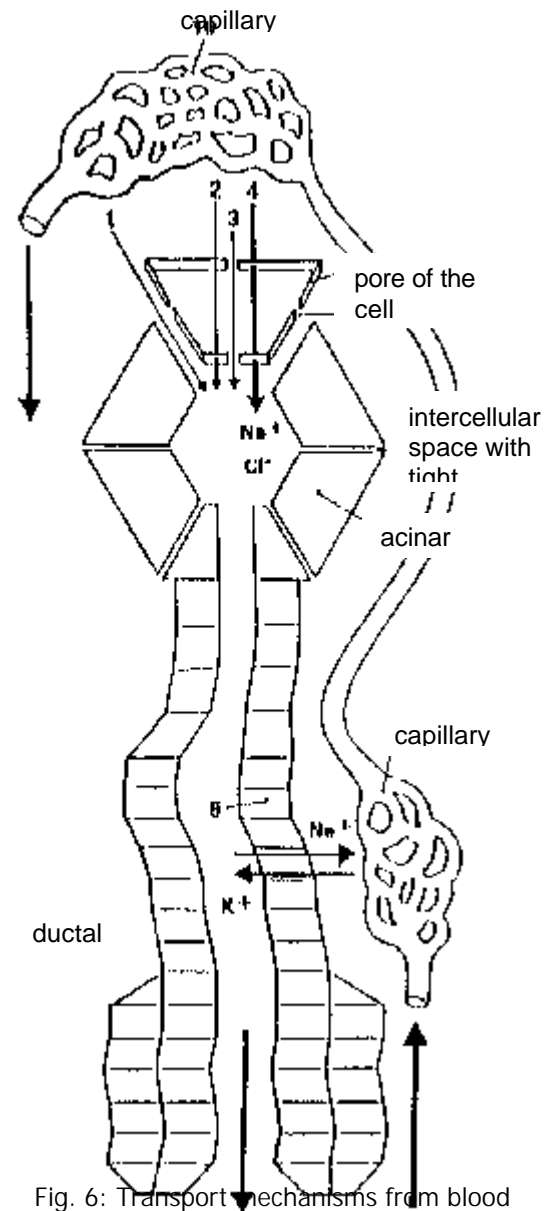


Fig. 6: Transport mechanisms from blood into saliva:
 saliva blood
 1 = Ultrafiltration along tight junctions
 2 = selected transport across the cell membrane
 3 = Filtration through pores of the cell membrane
 4 + 5 = active sodium pump
 (P. Haeckel and P. Hänecke, 1996)

In the following table the concentrations of analytes other than hormones in saliva and blood mentioned in literature are summarized. Unfortunately, a precise description and evaluation of the analytical method often is not mentioned and therefore in some cases a measurement of artifacts has to be assumed (i.e. the level of SHBG or LDH in saliva probably derived from a contamination with blood or crevicular fluid).

Analyte	Mixed Saliva	Plasma	Unit
In General			
Water	97 – 99.5	90 - 93	%
pH	(5.6)6.4 – 7.4 (7.9)	7.4	
Substrates			
Albumin	246 – 344	34 000 – 48 000	mg/l
Cholesterol	3 - 15	150 – 300	mg/dl
Creatinine	0.07 – 0.2	< 1.1	mg/dl
Glucose	< 2	55 – 115	mg/dl
Protein	1.1 – 1.8 (6.4)	66 – 87	g/l
Urea	17 – 41	< 50	mg/dl
Uric Acid	0.7 – 6.0	< 7.0	mg/dl
Enzymes (37 °C)			
α-Amylase	11 900 – 305 000	< 220	U/l
AP	< 11	< 270	U/l
SGOT (ASAT)	< 43	< 38	U/l
SGPT (ALAT)	< 11	< 41	U/l
LDH	113 - 609	< 480	U/l
Lysozyme	6 - 12	3 – 9	mg/l
Electrolytes/Minerals			
Calcium	0.88 – 2.05	2.20 – 2.55	mmol/l
Chloride	5 – 40	96 – 108	mmol/l
Magnesium	0.08 – 0.56	0.70 – 1.05	mmol/l
Phosphate	1.4 – 13.2	0.87 – 1.45	mmol/l
Potassium	6.4 – 37	3.3 – 5.1	mmol/l
Sodium	2 – 21	133 – 145	mmol/l
Other			
IgA	42 – 174	850 – 4 000	mg/l
CBG, male	38 ± 18	39 700 ± 6 300	µg/l
CBG, female	72 ± 71	42 200 ± 5 600	µg/l
SHBG, male	19 ± 10 ??	15 – 100	nmol/l
SHBG, female	63 ± 60 ??	15 - 120	nmol/l
Transferrin	< 0.5	250 - 350	mg/dl

Table 1: Analyte levels in saliva and in plasma (from various literature)

In the following table the steroid hormone levels in saliva and blood in some physiological situations described in literature are summarized. It can be seen that the saliva/plasma ratio of the steroids is at minimum about 1:10 (e.g. cortisol) up to more than 1:100 (e.g. testosterone). For some hormones like estradiol very small concentrations are found in saliva, and this allows the development of highly sensitive methods for assessment of steroid levels in saliva. Blood contamination can cause false positive results in some salivary diagnostic applications, and should be taken into consideration for testing purposes.

Analyte	Remark	Mixed Saliva	Plasma	Unit
Aldosterone	non pregnant female	29 – 118	80 – 790	pmol/l
Androstenedione	adult men	140 – 630	1200 – 11000	pmol/l
	adult women	62 – 482	1400 – 11900	pmol/l
Cortisol	Cortisol peak in adults	13.8 – 48.9	190 – 690	nmol/l
	8 hrs. after peak	1.4 – 8.6	55 – 250	nmol/l
DHEA	premenop. women	0.3 – 1.0	4.5 – 34.5	nmol/l
	adult men	0.3 – 1.7	6.2 – 43.3	nmol/l
Estradiol	follicular phase	2 – 18	26 – 650	pmol/l
	midcycle „peak“	9 – 29	180 – 1420	pmol/l
Estriol	40 weeks gestation	4.5 – 9.8	330 – 1596	mmol/l
Estrone	adult women	10 – 21	92 – 1294	pmol/l
	adult men	10 – 21	92 – 555	pmol/l
17- OH Progesterone	adult men	50 – 360	150 – 4900	pmol/l
	luteal phase	140 – 320	600 – 8800	pmol/l
Progesterone	follicular phase	< 160	500 – 3500	pmol/l
	luteal phase	200 – 1600	4900 – 72000	pmol/l
Testosterone	premenop. women	10 – 52	200 – 2860	pmol/l
	adult men	95 – 205	9900 – 27800	pmol/l
5 α - Dihydrotestosterone	premenop. women	10 – 26	80 – 1270	pmol/l
	adult men	34 – 172	860 - 3410	pmol/l

Table 2: Steroid hormone levels in saliva and in plasma (from various publications)