Thin-section transmission electron microscopy of human saliva

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Thin sections (90–100 nm) of flash-frozen human saliva fractions and whole saliva were examined by transmission electron microscopy. Inside the major filamentous structural components numerous ultrastructural details were observed, especially for whole saliva and submandibular/sublingual fractions. An outer reticulated zone believed to contain the major salivary glycoproteins surrounded an inner core consisting of a continuous, more electron dense phase with multiple vacuoles and granules of different sizes, shapes, and electron densities. The observed structures suggest a complex microarchitectural model for whole saliva. □ *Glycoproteins; mixed saliva; organic components; ultrastructure*

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A recent method for instantaneous solidification of saliva in liquid nitrogen (1, 2) has made possible sectioning, fixing, staining, and microscopic examination of mixed saliva and saliva fractions, which previously were virtually inaccessible to structural examination.

Studies performed on mixed saliva with optical microscopy (2) and with transmission and scanning electron microscopy (SEM) (3) have shown the presence of several structural components, including a colloidal network of continuous filamentous structures with integrated dispersed lipoid droplets (2). Compositional variations were also recorded among different salivary structures (3) by means of energy-dispersive roentgenographic analysis (EDAX) performed simultaneously with the SEM examination.

The earlier microscopical studies were performed on comparatively thick sections (about 10 μm), to determine the main structural components of the specimens and the possible presence of a three-dimensional network within saliva. During examination of these 'thick' sections it became evident that the major structural components of mixed saliva had significant variations in their electron density. Because these variations might be the effects of ultrastructures within the components, it was considered worthwhile also to study thinner sections of mixed saliva and saliva fractions by means of transmission electron microscopy.

Materials and methods

Using the methods described by Curby (4) and Block & Brotman (5), we collected approximately 2 ml of parotid and submandibular–sublingual saliva fractions from a 32-year-old female donor. In addition, a mixture of these two fractions was prepared on the basis of their approximate volumetric relationship noted during this sampling period (2 ml of parotid saliva to 3 ml of submandibular–sublingual saliva).

Two to 3 ml of stimulated whole saliva was also donated during empty-mouth chewing movements by this donor and by a 51-year-old man. Both donors were subjectively healthy, fully dentate, and in good oral health at general dental examination. The samples were collected between 1000 h and 1100 h, and no food or drink was consumed

by the donors for at least 2 h before sampling.

Droplets of all samples and mixtures were collected promptly (within 5 min) on microtome test piece holders by immersing the holder heads into foam-free saliva samples. Each holder was then carefully withdrawn through the saliva–air interface, thereby collecting a drop, and then—still held upside down—immediately immersed with a stainless steel tweezer into a thermoflask containing no less than 1000 ml of liquid nitrogen (−195.8 °C). After 1–2 sec of immersion with the tweezer, the holder was released, to immediately sink to the bottom of the thermoflask with its attached, solidified saliva droplet. The samples were stored in the thermoflask until retrieved with another stainless steel tweezer for mounting in a cryo-ultramicrotome (Reichert Ultracut-E Ultramicrotome, Reichert-Jung, Buffalo, N.Y., USA), equipped with a freezing stage (Reichert F C 4-D). Saliva sections 90–100 nm thick were then cut with freshly made glass knives at an environmental temperature of about −120 °C in the cutting chamber and allowed to sublime to dryness in this environment.

The sections were then transferred to Butvar-precoated (Butvar B-98 solution, Electron Microscopy Science, Fort Washington, Pa., USA) transmission electron microscope grids (TEM Grids 200 mesh, Electron Microscopy Science). The grids with their mounted sections were then kept in a silica gel containing desiccator for about 24 h to remove any retained moisture before carbon coating and examination in a transmission electron microscope (Hitachi HS-8, Hitachi Instruments, Tokyo).

Results

The presence of many ultrastructural components was observed in the whole saliva samples, the submandibular–sublingual samples, and the mixture of submandibular–sublingual and parotid samples. No reproducibly distinct ultrastructures were, however, observed for the parotid samples alone. Most of the ultrastructural components were detected inside the main filamentous salivary structures, especially in those with outer diameters greater than 0.1–0.2 μm. One characteristic organization in the whole saliva is shown in Fig. 1.

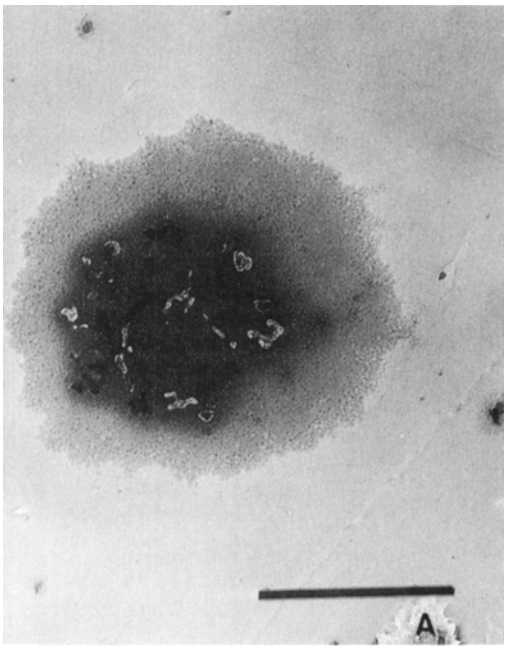

Fig. 1. Transmission electron micrograph of a 90- to 100-nm-thick section of whole saliva from a subjectively healthy 51-year-old male donor. One major and several minor structural components, in various sections, can be seen against a background with some artifacts (A) in the section carrying polyvinylbutyral resin film. The minor components have thin, foam-like outer zones and inner, more electron-dense, also foam-like cores. The major component has a comparatively thicker outer zone and also shows several granules with different shapes, sizes, and electron densities in the core phase. Bar = 1 μm.

The central part is dominated by irregular, electron-dense droplets (0.1 μm) in a closely packed dispersion of small (0.01–0.03 μm) polyhedral droplets with relatively electron-dense membranes. This pattern is reversed with increasing radius: the outer part consists of a dilute dispersion of 0.01-μm droplets of the electron-rich material in the less electron-rich one. The border of the background shows membranes of the less electron-dense material occluding the surrounding substances.

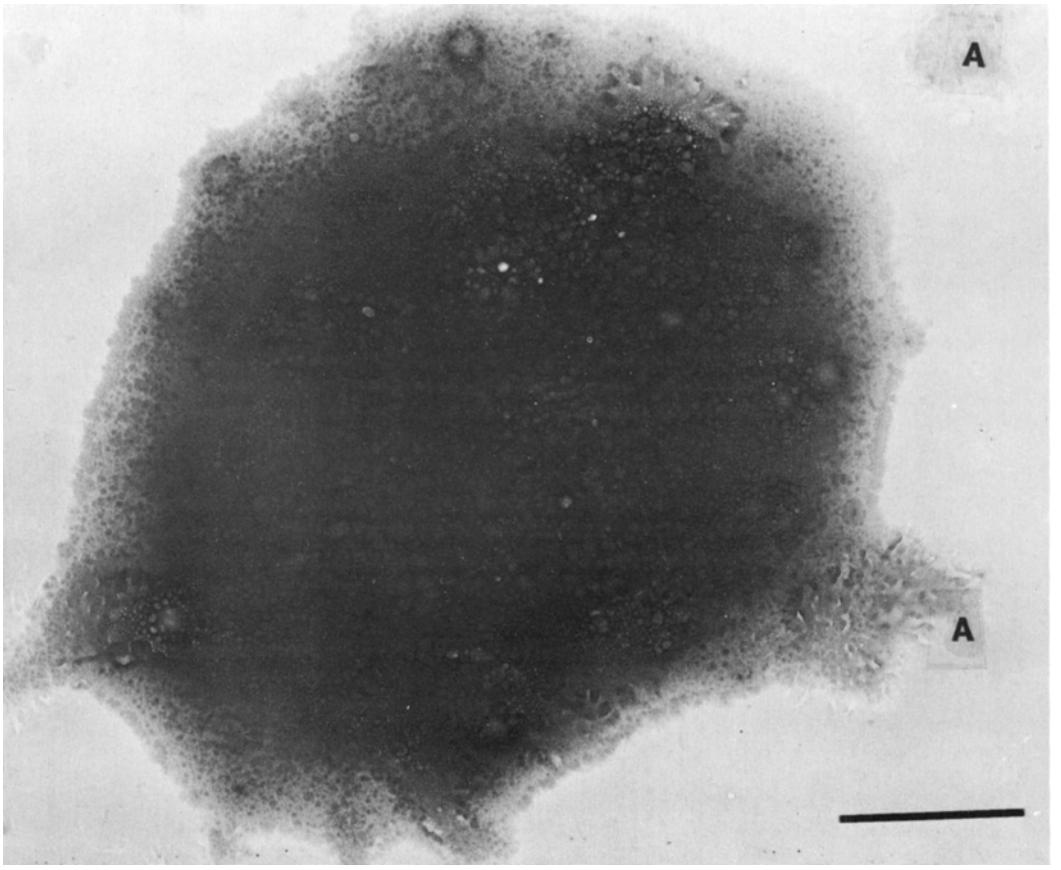

Fig. 2. Transmission electron micrograph of a 90- to 100-nm-thick section of whole saliva from a subjectively healthy 51-year-old male donor. The picture, probably an oblique section, shows one major rounded structure component with short extensions. These components have thin, foam-like outer zones and inner, more electron-dense, also foam-like cores. In the core of the major component there are multiple granules with different shapes, sizes, and electron densities. Artifacts (A) can also be seen in the section carrying resin film. Bar = 1 μm.

A similar arrangement in another whole saliva sample is illustrated in Fig. 2, in which the outer droplet layer is somewhat thinner and less electron dense. In this figure the central droplets also have less electron-dense central portions.

An example of the ultrastructures observed in submandibular–sublingual samples is given in Fig. 3, whereas Fig. 4 shows a sample of mixed submandibular–sublingual and parotid fractions.

In certain whole saliva sections (Fig. 5) larger (0.8 μm), electron-dense objects could be observed inside the major salivary network components. These objects were sometimes observed in the vicinity of the terminal ends of a seemingly swollen extension to a major structural component, in which the previously mentioned internal configurations appeared influenced by shear or concentration gradients in the medium.

Discussion

The present results merit both an analysis of the methods used and a preliminary evaluation of the results from a dental point of view.

The methods used in this study have

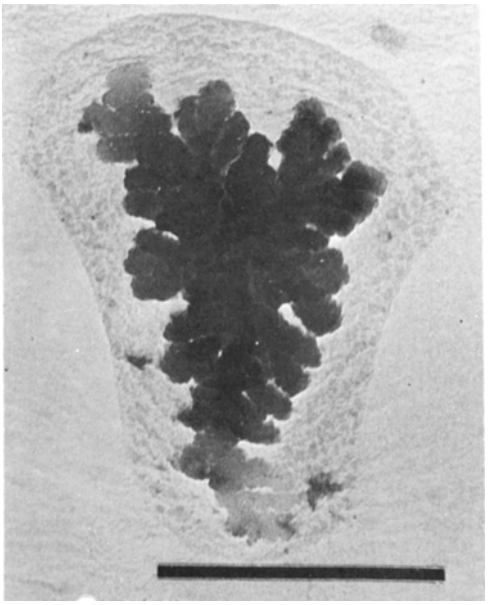

Fig. 3. Transmission electron micrograph of a 90- to 100-nm-thick section of submandibular–sublingual saliva from a subjectively healthy 32-year-old female donor. The picture, probably an oblique section of a major structural component, shows an outer less electron-dense zone encapsulating more varied shapes. Bar = 1 μm.

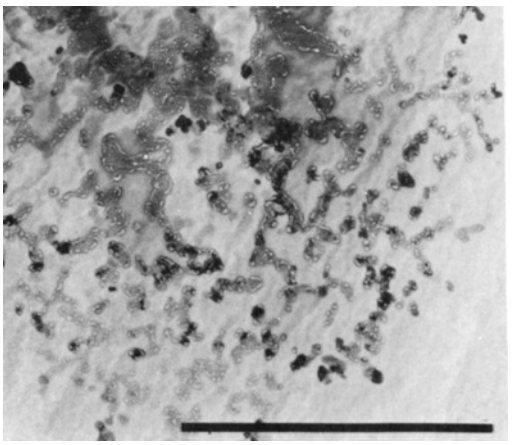

Fig. 4. Transmission electron micrograph of a 90- to 100-nm-thick section of a mixture of approximately 2 ml of parotid saliva with 3 ml of submandibular–sublingual saliva from a subjectively healthy 32-year-old female donor. Parts of a rounded major structural component can be seen. It has a thin, less electron-dense background structure with multiple granules of various sizes, shapes, and electron densities. Bar = 1 μm.

already been shown to be valid and accurate by recent morphologic examinations of sections of frozen mixed saliva samples about 10 μm thick (1–3). It was therefore considered worthwhile to use them also in an attempt to examine sub-micrometer-thick sections of the same type of samples. Again, the method turned out to be useful and to provide material for evaluation of internal salivary structures.

The examined saliva samples were donated by two test persons. Since there may always be individual variations between biologic structures and functions, the validity can be questioned for studies run on few subjects. In this case, however, these risks were considered to be small. The sampling conditions were well controlled, and previously reported light and electron-microscopic examinations of mixed saliva sections from these test persons displayed structures much the same as for other subjectively healthy persons (2, 3). The general nature of the observed structures will be evaluated during additional examinations of many more subjects, already in progress. The structures observed clearly show that the internal architecture of especially whole saliva but also some saliva fractions is both detailed and complex. Thus, in cross sections, at various angles to the filament axes, the major whole salivary structures had several different components; an outer honeycomb, apparently proteinaceous (based on electron density) material is of primary interest. This fenestrated membrane-like material had a thickness that varied from 0.01 to 0.1 μm from spot to spot. In the smallest aggregates this continuous material appeared as the major (sometimes only) structural component.

It is noteworthy that similar honeycomb structures were observed in transmission electron microscopic studies of thicker, OsO_4–vapor-postfixed sections of mixed saliva (3). The earlier noted network structures appeared in this study only as either multiple short extensions (Fig. 2) or as single longer ones (Fig. 5), because the thickness of the sections was only a 100th of that required for observation of the entire filamentous networks (3).

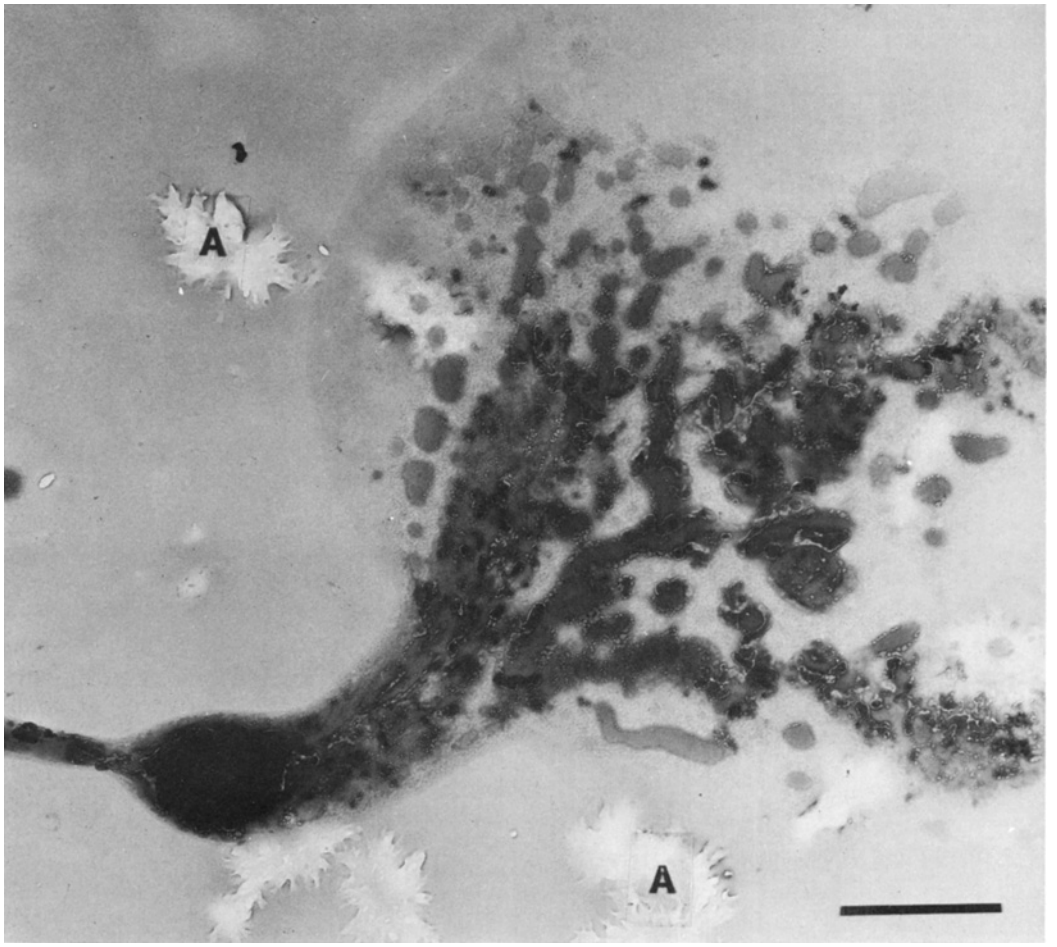

Fig. 5. Transmission electron micrograph of a 90- to 100-nm-thick section of whole saliva from a subjectively healthy 51-year-old male donor. The picture shows a rounded, major structural component with an extension that originally is relatively wide but then, at an approximate distance of 2 μm from the edge of the main component, narrows into smaller dimensions. The component has a thin, less electron-dense background structure with multiple granules of various sizes, shapes, and dimensions. Of special interest are the large electron-dense particle close to the terminal end of the wider portion of the extension and the ensemble of smaller granules located between the large particle and the interior of the main component. The figure also shows artifacts (A) in the section carrying resin film. Bar = 1 μm.

The outer structure, which obviously forms a separating barrier or membrane between the exterior continuous, aqueous electrolyte and the interior core phase, must incorporate the major salivary glycoproteins. Only these salivary components possess the proper surface and the colloid chemical characteristics for such an interfacial function. They have been confirmed to be the most surface-active salivary components during studies of interfacial phenomena occurring between saliva and clean solids (6, 7). The small core and barrier (0.1–0.3 μm) droplets seen in Figs. 1 and 2 appear to have the same or very similar compositions. Their variations in electron density may be due to shifts in the internal arrangements of their probably amphiphilic main molecular components.

The potential significance of the glyco-

protein-producing minor salivary glands located in the oral mucosa is enhanced by these findings. The extreme surface activity of the glycoprotein components enables structural salivary components to accommodate dilution and expansion. The glycoproteins' ability to form surface coatings and membranes makes them able to provide protective coatings to core materials produced and excreted from the major salivary glands.

Whether or not the outer zones of major salivary components are formed mainly from markedly amphiphilic glycoproteins, it appears that there are considerable differences between the compositions and physicochemical characteristics of the outer electrolyte and the inner, core material. In spite of the complex composition of whole saliva, it is likely that the inner-core portions of the major structural elements are dominated by protein concentrates. Some of the internal structures—shown, for example, in Figs. 1–5—may have enzymatic properties that require a protected environment to perform their functions. The presence of a protected salivary region with enzymatic functions explains how saliva can execute its known range of specific biologic functions (through highly specialized but environment-sensitive molecules) in spite of having an electrolyte as its main component. Complicated macromolecules do not otherwise maintain long-term precise functions when in an open electrolyte constantly mixed with the most varied substances.

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