Histochemical and biochemical properties of the labial and palatine glands and their secretions in the *Macaca irus* and *Cercopithecus aethiops* monkeys

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The increasing use of non-human primates in experimental studies in dentistry demands detailed knowledge of their basic oral biology. The labial and palatine glands and their secretory products were compared in two commonly used monkey species. The mucous cells of both gland types appeared to elaborate sulphated mucosubstances and the serous demilunes of the labial glands neutral mucosubstances. The secretions of these glands, as obtained by *in vitro* cultivation of glandular tissue were subjected to isoelectric fractionation. The labial and palatine secretions of the *C. aethiops* contained an acidic component which exhibited the viscous properties of the original secretions, showed incorporation of $^{35}SO_4$ and had high titres of virus-haemagglutination inhibition activity. A similar component has been found in *M. irus* labial and palatine secretions. The two monkey species were similar in that the labial gland secretions of both contained small amounts of lysozyme activity, which the palatine gland secretions did not. They differed in that alpha-amylase activity was present in the labial and palatine secretions of the *C. aethiops*, which corresponds to the human situation. The minor glands of the *M. irus* did not show traces of alpha-amylase activity. The distribution of several chemical characteristics of the salivary gland secretions of both monkey species indicated that neither of them were identical to man, even though a general similarity was evident.

Key-words: Labial and palatine salivary glands; monkeys; histochemistry; biochemistry.

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The increasing use of non-human primates for experimental studies in dentistry demands detailed knowledge of their basic oral biology. The marmoset (*Callithrix jacchus*) has a dentition similar to that of man and has been used in many studies related to periodontal diseases (*Levy, Dreizen & Bernick*, 1972). The marmoset is, however, relatively insusceptible to caries, which could be due to chemical peculiarities of its saliva (*Dreizen, Goodrich & Levy*, 1968). One of the factors involved might be an effect of differences in the autonomic innervation of the salivary gland duct system of this animal as compared to man (*Rossoni, Machado & Machado*, 1973).

Monkeys from different species such as

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the vervet (*Cercopithecus aethiops*), macaque (*Macaca irus*) and rhesus (*Macaca mulatta*) develop carious lesions similar to the ones observed in man when fed a cariogenic diet (*Shaw & Sognnaes*, 1955; *Ockerse & de Jager*, 1957; *Cohen & Bowen*, 1966). Histochemical observations indicate a great similarity between the major salivary glands of man and the rhesus monkey (*Munger*, 1964; *Leppi & Spicer*, 1966; *Shackleford & Wilborn*, 1968) and between the vervet and macaque monkeys (*Jacobsen & From*, 1973).

The morphology of the minor salivary glands of the macaque is comparable to that of man (*Cohen*, 1967) and it has been shown that they produce sulphated mucosubstances (*Hensten-Pettersen & Jacobsen*, 1975) which may be of importance in the formation of the acquired tooth pellicle (*Sønju*, 1975) and plaque (*Rølla, Melsen & Sønju*, 1975). The corresponding glands of the vervet have not been investigated.

The present investigation was designed to correlate information about the histochemistry and the secretory products of the minor salivary glands of the macaque and vervet monkeys.

MATERIALS AND METHODS

Monkeys

Salivary gland tissue was obtained from 6 male *M. irus* and 11 male *C. aethiops* monkeys of 2 to 6 kg, which were sacrificed for other purposes at the National Institute of Public Health, Oslo.

Histochemistry

Specimens of glandular tissue from the upper and lower lips and of the posterior portion of the hard palate were obtained from 2 *M. irus* and 6 *C. aethiops monkeys.* Specimens of parotid, submandibular and sublingual tissue were included as controls. In 5 monkeys (2 macaque and 3 vervet) the specimens were removed after the animals were bled from the throat under sodium pentobarbital anaesthesia, and fixed in Lillie's neutral buffered formalin. The glandular tissues from the last three vervet monkeys were extirpated after the animals were perfused with 1.7 per cent glutaraldehyde in 0.1M cacodylate buffer pH 7.3, and postfixed in the same solution.

All specimens were dehydrated in alcohol, cleared in naphtha and embedded in paraffin. Five-micron sections were subjected to the following stains: Haematoxylin and eosin, periodic acid-Schiff (PAS), alcian blue-Ehrlich's haemalum, alcian blue pH 1.0, alcian blue-critical electrolyte concentration (CEC) utilizing 0.1 and 1.0M $MgCl_2$, alcian blue pH 2.5-PAS. These methods were applied as described by *Pearse* (1968). In addition the sections were stained with Heidenhain's modification of Mallory's staining method, using azocarmine and aniline blue as described by *Preece* (1972).

Minor gland secretions

Four samples of *M. irus* and 5 of *C. aethiops* labial and palatine gland secretions were obtained by *in vitro* cultivation of extirpated glandular tissue in a synthetic medium (*Hensten-Pettersen & Jacobsen*, 1975). Labial gland tissue was taken from the upper and lower lips at the area opposite to the incisors and palatine gland tissue from the posterior portion of the hard palate. In some cultures radioactive sulphate ($Na_2{}^{35}SO_4$, Institutt for Atomenergi, Kjeller, Norway) was added to the medium at a concentration of 1 μCi per ml medium.

Biological activities

Alpha-amylase was estimated by an amyloclastic method (*Jacobsen & Hensten-*

Pettersen, 1970) and lysozyme by a spectrophotometric method (*Shugar*, 1952) with a suspension of cell walls from *Micrococcus lysodeikticus* as substrate.

Virus-haemagglutination inhibition activity was titrated on WHO perspex plates by the method of *Isaacs, Gledhill & Andrewes* (1952), using B/Lee influenza virus and chicken red blood cells.

Isoelectric fractionation

Dialysed samples of culture medium containing 10 to 15 mg protein were subjected to isoelectric fractionation on a 110-ml column (LKB Products, Sweden) coated with a thin layer of polytetrafluoroethylene to minimize adhesion of the mucosubstances to the column walls (*Hensten-Pettersen*, 1974). The experiments were performed at 4°C in a sucrose density gradient with 2 per cent LKB ampholine carriers pH 3 to 10 or pH 2.5 to 4, at a constant voltage of 300 V for 48 hr with the anode at the lower end. Fractions of 5 ml were collected and the pH determined at 4°C. The fractions were dialysed against 10 l of 0.15 M NaCl with 0.005 M $CaCl_2$ for 4 days with daily changes. The protein content of each fraction was estimated at $O.D._{215\ nm}$ (*Arneberg*, 1971). Radioactivity was determined by liquid scintillation counting of 1 ml aliquots in 10 ml Insta-Gel (Packard Instruments Co., Ill., USA) using a Packard Tri-Carb spectrometer model 3365.

Control experiments

The distribution of radioactive sulphate in the fractions obtained by isoelectric focusing was determined before and after dialysis. The dialysed fractions which still exhibited radioactivity were subjected to gel filtration on a Pharmacia K 9/30 column with Bio-Gel P-100 as bed material. Elution was performed with a 0.05 M tris-HCl buffer pH 7.2 with 0.1 per cent sodium dodecyl sulphate and 0.005 M 2-mercaptoethanol. Fractions of 0.5 ml were collected and the radioactivity estimated as described above.

Isoelectric focusing was performed with culture media which had been obtained without the use of radioactive precursors. Addition of radioactive sulphate to these culture media followed by isoelectric fractionation and dialysis was also performed.

Glucuronic acid

The fractions from the isoelectric focusing experiments that exhibited $^{35}SO_4$-activity were pooled, lyophilized and hydrolysed in 2 ml 4 N HCl for 4 hr at 100°C. The residues were taken to dryness under reduced pressure and tested for glucuronic acid by the method of *Nir* (1964), using a naphthoresorcinol reagent.

RESULTS

Histochemistry

The general histology and staining characteristics were similar in the corresponding glands of the two monkey species. The total size of the labial glands appeared to be somewhat larger in the *M. irus* than in the *C. aethiops*. Control sections of parotid, submandibular and sublingual gland tissue evinced the same pattern as described by *Jacobsen & From* (1973). The staining reactions of the labial and palatine glands are summarized in Table I.

Palatine glands

The palatine glands appeared as a continuous mass of purely mucous acini with a few strands of connective tissue. No serous demilunes or seromucous acini

Table I. *Staining reactions of the minor salivary glands of M. irus and C. aethiops*

	Labial glands		Palatine glands
	Mucous acini	Serous demilunes	Mucous acini
PAS	Magenta, granular appearance	Magenta, even colour	Magenta, granular appearance
AB/ Haemalum	Blue	Light pink	Blue
AB pH 1.0	Blue	No colour	Blue
AB-CEC 0.1 M $MgCl_2$	Blue	Light blue	Blue
1.0 M $MgCl_2$	No colour	No colour	No colour
AB-PAS	Deep blue	Magenta	Deep blue
Heidenhain	Unstained cytoplasm, Red nuclei and cell membranes	Red	Unstained cytoplasm, Red nuclei and cell membranes
Notes	Acidic muco-substances	Neutral muco-substances	Acidic muco-substances

were observed in any of the specimens. The convoluted duct system and intercalated and striated ducts which are a normal feature of the major salivary glands appeared to be poorly developed, whereas larger sinusoid lumina were regularly seen (Fig. 1a, b).

The mucous acini were PAS-positive with a granular appearance of the cytoplasm and luminal contents. They stained deep blue with alcian blue-PAS; these two stains overlapped completely in the acini and ducts. The mucous acini stained blue with alcian blue-haemalum, alcian blue pH 1.0 and alcian blue-CEC 0.1 M $MgCl_2$. With azocarmine-aniline blue the nuclei and cell membranes stained red, whereas the cytoplasm and lumina showed no colour.

Labial glands

The labial glands were situated in the deep submucosa as clusters of acini separated by wide strands of connective tissue and a few were also located in between the muscle bundles of the lips. The acini diameters were generally smaller than those of the palatine acini. Intercalated and striated ducts were seen, but were not so prominent or so well demarcated as those of the major salivary glands (Fig. 1c, d).

The mucous cells of the labial gland acini exhibited the same staining characteristics as those found in the palatine glands.

The serous demilunes of the labial glands stained magenta with PAS and alcian blue-PAS and appeared evenly stained in contrast to the granular appearance of the mucous cells. With alcian blue-haemalum the demilunes appeared pink and with alcian blue-CEC 0.1 M $MgCl_2$ pale blue. No colour was seen with alcian blue pH 1.0 or with alcian blue-CEC 1.0 M $MgCl_2$. The demilunes stained red with azocarmine-aniline blue.

Minor gland secretions

The culture media obtained by *in vitro* cultivation of minor salivary gland tissue exhibited the viscoelastic properties characteristic of minor gland secretions *in vivo*. These properties were retained when the media were dialysed against 0.15 M NaCl with 0.005 M $CaCl_2$, but were lost when $CaCl_2$ was omitted from the solution.

Biological activities

The distribution of alpha-amylase, lysozyme and virus-haemagglutination inhibition activities is summarized in Table II.

The undialysed culture media of both monkey species showed small amounts of

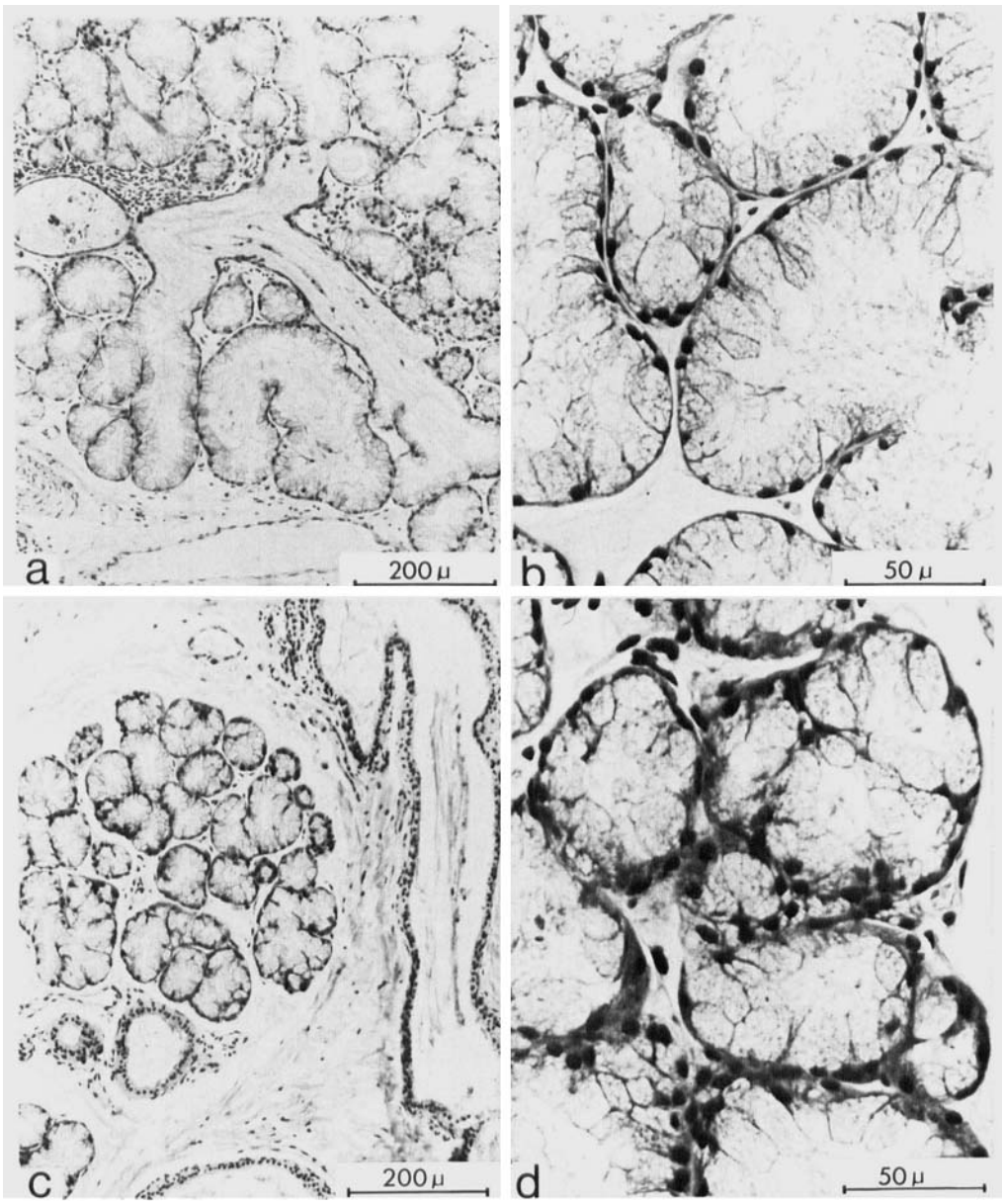

Fig. 1. Salivary glands of *Cercopithecus aethiops*. The corresponding glands of *Macaca irus* were identical and are not shown.

a) Palatine glands. Striated ducts absent. Poorly defined duct system. HE × 120.
b) Palatine glands. Mucous acini. Serous demilunes were not observed. HE × 480.
c) Labial glands. Striated ducts and convoluted duct system. HE × 120.
d) Labial glands. Predominantly mucous acini with small serous demilunes.

Table II. *Biological activities in M. irus and C. aethiops minor salivary gland culture media*

C. aethiops	Labial glands*)	Palatine glands*)
Alpha-amylase (μg/ml)	6—8	6—25
Lysozyme (units/ml)	15—35	0
Virus-HI**) (titre)	1/1600—1/3200	1/512—1/2048
M. irus		
Alpha-amylase (μg/ml)	0	0
Lysozyme (units/ml)	65—90	0
Virus-HI (titre)	1/1024—1/2048	1/256—1/4096

*) The ratio 5 ml medium per 0.2 g tissue was used in each experiment (n = 4).

**) Virus-haemagglutination inhibition activity.

Table III. *Isoelectric point of main protein fractions. Electrofocusing of C. aethiops palatine and labial gland culture media in a pH 3 to 10 gradient*

	Isoelectric point of protein peaks				
Labial glands					
1.	2.1*	3.7_m	4.8a	6.2	
2.	$2.9*_m$		4.7a	6.9	
Palatine glands					
1.	2.2*	3.7_m	4.8a	7.0	
2.	$2.1*_m$		4.8a	6.6	
3.	$2.3*_m$		4.7a	7.4	
4.	$2.8*_m$		4.4a	6.8	12.1
5.	$2.0*_m$		5.0a	8.0	

* Denotes incorporation of $^{35}SO_4$ and the presence of virus-haemagglutination inhibition activity.

m Denotes major protein peak in each experiment.

a Denotes presence of alpha-amylase activity.

lysozyme activity only in the labial gland specimens. Lysozyme activity was not detectable after dialysis.

Alpha-amylase activity was found in small amounts in the labial and palatine gland culture media of the *C. aethiops*, not in any of the *M. irus* cultures tested.

Virus-haemagglutination inhibition activity was present in high titres in the labial and palatine gland cultures of both species.

Isoelectric fractionation

Electrofocusing of *C. aethiops* labial and palatine culture media in a pH 3 to 10 gradient resulted in 3 to 4 protein peaks. The major proportion of protein focused in the acidic range, only in one palatine gland culture was a small peak of pH 12.1 observed. All cultures showed the incorporation of $^{35}SO_4$ in an acidic component (pI<3), which was also the largest one in most experiments (Table III).

The viscoelastic properties of the original secretions and virus-haemagglutination inhibition activity were confined to this component. The use of ampholine carriers in the pH range 2.5 to 4.0 did not cause a further separation of the components with virus-haemagglutination inhibition activity and with radioactive sulphate.

In addition, a variable pattern of minor components was observed. The most consisted one focused around pH 4.7 and exhibited alpha-amylase activity.

Control experiments

The distribution of radioactive sulphate in the undialysed fractions of electrofocusing experiments showed that the major portion was in the anode solution, i.e. pI < 1.3. After extensive dialysis, the residual radioactivity closely followed the OD_{215} nm pattern of the most acidic component.

Gel filtration of this dialysed component on Bio-Gel P-100 indicated that more than 95 per cent of its total radioactivity

was eluted in the void volume, the rest at the total volume.

In isoelectric fractionation experiments performed with culture media without radioactive precursors, the same protein distribution was observed.

Addition of radioactive sulphate to spent, non-radioactive culture media, followed by isoelectric fractionation and dialysis, gave no residual radioactivity in any fraction, and did not influence the protein distribution.

Glucuronic acid

Glucuronic acid was not found in any of the specimens in the amounts tested (1.0 to 2.0 mg dry weight).

DISCUSSION

The morphology of the corresponding minor salivary glands of the macaque and vervet monkeys as seen by staining with haematoxylin-eosin and azocarmine-aniline blue did not reveal any differences between the two monkey species. Unless otherwise stated the results will therefore be discussed together.

The labial glands were mixed, predominantly mucous with serous demilunes and a convoluted duct system, whereas the palatine glands were purely mucous and had a poorly defined interacinar duct system. The same pattern is seen in the sublingual glands of these monkeys (*Jacobsen & From*, 1973), where the major lobe (posterior sublingual gland) appears as a mixed, predominantly mucous area with serous demilunes, corresponding to the morphology of the labial glands, and the minor sublingual lobe (anterior sublingual gland) is purely mucous with no convoluted duct system, corresponding to the morphology of the palatine glands.

The histochemical staining reactions of the corresponding minor glands of both species were very similar, and showed little variation from gland to gland. Variation in the mucosubstance histochemistry of the acinar secretory cells of human minor salivary glands from different subjects has been observed by *Eversole* (1972) and *Harrison* (1974), but the minor glands of the vervet and macaque appeared more homogenous in this respect. The staining of mucous acini with alcian blue at pH 1.0 and the complete overlapping of alcian blue when followed by the PAS procedure indicated that these cells produce sulphated mucosubstances with *vic*-glycols. The positive reaction with alcian blue-CEC 0.1 M $MgCl_2$ might indicate that the mucous cells produced carboxylated, non-sulphated mucosubstances (*Scott & Dorling*, 1965). However, in a recent investigation into the general applicability of the CEC theory *Horobin & Goldstein* (1974) showed that the staining patterns obtained by this procedure do not primarily depend on the identity of the polyanions in the tissue section, i.e. whether they are carboxylates, phosphates or sulphates.

The minor gland secretions of the vervet and macaque monkeys as obtained by *in vitro* cultivation of salivary gland tissue exhibited the viscoelastic properties characteristic of these secretions *in vivo*. After isoelectric fractionation of vervet minor gland secretions the viscoeleastic properties of the mucosubstances were recovered in the acidic fraction (pI < 3), which also exhibited virus-haemagglutination inhibition activity and incorporated radioactive sulphate. This finding, as well as the protein distribution in general, was similar to the pattern observed earlier with macaque minor gland secretions (*Hensten-Pettersen & Jacobsen*, 1975). The virus-haemagglutination inhibition activity, a result of

sialic acid in terminal position in glycoproteins (*Gottschalk*, 1972), and the radioactive sulphate in the fractionated secretions were not separable by the methods employed, even when these fractions were refocused in a pH 2.5 to 4.0 gradient.

When correlated with the negative reaction for uronic acids in the acidic, sulphated component, and the histochemistry of the mucous acini, the present results made it likely that the mucous cells of the labial and palatine glands of the vervet and macaque elaborate sulphated sialoglycoproteins.

Both monkey species exhibited lysozyme activity in the labial gland secretions, whereas their palatine gland secretions did not. This pattern is also found in the human labial and palatine gland secretion (*Hensten-Pettersen*, 1975). Using immunohistochemical staining techniques, *Kraus & Mestecky* (1971) found that lysozyme is produced by distinct epithelial cells of intralobular and larger ducts in the human parotid glands, and the nuclei of these cells were seen to lie peripherally to the nuclei of the striated cells. The difference in the lysozyme production by labial and palatine glands might thus reside in the differences in the secretory duct systems that were seen between these two gland systems.

Small amounts of alpha-amylase were present in the labial and palatine gland secretions of the vervet, which is common to what is found in human minor gland secretions (*Hensten-Pettersen*, 1975), but was not found in any of the macaque minor gland secretions. The immunohistochemical study of *Kraus & Mestecky* (1971) shows that human parotid alpha-amylase is produced in acinar and adjoining intercalated duct cells. The monkey labial glands had serous demilunes which might conceivably elaborate alpha-amylase, but no such structures were evident in the palatine glands. It follows that the difference between the vervet and macaque cannot be explained by differences in their glandular morphology. A similar situation is found in the alpha-amylase producing capacity of the sublingual and submandibular glands of these two monkey species. In the macaque the parotid and submandibular glands secrete the highest amounts of alpha-amylase, whereas in the vervet the parotid and sublingual glands are the richest sources of this enzyme (*Jacobsen*, 1970). These differences are not matched by a higher proportion of serous cells in the sublingual glands of the vervet, in fact no differences in the histochemistry of the corresponding gland systems of these two species have been observed (*Jacobsen & From*, 1973).

Interspecies differences in the isoelectric points of salivary isoamylases have been reported. In man, alpha-amylases are isoelectric at pH 5.3, 5.6, 5.8 and 6.6 (*Andjic et al.*, 1970), whereas the corresponding values are 5.2, 6.0, 7.3 and 8.5 in the macaque (*Jacobsen*, 1973). The vervet appears to have only two isoamylases, isoelectric at pH 4.7 and 5.5 (*Jacobsen & Hensten-Pettersen*, 1974). With regard to the alpha-amylase secreting properties of the salivary glands, the secretory patterns of the major salivary glands appeared to be similar in man and the macaque, whereas the vervet was more similar to man with respect to the minor gland secretions.

The pH of saliva also shows interspecies differences. In man, saliva is usually neutral or slightly acidic (*Mandel*, 1974), whereas in the macaque the pH of parotid saliva is in the range of 7.97 to 8.24 (*Bowen, Eastoe & Morris*, 1964) and in the vervet around pH 7.8 which rised to 8.3

16 months after capture (*Ockerse & de Jager*, 1957).

In man, the acquired dental pellicle is formed by the deposition of salivary glycoproteins on the enamel surface (*Mayhall*, 1970) and is assumed to lay the foundation for the colonization of microorganisms to the tooth surfaces. Lysozyme, IgA and alpha-amylase (*Ørstavik & Kraus*, 1973) and virus-haemagglutination inhibition activity and blood group substances (*Sønju et al.*, 1974) have been detected in the acquired 2-hr dental pellicle. In view of the biological activities present, the viscosity and close contact with the tooth surfaces, it seems reasonable to assume that the minor gland secretions may play an important part in pellicle formation. In fact, all of the above-mentioned activities have been observed in human labial gland secretions (*Milne & Dawes*, 1973; *Crawford, Taubman & Smith*, 1975; *Hensten-Pettersen*, 1975), and it has also been shown that sulphated substances, probably originating in the labial glands, participate in the formation of dental plaque in the macaque (*Rølla et al.*, 1975).

The distribution of several characteristics of the salivary secretions, as reviewed above, indicate that neither the macaque nor the vervet has salivary gland systems identical to man, even though a general similarity is evident. Whether these variations extend to differences of importance in the etiology of caries and periodontal disease is not known. The ecological conditions in different parts of the oral cavity may, however, be influenced by salivary proteins with somewhat different characteristics in the vervet and macaque monkeys, and in man.

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