passages.

Nasal secretions or washings collected from 6 adult donors with illnesses resembling the common cold have been passed 4 to 5 times in both DMB and DHov cells. A type 4 adenovirus was isolated from an individual who had just started to work with this agent in the laboratory, and who had an increase in titer of type 4 neutralizing antibody. Cytopathogenic agents have not been isolated from the other specimens.

Summary. (1) Two strains of epithelial-like cells derived from nasal mucosa have been found to be susceptible to a number of viruses. The fibroblasts which predominated in the early passages of these cell lines were susceptible, although degeneration occurred at a slower rate, to type 4 adenovirus. (2) Cytopathogenic effects were produced by 7 adenoviruses, 3 polioviruses, 2 ECHO viruses, a Group B Coxsackie virus, herpes simplex, measles, "CA", and "chimp rhinitis" viruses. Titers obtained in the nasal epithelial-like cells are similar to those measured with HeLa cells. Multiplication of certain of these agents was confirmed by titration of passage material. (3) Influenza and mumps viruses and nasal secretions from individuals with common colds were not cytopathogenic.

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Lysozyme: Its Characteristics in Human Parotid and Submaxillo-Lingual Saliva. (22640)

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Lysozyme activity in human saliva was reported by Fleming along with his discovery of the enzyme in 1922(1). It has been shown that human parotid saliva exhibits greater lysozyme activity than the corresponding whole saliva(2). This has led to the conclusion that the parotid glands are the major contributors to the total salivary lysozyme titer, if not the sole contributors. We were able to corroborate this finding employing turbidimetric assay methods(3). However, the lower lysozyme activity in whole saliva was due, in part, to increases in optical density resulting from differing concentrations of salivary sediment in the reaction systems. In this study, to avoid this source of error, salivas were collected by methods other than expectoration. Lysozyme activity was determined in sediment-free individual salivary gland secretions. As a result, lysozyme titers in the mixed secretions of the submaxillary and sublingual glands were found to be considerably higher than those in the corresponding parotid salivas. Data are presented regarding some of the characteristics of parotid and submaxillo-lingual* salivary lysozyme. Special emphasis is placed upon the interaction of parotid and SM-L saliva and the effect of this interaction upon the resulting total salivary lysozyme titer.

* Henceforth submaxillo-lingual will be abbreviated as SM-L.

Methods. (a) *Lysozyme quantitation*: The turbidimetric lysozyme assay method of Smolelis and Hartsell(4) was adapted for our studies of salivary lysozyme. The optical density of a suspension of *Micrococcus lysodeikticus* cells, subsequent to exposure to an unknown saliva sample, was related to a previously prepared standard curve employing chromatographically homogeneous crystalline egg white lysozyme† as the primary standard. Accordingly, salivary lysozyme activity could be expressed as micrograms of enzyme per ml of saliva. These quantitative values, however, were not absolute, but represented egg white lysozyme equivalents. (b) *Saliva collections*: Parotid saliva was collected with the device and according to the method described by Curby(5). SM-L saliva was considered that secretion drawn from the floor of the mouth by gentle negative pressure into a sterile filtration tube after placing Curby caps over the right and left Stenson duct orifices. This method of collection yielded a clear mucoid fluid considered to be an expression of the submaxillary and sublingual glands. Parotid saliva, when collected singly, was stimulated by chewing sweetened gum. SM-L and parotid salivas, when obtained simultaneously, were stimulated by applying a small quantity of 3% acetic acid to the tip of the tongue.

Results. (a) *Parotid saliva*: Parotid saliva lysozyme tests were made at dilutions of 1:10 in the reaction system. The mean lysozyme titer in the secretions of the right glands of 100 normal male Naval personnel,‡ age 17 to 26, was 23.1 ± 14.3§ µg/ml. Values ranged from 4.5 to 80.0 µg/ml. The mean lysozyme titer in the mixed right and left gland secretions, collected simultaneously with SM-L saliva, in 30 individuals, was 15.0 ± 9.2 µg/ml. Values ranged from 5.0 to 42.0 µg/ml. The pH optimum for parotid lysozyme activity was 6.2 (Sørensons M/15 phosphate buffer). Total loss of enzyme activity was encountered upon heating parotid saliva at 60°C for 45 minutes (Fig. 1). (b) *Sub-*

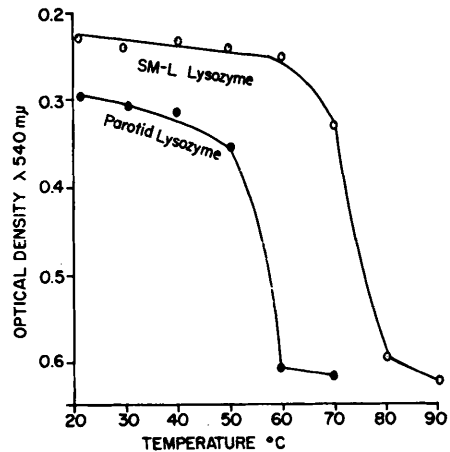

FIG. 1. Effect of heating on salivary lysozyme activity. Saliva samples were held at each temperature for 45 min. before activity tests.

maxillo-lingual saliva: SM-L saliva lysozyme tests were made at dilutions of 1:20. The mean lysozyme titer for 30 individuals was

TABLE I. Lysozyme Titers in Parotid, Submaxillo-Lingual (SM-L) and Equimixtures of Both Salivas.

Lysozyme (µg/ml)				
		Parotid and (SM-L)		
Parotid	(SM-L)	Expected	Observed	Difference
5.0	38.0	43.0	42.5	− .5
5.0	38.0	43.0	41.5	− 1.5
5.5	47.0	52.5	50.0	− 2.5
5.5	90.0	95.5	88.0	− 7.5
5.7	62.0	67.7	64.0	− 3.7
6.0	9.0	15.0	20.5	+ 5.5
6.4	64.0	70.4	50.0	−20.4
8.0	76.0	84.0	76.0	− 8.0
8.5	90.0	98.5	87.0	−11.5
9.0	50.0	59.0	53.0	− 6.0
9.5	68.0	77.5	76.0	− 1.5
9.6	88.0	97.6	88.0	− 9.6
10.5	74.0	84.5	66.0	−18.5
10.5	148.0	158.5	130.0	−28.5
11.0	82.0	93.0	82.0	−11.0
11.0	87.0	98.0	87.0	−11.0
12.0	92.0	105.0	90.0	−15.0
13.0	50.0	63.0	65.0	+ 2.0
16.6	37.0	53.6	64.0	+ 9.4
18.7	82.0	100.7	76.0	−24.7
20.0	70.0	90.0	72.0	−18.0
20.0	69.0	89.0	73.0	−16.0
20.5	65.0	85.5	74.0	−11.5
21.0	70.0	91.0	70.0	−21.0
23.2	94.0	111.2	94.0	−23.2
25.0	148.0	173.0	148.0	−25.0
25.0	80.0	105.0	80.0	−25.0
27.0	94.0	121.0	97.0	−24.0
34.0	38.0	72.0	44.5	−27.5
42.0	104.5	146.0	104.0	−42.0

† Difco Laboratories, Detroit, Mich.

‡ Henceforth all cases fall into this classification.

§ Group mean with standard deviation.

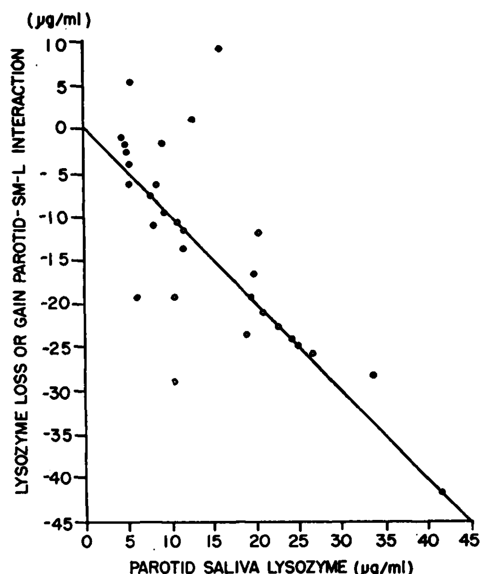

FIG. 2. Relationship of parotid lysozyme titers in each of 30 individuals to loss or gain in lysozyme activity in equimixtures of their parotid and submaxillo-lingual saliva.

73.4 ± 29.3 µg/ml. Values ranged from 9.0 to 148.0 µg/ml. The pH optimum was 6.2. Fig. 1 shows that total loss of enzyme activity occurred upon heating SM-L saliva at 90°C for 45 minutes. This remarkable thermostability of the SM-L enzyme is undoubtedly due to high concentrations of protein in the secretion. (c) *SM-L-parotid saliva interaction*: Lysozyme quantitations were made in equimixtures of SM-L and parotid salivas (1:20 dilutions) of the 30 individuals previously mentioned. The results of these tests appear in Table I. The observed lysozyme titers of the SM-L-parotid saliva interaction were, in all but 3 cases, less than the calculated expected titers. A plot of each parotid lysozyme value *vs.* the corresponding difference between the observed and expected value of the SM-L-parotid saliva interaction revealed a positive correlation (Fig. 2), suggesting existence of a component in the SM-L saliva capable of selectively inhibiting parotid saliva lysozyme activity.

Kaiser(6) has shown lysozyme inhibitory powers in electronegative chain molecules containing sulfur, *e.g.*, heparin, sulphonated hyaluronic acid, and sulphonated carboxy cellulose. Mucoitin sulfate, a monosulfuric acid ester of hyaluronic acid, occurs as the prosthetic group associated with gastric and salivary mucin(7). Accordingly, the "mucopolysaccharide"‖ component of 4 ml of SM-L saliva was isolated by a modification of the method described by Winzler, Devor, Mehl and Smyth(8). By dry weight, 5.8 mg of the material was obtained. This material was suspended in M/15 phosphate buffer, pH 6.2, and added to parotid lysozyme reaction systems in concentrations ranging from 0.1 to 1.0 mg/ml of parotid saliva. The SM-L "mucopolysaccharide" inhibited parotid lysozyme activity in a linear fashion up to 0.4 mg/ml (Fig. 3). At the higher concentrations a consistent reduction of approximately 75% in parotid lysozyme activity occurred. The SM-L "mucopolysaccharide" exhibited no lysozyme activity *per se*.

Discussion. The difference in the group means of the parotid saliva lysozyme titers found in the 100 right gland tests and in the 30 mixed right and left gland tests was significant. The values in each group, and particularly in the right gland group, were abnormally distributed so that the means were

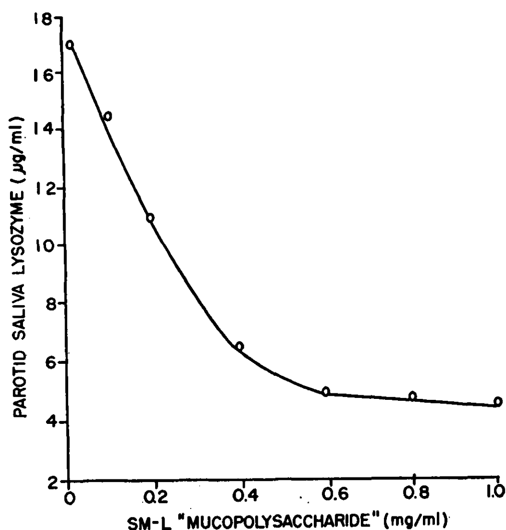

FIG. 3. The inhibitory effect of isolated "mucopolysaccharide" in submaxillo-lingual saliva on parotid saliva lysozyme activity.

‖ Mucopolysaccharide is placed in quotations since absolute identification and purity have not been determined.

not representative of the groups. Modal values, as determined by the Δ method(9), were essentially the same; right gland group 10.0 μg/ml and mixed right and left gland group 9.0 μg/ml. The apparent variability in parotid saliva lysozyme titers encountered in this study could be accounted for by the different methods of gland stimulation and/or by the ability of the parotid gland to produce lysozyme in varying amounts according to the particular nervous stimulation present. In the course of our work with SM-L-parotid saliva interaction, it was observed that heat inactivated SM-L saliva (90°C for 45 minutes) lost its ability to inhibit parotid saliva lysozyme activity. This finding would tend to discount an inhibition of a non-competitive nature. On the other hand, to call the SM-L "mucopolysaccharide" inhibition of the parotid lysozyme selectively competitive would imply greater specificity for the inhibitor than for the substrate in the *Micrococcus lysodeikticus* cells and this would be invalid without knowing more of the direct mechanism of the inhibitory reaction. Lysozyme activity in SM-L saliva does not appear to be inhibited by the "mucopolysaccharide" component present therein. In this regard, Lindley(10) draws attention to the fact that 2 enzymes, both requiring the same group for activity may nevertheless show marked differences in behavior toward the same inhibitor. It is probable that such a system exists in the SM-L-parotid saliva interaction. Recently, lysozyme has been implicated as a determinant in the integrity of mucous membranes; especially those of the alimentary canal(11). Meyer, Prudden, Lehman, and Steinberg found high titers of gastric and intestinal lysozyme occurring concomitantly with (and many times preceding) ulcerative gastrointestinal disease. Therefore, it is tempting to speculate upon the role of salivary lysozyme in the etiology and course of ulcerative gingival disease. A possibility exists that a diminished quality or quantity of salivary mucoid and/or an abnormally high parotid lysozyme titer could play an important part in the etiology of ulcerative gingival disease.

Summary. 1. Lysozyme titers in secretions of the submaxillary and sublingual salivary glands were found to be substantially higher than those in the parotid gland secretions. 2. Parotid saliva lysozyme titers were variable. Means of the groups tested were considered equivocal. 3. A component of submaxillo-lingual saliva, isolated as the "mucopolysaccharide" group, exerted marked inhibition upon lysozyme activity in parotid saliva. This inhibition was probably competitive in nature.

The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Navy, or the Naval Service at large.

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