Rheological and Transport Properties of Middle Ear Effusions from Cleft Palate Patients

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Middle ear effusions represent respiratory secretions which are physiologically important to functional *mucociliary transport systems.* Knowledge of middle ear mucus and methods of facilitating clearance continue to be important to cleft palate patients.

Middle ear mucus was collected from *cleft palate* children before surgical correction. After dialyzing and lyophilizing, this mucus was reconstituted at various nondialyzable solids (NDS) concentrations in Tris-Cl buffer. A physiochemical study was then undertaken since a mucociliary transport defect leading to serous otitis media is an etiologic possibility.

The *viscoelastic properties* of reconstituted middle ear mucus were determined using a magnetic microrheometer. The relationship to nondialyzable solids concentrations is described. The mucociliary transport rate as a function of NDS concentrations was recorded on the toad palate model. Results suggest a *maximum transport rate* at a specific NDS concentration. The viscoelastic properties also correlate well with the mucociliary transport rate. These may have clinical and theraputic relevance.

Introduction

It is estimated that at least 50 per cent of patients with cleft palates have an auditory deficit, most commonly of the conductive type (Bess et al., 1976; Bluestone, 1971; Caldarelli, 1975; Moller, 1975; Pannbacker, 1969; Paradise, 1975, 1976; Yules, 1975). An even greater percentage have otologic pathology, most frequently involving the tympanic membrane and middle ear (Bess et al., 1976; Bluestone, 1971; Caldarelli, 1975; Moller, 1975; Paradise, 1975; Yules, 1975). Middle ear effusions are universal in cleft palate infants (Bluestone, 1971; Koch et al., 1970; Paradise et al., 1969; Paradise, 1975; Soudijn and Haffstadt, 1975; Stool and Randall, 1967). This complication is so invariable that it has been found at one week of life (Paradise et al. 1969; Paradise and Bluestone 1969) and has even been hypothesized to be present antenatally (Paradise and Bluestone 1969).

Several investigators have suggested that production of middle ear effusions in cleft palate patients may be due to a dysfunction of the eustachian tube which prevents ventilation of the middle ear-mastoid system (Bennett et al., 1968; Cole et al., 1974; Dickson, 1975; Moller, 1975; Paradise, 1975; Yules, 1975). Bluestone (1971) proposes that dysfunction of the tensor veli palatini muscle results in functional obstruction of the nasopharyngeal end of the eustachian tube. Hypoventilation and negative pressure would

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then occur in the middle ear. This altered environment would cause middle ear mucosa abnormalities resulting in fluid accumulation and poor clearance (Buckingham and Ferrar, 1973; Sade, 1966; Sade et al., 1976). Impairment of clearance is characteristic of all types of secretory otitis media, not only of cleft palate. We have previously discussed possible mechanisms and factors in producing poor clearance (McCall et al., 1977) including, in particular, possible changes in the rheological (flow) properties of the effusions. Such changes, which may be due to altered physicochemical environment (pH, ionic strength) or biochemical composition of the macromolecular mucin components, have been shown to result in malfunction of mucociliary flow (Litt, 1970; Litt et al., 1976).

Mucus secretions, including those from trachea, cervix, salivary glands, and ear of the human and a number of other species which have been studied, contain high molecular weight glycoproteins (mucins) which are responsible for the physicochemical behavior (Litt et al., 1976). Rheologically, such secretions behave as viscoelastic fluids. That is, they possess both elastic behavior, characteristic of solids, and viscous behavior, typical of pure fluids. We have previously given an introduction to the behavior and modeling of such materials (Litt, 1973). Their behavior can be characterized in terms of two dynamic moduli: G' (storage modulus), which measures the elastic behavior, and G'' (loss modulus), which is a measure of viscosity. These parameters are not constant but depend on a flow rate or time of flow. Generally, this dependence is expressed in terms of a frequency. A detailed background has been published (Lutz et al., 1973) with reference to mucociliary flow.

Recent studies have shown that elasticity is essential to the flow of mucus in a ciliary propulsive system (Dulfano and Adler, 1975; King et al., 1974). Materials which have no gel (elastic) properties are not transported by cilia. We have previously shown that there exists an optimal elastic modulus for the transport of tracheal mucus (Shih et al., 1977) and that similar behavior is found using generalized disease middle ear effusions (McCall et al., 1977). In this paper, we extend these studies to mucus collected from patients with cleft palate suffering from secretory otitis media.

Materials and Methods

A. Collection and Preparation of Mucus Samples

Middle ear effusions were collected from 13 children (15 ears) with serous otitis media (SOM) immediately prior to reconstructive plastic surgery for cleft palate. At therapeutic myringotomy, mucus was removed from the middle ear under direct vision with a suction tip connected to a glass trap.

After collection, the samples were dialyzed against deionized water for 24 hours and lyophilized. The nondialyzable solids (NDS) content was determined by direct gravimetric methods. The lyophilized powder was stored at −20°C for later use.

For rheologic and mucociliary transport studies, samples were prepared by reconstituting dry mucus powder to predetermined NDS concentrations by addition of Tris-Cl buffer (0.10 M Tris, 0.05 M NaCl, pH 7.0) (McCall et al., 1977). Reconstituted mucus was stirred with a spatula twice daily and maintained at 4°C. After 36–48 hours, the mucus gelled as a hemogeneous solution and was ready for experimentation. The samples were used for both rheologic and transport studies.

For fractionation the mucus was reconstituted in Tris thiocyanate buffer and placed on a Sepharose 6B column (Wolf et al., 1977). The high molecular weight glycoprotein (mucin) fraction and the serum-like fraction were quantitated both spectrophotometrically in the column eluants and gravimetrically after dialyzing and lyophilizing.

Amino acid and sugar analysis was performed on the glycoprotein (mucin) fraction. The mucin was hydrolyzed in 6 N HCl at 110°C for 24 hours. The sample was then dried on a rotary evaporator at 45°C and reconstituted in 0.1 N HCl. A solution of sample applied to the chromatography column (Technicon Amino Acid Analyzer) was made with 0.2 ml reconstituted mucin sample mixed with 0.2 ml internal standard (norleucin) and 0.1 ml 62.5 per cent sucrose. A small amount (0.2 ml) of this solution, containing 0.18 mg hydrolyzed mucin, was applied to the chromatography column.

B. Rheologic Measurements

Viscoelastic measurements of mucus samples were made with a magnetic microrheometer (Lutz et al., 1973). In this instrument, a 100 micrometer iron sphere is oscillated in the mucus sample (0.1–0.2 ml) by application of a sinusoidally varying magnetic field gradient. The motion of the iron sphere is observed through an inverted light microscope and transduced into an electrical signal with an optical follower. The result is an output signal of the motion which can be compared in both amplitude and phase with the input force signal. A complete spectrum of response is obtained by varying the frequency of oscillation. Viscoelastic fluids will change both the amplitude and the phase of the output signal relative to the input signal.

Results of rheologic measurements are presented in terms of dynamic shear moduli G′ and G″ as a function of frequency, giving a spectrum of the elastic and viscous impedance characteristics of the sample. Details of calibration and calculations are available (Lutz et al., 1973). The instrument can reproducibly measure values of G′ and G″ on different aliquots of the same sample to 5 per cent.

C. Mucociliary Transport

Mucociliary transport was studied *in vitro* using a modification of the frog palate model of King et al. (1974). The upper palate of a 300–400 g marine toad (*Buffo marinus*)[1] was dissected free after pithing. The palate was placed in saline at 4°C for five hours before mounting in a constant temperature and humidity box (25°C and 100 per cent humidity). A stereomicroscope with a calibrated ocular micrometer was mounted over the palate, through which palatal ciliary activity and mucus transport could be observed. The field of view was fixed centrally in the mucociliary stream of the palate. The flickering light on the mucosal surface indicated continued ciliary activity.

Iron spheres (100 micrometers) were used as markers for mucociliary transport. The palate continued to secrete mucus and to transport for 24 to 30 hours. When the palate was mucus-depleted, no transport of markers occurred despite continued ciliary function. At this point, transport was dependent upon the addition of exogenous mucus. The ocular micrometer scale was graduated from zero to 10. Mucus samples were placed with a 25-gauge needle under direct vision at the zero point on the scale. The time interval for transport from six to 10 (4.4 mm) on the ocular micrometer was measured. Mucus samples of decreasing NDS were run in sequential order over the same ciliary transport track. Measurements for at least three runs on each sample were averaged to give an *in vitro* transport rate. Aliquots of the same samples were run at the beginning and end of each experimental session on a given palate. Significant change in ciliary activity marked by a greater than 10 per cent difference between beginning

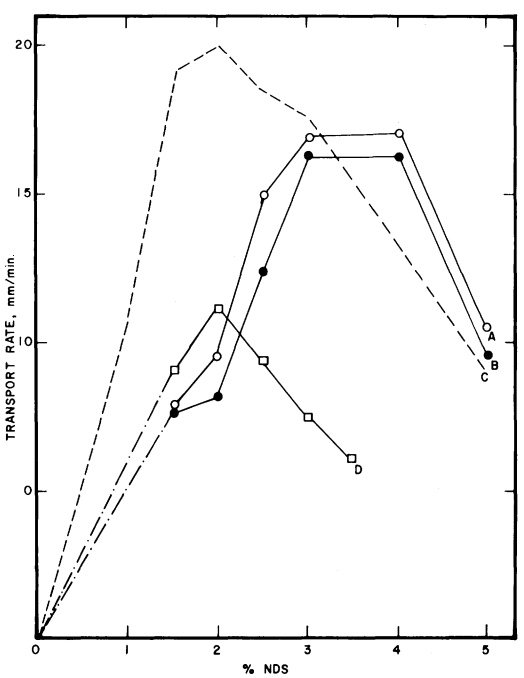

FIGURE 1. Transport Rate vs % NDS. A—Cleft palate mucus pool, toad palate 1; B—Cleft palate mucus pool, toad palate 2; C—SOM mucus pool, frog palate (ref 20); D—Canine tracheal pouch mucus, toad palate 3.

[1] In our previous studies, bullfrogs (Rana Cantesbiana) were used (McCall et al., 1977; Shih et al., 1977). These were not available at the time of these experiments and the toads were substituted. Previous investigators (King et al., 1974) had reported good results with both species.

and ending measurements necessitated the discarding of data collected on that palate.

Results

Figure 1 shows transport rate as a function of NDS content of various reconstituted mucus samples. Buffer solution was used as 0 per cent NDS, which showed no transport on a mucus-depleted palate. Curves A and B represent two separate toad palates on which the same samples of cleft palate mucus were run. Curve C is data from McCall et al., (1977) for transport rates of serous otitis media effusions on a bullfrog palate. Curve D is reconstituted canine tracheal pouch mucus transported on a toad palate.

Viscoelastic spectra were obtained for three, four, and five per cent NDS mucus samples. Figures 2 and 3 give the storage modulus (G′) and the loss modulus (G″) as a function of frequency. Also shown are the values for three per cent solution of secretory otitis media mucus from McCall et al., (1977). The cleft palate samples have much lower moduli than the equivalent concentrations of tracheal or cervical mucus (King et al., 1974; Lutz et al., 1973) and do not display a pronounced "plateau" region which is indicative of extensive entanglement couplings or temporary cross-links. For this reason also, reproducible rheologic measurements at concentrations below three per cent NDS were not possible because

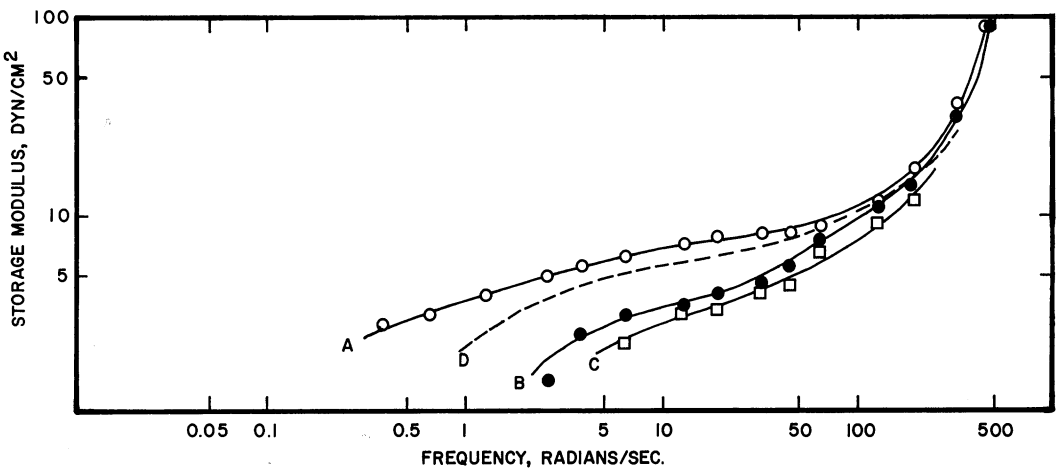

FIGURE 2. Storage modulus vs frequency. C—3% Cleft palate; B—4% Cleft palate; A—5% Cleft palate; D—3% SOM (ref 20).

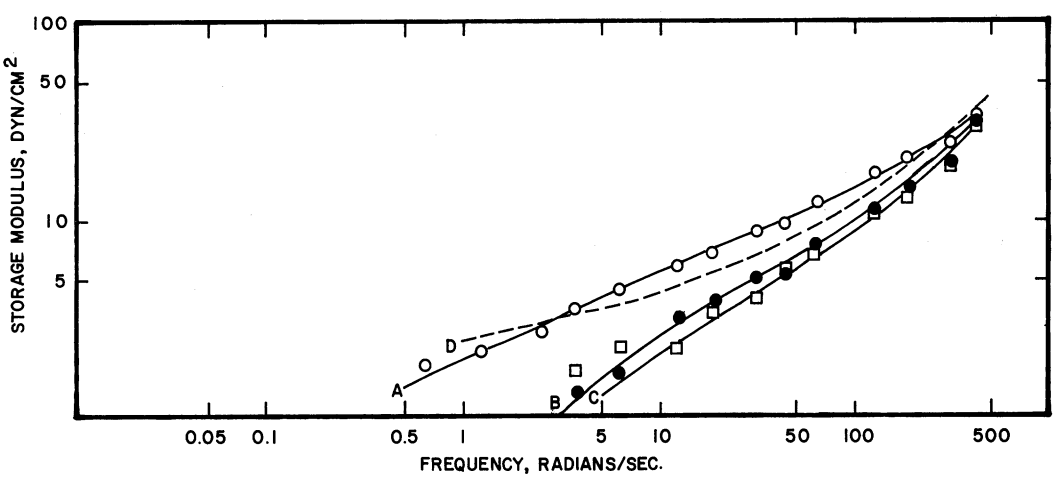

FIGURE 3. Loss modulus vs frequency. Same legend. C—3% Cleft palate; B—4% Cleft palate; A—5% Cleft palate; D—3% SOM (ref 20).

of settling of the iron sphere in the test chamber, also indicative of a very loosely cross-linked gel. G′ values for these solutions were estimated by extrapolation.

Fractionation of ear mucus revealed a Pool I (mucin or high molecular weight glycoprotein) concentration of 22.4 per cent and a Pool II of 77.6 per cent.

Table I lists the amino acid analysis of Pool I cleft palate middle ear effusions and effusions of secretory otitis media from our laboratory. Results are given as mole percentages. Pool I total amino acid content, glucosamine, and galactosamine are reported as weight percentages.

Discussion

The curves in Figure 1 demonstrate an increasing transport rate with increasing NDS content until a maximum is reached, after which the transport rate drops with increased solids content. The maximum for cleft palate mucus is approximately 3.5 per cent NDS. The maximum for SOM mucus and canine tracheal pouch mucus is 2 to 2.5 per cent (McCall et al., 1977; Shih et al., 1977).

Cleft palate mucus, like canine tracheal pouch and secretory otitis media mucus, showed a transport maximum at a specific NDS content (3.5 per cent). Therefore, NDS contents greater than 3.5 per cent will transport faster when diluted towards a 3.5 per cent NDS. NDS contents lower than 3.5 per cent will move slower when diluted.

This maximum was distinctly different from SOM (2 per cent NDS) or canine tracheal pouch (2.5 per cent NDS) mucus, occurring at a significantly higher NDS concentration. Based on mucin concentration, maximum transport occurs at 0.4 per cent in SOM mucin, 0.5 per cent in canine tracheal mucin, and 0.78 per cent in cleft palate mucin. Thus, almost double the concentration of CP mucin is required to obtain optimal ciliary transport, indicating that the "activity" of the mucin for transport is much less than that of the other types studied. This result is consistent with the rheological data. At 3 per cent NDS, cleft palate mucin is much less active rheologically than SOM (Figure 2).

In Figure 4 transport rate is plotted against G′ at 100 radians/sec. This frequency was chosen because it is the order of magnitude of ciliary beating frequency. The transport rate increases with increasing elastic modulus to a maximum rate at 8.5 dynes/cm^2, after which increasing elastic modulus results in decreasing transport rates. In SOM effusions this maximum rate is achieved at 5 dynes/cm^2 (McCall et al., 1977). In tracheal pouch mucus Shih et al. (1977) obtained an optimal transport at 10 dynes/cm^2. Thus it would

TABLE 1. Ear Mucin

	Mucoid		Cleft Palate	
	% (w/w)	% (m/m)	% (w/w)	% (m/m)
Aspartic Acid	2.96	7.21	0.904	8.13
Threonine	3.30	9.00	0.934	9.47
Serine	2.83	9.00	0.986	11.41
Glutamic Acid	4.08	9.00	1.206	9.95
Proline	1.91	5.39	0.747	7.88
Glycine	2.08	9.00	0.696	11.29
Amanine	2.20	8.12	0.406	5.58
Valine	2.60	7.20	0.652	6.67
Cystine	0.84	2.24	0.094	1.09
Methionine	0.49	1.07	0.156	1.21
Koleucine	1.31	3.25	0.484	4.49
Leucine	3.13	7.76	0.703	6.55
Tyrosine	1.87	3.35	0.305	2.06
Phehylalanine	2.18	4.32	0.520	3.88
Lysine	2.28	5.07	0.779	6.43
Histidine	1.55	3.25	0.505	3.88
Argenine	3.08	5.75	N.D.	
Galactosamine	8.85		1.144	
Glucosamine	4.53		0.622	
Total Protein Recovered %	38.77		10.077	

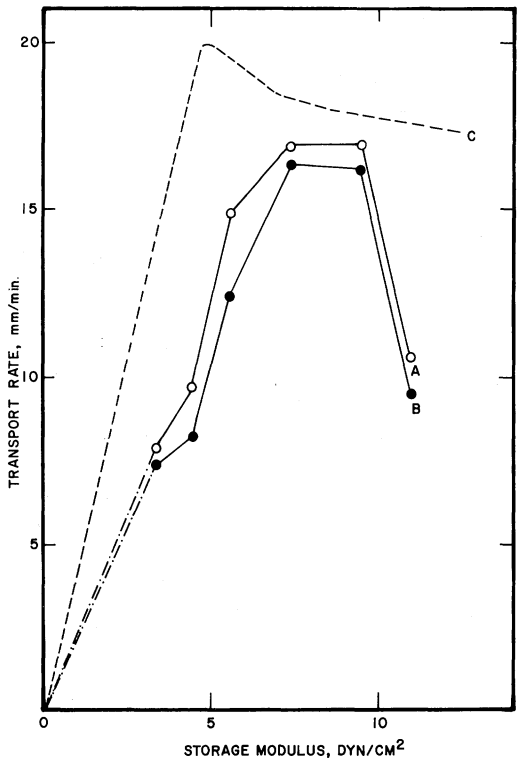

FIGURE 4. Transport rate vs storage modulus. A—Cleft palate, toad palate 1; B—Cleft palate, toad palate 2; C—SOM, frog palate (ref 20).

appear that the criterion for optimal transport is that the solution must have a G′ in the range of 5–10 dynes/cm^2. This value may occur at different mucin concentrations depending on the activity of the specific mucin. Cleft palate mucin appears to be relatively inactive requiring a much higher mucin concentration to achieve the necessary elasticity.

The chemical composition data of Table I provide some insight into this result. Although on a molar basis SOM and cleft palate mucin have similar amino acid compositions, the cleft palate mucin has a much lower hexosamine content, which should result in lower rheological activity. Also, for reasons not yet clear, a much lower weight of protein was recovered from cleft palate mucin. Usually the mucins are 25–40 per cent protein, but only 10 per cent was recovered for the cleft palate mucin pool.

The source of these differences is not certain at this time. The results indicate significant difference between the cleft palate pool and SOM pool which may be due to the different disease states. The SOM samples were of consistently higher (4.3–50 per cent) solids content indicating fewer serous contributions. The lower solids content of cleft palate samples (4.2–16.5 per cent) may reflect a higher serous component, with fewer glycoproteins, in this situation. In both cases, however, the concentrations are significantly beyond those needed for optimal clearance, so that these results are consistent with the hypothesis that impaired clearance is a major factor in the retention of ear effusions.

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