# PAROTID SECRETIONS IN CYSTIC FIBROSIS

## EFFECT ON ZETA POTENTIAL OF TRACHEAL MUCUS

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SUMMARY: Parotid saliva of cystic fibrosis patients demonstrates a significantly different effect on zeta potential of mucus than that of normals. The effect is not due to high electrolyte levels found in cystic saliva. A long time increase in zeta potential is probably due to a high α-amylase content of cystic saliva;however a significant initial decrease is due to adsorption of a basic polyelectrolyte on the glycoprotein substrate. This factor may be the same as those identified by other investigators in cystic serum and secretions.

Serum and secretions obtained from patients with cystic fibrosis (CF) are reported to contain specific factors which can be distinguished from those of normal subjects by various biological tests. Mangos and his co-workers(1) have shown that CF sweat and saliva contain a factor which interferes with sodium reabsorption in the parotid gland of the rat. Spock(2) and Bowman(3) have shown that CF serum interferes with the beating of ciliary explants. It is also well known that saliva and sweat from CF patients contain an abnormally high electrolyte content, particularly sodium chloride, as well as a relatively high organic content including components of enzymic nature(4). The relationship of these facts to the observation that bronchial secretions in CF are abnormally viscid and elastic is unclear at this time(5).

We have conducted a number of in vitro experiments based on the hypothesis that there is a relationship between the factors in CF secretions and the abnormal character of the mucus in CF.

The experiments are designed to ascertain whether addition of salivary secretions from CF patients to a substrate containing tracheal mucus would behave differently from that of normals. The approach taken was based on the expectation that any such effect would originate from an increase in agglomeration of the polymeric mucus material; therefore the primary measurement made was to follow the change of the zeta potential of the solution with time. The zeta potential, which is related to the electrophoretic mobility, reflects such factors as the shape of the molecule and the charge interactions between the molecule and the solvent. That is, changes in the molecular structure which should lead to decreased repulsion between charged polyelectrolyte molecules or be due to shape changes of the molecule should be reflected in changes of the zeta potential.

METHODS

The test colloid used was lyopholized tracheal mucus obtained by the pouch technique from healthy beagles(6). This material is sterile, essentially cell free and after lyopholization can be kept indefinitely at 5°C. Twenty milligrams of this material is suspended in 100 ml of phosphate buffer (.025M $Na_2 HPO_4$; .025M $KH_2 PO_4$; .1M NaCl) at pH 8. The test solution consisted of 10 ml of the dog mucus solution, 9.8 ml of the phosphate buffer and 0.2 ml of the test secretion, for a total volume of 20 ml. The electrophoretic mobility was measured in microelectrophoresis cell equipped with molybdenum and platinum electrodes (Zeta Meter, Inc., New York), and the zeta potential calculated from the Helmholtz-Smoluchowski equation(7). The zeta potential was followed with time for a period of approximately 90 minutes after which it usually attains a steady value. Samples of parotid saliva were obtained from patients and subjects using a Kerby

cup (Drummond Scientific Co., Broomall, Penna.). Salivary flow was stimulated using a colored Life-Saver; the clarity of the resulting secretion demonstrated that only material from the parotid duct was collected. Samples from CF patients and from heterozygotes were obtained at the CF Clinics of the Children's Hospital of Philadelphia and Hahnemann Hospital, as well as from patients at the Allergy Clinic of Children's Hospital.

## RESULTS AND DISCUSSION

Typical results are shown in Fig. 1. The CF samples show an initial rapid decrease in the zeta potential followed by a slow increase in constant value after 90 minutes. In a very few cases the initial decrease was not observed but the zeta potential continuously increased to its final value. The final increase in the zeta potential of the CF samples was unexpected. Normal samples show a small initial decrease which thereafter usually remains constant. Curves of heterozygotes have the same general shape as that of the cystics, although the initial decrease is smaller and more like the normal.

To date a total of 24 samples from cystics have meen measured; 19 of these follow the general behavior shown, two did not show the initial decrease and three did not show a final increase although they did show the initial decrease. Eight normal samples have been run, with results similar to that shown. The eleven heterozygotes studied gave more inconsistent results- seven resembled the data in Fig. 1C; the others more closely resembled the normal. Results for five allergy clinic patients were indistinguishable from the normals.

Since the most significant characteristic of the curves was the reduction in zeta potential after five minutes and the change after 90 minutes, these two parameters have been analyzed statis-

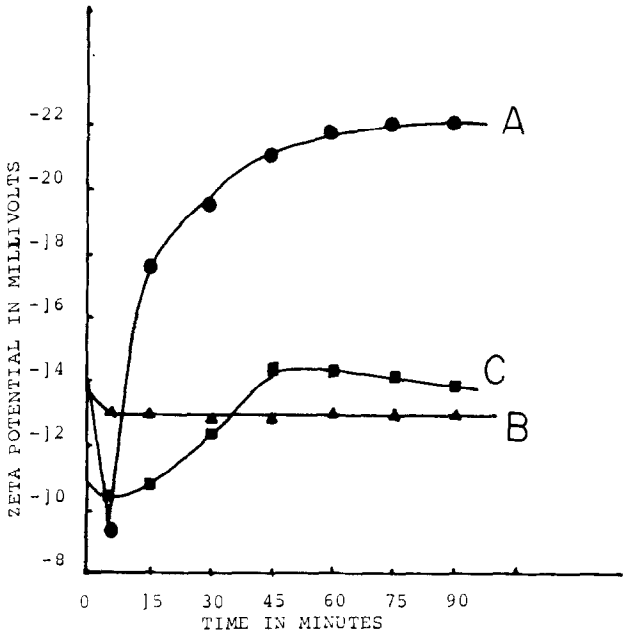


Figure 1

Time Response of substrate zeta potential to added Parotid Secretion. A, cystic fibrosis; B, normal; C, heterozygote.

tically with results given in Table 1. For the 5 minute change, ($\Delta\zeta_5$) the difference in the means of the cystics and normals is just significant at the 95% level whereas the normals and heterozygotes cannot be distinguished. The change after 90 minutes ($\Delta\zeta_{90}$) is more striking since the cystics have all increased whereas the normals have all decreased. The difference between cystics and normals is significant at the 99% level. Although the difference between heterozygotes and normals is not as striking, the heterozygotes are significantly different from the normals at the 95% level. Table 1 also shows results of tests which demonstrate that the behavior observed is not due to the increased electrolyte levels of CF saliva. However, addition of several commercial enzymes showed that several, including trypsin and in particular α-amylase, produced an in-

Table 1

Per Cent Change in Zeta Potential Upon Addition of Saliva and Various Reagents

| Material Added | $\Delta\zeta_5/\zeta_i$ | s | $\frac{ts}{\sqrt{n}}$ | $\Delta\zeta_{90}/\zeta_i$ | s | $\frac{ts}{\sqrt{n}}$ |
|---|---|---|---|---|---|---|
| CF Saliva (19) | -22.04 | 13.2 | 6.4(P=.05) | 36.1 | 19.5 | 13.0(P=.01) |
| Normal Saliva (7) | -12.1 | 7.0 | 6.8(P=.05) | -12.1 | 5.0 | 7.5(P=.01) |
| Heterozygotes (7) | -12.4 | 9.8 | 9.2(P=.05) | 26.9 | 22.4 | 21.7(P=.05) |
| .5M NaCl | - 1.2 | - | - | 0 | - | - |
| .5M KCl | 0.6 | - | - | - 1.2 | - | - |
| .05 $CaCl_2$ | - 4.2 | - | - | - 2.4 | - | - |
| Normal Saliva and 0.5M NaCl | - 5.8 | - | - | - 6.7 | - | - |
| Normal Saliva and 0.5M KCl | - 2.5 | - | - | - 7.5 | - | - |
| Normal Saliva and 0.05 M $CaCl_2$ | - 6.6 | - | - | - 9.1 | - | - |

$\Delta\zeta_5/\zeta_i$ = change in zeta potential after 5 minutes X 100/initial zeta potential

$\Delta\zeta_{90}\ \zeta_i$ = change in zeta potential after 90 minutes X 100/initial zeta potential

s = standard deviation for number of samples (n) given in parenthesis in first column

t = student's t for probability level given in parenthesis

In first six series of experiments, 0.2 ml of test material was added to 10 ml of substrate plus 9.8 ml of buffer. In last 3 experiments, 0.2 ml of saliva and 0.2 ml of electrolyte were added to 10 ml of substrate and 9.6 ml of buffer, for total of 20 ml in all cases.

crease in zeta potential similar to that observed with CF saliva.

Addition of chloranil, a known inhibitor of α-amylase(8) to the secretion, completely eliminated the $\Delta\zeta_{90}$ effect. The Chloranil inhibited curves resemble those for normals, except that $\Delta\zeta_5$

is much larger for the cystics. In five samples the decrease in zeta potential ranged from 22.3% to 30.7% of the initial value. In addition, in all cases the $\Delta\zeta_5$ when inhibited was greater than in the uninhibited run for the same sample. Runs with normal saliva showed no significant effects of chloranil on the zeta potential curves.

These data support the conclusion that the zeta potential behavior noted with CF saliva is controlled by two effects: an initial adsorption of a cationic material on the substrate leading to a decrease in zeta potential, and a simultaneous breakdown of substrate by α-amylase or other enzymes. This conclusion is also supported by the observation that the $\Delta\zeta_{90}$ effect is lost on boiling, and that contact with glass reduces the $\Delta\zeta_5$ effect. The tests show that cystic saliva contains significantly greater amounts of both the cationic basic material and of the enzyme.

Whether the factor causing the zeta potential decrease is the same as those of Mangos and Spock remains to be established. However, curves shown in Fig. 2 appear to lend credence to this possibility. The substance used, polyethyleneimine (PEI), is a basic polymer that has been demonstrated by Mangos to mimic the cystic fibrosis factor(1). We observe that a mixture of PEI and α-amylase behaves in very similar fashion to the CF saliva, and that inhibition of the enzyme by chloranil addition gives results quite similar to those obtained with inhibited CF saliva. The extent of decrease in zeta potential induced by PEI is a function of its concentration. The zeta potential reduction measured with chloranil-inhibited cystic saliva should therefore serve as an assay for the adsorbed cationic material which may possibly be identical to Spock and Mangos' factor. Bowman, McCombs and Lockhart(9) have characterized the ciliary inhibition

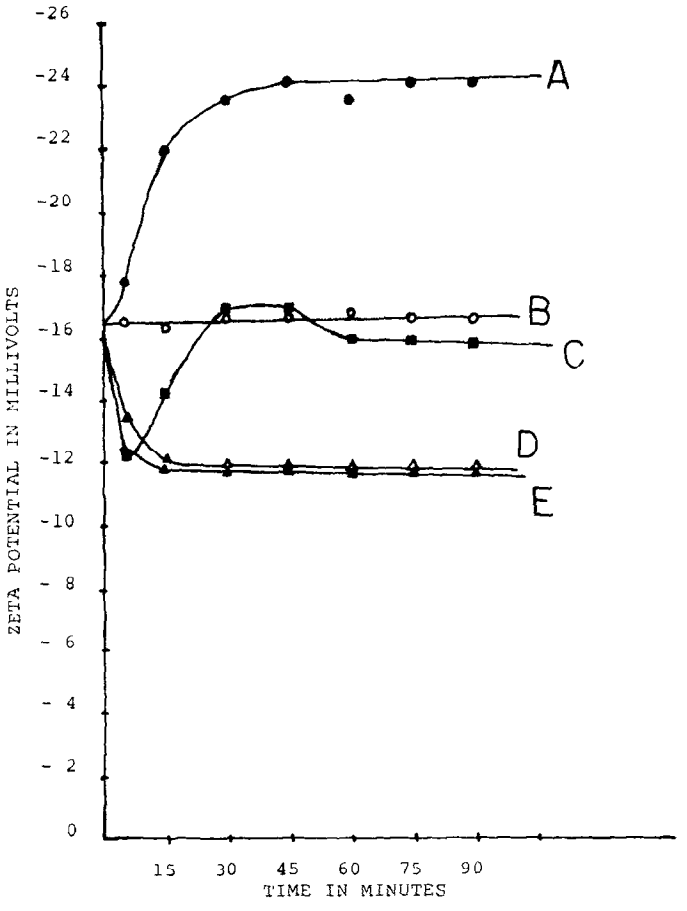


Figure 2

Time Response to substrate zeta potential to various Added Reagents. A, α-amylase, .0037 mg/ml; B chloranil, 0.5 x $10^{-6}$ M; C, polyethyleneimine 1.56 x $10^{-5}$ mg/ml and α-amylase, .0037 mg/ml; D, polyethyleneimine, 1.56 x $10^{-5}$ mg/ml; E, polyethyleneimine 1.56 x $10^{-5}$ mg/ml, α-amylase, .0037 mg/ml and chloranil, 0.5 x $10^{-6}$ M.

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factor and shown it to be a cation with 125-200,000 molecular weight, which would agree well with our observations.

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