Effect of cystic fibrosis and non-cystic fibrosis plasma on the movement and retention of $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$ in guinea-pig stomach and small intestine

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SUMMARY The effect of cystic fibrosis plasma on the net fluxes of $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$ across the guinea-pig stomach and small intestine was investigated, using an automatic short-circuit current apparatus.¹⁰ A significant increase in net fluxes across the stomach and small intestine for $^{45}\text{Ca}^{2+}$ in the presence of cystic fibrosis plasma compared with non-cystic fibrosis plasma was observed. There was an increase in net flux for $^{35}\text{SO}_4^{2-}$ across the stomach in the presence of cystic fibrosis plasma when compared with non-cystic fibrosis plasma. However, there was a more highly significant increase in net fluxes for $^{35}\text{SO}_4^{2-}$ across the small intestine in the presence of cystic fibrosis plasma when compared with non-cystic fibrosis plasma. The amount of $^{45}\text{Ca}^{2+}$ activity retained by the stomach and small intestine is more highly significant in the presence of cystic fibrosis plasma than in the presence of non-cystic fibrosis plasma. The retention of $^{35}\text{SO}_4^{2-}$ activity by the stomach and small intestine in the presence of cystic fibrosis plasma when compared with non-cystic fibrosis plasma was also highly significant. These findings indicate that cystic fibrosis plasma increases the net fluxes and raises retention of $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$ in guinea-pig stomach and small intestine.

Cystic fibrosis is one of the most common genetic diseases among Caucasian children and young adults. The disease manifests itself as a generalised disorder primarily affecting exocrine glands and the pulmonary and gastrointestinal system. Although the basic molecular defect is not known, a secretory dysfunction has been assumed to be an explanation for the pathogenesis of the disease.¹

Morrissey and Tymvios² have shown that there is an increase in sulphated glycoproteins from the duodenum to the ileum in cystic fibrosis patients. In addition, the intracellular distribution of these sulphated glycoproteins has been shown to be more concentrated towards the tips of the villi than crypts.² It has also been demonstrated that, in the presence of the cystic fibrosis plasma, there is an increase of sulphate uptake and retention by the frog's gastric mucosa.³

There are viscosity changes in cystic fibrosis mucus but raised sulphated glycoproteins alone

may not be the cause of this stickiness. An interaction between inorganic ions, such as Ca^{2+} , Mg^{2+} , Zn^{2+} , and Mn^{2+} , and mucus glycoproteins could result in mucus becoming viscous and impermeable to water and other ions. This imbalance of the secreted mucus would then change the functional and rheological properties.⁴⁻⁶

Recent evidence in the isolated hen trachea has shown that one type of goblet cell is more sensitive to external Ca^{2+} , resulting in an increase in the rate of mucus secretion.^{7,8} Furthermore, in the presence of high Ca^{2+} concentration, a sulphated mucus component is released by goblet cells.⁹

The cellular control mechanism responsible for electrolyte, water, and macromolecular secretion in exocrine tissue is not fully understood. In particular, very little information is presently available regarding the molecular events involved in the secretion of mucus.¹ We have used guinea-pig isolated tissue to follow the effect of cystic fibrosis and non-cystic fibrosis plasma on the movement and retention of $^{35}\text{SO}_4^{2-}$ and $^{45}\text{Ca}^{2+}$. The method uses a modified Ussing chamber and an automatic short-circuit current apparatus.¹⁰

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Methods

The cystic fibrosis plasma was obtained from cystic fibrosis patients. The non-cystic fibrosis plasma was obtained from patients with cardiothoracic diseases and other forms of pancreatic insufficiency, who were proven non-cystic fibrosis patients. Both the cystic fibrosis and non-cystic fibrosis plasma were kept at -4°C . All the patients were on normal hospital diets.

Radioisotopes, $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$, were obtained from the Radiochemical Centre, Amersham. $^{45}\text{Ca}^{2+}$ was obtained as calcium chloride in aqueous solution. The solution was diluted to 1 mCi/ml for the experiment. $^{35}\text{SO}_4^{2-}$ was obtained as an anhydrous salt of sodium sulphate. The salt was diluted to 1 mCi/ml for the experiment. Guinea-pigs (180–200 g) were allowed free access to a standard diet and water, and kept in the animal house. They were killed by a sharp blow to the neck. Samples of both stomach and small intestine were removed from the same animal for the various flux experiments.

GUINEA-PIG STOMACH

The stomach was removed and rinsed with Ringer solution maintained at 37°C . The stomach was carefully stripped of the outer muscular coat⁹ and the mucosa with its thin layer of muscularis mucosa was clamped between two halves of a modified Ussing chamber (Fig. 1). Both sides of the mucosa were bathed with 9 ml Ringer solution containing 110 mM NaCl, 5.0 mM KCl, 3.6 mM $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 1.2 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 26.0 mM NaHCO_3 , and 16.7 mM glucose, and was bubbled with 95% O_2 and 5% CO_2 . The pH of the bathing Ringer was pH 7.4. The modified Ussing's chamber had a water jacket with water circulating at 37°C which was pumped from a water bath. The whole chambers were then connected to a pre-calibrated

automatic short-circuit current (scc) apparatus.¹⁰

To study the net fluxes, at time zero, 200 μCi $^{45}\text{Ca}^{2+}$ was added to the serosal side of the preparation in the first chamber (A) and 200 μCi $^{45}\text{Ca}^{2+}$ to the mucosal side of the preparation in the second chamber (B) (giving the unidirectional serosal-to-mucosal and mucosal-to-serosal fluxes). 100 μl samples were collected every 15 minutes from the mucosal side of the first chamber (A) and from the serosal side of the second chamber (B). After 60 minutes 0.5 ml cystic fibrosis or non-cystic fibrosis plasma was added to the serosal side of the first chamber (A) (to give a final dilution of 1:10 ml) and 0.5 ml to the mucosal side of the second chamber (B), to study its influence on the unidirectional serosal-to-mucosal and mucosal-to-serosal fluxes. The net flux of tissue pair is the arithmetical difference between the unidirectional mucosal-to-serosal and serosal-to-mucosal fluxes.

At the end of 240 minutes the tissues were removed from the chambers and digested in 2 ml NCS tissue solubiliser (Amersham). The samples so obtained were counted in 10 ml scintillation fluid in the ^{14}C channel of Packard liquid scintillation spectrometer. The oxygenated preparation remains in good experimental condition for more than four hours. This is shown by the short-circuit current trace, which remains unaltered.

GUINEA-PIG SMALL INTESTINE

Isolated segments of guinea-pig small intestine taken from the region of the intestine distal to the hepatopancreatic duct were used. They were mounted without removing the muscular coat as flat sheets between the two halves of the modified Ussing chamber. The net flux studies of $^{45}\text{Ca}^{2+}$ were carried out as described above for stomach.

In a separate series of experiments 200 μCi $^{35}\text{SO}_4^{2-}$ was used to study its net fluxes in stomach

Fig. 1 Diagrammatic representation of the modified Ussing chamber, showing the water jacket around the chamber.

and small intestine, using the same experimental procedure as described previously for $^{45}\text{Ca}^{2+}$.

Results

The results of net fluxes for $^{45}\text{Ca}^{2+}$ are shown in Fig. 2A and B. No significant changes were observed in net flux of $^{45}\text{Ca}^{2+}$ in the presence of 0.5 ml non-cystic fibrosis plasma (whole plasma) for both stomach ($n=5$) and small intestine ($n=5$).

Fig. 2 Graphs of net $^{45}\text{Ca}^{2+}$ fluxes across (A) guinea-pig stomach expressed as $\mu\text{mol}/\text{cm}^2/15\text{ min}$; (B) guinea-pig small intestine expressed as $\mu\text{mol}/\text{cm}^2/15\text{ min}$. In the presence of cystic fibrosis and non-cystic fibrosis plasma. The net fluxes across the stomach were from serosal-to-mucosal and in the small intestine from mucosal-to-serosal.

In the presence of cystic fibrosis plasma there was a significant increase in net fluxes of $^{45}\text{Ca}^{2+}$ in the stomach ($n=5$) ($P < 0.01$) and a more significant increase in net fluxes for the small intestine ($n=5$) ($P < 0.001$) when compared with net fluxes in the presence of non-cystic fibrosis plasma.

Figure 3A and B shows no significant difference in retention of $^{45}\text{Ca}^{2+}$ for both stomach ($n=5$) and small intestine ($n=5$) in the presence of non-cystic fibrosis plasma. There is, however, a significant difference in the amount of $^{45}\text{Ca}^{2+}$ retained at the end of the experiment by stomach (A) ($n=5$) and small intestine (B) ($n=5$) when 0.5 ml of cystic fibrosis plasma was present, when compared with the retention in the presence of non-cystic fibrosis plasma ($P < 0.001$).

The effects of 0.5 ml of cystic fibrosis and non-

Fig. 3 Histograms showing the retention of $^{45}\text{Ca}^{2+}$ by (A) guinea-pig stomach expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$; (B) guinea-pig small intestine expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$. In the presence of cystic fibrosis and non-cystic fibrosis plasma.

cystic fibrosis plasma on the net fluxes of $^{35}\text{SO}_4^{2-}$ in both the stomach and small intestine are shown in Fig. 4A and B. In the presence of cystic fibrosis

Fig. 4 Graphs of net $^{35}\text{SO}_4^{2-}$ fluxes across (A) guinea-pig stomach expressed as $\mu\text{mol}/\text{cm}^2/15\text{ min}$; (B) guinea-pig small intestine expressed as $\mu\text{mol}/\text{cm}^2/15\text{ min}$. In the presence of cystic fibrosis and non-cystic fibrosis plasma. The net fluxes across the stomach were from serosal-to-mucosal and in the small intestine from mucosal-to-serosal.

plasma there was an increase in net fluxes for stomach ($n=5$) ($P>0.01$) and a more significant increase in net fluxes for the small intestine ($n=5$) ($P<0.001$) when compared with net fluxes in the presence of non-cystic fibrosis plasma. No significant changes in net fluxes were observed for both stomach ($n=5$) and small intestine ($n=5$) in the presence of non-cystic fibrosis plasma.

Fig. 5 Histograms showing the retention of $^{35}\text{SO}_4^{2-}$ by (A) guinea-pig stomach expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$; (B) guinea-pig small intestine expressed as $\mu\text{mol}/\text{cm}^2/\text{h}$. In the presence of cystic fibrosis and non-cystic fibrosis plasma.

Figure 5 shows the amount of $^{35}\text{SO}_4^{2-}$ retained at the end of the experiment by stomach (A) and small intestine (B). No significant difference was observed for either stomach ($n=5$) or small intestine ($n=5$) in the presence of non-cystic fibrosis plasma. However, a significant increase in retention was seen for both stomach ($n=5$) and small intestine ($n=5$) in the presence of cystic fibrosis plasma when compared with the retention in the presence of non-cystic fibrosis plasma ($P<0.001$).

There was also a significant difference in the amount retained by the stomach ($n=5$) and small intestine ($n=5$) in the presence of cystic fibrosis plasma.

Discussion

Di Sant'Agnese¹¹ emphasised the importance of investigating a possible relationship between cystic fibrosis plasma and the mucus abnormality ob-

served in cystic fibrosis. Earlier work by Farber¹² also indicated that a possible defect may be in the secretion of mucus in cystic fibrosis patients. The importance of the influence of whole cystic fibrosis plasma on the transport and retention of $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$ which is reported here may contribute towards a better understanding of some aspects of exocrine biology with special reference to cystic fibrosis.

It has been postulated by many workers that active transport of calcium is a physiologically significant mechanism in the intact animal. Calcium can also be absorbed by passive diffusion in the small intestine down a concentration gradient, from the intestinal lumen to the blood stream.¹³⁻¹⁷ Schacter *et al.*¹⁸ have suggested that at least two steps appear to be involved in calcium transport. The first is the relatively rapid absorption of calcium from the fluid bathing the mucosal surface. This process may result from the binding of calcium ions to anionic receptor sites on or in the mucosal cells and the binding may be influenced by the concentration of extracellular K^+ . The second step is the subsequent transfer of calcium to the fluid bathing the serosal surface, a transfer which may be complex and involve several processes. Accumulation at the serosal surface is relatively slow and represents the rate-limiting step in the transfer across the intestinal wall.

Schacter *et al.*¹⁸ using rat everted gut-sac preparation, have reported that each gut-sac in the experiment absorbed a maximum of $0.4 \mu\text{mol}$ calcium from the outside medium, and the absorption was completed in one hour. In contrast, only $0.2 \mu\text{mol}$ calcium was transferred to the inside medium in one hour and this transfer continued for three hours to result finally in the accumulation of $0.3 \mu\text{mol}$ calcium in the fluid bathing the serosal surface.

Our net flux result of $0.4 \pm 0.04 \mu\text{mol}/\text{cm}^2/15$ min in the small intestine (Fig. 2B) in the presence of non-cystic fibrosis plasma shows parallel similarities with these results. In the small intestine the presence of cystic fibrosis plasma increases net flux to a value of $0.98 \pm 0.8 \mu\text{mol}/\text{cm}^2/15$ min. This is a significant increase and can be seen in Fig. 2B. There is little published work on calcium transport in the stomach. This net transport is significantly greater in the presence of cystic fibrosis plasma when compared with the net transport in the presence of non-cystic fibrosis plasma. The cystic fibrosis plasma may have an influence on one or both of the steps involved in the active transport of calcium, suggested by Schacter *et al.*¹⁸

Very little has been reported on the mechanism

of SO_4^{2-} transport across the guinea-pig small intestine and the stomach. Also the interaction of Ca^{2+} and SO_4^{2-} transport has not been fully described. The fact that the net fluxes of Ca^{2+} (Fig. 2B) and SO_4^{2-} (Fig. 4B) across the small intestine are virtually similar would suggest that SO_4^{2-} could have a similar transport mechanism as reported for Ca^{2+} in the small intestine. It has been suggested that the interaction between these two ions could in some way result in morphological changes of the mucus glycoprotein.⁶ Other ions such as Zn^{2+} , Mn^{2+} , and Mg^{2+} have been shown to reduce the solubility of mucins.⁶

The other interesting finding is the significantly greater amounts of both $^{45}\text{Ca}^{2+}$ and $^{35}\text{SO}_4^{2-}$ retained by the stomach and small intestine in the presence of cystic fibrosis plasma when compared with non-cystic fibrosis plasma ($P < 0.001$). Recent evidence^{7,8} from studies using the isolated hen's trachea suggest that Ca^{2+} could play an important role in regulating secretion of mucus glycoproteins. Some goblet cells are more sensitive to Ca^{2+} than others. It has also been suggested by Kent and Anderson⁷ that in cystic fibrosis patients leakage of calcium from adjacent systems could increase the mucus secretion by the goblet cells. Increase in Ca^{2+} retention by the small intestine and the stomach and also increases in the net fluxes observed suggest that an alteration in intracellular calcium levels and calcium transport activity, may, in general, have a number of implications that may explain some of the manifestations of the disease.

From the results obtained in this investigation it has been shown that the retention of labelled sulphate by the stomach and small intestine was increased under the influence of cystic fibrosis plasma. As cystic fibrosis plasma increases the uptake of sulphate by the tissue, it is possible that it could also increase the incorporation of sulphate into mucus glycoproteins and explain the increased level of intracellular sulphated glycoproteins found in the small intestine of cystic fibrosis patients.²

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