J. Pharm. Pharmacol. 1991, 43: 124–127
Communicated September 17, 1990

Electrochemical determination of the permeability of porcine mucus to model solute compounds

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Abstract—An electrochemical approach to the determination of permeability through native mucus gel of simple electrochemically active solutes is reported. For all the the solutes studied, a reduction in effective diffusion coefficients was observed, with retardation of solute flux by a factor of at least two. However, NADH and the dicarboxylic acid derivative of ferrocene demonstrated a substantial, almost ten-fold, reduction in permeability through mucus. Results for the controls were in reasonable agreement with literature values where available. No consistent effect of molecular weight was evident with regard to the barrier properties of mucus over the molecular weight range of solutes investigated (34–660 daltons). The results suggest that mucus is acting more than as a gel support for an unstirred water layer.

Mucus forms a continuous adherent gel layer over intestinal surfaces. In particular, it constitutes an extracellular barrier to the diffusion of nutrients and therapeutic agents to the absorptive epithelial surface. The thickness of the gel has been estimated to be 100–500 μm (Kerss et al 1982; van Hoogdalem et al 1989) and its key gel forming constituent has been considered to be a 2×10^6 dalton glycoprotein subunit (Carlstedt & Sheehan 1984) with a high (70% w/w) carbohydrate component (Allen 1978), mainly occuring as oligosaccharides and confined to certain regions along the protein core of the macromolecules. Rheological properties are important for the action of mucus as lubricant, mixing barrier and particle trap, and these have been the focus of much recent attention (Meyer et al 1975; Crowther et al 1984). There is less information available about mucus as a diffusion barrier, and some controversy exists as to whether mucus stabilizes an unstirred water layer (DeSimone 1982; Morris 1985), or could present an additional, more potent, and perhaps more selective barrier to the transfer of low molecular weight nutrients and drugs in the small intestine (Smithson et al 1981).

Peppas et al (1984) have developed a theory for solute diffusion in intestinal mucus, which takes into account the concentration of the constituent glycoprotein, size of diffusing species and density of macromolecular crosslinks. However, direct practical observation is necessary to determine the influence of mucus upon the diffusion of specific solutes. There are some indications that the diffusion of certain species such as the ergot alkaloids (Nimmerfall & Rosenthaler 1980), aminoglycosides (Niibuchi et al 1986) and some other antibiotics (Cheema et al 1986; Kearney & Marriot 1987) is significantly retarded.

Here we report an electrochemical approach to the determination of flux through native mucus gel, of simple electrochemically active inorganic, organic and organometallic species.

Theory

The application of a polarizing voltage at noble metal working electrodes leads to surface decomposition of an appropriate electrochemically active solute, with the resulting development of solute concentration gradient through to the bulk solution.

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Provided a stable, unstirred diffusion layer can be created over the electrode surface, a stable flux of solute results, with the generation of a stable, steady state current as described by the Cotterell equation:

$$i_{ss} = nFAf \quad (1)$$

where i_{ss} is the steady state current, n is the number of electrons transferred, F is the Faraday constant, A the electrode surface area, and f the solute flux. By incorporating Fick's first law of diffusion

$$i_{ss} = \frac{nFAD_eC}{d_m} \quad (2)$$

where D_e is the effective solute diffusion coefficient, C the bulk solute concentration and d_m the membrane thickness; an estimate of effective diffusion coefficient can be obtained based on steady state current measurement.

An expression can also be derived (Bowers & Wilson 1958) which relates the current at time t (i_t) following an incremental jump in bulk solute concentration. A simplified version (Chien et al 1973) of this gives

$$i_t = i_{ss}[1-2\exp(-\pi^2 D_e t/d^2)] \quad (3)$$

A plot of ln $(i_{ss}-i_t)$ against t should give a slope of $\pi^2 D_e/d^2$ which permits assessment of whether the system obeys the simple unidirectional diffusion governed by Fick's law.

Materials and methods

Reagents. Hydrogen peroxide, uric acid, sodium ascorbate, paracetamol and catechol of Analar grade were purchased from BDH Chemicals (Poole, UK). The remaining chemicals of highest grade available were purchased from Sigma, MO, USA. Track etched porous polycarbonate membranes (1·0 μm pore size) were obtained from Nuclepore (Pleasonton CA, USA). An isotonic phosphate buffer was used consisting of (g L^{-1}): NaH_2PO_4 2·44, Na_2HPO_4 7·5, NaCl 3 and EDTA 0·6, pH 7·4.

Porcine gastric mucus was obtained from an abattoir using animals immediately after slaughter. The stomach was opened, the luminal surface washed with water and the gel collected by scraping the intact mucosal surface with a spatula (Williams & Turnberg 1980). The mucus obtained was frozen at −20°C until required for electrode experiments.

Experiments on HCl diffusion through fresh and frozen porcine gastric mucus gave similar responses over the entire pH range (unpublished work). Membrane laminates containing fresh and frozen mucus gave similar results with regards to solute diffusion at room temperature (20°C). Hence, frozen mucus was used to represent fresh mucus for convenience and ready availability.

Electrode system. A Rank (Rank Brothers, Cambridge, UK) O_2 electrode (Fig. 1). was used as the electrochemical cell, with a platinum working electrode and a silver reference electrode. An anodic (oxidizing) potential (+0·65 V) was applied against the silver cathode, except for the case of ferrous and ferric ions where voltages of +0·80 and −0·40V were used, respectively, in

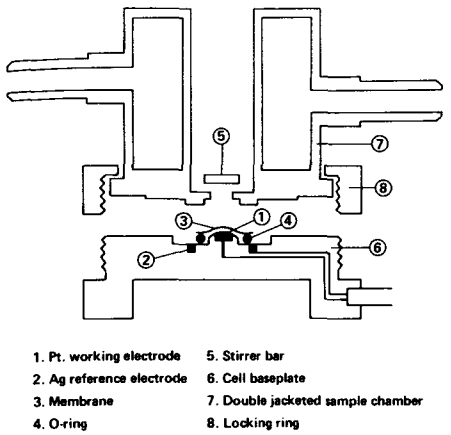

FIG. 1. Rank electrode assembly.

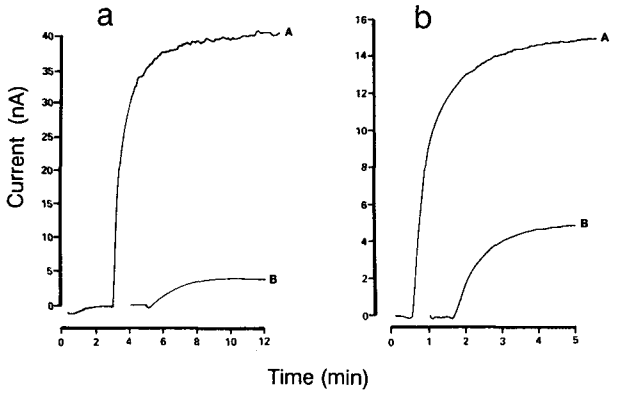

FIG. 2. a. The dynamic response of the Rank electrode to a step change in NADH concentration (0 to 0·1 mM) in the absence (A) and presence (B) of mucus. b. The dynamic response of the Rank electrode to a step change in paracetamol concentration (0–>0·02 mm) in the absence (A) and presence (B) of mucus.

O_2-free buffer. Rapid stirring by means of a magnetic stirrer ensured that current was independent of stirring rate. The electrode assembly was filled with internal buffer matching the external bulk solution, and then mounted with a nylon netting (100 μm thickness) which acted as spacer. Mucus gel was applied to the netting, and retained as a uniform layer behind a 1·0 μm pore polycarbonate membrane which was then securely clamped in position. For the control equipment, the spacer layer contained only buffer.

In all cases, the electrode assembly was allowed to achieve a baseline current; this was followed on a strip chart pen recorder (type CR6525, Lloyd Instruments, Fareham, Hants). Addition of a stock solution containing 1·0 to 10 mmol of electroactive reagent resulted in a sufficiently slow rise in current at the membrane covered electrode (steady state values at 3–5 min) to permit reliable monitoring using the strip chart recorder.

Results

The dynamic response of the electrode to a step change in NADH and paracetamol concentration is shown in Fig. 2 (a and b, respectively). The effect of mucus is to substantially diminish the maximal signal amplitude. By plotting ln (i_{ss}-i_t) against t (Fig. 3) it can be seen that the relationship is linear and that eqn 3 applies for the response in the presence and in the absence of mucus (Fig. 3).

Table 1 shows the effect of including mucus within the 100 μm nylon spacer. For all the solutes studied, a reduction in effective diffusion coefficients was observed, with retardation of solute flux by a factor of at least two. Over the molecular weight range of the solute investigated (34–660 daltons), no consistent effect of molecular weight was evident with regard to the retarding effect of mucus. Results for the controls are seen to be of similar order of magnitude to literature values, where available. However, the consistent overestimate by the present method is probably due to differences in both methodology and diffusant concentrations used. The ferrocenes have effective diffusion coefficients of a similar order of magnitude for the control and mucus experiments, but with the dicarboxylic acid derivative, a substantial, almost ten-fold reduction in permeability through mucus was observed; a similar degree of reduction in the diffusion of NADH through mucus was observed.

A major change of diffusion through mucus was observed for H_2O_2 (Table 1), however, H_2O_2 incubation with mucus, led to rapid H_2O_2 decomposition (checked electrochemically), indicating that the observed signal attenuation at the electrode was the result of decomposition of H_2O_2 rather than a retarded diffusion as suggested by Fig. 4. Corresponding incubation of NADH with mucus did not lead to decomposition, indicating a true retarded diffusion.

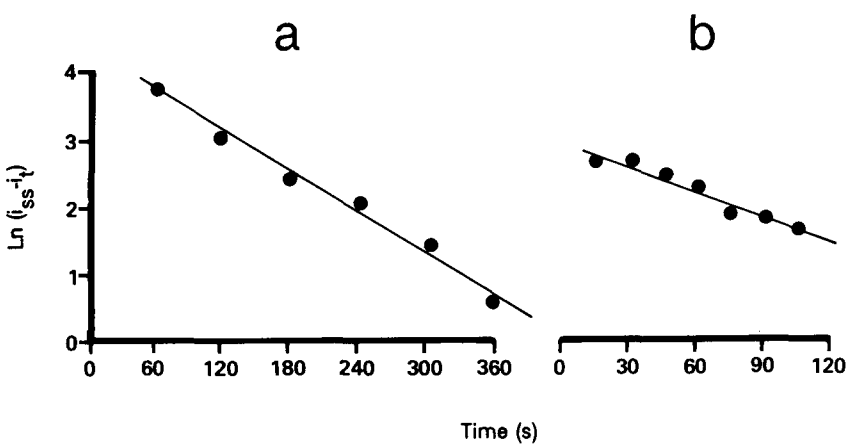

FIG. 3. A plot of ln ($i_{ss} - i_t$) against t from the dynamic response curves of NADH diffusion (Fig. 2a) showing linear slopes in the absence (a) and presence (b) of mucus, indicating that the system obeys simple unidirectional diffusion governed by Fick's law.

Table 1. Effective diffusion coefficients of selected solutes through aqueous layer and native porcine mucus.

Solute	D_e ($cm^2 s^{-1}$) Aqueous layer	Mucus layer	Literature values	Ref.	D_e Aqueous / D_e Mucus
H_2O_2	$5{\cdot}6\times10^{-5}$	$5{\cdot}20\times10^{-8}$	$1{\cdot}35\times10^{-5}$	Borggaard (1972)	1076[b]
Ascorbate	$2{\cdot}52\times10^{-5}$	$8{\cdot}50\times10^{-6}$	$7{\cdot}9\times10^{-6}$	Shamim & Salah (1980)	3·0
Urate	$2{\cdot}48\times10^{-5}$	$5{\cdot}60\times10^{-6}$	$7{\cdot}9\times10^{-6}$	Colton et al (1971)	4·4
Ferrocenecarboxaldehyde	$6{\cdot}84\times10^{-6}$	$2{\cdot}80\times10^{-6}$	—	—	2·4
Ferrocenecarboxylic acid	$8{\cdot}80\times10^{-6}$	$4{\cdot}25\times10^{-6}$	—	—	2·1
Ferrocenedicarboxylic acid	$7{\cdot}46\times10^{-6}$	$8{\cdot}29\times10^{-7}$	—	—	9·0
Acetyl ferrocene	$7{\cdot}36\times10^{-6}$	$3{\cdot}52\times10^{-6}$	—	—	2·1
Phenol red	$3{\cdot}72\times10^{-6}$	$1{\cdot}66\times10^{-6}$	—	—	2·2
Paracetamol	$2{\cdot}48\times10^{-5}$	$4{\cdot}14\times10^{-6}$	[a]$5{\cdot}7\times10^{-6}$	Sharma & Kalima (1977)	6·0
Catechol	$4{\cdot}98\times10^{-5}$	$2{\cdot}48\times10^{-6}$	$6{\cdot}6\times10^{-6}$	Sharma & Kalima (1977)	2·0
Ferrous sulphate	$1{\cdot}35\times10^{-5}$	$2{\cdot}59\times10^{-6}$	—	—	5·2
Ferrous chloride	$1{\cdot}35\times10^{-5}$	$2{\cdot}07\times10^{-6}$	—	—	5·2
Ferric sulphate	$1{\cdot}24\times10^{-5}$	$2{\cdot}07\times10^{-6}$	$4{\cdot}3\times10^{-3}$	Borgaard (1972)	6·0
NADH	$7{\cdot}66\times10^{-6}$	$8{\cdot}28\times10^{-7}$	—	—	9·0

[a] For m-aminophenol.[b] High value as a result of H_2O_2 decomposition in the presence of mucus.

Discussion

The incorporation of protein reagent layers and artificial polymeric membranes into electrochemical devices has become an established approach to the fabrication of medically usable biosensors (McDonnell & Vadgama 1989). All such devices have a response that is at least partly governed by membrane permeability, and it has proved possible to measure the mass transfer of simple redox compounds (Marrese et al 1987) in such systems. The use of amperometric electrodes mounted with a mucus gel provides a similar direct-reading system for mass transfer estimation; this not only simplifies the analytical procedure in comparison with the conventional diffusion chamber systems (Smith et al 1986), but approaches the physiological situation, with the working electrode acting as a 'sink' for the diffusing solute in an analogous fashion to the epithelial surface. The latter aspect could be of importance where the diffusion coefficient is affected by the actual concentration profile achieved by the solute within the gel phase (Cussler 1984).

Gastric mucus used here served as a readily available model gel, though some site-dependent differences in mucus in the small intestine are likely (Etzler & Braustrator 1974; Filipe & Branfoot 1976). Also, native mucus has non-glycoprotein constituents, notably lipids and proteins; these contribute to mass transfer resistance (Slomiany et al 1988), making it appropriate to use the unmodified gel.

Nimmerfall & Rosanthaler (1980) found that diffusion through mucus was consistent with the gel acting as a weak cation exchanger. The reduced transfer of the more anionic ferrocene dicarboxylic acid through mucus in our studies (Table 1) supports this contention; in some agreement with this are the generally higher relative diffusion rates observed for neutral species (catechol, phenol red) compared with anions (ascorbate, urate).

This study has found anomalously high diffusion coefficients for Fe(III) ions in aqueous buffer. Though it is not possible at present to explain the discrepancy, it is necessary to exclude the possibility of a modified electrochemical reaction at the working platinum electrode in the restricted electrolyte film used in the electrochemical cell (Fig. 1). The high diffusion resistance to paracetamol raises the possibility of binding of this agent by mucus in a similar manner to some antibiotics bearing nitrogen groups (Niibuchi et al 1986). Similarly, the mucus also shows high affinity for Fe(II) ions as demonstrated by their corresponding diffusion coefficients. Affinity of pig gastric mucus glycoprotein to counter-ions of various valencies has been demonstrated previously (Crowther & Marriott 1984), where ions of high valencies (such as Fe(III)) showed highest avidity for mucus. The gel used was likely to contain epithelium-derived enzymes, in addition to hydrolases known to be incorporated in mucus (Bandurko et al 1984). The presence of catalase undoubtedly accounts for the decomposition of H_2O_2, but the lack of any effect on NADH stability, indicates that dehydrogenases may not be present in significant quantities.

The high diffusional resistance demonstrated for mucus here is of relevance to the adsorption of a range of therapeutic and other exogenous compounds to which the small intestine is exposed. The results suggest that mucus is acting more than simply as a gel support for an unstirred water layer.

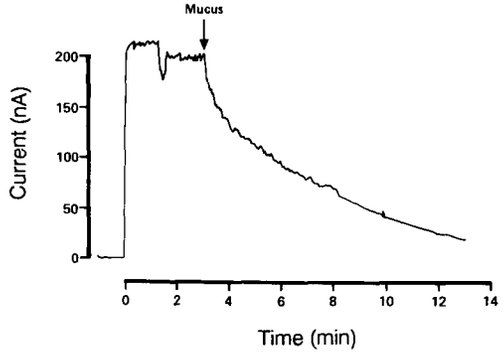

FIG. 4. Dynamic response of the Rank electrode to a step change in H_2O_2 concentration (0 to 0·1 mM) and subsequent decay of signal after the addition of mucus reflecting decomposition of H_2O_2 in the presence of mucus.

The authors wish to thank the Medical Research Council for support of this study.

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