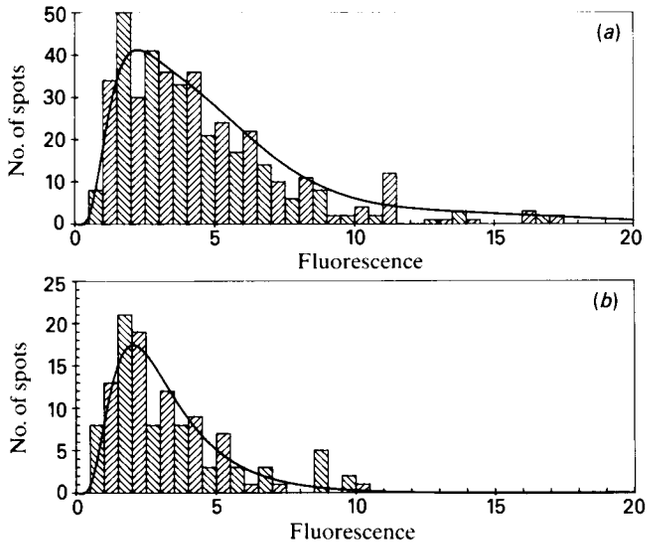

Fig. 1. *Histogram of spot fluorescent signal; artefacts have been distinguished from spots as described in the text*

(*a*) For six fibroblasts treated with DiI-labelled LDL. The continuous line is the best fit of the log-normal distribution found in (*b*) for single particles, convolved to give up to five particles per spot. (*b*) For DiI-LDL on a polylysine-coated slide. The continuous line is the best fit of a log-normal distribution.

apparent. Instead we found a broad distribution of fluorescent power.

To ascertain the fluorescent power corresponding to one DiI-LDL-receptor complex, we attached labelled LDL particles to a polylysine-coated slide and imaged under identical conditions to the cells. The fluorescent power histogram is shown in Fig. 1(*b*).

The histogram shows a long-tailed distribution; this is at variance with the Poisson distribution assumed by Gross & Webb [4] in their analysis. Electron micrographs, however, of the DiI-LDL showed variability in the particle sizes and a histogram of their corresponding surface areas gave a similar shape to that of Fig. 1(*b*). This would reasonably suggest that the amount of label incorporated into the lipid shell of an individual LDL particle is dependent on surface area.

The data for LDL attached to a polylysine-coated slide can be fitted by a log-normal distribution and the peak position and width obtained used to convolve and fit the cell data. We can quantify the single and double receptors reasonably accurately, but the long tail of this model leads to large errors in the values for cluster sizes of three or more.

In view of the inaccuracies involved when using a variably labelled marker such as DiI-LDL to determine cluster sizes, it should be possible instead to use LDL-receptor antibodies labelled with a uniform, unit number of probes to obtain precise values. Further work adopting this approach is envisaged to establish these values.

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On the interaction in solution of a candidate mucoadhesive polymer, diethylaminoethyl-dextran, with pig gastric mucus glycoprotein

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There is presently considerable interest in the evaluation of favourable mucus–polymer interactions for increasing the transit time of oral polymer drug delivery systems [1]. In this study, we investigate the interaction of the polycationic polysaccharide DEAE-dextran with a mucus glycoprotein by assaying for co-sedimentation in the analytical ultracentrifuge.

Pig gastric mucus glycoprotein (PGM) was partially purified from pig gastric mucus by gel permeation chromatography on a Sepharose CL-4B column using phenylmethanesulphonylfluoride as the proteinase inhibitor [2] and concentrated by ultrafiltration. DEAE-dextran [a derivative of T-500 dextran, weight average molecular mass ($M_{r,w}$) ~ 0.5×10^6] was obtained from Sigma U.K. The solvent used for all ultracentrifuge experiments was a phosphate/chloride buffer, pH 6.8, I 0.10 [3].

$M_{r,w}$ values of the PGM and DEAE-dextran were obtained by low-speed sedimentation equilibrium using procedures of Creeth & Harding [4] in a Beckman Model E incorporating a 5 mW He–Ne laser light source, at very low loading solute concentrations (~ 0.2–0.6 mg/ml) to minimize the effects of thermodynamic non-ideality. Values for $M_{r,w}$ of $(2.50 \pm 0.12) \times 10^6$ and $(0.53 \pm 0.02) \times 10^6$ were obtained for the PGM and DEAE-dextran, respectively. The value consistently obtained for PGM in the solvent specified is lower than that for PGM in 6 M-guanidinium chloride obtained by Creeth & Cooper [5] and probably is a consequence of some proteinase degradation; indeed, our value for the M_r corresponds to the 'subunit' size obtained by Carlstedt & Sheehan [6].

The interaction studies and apparent sedimentation coefficient (s_{20}) determinations were carried out at 20.0°C and rotor speeds of 19000 rev./min (to follow the sedimentation of the PGM component) and 44000 rev./min (DEAE-dextran) using sedimentation velocity in a MSE Centriscan equipped with a monochromator and using scanning Schlieren optics. Cells (10 mm path length) in a multi-hole rotor (to facilitate comparisons under identical run conditions) were employed. Interaction between the candidate polymer and PGM was assessed by evaluating s_{20} of the PGM and DEAE-dextran components (comparing individual controls with mixtures). The PGM and DEAE-dextran controls sedimented as single boundaries with $s^0_{20,w}$ values of (29.8 ± 0.9)S and (5.3 ± 0.1)S, respectively.

Abbreviations used: PGM, pig gastric mucus glycoprotein; (s_{20}) apparent (i.e. at a finite concentration and not corrected to standard solvent conditions) sedimentation coefficient at 20.0°C.

Table 1. *PGM/DEAE-dextran interaction data*

(*a*) The effect of increasing DEAE-dextran concentration. (*b* and *c*) The effect of increasing ionic strength.

	PGM control/mixture concn. (mg/ml)	DEAE-dextran control/mixture concn. (mg/ml)	Ionic strength	PGM control s_{20} (S)	PGM/DEAE-dextran mixture s_{20} (S)
(*a*)	2.0	0.6	0.1	18.3±0.4	21.7±0.4
	2.0	1.3	0.1	17.3±0.4	20.8±0.3
	2.0	1.6	0.1	18.3±0.4	23.4±1.0
	2.0	1.9	0.1	17.9±0.3	25.0±0.9
	2.0	2.6	0.1	18.3±0.5	21.2±0.9
	2.0	3.4	0.1	18.4±0.5	21.1±0.6
(*b*)	1.3	1.0	0.1	21.9±1.1	25.9±1.0
	1.3	1.0	0.2	—	21.7±1.1
(*c*)	1.3	1.0	0.1	21.7±0.9	25.8±0.6
	1.3	1.0	0.3	—	21.6±0.9

Under a given set of conditions (including the relative concentrations of the macromolecular components), the polymer was said to interact with PGM if the components in the mixture sedimented with a larger sedimentation coefficient than the controls after concentration effects had been taken into account (Table 1*a*). PGM concentrations were kept fixed (2.0 mg/ml) and the DEAE-dextran concentrations were varied (from 0.6 to 3.4 mg/ml): up to a DEAE-dextran concentration of 1.9 mg/ml, s_{20} values for the faster moving PGM component showed a steady increase (Table 1*a*) compared with the PGM controls, strongly indicative of an interaction. The increase in sedimentation coefficient of the PGM in the mixture cell (compared with the controls) was in the same sense as increase in turbidity of the suspension and loss of apparent areas under the Schlieren boundaries.

At higher values of DEAE-dextran concentration the s_{20} fell back to near the 'control' value again: this would appear to suggest that the interaction is very concentration sensitive, although the effects of the increased viscosity on increasing the concentration of the slower moving component (DEAE-dextran) cannot be excluded. Increase of the ionic strength in two separate experiments (Table 1*b* and 1*c*) also showed loss of interaction, indicating that the interactions between PGM and the polymer are electrostatic in nature.

In conclusion, the increases in (apparent) sedimentation coefficient of the PGM (and also DEAE-dextran) observed could be a direct result of the loss of concentration of PGM and DEAE-dextran in forming large turbid aggregates. Attempts at a more exact quantitative description of the interaction is difficult because of the presence of Johnston-Ogston [7]-related effects. These aspects, together with a consideration of the complications arising from the presence of other potentially interacting substances in the gastrointestinal tract (e.g. bile salts) and a comparison of interactions with other cationic and bifunctional polymers (and also non-interaction with polyanionics) will be considered in a future publication.

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Brain microsomes bind ryanodine and contain ryanodine-sensitive calcium channels

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Ca^{2+} channels from striated muscle sarcoplasmic reticulum (SR) have been functionally reconstituted in planar lipid bilayers [1] and purified as high-affinity ryanodine-binding proteins [2]. A deduced amino acid sequence for a skeletal muscle ryanodine-binding protein is now available from cloned cDNA [3]. All the reconstituted channels (including the purified protein) are activated by Ca^{2+}, ATP and caffeine, which increase the likelihood of the pore being open without altering the actual rate of ion transport. Some muscle channels are also activated by inositol trisphosphate ($InsP_3$) [4]. Given that the SR is merely a specialized endoplasmic reticulum, are similar channel proteins, possibly gated by chemical messengers, also present in non-contractile cells?

Rat forebrain microsomes were prepared by homogenization and differential centrifugation and binding isotherms for [^{3}H]ryanodine (0.5–50 nM, 54.7 Ci/mmol) were constructed following a protocol based on that in Table 2 of [5]. Unlabelled ryanodine (50 μM) was added to parallel samples to measure non-specific binding (the signal/noise ratio was at least 5:1 at the measured k_d). A typical binding isotherm, and associated Hill plot, are presented in Fig. 1(*a*). Channels

Abbreviations used: SR, sarcoplasmic reticulum; $InsP_3$, inositol trisphosphate.

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