Table 1. *ADCC of human mononuclear cells using rat monoclonal antibodies*

Human mononuclear cells, labelled with sodium [^{51}Cr]-chromate, were resuspended at 2×10^5 cells/ml in culture medium (Hale *et al.*, 1983) and 50 μl aliquots were incubated in microtitre plates with 50 μl of monoclonal antibody (1–10 μg/ml) at 20°C for 30min. The cells were washed and resuspended in 200 μl of medium containing 10^5 effector cells and incubated at 37°C for 4h. Radioactivity released was expressed as a percentage of the total input radioactivity.

Isotype	Number	Released radioactivity (%)
IgM	7	1,1,1,1,1,2,2
IgG2a	18	1,1,1,1,1,1,1,1,1,2,2,2,2,2,2,2,3,3
IgG2b	13	3,17,19,19,22,22,32,35,36,38,43,48,51
IgG2c	1	2

If these *in vitro* tests of ADCC represent clearance mechanisms which might operate *in vivo*, then rat IgG2b antibodies are a logical choice for therapy. We already have evidence that this subclass is the most effective at clearing cells in mice (Cobbold *et al.*, 1984). Furthermore, it has also been shown that IgG2b antibodies fix human complement (Fust *et al.*, 1980) and are particularly effective in complement-mediated lysis of cells (Hughes-Jones *et al.*, 1983). There is now a need to evaluate the role of rat IgG2b antibodies for serotherapy in man.

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A highly expanded spheroidal conformation for a mucin from a cystic fibrosis patient: new evidence from electron microscopy

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It is now widely accepted that mucus glycoproteins from a wide range of secretions are made up of a 500000–600000-M_r 'basic unit' consisting of a heavily glycosylated central core region and one or two end peptide regions (Silberberg & Meyer, 1982). These basic units are linked, supposedly into a linear array (Carlstedt *et al.*, 1983; Harding *et al.*, 1983*a*), via the end peptide regions to form the polymeric or 'macrostructure' of the higher M_r mucins (M_r 1×10^6–16×10^6). What is the gross conformation of these 'macrostructures' in solution?

Recently (Harding *et al.*, 1983*b*) evidence from solution studies has been presented to show that mucins occupy highly expanded spheroidal domains in solution. Attempts to support this by electron microscopy on a well-characterized cystic fibrosis bronchial glycoprotein 'CF PHI' (M_r 2×10^6) were hampered by the fact that the air drying of the specimens sprayed onto mica before platinum shadowing resulted in a high degree of flattening and also extension. If the negative-staining technique was used as an alternative, poorly defined patchy areas were evident, and although not inconsistent with this model, were rather ambivalent in interpretation.

Abbreviation used: CPD, critical point drying.

In this new study we now present evidence that if high shearing and surface tension forces are avoided on drying by using the technique of CPD, the mucin molecules appear to remain as intact swollen spheroids.

It is apparent from electron micrographs of unidirectionally platinum shadowed CF PHI (Fig. 2 of Harding *et al.*, 1983*b*) that the air-drying process results in a high degree of flattening, with each molecule revealing on average three 'low profile' highly flattened globular regions and two extended regions. The highly flattened regions may, for example, correspond to the originally highly expanded glycosylated regions, the extended regions corresponding to regions of naked peptide.

The technique of CPD, although widely used in tissue preparation for scanning electron microscopy, has found to date virtually no application in the study of macromolecules and small macromolecular assemblies (Willison & Rowe, 1980). It would appear, however, to have potential in this area, since three-dimensional structure must be sensitive to the large interfacial forces developed when phase boundaries (liquid/gas for air-drying, liquid/solid followed by solid/gas for freeze-drying) pass through the system.

Specimens are exposed though to considerable adverse chemical conditions (dehydration) during CPD, and fixation is therefore necessary. In some very recent work (A. J. Rowe, unpublished work) on myosin filaments, a modified CPD technique employing careful fixation has led to visualization of ordered structure not seen by other methods. We have therefore applied this new approach to mucins, with a further modification in using potassium permanganate as a fixative. Our final procedure gives consistent, reproducible images, showing the CF PHI molecules to

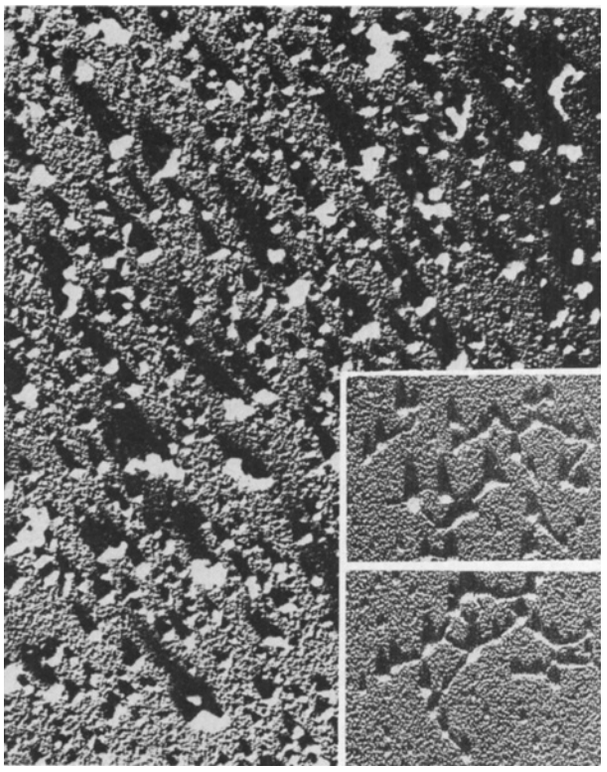

Fig. 1. *Electron micrograph showing a typical field of molecules of CF PHI, unidirectionally shadowed with platinum after fixation (3min in 2% $KMnO_4$) and critical point drying via acetone and liquid CO_2*

Magnification × 75000. Inset: selected area showing an effect frequently seen when glutaraldehyde was used as a fixative, with partial 'unravelling' of the molecules (magnification × 150000).

be large, roughly spherical particles (Fig. 1), of dimensions reasonably consistent with prediction (Harding *et al.*, 1983*b*). Only with less complete fixation was any evidence of elongated structure apparent (Fig. 1 inset).

The exact dimensions of the particles are a little difficult to determine, since the correction for 'metal cap' is not easily specified, and the use of internal standards to calibrate shadowing angle was difficult, as mucins tend to interact with added particles. Relying on shadowing geometry and ignoring the (probably small) effect of 'metal cap', we find the width of (non-aggregated) particles to be 61.5 ± 9.7 (S.D.)nm and height close to 59nm. Within experimental error they are therefore spherical, and using published values of M_r and $\bar{v}$ (Harding *et al.*, 1983*a*) a swelling ratio $\bar{V}_s/\bar{v}$ of 100 is calculated, in agreement with prediction ($\bar{V}_s/\bar{v} = 120$, loc. cit.). Insufficient fixation apparently allows the particles to 'unravel' during preparation (Fig. 1) and we consider it likely that this together with gross flattening is the explanation for the various images obtained in earlier studies (Lamblin *et al.*, 1979; Harding *et al.*, 1983*a*)

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Electrogenic reactions of the chloroplast cytochrome *b/f* complex

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In an earlier study we investigated the mechanism of quinol oxidation by the cytochrome *b/f* complex in chloroplasts (Selak & Whitmarsh, 1982). The effect of HQNO and DBMIB on the extent and kinetics of the flash-induced absorbance changes associated with the electrochromic shift and cytochrome b_6 and *f* were most simply accounted for by assuming that a modified Q-cycle (Mitchell, 1976) was operating under conditions of non-cyclic electron transport (Fig. 1). The question of which charge-transfer reaction gave rise to the slow electrochromic change was answered to a limited extent. We showed that electron transfer from the quinol oxidative site to cytochrome b'_6 was not electrogenic. In the context of a Q-cycle this left (i) electron transfer from cytochrome b'_6 to b''_6, (ii) electron transfer from cytochrome b''_6 to the quinone reductive site, and (iii) proton uptake associated with quinone reduction as possible electrogenic reactions. In the present study we address the question of the origin of the slow electrogenic step in more detail using NQNO and DBMIB to inhibit the slow electrochromic change. Comparison of the data with a simple kinetic model leads us to suggest that the slow electrogenic step associated with quinol oxidation is composed of at least two consecutive reactions.

Abbreviations used: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; NQNO, 2-n-nonyl-hydroxyquinoline *N*-oxide.

Isolation of chloroplasts from spinach leaves and spectrophotometric measurements were made as described previously (Selak & Whitmarsh, 1982).

We investigated the mechanism of quinol oxidation by spectroscopic measurements of the electrochromic shift at 515nm. Duroquinol in the presence of DCMU was used to support photosystem I-driven electron transport using short flashes. After a flash the cytochrome *b/f* complex is oxidized by photosystem I and in turn oxidizes quinol. We have proposed that quinol oxidation proceeds in two steps, with the first electron going to cytochrome b'_6 and the second electron to the Rieske FeS protein (Selak & Whitmarsh, 1982). Here we are concerned with electrogenic reactions subsequent to the reduction of cytochrome b'_6. The rise of the electrochromic shift is shown in Fig. 2*a* and is composed of both a fast and a slow kinetic phase. The fast phase arises from charge separation in photosystem I and the slow phase is associated with transmembrane charge transfer mediated by the cytochrome *b/f* complex. The slow phase is inhibited