A NEW FORM OF CARBON

UNDERSTANDING ANTARCTIC OZONE DEPLETION

The cellular defect behind cystic fibrosis

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ARTICLES

Solid C60 : a new form of carbon

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A new form of pure, solid carbon has been synthesized consisting of a somewhat disordered hexagonal close packing of soccer-ball-shaped C_{60} molecules. Infrared spectra and X-ray diffraction studies of the molecular packing confirm that the molecules have the anticipated 'fullerene' structure. Mass spectroscopy shows that the C_{70} molecule is present at levels of a few per cent. The solid-state and molecular properties of C_{60} and its possible role in interstellar space can now be studied in detail.

FOLLOWING the observation that even-numbered clusters of carbon atoms in the range C_{30}-C_{100} are present in carbon vapour[1], conditions were found[2-4] for which the C_{60} molecule could be made dominant in the large-mass fraction of vapourized graphite. To explain the stability of the molecule, a model was proposed of an elegant structure in which the carbon atoms are arranged at the 60 vertices of a truncated icosahedron, typified by a soccer ball. The structure, dubbed buckminsterfullerene[2] because of its geodesic nature, has been the subject of several theoretical stability tests[5,6] and has been discussed widely in the literature. Calculations of many physical properties have been made, including electron energies[7-9], the optical spectrum[9], vibrational modes[10-15], and the electric and magnetic properties[16,17]. There has been speculation on the possible chemical and industrial uses of C_{60} (ref. 2), and on its importance in astrophysical environments[18-20]. Until now, it has not been possible to produce sufficient quantities of the material to permit measurement of the physical properties, to test the theoretical calculations, or to evaluate the possible applications.

Some of us have recently reported evidence[21,22] for the presence of the C_{60} molecule in soot condensed from evaporated graphite. The identification was based primarily on the observed isotope shifts of the infrared absorptions when ^{12}C was replaced by ^{13}C, and on comparison of the observed features with theoretical predictions. The measured infrared and ultraviolet absorption bands were superimposed on a rather large continuum background absorption from the graphitic carbon which comprised $\geq 95\%$ of the sample. Here we report how to extract the carrier of the features from the soot, how to purify it, and evidence that the material obtained is in fact primarily C_{60}.

Method of production

The starting material for our process is pure graphitic carbon soot (referred to below as simply soot) with a few per cent by weight of C_{60} molecules, as described in refs 21, 22. It is produced by evaporating graphite electrodes in an atmosphere of ~100 torr of helium. The resulting black soot is gently scraped from the collecting surfaces inside the evaporation chamber and dispersed in benzene. The material giving rise to the spectral features attributed to C_{60} dissolves to produce a wine-red to brown liquid, depending on the concentration. The liquid is then separated from the soot and dried using gentle heat, leaving a residue of dark brown to black crystalline material. Other non-polar solvents, such as carbon disulphide and carbon tetrachloride, can also dissolve the material. An alternative concentration procedure is to heat the soot to 400 °C in a vacuum or in an inert atmosphere, thus subliming the C_{60} out of the soot (W. Schmidt, personal communication). The sublimed coatings are brown to grey, depending on the thickness. The refractive index in the near-infrared and visible is about two. To purify the material, we recommend removing the ubiquitous hydrocarbons before the concentration procedure is applied (for example, by washing the initial soot with ether). Thin films and powder samples of the new material can be handled without special precautions and seem to be stable in air for at least several weeks, although there does seem to be some deterioration with time for reasons that are as yet unclear. The material can be
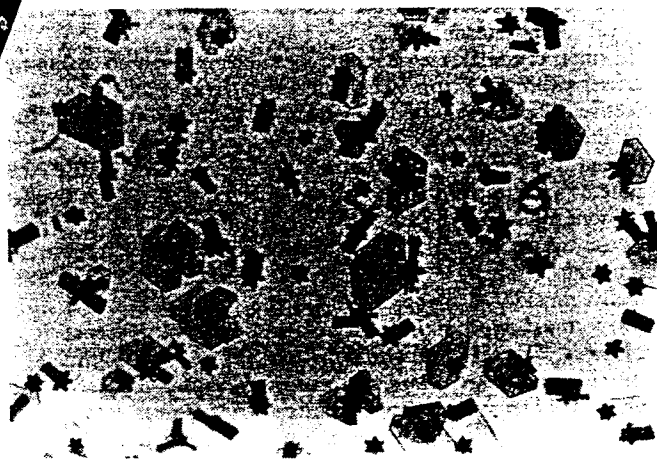
FIG. 1 Transmission micrograph of typical crystals of the C_{60} showing thin platelets, rods and stars of hexagonal symmetry.

sublimed repeatedly without decomposition. Using the
  ıratus described, one person can produce of the order of
'00 mg of the purified material in a day.

Studies by optical microscopy of the material left after
evaporating the benzene show a variety of what appear to be
crystals—mainly rods, platelets and star-like flakes. Figure 1
shows a micrograph of such an assemblage. All crystals tend to
exhibit six-fold symmetry. In transmitted light they appear red
to brown in colour; in reflected light the larger crystals have a
metallic appearance whereas the platelets show interference
colours. The platelets can be rather thin and are thus ideally
suited for electron-diffraction studies in an electron microscope
(see the inset in Fig. 3).

Mass spectroscopy

The material has been analysed by mass spectrometry at several
facilities. All mass spectra have a strong peak at mass 720 a.m.u.,
the mass of C_{60}. Significant differences in the spectra occur only
at masses lower than 300 a.m.u. Most of these differences seem
to originate from the different ionization techniques and in the
different methods of desorbing molecules from the sample. Mass
spectra recorded at low and high resolution are shown in Fig.
2. The spectra were obtained using a time-of-flight secondary-ion
ss spectrometer[23] and a C_{60}-coated stainless-steel plate. In

the mass range above 300 a.m.u., the spectrum is dominated by
C_{60} ions and its fragments (even-numbered clusters of atomic
carbon), and C-n ions. In this sample, the ratio of C-n to C_{60} is
~0.1. The high-resolution mass spectrum shows approximately
the expected isotope pattern for C_{60}. The increasing background
in the low-resolution mass spectrum is not produced by the
sample—such backgrounds also occur in blank measurements
on uncoated stainless-steel substrates.

So far, the cleanest mass spectra have been obtained when
the material was evaporated and ionized in the vapour phase
by electrons. In such spectra the low-mass background is sub-
stantially reduced and the entire mass spectrum is dominated
by C_{60} ions and its fragments. The ratio of C-n to C_{60} in these
mass spectra is ~0.02 and seems to be smaller than that shown
in Fig. 2. Both ratios are of the order of those reported from
laser-evaporation experiments[2,3]. We assume, as previously sug-
gested[2], that the C-n molecule also has a closed-cage structure,
either elongated[4] or nearly spherical[5]. Further details of the
mass spectroscopy of the new material will be published else-
where.

Structure

To determine if the C_{60} molecules form a regular lattice, we
performed electron and X-ray diffraction studies on the
individual crystals and on the powder. A typical X-ray diffrac-
tion pattern of the C_{60} powder is shown in Fig. 3. To aid in
comparing the electron diffraction results with the X-ray results
we have inset the electron diffraction pattern in Fig. 3. From
the hexagonal array of diffraction spots indexed as shown in
the figure, a d spacing of 8.7 Å was deduced corresponding to
the (100) reciprocal lattice vector of a hexagonal lattice. The
most obvious correspondence between the two types of diffrac-
tion is between the peak at 5.01 Å of the X-ray pattern and the
(110) spot of the electron diffraction pattern, which gives a
spacing of ~5.0 Å. Assuming that the C_{60} molecules are behav-
ing approximately as spheres stacked in a hexagonal close-
packed lattice with a c/a ratio of 1.633, d spacings can be
calculated. The results are shown in Table 1. The values derived
from this interpretation are $a = 10.02$ Å and $c = 16.36$ Å. The
nearest-neighbour distance is thus 10.02 Å. For such a crystal
structure the density is calculated to be 1.678 g cm^{-3}, which is
consistent with the value of 1.65 ± 0.05 g cm^{-3} determined by
suspending crystal samples in aqueous $GaCl_3$ solutions of
known densities. Although the agreement shown in Table 1 is
good, the absence of the characteristically strong (101) diffrac-
tion of the hexagonal close-packed structure, and the broad
continuum in certain regions suggest that the order is less than

FIG. 2 Low-resolution (top) and high-resolution time-of-flight
mass spectra of positive ions obtained from coatings of solid
C_{60}. A 5-keV Ar$^+$ ion beam was used to sputter and ionize
the sample. The isotope pattern (bottom) is approximately
that expected for C_{60} molecules composed of ^{12}C and ^{13}C
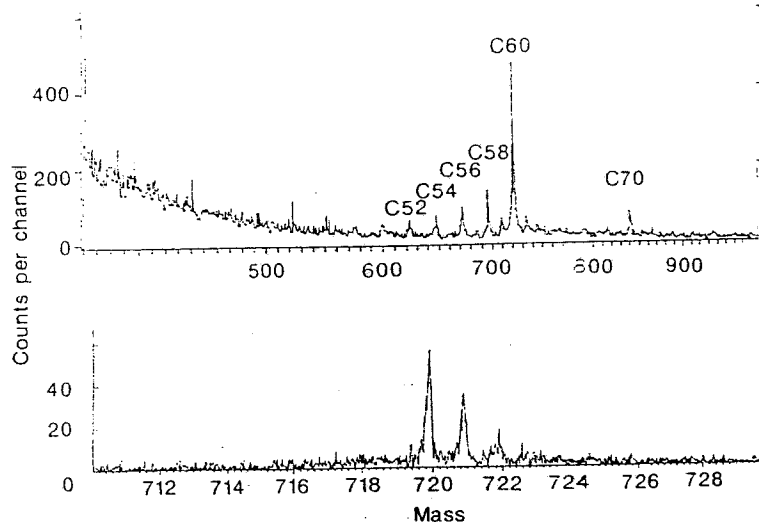isotopes of natural abundance.

TABLE 1 X-ray diffraction results

Measured 2θ (deg)	Measured d spacing (Å)	Calculated d spacing (Å)	Assignment (hkl)
10.2 shoulder	8.7	8.68	(100)
10.81	8.18	8.18	(002)
		7.68	(101)
17.69	5.01	5.01	(110)
20.73	4.28	4.28	(112)
21.63	4.11	4.09	(004)
28.1	3.18	3.17	(114)
30.8	2.90	2.90	(300)
32.7	2.74	2.73	(006)

Assignments for a hexagonal lattice using $a = 10.02$ Å, $c = 16.36$ Å. $(1/d^2) = \frac{4}{3}[(h^2 + hk + k^2)/a^2] + l^2/c^2$.

perfect. Further, X-ray diffraction patterns from carefully grown crystals up to 500 μm in size with well developed faces yielded no clear spot pattern (in contrast to the electron diffraction pattern on micrometre-sized crystals). It therefore appears that these larger crystals do not exhibit long-range periodicity in all directions.

A likely explanation for these facts lies in the disordered stacking of the molecules in planes normal to the c axis. It is well known that the positions taken by spheres in the third layer of stacking determines which of the close-packed structures occurs, the stacking arrangement in a face-centred cubic structure being ABCABC... whereas that in a hexagonal close-packed structure is ABABAB... If the stacking sequence varies, the X-ray lines owing to certain planes will be broadened by the disorder whereas other lines will remain sharp. Such disordered crystalline behaviour was observed long ago in the hexagonal close-packed structure of cobalt[26-28] where X-ray diffraction lines such as (101), (102) and (202) were found to be substantially broadened by the stacking disorder. Reflections from planes such as (002) remain sharp because these planes have identical spacings in the face-centred cubic and hexagonal close-packed structures. For the planes producing broadened diffraction peaks because of this kind of disorder, the following condition for the Miller indices (hkl) has been shown to apply[27,29]: $h - k = 3t \pm 1$ (where t is an integer) and $l \neq 0$. None of these broadened reflections are apparent in the X-ray pattern of Fig. 3. This may explain the weakness of the characteristically strong (101) peak. Whether or not this stacking disorder is

related to the presence of the possibly elongated C_{70} molecule has yet to be determined.

In small crystals at least, the C_{60} molecules seem to assemble themselves into a somewhat ordered array as if they are effectively spherical, which is entirely consistent with the hypothesis that they are shaped like soccer balls. The excess between the nearest-neighbour distance (10.02 Å) and the diameter calculated for the carbon cage itself (7.1 Å) must represent the effective van der Waals diameter set by the repulsion of the π electron clouds extending outward from each carbon atom. Because the van der Waals diameter of carbon is usually considered to be 3.3–3.4 Å the packing seems a little tighter than one might expect for soccer-ball-shaped C_{60} molecules. The reason for this has not yet been determined.

In summary, our diffraction data imply that the substance isolated is at least partially crystalline. The inferred lattice constants, when interpreted in terms of close-packed icosahedral C_{60}, yield a density consistent with the measured value. Further evidence that the molecules are indeed buckminsterfullerene and that the solid primarily consists of these molecules comes from the spectroscopic results.

Spectroscopy

The absorption spectra of the graphitic soot[21,22] showed evidence for the presence of C_{60} in macroscopic quantities. Following the purification steps described above the material can be studied spectroscopically with the assurance that the spectra are dominated by C_{60}, with some possible effects from C_{70}. Samples were prepared for spectroscopy by subliming pure C_{60} onto transparent substrates for transmission measurements. Depending on the pressure of helium in the sublimation chamber, the nature of the coatings can range from uniform films (at high vacuum) to coatings of C_{60} smoke (sub-micrometre microcrystalline particles of solid C_{60}) with the particle size depending to some extent on the pressure.

Figure 4 shows the transmission spectrum of an ~2-μm-thick C_{60} coating on a silicon substrate. The infrared bands are at the same positions as previously reported[21,22], with the four most intense lines at 1,429, 1,183, 577 and 528 cm^{-1}; here, however, there is no underlying continuum remaining from the soot. In many of our early attempts to obtain pure C_{60}, there was a strong band in the vicinity of 3.0 μm, which is characteristic of a CH-stretching mode. After much effort this contaminant was successfully removed by washing the soot with ether and using distilled benzene in the extraction. The spectrum in Fig. 4 was obtained when the material cleaned in such a manner was sublimed under vacuum onto the substrate. The spectrum shows
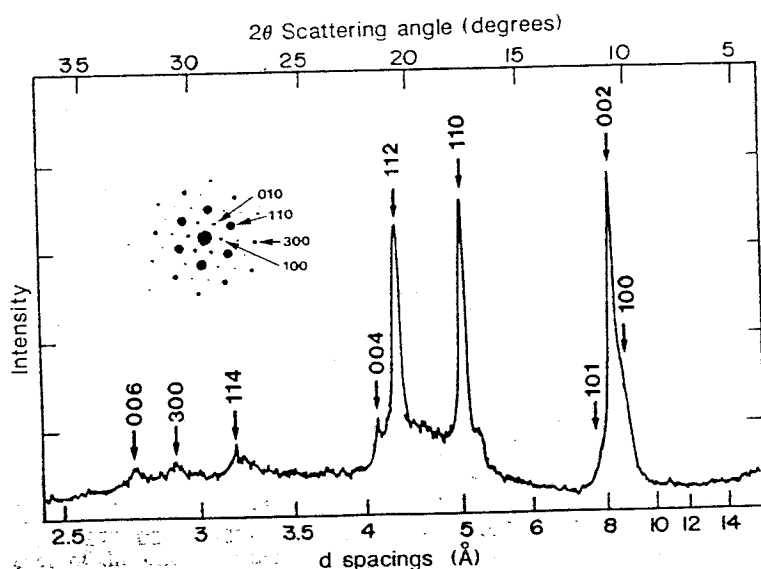

FIG. 3 X-ray diffraction pattern of a microcrystalline powder of C_{60}. Inset (upper left) is a single-crystal electron diffraction pattern indexed with Miller indices compatible with the X-ray pattern. The pattern is from a thin platelet such as those in Fig. 1 with the electron beam perpendicular to the flat face.
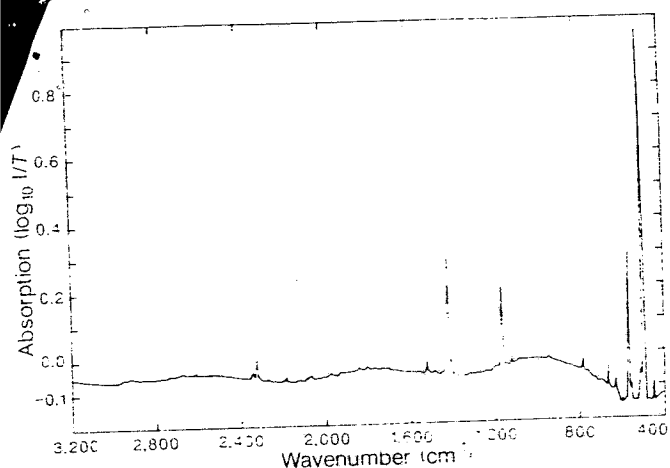
FIG. 4 Infrared absorption spectrum of a coating. ~2 μm thick. of solid C_{60} on a silicon substrate. referenced to a clean silicon substrate. Apparent negative absorptions are due to the coating acting in part as a non-reflecting layer.

very little indication of CH impurities. Vibrational modes to cc 'are with the measured positions of the four strong bands ha been calculated by several workers[10-15]. As noted pre- usly, the presence of only four strong bands is expected for tne free, truncated icosahedral molecule with its unusually high symmetry. Also present are a number of other weak infrared lines which may be due to other causes, among which may be absorption by the C_{70} molecule or symmetry-breaking produced (for example) by isotopes other than ^{12}C in the C_{60} molecule or by mutual interaction of the C_{60} molecules in the solid. Weaker features at ~2,330 and 2.190 cm^{-1}. located in the vicinity of the free CO_2 and CO stretching modes. may imply some attachment of the CO_2 or CO to a small fraction of the total number of C_{60} molecules. Another notable feature is the peak at 675 cm^{-1}, which is weak in the thin-film substrates but almost as strong as the four main features in the crystals. We suspect that this vibrational mode may be of solid state rather than molecular origin.

Figure 5 shows an absorption spectrum taken on a uniform film coated on a quartz glass substrate. The ultraviolet features are no longer obscured by the graphitic carbon background as in our previous spectra[22]. Broad peaks at 216, 264 and 339 nm dominate the spectra. Weaker structures show up in the visible, dominate the spectra. Weaker structures show up in the visible, : cluding a plateau between ~460 and 500 nm and a small peak ir 625 nm. At the bottom of Fig. 5 we have shown positions
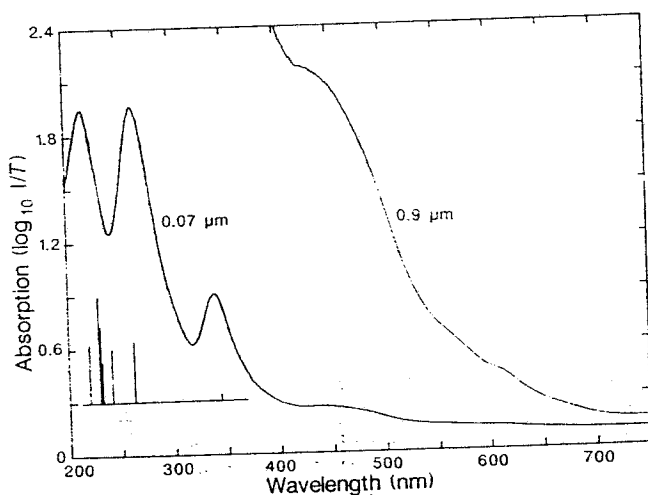

FIG. 5 Visible-ultraviolet absorption spectra of two thicknesses of solid C_{60} on quartz. The calculated[9] positions and relative oscillator strengths for allowed transitions of C_{60} are shown on the bottom.

and relative oscillator strengths taken from Larsson, Volosov and Rosén[9] calculated for the C_{60} molecule. They also reported a variety of forbidden bands with the lowest energy ones in the vicinity of 500 nm. There seems to be a rough correspondence between our measurements on solid films and the allowed transi- tions predicted for the molecule. The possibility exists, however. that one or more of the absorption features shown in Fig. 5 are due to C_{70}. We still do not observe a band at 386 nm in our films, as observed[30] using a laser depletion spectroscopy method and attributed to the C_{60} molecule. Quite similar spectra to that in Fig. 5 have been recorded for microcrystalline coatings deposited at helium pressures of 100 torr. for example. The peaks occur at the slightly shifted positions of 219. 268 and 345 nm.

Possible interstellar dust

The original stimulus for the work[2] that led to the hypothesis of the soccer-ball-shaped C_{60} molecule. buckminsterfullerene. was an interest in certain unexplained features in the absorption and emission spectra of interstellar matter. These include an intense absorption band at 217 nm which has long been attributed to small particles of graphite[31], a group of unidentified interstellar absorption bands in the visible that have defied explanation for more than 70 years[31,32], and several strong emission bands attributed to polycyclic aromatic hydrocarbons[33,34]. Based on the visible and infrared absorption spectra of Figs 4 and 5, we do not see any obvious matches with the interstellar features. The ultraviolet band at 216-219 nm has a similar peak wavelength to an interstellar feature, although the other strong bands of the spectrum have no interstellar counterparts. As the influence of C_{70} absorptions on the spec- trum is not yet known, a conclusive comparison with the 217-nm interstellar band is difficult. We note that the visible-ultraviolet spectrum presented here is characteristic of a solid, rather than of free molecules. In addition, these new results do not relate directly to absorption in the free C_{60}^+ molecular ion. which has been envisaged[19] to explain the diffuse interstellar bands. Nevertheless, these data should now provide guidance for poss- ible infrared detection of the C_{60} molecule. if it is indeed as ubiquitous in the cosmos as some have supposed.

Summary

To our method for producing macroscopic quantities of C_{60}. we have added a method for concentrating it in pure solid form. Analyses including mass spectroscopy, infrared spectroscopy. electron diffraction and X-ray diffraction leave little doubt that we have produced a solid material that apparently has not been reported previously. We call the solid fullerite as a simple extension of the shortened term fullerene, which has been applied to the large cage-shaped molecules typified by buckmin- sterfullerene (C_{60}). The various physical and chemical properties of C_{60} can now be measured and speculations concerning its potential uses can be tested.   □

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Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells

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The cystic fibrosis transmembrane conductance regulator (CFTR) was expressed in cultured cystic fibrosis airway epithelial cells and Cl⁻ channel activation assessed in single cells using a fluorescence microscopic assay and the patch-clamp technique. Expression of CFTR, but not of a mutant form of CFTR (ΔF508), corrected the Cl⁻ channel defect. Correction of the phenotypic defect demonstrates a causal relationship between mutations in the CFTR gene and defective Cl⁻ transport which is the hallmark of the disease.

CYSTIC fibrosis (CF), the most common lethal genetic disease in Caucasians[1], is characterized by abnormal electrolyte transport in several organs, including lung, sweat gland, intestine and pancreas[1,2]. Defective regulation of the apical membrane Cl⁻ channels that control the rate of transepithelial Cl⁻ transport is well documented in CF epithelia[3–5]. In normal airway epithelia, Cl⁻ channels are activated (opened) by an increase in intracellular levels of cyclic AMP. Cyclic AMP opens Cl⁻ channels by activating cAMP-dependent protein kinase which is thought to phosphorylate either the Cl⁻ channel itself or an associated regulatory protein. As a result, the rate of transepithelial Cl⁻ secretion increases. In CF airway epithelia, Cl⁻ channels are present in the membrane but they cannot be activated by cAMP or cAMP-dependent protein kinase[6,7]. As a result, CF epithelia fail to secrete Cl⁻ (and thereby fluid) towards the airway lumen. Defective Cl⁻ secretion probably contributes to the abnormal mucociliary clearance and lung disease that is the main cause of morbidity and mortality in patients with CF.

The CFTR gene sequence was recently identified and shown to be mutated in patients with CF[8–10]. A 3-base-pair deletion

resulting in the loss of a phenylalanine residue at position 508 (ΔF508) occurs in ~70% of CF chromosomes[10,11]. In the accompanying manuscript[12] we describe construction of a full-length CFTR coding sequence. By in vitro and in vivo synthesis we have shown that CFTR associates with membranes, is a glycoprotein and can be phosphorylated; these properties are consistent with those predicted from the DNA sequence[9]. This work enabled us to test the hypothesis that expression of normal CFTR will complement the CF phenotype and should eventually allow an assessment of the function of CFTR.

Expression of CFTR in CF cells

In evaluating expression systems, we had two main considerations: the protein product should be processed appropriately and most of the cells should express the protein. We chose the vaccinia-T7 hybrid expression system developed by Moss and co-workers[13,14] because it has several advantages, including: (1) infection and expression occur in a wide variety of eukaryotic cells; (2) the virus provides the enzymes necessary for transcription in the cytoplasm of the infected cell, thereby elimininating the need for nuclear processing; (3) the gene product can be correctly processed and targeted to the cell surface; and (4) a high percentage of cells express the recombinant DNA. We were also encouraged to adopt this system because it has been successfully used to express a functional K⁺ channel in several cell types[15], including epithelial cells (J.D.McC. and M.J.W., unpublished observations).

In the accompanying manuscript[12] we showed that HeLa cells produced CFTR when they were infected with a recombinant vaccinia virus expressing bacteriophage T7 RNA polymerase, and subsequently transfected with a plasmid containing the T7 promoter and terminator sequences flanking the CFTR coding sequence. We transfected a CF airway epithelial cell line (JME/CF15)[16] with constructs containing either the normal CFTR coding sequence (pTM-CFTR-3), or the mutated CFTR coding sequence (pTM-CFTR-3ΔF508). Figure 1a shows expression of CFTR in JME/CF15 cells at 6.5 hours after

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