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Immunodetection of planktonic algae

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Interest in the identification and characterisation of cyanobacteria and dinoflagellates in aquatic environments is increasing rapidly due to the perceived roles of these organisms in primary production and nuisance aspects in terms of water treatment and public health. Techniques for the identification and quantification of these organisms currently are limited, and the application of molecular approaches provides fundamental taxonomic information and techniques of practical value. Antigenic properties of algal cells may be useful taxonomic markers. Immunodetection techniques utilise the specificity of the antibody/antigen association as a probe for recognising and distinguishing between microorganisms according to their cell-surface chemistry.

Immunofluorescent detection of unicellular cyanobacteria and dinoflagellates has been studied with success in marine and freshwater ecosystems and a range of techniques and results are presented and discussed. The most recent advances in the study of planktonic algae have come with the application of continuous flow cytometric methods (CFC). Flow cytometry makes use of the autofluorescence properties of the algal cells, which alone can be used to demonstrate their presence and permit their quantification in natural water samples. When used in conjunction with immunolabelling techniques, the potential of CFC analysis is broadened to study the serological/strain composition of plankters in natural populations. Changes in algal strains represented within and between waters over periods of time are reported and discussed, along with the ecological issues thus raised.

Immunodetection in aquatic environments: cyanobacterial background

Immunodetection techniques utilise the specificity of the antibody/antigen association as a probe for recognising and distinguishing between microorganisms according to their cell-surface chemistry. The history of immunodetection in aquatic systems has developed through the study of prokaryotic organisms, and immunofluorescent detection of unicellular cyanobacteria has been studied with success in marine environments. The cyanobacteria (formerly known as blue-green algae) are Gram negative phototrophic prokaryotes which vary in size, morphology, physiology and ecology. Their taxonomy has been studied morphologically, physiologically and biochemically (Rippka *et al.* 1979; Rippka & Cohen-Bazire 1983; Anagnostidis & Komarek 1985; Komarek & Anagnostidis 1986; Golden *et al.* 1989). While these studies are helpful with most taxa of cyanobacteria, difficulties exist with very small unicellular forms (0.2 to 2.0 μm), which possess very few morphological distinguishing features. In aquatic systems these small forms of cyanobacteria, together with similar-sized heterotrophic bacteria, comprise the picoplankton, a term first coined by Sieburth

et al. (1978) and updated by Sicko-Goad & Stoermer (1984), for both marine and freshwater ecosystems. Cells of the genus *Synechococcus* are perhaps the major constituents of marine and freshwater populations of cyanobacterial picoplankters, and Waterbury *et al.* (1979) observed the occurrence of phycoerythrin-rich unicells of this genus present in a variety of geographical locations. It is the ubiquity and undoubted contribution of these unicells to primary productivity in both marine and fresh waters that makes their ecology and the dynamics of their populations of interest.

Antibodies have been used to specifically identify microorganisms in aquatic systems, by Fliermans & Schmidt (1977) in fresh waters and by Campbell *et al.* (1983) and Shapiro *et al.* (1989a,b) in the sea. In these studies, genus-specific polyclonal antisera raised against algal cultures were produced and in most cases the antisera were also species-specific. This approach, using antisera, has been used in conjunction with pigment signatures, as defined by high-performance liquid chromatography on cultured algal strains, by Shapiro *et al.* (1989a), who found a correlation between these two properties. This has been substantiated within the cyanobacteria by Campbell & Iturriaga (1988), who report that serogroupings (antibody cross-reactivity groupings) of marine cyanobacteria rich in either phycocyanin or phycoerythrin are mutually exclusive.

The aim of my recent work (Taylor 1993 and Taylor *et al.* 1995) has been to develop immunological methods to assist in the identification of strains, taxa or species of freshwater picoplankton-sized cyanobacteria. The antibodies should provide an additional tool for the identification of these small forms and consequently assist in the study of their biology and give an insight into the diversity of their population compositions.

The immunodetection method: theoretical basis

Prokaryotic cyanobacterial cells are Gram negative-staining organisms. Their cell-wall structure includes lipopolysaccharides (Weise *et al.* 1970). With the potential for unique species-specific polymers and the absence of mucilagenous coverings (in the case of unicells), immunodetection techniques seemed appropriate to study cell surfaces. Dinoflagellate cells may or may not be armoured. Some genera are bounded by cell membranes and covered in polysaccharide thecal plates or scales; others are not. The vaccination of killed whole cyanobacterial/algal cells into the blood-stream of a mammal elicits an immune response from that animal, including immunoglobulin (Ig) molecules, which have been specifically produced to negate the "foreign" polymers in the vaccine. These molecules are complementary to those responsible for their production, and recognise them and bind to them *in vitro*. This protocol results in the production of polyclonal antisera against whole cells, so that the entire surface area of the cyanobacterial or dinoflagellate cell is presented to the immune system. As a consequence, many different IgG (G class immunoglobulins) molecules are produced against the entire variety of cyanobacterial and dinoflagellate surface antigens. This results in a repertoire of immunoglobulins which specifically recognise many different surface-located molecules by virtue of their conformation. The value of such polyclonal antisera in a detection system is that they indicate degrees of homology (by their cross-reactivities) between strains of cyanobacteria, dinoflagellates and other organisms. The homologous organism, when confronted with its polyclonal antiserum, gives a response which may be visualised by a variety of detection techniques. These include fluorescence microscopy using fluorochrome dyes such as fluorescein isothiocyanate (FITC), with which positively-labelled cyanobacterial and dinoflagellate cells fluoresce green at their cell surfaces (Chantler & McIllmurray 1987).

Immunodetection of freshwater *Synechococcus* strains

Investigations of the cross-reactivities of polyclonal antisera raised against strains of freshwater *Synechococcus* were carried out with a range of microorganisms isolated from a range of waterbodies, so that the technique could be evaluated as a detection and taxonomic tool for the study of these organisms. Immunological cross-reactivity between unicellular cyanobacteria and polyclonal antibodies was visualised by four methods: fluorescence microscopy, Ouchterlony gel immunodiffusion, polyacrylamide gel electrophoresis (PAGE) with western blotting, and continuous flow cytometry (CFC).

These four methods gave grossly similar patterns of reactivity, indicating that the three antisera raised against strains of *Synechococcus* specifically recognised cells of the genus *Synechococcus*, both amongst a range of cultured microorganisms and in natural phytoplankton samples. The anti-*Synechococcus* polyclonal antisera showed no cross-reactivity with other cyanobacteria from the five groups described by Rippka *et al.* (1979), and none with *Synechocystis* strains, marine *Synechococcus* strains (generally), green algae, and aquatic freshwater heterotrophs (generally), but varied cross-reactivity occurred with a range of *Synechococcus* isolates from world-wide freshwaters including Windermere, Buttermere, Derwent Water and Bassenthwaite Lake in the English Lake District (Cumbria, UK), several Scottish reservoirs, an Irish lough, and lakes in Wales, Finland, Russia and Australia. The polyclonal antisera were raised against three strains of *Synechococcus*: two against Culture Collection of Algae and Protozoa (CCAP) strains 1479/5 and 1405/1 (Antisera AS1 and AS3 respectively) and one against an isolate from the north basin of Windermere (AS2). The clearest labelling was evident between two isolates with their respective homologous antisera, viz. CCAP1479/5 and CCAP1405/1. The intensity of fluorescence for fourteen other *Synechococcus* strains was assessed relative to strains CCAP1479/5 and CCAP1405/1. With one exception (CCAP1479/7), cross-reactivity with AS1 was mutually exclusive to cross-reactivity with AS3, dividing strains of the genus *Synechococcus* into two serogroups. AS1 cross-reacted to a lesser extent with *Synechococcus* isolates Pasteur Culture Collection (*PCC) 6307, CCAP1479/1A, CCAP1479/1B and CCAP1479/7, compared with the homologous antigen CCAP1479/5. AS3 cross-reacted strongly with *PCC7942, *PCC7943, *PCC6311 and CCAP1479/7. AS2 appeared to be the least selective of the polyclonal antisera tested; it was found to cross-react with both AS1- and AS3-positive organisms. Antisera cross-reactivities with *Synechococcus* strains other than homologues indicated that there are similarities between surface determinants of all the *Synechococcus* strains examined, and to a degree provided support for existing classifications such as the division of the genus *Synechococcus* by Rippka & Cohen-Bazire (1983).

Antisera raised against freshwater strains did not generally recognise marine strains, a fact vindicated by Suttle & Chan (1993) who found that cyanophage from the marine environment could not infect freshwater hosts which were cyanophage-susceptible to freshwater cyanophage. Results suggest that cyanophage receptors found on the surfaces of marine strains are not present on freshwater strains, thus supporting a theory of antigenic differences between marine and freshwater *Synechococcus* cells. Anti-*Synechococcus* antisera indicated similarities and differences in unicell populations within and between waterbodies, by their differing cross-reactivities in these studies. Suttle & Chan (1993) comment that "A single isolate of a *Synechococcus* species can be infected by viruses that occur in widely separate regions". Thus, it is logical that *Synechococcus* cells have conserved viral receptors which may be antigenic outer-membrane proteins. This provides a vindication for regarding outer-membrane proteins as potential taxonomic markers, a fact supported by the observation that *Synechococcus* cells from diverse geographical locations are antigenically similar, as perceived by these antibodies.

Methods of visualising immunolabelled samples

The methods of visualising antibody/antigen interaction vary in their sensitivities of detection. Fluorescence microscopy indicates cross-reactivity which requires subjective evaluation by the microscopist, whereas Ouchterlony gel immunodiffusion, PAGE and PAGE with western blotting, and CFC, are considered to be more objective and sensitive. Overall, when *Synechococcus* antiserum cross-reactivity was assessed by these methods, similar patterns of cross-reactivity emerged. Ultimately, the four immunodetection techniques examined here are considered to be valid methods for the study of unicellular cyanobacteria.

Ecological applications of immunodetection

The objectivity of evaluation of antisera cross-reactivities with *Synechococcus* strains was improved by employing CFC. Analysis of three microscopically identical immunolabelled *Synechococcus* isolates from the south basin of Windermere showed immunolabelling variability, indicating the sensitivity of this detection method. Natural samples from other Cumbrian lakes, including Buttermere, Derwent Water and Bassenthwaite Lake, contained cyanobacterial picoplankters which cross-reacted with these antisera. CFC analysis indicated seasonal variations in these populations, as perceived by the immunolabelling method. Thus populations that were cross-reactive with antiserum were present one month but absent the next. Figure 1 shows an example from Buttermere, for surface dip samples of picoplankters collected in June and July 1991. The overlay histogram of FITC fluorescence is plotted against events recorded for each of three polyclonal anti-*Synechococcus* antisera and a null serum with picoplankton samples. In the June sample (Fig. 1, *left-hand column*), evidence suggests the presence of a population of unicells which was weakly cross-reactive with antiserum AS1 (raised against CCAP1479/5) and perhaps to a lesser extent with AS3 (raised against CCAP1405/1). In the histograms (Fig. 1), a particular region representing highly fluorescent material was examined. Of the total numbers of recorded fluorescing events, 34.84% were in this particular area in the AS1-treated sample, compared with 22.30%, 24.05% and 29.75% in the null-treated, AS2-treated and AS3-treated samples respectively. The amount of cross-reacting material in a sample taken from Buttermere in July (Fig. 1, *right-hand column*) was less than that observed in the June sample, and there was a smaller difference between the null serum-treated sample and antisera-treated samples. Samples treated with NS, AS1, AS2 and AS3 contained *ca.* 10 to 14% of the total collected events within the same decades of the histograms, representing highly fluorescent material.

From these results, and the speed at which picoplanktonic populations were perceived to change (with respect to their immunological properties), it is speculated that the contribution of cyanobacterial picoplankton to primary productivity in some freshwater lakes may be mediated and controlled by cyanophages which identify their host according to antigenic surface determinants. Cyanophage infection can lead to a rapid "crash" of large cyanobacterial populations. Cyanophage-induced lysis could thus liberate nutrients quickly and enable the resurgence of subsequent populations with differing antigenic characteristics.

Immunodetection of potentially toxic genera of marine dinoflagellates

Immunodetection techniques have been applied to the identification of marine dinoflagellates, which pose a threat to human health. The aim is to evaluate whether polyclonal antisera can identify and detect potentially toxic dinoflagellate genera in natural (fresh and preserved) phytoplankton populations. Sako *et al.* (1993), Vrieling *et al.* (1993) and Adachi *et al.* (1994, 1995) have investigated the immunology and molecular genetics of cultured *Alexandrium*

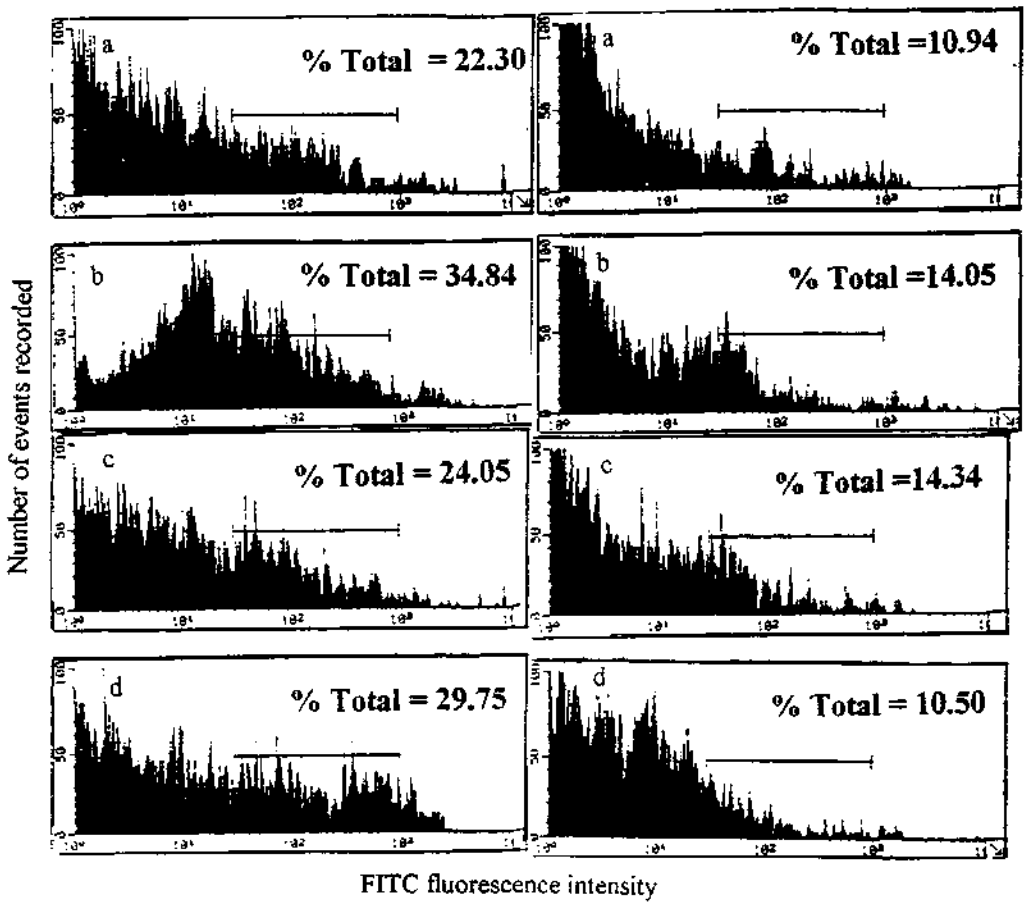

Figure 1. CFC histogram set region analysis of natural picoplankton samples from Buttermere (English Lake District) in June 1991 *(left-hand column)* and July 1991 *(right-hand column),* immunofluorescently labelled with three anti-*Synechococcus* polyclonal antisera and a null serum: (a) Null serum; (b) AS1 (anti-CCAP1479/5); (c) AS2 (anti-0mNBW2 isolate); (d) AS3 (anti-CCAP1405/1). The abscissa indicates FITC fluorescence intensity and the ordinate shows the number of fluorescing events recorded; horizontal bars indicate the region of intensities used for comparisons. Results for June 1991 *(left)* show AS1- and AS3-positive populations; results for July 1991 *(right)* show no cross-reactive populations.

species, but little work has been completed on the application of these techniques to ecological questions, and on natural phytoplankton samples.

Polyclonal antisera raised for several genera and strains of laboratory-cultured marine dinoflagellates

Antisera have been raised to cultures of *Alexandrium, Prorocentrum, Amphidinium* and samples of *Dinophysis*. These antisera were tested for the specificity with which they recognised cultured cells and samples. The polyclonal antisera appeared to specifically recognise the genus of algae to which they were raised. The immunolabelling reaction was visualised by three methods. All three gave grossly similar results for cross-reactivity. The intensity of the immunolabelling reaction between algal cells and antibody indicated the avidity of the recognition; homologous antiserum and algal strains gave the most intense

reactions. Strain-specificity of polyclonal antisera was ascertained (Taylor & Lewis 1995), and antiserum raised to a strain of *Alexandrium tamarense* from the River Tamar in southwest England (Plymouth culture collection PCC173a), was tested against a range of *Alexandrium* sp. isolated from the west coast of Scotland.

The effects of preservation on antigenicity (as perceived by antisera) and mixed sample composition were studied. Polyclonal antisera raised against *A. tamarense* from the Tamar (PCC173a) did not recognise *Prorocentrum, Amphidinium,* or *Gyrodinium,* but did recognise both live and preserved cultures of *Alexandrium* derived from cysts recorded from the west coast of Scotland; i.e. University of Westminster (UoW) strains 1, 2, 2c and 4. Variability in the intensity and surface location of the immunolabelling on cell surfaces indicated there are differences and homologies amongst these Scottish cultures, and suggested that this anti-*Alexandrium* antiserum is a useful tool for the rapid identification of potentially toxic dinoflagellates.

Photographs shown in Plate 2 (p. 106) illustrate the range of cross-reactivity. PCC173a *Alexandrium tamarense* (Plate 2, *top left*) labelled with antibodies, has clearly defined plates and a girdle zone. For UoW2c (Plate 2, *top right*) the junctions between the surface plates of the specimen shown are clearly visible, notably in the girdle area. Plate 2 (*bottom left*) shows an ecdysed cell of UoW4, bounded by a continuous membrane which is strongly and uniformly labelled throughout (fluorescing green). Plate 2 (*bottom right*) illustrates the genus specificity of the anti-*Alexandrium* antiserum's reactivity with respect to other dinoflagellates. In a sample containing a mixture of three genera, the only cells labelled by the anti-*Alexandrium* antiserum were those of *Alexandrium.* The antiserum recognised UoW2 and UoW4 well (but not as strongly as the homologous strain) and also recognised UoW2c. CFC analysis of this material indicated that reactivity of UoW2 and UoW2c was quite weak, but the reactivity of UoW4 was stronger; this indicates variability in immunolabelling intensity and thus CFC may be considered as a more objective evaluation method. It may be the case that all these strains are identical; PAGE could verify this. The cell-labelling of plates and ecdysed membranes may be a consequence of stationary phase culture, from which the vaccine was made, containing cysts and vegetative cells which would have a variety of algal antigens capable of eliciting an immune response. Antibodies resulting from a vaccination of such material would recognise a variety of surface features, suggesting an advantage of polyclonal over monoclonal antisera in the detection of organisms with complex surfaces. Whether strain identity is the case or not, the original antiserum strain was isolated from the River Tamar in Devon, whereas these new strains came from the west coast of Scotland; therefore some *Alexandrium* antigens must have been conserved between these isolates.

Application of polyclonal antisera to samples collected from natural dinoflagellate populations

The techniques used in the previous section were also applied to natural phytoplankton samples taken from UK coastal waters during 1993 and 1994, including the estuary of the River Wansbeck near Morpeth, Craster Rocks and the harbour at Berwick-upon-Tweed (all in N.E. England), the Firth of Forth (eastern Scotland), and Shapinsay Sound, Waulkmill Bay and northern and central Scapa Flow (all in the Orkney Islands). *Alexandrium* cells were found in samples from Craster Rocks and Berwick harbour, and both *Alexandrium* and *Scrippsiella* cells were found at a variety of locations in the Firth of Forth. CFC analysis indicated there are differences in the sizes of *Alexandrium* populations between the sites examined. A sample from Morpeth was more cross-reactive with the antiserum raised against UoW2c than that against PCC173a. This indicates that the cells of *Alexandrium* sp. from the sample were more like those taken from the west coast of Scotland than those in the River Tamar, Devon.

Anti-*Alexandrium* and anti-*Scrippsiella* antisera were tested against a sample from Shapinsay Sound, Orkney. CFC analysis showed that the population was *Scrippsiella*-positive. The Waulkmill Bay sampling site apparently did not contain *Alexandrium* cells on either of two sampling dates in 1993, when tested with anti-dinoflagellate antisera.

Phytoplankton-net tow samples from Scapa Flow in the Orkney Islands provided some interesting results; photographs of labelled cells are shown in Plate 3 A–D (p. 107). When collected, most of the samples were immediately preserved in Lugol's Iodine (Plate 3, A–C), and this has caused the chloroplast and cell contents to degrade; therefore no autofluorescence is evident. The cell surfaces appear smooth and unarmoured, possibly as a result of the preservation and immunolabelling techniques which may have caused ecdysis to occur, (shedding the cell membrane). Conversely, a fresh dinoflagellate cell shown in Plate 3D has retained its armour (plates and girdle are evident) and its autofluorescence. Such variety of surface labelling strengthens the case for using polyclonal antisera in these tests, as there is potential for recognition of a greater array of cellular antigens.

CFC analysis, shown in Figure 2, verified these findings and indicated the presence of an anti-*Alexandrium* antiserum cross-reactive population. Figure 2 (*lower*) shows a peak of events collected in the area of the histogram representing highly fluorescently-labelled material for the anti-*Alexandrium* treated sample; this peak was absent in an analogous area of the null serum treated sample (Fig. 2 *upper*).

Results on marine dinoflagellates from algal blooms

The most impressive results on marine dinoflagellates have been those involving large assemblages of algal cells – the so-called algal blooms. In recent summers, high concentrations of cells have discoloured water in the harbour at Weymouth in southern England. A sample of phytoplankton from Weymouth inner harbour was provided by the National Rivers Authority (Environment Agency). This 1994 material was considered to contain large numbers of *Alexandrium tamarense* cells (verified according to classical taxonomy, by Jane Lewis *personal communication*). The material was tested with both anti-*Alexandrium* antisera and a *Scrippsiella* antiserum. Cross-reactivity was assessed by fluorescence microscopy, CFC and a nitrocellulose-based dot test. The antisera cross-reacted with the algal cells in the sample to varying degrees. The anti-*Alexandrium* antisera cross-reacted, whereas the control and anti-*Scrippsiella* antisera did not. The three methods of visualising immunolabelling were in general agreement. Plate 3 E–H shows cells that have been immunolabelled with the three anti-dinoflagellate antisera and a control null serum. The cell shown in Plate 3F, which was treated with AS1 antiserum, is clearly immunolabelled. The cell has a green halo over its surface, whereas cells treated with other antisera have no such edging. CFC analysis of immunolabelled samples showed that the aliquot treated with AS1 contained many highly fluorescent particles; 9.45% of fluorescing events were recorded in the area representing highly fluorescent material, whereas only 5.10% were recorded from the same area of the AS2-treated aliquot (Fig. 3, *top right and bottom left*). The control and anti-*Scrippsiella* treated samples did not appear to contain cross-reactive cells at all, and only 2.25% and 2.90% of events were recorded in the areas of the plots representing high fluorescence (Fig. 3, *top left and bottom right*).

The nitrocellulose membrane dot test indicated that the anti-*Alexandrium* antiserum AS1 cross-reacted strongly with the 1994 sample from Weymouth harbour, and the second anti-*Alexandrium* antiserum AS2 cross-reacted to a lesser extent. Null and anti-*Scrippsiella* antisera were unreactive.

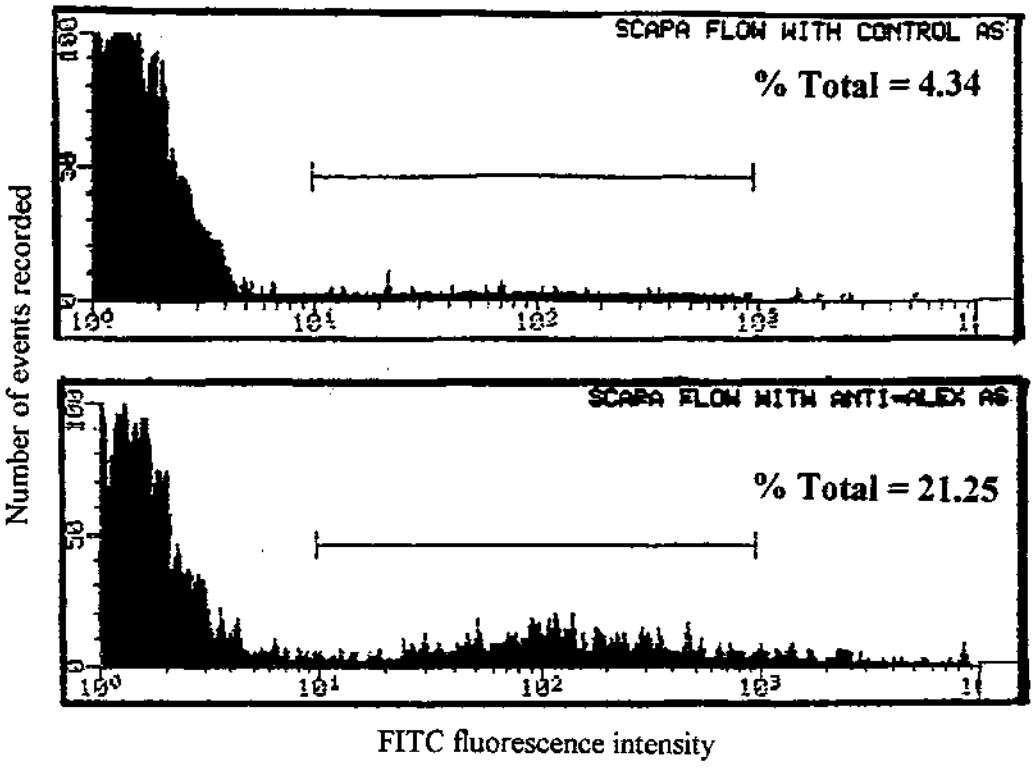

Figure 2. CFC histogram analysis of a phytoplankton-net tow sample from Scapa Flow, Orkney, on 4 May 1993, immunofluorescently labelled with null serum *(upper figure)* and polyclonal antisera AS1 (anti-PCC173a *Alexandrium tamarense) (lower figure).* The abscissa indicates FITC fluorescence intensity and the ordinate shows the number of events recorded; horizontal bars indicate the region of intensities used for comparison. Highly fluorescent material is present in the antiserum-treated sample, but not in the null-treated sample.

A phytoplankton sample collected from Weymouth harbour, on 28 June 1995, was weakly cross-reactive with antiserum AS1 (anti-PCC173a *Alexandrium tamarense*) and also with antiserum AS2 (anti-UoW2c *Alexandrium* sp.) by fluorescent microscopic examination, but CFC analysis indicated anti-UoW2c cross-reactivity (data not shown). Five strains of *Alexandrium* sp. were isolated from this 1995 sample. All five were cross-reactive with antiserum raised against PCC173a to varying intensities, but not with antiserum raised against UoW2c. These results suggest there is diversity amongst populations of *Alexandrium* cells which may be perceived by antisera, but which may be imperceptible by classical identification methods. These antigenic differences within natural populations may reflect differences between strains of the same species, and this could account for changes in the toxic status of recurrent algal blooms in some locations.

Antisera for recognising strains of Alexandrium *and* Dinophysis

Phytoplankton samples containing *Alexandrium* cells were collected from various geographical locations around Britain, and *Alexandrium* cultures were derived from the samples. These included the estuary of the River Avon in Dorset, where a preserved *Alexandrium* sample was shown to be cross-reactive with the antiserum raised against PCC173a, but not with that raised

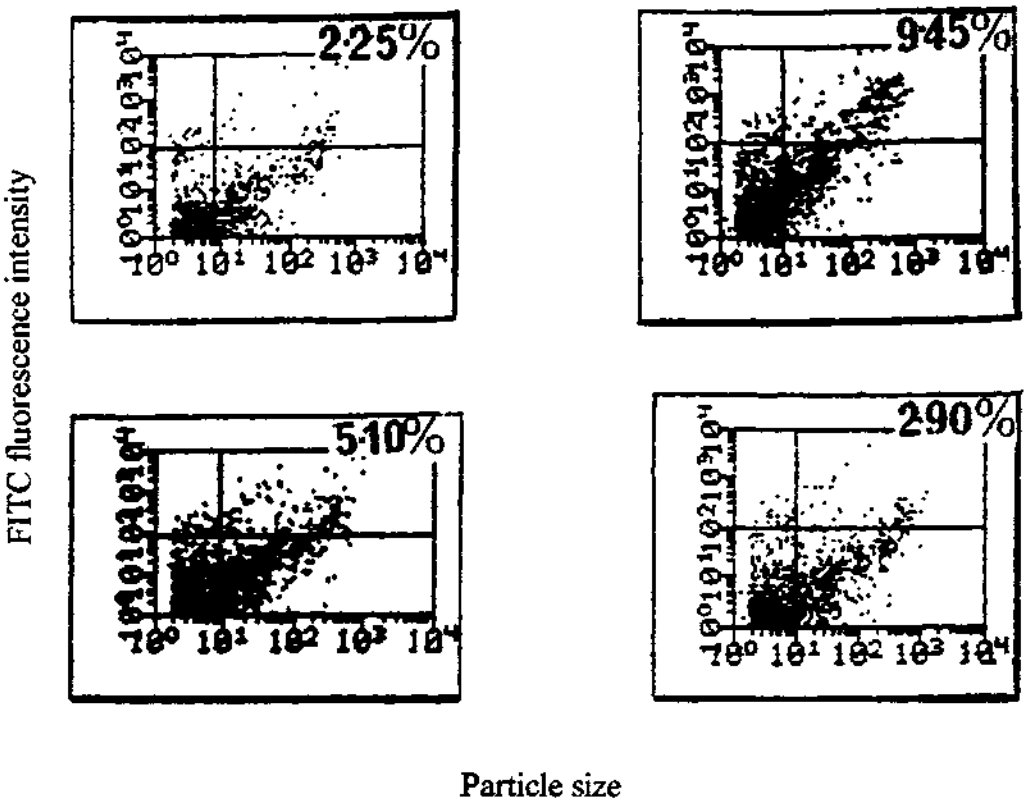

Figure 3. CFC analysis of a preserved phytoplankton-net tow sample from Weymouth harbour, Dorset, in 1994, immunofluorescently labelled with three polyclonal antisera and a null serum. *Top left:* Null serum; *top right:* AS1 (anti-PCC173a *Alexandrium tamarense); bottom left:* AS2 (anti-UoW2c *Alexandrium* sp.); *bottom right:* AS3 (anti-PCC104 *Scrippsiella trochoidea*). The abscissa indicates particle size and the ordinate shows FITC fluorescent intensity. Results suggest the presence of material cross-reactive with AS1 and AS2 but not with null serum or AS3.

against UoW2c; a Firth of Forth isolate, poorly reactive with both anti-*Alexandrium* antisera; three Belfast Lough isolates cross-reactive with anti-PCC173a antiserum; and a Portland inner harbour isolate which showed very good anti-PCC173a cross-reactivity, but none with anti-UoW2c antiserum.

Antisera were raised against cells of the species *Dinophysis accuminata* (from Arrochar, Scotland) and *Dinophysis norvegica* (from Blyth, N.E. England). Screening of these antisera against original vaccine material revealed that the anti-*D. accuminata* antiserum was poor at recognising cells of *D. accuminata*, but good at recognising cells of *D. norvegica*. Anti-*D. norvegica* antiserum recognised *D. accuminata* cells poorly and recognised *D. norvegica* cells well, so this antiserum appeared to be the most discerning of the two. CFC analysis of a Blyth harbour net-tow sample treated with the antiserum is presented in Figure 4. The CFC dot plot shows that, of the fluorescence events recorded for the sample, 7.58% occurred in the upper left quadrant in the anti-*Dinophysis* antiserum treated sample (Fig. 4, *right*), whereas only 3.78% were recorded (in this quadrant) in the null serum treated sample (Fig. 4, *left*). This suggests the presence of material recognised by the anti-*Dinophysis* antiserum.

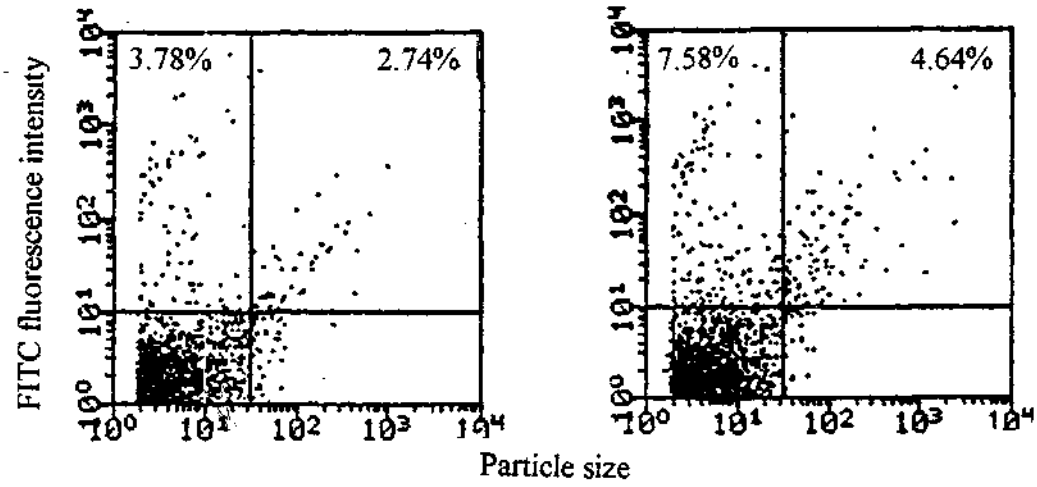

Figure 4. CFC dot plot analysis of a phytoplankton-net tow sample from Blyth harbour, Northumberland, immunofluorescently labelled with null serum *(left)* and anti-*Dinophysis norvegica* antiserum *(right)*, where 7.58% of recorded fluorescence events occurred in the quadrants relating to highly fluorescent particles, compared with 3.78% for the null serum sample. The abscissa indicates particle size and the ordinate shows FITC fluorescent intensity. Results suggest the presence of material cross-reactive with anti-*Dinophysis norvegica* antiserum.

General conclusions

Polyclonal antisera have been used to probe natural phytoplankton samples from various localities on the British coastline, in order to assess potential applications of the technique as a tool for (a) rapid identification of large numbers of dinoflagellate cells and (b) detection of low numbers of potentially toxic dinoflagellates in a preserved sample. The dot nitrocellulose paper test is relatively insensitive and only identifies target cells when these are present in large numbers. CFC is a more sensitive, predictive technique, as it reveals particular dinoflagellates when these are present in small numbers, and thus can identify potential algal blooms (and waning blooms).

Overall conclusions and future applications and uses of immunodetection techniques for investigating cyanobacteria in aquatic systems

Immunodetection techniques have been validated for the study of freshwater cyanobacteria, through the range of findings described herein and the work of Campbell *et al.* (1983, 1988), Caron *et al.* (1985) and Shapiro *et al.* (1989a,b). The world-wide conserved nature of synechococcal antigens, and antigenic variations in *Synechococcus* populations within waterbodies with time, have raised some interesting ecological questions concerning changes in strain composition of cyanobacterial assemblages. Future studies with antisera may indicate antigenic recognition links between cyanophages and their host cyanobacteria, and yield information on the cycling and turnover of carbon within systems. Immunodetection of viruses infecting cyanobacterial populations may lead to their quantification and thus suggest their contribution to carbon turnover in aquatic systems. The future may bring the development of new techniques for the detection of toxic organisms in fresh samples and improvements to existing techniques for the detection of freshwater cyanobacterial toxins.

Studies of marine dinoflagellate cells by Adachi *et al.* (1994), investigating *Alexandrium* sp. from Japanese coastal waters, found six divergent types, based on analysis of ribosomal DNA

by restriction fragment-length polymorphism. This differentiation of distinct genotypes within the genus *Alexandrium* agrees with results obtained by Sako *et al.* (1993), who found similar divisions in isolates of *A. catanella* and *A. tamarense*, by isoenzyme, genetic and monoclonal antibody analysis. Sako *et al.* found that for their studied *Alexandrium* group, all except a group named "WKS-1 group", were toxic, and "WKS-1" was considered to have a distinct genetic background and antibody cross-reactivity pattern compared with the other isolates. Such close agreement between genetic and antigenic strain differences is further validation of immunodetection techniques.

Dinoflagellate paralytic shellfish-poisoning toxins are presently detectable by immunological methods (Johnson & Mulberry 1966; Cembella *et al.* 1990). Future studies may develop antibodies which might discern toxic and non-toxic strains, and detect these differences in apparently homogenous populations. Adachi *et al.* (1994) conclude that polymerase chain reaction analysis of single cells from dinoflagellate populations may be possible, so that antigenic differences between individual cells, perceived by antisera, can be investigated genetically.

With the development of a greater array of anti-algal antisera we may be capable of predicting and diagnosing toxic events in waterbodies, broadening immuno-taxonomic studies to show relatedness between strains of a genus or species, or defining geographically distinct strains antigenically (thus showing transfers between populations, effected by transportation in ballast waters). Perhaps even the physiology, ecology and distribution of cyanobacterial strains with specific nutritional requirements could be derived from the immunodetection of outer-membrane proteins associated with nutrient transport (e.g. iron).

Moreover, fluorescence activated cell sorting, which allows particles of different sizes or fluorescence to be separated from a mixed sample, could separate immuno-reactive from non-reactive cells, and thus allow cells of differing antigenic properties to be cultured for closer genetic scrutiny. Approaches such as these will allow us a greater insight into the diversity and poise within apparently homogenous algal populations, perhaps answering and undoubtedly posing more interesting ecological considerations.

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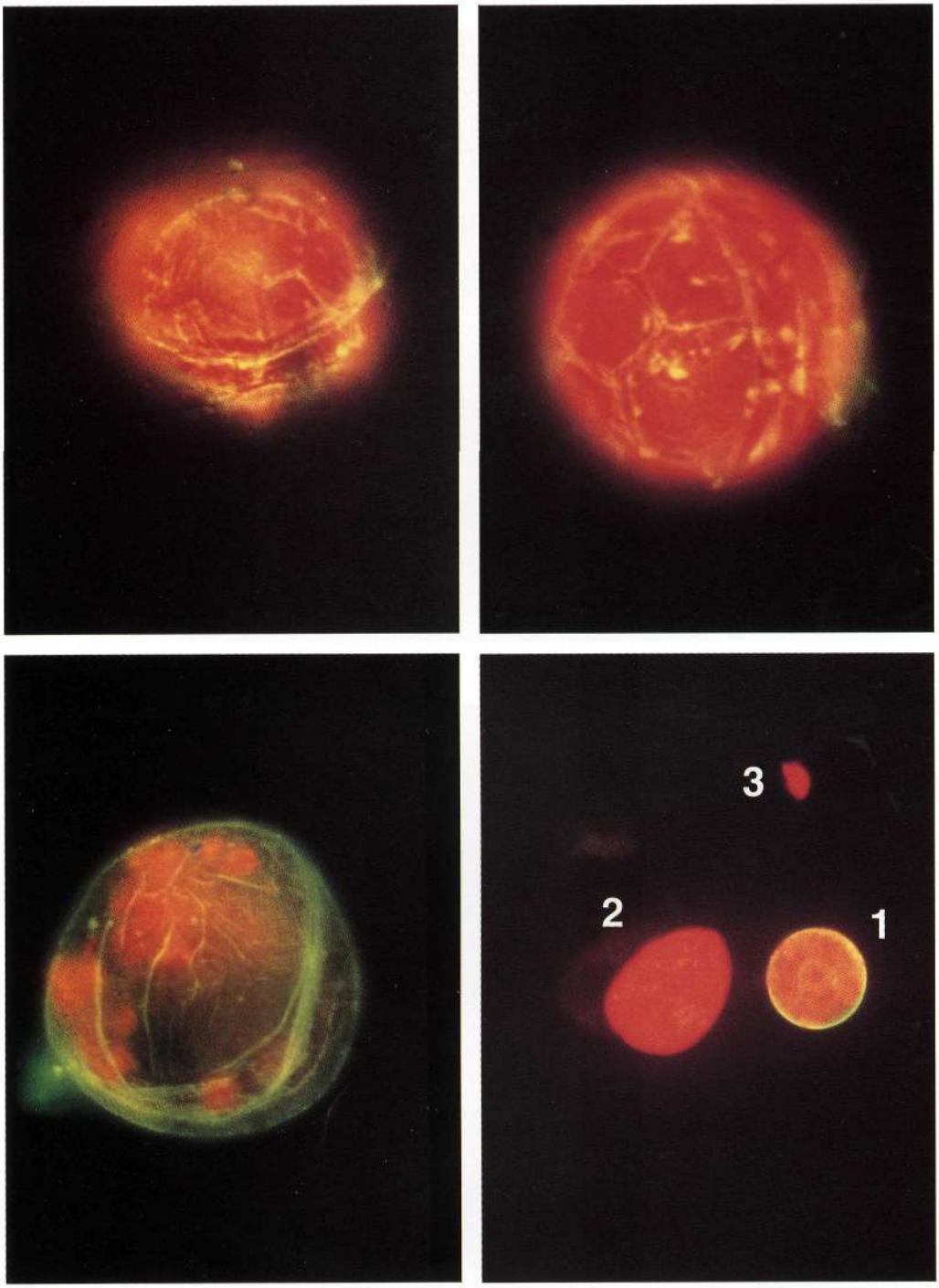

Plate 2. Various cultures of marine dinogflagellates immunofluorescently labelled with antiserum raised against PCC173a *Alexandrium tamarense* and observed by fluorescence microscopy at x1250 magnification. *Top left:* PCC173a *Alexandrium tamarense; top right:* UoW2c *Alexandrium* sp.; *bottom left:* UoW4 *Alexandrium* sp.; *bottom right: Alexandrium tamarense* (1), *Prorocentrum micans* (2) and *Amphidinium* sp. (3). The *Alexandrium* cell appears to have been surface-labelled with the fluorochrome dye FITC. (See Taylor, pp. 92-103).

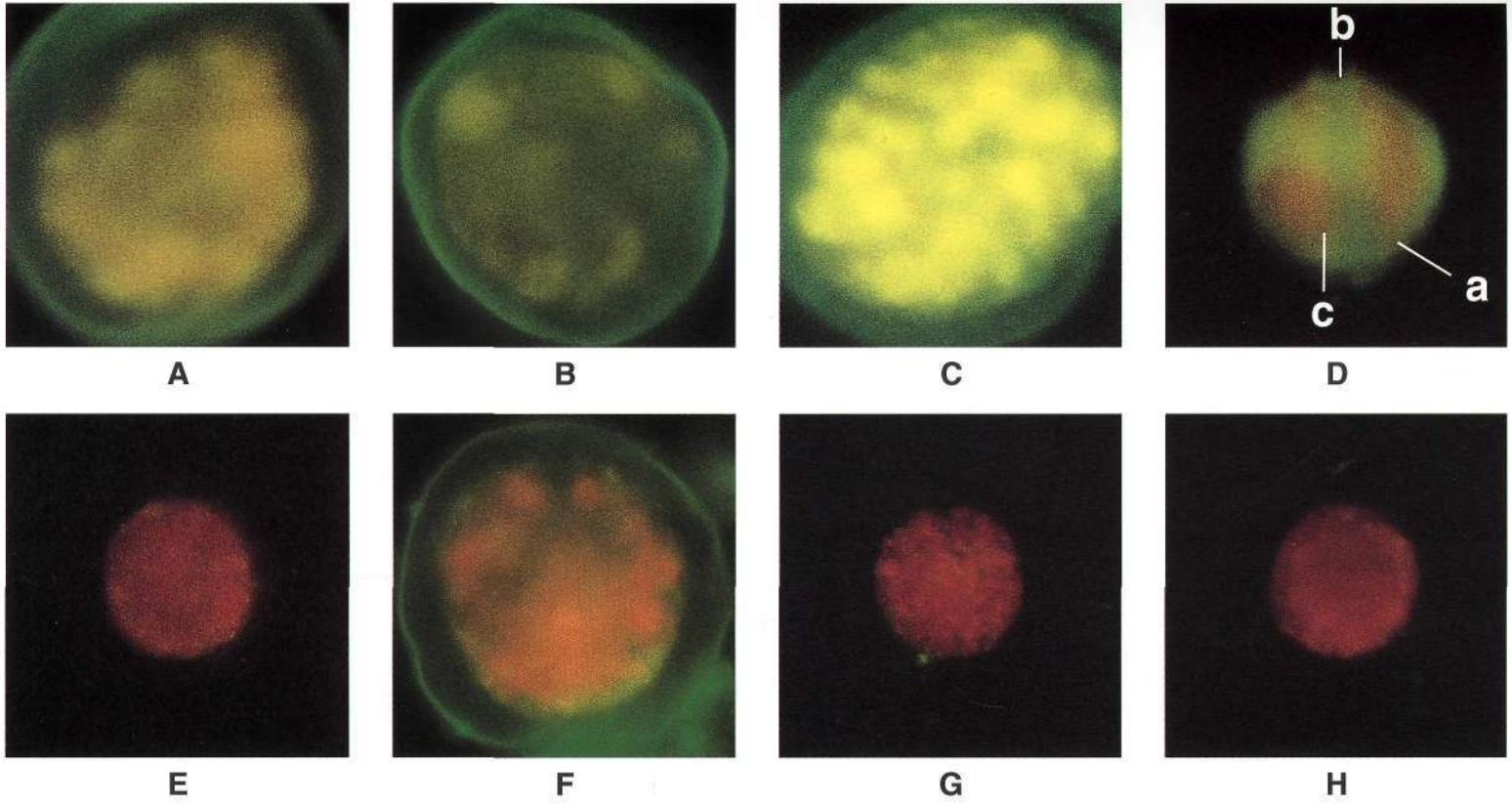

Plate 3. A–D. Photographs of marine dinoflagellates from Scapa Flow, Orkney, collected on 18 August 1994 and preserved in Lugol's Iodine (A–C, x800 magnification), and a fresh cell collected on 4 May 1993 (D, x400 magnification; a = cellular autofluorescence, b = plate junctions, c = girdle area). All cells were immunolabelled according to the indirect immunolabelling protocol with AS1 raised against PCC173a *Alexandrium tamarense*. Blue excitation flourescence shows cell membranes that are clearly labelled (green).

Plate 3. E–H. Photographs of marine dinoflagellates from Weymouth harbour, southern England, 1994, respectively immunolabelled with: null serum (E, red cell); AS1 (anti-PCC173a *Alexandrium tamarense)* (F, green and yellow cell); AS2 (anti-UoW2c *Alexandrium* sp.) (G, red cell); AS3 (anti-PCC104 *Scrippsiella trochoidea*) (H, red cell). Cells were observed under fluorescent blue excitation at a magnification of x400 and appear red/orange due to the presence of chlorophyll; the cell (F) which has been recognised by AS1 has a green/yellow edging, due to the presence of the fluorochrome dye FITC. Cells (red E, G, H) that were not recognised by the antisera have no such edging. (See Taylor, pp. 92-103).