The search for relevant cell culture research in cystic fibrosis: one researcher’s opinion*

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Recently there has been a plethora of research approaches using cell culture to study cystic fibrosis. Although these efforts can be lauded as attempts to understand one of the most common genetic disorders affecting the Caucasian population, such research should be relevant to the clinical entity known as cystic fibrosis. The ultimate objectives of this research should be to determine the, as yet unknown, basic defect and to develop an assay for heterozygote detection.

The clinical phenotype for cystic fibrosis (chronic bronchiolar obstruction and pulmonary infection, steatorrhoea, and azotorrhoea) is universally recognized (di Sant’Agnese and Talamo, 1967). All these features could be explained by changes in structure and function secondary to the accumulation of mucus on cell surfaces and passages. A widespread defect in mucus secretion would secondarily cause inhibition of movement of water and ions through the secretory glands. Although this hypothesis has been proposed (Farber, 1945; Johansen et al, 1968; Gibson et al, 1970), attempts to demonstrate an abnormality in mucus structure or function have not been successful. Thus, the search for the basic defect in cystic fibrosis continues.

Since the recognition that a cell phenotype is expressed, at least in culture, in all cystic fibrosis somatic cells (Danes, 1972), it has been interpreted by some that any consistent characteristic, whether morphological or metabolic, of such cystic fibrosis cultured cells reflects the basic defect. This, of course, is incorrect. The culture phenotype of the cystic fibrosis cell may be quite distinct from that of cells without this genetic endowment and still bear no direct relationship to the cystic fibrosis defect.

Cultured cells with mutant genotypes invariably show some culture deviations. Such is the case also for the cultured cystic fibrosis cell (Table). All the features of the cultured cystic fibrosis cell, so far described, have not been demonstrated to be specific for the cystic fibrosis gene. Unfortunately such observations probably do not lead us closer to an understanding of the basic defect in cystic fibrosis.

<table>
<thead>
<tr>
<th>Culture abnormality</th>
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<tr>
<td>Metachromasia</td>
<td>Danes (1969b)</td>
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<td>Heterogeneity in staining metachromasia and ametachromasia</td>
<td>Danes (1972); Nadler et al (1969)</td>
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<td>Variable mucopolysaccharide content intra- and extracellular</td>
<td>Danes and Bearn (1969b); 1969c); Matalon and Dorfman (1968)</td>
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<td>Increased glycosgen storage</td>
<td>Pallavicini et al (1970)</td>
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<td>Decreased collagen synthesis and collagenolysis</td>
<td>Houch and Sharma (1970)</td>
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<td>Longer generation time</td>
<td>Burtman et al (1970)</td>
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<td>Increase in lysosomes number and size</td>
<td>Danes and Bearn (1969b); Conover et al (1973c)</td>
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<td>Alteration in total hexosaminidase and ratio of isoenzymes A to B</td>
<td>Danes (1972); Bowman et al (1973); Conover et al (1973a), (1973b), (1973d)</td>
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<td>Cystic fibrosis factor activity (CFFA)</td>
<td>Danes (1973)</td>
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<td>Abnormality in complement</td>
<td>Danes and Bearn (1969a, b); Robertson et al (1974); Caudill et al (1974); Danes et al (1974)</td>
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<td>Metabolic cooperation between fibroblasts of normal (non-CF) genotype and CF (metachromatic, CFFA positive) but not between the latter and CF (ametachromatic, CFFA negative)</td>
<td>Rennert et al (1973)</td>
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<td>Increased sulphate and heparin incorporation</td>
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<td>Relative increase in dermatan sulfate with a normal total mucopolysaccharide content in CF lymphoid lines derived from families whose cultured fibroblasts have either culture phenotype</td>
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<td>Undermethylation of RNA in cultured lymphocytes and fibroblasts</td>
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A recent hypothesis has been that there is a humoral factor in body fluids which alters cellular function in the cystic fibrosis cell (Spock et al., 1967). A substance (cystic fibrosis factor) has been recognized in the serum and medium from cultured cells established from the majority of cystic fibrosis patients which alters the motility of rabbit tracheal cilia (Spock et al., 1967; Conover et al., 1973a) and oyster cilia (Danes, 1972; Danes and Bearn, 1972; Bowman et al., 1973). Although such a factor may by causally related to the pathological changes seen in cystic fibrosis (Farber, 1945; di Sant’Agnese and Talamo, 1967; Gibson et al., 1970), it has not been experimentally demonstrated. Research on this factor (Table), moreover, has not addressed itself to the role of this humoral factor in the cellular changes in cystic fibrosis — that is, accumulation of mucus on cell surfaces, changes in water and ion transport, and other alterations in organ functions.

Culture systems, so far studied, have not shown the cellular changes seen in biopsy and necropsy specimens of cystic fibrosis tissue. The search for relevant research would be enhanced by identification of a culture model which demonstrates these cellular abnormalities so well documented in vivo. The long-term lymphoid lines established from peripheral blood from homozygotes and heterozygotes for cystic fibrosis may be just such a cell model (Danes et al, 1974). Cystic fibrosis lymphoid lines can be distinguished from normal lymphoid lines by a relative increase in dermatan sulphate with a normal total mucopolysaccharide content. This feature suggests that the cystic fibrosis lymphoid cell growing in suspension shows a cell surface abnormality which may be the culture model of the in-vivo cell defect. The availability of such a cell model should help direct basic research to the study of the cultured cell with the cystic fibrosis genotype.

The abnormalities assigned to the cultured cystic fibrosis cell (Table) have all been considered at some time for use as a possible assay for the heterozygote. None has been proven specific for the cystic fibrosis gene and the reliability and reproducibility of their assays sufficient to warrant consideration for use in detecting the cystic fibrosis gene in the general population (Wood and di Sant’Agnese, 1973).

Cystic fibrosis factor has been widely proposed for use in screening for the heterozygote and thus the most thoroughly studied. At present there are two bioassays (rabbit tracheal ciliary assay (Spock et al. 1967; Conover et al., 1973b) and oyster cilia test (Bowman et al., 1969)). The developers of both assay systems agree that there are technical problems which limit the use of their assay systems to basic research aims but they do not agree on their reproducibility and significance. Although numerous biological fluids have been tested using one of these two bioassays, there is, at present, no agreement on which fluid should be used as the test sample and in which bioassay (Wood and di Sant’Agnese, 1973).

The direction of cystic fibrosis research using cultured cells has become diffuse. It has become a ‘rush’ to describe any abnormality (morphological, metabolic, or immunological) of the cultured cystic fibrosis cell. One way to reduce the randomness of this search is to test any hypothesis on cystic fibrosis families rather than on individuals. When a cell abnormality may be of possible use in screening for the gene, it is suggested that samples from families rather than individual cystic fibrosis patients and normal subjects be used to establish its specificity for the cystic fibrosis gene. As cystic fibrosis is an autosomal recessive disorder, it should be possible to trace a cellular abnormality, specific for the gene, through consecutive generations and to demonstrate its segregation.

The cultured cystic fibrosis cell will eventually ‘reveal’ its basic defect. Until then, the more that is learned about the expression of the cultured cystic fibrosis cell should enhance our understanding of the cellular pathology in vivo.

REFERENCES


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