Cystic fibrosis: current concepts

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Cystic fibrosis of the pancreas, the most frequently inherited disease affecting the Caucasian population, is an autosomal recessive disorder affecting principally the exocrine glands. It is of interest that despite the steadily increasing number of cellular and biochemical abnormalities reported in the disease even the most intrepid investigator has stopped short (sometimes only just) of staking claim to having identified the primary defect. Reported abnormalities include increased intracellular glycogen (Pallavicini *et al*, 1970), decreased collagen synthesis, and collagenolysis (Houck and Sharma, 1970). Disturbances in RNA methylation (Rennert, Julius, and LaPointe, 1972), serum kallikrein-like activity (Arvanitakis *et al*, 1973), serum hexosaminidase (Conover, Conod, and Hirschhorn, 1973a), and arginine esterase (Rao and Nadler, 1974) have also been described. Since the disease is clearly inherited in a simple recessive fashion, it is genetically certain that the many reported biological perturbations are secondary effects which will be fully understandable only when the primary, presumably enzymatic, defect is disclosed (Bearn, 1973a).

Intracellular metachromasia

The possibility of heterogeneity of the cystic fibrosis locus has been recently reopened by biochemical and cellular studies of fibroblasts derived from patients with cystic fibrosis and their families (Bearn, 1972). Systematic examination of these fibroblasts has enabled at least two clear-cut categories of cystic fibrosis to be defined at the cellular level (Bearn and Danes, 1969; Danes and Bearn, 1969; Conover, Conod, and Hirschhorn, 1973b). In some kinships the cells of affected individuals and their parents, who are obligate heterozygotes, when grown in culture demonstrate marked intra-

cellular metachromasia. Some, but not all, of the unaffected sibs of patients show metachromasia which does not apparently differ in degree from the metachromasia seen in those clinically affected. In other families with equally clear-cut evidence of clinical cystic fibrosis, no trace of intracellular metachromasia can be detected in affected individuals, heterozygous parents, or unaffected sibs.

In the few populations thus far studied, the ametachromatic patients are a clear minority and represent approximately 20% of the total (Danes and Flensburg, 1971; Danes, 1972), but more studies by other investigators are required before this figure should be taken too literally. The cellular and biochemical data supporting the concept of genetic heterogeneity appear at first blush to be at variance with earlier population data which gave no evidence for heterogeneity. The discrepancy may be more real than apparent; the methods utilized to detect heterogeneity at a population level are barely sensitive enough to detect genetic heterogeneity at the 20% level. More data are needed at the clinical, biochemical, and population level. The occurrence of certain critical matings could add additional weight to the existence of genetic heterogeneity (Bearn, 1973b).

Cystic fibrosis 'factor'

Recent studies (Danes and Bearn, 1972; Bowman *et al*, 1973b; Conover *et al*, 1973b; Danes *et al*, 1973) have focused on the functional characteristics and biochemical nature of the cystic fibrosis factor (CFF) in the serum of patients with the disease, the tinctorial (metachromasia) characteristics of cultured fibroblasts and long-term lymphoid cell lines as well as the presence of CFF in the medium in which fibroblasts derived from patients with cystic fibrosis have been grown.

Some association between the cystic fibrosis

factor and the metachromasia of cultured cystic fibrosis fibroblasts was recently described (Danes, 1973). In her experiments with cultured fibroblasts from cystic fibrosis patients and normal controls, Danes found that neither expression of the cystic fibrosis genotype in culture (metachromasia or CFF) could be affected by growing CF positive cells in used medium from normal or CFF negative cultures. If normal or CFF negative cystic fibrosis cells were grown in used medium from a CFF positive culture, the cells did not show metachromasia and CFF factor could not be detected after 3 hours of culture.

Sera from both homozygotes and heterozygotes for cystic fibrosis have been shown to inhibit the ciliary motion of rabbit tracheal explants (Spock *et al*, 1967), oyster gills (Bowman, Lockhart, and McCombs, 1969), and fresh water mussels (Besley, Patrick, and Norman, 1969). A second long-recognized defect in this disease is the observation that saliva and sweat from affected individuals markedly inhibit the reabsorption of sodium when they are infused into the parotid ducts of rats. Whether these two biological activities are caused by the same or different factors is not known, and their relationship to the basic defect has not been clarified. It is not even clear at the moment whether the factor causing inhibition of cilia motion of rabbit tracheal explants is the same as that which inhibits oyster cilia. An accurate comparison between the two factors is not currently available, since one group of workers has used only the rabbit tracheal system, whereas we and others have thus far restricted our bio-assay to oyster cilia. Even the most enthusiastic supporters of the ciliary inhibition system would agree that it is less than ideal since not only does it share with all biological tests a certain inherent variability but, at certain times of the reproductive cycle of the oyster, the cilia are particularly unreliable as a test system. Although all of those who have used the test agree that it cannot be used in mass screening to detect heterozygotes or for prenatal diagnosis, within an individual laboratory, the test is a most powerful and useful monitoring system for the purification of the factor (Bowman, Hirschhorn, and Bearn, 1974).

The observation (Bowman, McCombs, and Lockhart, 1970) that the oyster ciliary factor migrates in the γ -globulin fraction of the serum raised the interesting possibility that a specific antibody might be involved. This, however, does not appear to be the case (Bowman, Barnett, and Matalon, 1973a) and current evidence from our laboratory (Danes *et al*, 1973) also indicates that the factor is simply bound to certain γ -globulins. In further

studies, it was shown that this factor was produced *in vitro* by cultured skin fibroblasts of individuals affected with this disease but not by fibroblasts from normal individuals (Danes and Bearn, 1972; Bowman *et al*, 1973b). This factor could be detected in the culture medium of affected cells whether or not fetal calf serum was present in the medium. The factor was shown by dialysis, gel filtration, and electrophoretic studies to be positively charged and to have an approximate molecular weight of 7500 (Barnett *et al*, 1973) and was found to complex with γ -globulin subclasses IgG1 and IgG2 but not with IgG3 or IgG4 (Danes *et al*, 1973). Further, the interaction between immunoglobulin and cystic fibrosis factor was found to occur in the constant region of IgG1 and IgG2 heavy chains, since papain or pepsin fragments of these molecules did not complex with the factor. IgA1, IgA2, and IgD were also found not to complex with this factor. Similarly no interaction was found between the dyskinensis factor and purified serum haptoglobin (Hp1-1) or with group-specific component (Gc2-1) (Danes *et al*, 1973). However, serum β_2 microglobulin (Berggard and Bearn, 1968) was found to complex with this factor (Danes *et al*, 1973). This observation could be highly relevant, for although cultured fibroblasts do not synthesize γ -globulin they do synthesize β_2 microglobulin in significant amounts (Hütteroth *et al*, 1973).

The complement system

More recently, data have been obtained which suggest that the oyster cilia inhibition factor may be related to the complement system. Preliminary data from our laboratory suggest that the factor has properties similar to C3 anaphylatoxin (C3a) which has been made inactive by cleavage of its C-terminal amino acid, arginine. Normal serum contains a carboxypeptidase-B-like enzyme which specifically cleaves the C-terminal arginine from this biologically active peptide, thereby rendering it inactive in tests devised to measure anaphylatoxin activity (Bokisch and Müller-Eberhard, 1970). In preliminary experiments, it has been found that biologically active C3 anaphylatoxin does not affect ciliary activity, although ciliary dyskinesia was regularly observed after the C-terminus arginine was removed from the molecule.

In studies in our laboratory of complement levels in sera of 13 cystic fibrosis patients (Table I), whole complement (CH₅₀) and C2 haemolytic activity levels were found to vary over a wide range. However, in most of the patients, C3, when tested by its haemolytic activity or by immunochemical quantitation

TABLE I
COMPLEMENT ASSAY IN 13 CYSTIC FIBROSIS
PATIENTS

CF Patient	Haemolytic Activity*			Protein Concentration† C3
	CH ₅₀	C2	C3	
Do	180	140	135	151
Ha	91	87	102	99
Le	86	83	101	106
Ma	137	92	134	133
Del	120	95	166	163
Dec	114	106	68	84
Mey	72	51	102	124
Ma	101	102	119	111
Th	112	110	98	106
Po	66	47	113	111
Gl	71	61	138	150
Mo	108	94	126	132
Ca	108	104	164	166

* Haemolytic activity was measured as the number of units per ml. (1 unit being that amount of serum required to produce 50% lysis in the test system employed). Data presented in this table are expressed as the number of units of whole complement (CH₅₀) C2 and C3 present in the cystic fibrosis serum as a percentage of the number of units found in a pool of four normal controls.

† Protein concentration of C3 was measured in mg/ml; here the protein concentration of C3 in the cystic fibrosis sera is expressed as the percentage of that found in a pool of four normal controls.

Standard deviation in all assays performed on the four normal control sera was within $\pm 10\%$.

of protein, was found in most cases to be increased. These data differ from those of Conover, Conod, and Hirschhorn (1973c) who found that C3 protein was increased in CF patients although the haemolytic activity of the component was normal.

In 11 out of 13 CF patients, activity of the alternate mechanism of complement was measured by electrophoretic conversion of C3 pro-activator (C3PA) and C3 by activation with inulin (Götze and Müller-Eberhard, 1971). In 10 out of 11 cases, the conversion of C3PA to C3 activator (C3A) and C3 to its haemolytically inactive product (C3i) was less than 50% of that obtained with normal controls.

The observation that the dyskinesia factor found in the serum of cystic fibrosis patients might be related to the complement system raises several interesting questions. Is a specific antibody involved? If so, what is its specificity? Alternatively, is the activation of complement achieved via the alternate mechanism? If so, what is the mechanism which triggers this system? Since cystic fibrosis individuals are known to have increased levels of γ A in their serum (South *et al*, 1967) and because it is known that γ A will activate the alternate mechanism (Götze and Müller-Eberhard, 1971), does γ A trigger complement activation? A further involvement of γ A was suggested by experiments in which alveolar macrophages from patients with cystic fibrosis failed to

support phagocytosis of *Pseudomonas aeruginosa* (Biggar, Holmes, and Good, 1971). Yet another possibility which has to be considered is whether a C3-ase is involved which is not related to the known complement factors.

This brief review has emphasized the paradox of cystic fibrosis. A plethora of abnormalities have been disclosed yet none can be accorded the pride of place as the primary inherited abnormality. It is nevertheless hoped that in the near future, as a result of collaboration and integration of highly specialized areas of research, meaningful and significant data can be obtained which will ultimately be of value not only in identifying heterozygotes with this condition but also in the handling and treatment of patients with this common and disabling inherited disease.

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