ducer of antibodies to nucleic acids made it possible to pursue these therapeutic concepts.

The administration of cyclophosphamide 24 hours after antigen administration leads to a state of specific immunologic tolerance, apparently by destroying rapidly proliferating sensitized cells (*7*). Animals treated in this manner show a diminished response to subsequent antigenic challenge. We treated a group of B/W female mice (1 month old) with a single injection of poly I • poly C (100 μg) followed on the next day by 60 mg of cyclophosphamide per kilogram of body weight intraperitoneally (one course). In other groups of B/W females (1 month old) this was repeated two or three times separated by 4 to 5 days. Control mice were treated with either cyclophosphamide alone (60 mg/kg) two or three times, or with poly I • poly C alone (100 μg) two or three times. All mice were challenged with poly I • poly C in complete Freund's adjuvant 11 days after the last injection of cyclophosphamide. Equal volumes of poly I • poly C (1 mg/ml) and complete Freund's adjuvant were emulsified, and 0.3 ml of the mixture injected intraperitoneally. The mice were bled 2 weeks later for assay of antibodies to RNA.

Serum poly I • poly C binding was determined by a modification of the Farr technique with ^{14}C-labeled poly I • poly C (Miles Laboratories) as antigen and with 35 percent ammonium sulfate in the final reaction mixture (*4*). Each serum was titered with the ^{14}C-labeled poly I • poly C. The 50 percent binding point was considered the antigen-binding capacity of the serum. Animals that received two or three courses of poly I • poly C and cyclophosphamide made no detectable antibody to poly I • poly C after challenge (Table 1). By contrast, animals treated with either poly I • poly C alone or with cyclophosphamide alone were immunized by the challenge dose. Thus, the combined use of poly I• poly C and cyclophosphamide in this schedule led to a profound state of immunologic tolerance. A single course led to a 90 percent reduction in binding capacity. The specificity of the tolerance to poly I • poly C was demonstrated by the normal response to immunization with sheep erythrocytes of animals made tolerant to poly I • poly C.

This is the first demonstration of experimentally induced tolerance to a nucleic acid antigen. The effectiveness of this therapy in either the prophylaxis or therapy of murine or human systemic lupus needs to be determined. This technique may have widespread application in various "autoimmune" diseases as well as in the field of transplantation.

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Cystic Fibrosis: Characterization of the Inhibitor to Ciliary Action in Oyster Gills

Abstract. *The inhibitor to oyster ciliary activity was isolated from serum of cystic fibrosis patients and heterozygotes. The inhibiting protein fraction was a cation as judged by electrophoresis; it had a molecular weight of 125,000 to 200,000 as judged by gel filtration; and on diethylaminoethyl-cellulose chromatography it eluted with the immunoglobulin G fraction. The analogous fraction in normal individuals did not inhibit oyster ciliary activity.*

Serums from cystic fibrosis (CF) patients and their parents inhibit ciliary activity in preparations of rabbit trachea (*1*), oyster gills (*2*), and freshwater mussels (*3*).

We report the characterization of the serum fraction that inhibits oyster gill cilia. The results from separations on preparative starch-block electrophoresis, on diethylaminoethyl (DEAE) cellulose chromatography, and on Sephadex G200 gel filtration indicated that the inhibiting factor is present in the γ-globulin fraction of serum from CF

Table 1. Time required by electrophoretic serum fractions to stop cilia. Fractions designated "N.T." were not tested because they were not properly separated by electrophoresis or because they developed precipitates. "Ex" indicates fractions that produced expulsion of debris.

Subjects	Time required (min)					
	Whole serum	Fractions				
		G	E	D	C	B
			Patients			
E. D.	10	60	55	50	44	25 Ex
J. A.	8	N.T.	N.T.	60	40	15 Ex
J. B.	15	N.T.	50	15	55	20 Ex
P. C.	10	55	N.T.	50	45	5 Ex
M. E.	12	50	35	0	55	22 Ex
J. W.	15	50	40	50	40	30 Ex
J. L. M.	10	45	50	N.T.	40	2 Ex
S .S.	20	50	50	50	50	1 Ex
S. T.	20	33	45	45	40	30 Ex
P.P.	N.T.	65	N.T.	50	50	22 Ex
			Heterozygotes			
Mrs. W.	20	45	50	50	50	24 Ex
Mr. W.	15	25	55	50	40	35 Ex
Mrs. D.	17	N.T.	33	N.T.	60	38 Ex
Mrs. M.	8	40	40	50	N.T.	18 Ex
			Presumed heterozygote			
M. L. M.	15	50	50	50	30	7 Ex
			Normals			
L. L.	50	50	42	50	50	50
P. B.	50	25	16	N.T.	50	50
D. B.	50	50	50	50	50	50
L. N.	50	35	50	47	50	47
B. J.	48	60	60	60	60	41
M. K.	50	N.T.	N.T.	N.T.	40	50

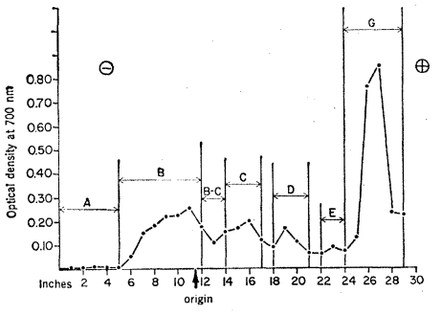

Fig. 1. Preparative starch-block electrophoresis (*4*) of human serum, *p*H 8.6.

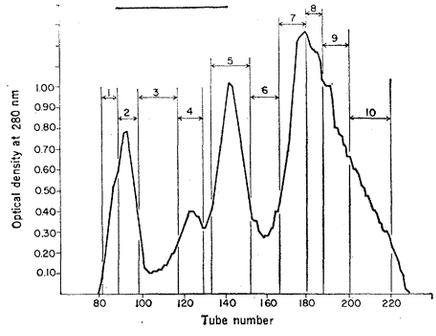

Fig. 2. Gel filtration of human serum on Sephadex G200. Each fraction was in 5.4 ml. The buffer was 0.005*M* tris-HCl. The column was 110 by 5 cm.

patients and heterozygotes. The same fraction from serum of 12 normal individuals failed to inhibit oyster cilia.

Serums (10 to 20 ml) collected from 10 CF patients, 4 heterozygotes, and 13 unaffected individuals were submitted to preparative starch-block electrophoresis or chromatography on DEAE cellulose (*4*). The electrophoresis separated individual serums into six or seven fractions (A to G) (Fig. 1). The corresponding fractions of each were pooled and concentrated by lyophilization or by ultrafiltration. After dialysis against 0.9 percent NaCl, enough saline solution was added to each fraction to make up the original volume of whole serum. The pooled fractions A to G were tested on oyster cilia (*2*). The time required for ciliary cessation and the serum's effect on the gill tissue were recorded (Table 1). Our earlier work (*2*) demonstrated that, although serums from unaffected individuals permitted oyster cilia activity to continue for 1 hour under a sealed cover slip, exposure to serums from CF patients and known CF heterozygotes caused ciliary action to stop within 38 minutes. When we tested the electrophoretic fractions on oyster cilia, we observed two classes of reactions: some fractions had negligible effect on the cilia; others caused the cilia to stop beating before 38 minutes.

The time periods required for the whole serums from CF patients, CF heterozygotes, and normal individuals to stop ciliary activity are also recorded in Table 1. In addition to inhibiting ciliary movement, some serums and some fractions when added to the preparation caused material to be expelled immediately from the cilia mounds. After cilia were exposed to the electrophoretic fractions from CF serums, the ciliary action was inhibited by fraction B, the protein group migrating most cathodally. Fraction B produced consistent inhibition of the gill cilia activity, limited the ciliary action to under 30 minutes, and produced expulsion of material from the gill mounds. All but three of the 35 remaining fractions from CF serums permitted ciliary activity to continue from 40 to 50 minutes. These three fractions appeared to exert an inhibition on the cilia that was not characteristic of the CF effect, and they never caused material to be expelled from the gill mounds.

Fraction B from the four CF heterozygotes tested also caused ciliary cessation and expulsion of material. No electrophoretic fractions from heterozygotes except fraction B expelled material, although two other fractions stopped ciliary activity before 40 minutes. The average protein concentration of fraction B in serums tested was 9.4 mg/ml. The fractions with highest protein concentrations did not necessarily have the greatest inhibitory effect.

The B fractions from serums of six normal individuals had no effect on ciliary action. Three other fractions from normals stopped ciliary activity before 38 minutes without expelling material.

Of particular interest was fraction B from a normal (M.L.M.), which reacted on gill cilia in the same way as did CF serum. This individual was one of two normals found in a previous study (*2*) whose whole serum stopped ciliary activity before 35 minutes. The inhibitory action of his fraction B was additional evidence that M.L.M. was a CF heterozygote.

Immunoelectrophoresis of fraction B from CF patients, heterozygotes, or normals, which had a protein concentration of 4.0 to 15 mg/ml, revealed the presence of γG, γA, and γM immunoglobulins. The C-reactive protein, also a basic protein, did not appear to be present in any of the serums of CF patients. The C'1q component of complement and lysozyme were present in trace amounts in fraction B after immunoelectrophoresis.

Table 2. Time required by DEAE fraction 1 to stop cilia.

Subjects	Time required (min)	
	Whole serum	Peak 1
Patients		
J. A.	8	15
E. D.	10	15
P. O.	N.T.	25
P. C.	10	7
Heterozygotes		
Mrs. M.	8	18
Mr. W.	15	7
C. L.	15	15
Normals		
B. B.	50	40
S. A.	50	43
D. B.	50	40
L. L.	50	40
P. G.	60	50
P. B.	50	50
D. K.	50	40
B. O.	50	60
G. A.	48	38
L. N.	50	50

Fractionation of serums from five CF patients, two CF heterozygotes, and ten normal individuals was performed on DEAE cellulose column chromatography with 0.005*M* tris-HCl buffer at *p*H 8.6 (*4*). Immunoelectrophoresis demonstrated that the first fraction, comprising the most cationic serum proteins, consisted mainly of γG immunoglobulins. Samples of fraction 1 from DEAE chromatography, which had a concentration of 3.3 to 8.6 mg/ml, did not react immunologically with antiserum to γM, γA, haptoglobin, ceruloplasmin, transferrin, albumin, or the group-specific protein after immunodiffusion or immunoelectrophoresis. After the first protein peak was pooled, it was dialyzed against 0.9 percent saline solution and was tested on oyster cilia. The periods necessary for ciliary cessation after exposure to DEAE peak 1 fractions were recorded for each sample and appear in Table 2. Characteristically, the first DEAE peak after chromatography of CF and CF heterozygote serum stopped cilia movement before 30 minutes and caused expulsion, whereas the analogous peak from ten unaffected individuals had little or no effect on the ciliary activity.

Serum subjected to gel filtration on

columns of Sephadex G200 separated into four major fractions. Serum from a CF patient (J.A.) was fractionated in this manner and divided into ten parts (Fig. 2). Ciliary inhibition was mainly demonstrated by fraction 5 from the gel filtration. The bordering fractions 4 and 6 had weaker inhibiting capacities. The remaining fractions had no inhibiting effect on the cilia. The inhibitory effect was observed with fraction 5 from gel filtration on Sephadex G200 of serums from a CF patient (J.A.), a CF heterozygote (Mrs. M.), and the presumed heterozygote (M.L.M.). When this fraction 5 from a nonaffected individual (D.B) was tested on oyster cilia, there was no inhibition of ciliary activity. As determined by calibration of the Sephadex column (*4*), eluted fraction 5 corresponded to a protein with a molecular weight of 125,000 to 200,000.

The proteins identified after immunoelectrophoresis of G200 fraction 5 were haptoglobin (trace amount), ceruloplasmin, γG (main component), and γA (trace amount).

This work indicates that a cationic protein or a substance bound to a cationic protein is the cystic fibrosis factor responsible for ciliary inhibition in oyster gill preparations. This protein appears to belong to the γG family of proteins or to have properties closely similar to them. Although the C'1q complement component and lysozyme migrate with the CF inhibitor during electrophoresis, they are not present in the CF fraction after gel filtration, owing to differences in molecular weight.

The component in the immunoglobulin fraction that is responsible for the response on oyster cilia observed in serums from CF patients and heterozygotes may be an altered protein or a protein carrying a bound compound which is the result of a mutated gene. This protein affects ciliated tissue in oysters and may be responsible for the defects in membrane transport, pulmonary function, and pancreatic enzyme secretion observed in patients with cystic fibrosis. This protein may act as an autoantibody or as a polycation, which is destructive to membrane integrity and energy exchange.

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6 FEBRUARY 1970

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13 November 1969

Insulin-Stimulated Glucose Uptake by Subcellular Particles from Adipose Tissue Cells

Abstract. *Microsomal particles prepared from isolated rat adipose cells take up* D-*glucose more rapidly than* L-*glucose. The rate of* D-*glucose uptake, but not that of* L-*glucose, is stimulated by incubation of the intact cells with insulin at concentrations as low as 10 microunits per milliliter before disruption and preparation of the microsomes.*

The particles (microsomal fraction) obtained as a result of high-speed centrifugation of disrupted, isolated adipose cells of the rat epididymal fat pad take up glucose in a way that resembles the transport system of intact cells (*1*). D-Glucose was both taken up and released more rapidly than L-glucose by these particles. The uptake of D- but not of L-glucose was decreased by a specific inhibitor of glucose transport, phlorizin. Moreover, prior addition of unlabeled glucose to the particulate preparation led to a more rapid uptake of labeled glucose when compared to a control with prior addition of sucrose, suggesting a counterflow phenomenon characteristic of transport systems (*2*). This subcellular preparation did not convert glucose to either CO_2 or lipid, and the bound sugar was recovered by elution and found to be unchanged D-glucose. These findings were interpreted to indicate that L-glucose entered the internal space of the microsomal particles by diffusion through membrane pores or "leaks," whereas D-glucose entered by a combination of diffusion and facilitated entrance by way of an intact glucose transport system.

It was of interest to see whether the uptake of D-glucose by this preparation was responsive to insulin, because stimulation of glucose transport is one of the primary actions of insulin on intact adipose tissue. When the intact isolated adipose cells were exposed to insulin before homogenization, a definite response was elicited. The subcellular "microsome" fraction prepared from the cells exposed to insulin showed an

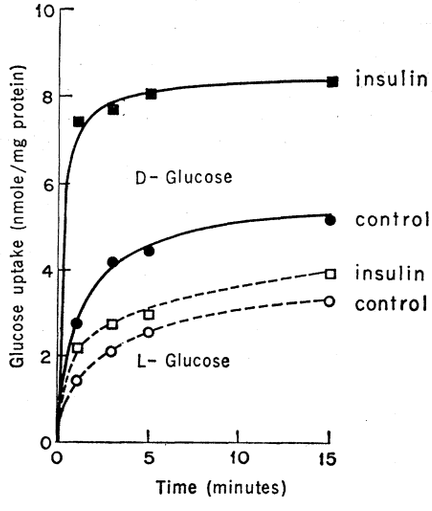

Fig. 1. Effect of insulin on glucose uptake by isolated microsomes. Isolated adipose cells were prepared from rat epididymal pads by incubation with bacterial collagenase. Cells were exposed briefly (5 minutes) to insulin (100 munit/ml) or an equal volume of H_2O and then disrupted in a Potter-Elvejhem homogenizer. Mitochondria and cell debris were removed by centrifugation at 10,000*g* for 15 minutes, and then the "microsomes" were isolated by centrifugation at 40,000*g* for 30 minutes. Microsomal particles suspended in Krebs-Henseleit phosphate buffer were incubated with D-[^{3}H]-glucose and L-[^{14}C]-glucose (each at 5 m*M*), and, at the indicated times, portions were rapidly filtered and washed with cold buffer on Millipore filters, which were then transferred directly to counting vials for determination of radioacivity retained in the particles. Sugar uptake is corrected for protein concentration of each pellet suspension.