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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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

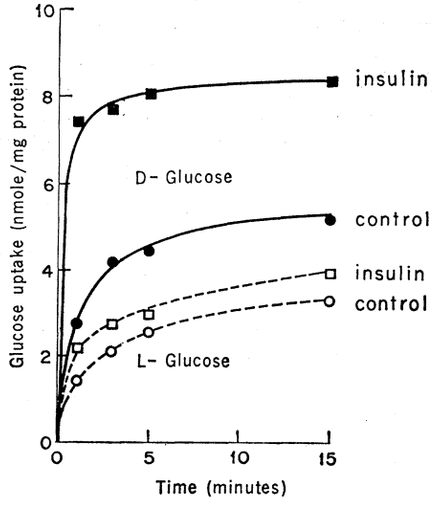

Fig. 1. Effect of insulin on glucose uptake by isolated microsomes. Isolated adipose cells were prepared from rat epididymal pads by incubation with bacterial collagenase. Cells were exposed briefly (5 minutes) to insulin (100 munit/ml) or an equal volume of H_2O and then disrupted in a Potter-Elvejhem 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.

Table 1. Effect of varying insulin concentrations on glucose uptake by subcellular particles. Experiments were performed as in Fig. 1 except that whole cells were exposed to varying insulin concentrations before disruption. Data for one time point are shown, and only net or "specific" uptake is recorded—that is, D-glucose minus L-glucose uptake. The differences observed are all significant ($P < .05$).

Insulin (μunit/ml)	Glucose uptake (nmole/mg protein)	
	Control	Insulin
100,000	1.91	5.13
1,000	3.20	5.29
100	2.09	3.57
10	2.43	4.37

increase in D-glucose uptake when compared to controls (Fig. 1). There was essentially no change in the (lower) rate of L-glucose uptake by the membranes. The response to insulin was seen when the cells were exposed to concentrations as low as 10 μunit/ml (Table 1), within the physiologic range and near the lowest concentrations at which responses can be detected in isolated intact cells (*3*). Response to 1 μunit/ml was variable.

The effect of insulin in these experiments was entirely on glucose transport into the microsomal particles. The membrane preparations from both control cells and those treated with insulin were unable to convert D-[^{14}C]-glucose to either ^{14}C-CO_2 or ^{14}C-labeled lipid. Furthermore, the radioactive sugar taken up by the particles was quantitatively eluted with distilled H_2O and was recovered entirely as the unphosphorylated glucose. Thus all of the insulin effect was on the rate of D-glucose uptake and not on any subsequent step in glucose metabolism.

Insulin can be inactivated by alkaline hydroylsis. Such a preparation was tested against whole fat cells and showed no significant stimulation of D-[^{14}C]-glucose conversion to ^{14}C-CO_2. Membranes prepared from adipose cells exposed to this inactivated insulin preparation showed no significant increase in D-glucose uptake when compared to membranes prepared from control cells.

Addition of high concentrations of insulin (100 munit/ml) to the subcellular preparations after the adipose cells were disrupted led to no stimulation in the rate of glucose uptake. This was not unexpected. Rodbell (*4*) has shown that carefully prepared "ghosts" of adipose tissue cells still respond to insulin but that the response is far less than in intact cells even though the "ghosts" are able to metabolize glucose. Thus, there was no reason to suppose that metabolically inert particles prepared after more stringent treatment of the whole cells should respond to the hormone.

It has been demonstrated with other cell types that the usual "microsomal" fraction contains particles of plasma membrane (*5*), and it seems likely that the glucose uptake system responsive to insulin demonstrated here is in these fragments.

Whatever change in the membrane-bound glucose transport system is affected by insulin apparently requires the presence of intact cells at the time of exposure to the hormone but is retained after disruption of the cells. Whether this is because of a specific membrane configuration required for insulin binding that is lost upon disruption or because of a required "soluble" component that is lost during separation and centrifugation is not clear. However, the fact that the isolated membrane preparation retains the effect of insulin stimulation indicates that the hormonally induced change is reasonably stable and should be amenable to study in this isolated preparation.

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Dextranase Activity in Coleoptiles of Avena

Abstract. *An enzyme activity similar to that of dextranase is associated with coleoptiles of* Avena sativa. *When subjected to purified dextranase, both the pure natural dextran and the cell walls of the* Avena *coleoptiles yield isomaltose and isomaltotriose. Thus, the cell walls contain dextrans or dextran-like compounds. Coleoptiles with low auxin content have a lower dextranase activity than coleoptiles with high auxin content. The enzyme activity is therefore in some way sensitive to the hormone.*

The occurrence of β-glucanases in coleoptiles of *Avena* has been reported (*1*). As part of a study of α-glucanases, I now report the presence of an enzyme activity very similar to that of dextranase in these coleoptiles. Dextranases break down α-glucans in which the glycosidic linkages consist of $\alpha(1,6)$ and, in smaller quantiy, $\alpha(1,3)$ bonds. Until now no such enzyme activity has been reported in higher plants (*2*).

As a specific substrate for demonstrating dextranase activity, I used a natural dextran produced by *Leuconostoc mesenteroides* NRRL B-512. This dextran has high purity, has a molecular weight of about 20 to 30 million, and contains 95 percent of $\alpha(1,6)$ linkages and 5 percent of $\alpha(1,3)$ linkages. Some of these $\alpha(1,3)$ linkages form the branching points of long side chains (*3*). In addition, I used a commercial

Table 1. Dextranase activity in pellets prepared from 20 sections of coleoptiles of intact and decapitated *Avena* plants as judged by viscosity of dextran solutions after 4 hours of digestion. Activity expressed as flow seconds. Numbers in parentheses represent percent decrease in flow time as compared with control substrate.

Type of pellet	Flow time of 2% dextran (m. w. 20 to 30 $\times$ 10^6)				Flow time of	
	Exp. 1 (sec)	Exp. 2 (sec)	Exp. 3 (sec)	Exp. 4 (sec)	2% dextran (m. w. 2×10^6) (sec)	4% dextran (m. w. 2×10^6) (sec)
None	447	450	444	460	475	260
Boiled	447					
From intact coleoptile	404 (9.5)	369 (10.0)	376 (15.5)	390 (15.2)	433 (9.2)	236 (13.1)
From decapitated coleoptile	445 (0.0)	423 (6.0)			475 (0.0)	247 (5.0)