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Cystic fibrosis of the pancreas
α-glucosidase
glycogen
lymphocytes
lysosomes
mitochondria

Cystic Fibrosis: Lysosomal and Mitochondrial Enzyme Activities of Lymphoid Cell Lines

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Extract

Seven lysosomal enzymes and three mitochondrial enzymes were studied in cultured lymphoid cells from four patients with cystic fibrosis, three heterozygotes, one borderline patient, and six healthy controls. The lysosomes and mitochondria were isolated by differential and density gradient centrifugation. The activity of α-glucosidase, which is a lysosomal enzyme degrading glycogen, showed a marked increase in activity in the lysosomes of cultured lymphoid cells obtained from patients with cystic fibrosis as compared with the healthy controls. Two substrates were used for measuring the activity of this enzyme, the synthetic *p*-nitrophenyl-α-glucoside (*p*-NP-α-glucoside), and the physiologic substrate glycogen. The values obtained with *p*-NP-α-glucoside were 32.6 (SD ± 8.6) and 13.8 (SD ± 12.2) μmoles/g lysosomal protein/hr and with glycogen 543.5 (SD ± 163.3) and 188.6 (SD ± 156.3) μmoles/g lysosomal protein/hr for patients with cystic fibrosis and controls, respectively. The values of α-glucosidase in the heterozygotes were also elevated, 17.2 (SD ± 5.5), controls 13.8 (SD ± 12.2) using *p*-NP-α-glucoside as substrate. When glycogen was used as substrate, the values were 226.7 (SD ± 85.3) in the heterozygote and 188.6 (SD ± 156.3) μmoles/g lysosomal protein/hr in the control. The activities of the other six lysosomal enzymes—β-glucosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, aryl-sulfatase, and acid phosphatase—were the same in patients with cystic fibrosis as in the controls. The three mitochondrial enzymes assayed, succinic dehydrogenase, glutamic dehydrogenase, and malic dehydrogenase, showed the same activity in all subjects tested.

Speculation

This study indicates that the metabolism of lymphoid cells obtained from patients with cystic fibrosis (CF) and maintained in culture for prolonged periods differs from that of cells of healthy controls. The abnormally elevated activity for α-glucosidase in lymphoid cells derived from patients with cystic fibrosis is noted when measured with synthetic substrate as well as with a physiologic substrate, glycogen. To date no defect in glycogen metabolism has been noted in cystic fibrosis although Pallavicini *et al.* did observe an increase in the glycogen content of cultured fibroblasts obtained from patients with cystic fibrosis. Bartman *et al.* found an increase in size of lysosomes in cultured fibroblasts from patients with CF by electron microscopy. These and our observations suggest the possibility of an impaired cellular metabolism in CF indicating that we may be dealing with a lysosomal disorder. Of some importance is the

observation that the activity of α-glucosidase is also elevated in the heterozygote, but not to the same extent as in the homozygote. Perhaps this could offer a means of detecting the heterozygote, a possibility which should appeal to the genetic counsellor. Although numerous techniques and reports have suggested methods of identifying the heterozygote, none is practical or reliable. To be of value the test would have to take less time than required in the technique used here. For this purpose we are in the process of studying the activity of the α-glucosidase from cells derived from the patient rather than from cells grown in continuous culture.

It is quite possible that patients suffering from malignant disorders or those receiving radiation or drugs may also show abnormal levels of lysosomal enzymes but may not have the same specificity; *i.e.*, many of the lysosomal enzymes may be elevated rather than an isolated one.

Introduction

Cystic fibrosis is an inherited, autosomal, recessive disease characterized by abnormalities of the mucous and sweat-secreting glands. The primary defect, however, remains undiscovered. Recent advances in cell culture techniques have made possible the study of this disease on a cellular level. Abnormalities have been recognized in tissue culture fibroblasts from such patients, as well as from heterozygotes [8]. Bartman *et al.* [2] studied the ultrastructure of cultured fibroblasts and found that the cells from four patients with CF had an increase in the number and size of lysosomes concomitant with a simultaneous increase in the amount of material stored within these subcellular particles. These findings suggested the possibility of an impaired cellular metabolism in cystic fibrosis which might be reflected in altered enzyme activities with possible changes in intermediary metabolites in subcellular fractions such as lysosomes and mitochondria.

The ability to isolate and establish permanent cell lines from peripheral blood lymphocytes has provided the tools necessary to grow sufficiently large quantities of cells to make such studies of subcellular organelles feasible. In previous studies [17], in which monolayer cultures were employed, it was found that homogenates of cultured fibroblasts from the skin of patients with cystic fibrosis showed no change in several lysosomal hydrolases, namely β-galactosidase, β-glucuronidase, aryl sulfatase, and acid phosphatase. Since this investigation had been carried out on whole homogenates of cells, it was possible that an actual lysosomal enzyme abnormality might be obscured by a number of interfering effects caused by other intracellular components. To eliminate those interferences, the present study deals with isolated lysosomes and mitochondria.

The activities of seven lysosomal enzymes (α-glucosidase [EC. 3.2.1.20], β-glucosidase [EC. 3.2.1.21], β-galactosidase [EC. 3.2.1.23], β-glucuronidase [EC. 3.2.1.31], *N*-acetyl-β-glucosaminidase [EC. 3.2.1.30], aryl sulfatase [EC. 3.1.6.1], and acid phosphatase [EC. 3.1.3.2]) and three mitochondrial enzymes (succinic [EC. 1.3.99.1], glutamic [EC. 1.4.1.2], and malic [EC. 1.1.1.37] dehydrogenases) were investigated in isolated lysosomes and mitochondria from established lymphoid cell lines obtained from patients with cystic fibrosis, heterozygotes, clinically healthy individuals, and one patient considered as "borderline" cystic fibrosis.

Materials and Methods

Patients

The four patients with CF were 7, 12, 19, and 22 years of age with an equal sex distribution. The borderline patient was a male 16 years of age and the heterozygotes were parents of CF children, aged 34, 41, and 46 years. Five healthy controls consisted of three males and two females between 20 and 55 years of age. One control, age 20, had had infectious mononucleosis and was well at the time of obtaining the specimen. The majority of cultures were taken between March 1970 and February 1971 [40].

Lymphoid Cell Lines

All the cell lines utilized in these studies were derived from peripheral blood buffy coats [19]. All of the cell lines were composed of cells which grow readily in suspension culture and have the morphology of lymphoblasts. Cells to be used for the isolation of subcellular organelles were grown as active suspension cultures in Erlenmeyer flasks. The growth medium was Eagle's minimal essential medium modified for suspension culture [10] and supplemented with "nonessential" amino

acids [18], sodium pyruvate (1 mM), and 10% whole fetal bovine serum. Actively dividing cultures were used throughout these studies.

Preparation of Subcellular Organelles

Six to 10×10^8 cells were harvested by centrifugation ($100 \times g$, 20 min) at room temperature. A modification of the techniques of Rahman and Lindenbaum [25] and Hogeboom [14] was used to prepare the organelles. The cells were resuspended uniformly by use of a Potter-Elvehjem homogenizer (Teflon-glass) in about 80 ml 0.25 mM sucrose solution containing 10 mM triethanolamine (TEA) and 2 mM ethylenediaminetetraacetic acid (EDTA) tetra sodium salt, pH 7.4. The cells were disrupted using the nitrogen decompression method described by Hunter and Commerford [15] in a nitrogen bomb [31]. This method was chosen because cells derived from CF patients and the borderline patient could not be broken by mechanical means (Potter-Elvehjem homogenizer with rotating pestle) without damaging subcellular particles. The nitrogen pressure was applied for 15 min at 900 psi on CF and borderline cells and 750 psi on control and heterozygote cells. Higher pressures were required to rupture the cells from the CF line and from the borderline case than from the control lines. Under these conditions, more than 90–95% of the cell membranes were found broken when examined microscopically. Differential and density gradient centrifugations were carried out (details are given in Fig. 1) in a refrigerated centrifuge [32] and in an ultracentrifuge [33], respectively. Linear sucrose density gradients (10–30%, w/v) were generated by mixing 2 and 50.5% sucrose media (10 mM TEA + 2 mM EDTA, pH 7.4) in a density gradient former [34]. The supernatant from the final centrifugation containing the lysosomes was carefully aspirated off. The mitochondrial sediment was resuspended in sucrose solution. Both fractions were centrifuged at 100,000 × *g* for 20 min. The resulting pellets were resuspended in 5 ml double-distilled water and disintegrated by gentle ultrasonification [35] (4×10 sec, 40 mv output energy, microtip). All steps were carried out at 4–6°. The lysosomal and mitochondrial homogenates as well as an aliquot of the whole cell homogenate were kept frozen at −20° in small quantitites. All determinations were carried out after a single thawing of the sample.

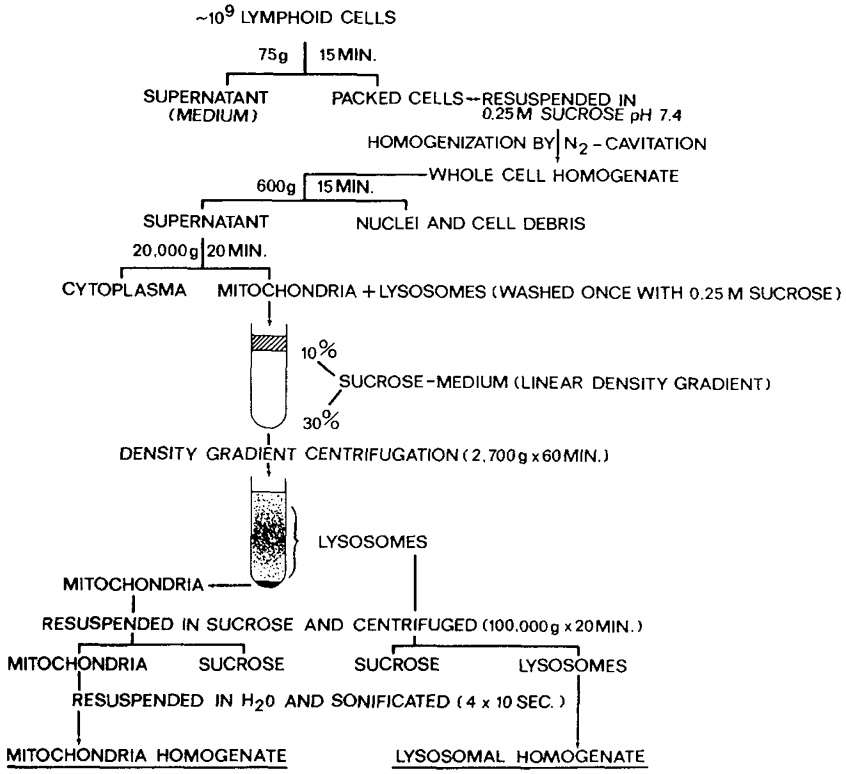

Fig. 1. Preparation of lysosomal and mitochondrial fractions from cultured lymphoid cells.

Enzymatic Assays

1. Lysosomal Enzymes

α-Glucosidase was measured using two different substrates: *1)* *p*-nitrophenyl-α-D-glucoside and *2)* glycogen. The method of Torres and Olavarria [30] was modified by substituting *p*-nitrophenyl-α-D-glucoside (*p*-NP-α-gluc) for α-phenylglucoside. The incubation mixture contained 0.100 ml 50 mM acetate buffer, pH 4.0; 0.100 ml 30 mM *p*-nitrophenyl-α-D-glucoside [36]; and 0.100 ml lysosomal homogenate. The mixture was incubated at 37° for 120 min, and the reaction was stopped by adding 0.100 ml 40% trichloroacetic acid (TCA) and then centrifuged at 2800 rpm for 15 min. Then 0.200 ml supernatant was pipetted into a small test tube to which 0.300 ml 2 N NaOH was added. The color was read at 420 mμ in microcuvettes. Standards of nitrophenol were run simultaneously with the samples. When glycogen was used as substrate, the incubation mixture consisted of 0.200 ml 50 mM acetate buffer, pH 4.0; 0.200 ml 10 mg/ml glycogen solution [37]; and 0.100 ml lysosomal homogenate. The mixture was incubated for 120 min at 37°. The reaction was stopped by immersing the tubes in boiling water for 2 min. Since the solution was turbid, precipitated protein was centrifuged at 2800 rpm for 15 min. Then 0.100 ml supernatant was used for glucose determination with glucostat special [32] according to the method of Dahlqvist [7]. The color was read at 420 mμ.

Determinations of β-glucosidase, β-galactosidase, and *N*-acetyl-β-glucosaminadase, in which in each instance liberated *p*-nitrophenol was measured, were based on Beck and Tappel's method [3] modified for microdetermination. The conditions for each determination were as follows: 0.100 ml McIlvaine's citrate-phosphate buffer, 0.100 ml substrate, and 0.100 ml lysosomal homogenate were incubated at 37° for 30–60 min. The reaction was stopped with 0.100 ml 40% (w/v) trichloroacetic acid and then centrifuged. Then 0.200 ml supernatant was transferred into small test tubes and 0.300 ml 2.0 N NH_4-OH (pH 10.7) was added for color development. The absorbance was read at 420 mμ. The substrates and the pH of buffer for each of the enzymes were as follows: β-glucosidase: 1 mM *p*-nitrophenyl-β-D-glucopyranoside [37], pH 5.0; β-galactosidase: 1 mM *p*-nitrophenyl-β-D-galactopyranoside [37], pH 3.0; *N*-acetyl-β-glucosaminidase: 2.4 mM *p*-nitrophenyl-*N*-acetyl-β-glucosaminide [37], pH 4.2. With each determination, suitable standards of *p*-nitrophenol were run.

β-Glucuronidase was measured using a modified method for microdetermination described by Talalay *et al.* [29]. The substrate used was 0.025 ml 12.5 mM phenolphthalein-β-glucuronic acid [37], with 0.200 ml 100 mM acetate buffer, pH 4.5, and 0.025 ml lysosomal homogenate. The reaction was stopped after 1 hr incubation with 0.25 ml 400 mM glycine-NaCl-NaOH buffer, pH 10.45, and centrifuged for 15 min at 2800 rpm. The color of the supernatant was read at 540 mμ. Suitable phenolphthalein standards were run with each determination.

Aryl sulfatase was determined by a modification of the methods of Roy [26] and Bowers *et al.* [5]. The incubation mixture contained 20 mM nitrocatechol sulfate dipotassium salt [37]; 50 mM acetate buffer, pH 5.0; lysosomal homogenate, 0.050 ml in a final volume of 0.500 ml. The incubation time was 120 min. The reaction was stopped by adding 1.5 ml 2.2% TCA. After centrifugation at 2800 rpm for 15 min 1.0 ml supernatant was transferred into a small test tube and 0.25 ml 5 N NaOH was added to develop the color which was read in microcuvettes at 540 mμ. Standards of nitrocatechol were run with each determination.

The acid phosphatase was determined by the modified method of Linhardt and Walter [20]. The incubation mixture consisted of 50 mM citrate buffer, pH 4.8; 5.5 mM *p*-nitrophenylphosphate [37]; and 0.050 ml lysosomal homogenate in a final volume of 0.500 ml. The mixture was incubated at 37° for 30 min in an agitated water bath and then stopped by adding 2.0 ml 0.1 N NaOH. The color was read at 405 mμ; *p*-nitrophenol standards were run parallel with the samples.

In all the enzyme determinations sample blanks were run simultaneously in which the lysosomal homogenate was added after incubation and stopping the reaction. The absorbance was read against the blank in a double beam spectrophotometer [39]. The enzymatic activities are expressed as micromoles of hydrolyzed substrate per gram or milligram of lysosomal protein per hour.

2. Mitochondrial Enzymes

Succinic dehydrogenase (SDH) activity was determined according to the method of Arrigoni and Singer [1], modified for measurement in a recording double beam spectrophotometer. A reference reaction mixture was used in which the substrate was substituted by H_2O to obtain a linear reaction. Malic dehydrogenase (MDH) and glutamic dehydrogenase (GluDH) activities were measured by the method of Bücher *et al.* [6], who used the modification introduced by Frieden [12]. Mitochondrial enzyme activities are expressed as micromoles

of either oxidized or reduced substrate per milligram mitochondrial protein per minute.

The protein content of the cellular, lysosomal, and mitochondrial homogenates was determined by the modified methods of Lowry *et al.* [21] and Eggstein and Kreutzer [11].

Results

The specific activities of seven lysosomal enzymes in isolated lysosomes of cultured lymphoid cells from six controls, four patients with cystic fibrosis, three heterozygotes and one borderline CF patient are shown in Table I. The values listed were obtained as an average of four to six individual subcultures and cell fractionations in each cell line. The activity of α-glucosidase was determined using two substrates, namely the synthetic *p*-nitrophenyl-α-glucoside and glycogen. A comparison between the mean value of the activities of the lysosomal enzymes with the recording of one standard deviation, the range of values, and the total number of determinations from which the mean was determined is shown in Table II. The specific activity of α-glucosidase is markedly higher with both substrates in the CF group, although individual values from controls overlap the cystic fibrosis group. No differences were found in the remaining six lysosomal enzymes. The specific activity of all the lysosomal enzymes were found to be highest in lysosomes obtained from the patient with borderline cystic fibrosis.

Table III presents the average activities of three mitochondrial enzymes (MDH, SDH, and GluDH) in isolated mitochondria from cultured lymphoid cells obtained from patients with cystic fibrosis, heterozygotes, borderline, and controls. No marked differences were found.

Two lysosomal enzymes, namely α-glucosidase and

Table I. Specific activities of lysosomal enzymes in isolated lysosomes from cultured lymphoid cells, obtained from four patients with cystic fibrosis (CF), three heterozygotes, one borderline, and six control subjects[1]

Group	Subject	α-Glucosidase		β-Glucosidase	β-Glucuronidase	β-Galactosidase	Aryl sulfatase	*N*-Acetyl-β-glucosaminidase	Acid phosphatase
		p-NP-α-gluc[2]	Glycogen						
		μmoles/g lysosomal protein/hr						μmoles/mg lysosomal protein/h	
Cystic fibrosis	*GCF*	29.5 ±11.3	402.0 ±144.0	17.0 ±7.1	65.0 ±22.0	168.0 ±37.0	115.0 ±31.0	5.7 ±1.3	3.2 ±0.5
	MBL	37.0 ±8.2	553.0 ±66.0	23.0 ±5.2	85.0 ±14.0	177.0 ±32.0	127.0 ±25.0	7.3 ±1.0	3.1 ±0.5
	KER	36.9 ±6.1	557.0 ±158.0	11.0 ±5.2	69.0 ±9.0	174.0 ±27.0	125.0 ±8.0	5.3 ±1.0	4.0 ±0.7
	PAC	26.9 ±4.1	661.0 ±185.0	15.0 ±4.6	93.0 ±15.0	100.0 ±14.0	103.0 ±4.0	5.8 ±0.8	3.4 ±0.7
Heterozygous for CF	*PGF*	21.9 ±3.7	201.0 ±108.0	21.0 ±7.7	91.0 ±12.0	181.0 ±27.0	122.0 ±18.0	8.3 ±0.7	4.2 ±1.8
	DSB	18.9 ±2.4	291.0 ±51.0	15.0 ±4.4	50.0 ±5.0	121.0 ±18.0	92.0 ±10.0	5.2 ±1.6	3.8 ±0.9
	RLF	10.8 ±1.0	187.0 ±57.0	16.0 ±3.2	85.0 ±24.0	143.0 ±13.0	123.0 ±14.0	6.1 ±0.3	3.3 ±1.2
Borderline CF	*TAM*	70.3 ±8.1	1140.0 ±163.0	21.0 ±3.3	105.0 ±16.0	294.0 ±51.0	127.0 ±23.0	7.3 ±1.0	4.4 ±0.4
Healthy controls	*EFB I*	3.6 ±0.9	106.0 ±74.0	19.0 ±10.0	57.0 ±20.0	165.0 ±43.0	109.0 ±47.0	5.7 ±1.7	2.7 ±0.4
	SLT I	4.2 ±1.6	86.0 ±52.0	26.0 ±10.0	62.0 ±7.0	156.0 ±24.0	98.0 ±21.0	5.4 ±0.3	3.1 ±0.8
	TOH	5.5 ±1.8	144.0 ±42.0	13.3 ±6.2	56.0 ±6.0	166.0 ±36.0	119.0 ±32.0	5.8 ±0.6	2.8 ±0.3
	RKB	6.6 ±2.7	103.0 ±24.0	9.8 ±3.1	62.0 ±12.0	129.0 ±27.0	131.0 ±17.0	5.3 ±0.7	3.2 ±0.7
	UCW I	29.7 ±6.2	289.0 ±77.0	15.6 ±1.4	92.0 ±18.0	114.0 ±18.0	138.0 ±16.0	5.6 ±0.4	2.7 ±0.7
	CB	31.8 ±5.3	455.0 ±98.0	11.9 ±8.8	118.0 ±28.0	153.0 ±46.0	137.0 ±16.0	5.3 ±1.2	4.2 ±0.8

[1] The values represent averages ± SD obtained from four to six individual subcultures and cell fractionations in each cell line.
[2] *p*-NP-α-gluc: *p*-Nitrophenyl-α-D-glucoside.

Table II. Comparison between average specific activities of lysosomal enzymes in isolated lysosomes from cultured lymphoid cells obtained from patients with cystic fibrosis, heterozygotes, borderline, and control subjects

Group	α-Glucosidase		β-Glucosidase	β-Glucuronidase	β-Galactosidase	Aryl sulfatase	*N*-Acetyl-β-glucosaminidase	Acid phosphatase
	p-NP-α-gluc[1]	Glycogen						
	μmoles/g lysosomal protein/hr						μmoles/mg lysosomal protein/hr	
CF (4)[2]								
Mean ± SD	32.6 ±8.6	543.5 ±163.3	16.0 ±6.8	78.0 ±18.7	154.7 ±41.7	117.2 ±22.0	6.0 ±1.2	3.4 ±0.7
Range	19.0–49.1	250.0–877.0	6.8–29.0	49.0–110.0	79.0–158.0	84.0–179.0	3.7–8.5	2.2–4.9
Total no. of determinations	20	20	21	20	20	22	20	22
Heterozygotes (3)								
Mean ± SD	17.2 ±5.5	226.7 ±85.3	17.4 ±5.8	75.4 ±23.6	148.5 ±32.0	112.2 ±20.2	6.5 ±1.7	3.8 ±1.3
Range	10.1–27.3	68.0–371.0	8.1–33.0	42.0–127.0	95.0–218.0	77.0–147.0	4.1–8.8	2.4–6.1
Total no. of determinations	15	15	15	15	15	15	15	15
Borderline (1)								
Mean ± SD	70.3 ±8.1	1140.0 ±163.0	21.0 ±3.3	105.0 ±16.0	294.0 ±51.0	127.0 ±23.0	7.3 ±1.0	4.4 ±0.4
Range	60.5–78.2	942.0–1358.0	15.9–24.1	68.0–127.0	233.0–368.0	90.0–148.0	5.8–8.6	4.0–4.8
Total no. of determinations	5	5	5	5	5	5	5	5
Control (6)								
Mean ± SD	13.8 ±12.2	188.6 ±156.3	15.3 ±8.3	74.1 ±27.8	145.9 ±36.5	122.8 ±29.1	5.6 ±1.0	3.1 ±0.6
Range	1.4–40.4	20.0–601.0	6.7–39.0	36.0–158.0	85.0–230.0	75.0–189.0	2.5–8.0	1.7–5.1
Total no. of determinations	29	30	27	31	28	29	30	30

[1] *p*-NP-α-gluc: *p*-Nitrophenyl-α-D-glucoside, CF: Cystic fibrosis.
[2] The number of individual cell lines is given in parentheses.

acid phosphatase, and one mitochondrial, malic dehydrogenase, were determined in the whole homogenates of the investigated cell lines. The results are presented in Table IV. As can be noted, a marked increase in the α-glucosidase activity was found in the cystic fibrosis group, which is comparable to the values obtained in isolated lysosomes. However, because of the very low activity of this enzyme in whole homogenate of control cells which lies below the limit of the micromethod's sensitivity, a high standard deviation was obtained. There is no marked difference between the activities of acid phosphatase and malic dehydrogenase between controls and cystic fibrosis. Here again the borderline case has the highest value for α-glucosidase activity.

Discussion

Danes and Bearn [9] studied cultured fibroblasts from patients with CF and heterozygous carriers of cystic fibrosis and observed an increased synthesis of mucopolysaccharides and their release into extracellular space. Matalon and Dorfman [22] noted an increase in total mucopolysaccharides with a distribution of the various fractions closely paralleling that observed in normal cell cultures. Johansen *et al.* [16] suggested that many tissues in cystic fibrosis are affected by a common defect which impairs cellular transport mechanisms and membrane permeability. As a result, excessive accumulation of secretory products and increased breakdown of cellular membrane components could occur, leading to an increased amount of mucopolysaccharides and other complex polyanionic macromolecules. This hypothesis is supported by the finding of Bartman *et al.* [2] that lysosomes in cultivated fibroblasts from patients with cystic fibrosis are more numerous and contain a larger amount of material. This accumulated material appeared in electron micrographs to consist of membrane-bound bodies, presumably lysosomes, containing a variable amount of material compatible with ultrastructural features of phospholipids and mucopolysaccharides. It is possible that this inability to metabolize excessive amounts of cellular substrates is enhanced in tissue cultures and induces the activity of lysosomal enzymes.

Table III. Specific activity of mitochondrial enzymes in isolated mitochondria from cultured lymphoid cells, obtained from patients with cystic fibrosis, heterozygotes, borderline, and control subjects[1]

Group	Subject	MDH	SDH	GluDH
		μmoles/mg mitochondrial protein/min		
CF	*GCF*	1.99 ± 0.40	0.044 ± 0.010	0.125 ± 0.030
	MBL	1.57 ± 0.51	0.020 ± 0.056	0.082 ± 0.021
	KER	2.30 ± 0.36	0.060 ± 0.009	0.149 ± 0.027
	PAC	3.33 ± 0.57	0.063 ± 0.020	0.156 ± 0.019
	Average	2.28 ± 0.78	0.047 ± 0.021	0.128 ± 0.037
	Range	0.42–4.00	0.012–0.086	0.055–0.178
	Total no. of determinations	21	20	21
Heterozygotes	*PGF*	2.87 ± 0.45	0.057 ± 0.010	0.138 ± 0.056
	DSB	2.60 ± 0.54	0.062 ± 0.017	0.150 ± 0.042
	RLF	2.19 ± 0.34	0.068 ± 0.022	0.126 ± 0.016
	Average	2.55 ± 0.50	0.063 ± 0.016	0.138 ± 0.030
	Range	2.07–3.34	0.041–0.087	0.060–0.161
	Total no. of determinations	15	15	15
Borderline	*TAM*, average	2.59 ± 0.15	0.095 ± 0.016	0.130 ± 0.027
	Range	2.40–2.87	0.072–0.111	0.091–0.161
	Total no. of determinations	5	5	5
Control	*EFB*	1.66 ± 0.24	0.051 ± 0.017	0.144 ± 0.034
	SLT	1.49 ± 0.61	0.044 ± 0.003	0.155 ± 0.008
	TOH	1.96 ± 0.28	0.067 ± 0.013	0.142 ± 0.023
	RKB	2.09 ± 0.41	0.072 ± 0.007	0.168 ± 0.023
	UCW	2.63 ± 0.68	0.062 ± 0.019	0.158 ± 0.044
	CB	2.04 ± 0.27	0.064 ± 0.015	0.156 ± 0.033
	Average	1.96 ± 0.56	0.061 ± 0.015	0.154 ± 0.029
	Range	0.82–3.47	0.027–0.087	0.103–0.224
	Total no. of determinations	31	28	28

[1] The values represent averages ± SD obtained from four to six individual subcultures and cell fractionations in each cell line. MDH: Malic dehydrogenase. SDH: Succinic dehydrogenase. GluDH: Glutamic dehydrogenase. CF: Cystic fibrosis.

In our study we found that α-glucosidase activity was consistently and markedly higher in lysosomes from cultured lymphoid cells of four patients with cystic fibrosis, average 32.6 with a range of 19.0–49.1 μmoles/g lysosomal protein/hr. The average value in the control group of six subjects was 13.2 with a range of 1.4–40.4 μmoles/g lysosomal protein/hr with two individuals falling in the CF range.

The reason for these two high values in the clinically healthy individuals (*UCW I* and *CB*, Table I) is difficult to interpret. It is possible that these two controls might be carriers of the CF gene, or these findings may indicate the wide range of "normal variation" for this enzyme. However, other studies dealing with brain and liver homogenates [24] did not show such a wide range of activity of this enzyme. Another possibility may be that we are measuring more than one enzyme or isoenzymes. Salafsky and Nadler [27] recently demonstrated the existence of different α-glucosidases in various tissue

Table IV. Activities of α-glucosidase, acid phosphatase, and malic dehydrogenase in whole homogenates of cell lines from cultured lymphoid cells obtained from patients with cystic fibrosis, heterozygotes, borderline, and control subjects[1]

Group	α-Glucosidase, μmoles/g protein/hr	Acid phosphatase, μmoles/mg protein/hr	MDH μmoles/mg protein/min
CF (4)[2]	4.22 ± 1.28	1.32 ± 0.38	0.76 ± 0.23
Range	2.80–7.40	0.65–2.03	0.40–1.15
Total no. of determinations	21	20	20
Heterozygote (3)	2.52 ± 1.06	1.44 ± 0.45	0.90 ± 0.29
Range	1.02–4.00	0.90–2.24	0.42–1.15
Total no. of determinations	15	15	15
Borderline (1)	6.10 ± 0.89	1.26 ± 0.35	0.74 ± 0.13
Range	5.00–7.50	0.80–1.57	0.54–0.87
Total no. of determinations	5	5	5
Control (6)	1.50 ± 1.60	1.12 ± 0.45	0.69 ± 0.18
Range	0–5.50	0.39–2.52	0.43–1.04
Total no. of determinations	31	31	31

[1] The values represent averages ± SD. MDH: Malic dehydrogenase. CF: Cystic fibrosis.
[2] The number of cell lines is given in parentheses.

from the same patient, using inhibition by turanose and pH optima. In the present investigation we studied the effect of turanose [37] (3-*O*-α-D-glucopyranosyl-D-fructose) as a specific and uncompetitive inhibitor of the lysosomal, as distinct from the neutral α-glucosidase activity. We obtained the same degree of inhibition using 5 and 30 mM concentrations of turanose (30–32% and 64–69% inhibition, respectively) with lysosomal homogenates of control and CF lysosomes. No inhibition was produced when pH 6.4 buffer was used. We, however, did not study the optimal pH for this enzyme. Alternatively, the substrates which were employed may not allow proper differentiation of closely related enzymes, a possibility which was proven to be the case in Krabbe's disease [28]. The possibility exists of two types of healthy populations—one with low values and the other with high values for α-glucosidase. Only the accumulation of more data would settle this question.

α-Glucosidase is a lysosomal (1→4) glucosidase which causes extensive degradation of glycogen by stepwise attack on the terminal residues. In addition to the synthetic substrate we used the physiologic substrate glycogen and obtained the same elevated activity of α-glucosidase in the patients with CF. Our findings are of special interest because of the recent observation of Pallavicini *et al.* [23] that the glycogen content of cultured fibroblasts from patients with cystic fibrosis is increased. The increased content of glycogen [23] might enhance the activity of α-glucosidase. In this sense, however, there appears to be no clinical evidence of glycogen deposition, or any suggestion of disturbed glycogen metabolism in patients with cystic fibrosis. The absence of clinical symptoms of glycogen abnormalities does not necessarily mean that on a cellular level glycogen metabolism is not affected. To elucidate this fact, the biochemical determination of glycogen content in CF lymphoid cells might be of great interest. α-Glucosidase activity determined in the whole homogenate of the investigated cell lines showed marked increase in the CF group comparable to the increase in isolate lysosomes. However, because of the very low activity of the enzyme in the whole homogenates of control cells a high standard deviation was obtained.

The estimation of other lysosomal enzyme activities, namely acid phosphatase, β-glucosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, aryl sulfatase, and β-glucuronidase, did not differ significantly in the patients with cystic fibrosis and controls. Griffin and Gibbs [13] reported that β-glucuronidase activity is decreased in short term cultures of PHA-stimulated CF lymphocytes on the 5th day of culture, while *N*-acetyl-β-glucosaminidase is decreased on the 7th day of culture. In our investigation we did not find any difference between the activity of these enzymes in the lysosomes of patients with CF and controls. However, in our study, continuously propagated lymphoid cell lines were used rather than short term cultures which had been established for only a brief period. Our cultures were grown for much longer periods of time, *i.e.*, from 3 to 12 months. In fact, we did not find any variation in the enzyme activities in these stable cell lines during a 12-month period. Recently, Kraus *et al.* [17] and Benke [4] reported no difference in β-glucuronidase activity in control and CF fibroblasts.

The three enzymes investigated (SDH, GluDH, and MDH) in the mitochondrial fraction were similar in the cystic fibrosis and control cells. This result indicates that energy-producing metabolism is probably not affected in these cells which are growing in culture under optimal conditions.

In our experience, continuously grown cell lines of lymphocytes appear to offer a useful system for the study of cellular metabolism in cystic fibrosis and heterozygous carriers of the disease. The question, however, arises as to whether the consistent increase in α-glucosidase activity is present only *in vitro*, after transformation and adaptation of cell growth, division, and metabolism to the specific conditions in continuous culture, or does it represent a metabolic abnormality existing *in vivo*? To answer this question it would be necessary to investigate the buffy coat cells of peripheral blood from patients with cystic fibrosis and controls. We are attempting to do this at present.

Summary

Seven lysosomal enzymes (α-glucosidase, β-glucosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, aryl sulfatase, and acid phosphatase) and three mitochondrial enzymes (malic, succinic, and glutamic dehydrogenases) were studied in isolated lysosomes and mitochondria in cultured lymphoid cells from four patients with cystic fibrosis, three heterozygotes, one borderline case of cystic fibrosis, and six normal control subjects.

The lysosomes and mitochondria were isolated by differential and density gradient centrifugation. Among the lysosomal enzymes, only the activity of α-glucosidase was markedly increased in the lysosomes of cultured lymphoid cells obtained from patients with cystic fibrosis. The activity of this enzyme was measured with two substrates (*p*-nitrophenyl-α-glucosidase and glycogen).

In both determinations, the difference in activity was markedly higher. The mean values for α-glucosidase activity, in micromoles per gram lysosomal protein per hour, were 32.6 (SD ± 8.6) and 13.8 (SD ± 12.2) for patients with cystic fibrosis and normal control subjects, respectively, with *p*-NP-α-gluc as substrate, and 543.5 (SD ± 163.3) and 188.6 (SD ± 156.3), respectively, with glycogen as substrate. Activities of the remaining six lysosomal enzymes as well as of the mitochondrial enzymes did not show any difference between the lymphoid cells of patients with CF and normal control subjects.

It was concluded that this increase in the α-glucosidase activity may suggest an impairment in cellular metabolism in cystic fibrosis.

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40. All procedures have been performed in accordance with the provisions set forth in the Declaration of Helsinki.

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