Pediat. Res. *13:* 764–768 (1979)

Cell culture fibroblasts
cystic fibrosis mitochondria

Altered Intracellular Calcium in Fibroblasts from Patients with Cystic Fibrosis and Heterozygotes

ROBERT J. FEIGAL AND BURTON L. SHAPIRO

Department of Oral Biology, H.S. Unit A 17-226, University of Minnesota, Minneapolis, Minnesota, USA

Summary

The importance of intracellular calcium (Ca) in secretion and transmembrane ion movement led us to study Ca in cells from patients with cystic fibrosis (CF) which is a lethal genetic exocrinopathy. Skin fibroblasts from patients with CF, obligate heterozygotes (HZ), and age- and sex-matched controls (C) were used in matched pair experiments measuring ^{45}Ca exchange into and efflux from the cells over time. CF cell lines and HZ cell lines exhibit increased ^{45}Ca exchange when compared with their respective controls ($P < 0.005$). The magnitude of this difference (approximately 30%) is not reduced when cells are washed with lanthanum chloride after the exchange period. This difference is likely attributable to an altered capacity of one or more of the intracellular Ca sequestering organelles. Further evidence for this explanation was seen in ^{45}Ca efflux experiments in which CF cells retained a higher percent of their initial 0-time ^{45}Ca than did C cells late in the efflux period ($P < 0.05$). The finding of an altered Ca pool size in both CF and particularly HZ cells suggests that altered Ca metabolism is related to the basic gene defect in CF.

Speculation

Increased intracellular Ca in CF cells, while necessarily secondary to the basic gene defect, may influence cellular metabolism sufficiently to be a basis for many events in the pathogenesis of the disease. The presence of the Ca pool size alteration in cells from obligate heterozygotes is evidence that this phenomenon is closely related to the basic gene defect.

CF is a lethal genetic exocrinopathy. The most apparent symptoms are chronic obstructive lung disease and gastrointestinal malabsorption. With one or both of these, the definitive diagnostic sign is elevated sweat chloride. This autosomal recessive trait affects approximately one in 1600 Caucasian newborns and has a carrier frequency of about one in 20 in Caucasian populations (20). While secretory anomalies in some exocrine glands of patients with CF have been reported, the metabolic basis for the disease is unknown.

Ca homeostasis involves carefully controlled systems that include transport and sequestration by cell membranes and intracellular organelles. Alterations in this homeostasis may cause changes in many associated intracellular systems. Regulation of cellular Ca by plasma membrane and intracellular organelles is recognized as a modulating factor in stimulus-secretion coupling, stimulus-contraction coupling, activation of several enzymes, and hormonal regulation (8, 24).

Altered Ca concentrations have been observed in some exocrine secretions from CF patients (9). Increased levels of Ca in submandibular saliva has been consistently confirmed (2, 28). Ca concentration in tears was found to be higher in subjects with CF than in their sibs (7). There were early suggestions concerning the role of increased Ca in CF pathogenesis (10). It was thought that increased Ca in secretions might lead to less soluble Ca-glycoprotein complexes and that subsequent precipitates cause tissue obstruction. Increased Ca will lead to increased fluid viscosity, but no progress in this relationship has occurred (10, 16). According to Di Sant'Agnese and Davis (9), there is little clinical evidence to suggest a Ca metabolic disorder in CF.

Recently, the role of Ca in the rabbit tracheal mucociliary bioassay system has been examined (3). A CF-like response was found when the Ca ionophore A23187 was added to the bathing medium. This response was abolished by Ca chelation with EGTA. A23187, when added to control sera, gave a CF-like response. EGTA eliminated the CF-like response when added to CF sera. Bogart (3) concluded that Ca is an important cofactor in the CF sera-induced rabbit tracheal mucociliary assay. Using isolated rat parotid acinar cells *in vitro*, CF saliva and a solution containing A23187 caused increased intracellular Ca in comparison with cells incubated in saliva from control subjects (21).

Until the report from this laboratory (26), relatively little and nothing definitive has appeared concerning intracellular Ca in CF. Fletcher and Lin (14) found no difference in the time course of Ca uptake by skin fibroblasts from one CF subject with whom they compared cells from one control. The use of glass coverslips as a substrate for their monolayers could have led to binding of much of the added Ca to negatively charged glass, thus, obscuring differences that might have existed. They did not preequilibrate with a cold Ca medium which we found to be a necessary condition to distinguish CF and carrier monolayers from the respective controls. In a preliminary study using X-ray spectroscopy (1), three pooled samples, one each of CF, carrier, and control fibroblast lines were examined: CF fibroblasts showed a greater Ca peak. Similarly, dissociated parotid acinar cells from CF subjects showed increased Ca and sodium in comparison with controls (21). The role of Ca in stimulus-secretion coupling (11, 24) and its relationship to the transport of sodium and hydrogen ions (19) suggested to us its potential involvement in critical events in CF.

Although fibroblasts are not exocrine cells, they are classically secretory cells. For this reason, as well as the ability to generate large numbers of genetically stable cells from small skin biopsies, we are using skin fibroblasts as a model system to study CF.

This report involves experiments on Ca exchange in fibroblasts from CF subjects, obligate HZ and their respective controls in which an unspecified intracellular Ca pool is observed to be markedly increased in CF and HZ cells.

METHODS AND MATERIALS

Skin fibroblasts obtained by biopsy of the medial surface of the upper arm were used to establish cell lines. Outpatients under care at the University of Minnesota CF Center were used as donors for CF lines while age- and sex-matched controls were people not under treatment for any illness. Parents of patients with CF were used to establish HZ cell lines, and age- and sex-matched controls were obtained for these lines also. Fibroblasts were routinely maintained in Eagles minimal essential medium with Earles salts plus L-glutamine, supplemented with 10% fetal calf serum, 100 units per ml of penicillin, and 100 μg/ml of streptomycin. Cells

were cultured in a humidified atmosphere of 5% CO_2 in air at 37°C, and were subcultured weekly with 0.5% trypsin plus 0.8% sodium citrate. Cells from each line were frozen and stored in liquid nitrogen for future use.

Cells were used between the third and the twelfth passage for all experiments. Multiwell trays (Linbro Plastics, Hamden, CN) with 24-1.5 cm diameter wells were seeded with 5×10^4 cells per well, and experiments were carried out between 6 and 10 days postseeding. On the day of the experiment, growth medium was removed from the wells and replaced with Krebs Ringer Bicarbonate (KRB) buffer (pH 7.4) containing 10 mg/ml dextrose and 0.1 mg/ml bovine serum albumin and gassed in 5% CO_2 in air. Preliminary experiments revealed that by varying either extrinsic Ca or phosphate, the ^{45}Ca exchange difference between CF and control cells could be amplified. Increasing increments of buffer Ca between 0.5 mM and 2.5 mM or increasing increments of buffer phosphate between 0.5 mM and 5.0 mM caused exchange in cells from subjects with CF and controls to increase. The increase in the CF cells was relatively greater than that in the control cells resulting in an amplified difference between the two groups at the higher concentrations of Ca or phosphate. Interaction between the Ca and phosphate levels was not analyzed. Because of this amplification, we added 2.5 mM $CaCl_2$ and 3.0 mM KH_2PO_4 to the KRB routinely. The cells were equilibrated in KRB in a 37°C CO_2 incubator for 60 min in the same Ca and phosphate concentration that would be present during the ^{45}Ca pulse. This equilibration period assured that the cellular compartments for Ca approached steady state with the buffer before the introduction of radioactive Ca. The buffer was then removed and identical buffer plus ^{45}Ca at 1 μCi/ml was added. At each sample period, exchange was stopped by aspirating the buffer from the respective wells and immediate washing of the monolayers with two 3 ml volumes of ice cold wash solution, either phosphate buffered saline (PBS) or 5 mM lanthanum chloride ($LaCl_3$) in 0.85% sodium chloride. $LaCl_3$ in the wash solution removes extracellular and glycocalyx bound ^{45}Ca while preventing any rapid efflux of Ca from the cell (25, 27). At the end of each experiment, wells were harvested by rinsing sequentially with 0.5 ml 0.1 N sodium hydroxide, 0.5 ml 0.1 N HCl, and 0.5 ml distilled water. All rinses from each well were delivered to a scintillation vial containing 10 ml Aquasol (New England Nuclear, Boston). At least 2 wells per cell line per tray were harvested and used to determine protein per well using a modified Lowry method (23). For some experiments, cell counts per well were determined with a Coulter Counter by harvesting cells in 1 ml of 0.05% trypsin.

^{45}Ca efflux data were obtained from fibroblasts in both monolayer and suspension systems using the same buffer as in influx experiments. The monolayer efflux experiments utilized the tray and seeding procedures as described for monolayer uptake. Growth medium was replaced with 1 ml KRB containing ^{45}Ca at 1 μCi/ml. The ^{45}Ca preloading was accomplished during a 90-min equilibration period after which the ^{45}Ca containing medium was removed and the monolayers were washed three times with 3 ml PBS. One ml of KRB without ^{45}Ca was added to each well. At the sample time points, the 1 ml buffer was removed for scintillation counting and was replaced with 1 ml fresh KRB. After the completion of the efflux, cell monolayers were harvested and radioactivity of cells determined as in uptake experiments.

For suspension efflux studies, 6×10^5 cells were seeded in 75 cm^2 (#3024 Falcon, Oxnard, CA) growth flasks and harvested 7 days later by the use of a soft rubber scraper. The cells were washed in 10 ml KRB and suspended in 13 ml KRB in plastic tubes (#2051 Falcon, Oxnard, CA). The buffer for the 90-min equilibration and ^{45}Ca loading period contained 1 μCi ^{45}Ca/ml. A water bath maintained the medium at 37°. Uniform cell suspension was assured by the use of a magnetic stirrer in each tube. Warmed and humidified 5% CO_2 in air was used to maintain pH. After ^{45}Ca loading, the cells were centrifuged, the radioactive buffer poured off, and the cells were washed with 10 ml KRB. Two ml KRB was added to each tube and cells resuspended in this efflux medium. At each time point, the magnetic stirrers were removed and the tubes were centrifuged at $2000 \times g$ for 45 sec. The medium from each tube was poured into a scintillation vial for counting in Aquasol and fresh KRB (2 ml) added to the cells. The entire procedure at each time point, from removal of cells from the water bath to resuspension, was accomplished in approximately 60 sec. At the completion of the efflux period, the cells were sonicated in 4 ml deionized water and aliquots taken for scintillation counting and protein determination.

Both monolayers and suspension efflux data were analyzed by taking the sum of all time points per minute (cpm) and the final cell cpm as the total radioactivity in the cells at time zero. Each efflux curve was plotted with percent of initial radioactivity left in the cells as a function of time.

Uncontrollable variablility in cell culture experiments is well known (4, 15). These experiments were designed to compare CF with control (or carrier with their control) cell lines. Therefore, experiments were performed with cell lines that were matched for age, passage number, day of experiment, multiwell culture tray, and, where possible, sex. This matched-pair design is the most powerful way to control against the random effects of extraneous variables. Comparison of means of samples in these experiments would be inappropriate. The only appropriate statistical tests for data derived from such a design are those comparing pairs of individuals such as matched pair Student's *t* tests (12). Therefore, matched pair Student's *t* tests were used with comparisons limited to cell lines on the same multiwell tray or in the case of suspension experiments, limited to matched tubes.

RESULTS

A representative experiment measuring the exchange over time of ^{45}Ca with cold Ca is shown in Figure 1. At 75 min, more intracellular Ca was replaced by ^{45}Ca in terms of cell protein in the CF sample than in controls. Matched pair analysis revealed that the difference at 75 min was highly significant (Table 1). In these experiments, cells were washed with phosphate buffered saline.

The experiments in which $LaCl_3$ was used to remove extracellular ^{45}Ca showed differences in ^{45}Ca exchange between CF and their controls (Table 2) as well as HZ and their controls (Table 3) at 75 min. Although the total monolayer radioactivity was reduced more than 60% when using lanthanum washes as compared with PBS washes, the increased ^{45}Ca exchange into both CF and HZ cells was apparent and continued to be statistically significant ($P < 0.005$ for the CF and control comparison, $P < 0.005$ for the HZ and control comparison). Those experiments in which HZ and CF

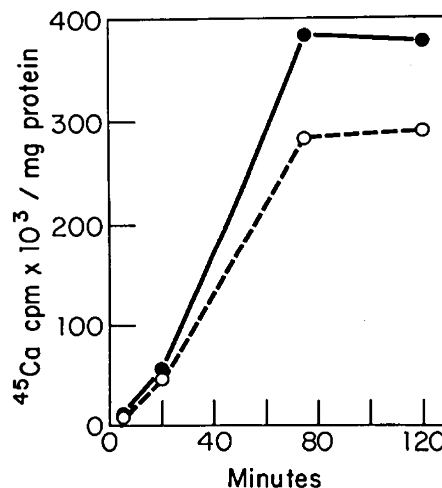

Fig. 1. ^{45}Ca exchange into monolayer cultures of CF (●) and control (O) fibroblasts. This is a graph of data from a representative experiment. Cells were equilibrated in KRB buffer containing 2.5 mM $CaCl_2$ and 3.0 mM KH_2PO_4 for 1 hr before the addition of fresh buffer containing ^{45}Ca (0-time). Monolayers were washed with cold PBS after the uptake period.

cells lines were on the same tray (Table 4) showed no differences between CF and HZ cells ($P > 0.25$) while both CF and HZ differed from the control values ($P < 0.01$).

A representative experiment measuring efflux of ^{45}Ca from preloaded cells in suspension is shown in Figure 2. These curves reveal a change in ^{45}Ca efflux from CF cells when compared to control cells. Statistical analysis of efflux time points from five matched pair experiments (Table 5) revealed significant differences after 150 min ($P < 0.05$). No differences between CF and control lines were observed in monolayer efflux experiments.

DISCUSSION

Ca exchange into both CF and HZ fibroblast cell lines is clearly greater than exchange into their respective control cells. The difference is observed after the uptake curves plateau indicating

Table 1. ^{45}*Ca exchange into CF and control fibroblast monolayers (monolayers washed with phosphate buffered saline)*

Time (min)	CF mean ± SE[1]	Control mean ± SE[1]	Pairs	Matched pair Student's t	P
5	9.47 ± 1.76	8.01 ± 0.85	12	0.8864	>0.10
20	58.21 ± 5.74	46.55 ± 4.95	12	1.657	0.07
75	279.91 ± 24.97	208.80 ± 19.52	12	3.779	<0.005
120	346.35 ± 17.12	246.81 ± 49.03	2		

[1] Cpm × 10^3/mg protein.

Table 2. ^{45}*Ca Exchange into CF and control fibroblasts 75 min after isotope addition (monolayers washed with* $LaCl_3$*)*[1]

	CF				Control			
Experiment number	Cell line	Age	Sex	Cpm/mg protein	Cell line	Age	Sex	Cpm/mg protein
1	46	16	M	17,330	33	13	M	12,308
2	48	19	M	14,048	52	17	M	9,674
3	45	7	F	16,358	51	8	F	9,668
4	45	7	F	26,670	51	8	F	20,830
5	44	10	M	14,631	31	13	F	13,680
6	45	7	F	19,202	30	14	F	14,483
7	46	16	M	12,515	33	13	M	11,700
8	45	7	F	19,718	31	13	F	17,300
9	26	14	F	7,314	30	14	F	6,913
10	46	16	M	25,700	33	13	M	16,876
11	45	7	F	19,684	31	13	F	12,363
12	46	16	M	19,390	33	13	M	19,506
13	46	16	M	15,107	33	13	M	11,827

[1] Matched pair comparison $t = 4.885$, $n = 13$, $P < 0.005$.

Table 3. ^{45}*Ca exchange into HZ and adult control fibroblasts 75 min after isotope addition (monolayers washed with* $LaCl_3$*)*[1]

	HZ				Control			
Experiment number	Cell line	Age	Sex	Cpm/mg protein	Cell line	Age	Sex	Cpm/mg protein
1	37	33	F	7,191	40	33	M	5,560
2	37	33	F	7,467	42	34	F	7,168
3	36	28	F	10,782	42	34	F	9,919
4	36	28	F	10,161	42	34	F	9,402
5	38	31	F	8,652	40	33	M	7,921
6	38	31	F	8,000	40	33	M	7,380
7	37	33	F	9,227	41	37	F	7,593
8	37	33	F	8,377	41	37	F	7,329
9	38	31	F	16,321	40	33	M	14,096
10	38	31	F	13,493	42	34	F	12,983
11	37	33	F	7,526	40	33	M	6,565
12	37	33	F	7,870	42	34	F	7,176

[1] Matched pair comparison: $t = 6.197$, $n = 12$, $P < 0.005$.

Table 4. ^{45}*Ca exchange into CF, HZ, and control fibroblasts 75 min after isotope addition (monolayers washed with* $LaCl_3$*)*[1]

	CF				HZ				control			
Experiment number	Cell line	Age	Sex	Cpm/mg protein	Cell line	Age	Sex	Cpm/mg protein	Cell line	Age	Sex	Cpm/mg protein
1	48	19	M	14,048	36	28	F	13,604	33	13	M	9,674
2	45	7	F	19,684	36	28	F	16,084	31	13	F	12,363
3	46	16	M	25,700	37	33	F	15,553	33	13	M	11,876
4	46	16	M	17,330	36	28	F	23,059	33	13	M	12,306
5	46	16	M	19,390	37	23	F	21,662	33	13	M	19,506
6	46	16	M	15,107	37	23	F	13,091	33	13	M	11,827

[1] Matched pair comparisons: $n = 6$, CF vs. HZ: $t = 0.0618$, $P > 0.25$; CF vs. C: $t = 2.927$, $P < 0.01$; HZ vs. C: $t = 3.102$, $P < 0.01$.

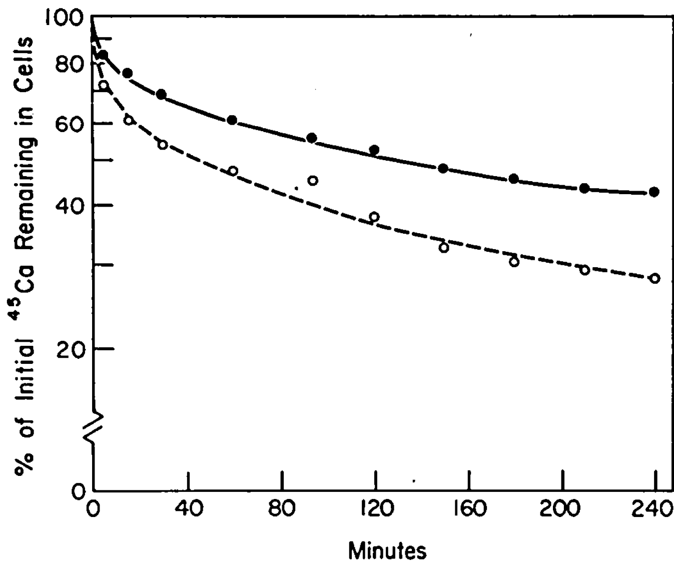

Fig. 2. ^{45}Ca efflux from CF (●) and control (○) fibroblasts. This is a graph of data from a representative experiment. Cells in suspension were prelabeled with ^{45}Ca for 90 min in KRB containing 2.5 mM $CaCl_2$ and 3.0 mM KH_2PO_4, then washed and resuspended in the same buffer without ^{45}Ca for efflux measurements. Each point represents the percent of total (0-time) ^{45}Ca remaining in the cells.

Table 5. *Match pair analysis of ^{45} Ca efflux from CF and control fibroblasts in suspension (n = 5 experiments)*

Time of efflux (min)	Matched pair Student's t	P
5	0.487	
15	0.343	
30	0.503	
60	1.369	
90	1.720	
120	1.973	
150	2.120	0.05
180	2.543	<0.05
210	2.886	<0.025
240	2.636	<0.05

a larger Ca pool size in the CF and HZ cells under these conditions. In order to be assured that possible protein differences between cell lines (13, 26) were not influencing the Ca data, ^{45}Ca exchange was expressed in units of mg protein and cell number. We found that with respect to either cell number or protein, the intracellular Ca pool size difference is significant.

While our cell lines were established from age- and sex-matched individuals, seeding for each experiment was done with cell lines randomly obtained from our tissue culture laboratory. Therefore, each experimental pair was not necessarily age- and sex-matched. The ages of matched pairs remained closely comparable because of the matching of the initial groups of individuals. Male to female comparisons within CF and control groups revealed no differences in Ca exchange attributable to sex differences.

Any change in cellular Ca metabolism or binding of the magnitude observed in these experiments (approximately 30%) is noteworthy. The finding of such a difference in CF cells and particularly in HZ cells, obtained from individuals who would be undetectable as carriers had they not had a CF child, suggests that altered Ca metabolism is related to the basic gene defect in CF.

In direct comparison experiments, the CF and HZ intracellular pool sizes were not different ($P > 0.25$), and in comparison with their respective control groups, the magnitude of the pool size increase seen in both CF and HZ lines were similar. Therefore, in this system, the carriers exhibit the same alteration as do the homozygotes for CF. It is generally assumed that in an autosomal recessive trait a detectable biochemical abnormality in heterozygotes will lie intermediately between the normal and the affected homozygous state. However, with an appropriate challenge to the genetically altered system, the heterozygous and homozygous states may be indistinguishable. For example, at sufficiently low oxygen tension, both homozygotes and heterozygotes for sickle cell hemoglobin will sickle (17). In Ca experiments preliminary to those reported here, it was found necessary to bathe cells with increased Ca and phosphate concentrations in our experimental media in order to observe consistent differences between CF and controls. The increased medium Ca or phosphate may be a challenge to a cellular system such that the ^{45}Ca uptake by both HZ and homozygous CF cells is essentially equal.

Our early studies on Ca efflux using monolayer cells showed no difference between CF and control cells (26). However, the suspension experiments showed clear differences of ^{45}Ca efflux. Multiple time point efflux experiments are classically done with cells in suspension (5). In order to measure parameters of the intracellular Ca sequestering pool or pools, the more readily available glycocalyx and cytoplasmic pools of Ca must exchange their ^{45}Ca into the media. An explanation for the apparent discrepancy between suspension and monolayer systems may lie in the measurable difference in ^{45}Ca efflux efficiency between suspension and monolayer experiments. In monolayer experiments, the percent of initial cellular ^{45}Ca left in cells after 4 hr of efflux never fell below 75%, while in suspension experiments this value approached 20%. The less efficient efflux seen in monolayer cells may have prevented detection of the difference observed in suspension.

Although there are no obvious morphologic or biochemical observations in CF fibroblasts to account for the observed Ca differences, several lines of evidence suggest that the altered Ca pool size is located in an intracellular Ca sequestering organelle system. When the extracellular and glycocalyx bound ^{45}Ca was removed with $LaCl_3$ washes, differences of approximately 30% were noted suggesting that the site of the difference is intracellular. Because the major portion of intracellular Ca is sequestered in organelles such as mitochondria (5) and microsomes (22), these are likely the site of the large pool size difference observed. This explanation for the data is supported by the fact that increased concentrations of Ca or phosphate ions in the experimental buffer amplified the difference between CF and control cells (see *Materials and Methods*) suggesting that the altered pool is one capable of sequestering high concentrations of Ca and phosphate or one which is sensitive to Ca or phosphate ion concentration. Ca uptake by mitochondria *in vitro* is greatly enhanced by the presence of phosphate as a permeant anion (18). Mitochondria *in vivo* have been shown to be capable of large accumulations of Ca in the form of calcium phosphate granules (6). Finally, data from the ^{45}Ca efflux experiments on cells in suspension show that the larger percent of initial ^{45}Ca remains in the CF cells as compared with controls. This suggests that, with our experimental conditions, a cellular calcium pool with slow efflux parameters such as mitochondria (6) contains more Ca in CF than in control cells.

This Ca pool size difference, while not characterized, is a consistent finding in repeated experiments comparing CF and HZ cells with their respective controls. The fact that the Ca pool size alteration is found using cells *in vitro* is strong evidence against the possibility that Ca differences are secondary to the disease process or treatment. The clear and consistent Ca pool size difference in HZ cells suggests the Ca alteration is not far removed from the abnormal gene product in CF.

REFERENCES AND NOTES

1. Baur, P. S., Bolton, W. E., and Barranco, S. C.: Electron microscopy and microchemical analysis of cystic fibrosis diploid fibroblasts *in vitro*. Texas Rep. Biol. Med., *34:* 113 (1976).
2. Blomfield, J., Warton, K. L., and Brown, J. M.: Flow rate and inorganic components of submandibular saliva in cystic fibrosis. Arch. Dis. Children, *48:* 267 (1973).

3. Bogart, B. I.: The biologic activities of cystic fibrosis serum. I. The effect of cystic fibrosis sera and calcium ionophore A23187 on rabbit tracheal explants. Pediatr. Res., *11:* 131 (1977).
4. Bolton, W. E., and Barranco, S. C.: Review of the growth characteristics of cystic fibrosis and normal human diploid fibroblasts *in vitro*. Texas Rep. Biol. Med., *34:* 97 (1976).
5. Borle, A. B.: Methods for assessing hormone effects on calcium fluxes *in vitro*. Methods Enzymol., *39:* 513 (1975).
6. Borle, A. B., and Anderson, J. H.: Control and regulation of calcium homeostasis and transport. Calcif. Tissue Res., *21* Suppl.: 21 (1976).
7. Botelho, S. Y., Goldstein, A. M., and Rosenlund, M. L.: Tear sodium, potassium, chloride, and calcium at various flow rates: children with cystic fibrosis and unaffected siblings with and without corneal staining. J. Pediatr. *83:* 601 (1973).
8. Carafoli, E.: Mitochondrial uptake of calcium ions and the regulation of cell function. Biochem. Soc. Symp., *39:* 89 (1974).
9. DiSant 'Agnese, P. A., and Davis, P. B.: Research in cystic fibrosis. N. Engl. J. Med., *295:* 481 (1976).
10. DiSant 'Agnese, P. A., and Talamo, R. C.: Pathogenesis and physiopathology of the cystic fibrosis of the pancreas. N. Engl. J. Med., *277:* 1287 (1967).
11. Douglas, W. W.: The role of calcium in stimulus-secretion coupling, In: R. M. Case, and H. Goebell; Stimulus-Secretion Coupling In The Gastrointestinal Tract. p. 17 (University Park Press, Baltimore, 1976).
12. Dunn, O. J.: In: Basic statistics: A Primer For The Biomedical Sciences. p. 72 (Wiley, New York, 1964).
13. Farrell, P. M., Pallavicinni, J. C., and Ulane, M. M.: Growth characteristics and protein content of tissue-cultured fibroblasts from cystic fibrosis patients. Proc. Soc. Exptl. Biol. Med., *149:* 340 (1975).
14. Fletcher, D. S., and Lin, T. Y.: Incorporation of L-leucine and D-glucosamine into skin fibroblasts derived from cystic fibrosis and normal individuals. Clin. Chim. Acta, *44:* 5 (1973).
15. Foster, D. O., and Pardee, A. B.: Transport of amino acids by confluent and nonconfluent 3T3 and polyoma virus-transformed 3T3 cells growing on glass cover slips. J. Biol. Chem., *244:* 2675 (1969).
16. Gibson, L. E., Matthews, W. J., Jr., Minihan, P. T., and Patti, J. A.: Relating mucus, calcium, and sweat in a new concept of cystic fibrosis. Pediatrics, *48:* 695 (1971).
17. Lehmann, H., and Huntsman, R. G.: The hemoglobinopathies, In: J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson: The Metabolic Basis of Inherited Disease; pp. 1398–1431 (McGraw-Hill, Inc., New York, 1972).
18. Lehninger, A. L.: Role of phosphate and other proton-donating anions in respiration-coupled transport of Ca^{2+} by mitochondria. Proc. Natl. Acad. Sci., *71:* 1520 (1974).
19. Lehninger, A. L., Carafoli, E., and Rossi, C. S.: Energy-linked ion movements in mitochondrial systems. Adv. Immunol., *29:* 259 (1967).
20. Lobeck, C. C.: Cystic fibrosis. In: J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson: *The Metabolic Basis of Inherited Disease.* pp. 1605–1626 (McGraw-Hill, Inc., New York, 1972).
21. Mangos, J. A.: Cystic Fibrosis, In: T. E. Adreoli, J. F. Hoffman, D. D. Fanestil: Physiology of Membrane Disorders. p. 941-953 (Plenum Medical, New York, N.Y., 1978).
22. Moore, L., and Pastan, I.: Regulation of intracellular calcium in chick embryo fibroblasts: calcium uptake by the microsomal fraction. J. Cell Physiol., *91:* 289 (1977).
23. Oyama, V. I., and Eagle, H.: Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). Proc. Soc. Exptl. Biol. Med., *91:* 305 (1956).
24. Rasmussen, H., Goodman, D. B. P., and Tenenhouse, A.: The role of cyclic AMP and calcium in cell activation. Crit. Rev. Biochem., *1:* 95 (1972).
25. Robblee, L. S., and Shepro, D.: The effect of external calcium and lanthanum on platelet content and on the release reaction. Biochim. Biophys. Acta, *436:* 448 (1976).
26. Shapiro, B. L., Feigal, R. J., Laible, N. J., Biros, M. H., and Warwick, W. J.: Doubling time, α-aminoisobutyrate transport and calcium exchange in cultured fibroblasts from cystic fibrosis and control subjects. Clin. Chim. Acta, *82:* 125 (1978).
27. VanBreeman, C., Farinas, B. R., Gerba, P., and McNaughton, E. D.: Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. Circ. Res., *33:* 44 (1972).
28. Wotman, S., Mandel, I. D., Mercadante, J., and Denning, C. R.: Parotid and submaxillary calcium in human cystic fibrosis. Arch. Oral Biol., *16:* 663 (1971).
29. The authors thank Dr. Warren Warwick for helping to provide access to clinical materials. We thank Ms. Nancy Laible for invaluable technical support in our tissue culture laboratory.
30. This research was supported by a State of Minnesota Special Allocation for Cystic Fibrosis Research, NIH Grant DE-00005 and the Cystic Fibrosis Foundation.
31. Received for publication April 7, 1978.
32. Accepted for publication June 29, 1978.

Copyright © 1979 International Pediatric Research Foundation, Inc.
0031-3998/79/1306-0764$02.00/0

Printed in U.S.A.