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Growth Characteristics and Protein Content of Tissue-Cultured Fibroblasts from Cystic Fibrosis Patients (38803)

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Tissue culture study of fibroblasts, lymphocytes, and other cells has provided key data for elucidating molecular defects in several inborn errors of metabolism (1, 2). Thus, the report of Danes and Bearn (3) that skin fibroblasts from cystic fibrosis (CF) patients show abnormal metachromasia upon toludine blue staining was greeted with great interest. Although this finding has not to date resulted in discovery of the underlying defect in CF, considerable research has been stimulated and other apparent alterations in CF fibroblasts have been described (4–6). Recently, Houck and associates reported that CF fibrobalsts in tissue culture show: (a) impaired synthesis of collagen upon ascorbate induction (7), and (b) a markedly reduced growth rate with a doubling time of 64 ± 7 hr (control = 34 ± 3 hr) (8). The latter change also appeared to be manifest in fibroblasts derived from amniotic fluid and has been used in genetic counseling (9).

Such an alteration in growth kinetics would seemingly imply a fundamental disturbance in CF cells. However, several years of experience with CF fibroblasts under the conditions of this laboratory suggest that these cells proliferate normally. In the present study to establish firmly growth capability and to further characterize the cells on a basic level, proliferation rates have been determined in several cell lines utilizing three measures of growth, viz., cell number, DNA levels, and total cellular protein. In addition, during growth, cell protein production has been assessed indirectly by calculation of the protein/DNA ratio.

Materials and Methods. Diploid fibroblast lines were readily developed from skin biopsies obtained from five normal volunteers and five patients with cystic fibrosis. The two groups were matched for age and the CF patients represented a broad spectrum in regard to onset and severity of disease. Cells were subcultured several times and for these studies were on the average in their tenth passage. Inocula of ca. 4×10^3 cells/cm^2 were routinely added to either 25-cm^2 or 75-cm^2 Falcon flasks containing Eagles' minimal essential medium fortified with nonessential amino acids, L-glutamine 10% fetal calf serum, and neomycin sulfate (50 μg/ml). The buffer generally used was $NaHCO_3$ but in a small number of experiments, Hepes (*N*-2-hydroxyethyl-peperazine-*N'*-2-ethanesulfonic acid), pH 7.4, was substituted; medium was changed every 3–4 days.

On a daily basis, sample flasks were removed from the 37°C incubator and fibroblasts were harvested mechanically, washed eight times with phosphate-buffered saline (pH 7.4), and homogenized for chemical analysis. Aliquots of the prepared homogenate were analyzed for DNA by the diphenylamine method (10) and for protein by the technique of Lowry *et al.* (11). For cell counts, separate flasks were either trypsinized, appropriately diluted, and counted with a hemocytometer, or in some instances, counted directly in the cultured flask with a Whipple calibrated eyepiece. Growth rates were determined as doubling times from data plotted semilogarithmically. Control and CF lines were cultured in pairs for each experiment and several lines were studied repetitiously with good reproducibility.

Results. In both groups average doubling time values showed some variation depending on the method of measurement (Table I); however, statistical comparison of data

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TABLE I. GENERATION TIMES OF CONTROL AND CF FIBROBLASTS AS MEASURED DURING LOGARITHMIC GROWTH.

Method	Generation times in hours (mean ± SE)		No. of paired cultures
	Control	Cystic fibrosis	
Cell count	27 ± 1.7	25 ± 1.5	9
DNA	36 ± 2.5	33 ± 2.9	6
Protein	36 ± 1.6	35 ± 1.4	13

obtained with cell counts vs DNA vs protein levels revealed that none of these differences were significant. The over-all mean generation time (average of all figures) for control fibroblasts was 31 ± 1.3 (SE) hr, a figure which agrees well with literature values (12). CF fibroblast lines were found to proliferate normally (Table I) with a mean doubling time of 29 ± 1.3 hr.

Although fibroblasts from cystic fibrosis patients showed a normal DNA content, examination of protein levels in paired cultures during active growth frequently revealed that CF cells were lower than the accompanying control cell line. The protein values were, therefore, expressed per microgram DNA and plotted as a function of time (days) after inoculation. It then became evident (Fig. 1) that the differences were highly significant ($P < 0.005$–$P < 0.02$) in preconfluent cells, i.e., during logarithmic growth. However, CF fibroblasts show statistically normal protein/DNA ratios in the postconfluent stage, when growth is contact inhibited.

Growth rates of fibroblasts vary as a function of the number of cells inoculated/unit area (13). For this reason, similar amounts of cells were used in these experiments from control and CF lines. There was, however, a tendency ($P < 0.4$) for the latter inocula to contain less protein. In order to rule out the possibility that this led to the apparent decrease in protein production during exponential growth, the inoculated volume (and protein) was progressively increased in a series of CF cells to levels exceeding those of control cells. The difference in protein/DNA ratio was maintained even when the starting amount of protein was higher in CF lines.

Two other points should be made. Protein analysis by the method of Lowry *et al.* (11) depends primarily on the chromogenic reaction of aromatic amino acid residues. It is conceivable, although unlikely, that a reduced level of these compounds would artifactually lower the protein level as determined. However, measurement of protein nitrogen in a limited number of cultures confirmed the abnormality of preconfluent cells. In addition, Fletcher and Lynn (14) have observed normal levels of tyrosine and phenylalanine in acid hydrolysates of CF fibroblasts. Another possible explanation for the lowered cellular protein is that one of several specific proteins are missing in CF cells. This was not found to be the case when soluble proteins from fibroblasts were subjected to polyacrylamide gel electrophoresis.

Discussion. There is abundant evidence that fibroblasts can be readily cultured from human tissues with maintenance of chromosomal stability and characteristic biochemical properties. It has been observed that through numerous subcultivations, these cells proliferate at reproducible rates, even when derived from individuals with various metabolic disorders (15). In this study, the data for generation time (Table I) indicate that CF fibroblasts are capable of normal growth, in contrast to the finding of Houck *et al.* (8). This discrepancy is puzzling, since, except for antibiotics, the culture conditions

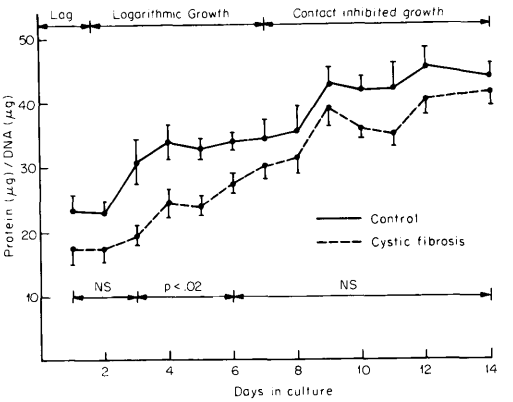

FIG. 1. Cellular protein content of control and CF fibroblasts during growth. Each point represents the mean of 14 evaluations on the average; standard errors of the means are indicated by brackets and the P values were derived from the paired t test with NS indicating $P > 0.05$.

of the two laboratories are similar (16). One possible explanation is that Houck and associates studied fibroblasts in early passages and used heavier inocula. Nonetheless, it must be concluded from our investigation that fibroblasts of the CF genotype are not fundamentally abnormal in growth potential as this would then be a characteristic easily reproducible in all systems.

In agreement with other reports (3–6), CF fibroblasts were found in this study to show evidence of metabolic dysfunction. The data of Fig. 1 indicate that CF cells exhibit a markedly reduced protein/DNA ratio during logarithmic growth. Since the DNA content of CF fibroblasts was found to be normal, the change in ratio indicates a decreased cell protein level. This was confirmed by expressing the protein figures on the basis of cell numbers, despite wider scatter of the data.

It has been demonstrated that exposure of normal fibroblasts to fresh, serum-containing medium leads to an increased rate of protein synthesis, possibly by induction on the genetic level (17). In this connection, it is of interest to note that the most pronounced difference ($P < 0.005$) in the slope of the protein/DNA curve (Fig. 1) occurs between days 2 and 4. This interval follows the well-described lag period (18) and overlaps the first feeding, i.e., the first substitution of fresh serum-fortified medium. Thus, it is plausible that CF fibroblasts temporarily fail to respond in a normal fashion to the inductive effect of serum. Such a failure would resemble the reported subnormal response of CF fibroblast cultures when ascorbate is added to enhance collagen synthesis (7). An impairment in protein production, if present, could conceivably result from an alteration in one of the many factors which control general cellular protein synthesis. Further study of CF fibroblasts in tissue culture, however, will be required to substantiate the hypothesis of an impairment in the regulation of protein synthesis.

Summary. Proliferation rates and cellular protein content have been measured in cultured fibroblasts derived from the skin of normal volunteers and cystic fibrosis patients. Three methods of measuring growth indicated that under our conditions, CF fibroblasts divide normally with a mean doubling time of 29 hr. During the logarithmic growth phase, however, lower cell protein/DNA ratios were observed consistently in CF cultures. This difference was not present in contact-inhibited, confluent fibroblasts. The finding of an apparent reduction in protein synthesis during rapid division, coupled with an observation by others that CF fibroblasts fail to normally induce collagen formation, suggests the possibility of a disturbance in the biochemical regulation of protein synthesis.

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