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Note

High-performance pyrolysis—gas chromatography: potential for differentiating cystic fibrosis cells

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Cystic fibrosis (CF) is the most prevalent biochemical genetic disease in Caucasian populations. Approximately one in a $2 \cdot 10^3$ live births is afflicted with this autosomal recessive disorder.

Despite a vast amount of research, the basic defect associated with CF has not been explained. Research directed toward elucidation of the defect has encompassed several diverse areas: metachromasia in cultivated skin fibroblasts, decrease in specific enzyme activity, increase in quantity of mucopolysaccaride or glycogen content, abnormality in collagen metabolism, accumulations of calcium ion, fucose uptake, and many more. Results of these investigations have been reviewed recently by Milunsky [1] and Nadler et al. [2].

Previously, by means of pyrolysis—gas chromatography (Py—GC) [3] we had investigated several genetic biochemical disorders by analyzing cultured skin fibroblasts. Despite the use of columns with low resolving power, one could clearly observe repeatable differences in the chromatographic profile of the cells.

More recently, we reported on Py—GC mammalian cell studies which incorporated both primary human tissues and cultured skin fibroblasts [4]. In this study, use of a microprocessor-controlled gas chromatograph equipped with a moderately efficient packed column rendered pyrochromatograms with much improved definition, compared to the 1972 study [3].

In the present paper, we have used a 43-m SCOT column with high resolving power in an attempt to delineate a cystic fibrosis "factor".

MATERIALS AND METHODS

Nine cultures were purchased from the Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, NJ, U.S.A.). Pertinent information is presented in Table I. Cells were cultured according to directions of the

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TABLE I

DERIVATIONS OF HUMAN SKIN FIBROBLASTS

GM No.*	Age**	Passage***	Sex	Race	Genetic status	
1011	7	11	M	W	CF	Son
1012	8	9	M	W	CF	Son
1009	34	10	M	W	Heterozygous	Father
1957	11	7	M	W	CF	Brothers
1959	10	5	M	W	CF	
668	10	12	M	W	CF	
38	9	6	F	B	Normal	

*Genetic Mutant Repository Catalog Number ascribed to a specific cell line from the Human Genetic Mutant Cell Repository, Camden, NJ, U.S.A.

**Age refers to the age of the patient from which the skin fibroblasts were derived.

***Passage indicates the number of times the culture was reinoculated and regrown.

suppliers. In brief outline, the method of culturing consisted of transferring cells to small tissue culture flasks with growth media containing 5% fetal calf serum. After a monolayer was formed, cells were removed from the glass surface by trypsinizing; they were then washed with sterile physiological saline, divided into two portions, and resuspended in fresh growth media. This procedure was continued until approximately 0.1 ml of packed cells were obtained. At that point, we washed the cells in glass-distilled water twice by simply centrifuging at 2000 *g* for 5 min and decanting the supernatant. Pellets were resuspended in glass-distilled water and lyophilized. Lyophilization was carried out in a Vertis Unit. Fine, filamentous, dry particles were the resultant product.

Pyrolysis—gas chromatography of skin fibroblasts

Small microgram quantities of sample were weighed on a Cahn Model G Electrobalance and transferred to a quartz boat (0.9 cm long, 0.24 cm wide, 0.14 cm deep with a 0.025-cm wall thickness). Each sample was placed in the platinum coil of a pyrolysis probe (Chemical Data Systems, Oxford, PA, U.S.A.), the latter being inserted into a quartz-lined interface connected to the gas chromatograph inlet. On pyrolyzing the sample in a stream of helium, thermal degradation products were swept into the gas chromatograph, separated, and detected. The resulting series of peaks, known as a pyrochromatogram, pyrogram (sometimes, as signature or fingerprint) were recorded, and results later compared.

Experimental details

Pyroprobe. Pyrolysis temperature, 801°C; duration of pyrolysis, 10 sec; pyrolysis temperature rise time, 12 msec; interface temperature, 200°C.

Gas chromatography. Varian Model 3700 gas chromatograph with CDS 111 data system. Temperature program, 65°C (4 min hold) then 6°/min up to 165°C (hold for 45—50 min); Carbowax 20M, 43 m × 0.5 mm I.D. SCOT column; $k' = 8.85$, $H_{Eff} = 37{,}600$ (using *n*-butylbenzene); helium carrier gas, flow-rate 39.8 cm/sec; detector, flame ionization (FID), $1 \cdot 10^{-12}$ a.f.s.

RESULTS

From a number of studies carried out in our laboratory on both primary and cultured cells of human, mouse, rat, hamster, as well as cells of microbial origin [3—6], we have found the Py—GC technique to be extremely reproducible in terms of retention time, detector response, and overall shape of profiles. For example, the first peak in a profile invariably emerged at 1.54 min. This fact is also borne out in the following illustrations, taken from duplicate analyses.

In Fig. 1 are depicted four chromatograms. The top three are of patients afflicted with the CF malady; the bottom is that of a normal subject. In comparing the two siblings, ages 11 and 10, we see only minor quantitative differences in areas designated A, B, C, D, E, out of a total number of peaks approximating 135. This number is about double that obtained from a good packed column. The unrelated CF pyrochromatogram differs from the two above in peak size in the areas generally designated B, a small additional peak at B1 and a somewhat larger peak at B2. However, on comparing the CF profiles to that of the normal, the latter shows distinct differences in two areas, 1 and 2. In the bracketed region designated Area 1, the normal profile has two peaks, the first of which is three times the amplitude of the second; in the CF profiles, however, these peaks have similar amplitudes. In Area 2, indicated by arrows, is the portion of the profile of a normal individual which distinguishes it from the CF. In the normal profile the first peak is higher in amplitude than the second. A reverse ratio is observed in CF profiles. Moreover, this Area 2 relationship is consistently reproduced in the three pyrochromatograms seen in Fig. 2.

In Fig. 2 are shown from the top, the Py—GC finterprints of two brothers, GM 1011 (age 7), and GM 1012 (age 8), both homozygous for CF. Their heterozygous father, GM 1009, has the profile shown at the bottom. Once again we see that the siblings' profiles are very similar with only minor differences. The greatest disparity between profiles of the brothers on one hand and the father on the other is indicated at peaks labelled 4 and 5. One observes in the profiles that ratios of principal peak amplitudes reflect a very high order of quantitative reproducibility.

DISCUSSION

One notable characteristic of the Py—GC technique is to give simple information in the form of a fingerprint or pyrochromatogram from biological material which originally existed in a highly complex matrix [6]. This characteristic has also been observed in Py—GC studies of man-made materials with intractable matrices [7].

In the present context, the precise qualitative and quantitative data obtained with the use of microprocessor-controlled instrumentation enable the investigator to differentiate and even to identify cells of biomedical importance.

Stored cells, grown two years earlier, gave essentially the same profiles as seen in the present study. Details were exactly repeated with one exception:

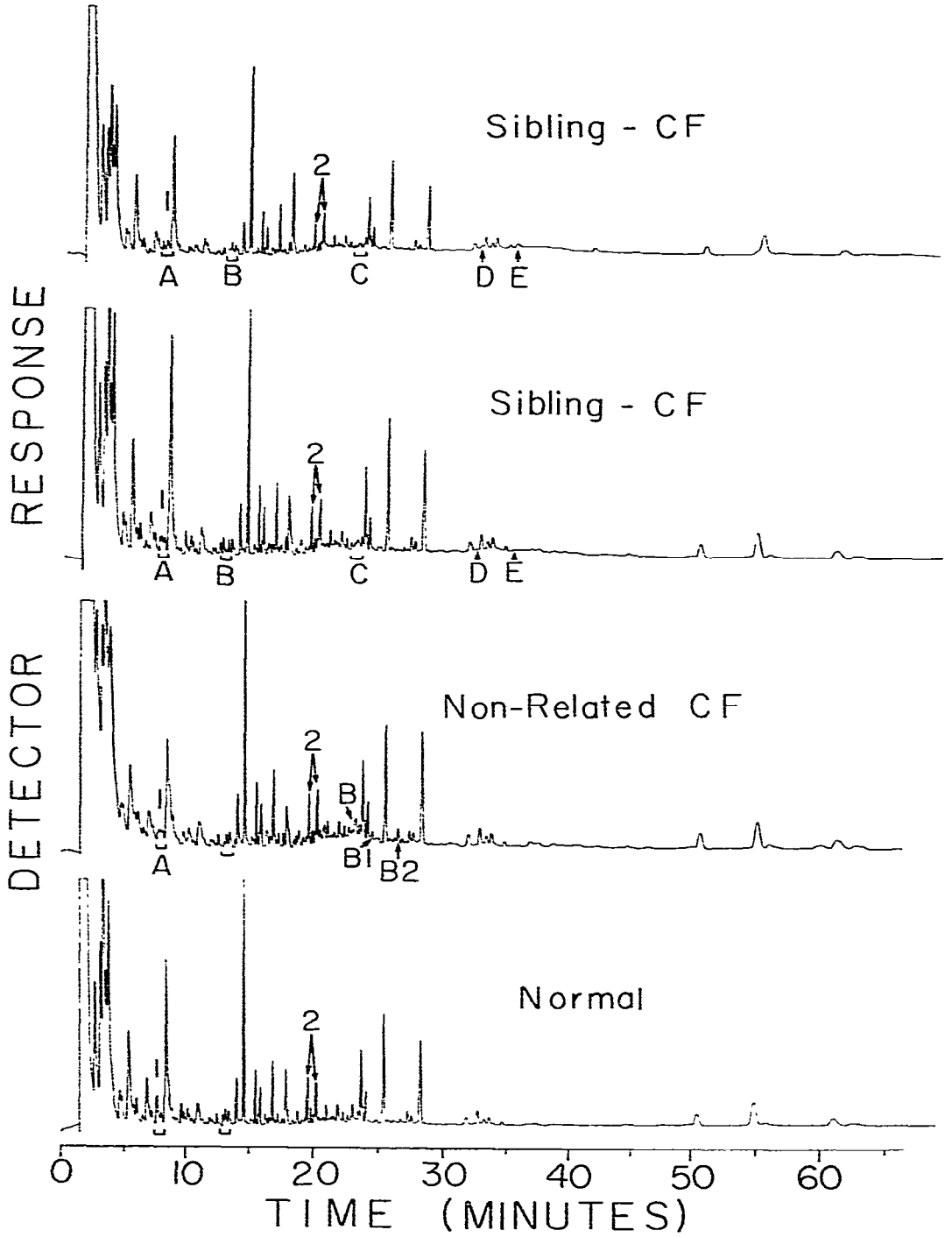

Fig. 1. Pyrolysis—gas chromatograms of human skin fibroblasts from cultured cells of various individuals from top to bottom; two brothers afflicted with cystic fibrosis (GM 1957, GM 1959), a non-related CF, GM 668 and a normal human diploid fibroblast GM 38. All profiles are displayed with a common elution time scale, e.g., bracketed area A appears at 8 min retention time on each chromatogram.

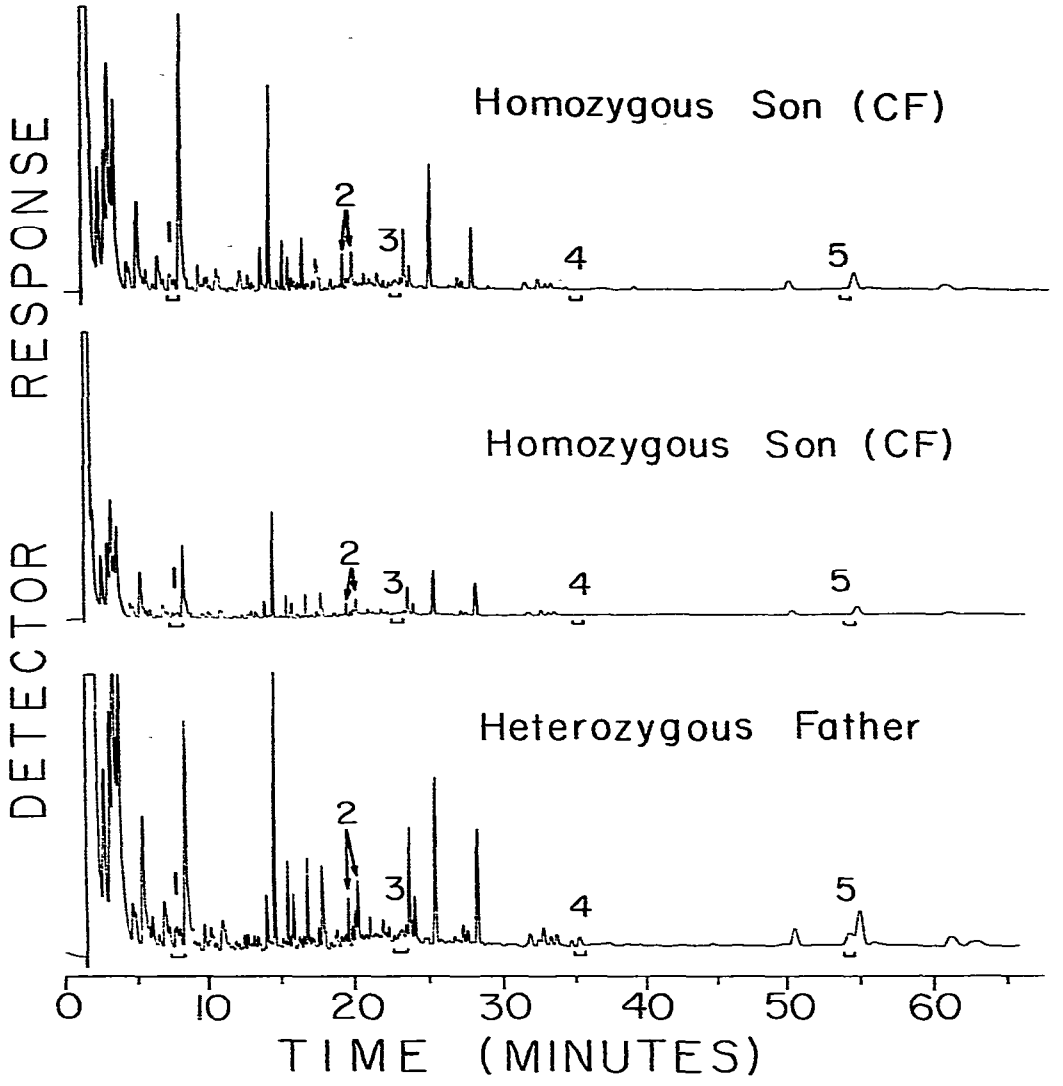

Fig. 2. Pyrolysis—gas chromatograms of human skin fibroblasts from cultured cells of homozygous son, GM 1011 (top), his younger brother, GM 1012 (middle) and their heterozygous father, GM 1009 (bottom). All profiles are displayed with a common time scale, e.g., area 3 covers the 22—23-min retention time period in the three profiles.

the two-year-old cultures always gave an extra peak at 28.50 min. It is possible that the extra peak could be ascribed to a change in pH, media formulation, e.g., use of a different calf serum, or that vital nutrients were present in excessive amounts [8]. The relative quantity of internally bound water in samples might also be a factor. In general, we have observed a certain immutability in pyrochromatograms of mammalian, as well as microbial cells.

The work described here is significant for a number of reasons. First, it paves the way for CF studies on the molecular level, such as those carried out in this institution on normal, primary mammalian cells derived from various human tissues [6]. Second, the pyrochromatograms are reproducible in all aspects, in-

dicating the possibility of delineating directly a cystic fibrosis "factor".

The congruency shown in the two sets of siblings' profiles as contrasted with the normal and heterozygous father may indicate a possible means of identifying heterozygous carriers of the disease.

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