the year before, but that is not the case in the remainder of the United States. By the end of fall this depression in number of births relative to the previous year was essentially over in both groups of dual-system states. The reduction was somewhat greater and lasted somewhat longer in the former Confederate states than in the other group, as would be expected.

The pattern of month-by-month variation within individual states further supports the evidence of Fig. 2. In order to reduce chance variation, we examined only the 34 states that averaged more than 2000 white births per month during this period. Of these, 16 were dual-system states. All but 2 of the 16 had fewer white births during the late spring and early summer of 1955 than in 1954. That was true of only 7 of the 18 unitary-system states, 6 of the 7 being states that border on one or more former Confederate states or other dual-system states.

In other words, we find the number of white births in the South to be lower than expected at almost the exact time we would predict, assuming the *Brown* decision demoralized prospective parents enough to cause some who would otherwise have stopped contracepting to continue and to cause others who had not been contracepting to start using contraception. The deflection is short-lived: it is concentrated in a period of 3 or 4 months. It is not large: Southern white birthrates were reduced by something on the order of 5 percent (*8*).

But even this small deflection is of considerable historical interest, if one accepts our explanation of it. It may, in addition, have some implications beyond that—implications for the study of fertility trends elsewhere in the developed world, and implications about the ability of social scientists to explain and predict such trends. Within the United States, for instance, since World War II there have been large, unprecedented, and unpredicted changes in fertility behavior, changes with significant consequences for many institutions in American society. The fact that these changes have been found within every social, economic, and racial group (*9*) suggests that they cannot be accounted for by changes in the composition of the population and that their explanation must be linked to historical events.

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References and Notes

1. The "South" here is the "census South," which includes the 11 former Confederate states, plus Delaware, Maryland, West Virginia, Kentucky, Oklahoma, and the District of Columbia.
2. R. R. Rindfuss, *Soc. Forces*, in press.
3. White Southern opposition to the decision hardly needs documenting, but it may be noted here that in a sample survey 2 years after the Court's ruling only 14 percent of white residents of the census South expressed support for school desegregation [H. R. Hyman and P. B. Sheatsley, *Sci. Am.* **211** (No. 7), 16 (1964)].
4. Psychological stress can trigger physiological subfecundity by blocking the release of luteinizing hormone necessary for ovulation [L. Mastroianni, in *Gynecology and Obstetrics*, S. L. Romney *et al.*, Eds. (McGraw-Hill, New York, 1975)]. It can also reduce male potency. But we are not suggesting that the *Brown* decision had those effects.

   We considered current events other than the court's decision as alternative explanations for the decline in Southern fertility. These ranged from Hurricane Hazel to the possibility of a short-run economic downturn in the South. However, after further reflection and investigation none of these alternative explanations seemed plausible.
5. This table is based on the absolute number of white births—in other words, on the numerator of a conventional fertility rate (the denominators are not available for individual states). This statistic is unsatisfactory in many applications [see, for example, M. Gomez B. and J. Reynolds, *Stud. Fam. Plann.* **4**, 317 (1973)], but it is unlikely that the three regions' denominators changed appreciably (and differently) in the short time span involved.
6. Of the other six, five were small states which exhibit considerable fluctuations in numbers of births from year to year: Idaho, Maine, North Dakota, Rhode Island, and Wyoming.
7. It has been suggested that the denominators for the data in Fig. 2 be based on a 3-year average rather than a single year. We have done this calculation; the differences between the dual-system states and the rest of the country are similar to those in Fig. 2 but less pronounced. However, using a 3-year average increases the problems involved in numerator analysis (*5*). The 3-year average may conceal substantial and differential changes brought about by the substantial migration from the South. Using only the preceding year minimizes this effect. Also, the effect of the upward fertility trend of the early 1950's is less with a 1-year base than with a 3-year average.
8. One should expect an effect like this not to be large. A deflection much greater than that observed would be, literally, incredible. The seasonally adjusted number of births in the former Confederate states for June 1955 was slightly more than 2 standard deviations below the average number of births (seasonally adjusted) in those states for the previous 13 months. (June was chosen because it represents the approximate beginning of a substantial deflection. If August or September had been chosen, the difference would be greater.) Alternatively, it is rather unlikely that the two groups of dual-system states would show declines for April, May, June, and July of 1955 (relative to the same months the previous year) while the rest of the country experienced increases, if chance variation were the only factor at work. Perhaps we should note that neither the Box-Tiao test [G. E. P. Box and G. C. Tiao, *Biometrika* **52**, 181 (1965)] nor the variation proposed by Glass [G. V. Glass, *Law Soc. Rev.* **3**, 55 (1968)] is appropriate here, because we are predicting a temporary change rather than a permanent one.
9. R. R. Rindfuss and J. A. Sweet, *Postwar Fertility Trends and Differentials in the United States* (Academic Press, New York, 1977).
10. Supported in part by a grant from the Carolina Population Center of the University of North Carolina. We thank Judy Kovenock for assistance in programming.

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Enhanced Dexamethasone Resistance in Cystic Fibrosis Cells: Potential Use for Heterozygote Detection and Prenatal Diagnosis

Abstract. *Cultured skin fibroblasts from patients with cystic fibrosis (CF) are more resistant to dexamethasone toxicity than are normal cells. We now report that, when fibroblasts cultured from obligate CF heterozygotes are exposed to dexamethasone, they have an intermediate survival compared to normal and homozygous CF cells. When dexamethasone survival was tested on cells from four patients undergoing amniocentesis, cells from a woman at risk of producing a child with CF showed significant dexamethasone resistance, similar to that of fibroblasts derived from known CF homozygotes; the other amniotic cell specimens showed dexamethasone sensitivity similar to that of normal skin fibroblasts. These data suggest that the dexamethasone resistance previously observed in skin fibroblasts may also be useful in the prenatal diagnosis of CF.*

Cystic fibrosis (CF) is the most common autosomal recessive disease among Caucasians, occurring once in every 1600 to 2500 live births. The clinical manifestations of the disease most commonly involve the pulmonary and gastrointestinal systems, resulting in a mean life expectancy for these patients of less than 21 years (*1, 2*). Although diagnosis of CF is aided by the finding of abnormally high concentrations of sodium and chloride ions in the sweat of affected individuals, the basic biochemical defect responsible for the disease is not yet known. In addition, there is no currently accepted method for detecting heterozygotes for CF or for making a prenatal diagnosis of the disease. In studies of skin fibroblasts cultured from many different individuals with CF, we observed that CF cells were more resistant to the cytotoxic effects of dexamethasone, a synthetic glucocorticoid, than normal fibroblasts were (*3*). We have also shown that CF cells exhibit cross-resistance to ouabain and the sex steroids, drugs whose molecular structures are similar to that of dexamethasone (*4, 5*). In each case, the resistance of CF cells to these drugs has not been due to any of the mechanisms commonly responsible for resistance to any single drug (*5, 6*).

We have now examined skin fibroblasts cultured from patients with CF

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and from their parents for the degree of dexamethasone resistance. The results show that fibroblasts from the obligate heterozygous parents of CF patients are somewhat more resistant than normal fibroblasts and considerably more sensitive than CF fibroblasts to dexamethasone cytotoxicity. In addition, the results reveal considerable dexamethasone resistance in amniotic fluid cells derived from a pregnant woman who already had a CF child, and a lack of this resistance in amniotic cells from pregnant women with a low risk of bearing a CF child.

To determine the dexamethasone response of cells from CF heterozygotes, cells from skin biopsies were obtained from individuals in five different CF families, including nine parents and seven affected children, as well as from eight unrelated normal individuals with no known family history of CF (*7*). The cultures were derived and maintained in a medium designated EMEM-B (*4*). For survival experiments, cells growing in monolayer were trypsinized, suspended in EMEM-B, and plated in 100-mm plastic tissue culture dishes (Falcon) with 9 ml of medium. Sufficient numbers of cells were plated at each drug dose to provide between 75 and 125 surviving colonies. After 24 hours, 1 ml of dexamethasone phosphate (Merck Sharp & Dohme) at a concentration tenfold higher than the desired final concentration was added to each dish. The cells were exposed to the dexamethasone for 24 hours; the dishes were then washed once with 10 ml of Earle's balanced salt solution and renewed with EMEM-B. The cultures were again renewed with EMEM-B 5 to 7 days after drug exposure, and incubated for a total of 10 to 14 days. When colonies contained a minimum of 65 to 75 cells, they were fixed, stained, and counted. Survival frequencies were calculated as the number of colonies surviving divided by the number of cells plated, and expressed relative to the plating efficiency of the strain in the absence of drug for each experiment.

Twenty-four cell strains (seven homozygous CF, nine obligate heterozygote, and eight normal) were tested in a total of 73 survival assays. For each experiment, cells from a normal, a heterozygote, and a homozygote were tested. The mean survival for each cell strain (as determined from one to nine experiments) at dexamethasone concentrations ranging from 10^{-10} to $10^{-6}M$ is given in Table 1. Survival at each drug dose was corrected only for the plating efficiency of each strain as determined in each experiment. In addition, the mean survival for all the different strains in each category (homozygous CF, heterozygous, and normal) were computed for all drug concentrations tested. The mean survival for all seven CF homozygotes at dexamethasone concentrations of 10^{-10}, 10^{-9}, and $10^{-8}M$ were approximately 100 percent; the mean survival of the eight different normal strains at the same concentrations were 58, 45, and 37 percent, respectively. The mean survivals of heterozygous cell strains at the same drug concentrations were 80, 60, and 51 percent, values between those of the normals and CF. The survivals at 10^{-7} and $10^{-6}M$ dexamethasone were 88 and 68 percent for the homozygous CF strains, and 27 and 20 percent for the normal strains; the survival frequencies for the heterozygous strains at these drug concentrations were 39 and 32 percent, again between those of the normal and homozygous strains. The differences between the mean survival frequencies for the three groups were statistically significant ($P < .001$) (*8*).

In a few survival experiments, the survival frequencies for a particular homozygous CF cell strain were unusually low or, in the case of a heterozygous strain, unusually high. For example, in one experiment, S-214 (heterozygote) showed survival frequencies at 10^{-10} and $10^{-7}M$ dexamethasone of 94 and 65 percent, respectively. However, a second analysis showed survivals of 85 and 26 percent, and a third analysis showed 70 and 35 percent. Thus, a single experiment may have occasionally yielded survival frequencies in which the distinction between heterozygous and homozygous CF was difficult, but the average of results from several determinations always yielded unequivocal results. Furthermore, in different experiments with some strains, the plating efficiency in the absence of drug may have varied from two- to threefold without affecting the relative survival of any strain in the presence of dexamethasone (*3*).

These data on survival after dexamethasone exposure (Table 1) indicated that cells from normals and CF obligate heterozygotes could be distinguished from homozygous cells. In order to determine if the dexamethasone survival method could be an intrauterine diagnos-

Table 1. Survival of skin fibroblasts at various dexamethasone concentrations. These data represent 73 survival assays. Each strain was tested between one and nine times, and the mean and standard deviation (S.D.) for each strain are shown. The mean survival (corrected for plating efficiency) and S.D. for each set of cells at each drug dose are also shown. The differences between the means for the groups were significant ($P < .001$, t-test) at all drug doses tested.

Strain	Family	N	Dexamethasone concentration (M)				
			10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
			Homozygotes				
S-215	1	6	1.06 ± 0.08	1.06 ± 0.06	1.11 ± 0.09	0.89 ± 0.08	0.68 ± 0.16
S-220	2	4	1.05 ± 0.05	1.09 ± 0.05	1.05 ± 0.08	0.82 ± 0.09	0.60 ± 0.08
S-223	3	1	1.14	1.04	1.14	0.98	0.68
S-244	4	5	1.01 ± 0.03	1.03 ± 0.05	1.02 ± 0.09	0.79 ± 0.10	0.69 ± 0.07
S-245	4	1	0.96	1.02	0.94	0.74	0.62
S-252	5	7	1.04 ± 0.04	1.10 ± 0.10	1.09 ± 0.07	0.89 ± 0.11	0.78 ± 0.15
S-253	5	3	1.08 ± 0.16	1.14 ± 0.12	1.07 ± 0.06	1.04 ± 0.17	0.74 ± 0.20
Mean ± S.D.			1.05 ± 0.06	1.07 ± 0.04	1.06 ± 0.07	0.88 ± 0.11	0.68 ± 0.06
			Obligate heterozygotes				
S-213	1	2	0.82	0.65	0.53	0.31	0.28
S-214	1	3	0.83 ± 0.12	0.63 ± 0.18	0.54 ± 0.19	0.42 ± 0.20	0.36
S-218	2	2	0.83	0.61	0.53	0.46	0.39
S-219	2	2	0.79	0.65	0.53	0.44	0.33
S-222	3	1	0.74	0.60	0.55	0.43	0.29
S-242	4	3	0.74 ± 0.07	0.59 ± 0.07	0.55 ± 0.03	0.42	0.31
S-246	4	2	0.84	0.60	0.49	0.40	0.40
S-248	5	3	0.76 ± 0.05	0.50 ± 0.10	0.38 ± 0.07	0.31	
S-249	5	2	0.83	0.59	0.48	0.31	0.20
Mean ± S.D.			0.80 ± 0.04	0.60 ± 0.04	0.51 ± 0.05	0.39 ± 0.06	0.32 ± 0.07
			Normals				
EX-25		2	0.58	0.40	0.35	0.30	0.26
GM-316		1	0.51	0.42	0.31	0.16	0.10
GM-1582		1	0.57	0.47	0.42	0.31	0.22
S-115		3	0.65 ± 0.11	0.50 ± 0.08	0.44	0.29	0.22
S-235		9	0.55 ± 0.04	0.41 ± 0.08	0.30 ± 0.08	0.22 ± 0.06	0.16 ± 0.04
S-238		2	0.59	0.46	0.39	0.30	
S-240		1	0.54	0.43	0.38	0.29	0.22
S-247		7	0.63 ± 0.09	0.49 ± 0.09	0.40 ± 0.07	0.26 ± 0.05	0.25
Mean ± S.D.			0.58 ± 0.05	0.45 ± 0.04	0.37 ± 0.05	0.27 ± 0.05	0.20 ± 0.06

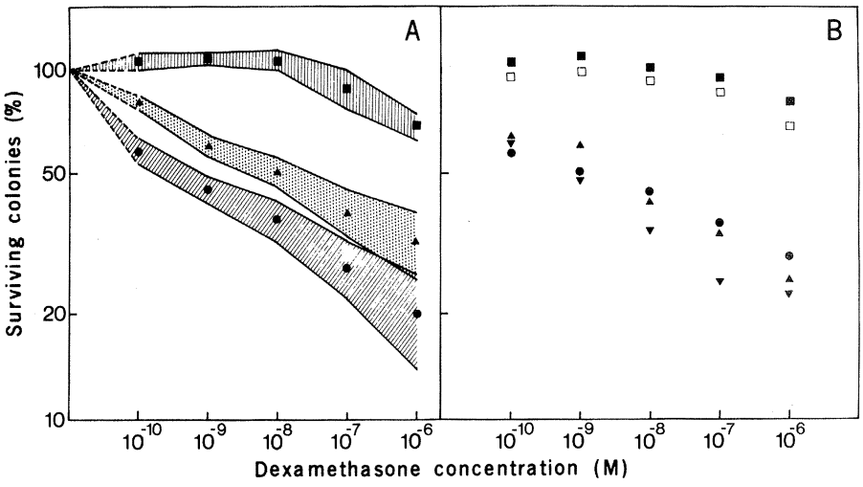

Fig. 1. (A) Survival of skin fibroblasts exposed to dexamethasone phosphate for 24 hours and allowed to form colonies. Each symbol represents the mean survival frequency (corrected for plating efficiency) at a given drug concentration for all of the strains in a particular category (■, CF homozygotes; ▲, obligate heterozygotes; ●, normal). Each strain was tested from between one and nine times in three replicate 100-mm petri dishes at each drug concentration in every test. The shaded areas represent 1 standard deviation around the mean survival frequencies for each category. (B) The survival of amniotic cell strains (■, A-256; ▲, A-257; ●, A-258; ▼, A-262) and a fetal skin fibroblast strain (□, F-256) exposed to dexamethasone. Strain A-256 was from the amniotic fluid of a pregnant woman who had a previous CF child, and strains A-257, A-258, and A-262 were from pregnant women at low risk of having a CF child. Strain F-256 was derived from the skin of the fetus corresponding to amniotic fluid strain A-256. Each point represents the mean survival frequency (corrected for plating efficiency) at each drug concentration determined in two experiments performed at different times. The survival frequencies in each experiment were the average of three replicate petri dishes.

tic test for CF with amniotic fluid cells, four mid-trimester amniotic fluid specimens were obtained, one (A-256) from a woman who already had a CF child, and three (A-257, A-258, and A-262) from women who were undergoing amniocentesis for indications other than a risk of CF (*7*). The cells in the amniotic fluid were pelleted by low-speed centrifugation (800*g* for 5 minutes), resuspended in EMEM-B, and plated in plastic petri dishes. After 2 to 3 weeks of growth, dexamethasone survival was assessed. Survival curves were determined for the four amniotic fluid cell strains (Fig. 1B) and compared with those of normal, heterozygous, and homozygous skin fibroblasts (Fig. 1A). The amniotic cell strains A-257, A-258, and A-262 (not at risk for CF) showed survival frequencies similar to the normal skin fibroblast strains. However, amniotic fluid strain A-256 showed significant dexamethasone resistance, similar to that seen with homozygous CF fibroblast cell strains; this strain was derived from the woman with one CF child. These results suggested that A-256 was from a CF homozygote whereas the other amniotic fluid cell strains were probably from unaffected fetuses. To test the reliability of these survival data, skin was cultured from the abortus corresponding to amniotic fluid specimen A-256, and the resultant fibroblast cell strain (F-256) also showed dexamethasone resistance comparable to homozygous CF skin fibroblast strains (Fig. 1B).

Our studies with amniotic fluid cells suggest the feasibility of using this method for the prenatal diagnosis of CF. However, many further tests need to be performed to establish the clinical reliability of the procedure and to determine possible problems. For example, even with optimal cell culture conditions, 2 to 3 weeks were required after the amniotic fluid was initiated in culture before enough cells were produced to perform the survival assay. An additional 10 to 14 days were required after dexamethasone exposure to allow formation of detectable colonies. It therefore requires approximately 4 to 5 weeks in our conditions to determine whether a fetus is affected with CF. It should also be noted that some amniotic fluid specimens yield cells of mixed morphology in culture, not all of which may respond to dexamethasone in the same manner as fibroblasts. In the amniotic strains in our experiments, the cells we encountered included fibroblastic, small epithelial, and amniotic cells, and no attempt was made to enrich or select for any single cell type in the survival tests. In some amniotic fluid specimens, contamination with maternal cells could be expected to cause some difficulty in assessing results, a problem inherent in most prenatal diagnoses.

Our studies indicate that the dexamethasone survival assay method may be useful in detecting CF with amniotic fluid cells and tissue culture techniques available in most centers performing prenatal diagnosis. Although the method is capable of detecting heterozygotes, the assay method is probably too cumbersome at present for large-scale screening for heterozygotes.

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7. Human specimens in this study were obtained under protocols approved by the Clinical Investigation Committees of the Children's Hospital Medical Center and the Boston Hospital for Women. Skin fibroblast biopsy material was obtained after receiving informed consent. Samples of amniotic fluid specimens were obtained only from those patients electing to undergo therapeutic abortion for reasons other than this study, and the experimental results obtained were not conveyed to them. The patients had no direct contact with the investigators involved in this study.
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