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Analysis of Fetal Intestinal Enzymes in Amniotic Fluid for the Prenatal Diagnosis of Cystic Fibrosis

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SUMMARY

Amniotic-fluid intestinal alkaline phosphatase activity, gamma-glutamyltranspeptidase activity, and leucine-aminopeptidase activity were quantitated to assess their reliability for the prenatal diagnosis of cystic fibrosis. The results indicate that for each of these enzymes an arbitrary cutoff point could be chosen that would enable one to correctly predict the outcome for the majority of at-risk pregnancies. However, some false positives and false negatives occurred with each enzyme. To obtain optimal diagnostic discrimination, the three enzyme values obtained for each sample were combined into a single linear discriminant function that proved to be a more accurate indicator of the outcome of the pregnancy. This was especially important for those cases in which the predicted outcome as based on the individual enzyme results was in disagreement. From the cases studied here, it appears that this method can be expected to give a correct prediction in ~96.5% of all 25%-at-risk pregnancies. False positives can be expected in ~1.4% of the pregnancies and false negatives in ~2.2%.

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INTRODUCTION

Attempts to establish a reliable method for the prenatal diagnosis of cystic fibrosis have been hindered by a lack of knowledge of the basic biochemical defect responsible for the disease. Instead, attention has focused on enzymes and other proteins present in amniotic fluid that might be used to distinguish affected from normal fetuses. These proteins have included disaccharidases: maltase, sucrase, lactase, and trehelase (Morin et al. 1983; Kleijer et al. 1985; Schwartz and Brandt 1985; Szabo et al. 1985); peptidases: leucine aminopeptidase and gamma-glutamyltranspeptidase (Baker and Dann 1983; Carbarns et al. 1983; Ben-Yoseph et al. 1984; Brock et al. 1984*b;* Aitken et al. 1985; Brock 1985; Muller et al. 1985); proteases: trypsin-like proteases and immunoreactive trypsin (Nadler and Walsh 1980*a,* 1980*b;* Schwartz 1982; Brock and Hayward 1983; Carbarns 1983; Poenaru and Vinet 1983; Brock et al. 1984*c*), as well as phosphodiesterase; and the alkaline phosphatases (Brock 1983, 1985; Brock et al. 1984*a,* 1984*b,* 1985; Muller et al. 1984*a,* 1984*b,* 1985; Aitken et al. 1985; Boué and Brock 1985).

Of all uses of the previously mentioned marker proteins, the most reproducible, reliable method for the prenatal diagnosis of cystic fibrosis has been that of quantitating the intestinal alkaline phosphatase isoenzyme activity present in amniotic fluid (Brock et al. 1984*a;* Brock 1985). In 1978, Mulivor et al. described a biochemical/heat denaturation method for quantitating the alkaline phosphatase isoenzymes in amniotic fluid and reported that second trimester amniotic fluids of 14–20 wk gestation had intestinal alkaline phosphatase as the major alkaline phosphatase isoenzyme (Mulivor et al. 1978*b*). Moreover, they showed that this isoenzyme was an electrophoretically unique fetal isoenzyme (Mulivor et al. 1978*a*) that probably had its origin in desquamated fetal intestinal mucosa and entered the amniotic fluid by passage of meconium from the fetal gut (Mulivor et al. 1979). By examining the alkaline phosphatase isoenzymes in amniotic fluid throughout gestation, they showed (1) that amniotic fluids of 14–22 wk gestation consisted, on average, of 81% intestinal, 15% liver/bone/kidney, and 4% placental alkaline phosphatase and (2) that after ~22 wk gestation <5% of the total alkaline phosphatase activity could be attributed to intestinal alkaline phosphatase. This observation was subsequently confirmed using an immunoprecipitation method employing murine monoclonal antibodies specific for each of the three main categories of human alkaline phosphatases (Mulivor et al. 1985). This disappearance of intestinal alkaline phosphatase from amniotic fluid coincided with the innervation of the anal sphincter muscle and led the authors to speculate on the usefulness of the measurement of fetal intestinal alkaline phosphatase as a sensitive method for quantitating the amount of meconium in amniotic fluid (Mulivor et al. 1979). Beginning in 1983, Brock (1983) and, shortly thereafter, Muller et al. (1984*a*) reported on the usefulness of amniotic-fluid alkaline phosphatases for the prenatal diagnosis of cystic fibrosis. Both authors described deficiencies of alkaline phosphatase and other amniotic-fluid microvillar enzymes in fetuses with cystic fibrosis. The most useful assays described to date include the measurement of L-

phenylalanine-sensitive and L-homoarginine-resistant alkaline phosphatase activity, intestinal alkaline phosphatase activity detected immunologically, gamma-glutamyltranspeptidase activity, and leucine-aminopeptidase activity. Deficiencies of these enzymes in amniotic fluids from fetuses with cystic fibrosis are thought to arise as a consequence of the retardation of the normal passage of these enzymes via meconium into the amniotic fluid owing to thick viscous meconium present in cystic fibrosis (Muller et al. 1984*a*, 1984*b*, 1985; Brock et al. 1985; Papp et al. 1985). This hypothesis has been supported by the observation that the majority of fetuses with microvillar enzyme deficiencies show evidence of intestinal obstruction both in utero, by ultrasonography, and in postmortem examination, by the finding of a meconium ileus equivalent (Shalev et al. 1983; Muller et al. 1984*b*, 1985; Brock et al. 1985; Papp et al. 1985).

The use of gene probes for the prenatal diagnosis of cystic fibrosis appears possible in the future but is not yet fully informative for all families with a 25% risk for cystic fibrosis (Knowlton et al. 1985; Tsui et al. 1985; Wainwright et al. 1985; White et al. 1985; Scambler et al. 1986). Many families currently desire prenatal diagnosis, and in this paper we present our experience with the quantitation of amniotic-fluid intestinal alkaline phosphatase, gamma-glutamyltranspeptidase, and leucine-aminopeptidase for this purpose.

Data are presented for 69 pregnancies with a 25% recurrence risk for cystic fibrosis with known outcomes. For all cases, the alkaline phosphatases were quantitated using both the biochemical/heat denaturation method and the immunologic method. The calculated percentages of intestinal alkaline phosphatase activity are combined with the results of gamma-glutamyltranspeptidase and leucine-aminopeptidase activity measurements to generate a discriminant function, *Zb*, that is a better predictor of the likelihood that the results indicate a normal or affected fetus than the results of any of the enzyme values judged independently.

MATERIAL AND METHODS

Amniotic-Fluid Specimens

Cell-free amniotic-fluid specimens that would normally have been discarded were acquired from local hospitals. The fluids were originally obtained by transabdominal amniocentesis from women whose pregnancies were being monitored for chromosomal or biochemical disorders as well as because of advanced maternal age. All of the control pregnancies described in the present paper are from pregnancies that have been delivered and been reported as having normal outcomes and normal karyotypes.

In addition, cell-free amniotic-fluid specimens were obtained from numerous sources from pregnancies with a 25% risk of giving birth to an infant with cystic fibrosis. This series consisted of both prospective and retrospective cases. All samples were stored at −20 C if not used immediately.

The following is a list of investigators who contributed amniotic fluids from pregnancies with a 25% risk for cystic fibrosis, along with the code for the fluids

that they submitted. Asterisks indicate pregnancies with unknown outcomes at the time the present paper was prepared.

Dr. A. Beaudet/Dr. G. Buffone (Houston)
FL,* GO,* MA,* SH,* WI,* MC,* LU,* SU,* MO,* PA,* CO,* BR,* GOR,* TH,* KA*

Dr. A. Boué (Paris)/Dr. F. Muller (Boulogne)
6, 11, 15, 20, 22, 25, 26, 27,* 34, 37, 38, 39, 40, 41, 43, 45, 46, 48, 49, 50, 52, 53, 54, 55, 56, 57, 60, 63, 64, 67, 71, 74, 78, 79, 81, 83, 84, 85, 86, 87, 88, 91, 95, 96, 97, 100, 103, 104, 106, 107, 108, 109, 111, 112, 113, 116, 117, 122,* 128, 131, 133, 135, 140, 141, 154, 155

Dr. L. Dallaire/Dr. M. Potier (Montreal)
82-0252, 84-0001, 84-0828

Dr. F. Gilbert (New York)
1280,* 1388,* GR,* 1584,* 1644,* PA,* VA,* AD,* 428, SE, 1691, GRI, DI, HO

Dr. M. Mennuti (Philadelphia)
WA,* BI, RA

Dr. H. Nadler (Detroit)
1818*

Dr. E. Pergament (Chicago)
DA,* NI,* PA,* LU,* BO,* NE,* WE,* HI

Dr. H. Punnett (Philadelphia)
SH*

Cystic Fibrosis Status for At-Risk Fluids

One hundred eleven at-risk pregnancies were studied. The amniotic-fluid samples all came from pregnancies between 16 and 20 wk gestation. In all but one of the families, at least one previous child had been found to have cystic fibrosis. The one exception was a pregnancy that resulted in a child with cystic fibrosis from whom an amniotic-fluid specimen that had been collected at 18 wk gestation for other purposes was still available for study. In 35 cases the pregnancy is still proceeding and the outcome not yet known.

Of 46 live borns, the child was judged normal in 36 cases and found to have cystic fibrosis in 10 cases. The outcomes of the live-born pregnancies were determined from the presence of signs and symptoms of the disease, from the results of sweat tests, or from the results of serum immunoreactive trypsin (IRT) determinations. Clinical symptoms included meconium ileus or severe pulmonary involvement. Serum IRT was determined at age 4–5 days. Blood was taken on a blotting card and sent by mail to the laboratory of Dr. G. Travert (Laboratory of RIA, Caen, France) for the determination of IRT. IRT was measured using a commercial kit: RIA gnost trypsin (Hoechst-Behring-France). Sweat tests were performed on babies after the age of 2 mo. The babies for whom only IRT results are presented all had normal physical examinations at 6 mo of age, but sweat tests were not performed.

In 30 cases the pregnancy had been terminated and the diagnosis of cystic fibrosis or normality had been made from autopsy findings such as gross ac-

TABLE 1

DIAGNOSES

Status	No. of Cases
Unaffected:	
Live borns:	
"Clinically normal" by physical examination, with no diagnostic testing performed	1
Clinically normal and normal sweat test	2
Clinically normal and normal IRT	26
Clinically normal and normal IRT and normal sweat test	7
Terminations of pregnancy: trisomy 21	1
Total	37
Affected:	
Live borns:	
Clinically affected with severe pulmonary involvement	1
Meconium ileus at birth	1
Abnormal sweat test	6
Abnormal IRT and abnormal sweat test	1
Abnormal IRT	1
Terminations of pregnancy:	
Meconium ileus	1
Meconium ileus and increased albumin	16
Abnormal meconium	2
Abnormal meconium and increased albumin	6
Increased albumin	4
Total	39

cumulation of meconium in the intestinal tract (so called meconium ileus equivalent), abnormal meconium and pellets, or elevated intestinal albumin concentration (Brock et al. 1985). The details are summarized in table 1.

Alkaline Phosphatase Isoenzyme Quantitation

Two independent procedures were employed to quantitate the fractions of total alkaline phosphatase activity representing the liver/bone/kidney, intestinal, and placental alkaline phosphatases in amniotic fluid. Both methods have been described previously (Mulivor et al. 1985). The biochemical/heat denaturation method uses a combination of heat denaturation and L-phenylalanine and L-homoarginine inhibition studies to quantitate the different alkaline phosphatases. The immunologic method utilizes murine monoclonal antibodies specific for each of the three main categories of alkaline phosphatases and rabbit anti-mouse IgG in an immunoprecipitation reaction for the quantitation.

Alkaline phosphatase enzyme assays were performed as previously described, except that the reaction conditions were modified so that the assays could be automated using a Baker Instruments CentrifiChem System 600. The reaction mixture consisted of 0.3 ml of 1.0 M diethanolamine (Fisher Scientific), pH 9.8, 0.28 M NaCl, 0.5 mM $MgCl_2$, 5 mM *p*-nitro-phenylphosphate (Sigma Chemical), and 0.02 ml of supernatant amniotic fluid. After a 1-min equilibration, the reaction was incubated for 12 min at 30 C and the change in absorbance at 405 nm was determined.

Gamma-Glutamyltranspeptidase Assay

Enzyme assays were performed using the Baker CentrifiChem reagent kit for gamma-glutamyltranspeptidase. The reaction mixture consisted of 0.415 ml of 4.1 mM gamma-glutamyl-*p*-nitroanilide, 41.2 mM glycylglycine, 1.1 mM glutamate, and 0.005 ml supernatant amniotic fluid. After a 1-min equilibration, the reaction was incubated at 30 C for 3 min and the change in absorbance at 405 nm was determined.

Leucine-Aminopeptidase Assay

The assay procedure described by Brock et al. (1984*b*) was modified for automation using the CentrifiChem System 600 analyzer. The reaction mixture consisted of 0.3 ml of 0.1 M Tris-HCl, pH 7.0, 4.4 mM L-leucine *p*-nitroanilide (Sigma), and 0.02 ml supernatant amniotic fluid. After a 90-s equilibration, the reaction was incubated at 30 C for 12 min and the change in absorbance at 405 nm was determined.

Enzyme Activity Calculations

All enzyme assays were performed at least in triplicate. Enzyme activity was calculated as milli-international units per milliliter of amniotic fluid. One enzyme unit is defined as the amount of enzyme that catalyzes the conversion of 1.0 μmol of substrate/min under standard conditions. The enzyme values reported for the alkaline phosphatases are the means of the biochemical and immunologic quantitations.

Discriminant-Function Analysis

Discriminant analysis of the data, based on Fisher's linear discriminant-function method, was performed using the STATGRAPHICS computer program (STSC Inc., Rockville, MD). This program generates a discriminant function from a data matrix. The discriminant function was calculated using the data collected for the control–normal outcome pregnancies as one population and the data collected from the 25%-at-risk-with-affected-outcome pregnancies as the second population.

RESULTS

The distributions of the percentage of total alkaline phosphatase activity as intestinal alkaline phosphatase activity (PCI), gamma-glutamyltranspeptidase activity (GGT), and leucine-aminopeptidase activity (LAP) for all of the control–normal outcome pregnancies are shown in figure 1. The distributions for GGT and LAP vary over a wide range and are skewed to the right. The lower 95% confidence cutoffs are at 155 mIU/ml and 30 mIU/ml, respectively. The distribution of PCI is more compact, though there is tailing to the left with 2% of the fluids falling below 3 SDs of the mean (44.5%). PCI was found to be more useful for the discrimination analysis (see below) than were the absolute values of intestinal alkaline phosphatase activity, which have a wider distribution and are skewed to the right.

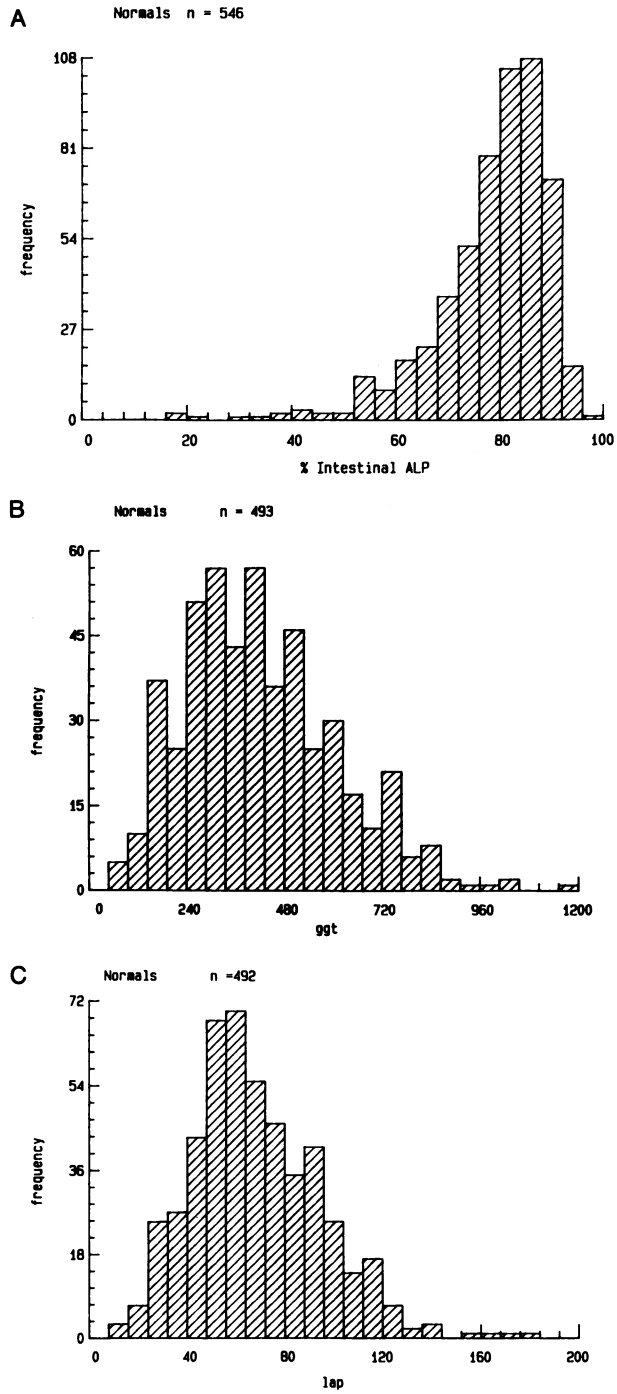

FIG. 1.—*A*, Distribution of PCI (% intestinal ALP) for 546 control–normal outcome pregnancies; *B*, distribution of GGT for 493 control–normal outcome pregnancies; *C*, distribution of LAP for 492 control–normal outcome pregnancies.

TABLE 2

ENZYME ACTIVITIES IN CONTROL–NORMAL OUTCOME PREGNANCIES

A. PCI

Weeks Gestation	No. of Samples	Mean % (SD)
15	17	76.91 (9.83)
16	212	78.36 (10.64)
17	198	78.54 (11.57)
18	70	78.81 (12.60)
19	41	81.66 (10.74)
20	8	69.94 (16.67)
Overall	546	78.56 (11.36)

B. GGT

		Mean Activity (SD) (mIU/ml)
15	15	419.20 (161.94)
16	201	455.05 (184.64)
17	183	411.70 (181.82)
18	58	391.57 (235.29)
19	32	386.50 (204.56)
20	4	181.00 (64.27)
Overall	493	423.73 (192.50)

C. LAP

		Mean Activity (SD) (mIU/ml)
15	15	66.87 (24.83)
16	201	71.03 (25.53)
17	183	68.17 (26.52)
18	58	66.53 (30.05)
19	32	65.63 (26.78)
20	3	29.67 (4.16)
Overall	492	68.71 (26.59)

The average values obtained for PCI, GGT, and LAP at various gestational ages for the control–normal outcome series of amniotic fluids are shown in table 2. PCI is fairly constant from 15 wk to 18 wk gestation with a rise at 19 wk and a decline at 20 wk. GGT and LAP decline from 16 wk to 20 wk.

Of the 76 at-risk pregnancies with known outcomes, complete data for PCI, GGT, and LAP were obtained for 69 samples. When the cutoff values stated above are assumed, the accuracy of each of the three enzymes independently for predicting the outcome of a pregnancy with a 25% recurrence risk for cystic fibrosis is as shown in table 3. PCI and LAP values are the best indicators of the genetic status of the fetus. Table 4 shows the concordance of the three marker enzymes both with each other and with the diagnosed outcome of the preg-

TABLE 3

CORRECT PREDICTIONS

Enzyme Activity	Unaffected Outcome	Affected Outcome	Overall
PCI	33/34 (97.1%)	30/35 (85.7%)	63/69 (91.3%)
GGT	32/34 (94.1%)	28/35 (80.0%)	60/69 (87.0%)
LAP	33/34 (97.1%)	31/35 (88.6%)	64/69 (92.8%)

nancy. PCI and LAP are the most concordant and offer the best accuracy in predicting the outcome of an at-risk pregnancy. But there are discrepancies.

To maximize the usefulness of these independently obtained enzyme values for predicting the outcome of an at-risk pregnancy, discriminant-function analysis was performed on the data. This analysis generates a single function that is a more reliable indicator of the likelihood that the results for a particular fluid belong to the normal or affected data populations than are the results for the three enzyme analyses judged independently.

The optimal discrimination has been obtained using the function *Zb*: $Zb = -9.23 + 0.052\ (\text{PCI}) - 0.210\ (\log \text{GGT}) + 3.271\ (\log \text{LAP})$.

The best cutoff point is $Zb = -2.60$, *Zb* values < -2.60 predicting cystic fibrosis and those > -2.60 predicting normality. Log GGT and log LAP were used because the nonlog distributions for each are skewed to the right and because log transformation tends to normalize the distributions.

Figure 2 shows the mean ± 1 SD for *Zb* for the control–normal outcome pregnancies at different gestational ages. *Zb* is fairly constant from 15 wk to 19 wk gestation.

The distribution for *Zb* for the same 492 control–normal outcome pregnancies is shown in figure 3. Nine samples or 1.8% of the total fall below a *Zb* value of -2.60. Also shown in figure 3 are the distributions of *Zb* for the 69 25%-at-risk fluids with known outcomes. All of the at-risk–normal outcome fluids have *Zb* values > -2.60, and all but three of the at-risk–affected outcome pregnancies have values < -2.60. *Zb* correctly predicts all 34 of the normal outcome pregnancies and 32 (91.4%) of the 35 affected outcome pregnancies.

Forty of the 111 at-risk amniotic fluids studied constitute a prospective study. The predictions for these fluids and the outcomes, if known, are shown

TABLE 4

CONCORDANCE

Combination	Unaffected Outcome	Affected Outcome	Overall
PCI + GGT	31/34 (91.2%)	23/35 (65.7%)	54/69 (78.3%)
PCI + LAP	32/34 (94.1%)	32/35 (91.4%)	64/69 (92.8%)
LAP + GGT	31/34 (91.2%)	26/35 (74.3%)	57/69 (82.6%)

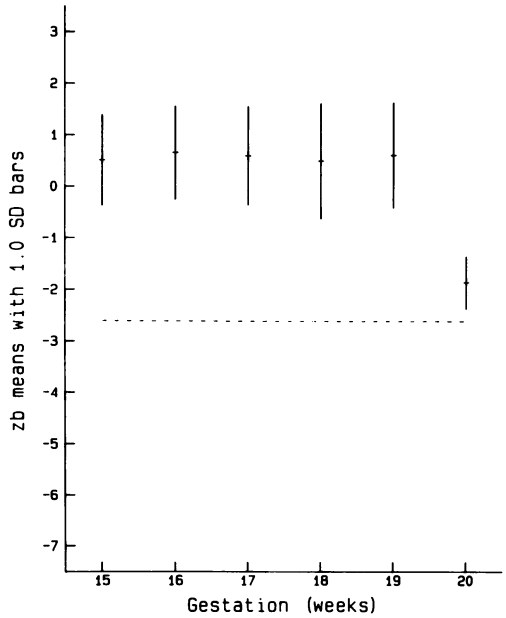

FIG. 2.—Mean ± 1 SD for *Zb* at 15–20 wk gestation for 492 pregnancies. The number of samples at each gestational age are as follows: 15 wk, 15; 16 wk, 201; 17 wk, 183; 18 wk, 58; 19 wk, 32; and 20 wk, 3. The cutoff line at −2.6 was established using the control–normal outcome and 25%-at-risk-with-affected-outcome data populations.

in table 5. Thirty-five percent (14 of 40) of the pregnancies are predicted to be affected and 65% (26 of 40) to be unaffected. Seven pregnancies have concluded, and the diagnosed outcomes agree with the predicted outcomes in all cases.

DISCUSSION

Ideally, the reliability of a prenatal diagnostic test should be based on a comparison of predicted and confirmed diagnoses in live borns or abortuses.

TABLE 5

PROSPECTIVE STUDY

Category	No. (%) of Cases
No. predicted to be affected:	
Liveborn affected	2
TOP-diagnosed affected	2
Outcome not yet known	10
Total	14 (35)
No. predicted to be unaffected:	
Liveborn unaffected	3
Outcome not yet known	23
Total	26 (65)

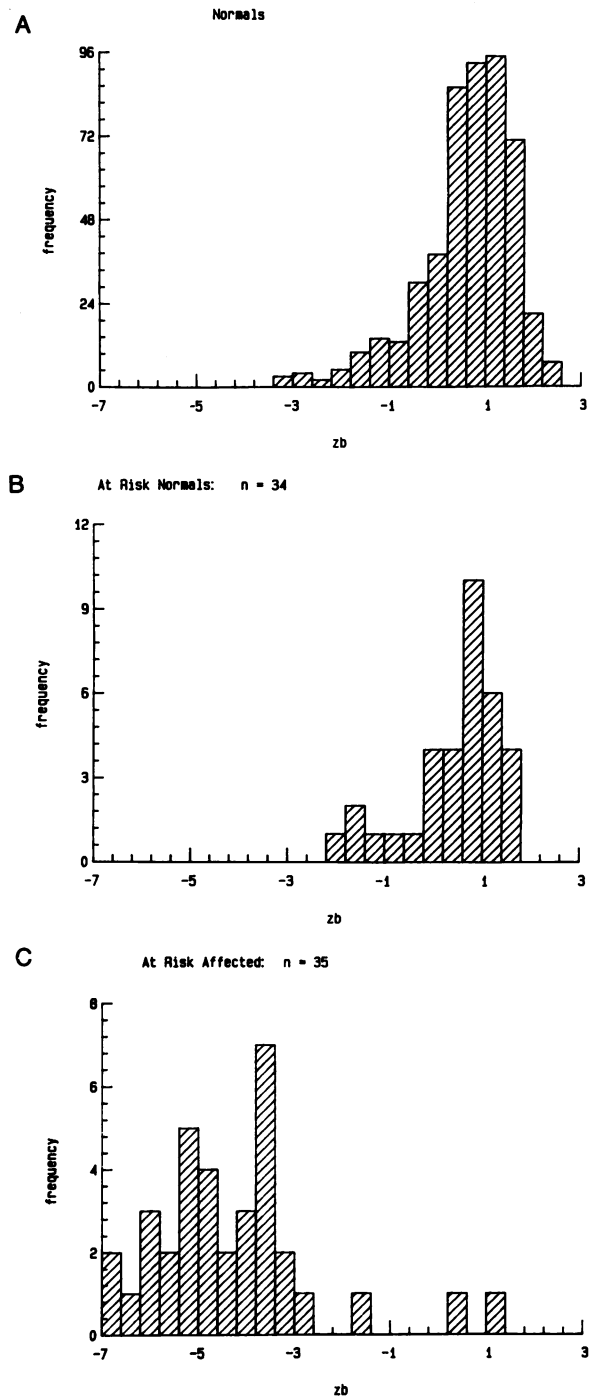

FIG. 3.—*A*, Distribution of *Zb* for 492 control–normal outcome pregnancies; *B*, distribution of *Zb* for 34 at-risk pregnancies with normal outcomes; *C*, distribution of *Zb* for 35 at-risk pregnancies with affected outcomes.

For cystic fibrosis, there is no unanimity as to the reliability of diagnosis in abortuses, and some would argue against using IRT or sweat-sodium and sweat-chloride testing as the sole criterion for diagnosing cystic fibrosis in live borns. The results presented here suffer from these criticisms.

Once the prospect of a reasonably reliable diagnostic test based on a limited number of retrospective cases had been published, a properly designed, double-blind prospective study with all pregnancies continuing to term failed to enroll a significant number of participants.

The at-risk fluids studied in this series consisted of retrospective cases, prospective cases, and cases studied prospectively in other laboratories and contributed for this study so that the alkaline phosphatase isoenzymes could be fully quantitated employing two different, independent methods and the results analyzed along with gamma-glutamyltranspeptidase and leucine-aminopeptidase activity values. Essentially the same results were obtained from alkaline phosphatase isoenzyme quantitations performed by the two different methods, thus confirming our previously reported observations on a smaller number of amniotic-fluid samples (Mulivor et al. 1985).

The results presented here are also in basic agreement with those published previously and show that PCI and LAP are reasonably reliable indicators of the occurrence of intestinal obstruction in cystic fibrosis. But, by combining PCI, GGT, and LAP into a discriminant function, *Zb*, a somewhat more accurate assessment of the genetic status of an at-risk fetus can be achieved. This could prove to be important in the small number of pregnancies in which the predictions based on the individual enzyme results are in disagreement.

Among the series of control–normal outcome pregnancies, *Zb* had values < -2.6 in 9 (1.8%) of 492 cases. This provided an estimate of false positives, since for all of the cases falling in the cystic fibrosis range, cystic fibrosis has been excluded on the basis of either sweat testing (four cases) or a lack of clinical symptoms (five cases). Among the at-risk cases who were diagnosed as having cystic fibrosis, 3 of 35 showed *Zb* values well above -2.6. Taking this approach with the data so far assembled, it appears that the method can be expected to give a correct prediction in ~96.5% of all 25%-at-risk pregnancies ([75% unaffected × .982] + [25% affected × .914]). False positives can be expected in ~1.4% of the pregnancies and false negatives in ~2.2%.

Three pregnancies that we predicted to be normal resulted in affected outcomes. All three cases came from Dr. André Boué's laboratory. Case 39 had amniocentesis at both 17 wk and 18 wk gestation. On both occasions PCI was normal. At 17 wk GGT was just under the 155 mIU/ml cutoff, and at 18 wk the value had fallen to 84 mIU/ml. LAP was twice the cutoff value of 30 mIU/ml at 17 wk gestation and 31 mIU/ml at 18 wk gestation. The *Zb* values for 17 wk and 18 wk were -0.20 and -1.59, respectively. Our interpretation of the data was that the fetus would have been unaffected. The pregnancy had been terminated, and the fetus was reported to show meconium ileus and elevated meconium albumin levels. The decision to terminate this pregnancy had been made on the basis of the results obtained for a 19-wk gestational age amniotic-fluid sample that showed low values for all enzymes (A. Boué, personal com-

munication). No 19-wk amniotic fluid was available for use in the present study.

Case 55 had amniocentesis at 19 wk gestation. PCI was 88.1%, GGT was 95 mIU/ml, LAP was 44 mIU/ml, and *Zb* was 0.31. Our interpretation was that the fetus would have been unaffected. The pregnancy resulted in a live born who tested affected as judged by IRT and by sweat-chloride test. Of special note is that both this infant and a previously affected sib have been described as having the so-called pulmonary form of the disease.

Case 133 had amniocentesis at 18 wk and 19 wk gestation. At 18 wk gestation PCI was 48.8%, which is just above the cutoff of 44.5%. At 19 wk this value rose to 94.2%. At 18 wk GGT was 160 mIU/ml, and at 19 wk it fell to 117 mIU/ml. At 18 wk LAP was 25 mIU/ml, and at 19 wk it rose to 72 mIU/ml. *Zb* values for 18 wk and 19 wk were -2.58 and 1.31, respectively. Our interpretation was that the fetus would have been unaffected. The pregnancy resulted in a live born who was clinically affected with severe pulmonary involvement.

These three cases illustrate some important issues in utilizing amniotic-fluid intestinal enzymes for the prenatal diagnosis of cystic fibrosis. In 26 of the 111 pregnancies reported here more than one amniocentesis was performed, both to help identify the most appropriate gestational age at which to perform the analysis and to clarify those initial amniocenteses that showed ambiguous results. The data reported here present the results for each pregnancy only once, and the data presented are those on which the final interpretation was made. This experience has led us to conclude that the best gestational age for amniocentesis is 17–18 wk. Amniocentesis at 17 wk is preferable so that a repeat tap at 18 wk can be performed if the results of the first tap are borderline. An accurate determination of the gestational age, preferably by ultrasonography, is therefore critical. At 16 wk and 19 wk greater ambiguity is encountered. Prior to 17 wk there are several examples of the intestinal blockage being only partial, so that an additional tap is necessary; and after 18 wk there are examples of intestinal blockage being confused with the normal disappearance of the intestinal enzymes from amniotic fluid, a development that occurs at 20–22 wk gestation concordant with the innervation of the anal sphincter muscle.

In case 133—and possibly case 55—it appears that there may have been a release of the intestinal obstruction in affected fetuses. At 18 wk there was apparent obstruction that at 19 wk had disappeared as judged by PCI and LAP. This is perhaps not surprising, since the results presented here indicate that the majority of affected fetuses show functional intestinal obstruction during the second trimester but only ~10% of affected fetuses have meconium ileus at birth (Park and Grand 1981; Muller et al. 1984*b*).

The results of the prospective study show a slight deviation from the expected 3:1 ratio of unaffected to affected fetuses, but this is not statistically significant ($\chi^2 = 2.13$, df = 1).

The analysis of amniotic-fluid intestinal enzymes for the prenatal diagnosis of cystic fibrosis is not 100% accurate. But with an overall accuracy of 96.5% it is probably acceptable. DNA probes for restriction fragment length–polymorphism analysis of linked polymorphisms in pregnancies with a 25% recurrence

risk for cystic fibrosis are now being tested and have shown great promise for the prenatal diagnosis of cystic fibrosis. This approach is, however, somewhat limited by the following: (1) a number of families are not fully informative; (2) a living affected proband or DNA sample must be available for study; (3) there are possible errors due to recombination; (4) some pregnancies present too late for the analysis to be performed; and (5) a good deal of time and labor (1.5–3 wk) is necessary for each case.

For the present, it seems desirable to perform amniotic-fluid enzyme studies and DNA studies concurrently (when this is practical) or individually, as the particular circumstances warrant. As long as we are dealing with linked DNA polymorphisms and not cystic fibrosis gene probes, each method provides independent confirmation of the other and thus enhances the accuracy of the diagnosis.

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