Cystic fibrosis screening in the newborn

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Robinson, P. G., and Elliott, R. B. (1976). *Archives of Disease in Childhood*, **51**, 301. **Cystic fibrosis screening in the newborn.** A new technique of measuring stool enzyme activity on dry specimens of faeces from newborn children at 4–5 days of age has detected 3 cases of cystic fibrosis in the first 6000 tests. No known cases of cystic fibrosis have been missed. Additionally, one case of pancreatic achylia of at least 4 months' duration has been detected. It is proposed that the detection of cystic fibrosis by this technique is sufficiently practical to be acceptable as a worthwhile newborn screening programme.

The screening test has been in use in Auckland for over a year and is now being set up in Hamilton, Wellington, and Dunedin (New Zealand), and Sydney (Australia).

Cystic fibrosis remains the commonest genetically determined disease of serious nature in children of Caucasian origin. In New Zealand it appears to affect about 1 in 3500 newborns, and accounts therefore for about 20 new cases per year in this country. If early treatment is helpful in preventing the often fatal complications of the disease (see below), it would be worthwhile establishing a reliable and cheap newborn detection programme. Previous attempts to diagnose the disease are either too expensive for screening (e.g. sweat salt concentrations) or lack specificity (gelatin digestion by stool). Recently meconium albumin concentration has been used (Prosser *et al.*, 1974). When 'dipstix' techniques are used (e.g. Prosser *et al.*, 1974) the results are open to observer error, and lack of quality control. The electrophoresis of proteins (Ryley *et al.*, 1974) requires special laboratory facilities and is not inexpensive. Our own recently described technique of specific stool enzyme assay (Robinson and Elliott, 1974) has been modified for a mass screening programme. The initial results of this programme are reported.

Material and methods

All patients born at two major maternity hospitals in Auckland (National Women's and St. Helen's) from August 1974 to June 1975 had a stool sample collected after day 4 on the specially prepared cards (see Fig.). These cards were forwarded approximately twice weekly to our laboratories. Retesting of all those showing a level of enzymes less than normal was carried

CYSTIC FIBROSIS SCREENING

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Name _____

D of B _____

Hospital _____

Hospital No. _____

Completely cover circle with faeces.
Fold paper on dotted line over
sample. Press firmly and staple
down.

FIG.—Faeces collection card (6×11 cm) made from blotting paper with plastic film stapled on the back.

out using the same technique. If the stool sample again showed less than normal concentrations of enzyme, a formal quantitative assay of the enzymes was carried out by a simple technique devised in our laboratory (Robinson, Smith, and Elliott, 1975). If still below normal values, a quantitative sweat test (see below) was performed.

A disc (approximately 6 mm in diameter) was punched from each faeces sample into an appropriately numbered hole in a dimple tray (corresponding to the last two digits of the serial number stamped on the card). Included in each set of tests were dimples containing 0.5 ml of standard enzyme (1 mg/ml) and the three working dilutions (1:20, 1:200, and 1:2000). A mixture of buffer, indicator, and substrate solutions was made (in the proportions of 6:2:1 for the trypsin assay) and pH adjusted to 8.2 with 0.1 mol/l NaOH. This was then added in 0.9 ml aliquots to each dimple. The trays were each briefly mixed on a vortex mixer and then incubated at 37°C until the weakest dilution of standard enzyme started to change colour (from purple to yellow). At this stage the colour in each of the holes was noted and the result entered in the book (+, change; ×, no change; and ±, little change).

A chymotrypsin assay was then done on those samples which showed little or no change in colour, with 5–10 randomly selected samples for controls and a set of standard enzyme solutions. The method was as for trypsin but different buffer and indicator solutions were used and double the quantity of substrate (ATEE) was added. Retest samples were then requested on those babies showing abnormal trypsin and chymotrypsin levels. Quantitative analyses were carried out using our previously reported method (Robinson *et al.*, 1976).

A cystic fibrosis screening card (Fig.) was completed and attached to the baby's chart at birth. On the fifth postnatal day a pea-size faecal sample was smeared on the circle on the card, which was folded, stapled down, and then forwarded to the screening laboratory.

If a specimen showed abnormal enzymes, a retest request with a screening card marked 'Retest' was sent to the nurse at the hospital where the baby was born, with details of name, date of birth, test date, and hospital number. Other details requested were gestational age, birthweight, the race of the parents, and any medication being given (pancreatic enzyme supplements interfere with the test). These details were sent to this laboratory by the nurse, who also forwarded the retest request, with the mother's address, to the District Nursing Division of the Auckland Extramural Hospital. The district nurse visited the mother and arranged for a repeat specimen to be put on the card which was then sent to the screening laboratory. Forms were forwarded through the hospital nurse to the district nurses who arranged collection and delivery of a 1–2 g sample of faeces. Where possible the sample was frozen soon after collection and delivery to the laboratory was usually within 24 hours.

Sweat testing. The apparatus and method of Gibson and Cooke (1959) was used. Sweat was

collected on prewashed 4.5 cm square Whatman No. 42 filter paper for 3 consecutive 10-minute periods and the sweat sodium concentration determined by atomic absorption spectroscopy. As pointed out by Schwarz and Thaysen (1956) the sweat sodium concentration is very dependent on sweat rate and so all our results are expressed as (Na⁺) in mEq/l at a given sweat rate in g/m² per min. Three periods of collection were used because, as stated by Schwarz and Thaysen (1956), results obtained at sweat rates of less than 5 g/m² per min may be anomalous and the results for the first collection period are atypical. Typical results obtained in our laboratory over a period of about 1 year are: cystic fibrosis patients (32) sodium 93.4 ± 18 mEq/l at sweat rate 6.2 ± 1.4 g/m² per min; control (15) sodium 12.4 mEq/l at sweat rate 6.2 ± 1.6 g/m² per min.

When a screening card arrived at the laboratory the following data were recorded in a book: surname, mother, date of birth, hospital number, and date of specimen. All samples were stamped with an automatic numerator (ENM Model 4513) and the serial number recorded to give a continuous record of the number of tests carried out. Retest samples were appropriately marked and the page ruled off when each batch of test was carried out. Columns for results of trypsin and chymotrypsin assays and comments were included.

All abnormal results were stored on a minicomputer system (Wang 2200B computer, 2216 CRT, 2217 Tap Cassette, 2222 keyboard, 2201 output writer) which kept a record of abnormal retests and quantitative stool assay results, as well as prepared form letters for retest requests and quantitative stool sample requests. Statistical data (number of tests, etc.) were also stored and automatically updated. Quantitative stool enzyme analyses, which were run as laboratory routine on material from many sources, were kept separately.

Trizma base (trishydroxymethylaminomethane, TRIS, Sigma T 1503), Trizma-HCl (TRIS-HCl, Sigma T 3253), p-tosyl-L-arginine methyl ester (TAME, Sigma T 4626), N-acetyl-L-tyrosine ethyl ester (ATEE, Sigma A 6751), sodium chloride and calcium chloride dihydrate (LR), bromothymol blue (Sigma B 0128), phenol red (Sigma P 4633), neutral red (George T Gurr Ltd. 22676), trypsin (Sigma T 0134), and chymotrypsin (Sigma C 4129), were obtained from the sources indicated.

Sample cards (Fig.) were prepared from blotting paper with polyethylene film (35 µm) stapled on as a backing. Tests were carried out in clear plastic, 96-hole dimple trays (Linbro Co., U.S.A.).

Trypsin. Buffer (pH 8.2, 0.005 mol/l) was prepared by dissolving TRIS-HCl (0.354 g), TRIS (0.334 g), sodium chloride (2.34 g), and calcium chloride dihydrate (2.94 g) in a litre of water. Substrate solution contained TAME (2.075 g) in 50 ml buffer. Indicator solution was made by mixing 5 ml 0.2% bromothymol blue and 5 ml 0.2% phenol red (both in 50% ethanol) with 50 ml buffer. Standard enzyme solution was 1 mg/ml buffer with working dilutions (prepared daily) of 1:20, 1:200, 1:2000 in buffer.

Chymotrypsin. Buffer (pH 7.8, 0.005 mol/l) was prepared by dissolving TRIS-HCl (0.532 g), TRIS (0.198 g), sodium chloride (2.925 g), and calcium chloride dihydrate (0.735 g) in a litre of water. Substrate solution was made by dissolving ATEE (0.452 g) in 25 ml methanol and making up to 50 ml with buffer. Indicator solution was prepared by mixing 5 ml 0.2% bromothymol blue and 5 ml 0.2% neutral red (both in 50% ethanol) with 50 ml buffer. Enzyme solutions were prepared as for trypsin.

Results

The results of the screening test to date are shown in Table I. Of the 6595 tests conducted, 3 cases of cystic fibrosis confirmed by sweat testing have been detected. One was the sib of a known case, but the laboratory processing was done by a technician unaware of this. A case of persistent (>4 months) pancreatic achylia (as shown by

TABLE I

Statistical data for screening test (as of 17 June 1975)

Total tests	6595
Retests requested	206 (3.1%)
Abnormal retests	40 (0.6%)
Normal retests	141
Retests not yet received	25
Confirmed cases of cystic fibrosis*	3†
Apparent incidence	1 in 2198

*Confirmed by further bulk stool samples and sweat testing.

†One case of persistent pancreatic achylia is also under investigation.

pancreozymin/secretin pancreatic function test) was also detected. This child had failed to thrive, but did not have the other symptoms commonly associated with pancreatic achylia. The progress of this case will be reported at a later date.

A false-positive rate of 3% was encountered on the first test, with a false-positive rate of 0.6% still apparent after the second screening test. To date we are not aware of any false-negative results. Samples of blank and test cards were punched and weighed. The results are shown in Table II.

TABLE II

Sample weights for screening test

Sample	No.	Mean weight ± SD (mg)	P
Blank paper	10	8.39 ± 0.38	$\left. \begin{array}{l} <0.01 \\ \text{NS} \end{array} \right\} <0.01$
Normals	47	13.93 ± 4.30	
Abnormals	14	16.80 ± 3.39	

Discussion

Our discussion must concern itself with three areas. Firstly, is the test worthwhile in terms of reliability and economy? Secondly, does it compare favourably with other screening tests? And thirdly, is it worthwhile in terms of the quality and quantity of life to detect such a disease in early infancy?

On the first count some objections could be raised to our high false-positive rate and the anxiety it may induce in parents. Our standards of normality are very high, being based on other workers' findings in older infants (Barbero *et al.*, 1966) and on the necessity of our research to define all results outside the 95% confidence limits as possibly abnormal. A false-positive rate of that same figure is usually acceptable in detecting galactosaemia—a disease with an occurrence rate in the newborn one order lower. Furthermore, it is not certain that all those with a 'false' positive test are free of symptoms. Our preliminary retrospective studies suggest that many of the children in this category do not thrive well initially. We are currently looking prospectively at this group.

Although it is said that about 10% of children with cystic fibrosis do not have pancreatic achylia (Shwachman *et al.*, 1956) and would thus not be detected by this programme, we suspect that this apparently normal pancreatic function may be a result of misleading measurements such as the stool proteolytic activity or nonlaboratory assessment of malabsorption. Even if the 10% rate is correct, this limitation in screening would have to be accepted for the recently described alternative test for meconium albumin (Prosser *et al.*, 1974). If a false-negative rate of about 10% should prove eventually to exist, this would imply about 1 in 35 000 births, or 0.005% of all births. Prosser's false-negative rate (8/20, 40%) is probably too high for routine use.

At the very worst, on both technical and theoretical grounds, the initial test has a false-positive rate of 3% and a false-negative rate of 10% (0.005% of all births). It is not entirely clear whether normal pancreatic function in the newborn period has survival value in cystic fibrosis or not. Pancreatic achylia is not however uniformly associated with a severe course of the illness (Swachman, Redmond, and Khaw, 1970).

Our second consideration, namely, is this test better than others?, must depend on reliability, convenience, and cost. A successful sweat test in the newborn would be ideal for reliability, but it is difficult to induce a high enough sweat rate to separate normal from abnormal. The sweat

test is also labour-intensive and quite impracticable for screening in all centres in New Zealand or in any other country with a rural area. Meconium albumin concentration has recently attracted some attention, particularly in the U.K. (Prosser *et al.*, 1974). When an individual 'dipstix' procedure is used it is not very clear that the many observers would always be able to detect subtle differences. Quality control cannot be invested in a large number of variably motivated observers, whereas quality control in a centralized biochemical newborn screening programme is readily obtained (Robinson and Elliott, 1974). Electrophoresis of protein in a central laboratory has been postulated as a way of overcoming these possibly uncontrolled meconium estimations. These assays however require expensive and committed apparatus with limited processing ability. Its specificity, reproducibility, and sensitivity has not yet been determined.

The third consideration is whether disease progress is altered by the early institution of treatment. The classical work of Shwachman *et al.* (1970) indicates that over a 20-year period the mean survival time has improved from approximately 1 year to 20 years of age in a group diagnosed early in life. The quality of life in many of the survivors is good. Our New Zealand survival figures over the 10 years contemporary with the latter period of the Shwachman study do not match these improvements (in preparation) and indicate that either treatment or time of diagnosis is not as good in New Zealand. A national screening programme may help to rectify this state of affairs.

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