Phenylketonuria

Mass Screening of Newborns in Ireland

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Phenylketonuria was, until recently, regarded as a very rare disease with an estimated incidence of between 1 in 18,000 and 1 in 40,000 (Centerwall, Berry, and Woolf, 1963). The results of mass-screening surveys of newborns in the United States showed that the incidence was approximately 1 in 10,000 (MacCready and Hussey, 1964), while a similar experience in Israel showed an incidence of 1 in 9000 (Cohen *et al.*, 1966).

The early detection of phenylketonuria, followed by dietary restriction of phenylalanine, provides the best hope of avoiding mental retardation in this condition. The introduction of a simple microbiological inhibition method for the estimation of blood phenylalanine has made it possible to carry out large-scale screening programmes on newborn infants (Guthrie and Susi, 1963).

The suitability of the Guthrie method was assessed in a pilot project in Dublin (Cahalane, 1964), and, subsequently, it was decided to make the method generally available, so that all newborn infants in the Republic of Ireland could be tested (*Lancet*, 1966). A description of the organization of this nation-wide testing programme, and of the results achieved in the first 16 months of its operation, is given in this report.

The State Department of Health decided on the establishment of the scheme and undertook the financial commitment. A central laboratory was established in the Pathology Department of the Children's Hospital, Temple Street, Dublin. Preliminary publicity was based on lectures and talks to medical societies and on announcements in appropriate medical journals. The press, radio, and television services co-operated in informing the general public.

Testing began on February 1, 1966, and those concerned with the newborn, i.e. paediatricians, obstetricians, public health authorities, hospital

authorities, family doctors, and nurses, were invited to participate. This participation was to be entirely voluntary and without remuneration to those collecting and submitting samples. It was free of charge to parents. The necessary materials for specimen collection were dispatched to all hospitals and individual practitioners through the co-operation of the county medical officers of health. Prepaid addressed envelopes for the return of samples were also supplied. A small staff consisting of one full-time laboratory technician and one part-time secretary under the direction of the hospital pathologist was responsible for the conduct of the entire programme.

Collection of Specimens

The blood was obtained by heel puncture with sterile disposable lancets and collected in the form of three dried spots on special filter paper. It was advised that collection be made between the ages of 3 and 7 days, and minimal descriptive data were written on the filter paper cards, which were returned by post to the Central Laboratory.

Laboratory Method

The Guthrie test is based on the fact that the inhibition of growth of *Bacillus subtilis* by β -2-thienylalanine is prevented by phenylalanine. Small discs punched from the blood samples are placed in rows on the surface of an agar medium to which has been added a suspension of *B. subtilis* spores in addition to β -2-thienylalanine. A series of standard phenylalanine concentrations in blood discs is included with each test-batch and, after overnight incubation, the size of growth surrounding the test disc is compared with the standards.

Specimens with a growth zone equivalent to or higher than the 4 mg./100 ml. standard were regarded as being presumptive positives, and the doctor or hospital concerned was immediately telephoned and requested to send repeat samples. Meanwhile, a one-dimensional chromatogram was run on an eluate of the initial blood spot using the method of Efron *et al.* (1964). If the

TABLE I

Cases of Phenylketonuria Detected Between February 1966 and May 1967

Case No.	Place of Birth	Domicile of Parents	Date of Birth	Age of Infant at Date of Sampling (days)	Approximate Levels of Phenylalanine (Guthrie) (mg./100 ml.)	Testing of Sibs		
						No.	Normal	PKU
1	Our Lady's Hospital Manorhamilton, Co. Leitrim	Co. Leitrim	1966 March 14	4	>20	2	1	1
2	Mount Carmel Hospital, Dublin	Co. Dublin	March 20	3	>20	5	3	2
3	St. Kevin's Hospital, Dublin	Co. Dublin	July 13	4	>20	4	2	2
4	Coombe Hospital, Dublin	Co. Dublin	November 8	4	>20	1	—	1
5	Our Lady of Lourdes International Training Hospital, Drogheda	Co. Louth	December 8	4	>20	2	2	—
6	Rock Hospital, Ballyshannon, Co. Donegal	Co. Donegal	1967 January 27	8	>20	1	1	—
7	Erinville Hospital, Cork	Co. Cork	February 11	3	10	—	—	—
8	County Hospital, Kilkenny	Co. Kilkenny	March 7	6	>50	3	2	1
9	Louth Hospital, Dundalk	Co. Louth	March 15	5	25	2	2	—
10	Rotunda Hospital, Dublin	Co. Dublin	March 22	5	12	2	2	—
11	Mount Carmel Hospital, Dublin	Co. Dublin	April 13	4	>30	1	1	—
12	District Hospital, Athlone, Co. Westmeath	Co. Westmeath	May 4	4	12	—	—	—
13	St. Vincent's Hospital, Tipperary	Co. Limerick	May 10	5	>20	4	4	—
14	Our Lady of Lourdes International Training Hospital, Drogheda	Co. Louth	May 25	4	>30	2	1	1

confirmatory tests were positive the physician-in-charge was notified so that treatment could be started without delay. At this stage it was suggested that serum and urine be sent for quantitative estimations of phenylalanine and other amino acids, and filter paper blood samples from parents and sibs were also requested. The treatment of the affected infant was then a matter of decision for the individual physician or hospital. Negative results were not reported on routinely unless specifically requested.

Results

During the 16-month period, February 1966 to May 1967, samples from 62,856 newborn infants were received. This represented approximately 72% of the total births for the entire country during that period. Samples from voluntary hospitals, local authority hospitals, private nursing homes, and domiciliary deliveries, were submitted. The distribution from the various sources was as follows: Local Authority hospitals and clinics, 22,958; voluntary hospitals, 21,153; private hospitals and nursing homes, 16,280; domiciliary deliveries, 2465, giving a total of 62,856.

An increased result equal to or greater than the 4 mg./100 ml. standard was obtained in 62 of the 62,856 samples. A persistent and significant

increase in blood phenylalanine was found in 14 cases (Table I); the remaining presumptive positive cases proved either normal on retesting (44 cases), or showed a raised serum tyrosine (4 cases), with a return to normal blood levels on follow-up.

There are 30 sibs of the 14 newly detected cases. Blood from these children was examined by the Guthrie technique and normal results were obtained in 21 of them. In each of 2 families (Cases 1 and 2) a hitherto unrecognized instance of classical phenylketonuria came to light. Increased blood phenylalanine levels were found in two sibs of Case 3, and both of these children were of normal intelligence. This family will be the subject of a report by Coffey and Moore. In 4 of the families (Cases 3, 4, 8, and 14), there was a previously diagnosed case of phenylketonuria.

In general the cases that were subsequently proved to be instances of phenylketonuria had a considerably higher initial blood phenylalanine level than did those that reverted to normal blood levels. This is not an absolute feature but, as is shown in Table II, no case of phenylketonuria had a blood level which was lower than 10 mg./100 ml. at the time of initial testing, and in 11 of the 14 cases it was greater than 20 mg./100 ml.

TABLE II

Diagnosis in 62 Cases where Blood Phenylalanine Level in Range 4-20 mg./100 ml.

	Approximate Phenylalanine Level (mg./100 ml.)						Total
	4	6	8	10	12	20	
No. of cases	21	11	8	6	4	12	62
Phenylketonuria . .	—	—	—	1	2	11	14
Tyrosinaemia	—	1	1	1	1	—	4

Discussion

Phenylketonuria was originally thought to lead inevitably to severe mental retardation, but it is now known that whereas the great majority of affected patients are mentally retarded, a small proportion have normal or near normal intelligence (Woolf *et al.*, 1961; Partington, 1962). Evidence has been presented that early administration of a low phenylalanine diet is effective in mitigating the mental deterioration seen in phenylketonuria (Knox, 1966). Therefore, early detection of the condition is essential (Centerwall *et al.*, 1963).

As a result of mass-screening of newborns for phenylketonuria, numbers of infants are now being treated on diets low in phenylalanine, and it is not possible to say what proportion of them might have developed normally without treatment. There is a very serious onus on physicians to investigate thoroughly each case detected by newborn screening methods, before subjecting the child and its parents to the difficulties and possible hazards of prolonged dietary restriction. Laboratory tests on blood and urine should be carried out without delay to confirm the presence of the abnormal metabolites that characterize the disease. It is important to realize that in some premature infants there is a delay in maturation of the phenylalanine hydroxylase system, and this may lead to quite high transient blood levels of phenylalanine in the neonatal period. Another cause of transient hyperphenylalaninaemia in the newborn is transfer of the amino acids across the placenta from a mother who, though normally intelligent, may be phenylketonuric (Mabry *et al.*, 1963). In fact, recent work tends to suggest that a positive screening test serves only as a signpost to what may be a number of phenotypically and genotypically distinct conditions (Anderson *et al.*, 1966; Schneider and Garrard, 1966; Auerbach *et al.*, 1966; Scriver, 1967).

The inference of this preliminary work is that phenylketonuria occurs much more frequently (at least in Ireland) than would have been expected,

since hyperphenylalaninaemia, which is neither transient nor maternally derived, has been found in 1 of every 4490 babies tested. The differential diagnosis principally devolves upon whether or not the infant is homozygous or heterozygous for phenylketonuria, and this can only be resolved by phenylalanine tolerance testing of parents and infant. Biopsy of liver, with assay of phenylalanine hydroxylase, is obviously an impractical step. It has not been possible to carry out loading tests in the cases detected in the present survey, but an otherwise complete examination of serum amino acids and urinary amino acids and phenols has been carried out before treatment has been started. Helpful information will be obtained by finding a raised serum phenylalanine in a sib of the index case, and for this reason Guthrie or other tests should automatically be carried out on all the sibs. There remains one further line of approach by which one may hope to avoid unnecessarily prolonging treatment, and that is to measure periodically the response of the serum phenylalanine to a temporary cessation of the low phenylalanine diet.

Since normal urinary metabolites may not be present in affected infants until several weeks of life have passed, it is important that any screening method for phenylketonuria in the newborn should employ blood as the medium for examination. The Guthrie technique, requiring as it does only small amounts of dried blood on filter paper, lends itself readily to the concept of mass screening. Furthermore, its ease of performance in the laboratory, its sensitivity, and its specificity make it an ideal technique. Scriver, Davies, and Cullen (1964) introduced a simple method by which a number of amino-acidopathies could be screened for from a small amount of whole blood collected in capillary tubes. A simple chromatographic technique using dried whole blood on a filter paper was described by Efron *et al.* Each of these techniques has the advantage of enabling recognition of more than one amino-acidopathy, but, unlike the Guthrie technique, their performance is somewhat complex. Fluorimetric determination of blood phenylalanine in small quantities of blood is a reasonably simple and reliable procedure (McCaman and Robins, 1962), and the adaptation of this technique to automation (Hill *et al.*, 1965) has provided a rapid procedure which is probably suitable for extensive screening.

It has been estimated that a single technician would be able to test at least 500 specimens per week (Guthrie and Whitney, 1964), but our experience has shown that an average of 1200 tests per week have been turned out with ease by one

technician, who has been able to carry out all the laboratory work involved in the survey, i.e. preparation, processing of samples, reading, and recording results, and carrying out chromatography on all specimens with raised values. Furthermore, a part-time secretary has been entirely responsible for comprehensive record-keeping, stock control, dispatch of collecting materials to doctors and centres throughout the country, and correspondence.

The logistics of the Guthrie test have been evaluated by two groups of workers (Partington and Sinnott, 1964; Cohen *et al.*, 1966). The cost was estimated at 10 cents (Canadian) per specimen by Partington and Sinnott, while Cohen and his colleagues estimated that it took 5–7 minutes to collect samples and write out the necessary details.

False positive and unduly raised results have not been a problem in the hands of most investigators (Cahalane, 1964; Partington and Sinnott, 1964; Baker *et al.*, 1964; Cohen *et al.*, 1966).

The prevalence of phenylketonuria in Ireland at present appears to be about 1 in 4500, but obviously such estimates are liable to change. It has been previously suggested that the frequency of the gene for phenylketonuria may be higher in people of Irish and other Celtic stock (Carter and Woolf, 1961).

Summary

A nation-wide screening of newborns for phenylketonuria has been in operation in the Republic of Ireland since February 1966. During the first 16 months of testing, an estimated 72% of the children born in the State have been tested during the first week of life, and 14 cases of phenylketonuria have been encountered, a frequency of 1 in 4490.*

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* Since submitting this paper for publication, a further 5 cases have been detected, making a total of 19, while the total number of tests has risen to 92,000, an incidence of 1 in 4842.