Papers and Originals

Present Status of Different Mass Screening Procedures for Phenylketonuria

Medical Research Council Working Party on Phenylketonuria*

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Summary: The Phenistix screening test for phenylketonuria in newborn infants, when used routinely at the recommended age of 4 to 6 weeks, passes as normal a substantial proportion—perhaps between a quarter and a half—of children with the disease, who are then diagnosed only after brain damage has occurred. Screening procedures based on three other tests—namely, the paper chromatography test for *o*-hydroxyphenylacetic acid in urine, the Guthrie test for phenylalanine in blood, and a modification of the Guthrie test for phenylalanine in urine—have been compared with each other and with the Phenistix test in a special field inquiry. Each of the three tests is more efficient than the Phenistix test for the detection of phenylketonuria at an early age.

A full appraisal indicates that the Guthrie test on blood would be the most satisfactory of the three tests to replace the Phenistix test as a screening procedure. Routine use of this would involve comprehensive arrangements for obtaining a single specimen of blood by heel-prick from every newborn infant at the age of 6 days or more, but preferably not later than 14 days, and for the rapid and economical processing of the specimens and skilled interpretation of the results at a few laboratory centres. These requirements have not proved difficult to meet in practice in the area where this procedure has been studied.

A positive screening test result does not signify a firm diagnosis of phenylketonuria, but is an indication for fuller investigation, most suitably at one of a small number of centres specializing in the management of phenylketonuria and other inborn errors of metabolism.

Introduction

The Medical Research Council's Working Party on Phenylketonuria has been studying the merits of different mass screening procedures for the early detection of phenylketonuria. This report contains a description of the principal tests for phenylketonuria which have been used on a substantial scale in Britain, together with a comparative review of their efficiency and of the administrative, technical, and practical problems involved in applying them. This review makes it clear that the screening procedure most commonly used in Britain at the present time, which is based on the Phenistix test on urine, is not sufficiently reliable for the detection of phenylketonuria, and that a procedure based on the Guthrie test on blood would be considerably more satisfactory. The relative merits of screening procedures for inborn errors of metabolism other than phenylketonuria have not been considered in this review.

The Disease

Phenylketonuria is characterized by an excessive level of phenylalanine in the serum, appearing within a few days of birth, and usually resulting, if left untreated, in mental retardation. The disorder is due to a block in the metabolism of phenylalanine to tyrosine. The frequency of occurrence of the disease in the general population in Britain is probably of the order of 1 in 10,000 births, but the precise determination of the frequency is difficult because it depends on completeness of ascertainment of the disease in large populations, and indeed there is evidence that the frequency differs in different population subgroups (Carter and Woolf, 1961). Because the condition is inherited as an autosomal recessive defect, the chance that another child will be affected in a family already having an affected child is one in four for each pregnancy.

Most methods for the direct determination of the serum phenylalanine level involve relatively lengthy and expensive laboratory procedures, and are therefore unsuitable for widespread use on newborn infants. Instead various relatively simple tests to indicate the presence of a relevant abnormality have been developed during recent years for use in mass screening procedures ; some detect a raised concentration of phenylalanine in the blood, one detects phenylalanine in the urine, and some detect, also in the urine, metabolites of phenylalanine rather than phenylalanine itself.

Four of these screening tests, which have been used on a substantial scale in various parts of Britain in recent years, are considered here: (1) the Phenistix test for phenylpyruvic acid in urine (Gibbs and Woolf, 1959 ; Boyd, 1961 ; Farquhar, Kansas, and Tait, 1962) ; (2) the paper chromatography test for *o*-hydroxyphenylacetic acid in urine (Armstrong, Shaw, and Robinson, 1955 ; Berry, Umbarger, and Sutherland, 1964) ; (3) the Guthrie test for phenylalanine in blood (Guthrie, 1961) ; and (4) a modification (E. G. Hall, unpublished) of the Guthrie test for phenylalanine in urine (Guthrie and Tieckelmann, 1960).

A number of other promising tests, which have not yet been used extensively in Britain, are referred to briefly at the end of the report.

Assessment of Screening Procedures

The purpose of all the screening procedures based on these tests is the same—namely, to enable abnormalities of phenylalanine metabolism to be detected at an early and presympto-

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matic stage. Experience suggests that in a patient in whom a screening test does not give a positive result the clinical diagnosis of phenylketonuria is likely to be delayed until the child is aged between 9 and 24 months, and possibly even longer. The purpose of the screening procedure is not to establish a diagnosis but to indicate which infants should have detailed diagnostic investigations: a positive test result means simply that further investigations are essential. Such investigations, which should be carried out in a centre specializing in the management of this and other disorders due to inborn errors of metabolism, may reveal no abnormality, they may reveal an abnormality other than phenylketonuria, or they may confirm a diagnosis of phenylketonuria.

It will be evident that the many outstanding problems in the diagnosis and treatment of phenylketonuria are irrelevant to an assessment of the relative merits of different screening procedures for the disease. None of these other problems therefore are considered in this review; the Medical Research Council is, however, at present considering methods of assessing the value of the dietary treatment of the condition.

It is possible to list certain desiderata for a screening procedure for phenylketonuria: (1) the procedure should not give negative results in children who have phenylketonuria; (2) it should be practicable, administratively and technically, to include all newborn infants in the screening procedure; (3) the procedure itself should be simple, practical, and economical both in money and in time; in particular, it should involve testing only a single specimen from the infant; (4) it should be possible to perform the test early in life without getting negative results from children with phenylketonuria, so that the diagnosis of phenylketonuria will be made before the occurrence of serious brain damage; (5) it would be an advantage if the specimen tested could also be used for screening tests for other inborn metabolic disorders; (6) the procedure should not give an unduly large number of positive results in children who have no metabolic disorder; and (7) the test should be suitable for central processing and interpretation, which, for consistency and reliability, should be the responsibility of a limited number of centres.

The way in which each of the four tests listed above and the screening procedures involving them have been applied in Britain are now described.

Phenistix Test

The Phenistix test uses absorbent paper strips, impregnated with buffered ferric ammonium sulphate and dried. If wetted with urine containing a sufficient concentration of phenylpyruvic acid the strip turns green. The strips may show different characteristic colour changes in the presence of various other substances, some of which may also be found in the urine of newborn children.

Following a conference arranged by the Medical Research Council in 1960, the Ministry of Health asked medical officers of health to consider undertaking, for the detection of phenylketonuria, routine screening of infants between 4 and 6 weeks of age, using the Phenistix test. By the end of May 1962 90% of the local health authorities in England and Wales had introduced routine screening procedures (Report, 1963); these now cover the whole of England and Wales (Ministry of Health, personal communication), though some of the current screening procedures are based on tests other than the Phenistix test.

In accordance with the Ministry of Health's recommendations the Phenistix test is usually made by a health visitor in the home or when the child is taken to a welfare centre, about four to six weeks after the birth. In some areas a first test is made at 10 to 14 days, followed by a second after four to six weeks. The aim is to wet the strip, either directly with urine or on a freshly wetted napkin; a further visit or visits may be needed to achieve this. The reaction is then assessed immediately by the health visitor.

The proportion of newborn infants who currently receive a Phenistix test in the routine schemes throughout the country is not known because the statistics refer to tests performed, not infants tested, and, as stated above, it is the policy in some areas to test each infant twice.

The Phenistix test will seldom be positive unless the phenylalanine level in the serum is above 15 mg./100 ml. (Armstrong and Low, 1957), but it may be negative with considerably higher levels. During the first two to three weeks of life the test is often negative in an affected child because the concentration of phenylpyruvic acid in the urine is not sufficiently high (Armstrong, Centerwall, Horner, Low, and Weil, 1961; Berry *et al.*, 1964). Since 1963 it has become clear that a considerable proportion of patients with phenylketonuria give no reaction to the Phenistix test in routine use even when this is made after the first weeks of life.

In 1964 the Working Party started a register of phenylketonurics in whom the diagnosis was made on or after 1 January 1964. This was at first confined to England and Wales; Scotland and Northern Ireland joined the scheme towards the end of 1965, and the register now contains information on more than 150 children with phenylketonuria. This register is probably not quite complete, and the information about some of the patients on the register is certainly not complete; however, it contains information about a total of 34 children with phenylketonuria who were born in England and Wales in 1964 or 1965 and who are known to have had a routine Phenistix test before the age of 3 months. In 23 of these patients the first (or only) test result was positive and led to a diagnosis of phenylketonuria, but in 11 (32%) the first test result was negative (and in six of these one or more later Phenistix test results were also negative). The diagnosis of phenylketonuria was made in 10 of these 11 patients only when they were aged between 8 and 23 months, by which time all 10 showed delayed development; the eleventh patient had a later Phenistix test which gave a very faint colour change, and a blood sample taken the next day showed a serum phenylalanine level of 47 mg./100 ml. Moreover, the frequency of negative Phenistix test results does not appear to be related closely to the age of the child when tested, at least within the first two months. Of the 13 patients first tested between eleven days and one month four were negative, and of the 18 patients first tested between one and two months seven were negative; the other three patients were first tested between two and three months, and all were positive.

Similar findings, on smaller numbers, have been reported elsewhere. A further 10 patients, mostly born in Scotland between 1960 and 1965, had a routine Phenistix test when aged under 3 months; in four the test was positive, in six negative (Stephenson and McBean, 1967). Seven patients born in Northern Ireland between 1960 and 1963 had a routine Phenistix test when aged from 3 to 6 weeks; in two the test was positive, in five negative (Carré, personal communication). Of nine patients in Australia reported by Pitt and Wilmott (1965) five were found negative on first being tested with the Phenistix test, and some of these gave negative results also on later occasions. If these four series are combined, 27 (45%) of 60 patients with phenylketonuria gave a negative result when first tested with the Phenistix test. In general, therefore, it would seem that a mass screening procedure based upon a single Phenistix test during the first three months of life would miss between a quarter and a half of the cases of phenylketonuria.

There are probably several reasons for these disappointing findings, such as variations in maturation of the relevant enzymes during the early weeks of life and shortcomings in the performance of the test. In particular, it is difficult to make sure that the strip has been applied to a freshly wetted napkin, and the multiplicity of testers militates against reliable interpretation of the results.

Paper Chromatography Test for o-Hydroxyphenylacetic Acid in Urine

This test has been developed, both in the laboratory and in its practical application in a mass screening procedure, by Dr. L. I. Woolf, at the Radcliffe Infirmary, Oxford (Woolf, 1967).

The test is made on filter paper which has been impregnated with urine and dried. The specimen is obtained by the mother, who is given a small supply of filter papers by the health visitor or welfare centre, with instructions on collecting the specimen, drying the paper, and writing identification particulars on it. The test paper or papers have in some areas been collected by a health visitor on a routine visit to the home and forwarded to the central processing laboratory; in others they have been posted by the mother to the local health authority and thence to the laboratory. The test has usually been performed, for administrative convenience, at an age of about 10 to 14 days.

The test paper is first checked visually, and if necessary chemically, for the presence of urine. Part of the test paper is then mounted on a standard filter paper sheet and a one-dimensional paper chromatographic test for urinary phenols (Armstrong, Shaw, and Wall, 1956) is run overnight. Ten test papers can be included on one standard sheet. If a suitable reagent is applied to the test paper the next day the appearance of a spot of distinctive colour and R_F value indicates the presence of an abnormal concentration of *o*-hydroxyphenylacetic acid in the urine. The paper chromatography test, as used, detects concentrations of *o*-hydroxyphenylacetic acid down to about 0.2 mg./100 ml. of urine. Negative or very weak positive results for other, normal, urinary components would provide an indication that another specimen was required.

This test has been used routinely in Oxfordshire, Cardiff, Bletchley, and Chesham since July 1965. By July 1967 more than 21,000 infants had been tested; the numbers tested in the first year represented 96.2% and those in the second year 98.7% of all live births in the area. The test was also introduced in Oxford City in January 1967. Apart from a few younger siblings of known patients with phenylketonuria, no child born in these areas during the period of use of this test is currently known to have the disease, whether routinely tested or not. The test has therefore not been used yet on a sufficient scale, or for long enough, in this area of low prevalence of the disease to assess reliably whether it misses patients with phenylketonuria, and, if so, how frequently.

With the urine chromatography test, about one non-phenylketonuric infant in 5,000 gave a weak positive reaction for o-hydroxyphenylacetic acid; in addition, eight infants per 1,000 showed tyrosyluria, necessitating examination of a second urine specimen for *o*-hydroxyphenylacetic acid.

The laboratory procedure outlined above also indicates the presence of abnormal concentrations of *p*-hydroxyphenyllactic acid in the urine (tyrosyluria and tyrosinosis) and of histidine in the urine (histidinaemia). Other portions of the same test paper may be used to test for conditions other than phenylketonuria, namely proteinuria, glucosuria, galactosaemia, cystinuria, and homocystinuria.

Guthrie Test on Blood

The practical application of this test in a screening procedure in Britain has been developed by Dr. J. S. Stevenson and Dr. J. Scott at Stobhill General Hospital, Glasgow. The test is made on filter paper which has been impregnated with several spots of blood obtained by heel-prick and dried. The specimen is obtained from infants delivered in hospital by the hospital medical or nursing staff; in the case of early discharges arrangements for testing have been made through the Public Health Department. The midwife, or exceptionally the general practitioner, obtains the specimen from infants delivered at home. In this country a single test has been performed at an age of 6 days or as soon as possible thereafter, the specimen then being forwarded to the central processing laboratory.

In the laboratory a small disc from one of the blood spots is incubated overnight in an assay dish containing spores of a strain of *Bacillus subtilis* and a substance which inhibits its growth, as well as a series of control discs impregnated with various concentrations of phenylalanine, which permits growth of *B. subtilis* in the presence of the inhibitor. One hundred test discs can be included on each assay dish. Comparison of the halo of growth of *B. subtilis* round the test disc with that round the control discs gives an assessment of the phenylalanine content of the test blood. The Guthrie test as used detects and estimates concentrations of phenylalanine down to about 2 mg./100 ml. of blood. A level of 6 mg./100 ml. or more is regarded as a result requiring fuller investigation. In Scotland in 1966–7 such levels were found in 162 out of 57,000 children tested (at an age of 5 days or more)—that is, in less than 3 per 1,000.

A series of surveys were made with this test in special schools, in mental subnormality institutions, and among the newborn in Glasgow between November 1964 and November 1965. Since then the test has been used routinely for all newborn infants in Glasgow, Edinburgh, and the surrounding counties; the area covered has expanded rapidly and now includes the whole of the South-east Scotland Region (Report, 1968). It is not known what proportion of live births in the area escape the Guthrie test on blood, but it is believed to be small. Between November 1965 and November 1967 more than 93,000 infants were tested, and 18 infants with phenylketonuria were found. No child in the area who is currently known to have the disease was missed by the Guthrie test on blood at an age of 5 days or more. Nearly all the patients with phenylketonuria came from West Scotland, where the prevalence of the disease is particularly high (Carter and Woolf, 1961).

The Guthrie test has been in use in parts of the United States since 1963, and about 2¼ million tests were made up to the end of 1965 (Guthrie, personal communication). The practice in the U.S.A. is for the infant to be tested before discharge from the maternity hospital (which in the U.S.A. is usually within the first three days of life), and many are tested again at an age of about 4 weeks. It has been estimated (Guthrie, personal communication) that at least 11% of infants with phenylketonuria would be missed in the U.S.A. if it were routine practice to make only the first of the two Guthrie tests. However, because the serum phenylalanine level in a phenylketonuric infant rises rapidly soon after birth (Hsia, 1967), a much smaller proportion of infants with phenylketonuria would be missed if the Guthrie test were performed as a routine towards the end of the first week of life. A very small number of cases have recently been reported in which this test gave a negative result when performed after the first week of life (Cahalane, personal communication).

Other blood spots in the specimen may be used to detect maple syrup urine disease, galactosaemia, and histidinaemia, but the appropriate techniques are at present more complicated than the corresponding techniques with the urine chromatography test.

Modification of Guthrie Test for Urine

A modification of the bacterial inhibition test for use with urine-impregnated paper was developed by Dr. F. P. Hudson and Dr. E. G. Hall at Alder Hey Children's Hospital, Liverpool, in 1964, because at the time there was reluctance to allow health visitors to collect blood specimens.

This test is made on filter paper which has been impregnated with urine and dried. The papers are distributed to mothers and the specimens collected in the same way as for the paper chromatography test, but at a minimum age of 3 weeks. In the

central processing laboratory all papers showing faecal contamination or giving a weak or negative result in a spot test for urea are discarded and another specimen is requested. A bacterial inhibition test similar to the Guthrie test on blood is then made, the standard discs having been cut from filter-paper of the same type as the specimen papers and impregnated in a napkin with concentrations of L-phenylalanine ranging from 8 to 20 mg./100 ml. A result of 10 mg./100 ml. or more for the test specimen, which is found in about 2.5% of tests, is regarded as a result requiring fuller investigation. A second specimen is requested if bacterial growth is totally inhibited by antibiotics in the urine.

This method of testing has been used in the Liverpool area since June 1964. By mid-1967 about 42,000 babies had been tested, and four cases of phenylketonuria had been detected; no patient with the disease is so far known to have been missed by this test.

The average proportion of live births tested has exceeded 80%, but in different districts and at different times the coverage has varied from 95% to 60%, the lower figures being associated with a shortage of health visitors.

Direct Comparison of the Four Tests

Early in 1966 a direct comparison of the above four screening tests was planned and started under the auspices of the Working Party. The aim was to obtain parallel specimens for all four tests from infants who were siblings of known phenylketonuric patients and from infants in whom a previous routine test had given a result which warranted further testing to confirm or exclude phenylketonuria. These specimens were to be taken before any dietary treatment was started. For the younger siblings of known patients several specimens were to be examined by each test at frequent intervals, starting if possible the day after birth. The Phenistix tests were to be made in the hospital, home, or welfare centre, and each of the other three tests in a laboratory which was experienced in the particular test. The paper chromatography tests on urine were all made by Dr. Woolf in Oxford, the Guthrie tests on blood by Drs. Stevenson and Scott in Glasgow, and the modified Guthrie test on urine by Drs. Hudson and Hall in Liverpool. The results of these tests were also compared with those of a direct determination of the phenylalanine level in serum (or plasma), a specimen of whole blood being taken for this purpose and tested by the hospital responsible for the care of the child.

So far a total of 21 children who are now known to have phenylketonuria have been investigated in this comparative study; eight were younger siblings of known patients and one was a first cousin, and 12 were patients in whom phenylketonuria was first suspected as a result of a routine test. The findings on each child before the start of dietary treatment are summarized in Table I, which shows the age in days when the last negative and the first positive results were obtained with each of the four tests. In the upper part of the Table, which refers to younger siblings of known patients (whose tests were usually started within a day or two of birth), the time of emergence of positive results to the various tests can be assessed fairly precisely for some of the children. Child 2, for example, became positive to the Phenistix test when aged 30 or 31 days, to the paper chromatography test and the modified Guthrie test on urine between 1 and 3 days, and to the Guthrie test on blood before the age of 3 days. Again, Child 3 became positive to the Phenistix test when aged 6 or 7 days, to the modified Guthrie test on urine between 3 and 5 days, and to the other two tests before the age of 3 days. It appears from these comparisons (coupled with the data for Children 4, 6, and 7) that the Phenistix test was on average the latest to become positive, the modified Guthrie test on urine and the paper chromatography test next, and the Guthrie test on blood probably the earliest.

It is important also to assess what the findings with each test would have been at different fixed intervals after birth. In making this evaluation it has been assumed that any test made previous to the last negative result would also have given a negative result, and that any test made (in the absence of treatment) after the first positive result would also have given a positive result; there is ample support for and no contradiction to these assumptions in the observed findings. Such an analysis was made at ages 5, 7, and 10 days, and 2, 3, and 4 weeks, and the results are shown in Table II. At 10 days,

TABLE I.—*Summary of Individual Findings with Four Screening Tests for Phenylketonuria Used in Parallel on 21 Children with the Disease (Before the Start of Dietary Treatment). A dash in the "Last Negative" Column Means that no Negative Result was Obtained with that Test Before the Start of Dietary Treatment; a Dash in the "First Positive" Column Means that no Positive Result was Obtained with that Test Before the Start of Dietary Treatment (or in the Next Two Days)*

Reference No.	Phenistix Test on Urine		Paper Chromatography Test on Urine		Modified Guthrie Test on Urine		Guthrie Test on Blood		Serum Phenylalanine Level		
	Age in Days at:		Age in Days at:		Age in Days at:		Age in Days at:		Age in Days at:		Level at First Positive Reading (mg./100 ml.)
	Last Negative Reading	First Positive Reading	Last Negative Reading	First Positive Reading	Last Negative Reading	First Positive Reading	Last Negative Reading	First Positive Reading	Last Negative Reading	First Positive Reading	
Younger Siblings of Known Patients											
1	7	8	—	9*	—	—	—	9*	—	5	45
2	30	31	1	3	1	3	—	3	—	9	> 20
3	6	7*	—	3	3	5	—	3	—	4	15
4	9	—	1	3	1	3	—	1	0†	3	25
5	—	9	—	11	—	11	—	10	—	10	33
6	9	—	3	5	3	7	—	3	—	9	15
7	16	18	4	6	4	6	—	0§	—	4	> 20
8	5	—	6	—	—	4	—	2	—	4	15
9‖	39	57	—	58*	—	51	—	57	—	57	67
Patients Detected by Routine Testing											
10	—	—	—	30	30	—	—	5‡	—	21	36
11	—	12	—	16	—	16*	—	5‡	—	14	62
12	—	—	—	5	—	5	—	5‡	—	—	—
13	—	19	—	21*	—	—	—	10‡	—	19	59
14	—	7	—	12	12	—	—	5‡	—	7	62
15	—	—	—	16	—	16	—	5‡	—	—	—
16	—	—	—	9	—	9	—	6‡	—	14*	66
17	—	9	—	9	—	9	—	4‡	—	9	76
18	—	—	—	8	—	—	—	6‡	—	—	—
19	—	—	—	28*	—	—	—	5‡	—	—	—
20	—	26‡	—	32*	—	28	—	32*	—	32*	62
21	—	12	—	10	—	10	—	4‡	—	11	45

* This was either one or two days after the start of dietary treatment; all other readings were obtained before the start of dietary treatment.
† 1·9 mg./100 ml. in cord blood.
‡ This was the routine test which led to the detection of the phenylketonuria.
§ On day of birth.
‖ A first cousin of a known patient.

for example, information on the Phenistix test result is available (according to the above criteria) for 8 of the 21 patients, and 3 of these 8 were judged to have been negative to the Phenistix test at that age. This may be compared with the data on the other tests at the same age—namely, 0 of 11 patients for the paper chromatography test on urine, 2 of 12 for the modified Guthrie test on urine, and 0 of 19 for the Guthrie test on blood.

It is clear from Table II that the Phenistix test on urine is not at all reliable during the first week or fortnight of life and is still not fully efficient at four weeks, and that the modified Guthrie test on urine may also give negative results as late as four weeks after birth. So far as this small inquiry goes, both the paper chromatography test on urine and the Guthrie test on blood were fully efficient from an age of 7 days onwards.

TABLE II.—*Assessment of Results of Four Screening Tests for Phenylketonuria at Ages of 5, 7, and 10 Days and 2, 3, and 4 Weeks*

Age when Tested (Days)	Phenistix Test on Urine		Paper Chromatography Test on Urine		Modified Guthrie Test on Urine		Guthrie Test on Blood	
	No. of Patients Tested*	No. giving Negative Result†	No. of Patients Tested	No. giving Negative Result	No. of Patients Tested	No. giving Negative Result	No. of Patients Tested	No. giving Negative Result
5	8	8	6	1	7	2	14	0
7	8	6	6	0	9	2	16	0
10	8	3	11	0	12	2	19	0
14	10	3	13	0	12	1	19	0
21	11	2	16	0	14	1	19	0
28	12	2	17	0	15	1	19	0

* Total of those patients known to have a positive result at the stated age or earlier and those known to have a negative result at the stated age or later.
† At the stated age or later.

It is instructive also to compare the findings with each test at the minimum age at which that test has been performed routinely in Britain. This has been taken to be 4 weeks for the Phenistix test on urine, 3 weeks for the modified Guthrie test on urine, 10 days for the paper chromatography test on urine, and 5 days for the Guthrie test on blood. At these respective times 2 out of 12 patients would have been negative to the Phenistix test, 1 out of 14 to the modified Guthrie test on urine, 0 out of 11 patients to the paper chromatography test on urine, and 0 out of 14 to the Guthrie test on blood. Thus at the minimum age of current routine use in Britain the paper chromatography test on urine and the Guthrie test on blood appeared to be fully efficient, whereas the other two tests showed lapses.

In addition to the findings reported in the Tables, parallel results with the four screening tests were also obtained for another group of 21 children, who are younger siblings of known patients but who were found *not* to have phenylketonuria themselves. Sixteen of these children consistently gave negative results with all the tests made, but five gave sporadic positive or "trace" readings—that is, below the level accepted as indicating a need for further investigation—in one or more of the tests. One of these five children was positive to the Guthrie test on blood at the age of 4, 8, and 12 days (and had trace readings at 2, 6, and 10 days); one was positive to the Guthrie test on urine at 7 and 9 days; one was positive to the Guthrie test on blood at the age of 1 day and to the modified Guthrie test on urine at 7 days; one who showed tyrosyluria consistently from 5 to 19 days by the paper chromatography test on urine was positive to the Guthrie test on blood at the age of 5 days (and had trace readings at 7, 9, 11, and 13 days); and one child had trace readings in the paper chromatography test on urine consistently from 2 to 20 days. The results of all the other tests made on these five children were negative.

In the assessment of these findings it must be remembered that these children are a special group, about two-thirds of whom will be heterozygotes for the phenylketonuria gene. The frequency of occasional positive readings in screening tests for phenylketonuria may be greater in such a group than in a general population group.

Relative Merits of Four Screening Procedures

The above test findings, and experience with the four procedures in this country, permit an assessment of the relative merits of the screening procedures. This assessment is made in relation to each of the seven desiderata for a screening test listed above.

Efficiency in Detecting Phenylketonuria

It has become increasingly evident during the past few years that the efficiency of the Phenistix test in detecting phenylketonuria is inadequate for a mass screening procedure. In a substantial proportion of phenylketonuric patients—perhaps between a quarter and a half—the Phenistix test, used routinely at about 4 to 6 weeks of age, gives a negative result and the child is regarded as normal, usually until obvious brain damage has taken place. One reason for this basic defect in the test is that there may not be a sufficiently raised level of phenylpyruvic acid in the urine of a phenylketonuric child to give a positive result during the early weeks of life. Practical difficulties in performing the test satisfactorily, and in reading the result, also contribute to the inefficiency of the test.

The Working Party's comparative study of the four screening tests has shown that in patients with phenylketonuria both the paper chromatography test on urine and the Guthrie test on blood are likely to be positive by the end of the first week of life, but it has confirmed that the Phenistix test may still give a negative result several weeks later. Because it is likely that a test which gives positive results from an early date will prove more efficient in practice than one which may only become positive later, these two tests may both be judged preferable to the Phenistix test as a basis for a screening procedure. The modified Guthrie test on urine appears to occupy an intermediate position.

No instance of a patient who was completely missed by any of these other three tests in routine screening practice in Britain has yet been reported. However, only the Guthrie test on blood has been used for long enough, and on a large enough scale, in an area with a high prevalence of phenylketonuria for it to be evident that its efficiency at an age of about 6 days, even if it eventually proves to be less than 100%, must be substantially greater than that of the Phenistix test.

Coverage of all Newborn Infants

Though numerical information is not available for two of the four screening procedures, it appears that a very high coverage of newborn infants can be attained with all of them, whether the method of collecting specimens is through hospitals and midwives or through mothers and health visitors. Some difficulties were experienced in the Liverpool area, however, during periods when there was a shortage of health visitors. In terms of coverage there is an inherent practical advantage in a screening procedure which is planned to take place at an early age, in that effective arrangements may (and should) be built into the procedure to ensure that infants who miss the screening test at the planned time are tested later, but before it is too late to prevent brain damage in those children with phenylketonuria.

Repeat Testing—Simplicity and Economy

There does not seem to be any advantage in a procedure involving repeat tests which would be commensurate with the

additional cost and effort. Because some patients repeatedly fail to give a positive result, patients missed by one Phenistix test may also be missed by a second (Woolf, Ounsted, Lee, Humphrey, Cheshire, and Steed, 1961 ; Mabry, Nelson, and Horner, 1962). Again, though it has been estimated (Guthrie, personal communication) that a single Guthrie test on blood at an age of about 3 days would miss at least 11% of patients, it would appear from the steep rise in the serum phenylalanine level in children with phenylketonuria during the first few days of life (Hsia, 1967), and from the Working Party's own findings, that to defer the Guthrie test on blood to an age of 6 days or more would effectively obviate the need to perform a second test. At present there is no reason to consider a procedure involving repetition of the paper chromatography test on urine, because there is no evidence so far that a single test at the age of routine performance (10 to 14 days) misses cases of the disease.

The simplest test procedure would appear to be that for the Phenistix test. However, it has several practical disadvantages, such as the difficulty of obtaining a satisfactory specimen on a first visit to the home, which necessitates a return visit by the health visitor. The multiplicity of persons reading the test results is a further disadvantage, and some health visitors do not like to have the responsibility of reading the test results themselves.

The paper chromatography test on urine has been found in the Oxford area to be more economical of the health visitors' time than is the Phenistix test. It requires central laboratory facilities, but it is relatively simple to perform large numbers of tests both quickly and cheaply and to read the results reliably.

The modified Guthrie test on urine involves the same administrative machinery as does the paper chromatography test. The laboratory procedure is rather more complex, but it is still simple to perform large numbers of tests quickly and cheaply, and to read the results reliably.

The Guthrie test on blood involves obtaining a blood specimen by heel-prick, and it requires central laboratory facilities ; the laboratory procedure is rather simpler than that for the Guthrie test on urine. Experience in Scotland has shown that large numbers of tests can be performed quickly, and the cost is not excessive.

It has been suggested that the Guthrie test on blood has the disadvantage, compared with the procedures based on urine specimens, that it involves a heel-prick for every infant, and that it might therefore be unacceptable either to parents or to the staff obtaining the specimens. The Working Party therefore wishes to emphasize that no difficulties of this nature have been experienced in the routine use of the Guthrie blood test in Scotland ; moreover, some of the staff concerned in the collection of urine specimens in other areas have expressed the view that it might be more expeditious to collect blood specimens instead.

Possibility of Early Performance of a Single Test

In order to avoid too many unreliable results the Phenistix test should not be performed routinely on specimens taken under the age of 4 weeks. There is less extensive evidence on which to base a recommendation for the timing of the other two urine tests, but the modified Guthrie test on urine should not be performed before the age of 3 weeks, and probably the paper chromatography test on urine should not be made before the age of 10 days (Berry *et al.*, 1964). In the comparative investigation the Guthrie test on blood was quite reliable from the age of 5 days, and it is recommended (Report, 1968) that the test should not be performed before the age of 6 days ; below the age of 4 days, as has been found in the United States, there is a risk that a phenylketonuric infant might be missed.

Because of the importance of early detection of phenylketonuria, a screening test which is reliable from an early age is to be preferred to one which becomes reliable only at a later age.

Screening Tests for Other Metabolic Disorders

The specimen for the paper chromatography test in urine, or for the modified Guthrie test in urine, lends itself more readily to screening for a variety of other metabolic disorders than does the specimen for the Guthrie test on blood. The Phenistix test (in the newborn) provides a screening test only for phenylketonuria.

Positive Results in Healthy Children

As indicated above, these occur to some extent with all the tests, and necessitate full investigation of a number of infants who ultimately prove to be healthy. However, this was not a major disadvantage with any of the four tests at their routine time of performance.

Processing and Interpretation in a Few Centres

In this respect the Phenistix test is at a disadvantage compared with the other three tests.

Other Possible Mass Screening Tests

A number of other approaches, all depending on the estimation of blood phenylalanine, have been made to the problem of devising a suitable screening test for phenylketonuria. With the exception mentioned below, none has yet been studied extensively in this country, but the potentialities of some or all of them are worth exploring and comparing with the Guthrie test on blood in future studies of mass screening techniques for phenylketonuria and other metabolic disorders. They are: (*a*) spectrophotofluorimetry of phenylalanine in blood (Hill, Summer, Pender, and Roszel, 1965) ; and (*b*) chromatography of amino-acids in blood (1) dried on filter paper (Efron, Young, Moser, and MacCready, 1964), (2) dried on filter paper or in heparinized capillary tubes (Scriver, Davies, and Cullen, 1964), and (3) in heparinized capillary tubes (Berry, 1962 ; Mellon and Stiven, 1966).

After the Working Party's comparison of the four screening tests had begun a pilot study of one of the above screening procedures was started in the Manchester area by Dr. G. M. Komrower, based on chromatography of a blood specimen (Scriver *et al.*, 1964). The aim was to include all babies born in Salford and in Administrative Health Division 15 (Lancashire). A blood specimen was collected by a health visitor on the first visit to the home following delivery of the child ; this was, on average, at the age of 12 days. The blood was collected in heparinized capillary tubes and sent, appropriately labelled, to collecting points and thence to the research laboratory in the Royal Manchester Children's Hospital. A total of 9,586 babies were available for testing, and specimens were obtained from 93% of them. It was necessary to repeat the test in 801 babies (8.4%) for technical and administrative reasons. Two cases of histidinaemia and one case of prolinaemia were detected, and there were many examples of transient tyrosinaemia (101) and several of transient phenylalaninaemia (28) and methioninaemia (31).

It was found, when known cases of phenylketonuria on dietary treatment were monitored, that serum phenylalanine levels of 4 mg./100 ml. or more may be detected and measured with this test. The method thus appears to be sensitive and reliable, but it was not used in the Manchester area in the first week of life. In Montreal, Scriver has employed it in

parallel with the Guthrie test and has detected phenylketonuria with it at the age of 4 days. It has the merit that it also covers conditions other than phenylketonuria.

Discussion

From the above considerations it is clear that, despite its simplicity of performance, the Phenistix test can no longer be regarded as a satisfactory basis for a screening procedure for phenylketonuria. The overriding reason is that the test passes as normal a substantial proportion of patients with the disease when it is used routinely between 4 and 6 weeks of age, and at an earlier age it would be even less reliable.

Of the other three tests considered, all of which have been used in routine screening procedures in this country, though for a relatively short period of time, the modified Guthrie test on urine would appear to have no special advantages over the other two, and may be rather less efficient in detecting phenylketonuria at an early age. Because it is a test on urine the results are likely to be more variable than are those of the Guthrie test on blood, and the laboratory procedure is rather more complex than that for the paper chromatography test.

Both the paper chromatography test on urine and the Guthrie test on blood are substantially more reliable than is the Phenistix test, and both involve setting up comprehensive field arrangements and streamlined central laboratory facilities. The Guthrie test on blood appears to be highly efficient in practice, but because smaller numbers of subjects have so far been tested there is less information on the efficiency of the urine chromatography test ; both tests probably have very occasional lapses. The Guthrie test on blood therefore has to be preferred, because there is more assurance of its high efficiency as a result of its wider use. It also has the advantage of being a direct test for the presence of excess phenylalanine in the blood. Moreover, it has been shown to be practicable to collect specimens for the Guthrie test on blood at an age of about 1 week, whereas the paper chromatography test has generally been carried out later—namely, at about 2 weeks ; because it is not known how early brain damage begins to take place in a phenylketonuric child there is an advantage in detecting the disease as early as possible. It appears to be no more difficult to obtain blood specimens from all infants than to obtain urine specimens.

When there is a general change in this country away from a screening procedure based on the Phenistix test, as would now appear to be desirable, the most satisfactory screening procedure to change to would be one based on the Guthrie test on blood. It seems unlikely that a screening procedure based on this test would require any radical alteration for at least five years and possibly much longer. There may well be developments in the relevant laboratory techniques, but this should not affect the administrative machinery for collecting the specimens, because there seems little doubt that a test on blood requiring central laboratory facilities would still form the basis of any more satisfactory screening procedure that might be evolved.

The introduction of the Guthrie test on blood throughout Britain should not, however, be a deterrent to the continued use and further study of other screening tests. In particular, it would be valuable for the paper chromatography test on urine to be used in parallel with the Guthrie test on blood in certain areas, in order that more experience might be gained with this method.

At the present time the importance of screening for phenylketonuria depends largely on the value of dietary treatment. Now that the treatment has been in use for several years it should be possible to make some tentative evaluation of it, and the Medical Research Council is considering the ways in which this might best be approached.

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