PAPERS AND ORIGINALS

Screening for Inherited Metabolic Disease by Plasma Chromatography (Scriver) in a Large City

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Summary

Before introducing a more comprehensive screen such as plasma chromatography, with its potential to detect 20 amino-acid disorders—an advantage over screening methods which detect only phenylketonuria—the greatly increased problems of organization and the effect on the community, midwives, paediatric services, and laboratory should be considered. The three years' experience in Birmingham showed a three-fold increase in cases detected and suggests criteria for further investigation and treatment.

Introduction

The discovery by Bickel, Gerrard, and Hickmans (1953) at the Birmingham Children's Hospital that the severe degree of mental subnormality associated with phenylketonuria could be prevented by dietary treatment during the first three months of life made early detection of this condition necessary. On evidence from the Medical Research Council Working Party on Phenylketonuria (1968) the Phenistix method of testing has been replaced by the microbiological test for phenylalanine in blood (Guthrie and Susi, 1963). Alternative procedures designed to detect amino-acid disorders other than phenylketonuria include that for *o*-hydroxyphenylacetic acid in urine (Woolf, 1967), chromatography of whole blood (Efron *et al.*, 1964), and another in which blood is collected in heparinized capillary tubes and the plasma subjected to one-dimensional paper chromatography (Scriver *et al.*, 1964). This last method has been tried on a small part (since extended) of the population of Manchester yielding some 5,000 births a year since 1965 (Komrower *et al.*, 1968).

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In Birmingham the Scriver screen was initiated in July 1968 and by March 1969 covered the whole city (population one million, 20,000 births a year). This paper describes organization in the city and in the laboratory, discusses the problems that arose and their solution, assesses the effect on local paediatric outpatient and inpatient facilities, defines the requirements to be met before such comprehensive screening is introduced into other areas (both compact urban communities and more widespread rural populations), reports the results in terms of new cases of metabolic disease discovered, and attempts to evaluate the cost of such a programme so that it may be compared with its alternatives.

Collection of Blood Sample

AGE OF TESTING

The Medical Research Council Working Party reported that specimens of phenylketonuric blood collected not later than the fifth day might still give a fallaciously normal result. Testing was therefore delayed until the sixth to ninth days of life. The age is checked in the laboratory and the number of samples rejected because they were collected too soon diminished as the experience of the collectors increased.

COLLECTION OF CAPILLARY BLOOD

Midwives are provided with heparinized capillary tubes of uniform bore (Red Tip), tablets of sealing compound (Cristaseal), and plastic carrier tubes 8.7 by 1.3 cm fitted with plastic stoppers. These are labelled on the outside with the address of the laboratory only so that they may be used repeatedly until they are blood-stained, when they are discarded. The patient's heel is cleaned with disposable alcohol-saturated swabs (Medi-swabs). Unless the solvent evaporates before puncturing the skin haemolysis occurs, making it necessary to repeat the test.

Two glass capillaries are filled with blood and sealed at one end. Blood does not escape from the open end and this avoids the need to cut off one sealed end in the laboratory. The two capillaries are placed in the carrier tube together with the detached head of a form giving details of the patient. This is folded so that the surname can be read without opening the

tube and acts as a label which cannot be separated from the sample and supports the capillaries during transport.

Each specimen is associated with only one administrative record, which has been designed to minimize clerical work. It comprises a form 21 by 15 cm with a 5-cm detachable strip on the top for recording surname, home address, date and time of birth (time is necessary in multiple pregnancies), sex and maternity hospital. On the remainder of the form is recorded the family practitioner and his address, the mother's surname and forename (forename of infant may not be decided at the time of testing), home address, date and time of birth, sex, maternity hospital, date of specimen and, for premature infants only, the weight and the weeks of prematurity.

Most of the collections are made by midwives on domiciliary service and the remainder come from two large maternity hospitals. In the city 12 collecting centres have been designated to which the specimens are taken by the midwives during the morning. A van collects the box of specimens from each centre and from the two hospitals at about noon, leaving an empty box and any further supplies of capillaries or other materials requested by the centre. The total collection is delivered to the laboratory by 2 p.m. for analysis during the afternoon.

Special arrangements need to be made for infants transferred for medical reasons to hospitals not participating in the scheme and who are likely to remain there for several weeks or months; otherwise babies who escape examination are pursued by either midwives or health visitors responsible for the home area. Early discharge of a mother from hospital (before the designated time of testing her baby) is often followed by a succession of changes of address, as she often resides temporarily with relatives before returning to her own home. Thus ensuring that all infants are examined has called for more effort than was initially expected. The importance of pursuing any infants known to have escaped testing is emphasized by two patients in whom diagnoses were ultimately made—a case of tyrosinosis and one of phenylketonuria. These patients were first tested as late as 9 weeks and 3 months respectively.

Analytical Procedure

The technique of chromatography adopted is exactly as described by Scriver *et al.* (1964). The work area in the laboratory is an office desk 120 by 70 cm with two drawers at one side to accommodate carrier tubes and other materials (Fig. 1). On top is a microhaematocrit centrifuge, a 144-place rack for carrier tubes, space for chromatography papers, a hair dryer, a tray to receive the specimens for the day, and a rack for the accompanying forms. The tubes are handled in batches of 12 as there

FIG. 1—Layout of work desk showing centrifuge, specimen rack, form rack, chromatography rack, record file and index card box for abnormal specimens, and tray of tubes and forms as received. The chromatography tank is in the background.

are 24 numbered places in the head of the centrifuge and the specimen rack is made up of ranks of 12. Such a system is important for specimen identification, as the individual capillaries are not labelled and their position in the centrifuge must be recorded on the forms and the sequence must follow the order of the carrier tubes (which still contain the folded name strips) in the tube rack.

The capillaries, the plugged end covered by a small rubber cap (Critocap) placed against the outer rim, are centrifuged and a cut is made at the junction of the separated cells and plasma. A length of plasma (hence the importance of uniform-bore capillaries), equivalent to 10 μ l, from each of 12 specimens is loaded on to a 25-cm square of Whatman 3 MM paper 2.5 cm from one edge. The capillaries containing residual plasma are plugged and returned to their carrier tube. They are kept at 4°C overnight and those found to be normal are then discarded. Specimens subsequently found to be abnormal are taped to the back of a card bearing details of the abnormality and kept at -20°C. They are then available for further study and to compare with subsequent specimens.

The papers are chromatographed in butanol:acetic acid:water (120:30:50) for 16 hours overnight, dried at room temperature with a fan for one hour, and dipped in ninhydrin stain (2.5 g ninhydrin, 0.1 g isatin, 10 ml lutidine, 1 l. acetone) and developed at room temperature for 30 minutes followed by 2 minutes at 105°C. After examining the papers thus stained (Fig. 2) and noting any abnormality on the appropriate form a

FIG. 2—Typical Scriver chromatogram. The only "abnormalities" are a few cases of moderate tyrosinaemia.

cut is made between the histidine and glutamine spots and the lower part of the paper is stained in diazotized sulphanilic acid (Pauly's stain) and the upper part in Ehrlich's reagent (1 ml dimethylaminobenzaldehyde in concentrated hydrochloric acid, 10 g/100 ml, 4 ml acetone). This allows a better assessment of histidine and may show proline and citrulline if they are present. Any further abnormalities found by this staining are recorded, the whole procedure being completed before noon, 24 hours after the specimen is received.

Reporting Results

It has been found convenient to operate the scheme on five days each week, spreading the tests due during the weekend and

bank holidays over the first two days of the following week. The longest period without testing has been four days. Each day the laboratory reports to the midwives supervisor's office the name and date of birth of patients with normal results. The home address is given only where there may be confusion, as in the case of names such as Begum, Bi, Jan, and Kaur. The office is also sent a list (in greater detail) of those with abnormal findings together with a recommendation for further action, which depends on the nature of the abnormality.

In the midwives supervisor's office a clerk first notifies the appropriate midwife by telephone of the repeat tests required and the date when these should be done. Each is recorded in a day book and cancelled when the result of the repeat test is reported; at the same time the health centre is informed if the test has become normal. Birth notification lists, received daily from the medical officer of health's statistical department, are used to record the results of all the tests and the lists are checked weekly to see that no baby is missed. The results of tests on babies born and tested within the city but who have returned to their home outside the city are reported to the appropriate medical officer of health.

Types of Abnormality and Recommended Follow-up

Few abnormalities on the chromatogram indicate a specific diagnosis (phenylalanine, histidine, proline, and the creamy plasma of hyperlipidaemia) and most require further capillary specimens to be taken. Tests cannot be repeated more than once without inducing an unacceptable degree of parental anxiety, and even if the abnormality is thought unlikely to be significant the third and subsequent tests are combined with an outpatient visit, where the problem can be explained to the parents. Only when the first test shows hyperphenylalaninaemia is this procedure departed from.

PHENYLALANINE

Phenylalanine can be detected with the same sensitivity as with the Guthrie test, 2 mg/100 ml. Normally none is seen, and in cases of phenylketonuria there was no doubt in the first test that the concentration was abnormally high. Less striking increases, associated with variant hyperphenylalaninaemia (Anderson *et al.*, 1966; Schneider and Garrard, 1966; O'Flynn *et al.*, 1967; Menkes and Holtzman, 1970), have been seen in only one case (Case 2, Table I). When the concentration of phenylalanine is suggestive of phenylketonuria arrangements are made by telephone with the family practitioner for quantitative studies to be made and the patient is normally referred to a paediatrician within 24 hours.

HISTIDINE

Definite though moderate increases in histidine usually indicate histidinaemia. When confirmed by quantitative determination sweat is examined for urocanic acid, which is absent in histidinaemia. These patients are also referred, though less urgently, to a paediatrician.

PROLINE

Increase of proline alone indicates a specific disorder, prolineaemia. There are the two types, due to deficiency of either proline oxidase or pyrroline 5-carboxylic acid dehydrogenase activity. At present there is insufficient evidence that neonatal prolineaemia is necessarily associated with any clinical abnormality, but the cases reported in older subjects suggest that this may be so, and those detected by screening are being examined at regular intervals of about three months for signs of renal impairment

or deafness, the latter by auditory-evoked encephalographic response.

HYPERLIPIDAEMIA

The one case of hyperlipidaemia discovered by the creamy nature of the plasma, which contained 14.5 g total lipid/100 ml, was admitted promptly for further investigation and dietary treatment.

TYROSINAEMIA

This common abnormality is usually transient. It has been suggested that administration of 100 mg of ascorbic acid will correct transient tyrosinaemia within 24 hours but will not affect that due to the more serious tyrosinosis (Light *et al.*, 1965). This was tested by repeating the procedure on infants showing tyrosinaemia (27 full term, 8 premature) and immediately giving 100 mg of ascorbic acid in a small quantity of milk feed and repeating the test 24 hours later. Only two full-term and two premature infants still had tyrosinaemia before ascorbic acid. After ascorbic acid one in each group showed only a slight reduction in the amount of tyrosine and the other two were unchanged. This indicates that except in the rare presence of a deficiency of ascorbic acid any apparent effect of ascorbic acid on tyrosinaemia is more a result of the time that has elapsed than of any effect of the vitamin. This is in accord with the observations of Bloxam *et al.* (1960) and Avery *et al.* (1967). At first infants showing tyrosinaemia were examined at frequent intervals, and from these results it has been possible to determine the rate at which the abnormality disappears. Ninety-five per cent. of cases, whether premature or full term, become normal during the first 6 weeks of life (Fig. 3), and so provided increased tyrosinaemia is the only abnormality present at the first time of testing the infant is not retested before this age.

FIG. 3—Proportion of cases of neonatal tyrosinaemia becoming normal at different ages. Lower bars in diagram are calculated from 18 subjects tested at various and irregular intervals. Upper bars are from 339 subjects tested at intervals not greater than two weeks. The latter are thought to reflect the true rate of change more closely.

The one patient found with tyrosinosis was born 12 weeks prematurely. At 9 weeks the first test showed methioninaemia, which was confirmed one week later. Before this was investigated further she was admitted to another hospital as an ill child (with respiratory symptoms in no way suggestive of metabolic disease), where severe tyrosinuria was found. She died aged 3 months. The dilated renal tubules and fatty liver found at necropsy resembled those seen in tyrosinosis.

A more direct tyrosine problem occurred in a boy with tyrosinaemia which persisted for at least five months. Urine contained *p*-hydroxyphenylacetic and *p*-hydroxyphenyllactic acids and the ferric chloride test was positive at first but later became negative. The abnormality was corrected by a low

phenylalanine diet. This was progressively withdrawn, plasma levels remaining normal. The child, however, was mentally subnormal and had poor muscle tone. This suggested a diagnosis of tyrosine transaminase deficiency.

METHIONINE

Neonatal methioninaemia has been studied especially to evaluate its pathological significance. The most common cause is a high protein intake, but there are several other important considerations (Raine, 1971). Although the study is incomplete six out of 37 infants required hospital admission, two with rickets, one with hepatomegaly, two with anaemia, and the case of tyrosinosis referred to above. Many infants after going through a transient tyrosinaemia in the first three weeks proceed to a transient methioninaemia in the next three. These findings will be reported separately, but meanwhile we believe that infants showing methioninaemia should be followed until this has disappeared and all should have their urine examined for homocystine. Homocystinuria was not detected in the present study.

BRANCHED-CHAIN AMINO-ACIDS

Maple syrup urine disease has not been encountered on neonatal screening and the criteria for prompt action are still uncertain. Increases are usually associated with recent feeding, and the midwife merely checks that the baby is clinically well.

OTHER ABNORMALITIES

Occasionally certain other abnormalities are found to persist on the second test. These include increases in two or more amino-acids or of the whole pattern. Such infants attend the

special metabolic clinic as outpatients for clinical examination and further investigation of blood and urine. Almost always by this time the abnormality has disappeared.

QUANTITATIVE AMINO-ACID ANALYSIS

This is essential for confirming a diagnosis and for future follow-up. If the disorder can be treated dietetically or otherwise progress is followed by quantitative amino-acid levels. The Technicon Sequential Multisample Amino Acid Analyzer with a short programme is used when only phenylalanine and tyrosine are required (Cooke and Raine, 1970). The analyser may also be useful in the identification of unknown ninhydrin-positive substances occasionally found (Raine and Cooke, 1971).

Results of Screening Programme

SPECIFIC DIAGNOSES

The diagnoses made during the three years that the scheme has been applied to the whole city are given in Table I. Those made during the pilot study are not included nor are those made in patients referred to the Children's Hospital from areas outside that being screened by the present system. So far no child considered normal when tested has been referred with a disease that the screen was designed to detect. The figures can therefore be directly related to a given population and to the neonatal period, and something close to the true incidence can be calculated for these various abnormalities. This has not been possible from several previous reports of neonatal screening programmes (Turner and Brown, 1967; Levy *et al.*, 1968; Clow *et al.*, 1969; Applegarth *et al.*, 1970). The incidence of phenylketonuria was about that expected and these cases would have been detected by the Guthrie test. Although it is claimed that five different Guthrie tests will detect up to 16 diseases,

TABLE I—Specific Diagnoses made during Three Years of Testing All Neonates in the City of Birmingham

Diagnosis	Case No.	First Suspected		Age (days) when Diagnosis Confirmed	Age when Still Accepted
		Date	Age in Days		
Phenylketonuria	1	8/7/69	6	19	2½ years
	3	23/12/69	6	7	2 years
	2	6/3/70	18	23	1½ years
	4	8/6/71	8	9	6 months
	5	28/2/72	9	11	
Histidinaemia	6	14/10/69	6	22	Sweat test negative 67 days
	7	17/12/69	5	79	2 years
	8	11/5/70	18	76	1½ years
	9	16/7/70	8	36	1½ years
	10	24/8/71	9	38	4 months
Prolinaemia (type 1) ..	11	7/5/69	37	42	8 months
	12	20/4/70	7	38	7 months
	13	4/5/70	8	109	Normal at 1 year
	14	12/11/70	41	77	104 days (normal at 1½ years)
	15	13/7/71	10	27	5 months
Tyrosinosis	16	31/8/71	41	80	4 months
Tyrosine transaminase deficiency	17	6/3/70	65	94	Died 95 days
Hyperlipidaemia	18	26/3/70	29	93	5 months
	19	1/9/70	9	17	1½ years

TABLE II—Reasons for Making Further Test After Initial One

	Half-year Period						Total
	1	2	3	4	5	6	
No. of babies tested	9,536	9,150	9,965	9,056	9,295	8,713	55,715
No. requiring further tests	510	652	571	669	581	617	3,600 (6.5% of babies tested)
No. with abnormal patterns	364	479	468	574	472	539	2,896 (80.4% of further tests)
Tyrosine	284	303	280	299	191	168	1,525 (52.7% of abnormal patterns)
Histidine	37	63	18	31	52	54	255
Phenylalanine	13	22	4	9	28	25	101
Methionine	8	17	26	41	72	68	232
Proline	1	6	14	15	10	24	70
Branched chain	2	7	7	61	44	67	188
Glycine	5	0	1	0	0	0	6
Others	14	61	118*	118†	75	133	519
No. of technical failures	146	173	103	95	109	78	704 (19.6% of further tests)
Laboratory failure	31	41	42	11	18	11	154
Haemolysis	68	104	55	64	81	59	431
Unsatisfactory specimen (including age) ..	47 (11)	28 (4)	6 (2)	20 (4)	10 (4)	8 (4)	119 (29)

*Homocitrulline in 39 cases.

†Homocitrulline in nine cases.

the single test usually applied would not have recognized the two diseases which appear to be as common as phenylketonuria—prolinaemia and histidinaemia—and the three rarer diseases would have been discovered late or not at all.

Table I shows that the delay in confirming a suspected diagnosis can be considerable, and it is again emphasized that the significance of a particular and apparently specific chemical abnormality in the neonatal period is not always well established. Histidinaemia not supported by the absence of urocanic acid from the sweat requires further study, and the two subjects whose prolinaemia disappeared at about 1 year of age show that continuous review is necessary at least for the first two years of life.

REASONS FOR FURTHER TESTING

After the initial test a further test may be required either for technical reasons or because of an abnormal chromatographic pattern. These are further analysed in Table II, where the figures are given for periods of six months.

When tested at 6-9 days of age 6.5% of the infants required more than one test, and of these further tests 42.4% were occasioned by tyrosine. This proportion is higher than that reported by Komrower *et al.* (1968) and is believed to reflect the earlier age for the initial test. Repeat tests for other abnormalities are more rare and usually do not fluctuate. The two exceptions are for branched-chain amino-acids and the other abnormal patterns in the fourth and third half-year periods respectively. The first followed the admission to the hospital of a 7-month-old retarded child with maple syrup urine disease (who had not been screened at birth by this method), after which the observers became oversensitive to this abnormality. The second coincided with the inclusion in the survey of a maternity unit which regularly used tinned evaporated milk for feeding, which we now know contains homocitrulline formed during the preparation of the milk (Gerritsen *et al.*, 1961; Gerritsen *et al.*, 1963) and which gives a pink spot when the chromatogram is overstained with Ehrlich's reagent. For some time we were not aware of this explanation and continued to investigate these infants who all became "normal" when discharged to their homes.

The need to repeat tests for technical reasons was small (1.3% of infants) and hopefully will decrease. The number of specimens collected at the wrong age fell noticeably after the first half-year and emphasizes that the introduction of such a screening programme should be accompanied by opportunities for instruction and discussion with all the midwives involved.

Effects of Delivering Specimens by Post

The scheme is operated in a compact urban community and all specimens are examined the day they are collected. This would

not be possible in a rural community, and the possibility of conducting the scheme with the ordinary postal system was examined. For this purpose four capillary tubes of blood were taken from 115 babies. Two were sent to the laboratory the same day by the usual van and the other two were posted in their carrier tube, several being sent together in a standard cardboard specimen box. Tests were done over several months during extremes of the British climate.

Various difficulties were expected with the posted specimens, including haemolysis due to cold or excessive delay, broken tubes, and more variable patterns due to haemolysis or decomposition. The effects were evaluated in terms of whether the results of the fresh and posted specimens from the same infant differed in any way and in terms of the frequency with which the test on this initial specimen, delivered by the two methods, led to a request for a further test for any reason.

In 37% of posted specimens glutamine had converted to glutamic acid, making this spot much stronger, but once this difference was accepted as normal no other difference in pattern was seen between the two specimens. Repeat tests were called for in two babies for tyrosinaemia, the abnormality being recognized in both fresh and posted specimens. Specimens from two babies were haemolysed and had to be repeated. One of these was a posted specimen but the other was fresh; in each case the alternative specimen was satisfactory. Thus when a slight change in the normal pattern is allowed for delivery of specimens by post appears to be an acceptable alternative to organizing a more immediate delivery system.

Collection of Specimens and Time of Feed

Any reduction in the number of tests repeated for reasons unrelated to disease would be welcome and it was thought that some apparent abnormalities might be due to variations associated with feeding. Although collecting fasting specimens—that is, just before a feed—would have imposed an additional burden on the midwives the value of this in reducing the number of tests required to be repeated was determined by a small group of midwives who deliberately took specimens either not later than one hour or not less than three hours after a feed. These are referred to as the feeding (522 specimens) and fasting (567 specimens) groups. It was not thought justifiable to take the blood from the same infant in the two states. The chromatograms were examined by each of four observers and the occurrence of abnormalities requiring repeat tests noted (Table III).

The difference between the number of abnormal chromatograms in the two groups was not statistically significant (χ^2 test not significant at the 5% level). Tyrosine, however, was more commonly increased in the fasting group and branched-chain amino-acids were more commonly increased in the feeding group (χ^2 test significant at the 5% level in both cases). The other abnormalities were equally distributed between the two groups.

TABLE III—Effect on Chromatogram of Sampling in the Fasting State and Soon After a Feed

Abnormal Chromatograms	Chromatograms Judged Abnormal by at Least One Person			Score*		
	Fasting	Feeding	Total	Fasting	Feeding	Total
Tyrosine	12	3	15	10.25	2.25	12.50
Methionine	1	1	2	0.50	0.50	1.00
Glycine	5	3	8	2.00	1.25	3.25
Proline	1	1	2	0.75	0.25	1.00
Alanine	1	1	2	0.75	0.25	1.00
Histidine	2	0	2	1.25	0	1.25
Glutamic acid	1	0	1	0.25	0	0.25
Branched chains	5	10	15	2.00	6.00	8.00
Lower half of chromatogram	7	11	18	4.25	6.75	11.00
Amino-acids generally increased	4	1	5	2.00	0.50	2.50
Total abnormal	39	31	70	23.50	17.75	41.25
Total normal	528	491	1,019	543.50	504.25	1,047.75
Grand total	567	522	1,089	567.00	522.00	1,089.00

*To calculate the score the number of times a chromatogram was judged abnormal by any of the four observers is divided by 4. This value has been used in the statistical calculations.

Thus it is not necessary to collect the first specimen at a particular time in relation to feeding. Repeat tests, however, are always collected after a fast of three to four hours so that any dietary effects can be excluded.

Agreement of Observers Interpreting Chromatograms

Since the chromatograms in the feeding and fasting experiment were examined by four observers independently the observations can be used to assess the extent to which they agreed in this necessarily subjective interpretation. Observers with most experience reported fewer abnormal results. Increases in tyrosine levels were recognized most consistently. Eleven out of 15 cases were noted by all four interpreters, two cases by two, and two cases by only one. Most cases of increases in proline and branched-chain amino-acids, and general increases in the lower half of the chromatogram were noted by three or more observers. In areas of the chromatogram that are crowded (histidine, methionine, glycine, glutamic acid, alanine, and generally raised patterns) most of the abnormalities (about 20% of all abnormalities) were noted by only one or two observers. Each observer showed individual sensitivity to some amino-acids rather than others.

New observers should match their interpretation with that of their colleagues, and it is important that the frequency with which specific abnormalities are recorded should be reviewed every six months so that the sensitivity of observers may be adjusted to avoid unreasonable variation such as that which occurred for branched-chain amino-acids after the discovery of a case of maple syrup urine disease (Table II).

Effect of Screening Programme on Local Paediatric Facilities

For one million inhabitants, 20,000 births a year, about 50 children averaged three visits each to the outpatient clinic each year. Of these eight a year were admitted for investigation or treatment. With the help of a social worker more investigation and control of dietary therapy is being done on an outpatient basis. The laboratory must keep referral to a minimum by using the criteria described: it is not possible to assess all the curious abnormalities seen on the chromatograms.

Reactions of the Public and Midwives

There have been about 10 refusals each year, mostly for repeat tests. Only one or two have prevented the initial test. Publicity by the Press, radio, and local television when the scheme was extended to the whole city played an important part in its acceptance by the public. At first midwives were apprehensive of making the heel-pricks in front of the parents, but a recurring educational programme was provided and we have aimed to make all midwives feel personally involved in the scheme.

Cost of Screening

To establish the screening procedure involves capital expense of £350 and current expenditure of £400 a year. One technician

(average salary £1,000 p.a.) can perform up to 30,000 tests a year, making the cost about 5p per test. In addition a biochemist, a secretary, and a van driver give some time to this work, and a clerk in the midwives supervisor's office checks that all results are entered on the baby's record card. This cost is similar to that of the Guthrie test up to 30,000 tests a year. Above this the Guthrie test becomes cheaper, since one technician can deal with many more samples. The Sriver test, however, has found seven cases of disease a year, only two of which would have been recognized by the Guthrie test.

Discussion

There are already 103 inherited metabolic diseases for which the precise enzyme deficiency is known (McKusick, 1971). Many are already the subject of experimental treatments showing more or less promise. The present work is an attempt to extend the management of this group of diseases beyond the single entity, phenylketonuria (Raine, 1972).

The Sriver chromatographic system has proved remarkably efficient in that it is capable of detecting in a single procedure some 22 aminoacidopathies (Table IV). The question must therefore arise whether this method should replace the Guthrie system at present recommended as national policy in Great Britain. The answer will depend on several considerations already indicated and on others discussed by the McKeown Committee (Nuffield Provincial Hospitals Trust, 1968). One, the number of diseases being sought that are treatable will change with time; indeed the need to treat some is yet to be established.

This form of screening implies a search for disease that is unsolicited by the patient, and once a disease is detected there follows a complete commitment to deal with it as effectively as possible. It is therefore most important that such a comprehensive detecting system should not be introduced unless the laboratory and clinical facilities as well as the interest and enthusiasm of the paediatrician, dietitian, and biochemist can be ensured.

This work has required the help of many people. Miss S. T. Davy, Matron of the Sorrento Maternity Hospital, Birmingham, inspired confidence by agreeing to a pilot study. In the laboratory Mr. G. Trevis, Mrs. G. R. Haynes, Misses S. Almond, A. Pickup, and S. Watson, and Mrs. A. McKeon helped to work out technical problems. Dr. B. S. B. Wood, Consultant Paediatrician, Birmingham Children's Hospital, and Professor E. G. Knox, Department of Social Medicine, University of Birmingham, have given constructive advice and help at several stages, and throughout we have enjoyed the ready co-operation of Dr. E. L. M. Millar, Medical Officer of Health for Birmingham.

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TABLE IV—Inherited Metabolic Diseases Detectable by the Sriver Method

<ul style="list-style-type: none"> β-Alaninaemia Citrullinaemia Glycinaemia (vitamin B₁₂ sensitive methylmalonic acidemia) Glycinaemia (vitamin B₁₂ insensitive methylmalonic acidemia) Glycinaemia (propionic acidemia) Glycinaemia (non-ketotic-type) Histidinaemia Hydroxyprolinaemia Lysinaemia (classical) Lysinaemia (lysine intolerance) 	<ul style="list-style-type: none"> Lysinaemia (Saccharopinuria) Maple syrup urine disease (classical) Maple syrup urine disease (intermittent) Methioninaemia (?tyrosinosis) Methioninaemia (homocystinuria) Phenylketonuria (classical) Phenylketonuria (variant) Prolinaemia type I Prolinaemia type II Sarcosinaemia Tyrosinaemia Valinaemia
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Amoxycillin: A new Semi-synthetic Penicillin

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Summary

Amoxycillin (α -amino-*p*-hydroxybenzylpenicillin) is a new semi-synthetic penicillin with a broad spectrum of antibacterial activity similar to that of ampicillin. Penicillin-sensitive strains of staphylococci, streptococci, and pneumococci were sensitive to concentrations of 0.1 μ g or less of amoxycillin/ml. Strains of *Haemophilus influenzae* were inhibited by a level of 0.5 μ g/ml, and most strains of *Escherichia coli*, *Proteus mirabilis*, *Shigella sonnei*, *Salmonella* species, and *Streptococcus faecalis* were sensitive to a concentration of 5 μ g or less of amoxycillin/ml. Penicillinase-producing strains of *Staphylococcus aureus* and strains of *Pseudomonas aeruginosa*, indole-positive *Proteus*, *Klebsiella*, and *Enterobacter* were insensitive to amoxycillin. The new penicillin was bactericidal in activity, as with other penicillins, and its antibacterial activity was not reduced in the presence of serum. After oral administration to volunteer subjects amoxycillin produced serum concentrations twice as high as those obtained with similar doses of ampicillin, and the penicillin was recovered unchanged in high concentrations in the urine. The absorption of amoxycillin was not greatly influenced by food, and administration of probenecid resulted in increased and more prolonged concentrations of amoxycillin in serum.

Introduction

Amoxycillin (Amoxil; BRL 2333; α -amino-*p*-hydroxybenzylpenicillin) is a new semi-synthetic penicillin synthesized in these laboratories (Long *et al.*, 1971). The compound has a chemical structure related to that of ampicillin (Fig. 1) and its antibacterial spectrum and level of activity are similar to those of ampicillin (Neu and Winshell, 1971a; Sutherland and Rolinson, 1971). However, amoxycillin is better absorbed than

ampicillin after oral administration to human subjects and produces serum concentrations considerably higher than those of ampicillin (Neu and Winshell, 1971b; Croydon and Sutherland, 1971). Results are reported here to compare the antibacterial activities and absorption and excretion in man of amoxycillin and ampicillin.

Materials and Methods

Amoxycillin is available as D(-)- α -amino-*p*-hydroxybenzylpenicillin trihydrate (Fig. 1) which, like ampicillin trihydrate, is relatively insoluble in water (0.4% v/w at room temperature),

Mol. wt. 419.46

Amoxycillin, BRL 2333

D(-)- α -amino-*p*-hydroxybenzylpenicillin trihydrate

FIG. 1—Structure of amoxycillin.

but aqueous solutions may be readily prepared in phosphate buffer, pH 8.0. The penicillin is relatively stable to acid and a 1% solution at 37°C has a half-life in simulated gastric juice (pH 1.5) of 17 hours compared with a half-life of 12 hours for ampicillin. Amoxycillin used in these studies had an assigned potency of 830 μ g/mg, and ampicillin trihydrate (Penbritin) a potency of 840 μ g/mg, both expressed in terms of anhydrous free acid.

Antibacterial Activity.—Minimum inhibitory concentrations required to inhibit growth of the test organisms for 18 hours at 37°C were measured by serial dilution in agar (Blood Agar Base, Oxoid) or nutrient broth (No. 2, Oxoid). Agar plates were inoculated with one drop (0.003 ml) of an undiluted overnight culture delivered with a multiple inoculating device. For tests in liquid medium the inoculum used was normally one drop (0.03 ml) of an overnight broth culture (about 10^7 cells) in 5 ml of medium.

Binding to Serum Protein.—The extent of binding of amoxycillin and other penicillins to protein of human serum was

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