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Progress in screening for inborn errors of metabolism

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Summary. The inborn errors of metabolism are a series of individually rare biochemical anomalies some of which cause serious clinical manifestations. They are of great interest to biochemists and geneticists, as well as to paediatricians and internist for whom they often present special diagnostic and therapeutic problems. The study of the inborn errors of metabolism also has implications in the fields of epidemiology and social medicine. The number of known inborn errors of metabolism has increased rapidly in recent years, and others, as yet unidentified, presumably await recognition. Only a few of these conditions can be treated now, but the realisation that early diagnosis is essential in order to achieve good results has stimulated interest in the possibility of examining either whole populations or selected predisposed groups of individuals for biochemical differences which characterise particular inherited metabolic diseases.

This article reviews some recent developments with particular reference to the indications for such screening programmes and progress in the identification of previously unknown inborn errors of metabolism in otherwise homogeneous population groups. – The inborn errors of metabolism are due to single gene mutations. – Recognition of the asymptomatic individuals who are heterozygous for the abnormal gene causing the disease may be important clinically and the identification of these individuals has to be considered as one aspect of metabolic screening for the inborn errors of metabolism.

Screening tests are diagnostic procedures which are applied without a specific clinical indication from the viewpoint of the individual who is being tested. The philosophy which underlies the development of screening methods for the inborn errors of metabolism is therefore the same as that which has led to programmes for the early diagnosis and treatment of other diseases, for example the correctable orthopaedic conditions which are routinely sought clinically by paediatricians in the neonatal period.

The inborn errors of metabolism present special problems in that they are individually rare with non-specific symptomatology, and treatment may be needed in the presymptomatic stage. This review concentrates on some general aspects of screening for the inborn errors of metabolism and does not aim to describe the detailed present position with respect to the individual diseases except where this is necessary to illustrate general concepts.

The motivation for the development of screening programmes is summarized in diagrammatic form in figure 1, this was drawn up with mental handicap particularly in mind but applies equally to the diseases where physical handicaps predominate.

The usual sequence of events is shown by the solid lines, namely that brain damage occurs in the presymptomatic stage and clinical suspicions arize when the child begins to show developmental delay, this is followed by a biochemical diagnosis and treatment which may be too late. The dotted lines show what is needed, namely biochemical diagnosis in the presymptomatic stage with treatment before the brain is damaged.

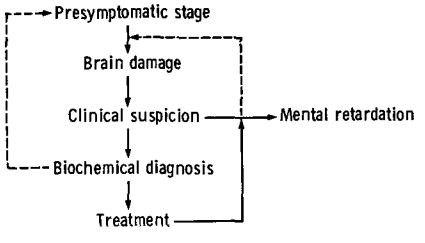

Fig. 1. Motivation for screening for inborn errors of metabolism. The sequence of events without presymptomatic screening is shown by the solid lines, namely that biochemical diagnosis and treatment are delayed until the metabolic lesion has produced irreversible secondary damage. The dotted lines show the results with an effective screening programme, namely diagnosis and treatment before there is secondary damage.

Metabolic screening may be non-selective and aim to cover the whole population, or it may be selective and aim to examine a segment of the population which may be defined on either clinical or genetic grounds. Screening programmes in which the population to be studied is defined on either geographical or ethnic grounds are regarded as being non-selective for the purposes of this discussion.

The development of metabolic screening programmes are usually considered in terms of seeking for single identifiable biochemical defects individually. However, the increasing number of such specific metabolic lesions which are being identified suggests that it would be rational in the future to develop screening methods for the simultaneous detection and differentiation of large classes of clinically related diseases. The recent work on screening for inborn errors of metabolism in which there is an abnormal non-amino organic aciduria[1] and for errors of glycoconjugate metabolism[2–5], shows that this approach is feasable, although it may require high level technology. The amino acidopathies are exceptional in that relatively simple, although labour intensive, chromatographic methods can be used for preliminary broad screening. The early successes with these diseases, which are a particularly well circumscribed group in terms of the chemical marker (an α-amino group) encouraged the view that similar fairly simple methods would serve to detect and differentiate other groups of metabolic diseases. However, this has not been wholly borne out by the results of later work, and recourse to more technologically advanced methodology has proved to be necessary[6–12].

Non-selective or whole population screening programmes are currently established to detect one particular disease, others may be detected incidentally, or by making minor modifications to the test procedure. The establishment of a screening programme should be preceeded by a careful study of the disease concerned with particular reference to its clinical severity and uniformity, its genetic homogeneity and the certainty of the biochemical and clinical correlations which have been claimed. The recent discussions about the significance of histidinaemia and the need to treat it with low histidine diets exemplifies the problems which arise from lack of basic knowledge about the disease or metabolic variant with which one is dealing[13]. Histidinaemia may be genetically heterogeneous, harmless in some cases and harmful in others. It is noteworthy that although phenylketonuria has been intensively studied and the principles of its dietary treatment have been known for more than 20 years, complete agreement on the details of management[14], and on the significance and inter-relationships of the different hyperphenylalaninaemic states is still lacking.

Screening programmes should only be set up for treatable or preventable diseases except for local programmes which may be established for research purposes.

A policy of widespread screening for inherited disease involves economic and social factors as well as purely scientific considerations. Komrower[15] showed that a policy of screening for phenylketonuria and treating the affected individuals by phenylalanine restriction was cost-effective when compared with the cost of life-long care for mentally handicapped untreated phenylketonuric patients. Seegmiller's[16] calculation led to the same conclusion in the case of Down's syndrome in which the cost of prenatal screening and abortion of affected foetuses can be offset against the financial cost of caring for a mentally handicapped patient for what may be a natural life span. Although Down's syndrome is an inherited chromosomal abnormality, it is analogous in this context to an untreatable inborn error of metabolism which can be diagnosed *in utero* at about the 16th week of pregnancy. These calculations of cost effectiveness exclude the invisible benefits in humanitarian terms, and are incomplete even in economic terms, in that they do not allow for the contribution which a patient with an effectively treated metabolic disease can make to the national economy.

Non-selective (whole population) screening for inborn errors of metabolism

Some requirements which are particularly relevant to non-selective or whole population screening are set out in table 1. It is difficult to be dogmatic about the incidence at which non-selective (whole population) screening for an inborn error of metabolism, which produces severe disability is justified by the incidence of the disease itself. The figure of 1:10,000 is the overall incidence of phenylketonuria in Caucasian communities although the value is almost twice as high in some parts of Europe. The problem of the lower incidence diseases will be best met in the future by developing methods which screen for large classes of diseases in a rather nonspecific way but which also have the capacity to differentiate the individual members of the group from one another as part of the initial screening process. It is doubtful if this sophisticated approach will be applicable to nonselective (whole population) screening in the foreseeable future for logistic and financial reasons.

Table 1. Requirements for a nonselective (whole population) screening programme

Reliable and simple test
No false negatives, few false positives
Incidence of abnormality reasonably high ($\gtrsim$ 1:10,000)
Ethically acceptable to the target population
Informed consent obtainable from the target population
Confidentiality safeguards for the target population

The diseases for which nonselective screening programmes have been most advocated are summarized in table 2. The longest experience in this field is with

Table 2. Nonselective (whole apulation) Screening for inborn errors of metabolism

Generally accepted
Phenylketonuria
Proposed by some authorities and/or applied locally
Other aminoacidopathies
Congenital hypothyroidism
Galactosaemia
Cystic fibrosis
Hyperlipidaemias
Tay-Sachs disease
Haemoglobinopathies

screening for phenylketonuria. This has become a public health matter in most countries and is still the only metabolic disease which is universally accepted as meriting nonselective screening. The Guthrie microbiological inhibition assay, which uses a drop of dried blood can be modified to detect abnormal concentrations of leucine, tyrosine, methionine, (this also detects homocystinuria), histidine and argininosuccinic acid. The alternative one-dimensional chromatographic method[17] can detect an abnormal pattern in the plasma amino acids including one which is quite unsuspected. It has therefore greater potential than the Guthrie test but the results need critical evaluation if the institution of elaborate treatment programmes for the management of harmless anomalies is to be avoided.

Whole population screening for homocystinuria, prolinaemia, histidinaemia, or tyrosinaemia individually, is not justified except in certain special circumstances, such as the Canadian genetic isolates where tyrosinaemia occurs in 1:700 of the population.

Congenital hypothyroidism is the disorder for which, after phenylketonuria, an effective whole population neonatal screening programme is most urgently needed. The incidence is between 1:3000 and 1:8500 in different parts of Europe, and in North America. There is general agreement that very early diagnosis and treatment of congenital hypothyroidism improves the final outcome and that hormone replacement beginning during the first days of life would be ideal[18–22]. However, the clinical diagnosis of congenital hypothyroidism is very unreliable even in cases of complete absence of the thyroid gland, and especially so in cases of ectopic thyroid, which is the more frequent cause of the disease. Chemical methods are available with which to screen for congenital hypothyroidism on the basis of the plasma concentrations of thyroxine (T_4), 3,5,3′-triiodothyronine (T_3), 3,3′,5′-triiodothyronine (reverse T_3) and thyroid stimulating hormone (TSH) either at birth or in association with screening for phenylketonuria using spots of blood dried on filter paper when the child is 6 days old. The best method to use is still uncertain. The American Thyroid Association[23] recommends that testing for congenital hypothyroidism should be combined with existing newborn screening programmes, that T_4 should be parameter which is measured and a TSH assay should be used to retest the suspicious specimen. Neither of these tests is suitable to use alone, T_4 being somewhat too insensitive and TSH tending to give too many false positive results. The Association recommends similar tests on umbilical cord blood if the chemistry is to be done locally, and that further developmental work on TSH and reverse T_3 assays should be undertaken. These recommendations mean that in many places screening for congenital hypothyroidism will be combined with the Guthrie test for phenylketonuria at about the 6th day of life. This would be administratively convenient, but it has not been proved to be the best time to screen for congenital hypothyroidism. It should be equally easy to organize screening based on the use of umbilical cord blood and this could be linked to screening for some other diseases for which treatment is needed during the 1st day or 2 of life. Unpublished data suggest that reverse

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T_3 would be the most suitable parameter for neonatal screening on cord blood, the normal levels being between 2 and 10 times the corresponding adult values with no overlap. It is of interest that T_4 and TSH values do not discriminate between neonates and adults and that T_3 concentrations are normally so low that is would not be possible to define a normal range against which to identify abnormally low values (Himsworth, unpublished data). Reverse T_3 measurements can also be applied to amniotic fluid[24] so that prenatal diagnosis may also be possible. This is particularly important because of the possibility that congenital hypothyroidism damages the brain before birth and may be treatable *in utero*.

Galactosaemia, congenital deficiency of hexose-1-phosphate uridylyl-transferase [galactose-1-phosphate uridyl-transferase (EC 2.7.7.12)], is treatable but some cases die from a fulminating illness complicated by *E. coli* septicaemia before they are old enough to have shown intolerance of milk, and before the results of a blood test taken at about 6 days of age would be available. The deficient enzyme is normally present in erythrocytes. Screening needs to be done on cord blood and could be combined with screening for congenital hypothyroidism. If whole population neonatal screening cannot be provided for this group, it should certainly be available for all acutely sick neonates and for the newborn siblings of an affected child.

Cystic fibrosis is the commonest autosomal recessive disorder in Caucasian populations, having an incidence of about 1:2000, and a carrier rate of about 1:23. Early diagnosis would be worthwhile because 10–15% of patients develop meconium ileus, at or shortly after birth. This complication has a high mortality. It is widely agreed[25] that early antibiotic treatment and physiotherapy lower the morbidity from respiratory complications. Some authorities consider that it would be justifiable to screen school-leavers in order to determine their carrier status if a suitable test were available. Considerable technological development and field trials are needed in order to develop ion specific electrodes for sodium and chloride in sweat or saliva to a point where they could be used by paramedical workers on all infants[26,27]. In addition, some infants cannot sweat fast enough for abnormalities in the sweat electrolytes to be detected. Tests based on the demonstration of excessive amounts of protein and certain enzyme in the meconium[28,29] appear more promising, but also require further validation. Both tests are about 85% sensitive and 95–99% specific, but the results have to be confirmed with the classical iontophoresis sweat test[30]. Tests based on the 'ciliary dyskinesia' factor[31,32] have not so far been reported. The development of a satisfactory diagnostic test for cystic fibrosis is an urgent paediatric and public health need in Europe and other countries with a predominantly Caucasian population.

There are clinical suggestions and indirect evidence that genetic factors contribute to the aetiology of atherosclerosis. The results of prospective epidemiological studies suggest that abnormalities of the plasma lipoproteins are a major risk factor for the development of atherosclerosis. Proposals to screen newborn infants for hyperlipidaemias and hyperlipoproteinaemias are still at the pilot stage and aimed at identifying the individuals who will be predisposed to atherosclerotic cardiovascular disease in later life. There is no agreement on the potentially most worthwhile parameters to study, and little knowledge of the background physiological changes against which abnormalities with predictive value must be identifiable[33–38]. If an association between a chemical abnormality in infancy and later atherosclerosis could be demonstrated, there would be strong pressure to institute a screening programme and to treat the affected individuals as early and as vigorously as possible.

Tay-Sachs disease (GM_2-gangliosidosis) is sufficiently common (about 1:2000) in Ashkenazi jewish communities for whole population screening on the basis of plasma hexosaminidase-A deficiency to have been instituted in order to identify carriers and hence families in which cases are expected to occur, so that the first pregnancies can be monitored by amniocentesis and hexosaminidase determination on amniotic fluid and cultured amniotic cells[39]. Automated methods of determining plasma hexosaminidase-A give reliable results and good quality control data but it should be noted that pregnancy and oral contraceptives can cause spurious low results so that normal values approach the range for the heterozygous carriers. The initial study of Kaback and his collaborators[40] in the USA yielded about 300 carriers in approximately 7000 subjects tested and 11 couples with both partners affected, none of whom had, at the time of the survey, an affected child. These workers also investigated the results of this screening programme from the viewpoint of the individuals who participated in it, and of their motives for having done so[41,42]. Almost all of the respondents were pleased that they participated and there were no adverse effects on their reproductive lives or inter-personal relationships. The success of carrier detection programmes depends on the cultural and educational background of the target population, and on the way in which it is introduced to the community. Non medical as well as medical channels for the dissemination of information may be necessary and this aspect requires careful consideration before the programme is launched.

Further studies by Kaback[43] have confirmed the validity of the earlier work, and he recently reported that more than 160,000 individuals had been screened on a world wide basis and nearly 7000 heterozygotes detected. Of 101,000 American Jews screened (without known carriers or affected offspring in their families),

1 in 27.3 Ashkenazi Jews are carriers, and 125 couples who were both carriers and therefore at risk for an affected offspring were identified. The carrier rate is about 1:100 for non-Ashkenazis. Tay-Sachs disease can be diagnosed *in utero*. The results of monitoring the pregnancies of the at risk group and of aborting the affected foetuses and allowing the unaffected to continue to term have been very satisfactory.

Except in Israel[44] attempts to introduce similar programmes have been less successful than in the USA. This was particularly so in Britain[45], and it may be that more good will be done by concentrating on family based testing programmes in the present state of public opinion at least in Europe.

The compliance rates in Kaback's original survey is uncertain, being between 10 and 40%. The size of the true target population is difficult to estimate in this type of survey, because it is strictly those individuals who actively wish to have children when the test is being offered.

The experience gained in introducing screening for Tay-Sachs disease carriers will provide a background body of information which will be valuable when it becomes possible and desirable to introduce whole population carrier screening for another inborn error of metabolism such as cystic fibrosis in Caucasian populations.

Sickle cell disease and Thallasaemia are major health problems in some countries, and the development of methods for early diagnosis, carrier detection and prenatal diagnosis would be desirable. Methods based on measurements of globin β-chain synthesis are currently being developed for prenatal diagnosis of these diseases[46,47]. Haemoglobin-S can also be demonstrated electrophoretically in foetal blood sometime between the 18th and 28th week of gestation. The urgent needs in this field are: firstly, the development of a flexible foetoscope so that blood can be obtained from a foetal vein on the surface of the placenta in all cases and blind needling of anteriorly situated placentae for blood sampling avoided; and secondly, the simplification of the biochemical tests which currently depend on studies of the incorporation of [^{3}H] leucine into the globin chains, chromatographic separation of the α, β and γ-chains, and determination of the ratio of the radioactivities of the β- and γ-chains.

Selective screening for inborn errors of metabolism

Selective screening means that the test is applied to a subgroup of the total population which is defined either on clinical or on genetic grounds. The latter is the blood relationship of the individuals being tested to known cases of the disease. In order to screen for overt cases of the disease, the male and female sibs of the index cases are studied in the autosomal recessive disorders, whereas the parents as well as the sibs are studied in the autosomal dominant disorders, and the male blood relations descended through the female line on the maternal side of the family in the sex-linked recessive disorders. Selective screening on genetic grounds includes carrier detection studies within the family of the propositus. The parents as well as the sibs are studied for this latter purpose in the autosomal recessive disorders. In the case of the sex-linked disorders, the carriers will be found among the female blood relations on the mother's side of the family and among the patient's sisters.

The routine testing of urine from all cases of urolithiasis for cystine is an example of selective screening on the basis of clinical criteria.

Multidisease screening techniques can be used for selected population screening even if they are unsuitable for whole population screening. A multidisease screening method must be comprehensive (able to detect all the variants), specific (able to differentiate all the variants), able to give an unambiguous identification

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on the basis of a single analysis and to detect unsuspected variation. Appropriate chemical grouping of the inborn errors of metabolism and the use of multidisease screening methods could remove some of the serendipity which has been a feature of this field of study in the past.

Multidisease screening methods have been developed for: a) diseases in which there is a characteristic non-amino organic aciduria; b) the abnormal oligosaccharidurias; c) the identification of metabolic blocks on the biosynthetic pathways of glycoconjugate metabolism.

a) Screening for non-amino organic acidurias

About 50 of the known 180 single enzyme defects which cause an inborn error of metabolism are associated with characteristic abnormal non-amino organic aciduria. The method which has been developed to screen for these abnormalities is summarized in figure 2, the only prerequisite is that the compound to be identified is acidic with an overall pK_a value of below about 5.5. The sensitivity of the analytical system is so arranged that it excludes consideration of urinary constituents with concentrations below about 5 mg per g of creatinine or roughly 10 mg/24 h for adults because in all of the known single enzyme blocks which produce clinical symptoms, gross amounts of an abnormal metabolite accumulate so that the concentration of the latter is of the order of at least tens of mg/l. The control data and prevalence of different acidic metabolites were established on 420 control unrestricted subjects who were appropriately grouped for age and sex[49–51]. The prevalence of excretion of detectable amounts of the main urinary acidic metabolites in the range defined for the study is shown in table 3. The consistent excretion of the deoxytetronic and tetronic acids which constitute 30–35% of the total organic acid excretion on a molar basis had not been previously recognized. This finding was incidental to the main objective of the work and could be significant in relation to, for example, the solubilisation of calcium salts in urine.

Table 3. Prevalence of excretion of detectable amounts of the major (and some minor), urinary acidic metabolites in 420 subjects age 1.5–83 years[49]

Metabolite	No. of subjects excreting detectable amounts	Prevalence, (%)
Oxalic acid	239	57
Sulfate	420	100
Benzoic acid	38	9
Phosphate	420	100
Succinic acid	58	14
4-Deoxytetronic acid	416	99
3-Deoxytetronic acid	395	94
2-Deoxytetronic acid	414	99
5-Hydroxymethyl-2-furoic acid	48	11
Tetronic acids*	420	100
2-Oxoglutaric acid	147	35
4-Hydroxyphenylacetic acid	382	91
Tartaric acid	117	28
2-Deoxypentonic acid	413	98
Aconitic acid**	406	97
Hippuric acid	289	69
Citric acid	413	98
Glucono-1,5-lactone	411	98
Glucuronic acid	270	64
Gluconic plus glucaric acids***	401	96

* Always both erythronic and threonic acids, with the former acid comprizing 55–75% of the combined peak. ** Assumed to be cis-aconitic acid, this being the naturally occurring form of this acid. *** Generally with glucaric acid as the major constituent.

The quantitative excretion patterns[51] of the normally occurring acidic metabolites could be classified into 4 groups: a) unimodal distribution with detectable values in all subjects; b) apparently unimodal distributions with some values below the defined limit of detectability; c) clearly bimodal distributions, comprizing a unimodal sub-group plus a block of undetectable values;

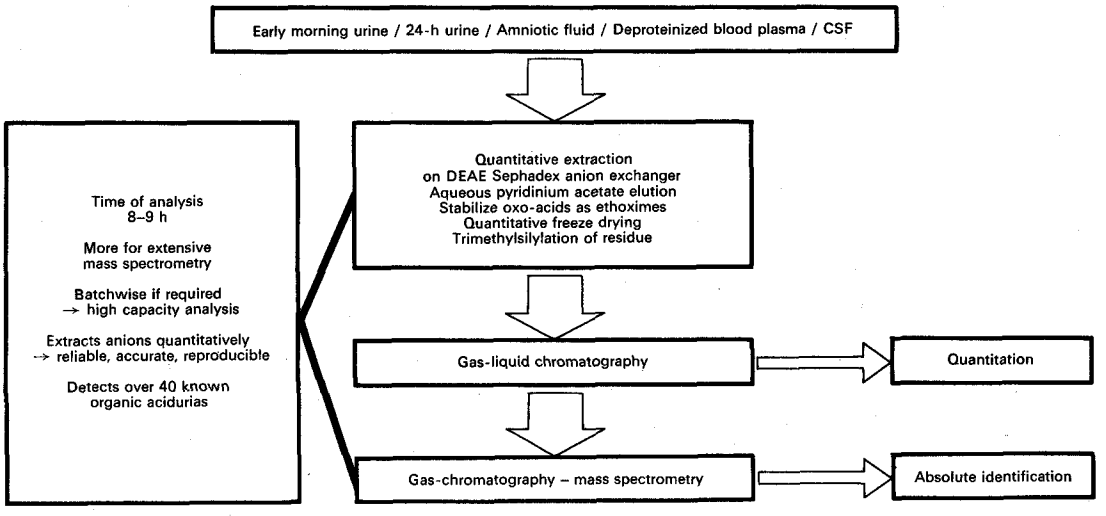

Fig. 2. Screening procedure for non-amino organic acidurias[48].

d) irregular distributions with a majority of undetectable values. Groups *a* and *b* contained most of the major metabolites; the bimodality of group *c* compounds raises the possibility of genetically-controlled polymorphisms and group *d* contains metabolites of exogenous origin.

It has been suggested on the basis of fragmentary studies that inborn errors of metabolism might contribute appreciably to the incidence of mental handicap[52]. A detailed investigation of this suggestion with respect to either known or previously unrecognized disorders, in which an abnormal organic aciduria could provide a biochemical marker of an inborn error of metabolism, was investigated using the coupled gas chromatographic-mass spectrometric method[49–51] referred to above. A group of 1778 permanently institutionalized patients with mental handicap, including 248 children[1], showed that about 5% of them had an abnormal organic aciduria on the basis of a single examination. The only consistent abnormality on retesting daily for 1 month was in the phenylketonuria patients who were detected at the anticipated level of about 1%.

The preliminary results of screening neonates and infants for organic acidurias because of non specific illness and failure to thrive suggest that the comprehensive study of non-amino organic acids may be diagnostically more useful than amino acid analysis and that acute non specific illness in infancy is a better pointer to inherited metabolic disease than longstanding mental handicap (Chalmers and Watts, unpublished data). These investigators studied about 400 hospitalized newborn babies and infants selected for these clinical reasons and found 8 patients with previously unrecognized metabolic diseases including new cases of methylmalonic aciduria, branched chain ketoaciduria and propionic acidaemia; 10 cases where a suspected organic acidopathy was confirmed and 21 cases with an abnormal organic aciduria which could either not be related to a recognised disease or could only be related to acquired disease. These figures do not include patients who had been diagnosed independently and who were used to validate the methodology. Only 4 of the 400 patients showed a characteristic abnormal amino aciduria which would have been diagnostically useful. A diagnosis of an inherited metabolic disease was confirmed by urinary organic acid analysis in a further 10 patients who were newly referred having been provisionally diagnosed elsewhere. 6 other patients showed an organic aciduria, which suggested an at present undefined metabolic disease. Lactic acidosis of unknown aetiology was observed in a further 11 patients and evidence of ketosis was observed in 4 others (Chalmers, unpublished data).

The detailed organic aciduria screening has been incorporated into an overall plan for the investigation of acutely sick infants (figure 3). This should permit the diagnosis of about half of the approximately 180 known inborn errors of metabolism and is being evaluated in practice. The presence of a metabolic acidosis is not demanded before examining the urine for acidic metabolites. A separate qualitative test for abnormal glycosaminoglycan excretion has recently been added to the scheme.

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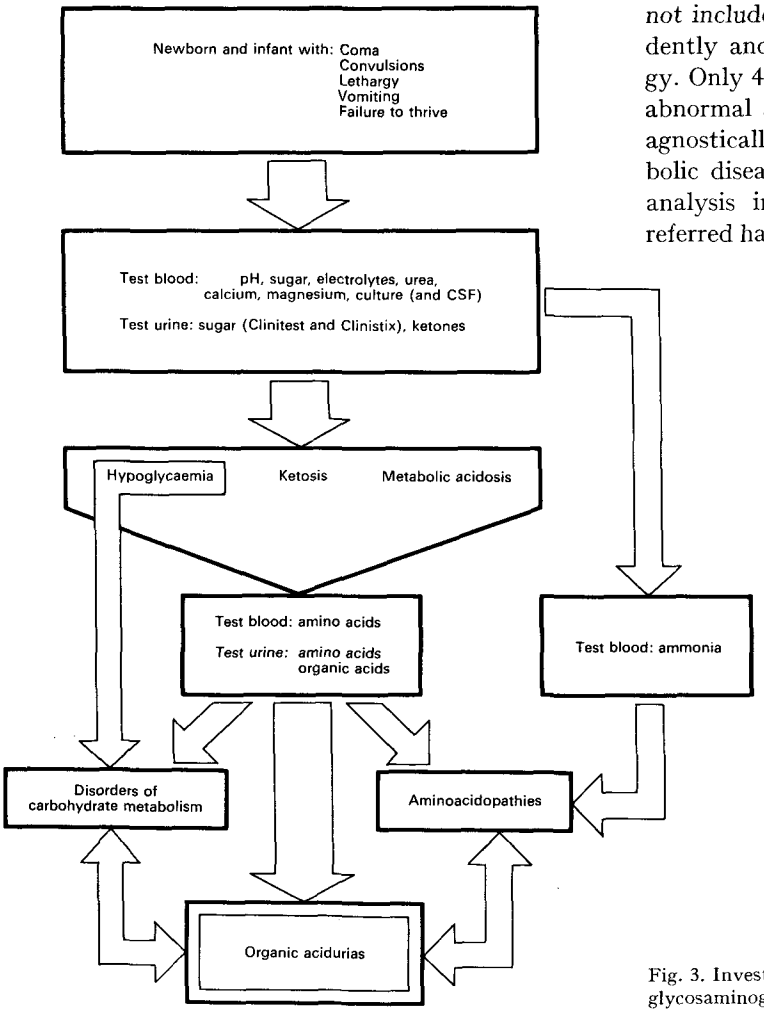

Fig. 3. Investigation plan for the acutely sick infant[48]. An abnormal glycosaminoglycan excretion should be specifically tested for.

b) Screening for oligosaccharidurias

A defect of an enzyme of either catabolism or biosynthesis of a specific group of glycoconjugates has been implicated in the glycoprotein storage diseases (e.g. mannosidosis and fucosidosis), the sphingolipidoses, the mucolipidoses and the mucopolysaccharidoses. In general, these diseases are characterized by excessive excretion of partly synthesized or degraded glycoconjugates and glycoconjugate fragments as well as storage of these materials in the tissues. Some examples of diseases due to defective degradative

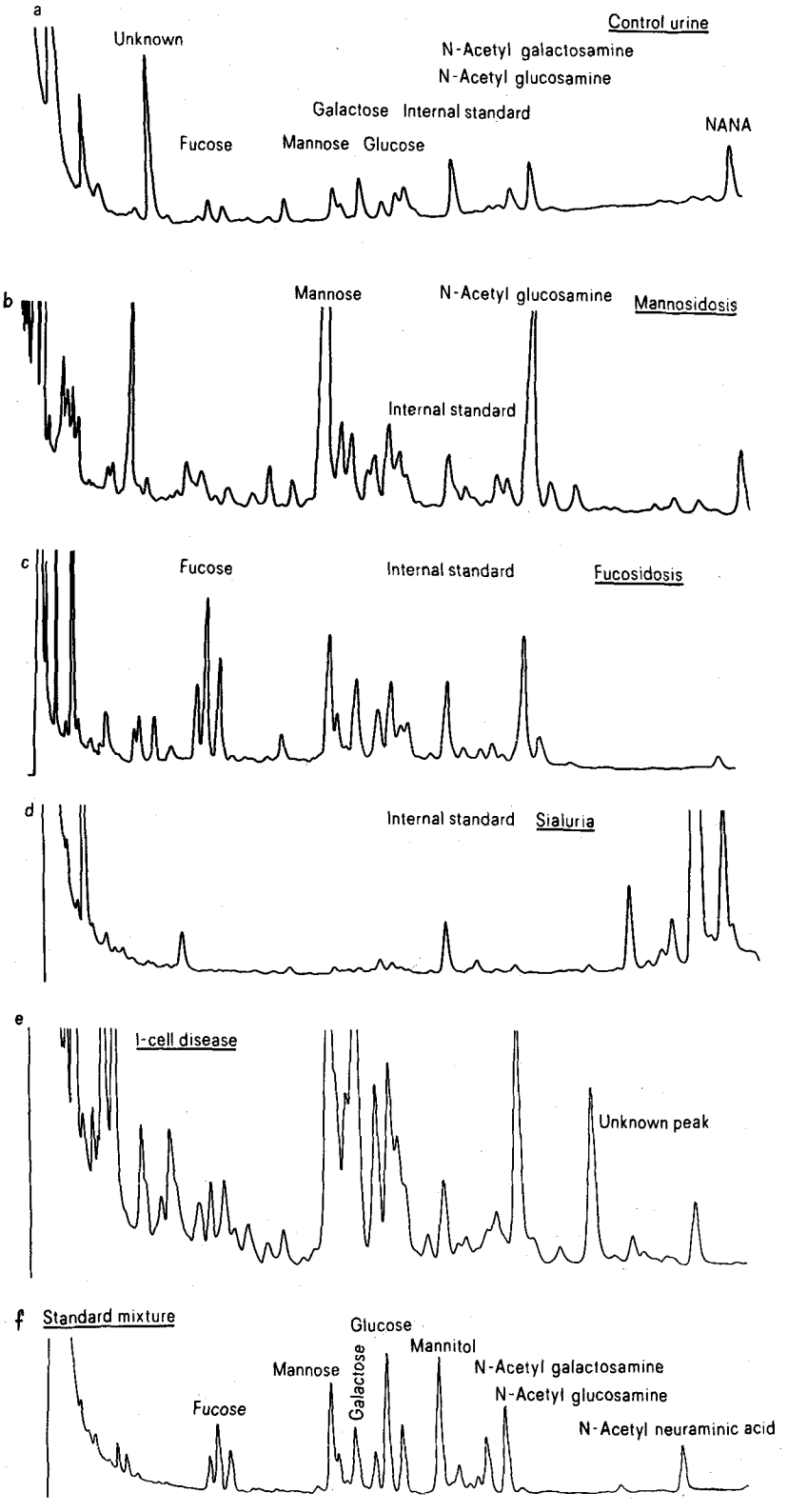

Fig. 4. Gas-liquid chromatogram of the trimethylsilylmethylglycoside derivatives of the carbohydrate constituents of the urinary oligosaccharides in **a** normal urine and in urine from cases of: **b** manosidosis, **c** α-fucosidosis, **d** sialuria, **e** I-cell disease. The result obtained with a mixture of reference compounds is also shown[3].

enzymes are: mannosidosis [α-mannosidase (EC 3.2.1.24)], Tay-Sachs disease [β-N-acetylhexosaminidase (EC 3.2.1.52)], GM_1 gangliosidosis [β-galactosidase (EC 3.2.1.23)], Hurler's disease [α-iduronidase (EC 3.2.1.76)]. Metabolic lesions on the anabolic pathways are exemplified by the deficiency of GM_3-UDP-*N*-acetylgalactosaminyl transferase in GM_3 gangliosidosis, and of galactose-1-phosphate uridylyl transferase (EC 2.7.7.12) in galactosaemia[5].

In order to screen for abnormal oligosaccaride containing fragments in the urine and to determine their composition, they are first separated from the salts and other low molecular weight substances by gel chromatography, and degraded by methanolysis to yield the constituent monosaccharides as methylglycosides, which are then silylated. The final trimethylsilylmethylglycosides are separated and quantitated by gas-liquid chromatography. The method detects all of the common carbohydrate constituents of glycoconjugates, namely: fucose, mannose, galactose, glucose, 2-acetamido-2-deoxyglucose (*N*-acetylglucosamine), together with siliac and glucuronic acids[2,3], and it should detect the results of 30–40 theoretically predictable different metabolic blocks. Some examples of the results obtained in certain independently diagnosed diseases, and with a mixture of known compounds, are shown in figure 4. Screening 763 hospitalized patients with severe mental handicap has shown that disorders characterized by abnormal excretion of glycoconjugates and/or oligosaccharides do not contribute significantly to the incidence of long term mental handicap in the United Kingdom[4].

c) *Screening for blocks on the pathways of glycoconjugate synthesis*

Although metabolic blocks on the anabolic sequences by which glycoconjugates are built up, can cause the urinary excretion of abnormal carbohydrate containing fragments, their identification on this basis is indirect. It has recently been shown[5] that glycoconjugate synthesis can be studied directly in lymphocytes isolated from human peripheral blood, and the defect of an anabolic sequence, deficient galactose-1-phosphate uridyltransferase activity, demonstrated. Different isotopically labelled sugars are incubated with separate portions $2 \times (10^5–10^6)$ cells of the lymphocyte preparation and their incorporation into the total acid precipitable cell constituents measured. The metabolic locations of the labelled sugars are shown in figure 5. This method tests for about 35 theoretical metabolic blocks in the glycoconjugate biosynthesis simultaneously, provided that they are expressed in lymphocytes. This novel approach is being used in the author's laboratory to search for metabolic lesions of this type in mentally handicapped patients.

Tissue enzymology and screening for inborn errors of metabolism

The demonstration of a specific metabolic lesion *in vitro* is an important objective in the study of an inborn error of metabolism, and often leads to the development of a specific diagnostic test for the overt cases and for the carriers of the abnormal gene concerned. Although many of the definitive and classical studies, of which only a few can be quoted, have been made on liver[53–55], jejunal[56], renal biopsies[57], and epidermis[58], most diagnostic work in this area is done on blood, and on fibroblasts grown from skin explants. The definitive diagnosis of the glycogenoses[59] and some

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Some enzymic steps in complex carbohydrate metabolism

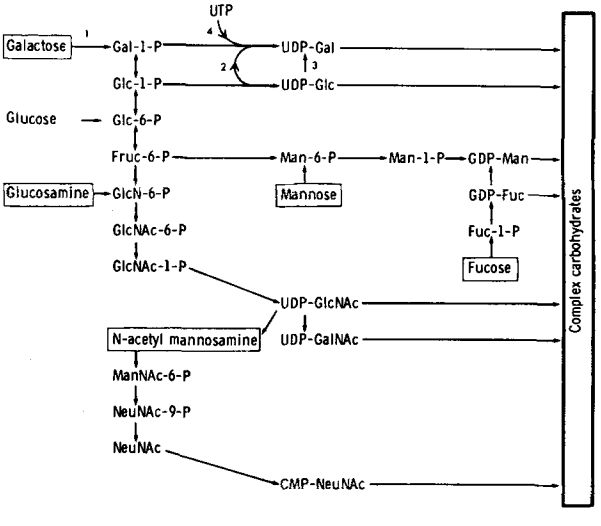

Fig. 5. Simplified diagram to show the flow of monosaccharide precursors into the acid precipitable macromolecules of human lymphocytes in vitro. The isotopically labelled precursors used to screen for metabolic blocks on the glycoconjugate biosynthetic pathways[5] are shown in the boxes, for example Mannose. The figures indicate the following enzymes: 1. galactokinase (EC 2.7.1.6); 2. hexose-1-phosphate uridilyltransferase [galactose-1-phosphate uridyl transferase (EC 2.7.7.12)]; 3. UDP-glucose-4 epimerase (EC 5.1.3.2); 4. galactose 1-phosphate uridilyl transferase [UDP-galactose pyrophosphorylase (EC 2.7.7.10)]. The final transfer of each monosaccharide from the activated sugar nucleotide into complex carbohydrates is catalyzed by separate glycosyl transferases.

urea cycle disorders[60] is an important exception to this generalisation.

Hair bulbs appear to have considerable potential for this type of work because they provide an easily accessible biopsy of a metabolically very active ectodermal tissue which does not require culture, and which is not therefore liable to the changes which may occur in fibroblasts when these are grown *in vitro* on glass or plastic surfaces and in media containing foetal calf serum. The latter may contribute enzymes or other biologically active materials to the cells. It is particularly difficult to establish the intermediate levels of enzyme activity which characterize the carriers of the lysosomal storage diseases using cultured fibroblasts because the cells take up some enzyme from the medium. Gibbs[61] showed that α-iduronidase (EC 3.2.1.76) assays on the hair follicles from the obligate heterozygous carriers of the abnormal gene which causes Hurler's disease differentiates these subjects clearly from normal individuals and from the clinically affected individuals. Similar results were obtained with arylsulphatase-A (EC 3.1.6.8) assays on hair follicles in the case of the obligate heterozygous carriers for metachromatic leukodystrophy[62]. Hair roots also show some degree of clonal growth and because of the random inactivation of the X-chromosome in females[63], 2 types of follicle, those with normal enzyme activity and those with reduced activity can be demonstrated in X-linked diseases. Except for the studies which differentiated the tyrosinase (EC 1.14.18.1) positive from the tyrosinase negative types of oculocutaneous albinism[64], measurement of the enzyme activity of hair follicles has been mainly used in clinical biochemical genetics to identify the carrier status of the maternal female blood relations of patients with sex-linked recessive disorders: glucose-6-phosphate dehydrogenase (EC 1.1.1.49) deficiency[65], the Lesch-Nyhan syndrome[66,67] and Fabry's disease[68,69].

Conclusion

Although the inborn errors of metabolism are individually rare, they constitute a considerable paediatric clinical case load. There have been several attempts to quantitate this and Raine[70] calculated that 8.5% of paediatric deaths and 4.7% of paediatric hospital admissions were due to autosomal recessive or sex linked recessive diseases. These diseases place a particularly heavy and prolonged burden on the parents and other family members. The happy and fruitful symbiosis of clinical medicine and basic biochemistry, which an interest in this field generates, has yielded a large body of information about these diseases which awaits application and further development. The extent to which preventive and therapeutic possibilities in relation to the inborn errors of metabolism can be developed depends mainly on the funds which the subject can attract in competition with other aspects of preventive and therapeutic medicine.

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