Organic Acids in Man

Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias

R.A. CHALMERS

and

A.M. LAWSON

Organic Acids in Man

Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias

R.A. CHALMERS

and

A.M. LAWSON

M.R.C. Clinical Research Centre,
Harrow, UK

LONDON NEW YORK
CHAPMAN AND HALL

First published 1982 by
Chapman and Hall Ltd
11 New Fetter Lane, London EC4P 4EE
Published in the USA by
Chapman and Hall
in association with Methuen, Inc.
733 Third Avenue, New York NY 10017

Printed in Great Britain at
the University Press, Cambridge

ISBN 0 412 14890 0

British Library Cataloguing in Publication Data

Chalmers, R. A.

Organic acids in man
1. Biological chemistry 2. Organic acids
I. Title II. Lawson, A. M.
612'.015 QP801.0/

ISBN 0-412 14890 0

Library of Congress Cataloging in Publication Data

Chalmers, Ronald Aubrey.

Organic acids in man.
Includes bibliographical references and index.
1. Acidosis—Diagnosis. 2. Acids, Organic—Analysis. 3. Acids, Organic—Metabolism—Disorders—Diagnosis. 4. Chemistry, Clinical—Technique.

I. Lawson, A. M. II. Title. [DNLM: 1. Carboxylic acids—Analysis. 2. Metabolism, Inborn errors—Diagnosis. QU 98 C4380]
RB147.C46 1982 616.3'99 81–11342
AACR2

Preface

The writing of this book was prompted by the need for a comprehensive collection of current data on organic acids suitable for both newcomers and established researchers in this field. The only previous text of the kind was the excellent review by Nordmann and Nordmann (1961), and at that time the main method of analysis was paper chromatography with liquid chromatography being used in a limited way. Only three diseases in which organic acids accumulate were known (primary hyperoxaluria, phenylketonuria and alcaptonuria). Since then, with the development of gas chromatography and mass spectrometry, and the further development of liquid chromatography, knowledge concerning the nature of the organic acids in physiological fluids has been greatly extended. At the same time, the number of organic acidurias has increased dramatically, there being now some 40–50 known diseases of this type. During the past 15 years or so, there have been several reviews, dealing with either specific diseases or groups of diseases (Gompertz, 1972, 1974; Tanaka, 1975), or presenting the proceedings of symposia (Stern and Toothill, 1972) or workshops (Mamer *et al.*, 1974). This present text deals comprehensively and in detail with the organic acids in human physiological fluids in health and in disease states, and is particularly concerned with the methods necessary for their separation, determination and indentification.

Our own personal approach and experience in this field predominates in several sections of the book, but we hope to have achieved a comprehensive representation of all other approaches to the subjects covered. We are indebted to the many other workers in this field, both present and past, on whose work we have drawn extensively, and without which this book would be a poor substitute of the present text.

Particular thanks are due to our collaborators and colleagues who have, through discussions and correspondence and with the gift and exchange of reference specimens of urine from patients with identified organic acidurias, greatly extended our knowledge and understanding of these diseases: especially Dr S. K. Wadman and Dr M. Duran, Wilhelmina Kinderziekenhius, Utrecht Universiteit Kinderkliniek, The Netherlands, Dr P. Beaune and Dr J. M. Saudubray, Hôpital NeckarEnfants Malades, Paris, Dr N. Gregersen, Aarhus Kommunehospital, Denmark, Dr N. Kennaway and Dr N. Buist, University of Oregon, Portland, Oregon, U.S.A., Dr K. Tanaka, Yale

University School of Medicine, New Haven, Connecticut, U.S.A., Dr V. G. Oberholzer, Queen Elizabeth Hospital for Children, Hackney, London, Dr N. Brandt and Dr E. Christensen, Rigshospitalet, Copenhagen, Denmark, Dr O. Borud, University of Tromsö, Norway, and Dr D. Gompertz, formerly of Hammersmith Hospital, London. We are also indebted to Dr Richard W. E. Watts who initiated our interest in this field and whose collaboration, advice, help and encouragement over many years have provided the stimulus and environment for the progression and success of our work. Responsibility for any errors of fact or statement are, however, exclusively our own. This is a rapidly expanding field and in the time taken to publish this manuscript we have no doubt that new advances in methods and techniques will have been made, new information on human diseases obtained, and previously unrecorded diseases reported. We will always be very pleased and interested to enter into correspondence and information exchange on new data, and any opinions and errors in this book.

We are most grateful to the many secretaries who have typed drafts and the manuscript at various stages, particularly Miss D. Wood, Miss A. Morgan, Mrs L. Lester, Mrs M. Moriarty and Mrs J. Setchell, and also Mr A. C. S. Thomas, Mr M. J. Madigan and Mrs B. M. Tracey for their help in assembling the appendix of mass spectral and gas chromatographic retention data. The Department of Medical Illustration at the Clinical Research Centre has been responsible for some of the illustrations used. Any work on patients described in this book and obtained in our own laboratories was approved by the Ethical Committee of Northwick Park Hospital and Clinical Research Centre.

We are very grateful to our publishers and to Mr R. Stileman for their patience over the time taken to produce the manuscript. We hope that this book will be useful not only as a reference work but also as a practical bench book for researchers, both present and future, clinical and non-clinical, in the field of the organic acidurias and of inherited metabolic diseases.

MRC Clinical Research Centre,
Harrow, London.
1981

R. A. CHALMERS
A. M. LAWSON

Contents

	Preface	xi
1	**Introduction**	1
	References to Preface and Chapter 1	6
	PART I: METHODS	9
2	**Extraction of Organic Acids from Physiological Fluids**	11
	2.1 Urine (and other protein-free fluids)	12
	2.2 Other physiological fluids	23
3	**Derivatives of Organic Acids**	27
	3.1 Derivatives of carboxylic acids and hydroxy acids	27
	3.2 Derivatives of oxo acids	39
4	**Separation, Quantification and Identification**	46
	4.1 Paper and thin-layer chromatography	48
	4.2 Liquid (column) chromatography	52
	4.3 Gas–liquid chromatography	55
5	**Gas Chromatography–Mass Spectrometry**	83
	5.1 Instrumentation	84
	5.2 Specialized MS techniques	94
	5.3 Mass spectra of organic acids and their derivatives	102
6	**Volatile Carboxylic Acids**	128
	6.1 Extraction of C_1–C_5 acids from physiological fluids	128
	6.2 Gas-chromatographic determination of free C_1–C_5 acids	129
	6.3 Gas-chromatographic determination of esters of C_1–C_5 acids	133
	References to Part I	136
	PART II: ORGANIC ACIDS IN PHYSIOLOGICAL FLUIDS FROM NORMAL SUBJECTS	161
7	**Organic Acids in Blood and Urine from Normal Healthy Adults and Children**	163
	7.1 Organic acids observed and quantified prior to GC–MS	163
	7.2 Organic acids observed in blood and urine using GC–MS	165
	7.3 Concentrations of organic acids in urine	176
	7.4 Concentrations of organic acids in blood	186

7.5 Volatile C_1–C_6 organic acids in blood and urine 187
7.6 Effects of diet, individual variation, drugs and artefacts on the apparent excretion of urinary organic acids 189

8 Organic Acids in Physiological Fluids Other than Urine and Blood 196
8.1 Amniotic fluid 196
8.2 Cerebrospinal fluid 200
8.3 Saliva 203
8.4 Other fluids 203

References to Part II 204

PART III: ORGANIC ACIDS IN HUMAN METABOLIC DISEASES (THE ORGANIC ACIDURIAS) 209

9 Organic Acids in the Study of Metabolic Diseases 211
9.1 Introduction 211
9.2 The acutely ill newborn and infant: screening for the organic acidurias 217
9.3 The prenatal diagnosis of the organic acidurias 221
9.4 Treatment of the organic acidurias 229
References 235

10 Disorders of Branched-Chain Amino Acid Metabolism 239
10.1 Introduction 239
10.2 Disorders affecting all three branched-chain amino acids: Branched-chain keto aciduria (maple syrup urine disease) 240
10.3 Disorders of L-leucine metabolism 244
Isovaleric acidaemia 250
3-Methylcrotonylglycinuria with 3-hydroxyisovaleric aciduria 258
3-Methylglutaconic aciduria 270
3-Hydroxy-3-methylglutaric aciduria 271
10.4 Disorders of L-isoleucine and L-valine metabolism 280
2-Methylacetoacetyl-CoA thiolase deficiency 283
References 289

11 Disorders of Propionate and Methylmalonate Metabolism 296
11.1 Propionic acidaemia 296
11.2 The methylmalonic acidurias 311
References 325

12 Specific Causes of Congenital Keto Acidosis in Infants 332
12.1 Succinyl-CoA:3-keto acid-CoA transferase deficiency 332
12.2 Acetoacetyl-CoA thiolase deficiency 333
12.3 Biochemistry of congenital keto acidosis 334
References 336

13 Disorders of L-Lysine Metabolism 338
13.1 2-Ketoadipic aciduria 340
13.2 Glutaric aciduria (glutaryl-CoA dehydrogenase deficiency) 344
References 347

14 The Dicarboxylic Acidurias 350
14.1 Oxidation of fatty acids in man 351
14.2 Congenital dicarboxylic acidurias 353
14.3 Systemic carnitine deficiency 361
14.4 Abnormal organic aciduria due to toxins 363
References 378

15 Congenital Lactic Acidurias and Disorders of Pyruvate Metabolism 383
15.1 Introduction: Normal metabolism of pyruvate and lactic acid production in man 383
15.2 Lactic acidosis and lactic aciduria due to uninherited disorders in the newborn and infant 387
15.3 Lactic aciduria as a secondary phenomenon in other organic acidurias 389
15.4 Disorders of pyruvate dehydrogenase and of the tricarboxylic acid cycle 390
15.5 Disorders of gluconeogenesis 394
15.6 Disorders of the respiratory chain 396
References 398

16 Organic Acidurias due to Disorders in Other Metabolic Pathways 405
16.1 L-Pyroglutamic aciduria (5-oxo-L-prolinuria) 405
16.2 D-Glyceric aciduria 408
16.3 Primary hyperoxaluria 409
16.4 D-Glucaric aciduria (saccharic aciduria) 414
16.5 Disorders of aromatic amino and organic acid metabolism 416
Disorders of L-phenylalanine metabolism: Phenylketonuria 420
Disorders of L-tyrosine metabolism: tyrosinaemia, tyrosinosis, tyrosyluria 428
References 436

Appendix of Mass-Spectrometric and Gas-Chromatographic Data 447

Index 497

1 Introduction

The 23 pairs of human chromosomes contain more than 50 000 different gene pairs or loci, certain genes specifying the sequence of amino acids in proteins and others controlling the rates or times of initiation of protein synthesis (Stanbury *et al.*, 1978). The structure of each gene is subject to ionation or mutation, the immediate effect of which is an alteration in the quality or quantity of a specific protein. The original concept of inborn errors of metabolism, of 'one gene–one enzyme' (Garrod, 1908, 1909; Harris, 1963) has been superseded by the modern concept of inherited metabolic diseases of one cistron – one polypeptide chain, the cistron being a functional unit of DNA controlling the synthesis of one polypeptide chain, many of which may go together to form a composite enzyme protein. At the time of writing, about 180 diseases are known in which a disorder of intermediary metabolism occurs as a result of an inherited single enzyme defect (Raine, 1972, 1974a; Watts *et al.*, 1975; McKusick, 1978; Brock, 1972; Stanbury *et al.*, 1978). Most of these diseases are of autosomal recessive inheritance, and, although the individual incidence of the homozygous or carrier state is low, the overall occurrence of inherited metabolic diseases is much higher. The morbidity and mortality of these diseases are high and, with severe mental and physical retardation often occurring in surviving cases of some disorders, the rarity of the individual disorders is no longer a valid reason for failing to consider them (Rosenberg, 1974). Additionally, the study of these diseases has been a primary factor in elucidating normal metabolic pathways in man.

Estimates of the overall incidence of inherited metabolic disease vary depending on the country concerned, the techniques and physiological fluids used in the screening programmes and the diseases included in the survey, but in live-born infants their occurrence may be conservatively estimated at about 3–4 per 1000 based on multinational surveys (for example, Council of Europe Report, 1973; Levy, 1974, 1976; Thalhammer, 1975; Chalmers *et al.*, 1977). The incidence of gene-influenced disease is much higher (Scriver, 1977) and it is to be remembered also that some population groups have an incidence of certain diseases higher than average (Stanbury *et al.*, 1978). The clinical case load is considerable with recessive and sex-linked genetic disease accounting for 5–8 per cent of paediatric hospital admissions and 8.5 per cent of paediatric deaths (Raine, 1974b; Stanbury *et al.*, 1978). Scriver (1977) places these figures as high as 30 per cent and 40 per cent respectively and also states

that at least 11 per cent of medical care for adults in hospital relates to gene-influenced disease. Several of the metabolic diseases are now treatable, early diagnosis followed by effective treatment often preventing the severe consequences of the diseases and allowing normal physical and mental development. In other diseases antenatal diagnosis and genetic counselling with the option of abortion of affected foetuses are of increasing importance when there is no adequate treatment and the affected individuals are severely handicapped or suffer distress and early death.

An abnormal pattern of amino acids in blood and/or urine has proved to be a valuable marker of one class of inherited metabolic disease, the amino acidopathies, and the systematic and detailed study of these metabolites in blood and urine has led to the recognition of several new disease states. The inborn errors detected by amino acid chromatography are limited to enzyme deficiencies affecting the first and sometimes the second stages of amino acid catabolism, and during the widespread screening programmes of the 1950s and 1960s the work done concentrated primarily on such disorders. This was due to some extent to the availability of suitable methods for screening and quantitative study such as established techniques of paper and thin-layer chromatography of the ninhydrin-positive amino acids and of the 2,4-dinitrophenylhydrazones of the deaminated keto acids. In their metabolic pathways, after removal of the α-amino nitrogen, the carbon skeletons of the amino acids undergo several degradative steps in order to introduce the modified molecule into the tricarboxylic acid (Krebs) cycle. The non-amino intermediates of these degradative pathways are generally all organic acids which often contain other functional groups such as hydroxyl, oxo and unsaturated bonds, and these acids have been studied relatively little until about 15 years ago, owing mainly to the lack of suitable analytical methods for their detection, identification and quantification. Thus, only relatively few organic acidurias were known until about 1966 (Table 1.1), and these were generally those associated with an amino acidopathy or detectable by other simple means, for example phenylketonuria and maple syrup urine disease (branched-chain keto aciduria), or associated with specific clinical symptoms, such as primary hyperoxaluria and alcaptonuria (homogentisic aciduria).

The identification in 1966 by Tanaka and his colleagues of isovaleric acidaemia, using gas–liquid chromatography and mass spectrometry (Tanaka *et al.*, 1966), opened up this new and challenging field to the potential of

Table 1.1 Organic acidurias known prior to 1966 (those associated with a diagnostic amino aciduria* or specific clinical symptoms† are indicated)

*Phenylketonuria	†Alcaptonuria (homogentisic aciduria)
*Tyrosinaemia/tyrosinosis	†Primary hyperoxaluria (type I)
*Branched-chain keto aciduria (Maple syrup urine disease)	Methionine malabsorption syndrome ('Oast house' disease)
*Histidinaemia	

systematic study, these techniques providing the most powerful tool available for the study of a wide variety of metabolites. The discovery of isovaleric acidaemia was soon followed by reports of methylmalonic aciduria (Oberholzer *et al.*, 1967; Stokke *et al.*, 1967), propionic acidaemia (Hommes *et al.*, 1968), pyroglutamic aciduria (Jellum *et al.*, 1970), and 3-methylcrotonoylglycinuria (Eldjarn *et al.*, 1970; Gompertz *et al.*, 1971). The rate of increase has been dramatic, and today more than 45 diseases are recognized in which organic acids accumulate in physiological fluids, including those with or without an associated amino acidopathy and some disorders of carbohydrate and lipid metabolism (Fig. 1.1 and see Tables 9.1 and 9.2 in Chapter 9). These diseases are collectively termed the organic acidurias and they are increasingly recognized to be of importance in paediatrics, metabolic medicine and clinical genetics.

The term organic aciduria is used here to mean metabolic diseases in which organic acids accumulate in the blood and urine. The terminology employed for a particular disease is often dependent on the nature of the physiological fluid under study at the time of discovery of that disease – if blood is being studied, the disease is often called an organic acidaemia, if urine, an organic aciduria. However, in our experience and that of others, and by study of the published literature, generally whenever an organic acid accumulates in the

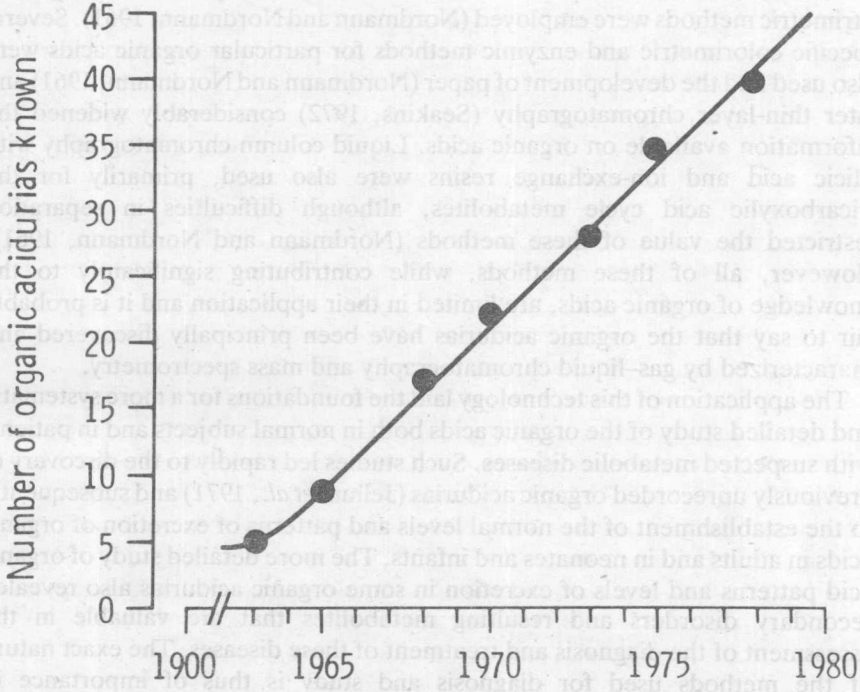

Fig. 1.1 Rate of increase in identification of organic acidurias.

blood at the levels observed in these metabolic disorders, the same acid and frequently several other important metabolites also appear in the urine. Urine is more commonly studied than blood and the nature of the metabolite observed in urine can often give a clue to secondary defects. In addition, in some diseases, the organic acids observed are found only in urine, and thus to avoid unnecessary confusion the organic acidaemias together with these diseases are called collectively the organic acidurias throughout the present text.

Before continuing further it is necessary to define the type of the organic acids to be discussed. The term has generally been considered to include all carboxylic acids with or without oxo, hydroxyl, or other non-amino functional groups. The amino acids are excluded, although other nitrogen-containing organic acids such as pyrrolidonecarboxylic acid (pyroglutamic acid) are included. Also included are short-chain fatty acids and nitrogen-containing amino acid conjugates, for example hippuric acid (benzoylglycine). The common features of these acids are high water solubility, acidity and ninhydrin-negativity (Tanaka, 1975), and thus the scope of the subject is wide and not limited to realtively few compounds as in the case of the physiological amino acids.

The aim of the first studies on organic acids in blood and urine was to determine the total amounts of these acids, and, in general, non-specific titrimetric methods were employed (Nordmann and Nordmann, 1961). Several specific colorimetric and enzymic methods for particular organic acids were also used and the development of paper (Nordmann and Nordmann, 1961) and later thin-layer chromatography (Seakins, 1972) considerably widened the information available on organic acids. Liquid column chromatography with silicic acid and ion-exchange resins were also used, primarily for the tricarboxylic acid cycle metabolites, although difficulties in separation restricted the value of these methods (Nordmann and Nordmann, 1961). However, all of these methods, while contributing significantly to the knowledge of organic acids, are limited in their application and it is probably fair to say that the organic acidurias have been principally discovered and characterized by gas–liquid chromatography and mass spectrometry.

The application of this technology laid the foundations for a more systematic and detailed study of the organic acids both in normal subjects and in patients with suspected metabolic diseases. Such studies led rapidly to the discovery of previously unrecorded organic acidurias (Jellum *et al.*, 1971) and subsequently to the establishment of the normal levels and patterns of excretion of organic acids in adults and in neonates and infants. The more detailed study of organic acid patterns and levels of excretion in some organic acidurias also revealed secondary disorders and resulting metabolites that are valuable in the assessment of the diagnosis and treatment of these diseases. The exact nature of the methods used for diagnosis and study is thus of importance in evaluating the results obtained; methods for extraction of the acids, for their

conversion into suitably stable and volatile derivatives for gas chromatography, for their separation, and for their absolute and unambiguous identification are fundamental to the study of the organic acidurias. In addition a detailed understanding of the qualitative and quantitative patterns of the organic acids in physiological fluids from normal subjects in all age groups are necessary in assessing the importance of metabolites observed in fluids from patients.

This book is therefore divided into three main parts, concerned with methods, data on normal subjects, and finally with the organic acidurias themselves. Preliminary screening for the organic acidurias may be done by paper or thin-layer chromatography and due reference to these techniques is made. However, the resolving power and scope of such methods is limited and while possibly suitable for screening for specific metabolites or for groups of metabolites that may approximately co-chromatograph [for example, the acylglycines (Tanaka, 1975)], the use of these techniques should be limited to preliminary screening only. Even then, some of the organic acidurias may not be detected by these means, and more comprehensive methods are essential. Liquid-chromatographic methods while having the advantage that derivitization may not be required, are still limited with respect to resolution and to methods of identification, detection and quantification, and generally gas chromatography–mass spectrometry is required additionally for this purpose (Sweetman, 1974). Liquid chromatography is presented, however, in some detail in this book, but the major emphasis is on gas chromatography and mass spectrometry and the techniques related to these analytical methods. Methods for the extraction from physiological fluids of the organic acids as a group prior to gas chromatography are discussed at length, as are the methods required subsequently to convert the acids into chemically and thermally stable but volatile derivatives. Gas chromatography is presented as the primary separation, quantification and preliminary identification technique with the use of mass spectrometry for absolute and unambiguous identification of the metabolites. These latter chapters are supported by an extensive compilation of gas-chromatographic and mass-spectrometric data on a wide range of physiological and pathological organic acids. Analysis of the C_1–C_5 organic acids requires separate techniques and these are dealt with in a chapter devoted to these compounds.

The second part of the book is concerned with the organic acids in physiological fluids from normal subjects and presents both qualitative and quantitative data on adults and on the normal infant and normal neonate. The prenatal diagnosis of organic aciduria by direct chemical analysis of amniotic fluid has been achieved and the potential of this technique is underlined by a section on the organic acids normally present in this fluid.

The final part deals with the organic acidurias themselves. In addition to their common chemical and biochemical features of the accumulation of water-soluble, ninhydrin-negative, organic carboxylic, hydroxy, oxo and other acids,

they often have common clinical features. The organic acidurias frequently present with acute symptoms in early life (neonatal and early infantile) with common signs of acidosis, ketosis, vomiting, convulsions and coma (Chapter 9). Early death may occur and survivors may often be physically or mentally handicapped. Early diagnosis by the comprehensive but specific methods detailed in this book, coupled with adequate therapy, may lead to survival and normal physical and mental development. Other organic acidurias may present with 'failure to thrive' or failure to pass developmental milestones, and careful screening of such patients for metabolic disease is important. The clinical heterogeneity of these diseases requires investigation of the patients in depth to define more precisely the nature of the underlying disorder, and organic acidurias due to nutritional or toxic factors need to be considered. All of these facets, including prenatal diagnosis and treatment, are dealt with in this section, with the major organic acidurias being discussed in some detail. The emphasis is on the diagnosis and study of these diseases by gas chromatography and mass spectrometry and details of clinical and enzymological studies are covered by references to relevant literature wherever possible, which it is hoped, will serve to introduce the interested researcher to a more detailed coverage of specific diseases.

References to Preface and Chapter 1

Brock, D.J.H. (1972), Inborn errors of metabolism. In *The Biochemical Genetics of Man* (eds. D.J.H. Brock and O. Mayo), Academic Press, London, pp. 428–460.

Chalmers, R.A., Watts, R.W.E. and Lawson, A.M. (1977), A comprehensive screening method for detecting organic acidurias and other metabolic diseases in acutely sick infants and children. *Ann. Clin. Biochem.*, **14**, 149.

Council of Europe working party to study hereditary metabolic diseases, Report (1973), Collective results of mass screening for inborn metabolic errors in eight European countries. *Acta Paediatr. Scand.*, **62**, 413.

Eldjarn, L., Jellum, E., Stokke, O., Pande, H. and Waaler, P.E. (1970), β-Hydroxyisovaleric aciduria and β-methylcrotonylglycinuria: a new inborn error of metabolism. *Lancet*, **ii**, 521.

Garrod, A.E. (1908), Inborn errors of metabolism (Croonian Lectures). *Lancet*, **ii**, 1, 73, 142, 214.

Garrod, A.E. (1909), *Inborn Errors of Metabolism.* (The Croonian Lectures delivered before the Royal College of Physicians, London, June 1908). Henry Frowde and Hodder and Stoughton, Oxford University Press, London.

Gompertz, D. (1972), Organic acidurias. In *Proceedings of 8th Symposium on Advanced Medicine* (ed. G. Heale), Pitman, London, pp. 270–284.

Gompertz, D. (1974), Inborn errors of organic acid metabolism. *Clin. Endocrinol. Metab.*, **3**, 107–130.

Gompertz, D., Draffan, G.H., Watts, J.L. and Hull, D. (1971), Biotin responsive β-methylcrotonylglycinuria. *Lancet*, **ii**, 22.

Harris, H. (1963), *Garrod's Inborn Errors of Metabolism* (reprinted with a supplement). Oxford University Press, London.

Hommes, F.A., Kuipers, J.R.G., Elema, J.D., Jansen, J.F. and Jonxis, J.H.P. (1968), Propionic acidaemia, a new inborn error of metabolism. *Pediatr. Res.*, **2,** 519.

Jellum, E., Kluge, T., Börresen, H.C., Stokke, O. and Eldjarn, L. (1970), Pyroglutamic aciduria – a new inborn error of metabolism. *Scand. J. Clin. Lab. Invest.*, **26,** 327.

Jellum, E., Stokke, O. and Eldjarn, L. (1971), Screening for metabolic disorders using gas–liquid chromatography, mass spectrometry and computer technique. *Scand. J. Clin. Lab. Invest.*, **27,** 273.

Levy, H.L. (1974), Neonatal screening for inborn errors of amino acid metabolism. *Clin. Endocrinol. Metab.*, **3,** 153.

Levy, H.L. (1976), Screening: perinatal aspects. In *Birth Defects, Risks and Consequences* (eds. S. Kelly, E.B. Hook, D.T. Janerich and I.H. Porter), Academic Press, New York, pp. 289–296.

Mamer, O.A., Mitchell, W.J. and Scriver, C.R. (1974), Application of gas chromatography–mass spectrometry to the investigation of human disease. (Proceedings of a workshop, Montreal, 1973). McGill University–Montreal Children's Hospital Research Institute.

McKusick, V.A. (1978), *Mendelian Inheritance in Man.* 5th edn., The Johns Hopkins University Press, Baltimore and London.

Nordmann, J. and Nordmann, R. (1961), Organic acids in blood and urine. In *Advances in Clinical Chemistry* (eds. H. Sobotka and C.P. Stewart), Academic Press, New York and London, vol. 4, pp. 52–120.

Oberholzer, V.G., Levin, B., Burgess, E.A. and Young, W.F. (1967), Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. *Arch. Dis. Childh.*, **42,** 492.

Raine, D.N. (1972), Management of inherited metabolic disease. *Br. Med. J.*, **2,** 329.

Raine, D.N. (1974a), Inherited metabolic disease. *Lancet*, **ii,** 996.

Raine, D.N. (1974b), The need for a national policy for the management of inherited metabolic disease. *J. Clin. Pathol.* **27,** [Supplement (Royal College of Pathologists) **8**], 156.

Rosenberg, L.E. (1974), Diagnosis and management of inherited aminoacidopathies in the newborn and the unborn. *Clin. Endocrinol. Metab.*, **3,** 145.

Scriver, C.R. (1977), A biomedical view of enzyme replacement strategies in genetic disease. In *Biomedical Applications of Immobilised Enzymes and Proteins, Vol. I* (ed. T.M.S. Chang), Plenum Press, New York and London, pp. 121–146.

Seakins, J.W.T. (1972), Chromatographic screening methods for organic acids in urine. In *The Organic Acidurias* (eds. J. Stern and C. Toothill), Churchill Livingstone, Edinburgh, pp. 175–182.

Stanbury, J.B., Wyngaarden, J.B. and Fredrickson, D.S. (eds.) (1978), *The Metabolic Basis of Inherited Disease.* 4th edn. McGraw-Hill Book Co., New York.

Stern, J. and Toothill, C. (eds.) (1972) *The Organic Acidurias* (*Proceedings of the 9th Symposium of the Society for the Study of Inborn Errors of Metabolism, 1971*), Churchill Livingstone, Edinburgh.

Stokke, O., Eldjarn, L., Norum, K.R., Steen-Johnson, J. and Halvorsen, S. (1967), Methylmalonic aciduria, a new inborn error of metabolism which may cause fatal acidosis in neonatal period. *Scand. J. Clin. Lab. Invest.*, **20,** 313.

Sweetman, L. (1974), Liquid partition chromatography and gas chromatography–mass spectrometry in identification of acid metabolites of amino acids. In *Heritable Disorders of Amino Acid Metabolism: Patterns of Clinical Expression and Genetic Variation* (ed. W.L. Nyhan), J. Wiley & Son, New York, pp. 730–751.

Tanaka, K. (1975), Disorders of organic acid metabolism. In *The Biology of Brain*

Dysfunction, Vol. 3 (ed. G.E. Gaull), Plenum Publishing Co., New York, pp. 145–214.

Tanaka, K., Budd, M.A., Efron, M.L. and Isselbacher, K.J. (1966), Isovaleric acidaemia: A new genetic defect of leucine metabolism. *Proc. Natl. Acad. Sci. U.S.A.*, **56,** 236.

Thalhammer, O. (1975), Frequency of inborn errors of metabolism, especially PKU, in some representative newborn screening centers around the world. *Humangenetik*, **30,** 273.

Watts, R.W.E., Chalmers, R.A. and Lawson, A.M. (1975), Abnormal organic acidurias in mentally retarded patients. *Lancet*, **i,** 368.

Part I Methods

Every scientific advance is an advance in method. The invention of a new specialized laboratory procedure brings about rapid conquests in new fields of science and technology; finally it exhausts itself and is replaced by a still more practical method.

Zechmeister and Von Cholnoky, 1943