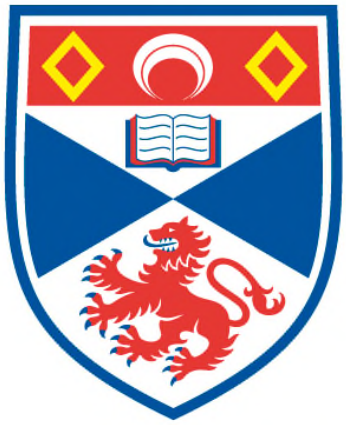
FIFTY YEARS IN INBORN ERRORS OF METABOLISM: FROM URINE FERRIC CHLORIDE TO MASS SPECTROMETRY AND GENE ANALYSIS

Neil R. M. Buist

A Thesis Submitted for the Degree of MD
at the
University of St Andrews



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Fifty Years in Inborn Errors of Metabolism: from Urine Ferric Chloride to Mass Spectrometry and Gene Analysis

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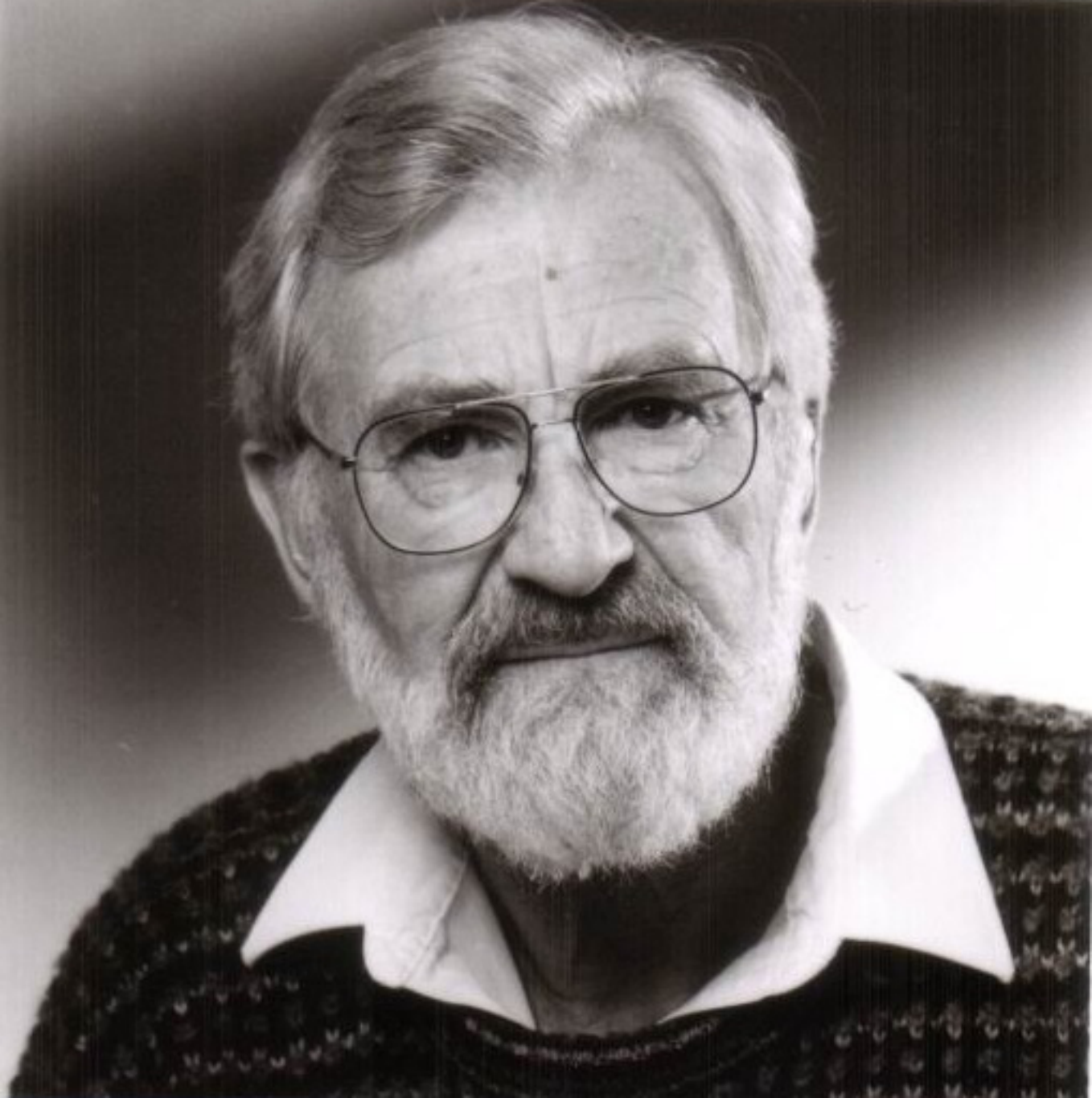

This Thesis has been awarded the 2014 Rutherford Gold Medal for life time achievement

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CURRICULUM VITAE

Birth: July 11, 1932, Karachi, India
Citizenship: British

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ARTICLES HIGHLIGHTED IN GREY ARE CITED IN THIS THESIS

UNDERGRADUATE EDUCATION:

Dalhousie Castle School, Scotland	9/40-12/45
Sedbergh School, England	1/46-7/50
University of St. Andrews, Scotland	0/50-6/56

PROFESSIONAL EXPERIENCE:

House Physician Internal Medicine, Arbroath Infirmary	8/56-2/57
Externe to Cardiopulmonary Hopital Marie Lannelongue, Paris, France	2/57-4/57
House Surgeon, Royal Hospital for Sick Children, Edinburgh, Scotland	4/57-10/57
Military Service: Regimental Medical Officer to various units, Far East	10/57-1/60
House Physician, Pediatrics, Royal Infirmary, Dundee, Scotland	1/60-8/60
Registrar, Internal Medicine, Maryfield Hospital, Dundee, Scotland	8/60-4/62
Lecturer in Child Health, University of St. Andrews, Scotland,	4/62-7/64
Research Fellow in Pediatric Microchemistry	7/64-7/66
University of Colorado Health Sciences Center	
Assistant Professor, Pediatrics, University of Oregon Medical School	7/66-7/70
Associate Professor of Pediatrics & Medical Genetics	
University of Oregon Health Sciences Center.	7/70-7/76
Professor of Pediatrics and Molecular & Medical Genetics.	7/76-7/96
Oregon Health & Science University	
Emeritus Professor of Pediatrics and Molecular & Medical Genetics	7/96-present
Oregon Health & Science University	
Clinical Faculty UCSF, [Children's Hospital of Central California].	2000-present

DEGREES/BOARD CERTIFICATION:

M.B., Ch.B., St. Andrews, with commendation	1956
D.C.H., (Diploma of Child Health), London.	1960
M.R.C.P.E., (Member, Royal College of Physicians)-Pediatrics	
Edinburgh, Scotland.	1961
E.C.F.M.G., Royal College of Physicians.	1964
Fellow, American Academy of Pediatrics	1980
Diplomate, American Board of Medical Genetics, Clinical Genetics.	1982

LICENSE:

UK.	1956
Oregon Medical License #MDO7658.	1970

HONORS:

Fellow, Royal Collage of Physicians, Edinburgh(FRCPE).	1975
Fogarty International Fellowship, NIH/Visiting Scientist to the Hopital Ste. Justine,	
University of Montreal, Montreal, Quebec, Canada.	1977-78
Listed in "The Best Doctors in America", by peer nomination.	1991-2006
OHSU/SOM, Basic Sciences Excellence in Teaching Award.	1993
Special Commissioners Award, US Food & Drug Admin. for special services.	1996
Presidents's Volunteer Service Award.	2007

VISITING PROFESSOR:

World Health Organization, China.	Mar.-Apr. 1988
University of Colorado Health Sciences Center, Denver,Colorado.	Apr 1990
Wesley Medical Center, Wichita, Kansas.	Jan 1991
Phoenix Children's Hospital, Phoenix, Arizona.	Feb 1991
Tucson Medical Center, Tucson, Arizona.	Feb 1991
University of Illinois, Chicago, Illinois.	June 1991
Kapoiolani Medical Center, Honolulu, Hawaii.	May 1992
Shriners Hospital for Crippled Children, Honolulu, Hawaii.	May 1992
Arkansas Children's Hospital, Little Rock, Arkansas.	Mar 1993
Visiting Professor, U of Rochester NY.	April 2003
Pago Pago, Western Samoa.	Mar 1994
Australian Society for Human Genetics, Auckland, New Zealand.	Aug 1994
Mahidol University, Bangkok, Thailand.	June 1996
University of PuertoRico.	Oct 1996
University of New Zealand, Auckland.	May 1997
University of New Zealand Auckland.	June 2004
Visiting Professor, UCSF to Valley Children's Hospital, Fresno	Dec 2000-present

SOCIETY MEMBERSHIPS:

British Medical Association.	1956-96
Western Society for Pediatric Research.	1967-present
Council Member.1972-75; 1988-91	
Pacific Northwest Pediatric Society.	1966-present
Society for Pediatric Research.	1973-78
American Pediatric Society.	1978-present
Society for the Study of Inborn Errors of Metabolism.	1973-present
U.S. Representative on Council. 1975-1993	
Society for Inherited Metabolic Disorders.	1977-present
Founding Member. 1977	
Treasurer. 1977-2001	
President.1998	
Oregon Pediatric Society.	1966-present
Oregon Diabetes Association	1976-1995
Portland Academy of Pediatrics.	1988-present
International Newborn Screening Society, Founding Member.	1988-present
Council Member. 1988-91	

TEACHING:

Medical & Molecular Genetics/1st Year courses. until 1996, Biochemistry/Cell organization & function/1st Year courses.until 1996, Pediatrics/3rd Year courses. until 1996. Electives & Clinic Rotations/Medical Students, Medical Genetics Students, Residents, Nutrition Course for paramedics.1990-present.
Nutrition course for medical Students.1985-present. Invited Hospital Grand Rounds around the country.1966-present.

SPECIAL EXPERIENCE:

Family Practitioner, UK Army Medical Services, Malaysia/Nepal.	1957-1960
Volunteer Medical Officer, International Rescue Committee	
Thailand/Cambodia Refugee Camps	1980
Volunteer Medical Officer, International Rescue Committee	
Sudan/Ethiopia Refugee Camps.	1985
Diabetic Children's Camp Physician, 1-3 wks, annually.	1967-92
Shriners Hospital for Crippled Children, Muscle Biopsy Clinic.	1989-96
Visiting Consultant, Children's Hospital of Central California,	2000-present
Volunteer Medical Officer, NW Medical Teams Int, Mazar-e-Sharif, Afghanistan	2002
Hosanna, Ethiopia. 2003. Thailand/Sri Lanka, [Tsunami relief].	2004-05
VolunteerMedical officer El Salvador, Cambodia, and Somalia.	2008-11

ADMINISTRATION: OHSU

Director, Pediatric Metabolic Laboratory. 1966-93.
Director, Metabolic Birth Defects Center. 1966-96

ADMINISTRATION: Other Organizations

Medical Consultant, Northwest Regional Neonatal Screening Program, 1970-present
Medical Advisory Committee, Tri-County March of Dimes. 1977-96
Oregon Diabetes Association Research Committee. 1977-80
Diabetic Children's Camp Foundation, Board of Directors. 1987-97
Board of Directors, Mize Information Enterprises 1987-97
Medical advisory Board Maple Syrup Disease Foundation. 1997-present

MAJOR PROFESSIONAL INTERESTS:

Metabolic Diseases, inborn or acquired
International nutritional problems

VISITING CLINICS/OUT OF STATE APPOINTMENTS:

Metabolic and Genetics Clinics in the following states and Medical Consultant, Pacific Northwest Newborn Screening:
Alaska, Nevada. 1990-1996
Idaho, Montana. 1980-2002

BUSINESS EXPERIENCE:

President & Chairman, Metabolic Nutritional Inc

GRANTS AND OTHER SUPPORT: (1992-2012)

Title: Consulting Contract for Metabolic Disorders
Agency: OREGON STATE HEALTH DIVISION (Renewed annually)
Dates: 7/1/96-6/30/97. Amount: $24,000/year.
Title: Quantitation of Oral Motor Function In Infants 1R43-HD/DK38234
Agency: NICHD
Date: 01/01 01-12/31/03. Amount: $100,000
Title: Quantitation of Oral-Motor Function in Infants 1R43-HD38234 [NICHD]
Date: 08/01-05-07/31-09. Amount: $750,000
Title: Quantitation of Oral-Motor Function in Infants 1R43-HD38234 [NICHD]
Date: 08/01/2010-6/30/2013. Amount: $2.5,000,000

US PATENTS AWARDED:

1. Title: A more Organoleptic Mixture of Aminoacids for Medical
Date: 1994 Patent #: 5,411,757
2. Title: A Pressure-or Flow-Sensitive Feeding Monitor
Date: 8/29/2000 Patent# 6,109,100

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Special Citation:

Roche, L.S.C. An unusual case of congenital pyloric stenosis. J. Royal Army Medical Corps., 455-457, Dec. 1933.

CHAPTERS IN BOOKS

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2. **Buist, N.R.M.,** Kennaway, N.G., Fellman, J.H.: Disorders of tyrosine metabolism in heritable disorders of amino acid metabolism. IN: Inherited Disorders of Amino Acid Metabolism, 2nd Edition.: W.H. Nyhan, Ed: Wiley & Sons, New York, 1974.

3. **Buist, N.R.M**., Kennaway, N.G., Fellman, J.H.: Inborn errors of tyrosine metabolism. IN: Inherited Disorders of Amino Acid Metabolism.: W.H. Nyhan; Ed. Wiley & Sons, New York, pp.160-176, 1974.
4. **Buist, N.R.M**.: Metabolic Screening of the Newborn Infant. IN: Clinics in Endocrinology and Metabolism. Aspects of Neonatal Metabolism. 2nd Edition. Ed: J. Forfar. Saunders & Co., London, 1976.
5. **Buist, N.R.M.** & Kennaway, N.G.: Inborn errors of amino acid metabolism. IN: Textbook of Pediatrics, 2nd Edition: J. Forfar and G. Arneil,Eds. Livingstone Press, Edinburgh, pp.1035-1063, 1978.
6. **Buist, N.R.M**.: Disorders of aromatic amino acid metabolism. IN: Practice of Pediatrics,: V. C. Kelley,Ed. Harper & Row, New York, 1980.
7. **Buist, N.R.M**.: Neonatal screening for metabolic abnormalities. IN: Management of High-Risk Pregnancy: John T. Queenan, Ed. Medical Economic Co., Oradell, N.J., 1980.
8. Weleber, R.G., & **Buist, N.R.M**.: Galactosemia and galactokinase deficiency. IN: Current Ocular Therapy: F.T. Fraunfelder & F.H. Roy, W.B. Eds. Saunders, Philadelphia, 1980.
9. **Buist, N.R.M**.: Principles of screening for amino acid disorders. IN: Practice of Pediatrics: V.C.Kelley, Ed. Harper & Row, New York, 1981.
10. **Buist, N.R.M**.: Metabolic Screening of the Newborn Infant. IN: Clinics in Endocrinology and Metabolism. Aspects of Neonatal Metabolism. 3rd Edition: J. Forfar. Ed. Saunders & Co., London, March 1981.
11. **Buist, N.R.M**., Kennaway, N.G., Kelly, T.E., Lloyd, J., Fernandes, J., Hume, R. and Forfar, J. O.: Metabolic Disorders. IN: Textbook of Pediatrics, Vol.2: J. Forfar and G. Arneil, Eds.
Livingstone Press, Edinburgh, pp.1196-1287, 1984.
12. **Buist, N.R.M**., Kennaway, N.G., Fellman, J.H.: Tyrosinemia type II: Hepatic cytosol tyrosine aminotransferase deficiency (The "Richner-Hanhart syndrome"). IN: Inherited Diseases of Amino Acid Metabolism: H. Bickel and U. Wachtel, Eds. Georg Thieme Verlag, Stuttgart, pp.203-235, 1985.
13. **Buist, N.R.M**. & Powell, B.R.: Hereditary disorders of fatty acid and carnitine metabolism. IN: Disorders of Fatty Acid Metabolism in the Pediatric Practice. Discovery International, pp.12-20, 1990.
14. Kennaway, N.G. & **Buist, N.R.M**.: Phytanic acid oxidase deficiency, infantile type. IN: Birth Defects Encyclopedia: M. L. Buyse, Alan R. Liss, Eds. New York, pp.1384-1385, 1990.
15. **Buist, N.R.M**., Kennaway, N.G. and Steinberg, D.: Phytanic acid storage disease. IN: Birth Defects Encyclopedia: M. L. Buyse, Alan R. Liss, Eds.New York, pp. 1385-1386, 1990.
16. **Buist, N.R.M**., Kennaway, N.G. & Steinberg, D.: Phytanic acid storage disease. IN: Birth Defects Encyclopedia, Ed., M. L. Buyse, Blackwell Scientific Publications, Cambridge, MA, 1991.
17. **Buist, N.R.M**., Prince, A.P., Huntington, K.L., Tuerck, J.M., Powell, B.R. & Waggoner, D.D.: Approaches to the dietary management of hyperphenylalaninemia. IN: Treatment of Genetic Disease, Ed.: Robert J. Desnick, Churchill Livingstone, New York, pp. 23-44, 1991.
18. **Buist, N.R.M**., & Tuerck, J.M.: The practitioner's role in newborn screening. IN: Pediatric Clinics of North America, Medical GeneticsII . Ed.: Judith G. Hall, W.B. Saunders Co., Philadelphia, Vol 39(2), pp. 199-212, 1992.
19. **Buist, N.R. M**., Kennaway, N.G., Powell, B.R., Fernandez, J., Hume, R., West, R. J., Lloyd, J. K., Besley, G. T. N., Kelly, T. E., Schutgens, R. B.H., Heymans, H.S.A. & Wanders, R. J. A.: Inborn errors of metabolism. IN: Forfar & Arneil's Textbook of Paediatrics, 4th Ed., Eds: Campbell, A. G. M., and McIntosh, N. Churchill Livingstone, Edinburgh, pp.1173-1255,1992.
20. Waggoner, D.D., Donnell, G.N., and **Buist, N.R.M.** Long-term prognosis in galactosemia: Results of a survery of 350 cases. IN: Galactosemia, New Frontiers in Research, Ed.: George N. Donnell. US Dept. Health & Human Services, Public Health Service, National Institutes of Health, February, pp.19-29,1993.
21. **Buist, N.R.M**. & Waggoner, D.D.: Galactosemia: Early treatment does not prevent long term problems. IN: New Horizons in Neonatal Screening.: Proceedings of the 9th International

Neonatal Screening Symposium and 2nd Meeting of the International Society for Neonatal Screening. J.-P. Farriaux and J.-L. Dhondt, Eds. Elsevier Science B.V., Amsterdam, pp.171-184,1994.
22. Tuerck, J.M. & **Buist, N.R.M**.: Problems in follow-up of abnormal hemoglobin (Hgb) screening results - U.S. data. IN: New Horizons in Neonatal Screening.: Proceedings of the 9th International Neonatal Screening Symposium and 2nd Meeting of the International Society for Neonatal Screening. J.-P. Farriaux and J.-L. Dhondt, Eds. Elsevier Science B.V., Amsterdam, , pp.207-209,1994.
23. **Buist, N.R.M**.: Concluding Remarks. IN: New Horizons in Neonatal Screening.: Proceedings of the 9th International Neonatal Screening Symposium and 2nd Meeting of the International Society for Neonatal Screening. J.-P. Farriaux and J.-L. Dhondt, Eds. Elsevier Science B.V., Amsterdam, pp.383-386,1994.
24. **Buist, N.R.M.** & Tuerck, J.M.: Quality control (QC) beyond the laboratory. IN: New Trends in Neonatal Screening: Proceedings of 1st Asian Pacific Regional Meeting, International Society for Neonatal Screening. N. Takasugi & H. Naruse, Eds. Hokkaido Univ. Press, pp. 43-46,1994.
25. Buist, A.S., Barker, A.F., D'Silva, R. & **Buist, N.R.M**.: Alpha-1-antityrpsin deficiency: Prevalence in the United States. IN: Alpha-1-Antitrypsin Deficiency, B. C. Trapnell, Ed. 1994.
26. **Buist, N.R.M**. & Tuerck, J.M.: Neonatal screening and carrier detection. IN: Diseases of the Fetus and Newborn, 2nd Ed., G. B. Reed, A.E. Claireau, F. Cockburn, Eds. Chapman & Hill, London, pp.1045-1054,1995.
27. **Buist, N.R.M.:** Disorders of Gluconeogenesis. IN: Inborn Metabolic Diseases: Diagnosis and Treatment, 2nd Edition. J. Fernandes, J.-M. Saudubray & G. van den Berghe, Eds. Springer-Verlag, Berlin, pp.101-106,1995.
28. **Buist, N.R. M**., Kennaway, N.G., Steiner, R., Fernandez, J., Besley, G.T.N.,Schutgens: Inborn errors of metabolism. In: Forfar & Arneil's Textbook of Paediatrics, 5th Edition, Eds.: Campbell, A.G.M. and McIntosh, N. Churchill Livingstone, Edinburgh, 1997.
29. **Buist, N.R. M**.: Disorders of amino acid metabolism. In: Textbook of Perinatal Medicine. Asim Kurjak, Ed. Parthenon Publishing Group, London, pp.911-933,1998
30. **Buist, N.R.M.** Seven chapters in The NORD guide to rare Disorders. Lippincott, Williams & Wilkins. Philadelphia. PA pp. 2003.
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32. Schrieber, D, Winter, S.C., **Buist N.R.M.** Carnitine Rx ETC

ARTICLES IN JOURNALS

1. Goodall, H.B., Guthrie, W., **Buist, N.R.M**.: Familial haemophagocytic reticulosis. Scott. Med. J. **10**:425-438, 1965.
2. **Buist, N.R.M**., O'Brien, D., Mijer, F.: Treatment of phaeochromocytoma with a B adrenergic blocking agent. Arch. Dis. Childh. **41**:435-436. 1966.
3. **Buist, N.R.M**., O'Brien, D.: The separation of peptides from amino acids in urine by ligand exchange chromatography. J. Chromatogr. **29**:398-402, 1967.
4. **Buist, N.R.M**.: A set of simple sideroom urine tests for the detection of inborn errors of metabolism. Brit. Med. J. **2**:745-749, 1968.
5. **Buist, N.R.M**., Strandholm, J.J.: Experience with combined amino acid peptide analysis of physiological fluid Technicon International Symposium, Mediad, Inc., New York, 321-326, 1970.
6. **Buist, N.R.M**., Higgins, J. V.: A simple gas pressure regulator to control elution rates during liquid column chromatography at pressures below 2 lb./inch2 . Clin. Chim. Acta **28:**368-369, 1970.
7. **Buist, N.R.M**. Campbell, J. J. , Castro, A., Brant, B. : Congenital islet cell adenoma causing hypoglycemia in the newborn. Pediatrics **47**:605-609, 1971.
8. Strandholm , J. J., **Buist, N.R.M**., Kennaway , N. G. : Homoargininosuccinic acid synthesis by an enzyme from pig kidney . Biochem. Biophys. Acta **237**:293-295, 1971.

9. Curtis , H. T., **Buist, N.R.M.** : Use of toluidine blue for sequential staining of urinary amino acid chromatograms. J. Chromatog. **57:**165-66, 1971.
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11. Tracy, W., Steen J. **, Buist, N.R.M.** : Analysis of dentine pathogenesis in Vitamin D - resistant rickets . Oral Surg, Oral Med , Oral Path, 32:38-44, 1971.
12. Strandholm, J. J., **Buist , N. R. M.** , Kennaway , N. G. , Curtis , H. T. : Excretion of ?-N- acetylcitrulline in citrullinemia. Biochem. Biophys. Acta **244**:214-216 , 1971.
13. **Buist, N. R. M.**, Jones, R. N. , Cavens , T. : Familial haemophagocytic reticulosis. Arch. Dis. Childh. **46**:728-729, 1971.
14. **Buist , N. R. M.** , Ramberg, D. A. , Strandholm, J. J. , Ferry, P. C. : Copper poppers : a benign cause of blue diapers. Arch. Dis. Childh. **46**: 873-874 , 1971.
15. Bellinger, J. F, **Buist, N. R. M.** : Rapid column chromatographic measurement of orotic acid. Clin. Chem. **17**:1132-1133, 1971.
16. Prescott, G.H., Magenis, R.E. and **Buist, N.R.M.**: Amniocentesis for antenatal diagnosis. Postgraduate Med. **51**:212-217, 1972.
17. Fellman, J.H., **Buist, N.R.M.**, Kennaway, N.G., Swanson, R.E.: The source of aromatic ketoacids in tyrosinemia and phenylketonuria. Clin. Chim. Acta **39**:243-246, 1972.
18. Kennaway, N.G., **Buist, N.R.M.**, Fellman, J.H.: The origin of urinary p-hydroxyphenylpyruvate in a patient with hepatic cytosol tyrosine aminotransferase deficiency. Clin. Chim. Acta **41**:157-161, 1972.
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24. Vidgoff, J., **Buist, N.R.M.**, O'Brien, J.S.: Absence of beta-N-acetyl-D-hexosamindiase A activity in a healthy woman. Am. J. Human Genet **25**:372-381, 1973.
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26 Fischer, G.W., Vazquez, A.M., **Buist, N.R.M.**, Campbell, J.R., McCarty, E., Egan, E.T.: Neonatal islet cell adenomas: case report and literature review. Pediatrics **53**:753-756, 1974.
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28. **Buist, N.R.M.**, Kennaway, N.G., Hepburn, C.A., Strandholm, J.J., Ramberg, D.A.: Citrullinemia: investigation and treatment over a four year period. J. Pediatr. **85**:208-214, 1974.
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30. **Buist, N.R.M.**: Neonatal screening tests for inborn errors of metabolism. Contemporary Ob/Gyn **4**:59-62, 1974.
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34. **Buist, N.R.M.**, Metabolic Screening of the Newborn Infant. Clin Endocrin Metab. **5:** 265-288. 1976.

35. Vidgoff, J., **Buist, N.R.M.**: Serum hexosaminidase activity in I-cell disease carriers. Hum. Genetics **36**:307-316, 1977.
36. Fellman, J.H., **Buist, N.R.M.**, Kennaway, N.G.: Pitfalls in human metabolic studies: The origin of urinary p-tyramine. Clin. Biochem. **10**:168-170, 1977.
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38. LaFranchi, S., **Buist, N.R.M.**, Murphey, W.H., Larsen, P.R., Foley, T.P., Jr.: Transient neonatal hypothyroidism detected by newborn screening program. Pediatr. **60**:538-541, 1977.
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43. LaFranchi, S.,Murphey, W.H., Foley, T.P., Larsen, P.R., **Buist, N.R.M.**: Neonatal hypothyroidism detected by the Northwest regional screening program. Pediatr. **63**:180-191, 1979.
44. Atkin, B.M., **Buist, N.R.M.**, Utter, M.F., Leiter, A.B., Banker, B.Q.: Pyruvate carboxylase deficiency and lactic acidosis in a retarded child without Leigh's disease. Pediatr. Res. **13**:109-116, 1979.
45. Illingworth, D.R., Connor, W.E., **Buist, N.R.M.**, Lin, D.S., Jhaveri, B.M. and McMurry, M.P.: Sterol balance in abetalipoproteinemia: Studies in a patient with homozygous familial hypobetalipoproteinemia. Metabolism **28**:1152-1160, 1979.
46. Kennaway, N.G., Weleber, R., **Buist, N.R.M.**: Gyrate atrophy of the choroid and retina with hyperornithinemia: biochemical and histologic studies and response to vitamin B6. Am. J. Hum. Genet. **32**:529-541, 1980.
47. **Buist, N.R.M.**: Is pyruvate carboxylase involved in the renal tubular reabsorption of bicarbonate? J. Inher. Metab. Dis. **3:**113-116, 1980.

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ABSTRACTS

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I would be remiss if I did not acknowledge the enormous influence that my medical ancestry played in determining from the age of seven that all I wanted to be was a doctor. My grandfather Robert Cochrane Buist was Chairman of the Department of Obstetrics and Gynaecology for St Andrews University in Dundee and my father, Lt Col Thomas Powrie Buist a dermatologist in the RAMC for 43 years. His two siblings were an ophthalmologist and a dentist respectively; two cousins were also physicians; all five trained at St Andrews. Since then our medical clan has grown considerably.

My career would never have been successful were it not for the aid of my spouse Dr Sonia Buist MD St And. And the support of my three daughters Catriona Buist PhD, Alison Buist PhD and Diana Buist PhD; all in the medical field but sadly none of whom were trained at St Andrews.

The real success of my programme in Oregon was due to two main support systems. The first was the charitable organization – The March of Dimes, started by President Roosevelt to support research and service for infants with paralytic poliomyelitis and subsequently extended to all infants with Birth Defects. For over 20 years, the March of Dimes supported me with grants that totaled over 1 million dollars. This enabled us to develop a world-class laboratory and the largest Metabolic Clinic in the US. The other critical support came in large part from the care and support with which my staff coddled and protected me from both administrative and scientific nightmares. Nancy Kennaway D. Phil. frequently saved my bacon in the laboratory, Judi Tuerck MS,RN was the bastion in both the clinic and in the newborn screening programme and Kathleen Huntington RD,MS my clinic nutritionist who kept the patients from damage due to my nutritional ignorance!. In all, we worked together as a team for a total of more than 90 years. All three independently developed internationally recognized reputations, Kennaway for her meticulous and seminal work in mitochondrial disorders, Tuerck in the field of newborn screening; in which she developed the concept of Newborn Screening Practice and Huntington in her political work in trying to get equitable insurance coverage for the nutritional care of patients with inborn errors of metabolism. Barbara Nagle and Leslie Lublink were the critical office support who, together, were with me for over 28 years.

CHAPTER I

INTRODUCTION:

"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable is hardly perceived unless we are deprived of them, or they become deranged in some way". [William Harvey: 1657, in a letter to a colleague].

This quotation may seem of strange origin and vintage to start this thesis. However, it embodies the very basis upon which my career eventually settled. In a rallying call that is anathema to Medical Students, Harvey applauds the study of "the rarer forms of disease", something that most Medical School teaching tends to avoid! It is serendipitous that Harvey also used the concept of "workings apart from the beaten path" thus predating the use of the concept of a biochemical pathway by several centuries! I have indeed used this quotation in many lectures to emphasize the point that a curious mind should be open to all sorts of new and unexpected wonders, that rare disorders can indeed inform us about many more mundane problems and that this applies particularly to areas of Medicine where almost everything still waits to be discovered.

Many of the reports in my CV represent either the first known case of some disorder or a very early case in which we had done some special studies. I make no apologies for having reported upon so many rare disorders nor for including them in this anthology. In each case, I believed that they offered something new to the emerging science of metabolism. In addition, when a rare disorder is "discovered" through a few case reports, it always turns out that additional cases have been lurking in other clinics around the world and also that, once recognized, even more additional cases are found.

Repeatedly in this collection, it can be seen how the initial reports of a condition, often little more than clinical observations, have led over the years into far more in depth understanding of physiology, enzymology, organelle pathophysiology, gene identification

and an understanding of gene function. In the future, I expect to see many new advances in gene therapy.

References derived from my CV are given as the numbers in the CV and highlighted in grey. Other references are included in the text identified by the name of the first author.

My background.

I arrived in University College, Dundee in 1950 to study Medicine. At that time it was still thought that humans had 48 chromosomes, [the correct number, 46 was only identified in 1956, the year I graduated]. I will recall the excitement when vitamin B12 and the the "mycin family of antibiotics were discovered. Many of my teachers had still been trained in the pre-antibiotic era and much of our teaching was coloured by pre- and immediate post-war medical practice. I was sure that I wanted to become a country GP similar to AJ Cronin, one of my boyhood heroes. By the time I graduated, however, I had become fascinated by the unusual, the rare and challenging cases – an interest fostered by the late Dr Walter Strauss in Arbroath Infirmary.

During my post-graduate training, this bent became entrenched and I mutated into becoming a University Professor, whose specialty was in the most rare and esoteric of areas – namely the nascent field of Clinical Genetics and especially Inborn Errors of Metabolism [IEM]. This might have seemed like an unlikely direction to follow especially since I recall that the sum total of lectures in physiological biochemistry we received in UCD had numbered only five! When my conversion started, there were no laboratories that specialized in this field, no analysers available to detect quantitative abnormalities plasma amino acids or other metabolites, there were only a handful of disorders that no physician really expected ever to encounter and there were no specialists in this field. The standard method for detecting most metabolites was by paper or thin layer chromatography. It was a triumph just to establish a real diagnosis.

In the course of my registarships in Dundee Royal Infirmary, it became apparent to me and my professors that I would be best suited to a career in some branch of academia. At that time, in order to achieve that, it was almost essential to obtain a "BTA" status [BTA – Been To America!] since there was still hardly any opportunity to do research in post-war UK. In 1963, I elected to try for a Fellowship in renal disease in the US, but Professor John Henderson [Department of Child Health] arranged a fellowship for me to work in Denver, Colorado with Dr Donough O'Brien in his new microchemistry laboratory. After my rudimentary basic lectures in biochemistry this prospect frightened

me so much that I armed myself with a slip of paper on which the formula for each amino acid was displayed; it stayed in my wallet for many years!

Once in Colorado, I became intimately familiar with all the workings of an amino acid analyser, [I could repair them and keep them running and even build one from components], and all of the exciting new diseases that such a machine could surely detect. When I first started, a complete run on this machine took 21 hours to complete. By the end of my fellowship we had reduced this time to six hours and with better resolution. Such analysers were just becoming more available in the US, designed for use in centres specializing in the nascent field of Biochemical Genetics. One technical achievement I did make was able to develop a method that separates the free amino acids from all peptides in urine and this is the technical article I have chosen for this treatise. [See reference 3 in Chapter XII].

At the end of my two-year fellowship it transpired that there would be no amino acid analyser available to me if I returned to Scotland and I thus reluctantly decided to stay in the US at least for a few years. I was appointed to a new position in the University of Oregon Medical School with the mandate to develop a clinical centre for the diagnosis, investigation, treatment and research into inborn errors of metabolism. To start with, this whole program was funded by a grant for $30,000 from the March of Dimes without which both I and my programme would never have been able to grow and to flourish.

Biochemical Genetics as a specialty gets started.

The first group to formalize the field of metabolic diseases was the Society for the Study of Inborn Errors of Metabolism [SSIEM] in the UK; it was only founded in 1963. Dr O'Brien was one of the originators of the specialty and also founding father of the Society for Inherited Metabolic Disorders [SIMD] in the US, but even this American counterpart to the SSIEM was only founded in 1987; I too was one of the founding members and was the treasurer for 25 years. Other Societies have followed with groups in Canada, South America, Europe, India, China, Japan and elsewhere.

It will be clear that my career in this specialty spans the entire academic history of the specialty. As would be expected, much of my bibliography relates to clinical detection and evaluation. It has been exciting to watch the evolution of this specialty from a kind of "Gee-Whiz" mentality into one of the most academic specialties in the whole of Medicine; my colleagues have been the pioneers in the application of DNA technologies to clinical situations, both for diagnostic reasons and also for potential therapies. I have had the great fortune to work all this time with the evolution of this specialty into a field that has

specialists in most countries and covers over 1000 disorders each with many genetic variants. A web site dedicated to sharing information about problems and questions in this field [Metab-L] has contributors from over 100 countries. The standard reference on the subject is a five-volume textbook, now only available online – The Online Metabolic and Molecular Bases of Inherited Disease edited by Scriver. Biochemical Genetics is now a full-fledged specialty in its own right. Several major reference textbooks are shown at the end of this chapter.

My role in Newborn Screening.

From the outset, I worked not only at the University but also with the state newborn screening laboratory where the world's first routine screening for phenylketonuria [PKU] was started early in 1961. This added another dimension to my interests in that I was integral to many of the decisions and developments that were occurring in newborn screening. The Oregon newborn screening programme was one of the pioneers in this field and it has maintained its preeminent position ever since. We became known for our work with aminoacidopathies but soon we were branching out to many of the other disorders that were being discovered and unraveled. Our programme introduced many novel tests, maple syrup urine disease, muscular dystrophy [See Reference 94 Chapter CC]. We also screened 100,000 infants for alpha-1-antitrypsin deficiency and detected 22 cases giving a far higher incidence than was thought at the time; as far as I know, no other screening programme has ever repeated our experience and none has reported on the outcome of such cases after 20 years [80].

We were the first place in the world to start screening for congenital adrenal hyperplasia which we did because of a very high incidence of this disorder in Alaskan Natives. We also included screening for maternal PKU in the state programme of prenatal testing for syphilis; we tested 330,000 women and found 15 cases with variably elevated blood levels of phenylalanine. Most had been born before the advent of routine newborn screening. This was a startling number that showed that not all patients with hyperphenylalaninaemia were grossly delayed and cemented the idea that many milder cases of this disorder were present in the community. The multiparous women who had previously had children that were developmentally delayed.

My clinic nurse, Judi Tuerck RN, developed the seminal idea that newborn screening must not be considered only a laboratory programme but as a complete system that included not only the initial collection and handling of specimens, the total activity in the screening laboratory, the follow-up and treatment and also the essential evaluation of the

success of all these components [69]. Thus was born the concept of Newborn Screening Practice and its essential role in quality assurance inthese programmes. This was surprisingly a hard concept for many since most screening programmes at the time were based entirely in state laboratories that had little or no contact with any clinical follow up. We also developed the first regional screening centre in the US eventually growing to include seven other states. At one stage it spanned from Delaware in the east coast of the US to Khatmandhu in the Far East, an area close to that of Western Europe but with a population of only about 8-10 million.

My greatest success in this field was to get screening for neonatal hypothyroidism started in the US, we became only the second centre in the world to do this [38,43]; it is now mandatory in most screening programmes around the world.

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My Clinic.

The clinic that I established at the University eventually became the largest of its kind in the US. This was partly because we had our own laboratory and because I decided from the start that we should include all referrals for potential metabolic disorders and see people of all ages. Soon I was holding clinics throughout Oregon and then in Idaho, Montana and Nevada as well as some in Alaska. Once again the area and the population were about the same size as that covered by the screening programme. I would travel up to 1000 miles for such clinics and some patients would still have to travel 300 miles just to see me in consultation – a true outreach system!

By the end of my tenure at the University, over 150 different metabolic disorders were represented in the clinic roster. Some were the only known cases either in the US or even the whole world. The oldest patients were both 90 years old, one with hereditary fructosaemia and the other with gyrate atrophy of the retina [a defect of ornithine metabolism].

Many specialist's careers tend to concentrate on one or only a few areas of special interest during their career. I however, being responsible for all metabolic diseases, was able to explore a wide variety of disorders. Such huge diversity led inevitably to endless potential topics for research much of which could be conducted in my own laboratory. With passing years and more experts available, it became increasingly advantageous to share patients and samples with people who had more in depth experience in specific problems. These days, when only a handful of cases of a disorder are known in the whole world, there is now usually someone who has made it their career interest and with whom it is best to collaborate for optimal results. This evolution is reflected throughout my CV in which over 40 different inborn

errors are represented and in which many studies are shared with other centres. To some extent, I viewed myself as a clinical pimp whose job it was to detect interesting cases and then get them studied elsewhere in more depth than we could ever hope to provide in Oregon.

My Laboratory.

Much of the credit for the growth and success of our programme must be attributed to Nancy Kennaway D. Phil. who was in charge of the laboratory and who all too frequently had to rein in my enthusiasm to study the newest and latest problem we had uncovered in one of the clinics. The mainstay of our testing was a batch of simple screening tests [4] accompanied by one-dimensional paper chromatography of blood or urine. This "Metabolic Screening" yielded huge dividends in diagnosis and the detection of unusual metabolic patterns. The laboratory had a great deal of freedom [before the advent of layers of administration] to be able to develop and explore any area that we thought to be of interest or value in the field. Dr Kennaway's fastidious attention to detail gave to laboratory a world-wide reputation [and often saved my bacon as well!]. It was the combined association of the newborn screening, the clinics and particularly my Metabolic Laboratory that helped to make this centre so successful in so many areas.

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CHAPTER II.

SOME BACKGROUND ON INBORN ERRORS OF METABOLISM

What are Inborn Errors of Metabolism?

So what exactly is this arcane field of Medicine that specializes in "Inborn Errors of Metabolism? The term was coined by Sir Archibald Garrod in the Croonian lectures in 1908. These lectures were all reprinted in The Lancet where their significance lay unrealized for several decades. Garrod was the first to connect a human disorder with Mendel's laws of inheritance. He also proposed the idea that certain diseases came about through disruptions in specific metabolic processes. With this great flash of insight, Garrod cemented together the two basic tenets of Biochemical Genetics, namely that they were caused by defects in enzymes or protein structure and that they were caused by genetic variants. He originally described four disorders that included Alcaptonuria, Albinism, Cystinuria and Pentosuria and presciently laid out pretty well the whole concept in two stunning paragraphs as follows:

> *"The view is daily gaining ground that each successive step in the building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose.*
>
> *"It may well be that the intermediary products formed at the several stages have only momentary existence as such, being subjected to further change almost as soon as they are formed; and that the source of metabolism along any particular path should be considered as in continuous movement rather than as a series of distinct steps. If any one step in the process fail the intermediate product in being at the point of arrest will escape further change, just as, when the film of a biograph is brought to a standstill, the moving figures are left with foot in air. All that is known about the course of catabolism tends to show that in such circumstances the intermediate product in being is wont to be excreted as such, rather than it is further dealt with along abnormal lines –with the result that products are formed which have no place in the normal body chemistry."*[Garrod].

The wider field of metabolic disease.

We should now turn attention to the wider field of metabolic disorders in order to demonstrate the breadth of the specialty and the pervasive presence that can manifest by dysfunction of almost every biochemical process, in every tissue and in every cell type. At the outset, it seemed as though the category of Inborn Errors of Metabolism would, or should, be restricted to disorders in which some biochemical abnormality could be detected. Thus, the results of a mutant enzyme function resulting in abnormal elevation of a metabolite was surely a metabolic problem but, for example, a defect of collagen structure was not, even though both such problems could mimic each other in certain cases. In the early days,

clinical geneticists were trained in chromosomal analysis and the recognition of dysmorphic syndromes. They rarely became involved biochemical genetics and thus our specialty became the "go-to" place when there was any search for a CAUSE as opposed to the mere recognition of a syndrome.

This field has not stood still. Since I retired in 1996, there have been great new technologic advances. Newborn Screening now routinely detects over 40 different conditions and detects far more cases than were found before screening was available. We are finding that the picture is more complex than was thought. Mild variants and genetic heterogeneity add greatly to the complexity of diagnosis and management.

From the outset, the people attracted to this specialty, have concentrated predominantly upon trying to tease out the underlying aetiology of a problem in a laboratory. Indeed there have always been many more research workers than clinicians in this field. Metabolic specialists, initially involved with the biochemistry of genetic disease, were soon embroiled in unraveling basic problems of cell biology. As the science of genetics and the unraveling of the human genome have exploded, our specialty has led the way in applying this information, not only to biochemical disorders but also to unraveling much of the basic science of clinical dysmorphic syndromes. What seemed decades ago as a clear separation between biochemical genetics and syndromic genetics, has narrowed. An example of this can be seen in what might be termed "ciliopathies". Disorders that have apparently have nothing in common with each other such as polycystic kidney disease, the Bardel-Biedet syndrome, Lowe syndrome, Jeune asphysiating thoracic dystrophy and the Alstrom syndrome are all associated with problems in genes that have to do with the early development of cilia [Saey]. While this is of little immediate clinical import, it does show how dysmorphism and biochemistry have intersected.

The explosion of high resolution DNA technology and its applicability to clinical practice and better biochemistry and have led to the possibility of screening either parts or even routine sequencing of the whole genome so that it is now possible to diagnose some disorders from the DNA pattern rather than from an abnormal biochemical profile. As our understanding of DNA improves, we an expect this approach will become ever more main-stream, not only in clinical genetics but in many other fields such a the diagnosis and management of malignant disease. Even at the present time, in making a genetic diagnosis these days, it is not appropriate just to recognize some specific pattern of physical or biochemical abnormalities; it is necessary to revert to the Internet to access the latest information about potential diagnoses, work-up, evaluation and even treatment.

Incidence of Metabolic Disorders.

Over one thousand disorders are now classed as inborn errors of metabolism: virtually every one of them is rare. Disorders such as PKU [~1:15,000] births and Galactosaemia [~1:50,000] are considered common. Some others are stock-in-trade conditions such as the lysosomal disorders Gaucher or Tay-Sachs diseases and should be well known, if not

clinically, at least by reputation, to all practitioners even if they never encounter a case during their whole career. Most metabolic disorders however, are much rarer with incidences that are usually < 1:100,000 and for many there are only a handful of cases known in the whole world. Even the most experienced metabolic specialist can never expect to encounter all of these in a full career. Taking all known metabolic diseases that can manifest in childhood, together, the overall incidence is considered to be ~1:1,400 [Applegarth] For a whole population, this is a marked underestimate since it does not include some common adult onset disorders such as gout [~1:100] idiopathic hypercalcuria [~1:100] let alone the primary hyperlipidaemias.

Major Categories of Inborn Errors of Metabolism.

In order to present the breadth of this specialty, these are laid out in Table I. Approximate numbers of disorders in each category are given.

Symptoms of Inborn Errors of Metabolism.

There is almost no function of any cell in any organ that cannot be affected by an inborn error of metabolism. It is because the manifestations are so protean that metabolic clinics tend to act as a resource last resort for clinicians stumped by cases within their own specialties. It must be said though, that even when a biochemical cause can be identified, the real primary, proximate cause of the symptoms is often not clear. In spite of decade of research and many millions of dollars, the aetiology of the brain damage in phenylketonuria is not known.

Table I

Classes of Inborn Errors of Metabolism

Disorders of carbohydrate metabolism ~40

E.g., Glycogen storage disease, defects of monocarbohydrate metabolism

Disorders of amino acid metabolism >40

E.g., Phenylketonuria, maple syrup urine disease, histidinaemia

Urea Cycle Disorders 10

E.g., Carbamoyl phosphate synthetase I deficiency, citrullinaemia

Disorders of organic acid metabolism (organic acidurias) >20

E.g., Glutaric aciduria type I

Disorders of fatty acid oxidation, carnitine >20

E.g., Medium-chain acyl-coenzyme A dehydrogenase deficiency, carnitine palmitmoyl transferase deficiency

Disorders of porphyrin metabolism >10

E.g., Acute intermittent porphyria

Disorders of purine or pyrimidine metabolism ~15

E.g., Lesch-Nyhan syndrome, gout

Disorders of steroid and hormone metabolism ~20

E.g, Smith-Lemli-Opitz syndrome, congenital adrenal hyperplasia

Disorders of mitochondrial function >60

E.g., Kearns-Sayre syndrome

Disorders of peroxisomal function ~15

E.g., Zellweger syndrome, infantile Refsum syndrome

Lysosomal storage disorders >50

E.g., Gaucher's disease, Tay-Sachs disease

Disorders of the Golgi apparatus >50

E.g., the Carbohydrate Deficient Glycoportein syndromes

Disorder of the sarcolemma ?10

E.g., Rippling muscle disease

Miscellaneous enzyme defects >20

E.g., Hypophosphatasia

Table Illsome generalia

I symptoms suggesting an Inborn Error of Metabolism

- Growth failure, failure to thrive, weight loss
- Recurrent vomiting, diarrhea, abdominal pain
- Developmental delay, seizures, dementia, encephalopathy, stroke
- Abnormal behavior, depression, psychosis
- Deafness, blindness, pain agnosia, Retinopathy
- Skin rash, abnormal pigmentation, lack of pigmentation, abnormal hair
- Dental abnormalities
- Immunodeficiency, thrombocytopenia, anemia, hepatosplenomegaly

- Some forms of cancer
- Polyuria, renal failure, dehydration, edema, Fanconi Syndrome
- Cardiomyopathy, myocardial infarction
- Hepatomegaly, jaundice, liver failure
- Dysmorphic facial and physical features
- Myopathy, muscle weakness, cramps
- Osteodystrophy
- Ambiguous genitalia, delayed puberty, precocious puberty
- Hypothyroidism, adrenal insufficiency, hypogonadism, diabetes mellitus

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CHAPTER III

Lessons from Phenylketonuria [PKU].

The specialty of Biochemical Genetics would probably have remained an academic curiosity had not a Swedish clinician, Dr Asborg Folling, in 1934, discovered a disorder of phenylalanine metabolism that he called *imbecillitas phenylpyruvica* - the disorder now called phenylketonuria [PKU]. These subjects were severely retarded and excreted large quantities of phenolic compounds in their urine, hence the name. They were later shown to have very elevated blood levels of phenylalanine.

It is worth while to expand a bit on this disorder since PKU became, and still is, the paradigm of inborn errors of metabolism and it has coloured our thinking for decades as we learned more about the pathophysiology, the variants, the genetics and the treatment.

Initially the search was for the primary enzymatic defect and phenylalanine hydroxylase deficiency was confirmed within a few years. The next order of business was to consider treatment by the use of a diet in which phenylalanine was largely excluded. By 1952 Dr Marvin Armstrong in the US was able to make such a product but the cost was many thousands of dollars and there were not enough amino acids available to sustain even a vestigial effort. In 1949 Dr Louis Woolf in Great Ormond Street proposed a method to make a product and some years later, Dr Horst Bickel persuaded him to make some to treat a child with PKU; it was quickly shown that she responded remarkably and rapidly to the treatment. This fueled the idea that detection in the newborn, followed by treatment, might prevent the brain damage.

This led Dr Robert Guthrie in Buffalo NY to develop the Guthrie blood spot test for phenylalanine. It was first deployed routinely in both Oregon and Massachusetts and was the standard method for newborn screening for PKU for 40 years until new technology replaced it. Guthrie's approach to the use of dried blood spots was totally revolutionary, it has facilitated the expansion of screening programmes around the world and is still the way in which blood samples are obtained. Once blood is dried on the paper, almost all metabolites, antibodies and even enzymes are stable for years and even most enzymes can be reliably assayed after a long time. Moreover, these samples can be collected in homes or villages and can be sent to a far-distant central testing laboratory. Early testing in institutions for the mentally retarded revealed that one percent of such inmates had indeed got PKU and this added hope to the basic idea of early diagnosis and treatment.

At the beginning, there was a very steep learning curve about every aspect of such screening and treatment. There were extraordinarily fierce arguments about whether the treatment was beneficial or harmful. Certainly quite a few infants did suffer and some even died from phenylalanine deficiency due to ignorance and over-zealous treatment before the general parameters of therapy were worked out. It took 20 years of a huge international collaborative study to sort out the major problems. Over decades, detection and treatment have become more routine and more standardized but the details of such therapy are still evolving and are still a cause for research.

This disorder cemented the idea that a disorder of metabolism could cause profound problems and finally opened curious eyes to the possibility of other disorders and also the possibility of treatment.

Originally we thought that since PKU caused brain damage, then surely every other similar disorder, with elevated blood levels of an amino acid or other metabolite, would do the same. It became the rage, in which I was also involved, to test whole populations of mental institutions using paper chromatography in the hope of finding additional disorders and patients who might be amenable to biochemical modification of their underlying disorder. Many new disorders were indeed uncovered and many patients were subjected to various forms of treatment to try and correct the biochemical abnormalities without any preliminary data suggesting that such an approach might work.

One disorder that illustrates the confusion about indiscriminate testing was histidinaemia for which newborn screening was deployed for decades. fierce debates soon evolved in two of the prime metabolic centres in the North America. One group maintained that screening and treatment were indeed warranted and beneficial and the other insisted that they were unnecessary and that the disorder was benign. This was not resolved until a huge study on 540 cases from Japan, where the condition is common, indicated that indeed histidinaemia was a benign condition and that screening and treatment should be stopped [Tada].

In the past ten years newborn screening has been revolutionized by the development of new technology. By the use of a single test, it is now possible to detect as many as 40 metabolic disorders and this is now universal in the US and in many other countries. The same situation that existed over histidinaemia still exists with the expanded newborn screening now in use. For several of the obvious metabolic abnormalities detected by this technology, treatment has become routine even before the significance of the abnormality has been evaluated. It will take many years more to sort out the whole situation.

Phenylketonuria has continued to teach us many new things. There was the discovery that high blood phenylalanine profoundly affects foetal development causing several birth defects including profound brain damage causing what is now known as the Maternal PKU Syndrome. Another big international study was required and now we know that it is essential that the blood level of phenylalanine in women with PKU be lowered almost to normal during the pregnancy and that such an approach can save the infant from such damage [Hanley].

When treatment was first available for diagnosed infants, it was thought that it was only needed until brain development was largely completed, perhaps about six or seven years of age. Not so, relaxing the diet during childhood leads to poor school performance and attention problems that can last until the diet is reestablished. The diet is now recommended for life. All very well for the prescribers but not so good for the subjects. Fifteen years ago the only products that were easily available were based on mixtures of free amino acids from which the offending amino acid was absent; all such were considered as “infant formulas”. These were, and indeed are, totally impossible for older patients to use especially if they are required to take 3-400 g/day of the product. I have worked for >20 years with industry to develop new approaches to this problem and this is addressed in Chapter XX.

The next part of the PKU saga came with the development of genetic testing and DNA technology. It was well recognized that there were several sub-varieties of PKU, usually classified as severe, moderate or mild. The genetic explanation is now evident; as of this time there are well over 700 mutations in the hydroxylase gene that are known; some are benign variants while others affect the

function of the enzyme to various degrees. Genetic heterogeneity now can fully explain these variations. Additional defects are known to occur in the cofactor [biopterin] metabolism of all aromatic amino acid hydroxylases. These also cause hyperphenylalaninaemia but require totally different treatment.

Finally, the newest approach to treatment of certain enzyme defects is to attempt to alter the 3-dimensional folding of the mutant protein in such a way as to improve its overall function. This can sometimes be done with specific "chaperones" that can rearrange the tertiary folding of the mutant proteins in such a way that they can function better. Efforts to achieve successful gene therapy are progressing but have not yet made it into human trials [Cerreto].

All the lessons learned about PKU can be applied equally well these days to almost every other inborn error of metabolism. Initial descriptions followed by detailed biochemical analyses, discussions regarding treatment and research into understanding the genetic basis and variability of the disorder.

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CHAPTER IV

My role in the development of a new approach to the composition of Medical Foods for the treatment of aminoacidopathies

The Problem:

Until the 1970s, the foods for the treatment of PKU and other aminoacidopathies were almost all based on infant formulas manufactured by the infant food manufacturers. The nitrogenous components of these products were assembled either from hydrolysed casein or free amino acids some of which have disgusting tastes and or smell. For infants and small children this was not considered a real problem but as soon as the diet was recommended for older patients there was real trouble. How could one expect a grown child, a teenager or an adult, especially if pregnant, to take these products in anything like enough quantity to have any meaningful effect? The rate of dietary non-compliance was almost total! At that time there was hardly any alternative products that could be used to provide calories let alone to satisfy both hunger and food acceptance.

The treatment of phenylketonuria [PKU] by dietary restriction of the amino acid phenylalanine was developed in the UK and first tried in 1959. In the initial cases, the trials produced what seemed to be a miraculous improvement. Within a few years such an approach became commercially and medically possible which fueled the interest in screening for PKU in the newborn; a programme that has become routine in many parts of the world.

In the early years of screening and treatment of PKU, treatment was started immediately on diagnosis and for this, phenylalanine-deficient milk products that could be used as replacement of normal infant feeds were the only products that seemed to be necessary. These were prepared as powdered infant formulas based either upon hydrolysed, processed casein or mixtures of all the amino acids, except phenylalanine. The amino acid profiles of these were based on the approximate amino acid profile of cow's milk proteins which was thought to be the most desirable composition. In fact, this profile was badly biased in that the standard values were obtained, as for all foods at the time, by the old, but standard, technology of acid hydrolysis of proteins. This process converts the amide amino acids [glutamine and asparagine] to the dicarboxylic acids glutamate and aspartate respectively. While the amides do not taste too bad, the dicarboxylic acids both have highly intrusive flavours. Glutamate is the basis of the condiment monosodium glutamate [MSG] and we all know how little of that is used to flavor a dish.

These infant formulas, without exception, tasted horrible; the dicarboxylic acids glutamate and aspartate were the most abundant and the sulphur containing amino acids methionine and cysteine also have extremely unpleasant organoleptic properties [tastes, smell and mouth-feel] that are totally impossible to mask. In fact, many infant formula products also have very unpleasant organoleptic properties to adult tastes so that the tastes

of these products did not raise much interest. At that time it was assumed that treatment would only need to be given during the time of brain growth namely the first 5-7 years.

But for many reasons this was not to be.

In the 1970s, it was found that if adult females with PKU became pregnant, the offspring were invariably severely damaged but if it were possible to lower the blood phenylalanine level sufficiently, the outcomes were improved.

The next bombshell was that if treatment stopped at the age of 6-7, most of the children began to demonstrate declining school performance and IQs. Moreover, treatment of older children and adults with little more than foul tasting baby formulae, was not a recipe for success and compliance with such restrictions was abysmal. The final straw was that adults with PKU seemed to have a very high incidence of progressive brain damage that was somehow linked to the underlying biochemical problem.

All these factors led to the concept that "diet-for-Life" was necessary even though the effects of this would not be known for decades. This led gradually to a nascent industry that has tried to make a wider range of foods that were protein-enriched and based at least theoretically on a more "normal" food product such as high protein energy bars and amino acids in capsules. However, these were still based upon the same general mixtures of amino acids that tasted so bad. At the same time a few companies had started to made low-protein foods that could be used to provide energy without excess protein and were intended to be facsimiles of regular foods available on the open market. These were intended to provide some variety and to enrich the nutritional experience which they did but they were mostly either very bland or did not appeal to the patients.

One day I decided that it should be possible to design a better amino acid mixture with optimized organoleptic properties and based on sound nutritional information. It is worthwhile to describe how I went about this since it was a one-day exercise followed by a three-year research validation study. One piece of critical information was the amino acid profile of cow's milk protein based on amino acid sequencing rather than on acid hydrolysis. Sequencing showed that at least 50% of the dicarboxylic acids had derived from their diamide precurors [glutamine and asparagine]. Here already was a way to reduce the most unpleasant compounds.

With all 20 amino acids arrayed on a table, I asked all those who passed, to taste each one and to rate them in order of acceptability or unpleasantness. The results were so consistent that we could develop a scale of organoleptic acceptability. It was immediately obvious that all the profiles used in the food products were entirely inappropriate with the two most awful tasting amino acids, glutamic and aspartic acids, both non-essential, being the most abundant. Both could safely be removed entirely without damaging the nutritional value of any new mix. Other changes that seemed obvious was to reduce or eliminate several other unpleaseant amino acids that were non-essential such as proline but present in unnecessary amounts and replace them with others that were less obnoxious but of

equivalent nutritional value. The profile of the essential amino acids had to be studied to ensure that it met all requirements for nutritional adequacy.

Thus was born what was then called the Oregon Mix and it was used, and still is, by several companies that completely changed their amino acid formulations. Products using this approach now constitute ~20% of all the relevant sales in the US.

Actually the effect of the revelation that these special foods did not have to taste horrible led to a further and even greater development. People realized that "Diet-for-Life" demanded a far wider selection of foods that could at least bear some resemblance to normal foods. This has spawned a huge expansion of the ancillary medical foods industry so that over 250 low-protein medical food products are now available for patients condemned to a life of phenylalanine or other nutrient limitation. [250 v. 30,000 for normal diets]. This is not a huge variety but for those patients who earlier were condemned to a pint of disgusting formula every day it is nirvana! Clearly more developments are in the wings and I am still working with this industry to try and develop more variety and better tasting products.

This work is something about which I feel very proud since it has had the opportunity to affect the quality of life for thousands of PKU sufferers.

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CHAPTER V

Benzyl alcohol poisoning in premature infants

The Problem:

Around 1980, a strange syndrome was being noted in several NICUs around the country including ours at the University. Very small premature infants were developing a peculiar breathing pattern termed "the Gasping Syndrome" and were becoming increasingly lethargic and then dying.

I should start this section by stating that unraveling this problem was by far the most important public health action of my whole career. We sorted out a disaster that was killing hundreds of premature infants every year. One would think that this merited a larger article than the rather meager letter to the Lancet that was the definitive report but by the time anything could have been written, the whole problem was past and solved.

In the decade before this problem was noticed, the survival rate for very premature infants was steadily improving thanks to huge improvements in diagnosis and care. During this evolution, many strategies were tried but not all were successful or even safe.

Amongst these was better attention to intravenous access and administration of medications and nutrition as well as greater access for better biochemical monitoring. It became commonplace to access such venous sites as many as 15-20 times a day. However each time the line was broached, it was standard practice to flush the line both before and after the procedure with a saline solution to prevent clotting and to ensure sterility. In this way, As much as 100 ml of flush solution could easily be administered every day. As was the practice for all patients, the saline was prepared with benzyl alcohol as a preservative and sterilizing agent.

In 1980, a strange syndrome was emerging in the smallest premature infants. It was noted that they would gradually become more lethargic, less responsive and start to develop a gasping form of respiration accompanied by profound acidosis; death was the usual outcome. The "Gasping Syndrome" as it became known was being noticed all over the country and was rampant in the NICU of my own hospital. It was estimated that 4-500 infants were dying in the US every year from this problem.

Urine specimens from several of these infants had indeed been submitted to our laboratory for our regular "Metabolic Screening" BUT without any clinical indications for the test; specifically no mention of an acidosis was ever made. Because of this, none of the specimens had been tested for organic acids by gas chromatography. One day, I received a phone call from a resident in an NICU informing me about the problem and that these infants were all dying with a profound acidosis. At last there was the indication for an organic acid analysis.

It took less than four hours for our laboratory to unravel the mystery. The urine chromatogram showed only two huge peaks that could easily be identified as benzoic and hippuric acid.

Benzoic acid is not a human metabolite therefore it MUST have originated externally. Hippuric acid is a metabolite created from many substrates that contain a benzene ring and thus is readily derived from benzoic acid. Clearly the infants were receiving something extraneous that contained a benzene ring. A phone call to the requesting NICU soon identified that the only source for this was in the IV flush solutions that were in general use. An emergency alert was sent out around the country and the whole epidemic came to an immediate halt. In Portland, at least 20 infants had died from it. Thus this was an example of iatrogenic poisoning from a benzoyl compound.

It is worth examining further why this catastrophe occurred. The volume of fluid given to flush ANY IV site was fairly similar regardless of the age of the subject. Thus the smallest babies were receiving by far the largest amounts per kg especially as they were usually sicker and thus merited more blood tests. Blood benzoate levels in excess of 100 mg/dl were present in several of the samples that we had stored in the laboratory. The molecular weight of benzoic acid is 122 so that the levels in the infant blood were about 10 mM.

It is now possible to explain the biochemical reason for the problem. The conversion of any organic acid to a conjugate, and therefore of benzoate to hippurate, requires an intermediary step that involves L-carnitine. Infants, especially if premature, are born with scant supplies of carnitine and if the system is called upon to handle large amounts of acid, the supply of carnitine is not sufficient to the task. Carnitine is an essential component of many intracellular reactions including aerobic metabolism, the transport of fatty acids into cells and fatty acid catabolism. Lack of it is known to cause severe problems including cardiomyopathy and energy failure. It is not known if adequate carnitine could have prevented the deaths. The syndrome was exclusively restricted to small premature infants in whom the volume/weight ratio was far greater than for older infants.

The resolution to this epidemic illustrates how important it can be for a laboratory to obtain accurate clinical information when receiving samples for analysis. Admittedly, our metabolic laboratory played a special role in that we also provided a direct consultative service and every report was examined and signed out either by myself or the laboratory director. Moreover we held all samples for over 20 years so were able to go back and show how many specimens from our own NICU showed the
characteristic organic acid pattern. This was an action much appreciated by certain lawyers but not by the clinicians in the NICUs!

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CHAPTER VI

Studies on Galactosaemia

The Problem:

It was known that infants with this disorder had an immediate postnatal risk of severe disease and death in the first week of life if they ingested galactose, one half of the disaccharide lactose, [milk sugar]. It also seemed to be the case that as a group, they suffered a high rate of long-term problems including developmental challenges and speech problems. However, the situation was not clear and the aetiology of the latter was totally unknown. Treatment was confusing in that it was impossible to bring the blood levels of galactose-1-phosphate down to normal, moreover a strictly galactose free diet was a practical impossibility!

I have selected this article on Galactosaemia because it was a watershed in our perception of this disorder and became the article that clarified the huge amount of misinformation that was current at the time. Galactosaemia is a relatively common metabolic error occurring in about 1:50,000 births. Our state newborn screening programme started screening for it in 1965, being, I believe, the first place to do so in the world. All suspected cases were of course referred immediately to our centre.

This disorder presents in the first week of life as soon as the infant starts to take any feed that contains lactose [a glucose-galactose disaccharide]. Within a few days the infant is deathly ill with profound liver and kidney damage and severe septicaemia; hyoglycaemia leads to convulsions and if the infant survives, cataracts develop. It was known by aficionados that in those infants that do survive, there was frequently considerable brain damage with a specific propensity to expressive verbal dyspraxia. Females might develop gonadal failure whereas males did not. The overall incidence of these complications was not known.

It was also known that infants who had never received galactose after birth, never developed any of the acute life-threatening symptoms so it seemed clear that galactose exposure was the reason for the short term problems although there was no idea how the galactose exerted these malign manifestations. It was assumed that the long-term problems were related to the severity of the neonatal crises with damage targeted at specific organs, ie, if the neonatal problems could be avoided, the long-term problems would not develop. This thinking was based upon the "model disorder" with PKU were preventable by severe and prolonged restriction of dietary phenylalanine. Not that the PKU model had been easy to establish. There had been

fierce arguments in the 1960s and '70s that the diet was the CAUSE and not the cure of the brain damage. However, the PKU model was persuasive because there was a steady stream of new aminoacidopathies emerging many of which seemed to be associated with mental

retardation or even worse. Thus a strict "galactose free" diet was the accepted treatment, was expected to be continued for life and it was assumed that this would prevent the long-term problems. Compliance with this regime was monitored by regular blood tests for galactose-1-phosphate that was somehow assumed to be at least a surrogate for whatever caused the lethal complications.

This model actually did not make logical sense since it was known from a few galactosaemic infants that high levels of galactose-1-phosphate were invariably present in the cord blood at delivery. If this metabolite was the problem, or even a reflection of the primary cause of the problems, then why were these infants born apparently completely normal? Moreover, why did infants that were started on galactose-free diets at birth escape all of the catastrophic newborn problems but still seem to have the same long-term complications? The difficulties were compounded by the fact that the only metabolite available to monitor the efficacy of the diet was the same galactose-1-phosphate and that was it was IMPOSSIBLE to eradicate from the blood. Frustrated specialists assumed that the patients were not being compliant enough with the diet, just in the same way that many patients with PKU on treatment had high blood levels of the amino acid and were known to be non-compliant. As the dietary science improved, it became obvious that it is actually impossible to develop a truly galactose-free diet. More recently still, it has been shown that endogenous galactose production occurs every day being essential to the maintainance of galactolipids, galactoproteins and other metabolites. In fact, the levels of galactose-1-phosphate have been used to try and calculate the daily endogenous production and turnover of galacto-compounds.

In order to address this mess I decided to develop a large survey of experience with this disorder from around the world and we were able to collect 350 useable responses. From this it soon became clear:

1 That all affected infants have very high cord blood levels of galactose-1-phosphate even if the mother was on a milk-free diet during the pregnancy.

2. If they never received any nutritional galactose in the first weeks there were NO crises and cataracts never developed.

3. If they ingested enough galactose later, they did develop the acute liver and kidney problems as well as cataracts. For adults, the former were as reversible as they are in newborn infants.

4. There was no difference between the incidence of the long-term problems or their intensity regardless of whether they had ever been exposed postnatally to galactose or not.

5. Develpmental problems were present in up to 80 percent of the cases, 50-60 percent of all the cases had a degree of verbal dyspraxia and 80 percent of the females had gonadal failure.

From this emerged an understanding that the cataracts and the neonatal crises were indeed caused by ingestion of galactose but even now, the primary cause of the neonatal liver and

kidney damage is still not known. From animal studies, It is known that fructosamine is highly toxic to both tissues so that it is my postulate that at least one of the toxic metabolites is galactosamine although this has not been shown to date.

The high incidence of the late-term complications was a shocker. It was clear that they were unrelated to any galactose restriction given either to the mother during pregnancy or to the infants after birth. It was now inescapable that the developmental delays, the verbal dyspraxia, the behaviour problems and the ovarian failure were caused by some biochemical disturbance that was present before birth. The highly characteristic speech defect of verbal dyspraxia remains an enigma. This is a problem in which the subject can hear and understand a word or conversation, knows how the word should be pronounced but is unable to create the proper sounds due to a disconnect between the processing cerebral cortex and the speech pathways. Why ovaries should be damaged but testes are not also remains unknown.

I am working with one of the big galactose centres in the US in trying to unravel some of these mysteries. In regard to the long-term problems, their primary cause is completely unknown.

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CHAPTER VII

A Set of Simple Sideroom tests for the detection of inborn errors of metabolism

I have chosen to include this article not because it represents any significant advance in science but because it represents the state of investigation for metabolic disorders that existed in the mid-1960s. At that time, not many true inborn errors of metabolism were recognized and even paper chromatography of amino acids was rather primitive, unreliable and not widely available. At that time there was no amino acid analyser dedicated to clinical service in the whole of Scotland. Metabolic disorders were rarely, if ever, considered in a differential diagnosis and, if they were, there was either no known biochemical test to screen for one, or such testing was not done locally. Where any such tests were available, they were the purview of a regular clinical laboratory where there was no basic knowledge of metabolic disorders.

At that time, urinalyses, blood counts and smears, erythrocyte sedimentation and other basic tests were routinely performed by house physicians in ward side-rooms so there was a model for thinking that some simple tests for metabolic disorders might provide a useful addition not only as a basic clinical tool but also for teaching purposes. By publishing this article in the British Medical Journal, I intended that interested practitioners could at least begin to think about the field and to feel that a set of simple screening tools could easily be deployed in their hospitals. Clearly they were not going to be performed in a general practice, but if they could be available in Paediatric wards they might facilitate the initial diagnoses of these rare disorders.

By the time this article was published, this set of tests was already in use in the few metabolic centres already established. This set of tests was used, as published, in my own laboratory in Oregon at least until I retired in 1996 and is still in use. They complimented the one-dimensional paper chromatography that was the work-horse of our, and others, "metabolic screening" [This was totally different technology than used in the State routine newborn screening programmes that were being started around the country and overseas]. As practitioners became familiar with the breadth of metabolic problems and that there was some way at least to try and find much of what was known at the time, these tests were in increasing demand. The panel of tests described in this article remained a central part of screening for IEM both in such centres for decades and some are still used routinely in biochemical genetic laboratories. Although some have been replaced by far more technically sophisticated methods, they remain a useful, simple and reliable panel with which to initiate a basic investigation.

In 1998 I was pleasantly surprised on a visit to Chulalongkhorn Hospital in Bangkok, to be proudly informed and shown my article and was assured that it was the basis of their urine metabolic screening and that they were still using the whole battery of tests in their basic metabolic screening programme.

What this panel does not do well is to provide much in the way of an algorithmic approach, at least not for todays much more complicated scene, but its importance was that for many, this was the first inkling that such an exotic world might be approachable and considerable information gleaned very simply.

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CHAPTER VIII

Experiences in war-torn areas with refugees:

Why is this chapter included in this thesis?

My experiences in several war-torn situations around the world has surely affected the way I think and practice Medicine. I have been most fortunate in being able to leave my academic position at very short notice and to go where there have been major problems with refugees usually arriving in some barren place with advanced malnutrition and a huge array of diseases including a wide selection of tropical diseases that are never encountered in a normal practice elsewhere. Not only is the clinical practice absorbing and challenging but it is a thrill to be able to proactice Medicine with no laboratory, no radiological support little in the way of medical or nutritional supplies and to be able to rely upon ones own skills circumstances. For me, it is easy to give up access to all laboratory and radiological assistance. It is, or should be, easy to fall back onto the firm tenets of clinical examination stilled into us at St Andrews and to call back out of the recesses of ones mind the litanies of tropical diseases and nutritional disorders that one had only heard about vaguely, if at all. To encounter and to recognize them in such circumstances is truly rewarding and has undoubtedly enhanced my career.

In early 1980, the political disaster that was Cambodia under the Pol-Pot regime finally exploded. As many as 3-4 million of the population [~50%] had died: unknown numbers had been systemically and brutally exterminated and the rest had died from a fierce medical and nutritional crisis that developed as the society disintegrated into agricultural and societal chaos. The well-educated and civilized society was destroyed.

The bubble eventually burst and hundreds of thousands of ill and starving people began to flood out into neighbouring countries especially to Thailand. Refugee camps were developed and I was fortunate enough to be able to arrange a 3-month stint with the International Rescue Committee to run a paediatric ward in a hospital in the camp Kao-I Dang on the border of Thailand and Cambodia. From day to day, this camp, still in a very unstable political position, was home to 110-130,000 people; there were two hospitals and our ward held about 100 beds. We had enough staff to work 12-hour shifts every day and expected to see about 100 patients a day. The standard clinical

work was fascinating; rare genetic syndromes were obvious and formed some grist for weekly hospital "Grand Rounds" that I organized. Much more interesting to me though, were all the extreme nutritional deficiences that I saw.

When I arrived, the International Red Cross had just done a survey for vitamin A deficiency in the community and reported that there was not much evidence for it. I went around the same area and found 40 cases of xerophthalmia in less than an hour. What did this imply not only for the ICRCs ability to recognize such problems but also what did it tell me about my own interests? I am not especially gifted, but this confirmed to me my interest in the unusual and exotic aspects of medicine and that I seemed to have a special bent in recognizing them. I had worked in Malaysia for two years in the army and was attuned to the medical problems that occur in the tropics. The daily flood of children and adults with terminal malnutrition and the accompanying deficiencies of vitamins and other nutrients was easy for me to accept, to recognize and to treat.

This experience was followed five years later, in Sudan during a similar exodus of terminally ill Tigrineans from Ethiopia in whom the nutritional conditions were even worse. On my arrival in the Sahara, I was placed in charge of a medical programme for a camp of 10,000 people and was immediately informed of an epidemic of oedema that had developed. On questioning the staff, I asked if anyone had considered the possibility of Kwashiorkor or beri-beri – "No" was the answer! This experience in running a 100 bed hospital ward as the only physician and being plied with 200-400 out patients per day, led to a personal epiphany that has remained with me ever since – wherever possible, teach and not "do". The variety of tropical diseases was huge and provided me with experience that few others have had. It segued into a secondary career and interest in nutritional diseases and their effect in tropical infections. I have become *de facto*, a local expert and lecturer in this field and still continue to travel to troubled areas such as Afghanistan, Ethiopia, Sri Lanka, El Salvador in 2010 and Somalia in 2012. It can thus be seen that these reports from the field reflect what were life-altering experiences that have moulded my career and life in profound ways.

References from my CV:

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CHAPTER IX

My first appearance in the medical literature!

This compilation of my experience in Medicine would hardly be complete if my first appearance in the medical literature were not included. I was the second case of pyloric stenosis reported in this article which was written by the army officer who supervised the operation on me. I do not think that my contribution to this opus was anything other than passive!

Roche, L.S.C. (1933) An unusual case of congenital pyloric stenosis. J. Royal Army Medical Corps, 455-457.

CHAPTER X

A wide selection of rare and unusual diseases

In the following chapters I describe a number of rare conditions, each case or family chosen from a wider selection of subjects to demonstrate some novel aspects of pathophysiology. I have chosen some of the unusual cases that we worked on and to explain why they are, or were, of special interest. Indeed, several of them led to the recognition of totally new classes of disorders of organelles such as in the peroxisomes, the Golgi or the sarcolemma. In all of the disease presented, it can be seen that our initial findings led us, or spurred others, to pursue further understanding of the pathophysiology and/or the basic gene defects. Certainly their recognition led to widespread collaboration with other researchers who were better able to take knowledge to a higher level than I could ever have done.

My success in detecting and exploring a wide variety of rare disorders was the result of a combination of several factors. The first was that my clinic was the only centre for metabolic disorders in several States in a geographic area roughly the size of Western Europe. With a population of ~8 million people, one would predict that there should be about 8 patients with disorders that occur only 1:1,000,000, and ~80 with disorders occurring in 1:100,000 let alone a far larger string of more common conditions.

The second factor was that my laboratory received around 3000 samples each year for metabolic testing from all over the country and indeed from overseas so that I had access to an even larger number of cases in whom the lab had detected a possible metabolic disorder. Such cases would never have come to attention if the urine specimens had not been sent to our laboratory in the first place.

The third factor included the fact that with my aid, the State Newborn Screening programme was able and willing to set up new screening tests that seemed attractive but that, at that time, had never been validated for use in a Public Health programme.

With my interests in the unusual, the rare, the bizarre, it was natural that I was more than usually willing to pursue intriguing openings that offered some hope of research and new knowledge. Given the early years of this specialty in which I was working, it may be hardly surprising that I was able to recognize at least six totally new disorders but in truth, I think that much of my success came from being more than usually curious.

This search for the exotic, was enormously aided by obsessional perusal of "*Current Contents*" each week. This addictive publication contained the frontispage contents of every major scientific publication from around the world. It was from this source that I often discovered some new disorder that had just been described only to recognize that I had seen a similar case in my own practice sometimes years earlier. Examples of this include the original descriptions of a new disorder of methionine metabolism [ChapterXXI], the original "CDG" syndrome [Chapter XVIII] and the original Infantile Refsum cases [Chapter XVI]; In each case, I immediately realized that I had seen such cases in my clinic in the past without a proper diagnosis being established.

I have also provided the Online Mendelian Inheritance of Man [OMIM] catalogue number and, when known, the gene location and name of the affected gene in brackets after the title.

CHAPTER XI

Tyrosinaemia Type II; "Oregon type". Tyrosine aminotransferase deficiency.

A new disorder that was extremely controversial.

[OMIM 613018; gene location 16q22.2].

The Problems in this case:

The patient who was the subject of this report, was presented to me on my first day in my new job in Oregon. He had very severe multiple congenital anomalies and was profoundly mentally retarded. He had also a very severe erosive keratopathy and peculiar blister-like lesions on his hands and feet. A very high level of plasma tyrosine was originally detected by newborn screening. As our studies progressed he presented several apparently insoluble controversies that included the excretion of large amounts of a putative enzyme defect, and an explanation of the congenital defects.

At the time [1966] only one disorder of tyrosine metabolism [tyrosinaemia] was known. It was a progressive disorder that severely damaged the liver and kidney in early infancy. The precise enzymatic defect in tyrosine catabolism was unknown but, because of high excretion of p-hydroxyphenolic acids, was thought probably to arise at the level of p-hydroxyphenylpyruvic acid oxidase. Such a defect would easily explain the large amounts of the substrate of this enzyme that were shown to be in the blood and urine in those cases. [It is now known that in this disorder, the primary enzyme defect is in fumarylacetoacid hydroxylase lower down the tyrosine catabolic pathway and that a very toxic metabolite causes disruption of several enzyme pathways].

In our case, there was neither liver nor kidney damage but there was marked increase in the blood and urine p-hydroxyphenylpyruvic acid thus ostensibly requiring that the primary enzyme defect should be at or below the level of p-hydroxy phenylpyruvate oxidase. This enzyme was known to be the next one down the path from tyrosine transaminase. This was antithetical to the standard thought process at the time regarding the other cases of tyrosinaemia. However, our studies clearly showed that the enzyme defect was at the first step of tyrosine catabolism in tyrosine aminotransferase for which the ketoacid is the direct product. Our proposed enzyme defect should theoretically have prevented any significant production of the ketoacid.

Thus this patient presented a truly fascinating biochemical conundrum in that the PRODUCT of the missing enzyme was present in vast excess in both plasma and urine. Skeptics insisted that our enzyme results were false and even refused, at first, to publish the case!

Further studies soon revealed that the primary defect was in the cytosolic tyrosine aminotransferase enzyme while the mitochondrial counterpart was totally normal. This led to

the concept that the cytosolic block resulted in large amounts of tyrosine within the cells that could either leak out or be actively transported into the mitochondria and then be converted to the deaminated ketoacid product p-hydroxyphenylpyruvic acid. This then was produced in such quantities there that it was escaping into the blood and thus making its way to the kidneys. At the time, this was a new concept of inter-organelle or inter-organ metabolic cooperation.

In regard to the aetiology of the congenital malformations, in 1966, there was a growing interest in the problems of Maternal PKU in which a foetus is irreversibly damaged from the first weeks of pregnancy by elevated maternal blood levels of phenylalanine. It was assumed therefore that the malformations in our case might have been caused by prenatal biochemical damage even though the mother's blood tyrosine was normal. Such thinking gradually faded but there was no explanation for the combination of problems. Twenty-five years after the initial diagnosis, the primary cause was finally revealed. There was a very small contiguous chromosomal deletion in the paternal chromosome 16 [where the gene for the missing enzyme is located]. The mother was then shown to be a heterozygote for the enzyme defect. The deletion explained the anatomic defects [and has indeed subsequently been described in another unrelated patient with similar physical findings].

Our group also showed in rats that the palmar-plantar keratosis and the keratitis are caused by extremely high plasma levels of tyrosine which, being very insoluble, readily crystallizes out in cold conditions. Tyrosine crystals could be seen in the corneas and in the dermis in the most exposed areas. The tissue distribution was thus explained. These symptoms are easily reversed by lowering the blood tyrosine levels.

Finally this patient became the explanation of another rare disorder that had been originally described 30 years before in remote Swiss mountain areas. It was known in ophthalmic circles as the Richner-Hanhart syndrome in which identical forms of the palmar-plantar keratosis and the keratitis were present. The biochemical cause of this was unknown until our studies came to light. Many such patients were developmentally delayed. All such cases have now been shown to be due to tyrosinaemia type II and the associated delays are probably due to local inbreeding in isolated alpine valleys. Yet another problem solved!
Why was this patient important?

This is the first patient known to have this disorder of tyrosine aminotransferase deficiency. At the time, we knew from newborn screening that an overgenerous intake of protein often led to very high levels blood of tyrosine, and often other amino acids, in the weeks after birth. But what were the possible consequences? Should they be treated? Should newborn screening always include tyrosine as well as phenylalanine? How was the severe liver and kidney damage in the condition then known as tyrosinaemia be explained? What was the normal catabolic pathway for tyrosine? These and other questions took many years to sort out. Once the validity of our findings was accepted, the original hepatorenal tyrosinaemia was allocated the subtype of Type I and our case became labeled as type II. A Type III is also now

recognized. The idea that tyrosine produced in the cytosol of a cell could migrate into the mitochondria and there be catabolized was new. Once again, the detection of a single patient led the way to a greater understanding of normal biochemistry.

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CHAPTER XII

Iminodipeptiduria due to prolidase deficiency

[OMIM 170100; gene locus: 19q13.11]

The Problem of this case:

Huge amounts of unknown compounds were detected in urine by both paper chromatography and on the amino acid analyser. These were tentatively identified as containing proline, an amino acid that constitutes about one third of the proteins in collagen. A tentative diagnosis had been made in another centre of a novel form of lathryism.

My enthusiasm for developing a method to separate amino acids from peptides would not have borne much fruit had I not come across the sample of urine that contained the putative abnormal proline compounds. From their chromatographic behavior, I suspected that these were possibly dipeptides containing proline. Low and behold, my peptide separation technique worked like a charm and I was able to isolate these unknowns without any contaminating free amino acids. Through hydrolysis of the peptide fractions, it was a matter of relatively short order to show that every one of these compounds was either an X-PRO di-peptide or an oligo-peptide containing proline.

Two enzymes are known to cleave iminodipeptides, prolinase cleaves PRO-X dipeptides and prolidase cleaves X-PRO links. Lack of prolidase activity seemed to be the only possible explanation for this unique iminodipeptiduria. Surely I had found a new class of defect – namely one in the catabolic pathways of peptides. Sadly there were no tissues on which this hypothesis could be confirmed but this case report was indeed the first description of hereditary prolidase deficiency – a rare disorder that most metabolic experts have heard of but few have seen. Since that time a number of such cases with identical peptiduria have been recognized and the enzyme defect and underlying gene defect have been established.

Why was this case important?

This was the first report of this disorder. Patients with prolidase deficiency present with a variable array of chronic leg ulcers, recurrent infections, some facial dysmorphism and mild to severe mental retardation. These are not findings that would seem to have a high dividend though urine metabolic screening but the pattern of peptiduria is so characteristic that it can easily be recognized. This report describes the first case of another novel class of defect, namely one in a peptide catabolic pathway. Unlike several of the other disorders presented in this thesis, the finding of prolidase deficiency has not revealed a whole new class of peptidase disorders although it may be that proline links present special conditions that require a special prptidase to break whereas most amino acid links do not have the same tertiary structures and so can be broken by any of a number of enzymes.

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CHAPTER XIII

Citrullinaemia. [OMIM 215700; gene location: 9q.34.11, Argininosuccinic acid synthase]

The patient:

In 1968, an infant was sent to our clinic with a history of lethargy, vomiting and declining head growth. Metabolic studies showed high amounts of citrulline in the blood and urine.

Citrullinaemia is a recessively inherited defect in the Krebs-Hensleit urea cycle. At the time this infant was diagnosed, only one other case with this diagnosis was known in whom profound hyperammonaemia in the days after birth had been fatal. There had been reports of other infants with hyperammonaemia with other defects of the urea cycle; almost all were seriously ill very soon after birth and most died. Moreover infants that did survive, had severe brain damage. Already in this patient, the glimmerings of variability were evident in that our patient was not diagnosed until about three months after birth. Little was known about the best way to treat these disorders during the acute phases let alone for the long term and very few products were available. Treatment required severe restriction and monitoring of ALL protein intake FOR LIFE. The ingestion of a normal amount of protein even for a day was accompanied by a metabolic deterioration and hyperammonaemia. My role in trying to ameliorate the dietary proteins used in this and other disorders is discussed Chapter IV. This patient did indeed survive and is still living, currently being on a waiting list for a liver transplant. This procedure, if successful will essentially cure her condition and should allow her to live a life without any protein restriction.

Why was this patient important?

At the time of diagnosis, this was the second known case of citrullinaemia. Other defects in the urea cycle and all were thought to be very rare. Since then, disorders of the urea cycle have been found to be far commoner than was thought four decades ago. Citrullinaemia, is now diagnosed in ~1:100,000 births most of whom are detected by routine newborn screening. The basic tenets of treatment have not changed but gene therapy has been tried not in citrullinaemia but in another defect of the urea cycle. As might be expected, liver transplantation "cures" the urea cycle problem but, since the enzymes are expressed in other tissues [where their function has not been much studied] the patients continue to excrete small quantities of their characteristic metabolite[s]. A number of different mutations in the citrullinaemia gene are now known that explain the milder presentation of this case. Defects of several other genes are now also known to cause different types of citrullinaemia.

28. **Buist, N.R.M.**, Kennaway, N.G., Hepburn, C.A., Strandholm, J.J., Ramberg, D.A.: Citrullinemia: investigation and treatment over a four year period. J. Pediatr. **85**:208-214, 1974.

CHAPTER XIV

Rippling Muscle Disease. [OMIM 606072;
Gene location in this family: 1:214,500,000-224,100,000].

The Family:

During a pilot screening programme for Duchenne Muscular Dystrophy by the State newborn screening programme, an infant was detected to have a mildly elevated level of creatine phosphokinase. The finding persisted but would not have been high enough to be caused by the muscular dystrophy and ordinarily the result would have been filed away. My curiosity once again paid off.

A family with an extremely rare dominantly inherited myopathy came to attention during this pilot study; only one other family was known at the time. Many members of our family had muscles that, when they were spontaneously stimulated by mechanical pressure such as a finger-poke, began to show a prolonged series of slow rolling movements lasting up to 30 seconds in the stimulated muscle AND into neighbouring ones. None of the affected people was weak, indeed several were very muscular and athletic. Several members had mildly elevated creatine phosphokinase levels and most had to do some stretching exercises before doing anything strenuous.

The movements of the muscles generated no electrical activity as assessed by electromyography and thus could not involve any depolarization of the muscle cell membrane. This means that the stimulus to contraction must have originated from within the cell, somehow bypassing the usual mechanism that is normally initiated by nerve stimulation at the cell membrane. I postulated that this was likely to be at the level of the sarcolemma where calcium release, normally stimulated electrically by nerves, initiates the depolarization that leads to contraction. One other family was known at the time but they had a tendency to develop cardiomyopathy so it seemed likely that more than one gene could cause this syndrome. Since the contractions were electrically silent, some intracellular stimulus must be triggering the release of intracellular calcium that is essential to initiate a contraction. Since an external stimulus such as tapping a muscle led to the rippling, it seemed likely that the mechanical pressures produced by a contraction in one muscle fibre could be detected in neighbouring ones and that if strong enough could equally well travel from muscle to muscle.

Why was this study important?

This was the second known family with this disorder. The finding of detectable, indeed visible muscle contractions in the absence of any electromyographic activity was a sure sign that the defect had to be intracellular, beyond the outer lining of the cell membrane, most likely at a site where calcium release was controlled – probably in the sarcolemma. This was a totally novel idea that, with time has proved to be correct; while the gene location in this family

has been mapped, in a few other families a different defect has been shown in the gene for sarcolemmal caveolin-3; but at this time the primary defect in our family is still not known.

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CHAPTER XV

A Fatal X-Linked Disorder of Diarrhoea, Diabetes Mellitus and immune Dysregulation; [XLAAD]. [OMIM 304790; gene location: Xp11.23. FOXP3]

The Family:

This story unfolded in a sad way after the presentation of a single male infant with untreatable, secretory diarrhoea, diabetes, and progressive decline to death.

Our report on this large family represents the first account of this disorder although occasional a few cases that sounded similar to this infant had been published. A family history was uninformative until one day the mother sheepishly confessed that she had researched her Mormon ancestry and discovered eight other dead male infants with a similar disorder! Clearly this was an X-linked disorder that had catastrophic consequences. A second, affected male infant was later born to the same mother by which time it was apparent from independent research that there was major disruption of the immune regulatory system. We therefore made attempts to perform a foetal bone-marrow transplant but never got this approved and this child also succumbed after a few years.

Why was this family important?

This was the first full report of a family with this disorder. The finding, not only of a new disease and one that can reappear through generations, but one that is deeply involved in the immune system, offered a huge opportunity for basic research into mechanisms that are still not totally understood. It took more than 20 years before the primary gene defect was elucidated [122]. The defect is in an immune-regulatory gene called FOXP3 and is comparable to a similar X-linked defect found in mice namely "Scurfy". In some more recent cases, there has congenital absence of the pancreas has been noted but in our index case the pancreas was present. Why the pancreas is singled out for such damage is not known; indeed the exact role of the gene in animals or man in the overall function of the immune system is not yet known.

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122. Ramsdell F, Peake J, Faravelli F, Casanova JL, **Buist, N**, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Dagna Bricarelli F, Byrne G, McEuen M, Proll S, Appleby M, Brunkkow ME, Wildin RS. (2001).X-linked neonatal diabetes mellitus, enteropathy, and endocrinopathy sydrome Is the clinical equivalent of mouse scurfy. Nat Genet. Jan:**27**:18-20.

CHAPTER XVI

Infantile "Refsum" Disease.

[OMIM 601539; gene location: 7q21.3. PEX1].

The Patient:

An infant who was referred to our clinic in 1981 had profound hearing loss, absent deep tendon reflexes, marked developmental delay, an unusual retinopathy and a complex of dysmorphic features.

I did not initially make a diagnosis, but shortly after I saw him, there was a report that I found in Current Contents that described an identical syndrome from a European clinic and also provided some basic biochemical information that I was able to confirm in this, our index case. On reflection, I was able to recall three other cases that seemed to be identical, was able find them and to confirm the same diagnosis in each. This report on four cases of Infantile Refsum Disease, was only the second and the first to originate in the US. The eponym came from a very rare disorder of adults [Refsum disease] in which some of the same features were present but clearly this infantile disorder was something far more serious.

Why was this report important?

This was only the second report of this syndrome. Over the past 25 years it has become clear that this disorder is only one of a group of more than 15 disorders that arise because of defects either in the biogenesis or specific enzymatic functions of peroxisomes. These organelles were only described in 1967 as a new type of intracellular structure and the possible existence of biochemical disorders of peroxisomes, [in X-linked adrenoleucodystrophy] was only postulated in 1981 by Moser. At the time of this report, "peroxisomopathies" were not a recognized class of disorder and their role in serious human disease was unsuspected. Once one or two disorders were described, whole laboratories have been devoted to studying this field.

65. Budden, S.S., Kennaway, N.G., Buist, N.R.M., Poulos, A., Weleber, R.G.(1986). Dysmorphic syndrome with phytanic acid oxidase deficiency, abnormal very long chain fatty acids, and pipecolic acidemia: Studies in four children. J. Pediatr. 108:33-39.

CHAPTER XVII

Hereditary Hypocalcuric Hypercalcaemia [HHH].

[OMIM 145980; gene location 3q.13-q21.1]

The Patients:

Two separate infants in two different families are presented. One came in with profound hypocalcaemic seizures and the other with severe bone abnormalities thought to be due to hyperparathyroidism.

The two reports that I have provided are interesting because they reflect entirely opposite ways in which an identical biochemical disorder can present depending upon which parent and which foetus has the mutant gene. In one case, an infant presented with profound and prolonged hypocalcaemic seizures; in the other affected infant, hypercalcaemia and severe bone disease were accompanied by high levels of plasma parathyroid hormone.

In this condition, which is dominantly inherited, the calcium sensing system is defective throughout the body and demands that the serum calcium be maintained at higher than normal levels, usually between 2.75-3.35 mM [11-13 mg/dl] [normal 2.0-2.75 mM]. At this level, all the calcium-related activities function normally so that, for example, there is no hypercalcuria and the parathyroid hormone levels are normal. Indeed, if the plasma calcium level is reduced, as would happen after an injudicious parathyroidectomy, the parathyroid glands respond by hypertrophy in attempts to raise the plasma calcium again.

Thus, if a mother has the gene, and the foetus does not, the foetus is bathed in inappropriately high levels of calcium and it will develop hypoparathyroidism *in utero* as these glands are shut down in the face of inappropriately high ambient calcium levels. This problem can persist for months postnatally, presenting with hypocalcaemic seizures. If both the mother and foetus have the gene, the calcium homeostatic system in the infant is totally unaffected.

Conversely, if a foetus inherits the gene from the father and the mother does not have it, the foetus requires a higher calcium level than the mother's blood can provide so it tries to obtain more by developing severe hyperparathyroidism prenatally. Postnatally, this presents with profound prenatal bone abnormalities and the hyperparathyroidism persists for many months after birth.

Why are these two cases important?

This disorder is not vanishingly rare, but the dangers of the foetal presentations have never been well recognized. In both of the scenarios presented above, most clinicians, even experienced endocrinologists, miss the true situation and focus upon the dysfunctional parathyroid functions.

Such families are often misdiagnosed as having familial hyperparathyroidism which sadly often leads to inappropriate surgery. This, of course, does nothing to improve asymptomatic hypercalcaemia in the adults nor the temporary hyperparathyroidism in the neonates, indeed surgery can aggravate the problems and usually worsens the clinical situation. I have seen both situations several times but still the condition is still not well recognized even by endocrinologists.

When we submitted these cases for publication, the primary aetiology was unknown and we proposed that the defect had to be in a calcium sensing system. This suggestion had to be removed from our texts on the demands of a reviewer. Since then, the gene has been cloned and the defect has indeed been shown exactly as we originally suggested. Reviewers are not always right! [Dr B.R. Powell was a fellow in my programme.]

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CHAPTER XVIII

A Congenital Disorder of Glycosylation. CDG Type IA.

OMIM 212065: Gene localization 16p13.2. Phosphomannomutase deficiency

The Patient:

In 1977, I was consulted on an infant with peculiar fat pads distributed over the buttocks and thighs. She was somewhat delayed but was not otherwise thought at the time to be dysmorphic.

Ten years later, I saw a photograph of a baby in the letter section of The Lancet. It was identical to the one I had seen years before; I just happened to remember the name and was able to find the family. Lo and behold by now, the patient had a sibling and both were extremely delayed and dysmorphic with a wide array of physical and biochemical abnormalities. Notable was the abnormal glycosylation pattern of transferrin [and indeed several other plasma transport proteins]. Correspondence with the Lancet author led to the unraveling of another completely new class of disorders namely disorders in which the post-ribosomal processing of nascent proteins is disrupted. Glycosylation of proteins occurs through a highly complex system in the Golgi apparatus of the cell in which side chains are built up, modified and eventually exported with different "Zip codes" destined for uptake by other cells and organs.

Why was this case important?

In the first place this story has led to delineation of a completely new class of disorder namely defects on Golgi based post-ribosomal glycosylation of proteins. At the start, we had no idea that this syndrome would be the entry to a whole new class of disorder or that there would be such a complex system of enzymes in the Golgi that are required for final structuring of so many different proteins. CDG IA was the first to be delineated biochemically and this fueled the development of whole laboratories devoted to studying what was a largely unknown area of biochemistry namely the post-ribosomal glycosylation of proteins. As of today, there are more than 45 distinct Congenital Disorders of Glycosylation - "Golgi-opathies" a number that equals the lysosomal disorders and one which is continuing to grow.

It is now clear that the exact structure of the carbohydrate side chains depends upon a complex system of synthesis, alteration and further synthesis of the nine membered side chains that define the final destination and function of all glycoproteins. It is hardly surprising that the symptoms in each disorder depend upon exactly what part of the glycosylation is defective; thus these diseases are multifaceted and unpredictable. Much remains to be learned about the system and the fine points of glycosylation.

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CHAPTER XIX

Thiamine responsive diabetes and deafness [TRMA].

OMIM 249270: gene locus: 1q23.3 SLC19A2]

The Patient:

I was referred an Alaskan native patient in the wards who had massive megaloblastic anaemia together with diabetes and profound nerve deafness. This combination had stumped the university haematologists but once again, thanks to "Current Contents", I knew that this combination had been previously reported in several families from Europe. Within a week of starting thiamine therapy, the anaemia had resolved but neither the diabetes nor the deafness seemed to respond at all.

Why was this family important?

As far as I know, this was the first case of this disorder to be discovered in the US. While we were not the first to report this syndrome, our family was one in which Neufeld was able to locate and characterize the gene and its putative function. At the time of our report, nothing was known about a possible thiamine transporter but this turned out to be the primary cause of the problems. It is not clear why these patients do not all suffer from B_1 deficiency in the form of beri-beri in which megaloblastic anaemia, diabetes and deafness are not usual features nor is it clear why the ear, bone marrow and the pancreas take the brunt of the pathology. Clearly there is more that is not known about the pathophysiology than is known.

Reference from my CV:

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CHAPTER XX

Folinic Acid Responsive Seizures. A false alarm!

The Patient:

A newborn infant was referred for uncontrollable seizures; a previous sibling had died, aged six months, with a similar problem. Spinal fluid was sent to a specialty laboratory where sophisticated analysis showed the presence of two unknown compounds. These had been seen once before in an infant whose seizures seemed to have responded to folinic acid. The seizures in our case did indeed respond to this, but the infant remains severely retarded.

Our report [99] includes another infant who had similar biochemical findings in the spinal fluid and who also responded to folinic acid. It has long been known that rare infants with recalcitrant seizures may respond totally to pharmacological doses of pyridoxine so the idea that seizures might respond to another vitamin was not unreasaonable. We got onto this disorder after a sample of spinal fluid, submitted to a specialty laboratory, showed a very high level of an unknown compound that they had seen only once before in an infant whose seizures had seemed to respond to large doses of folinic [but not folic] acid. This proved to be true in our infant as well and the search for a primary defect was on around the world. I did not participate in this research except as a supplier of more spinal fluid; eventually the basic biochemical defect was isolated. It came as a big surprise that this apparently "new" disorder was in fact a sheep in another guise!

Why was this patient important?

Seizures in the newborn are always serious and sometimes fatal. Our report was the first to describe this syndrome and stimulated a lot of neurologists to start examining the spinal fluid for neurotramsmitters, the technique developed by Hyland that originally revealed the unknown metabolites. As more samples were analysed, it seemed as if the same metabolites could be found in patients with another very severe seizure disorder, namely B_6- or pyridoxine-dependent seizures. This disorder, which responds dramatically to large doses of pyridoxine, had been well known for years but the primary biochemical defect was unknown. The interest fueled by the folinic acid responsive patients, fueled a lot of new research into B_6 metabolism and the primary defect of B_6 dependent seizures is now known. Indeed other defects of pyridoxine metabolism have also been uncovered.. Subsequent research in other centres has now shown that folinic acid responsive seizures and pyridoxine responsive seizures are in fact the same disorder and are caused by mutations in the same gene. The identity of the peaks discovered in the spinal fluid analyses is still unknown but they are probably a complex metabolite of pyridoxine resulting from dysfunctional peroxisomal catabolism of pipecolic acid.

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CHAPTER XXI

S-Adenosylmethionine Hydrolase Deficiency
[OMIM 613752: Gene location 20q11.22]

The Patient:

An infant was detected through the newborn screening programme to have persistently elevated levels of blood methionine. On examination he was hypotonic but not dysmorphic. Tests for homocystinuria were negative and the protein intake was not high. The plasma creatine phosphokinase level was modestly elevated as were the serum transaminases. He was treated with a methionine-restricted diet but without apparent benefit and it was stopped after a few years. He was followed for 25 years before it became practical to demonstrate the primary enzyme defect. He was obviously weak due to a marked myopathy and was also developmentally delayed.

From the outset I was certain that this patient must have had the same defect as had been reported briefly in a single patient from Hungary who had had high blood methionine and a myopathy. This baby had had the identical presentation and biochemical findings but had died without any definitive enzymology being available. Once again, careful perusal of Current Contents had provided a possible clue. Over the years several experts suggested that one or another enzyme might he the culprit but none of these panned out.

Eventually, workers in Hungary found another apparently identical patient and were also able to locate the family of the original case. They developed a major research interest in this area and were able to show that the primary defect was in S-adenosylmethionine hydrolase activity. During their research, I was contacted again by Dr Harvey Mudd at the NIH, who was, and still is, the doyen of methionine metabolism with whom I have remained in contact for 40 years regarding this and other patients. Samples from this patient were sent to the Hungarian group and the precise defect was confirmed. In addition they were also able to find the precise mutations in these subjects.

Why was this patient important?

Here was yet another patient with essentially a new disorder even though I had to wait 25 years for the defect to be identified. Hypermethioninaemia can occur in several disorders including some forms of homocystinuria, various defects of methionine metabolism, of which I have seen several, in chronic liver damage and, in infants if the protein intake is too high; this patient did not fit any of these. The cause of the marked destructive myopathy is not known but it is a constant finding in all cases described to date. There are numerous S-adenosylmethionine-dependent methyltransferases, which are inhibited to some extent by S-adenosylhomocysteine. As a result of this disorder, there is a whole new interest in methylation and its relationship to possible human disease. Of particular interest is in the area of DNA methylation which has a

profound effect on the expression of heritable traits and the expression of tissue-specific gene expression during embryogenesis and after birth. One thing we have learned from this case is that the myopathy, while debilitating, does not seem to be progressive. Undoubtedly, there is more to learn about the mechanism of cell damage in this disorder; presumably it is related to disruption of methylation which is a essential component in so many pathways.

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CHAPTER XXII

A Patient with a Defect in Complex III of the Respiratory Chain.

[OMIM 516020]

The Patient:

A nine-year-old girl was referred to my clinic with a history of profound weakness and poor muscle function associated with exercise induced dyspnea. It was immediately obvious that she had a marked lactic acidosis thus raising the probability of a disorder in the mitochondrial electron transport chain.

My role in this saga was as the clinician responsible for recognizing the class of problem she had, for managing the initial diagnostic tests and the ongoing clinical care. It was entirely due to Dr Nancy Kennaway's interest in mitochondrial disorders that we were able to learn so much from this one case. In our own laboratory, Dr Kennaway ran an internationally renowned mitochondrial research laboratory, and it was she who elaborated the primary defect in the mitochondrial genome in this patient. Not all studies could be done in Oregon so it was she also who contacted the prime researcher in respiratory physiology in the world Dr Britten Chance who at the time, was working on P_{31} spectrometry of muscles during exercise. He leaped at the opportunity to study a patient with a real defect in the electron transport chain in his own lab, and the patient travelled there more than once.

During the initial tests, it was apparent that the intramuscular levels of ATP were normal but that creatine phosphate was low and was easily depleted by even slight exercise. By using the P_{31} spectroscopy, it transpired that judicious use of both vitamin K, as a substitute quinone for Complex III and also vitamin C, could both clearly be shown to improve the function of the electron transport chain with more sustained muscle production of ATP and creatine presumably by enhancing the transport of electrons. Indeed these studies were the initial and best evidence that ANY vitamin cocktail might, in some cases, be beneficial in mitochondropathies. These days, many centres use a wide variety of vitamin cocktails for these patients that includes niacin, riboflavin, vitamins C and K, L-carnitine, all directed at various points in the electron transport chain where they are normally required.

The OMIM number provided above includes defects in the mtDNA cytrochrome C subunit of Complex III; it does not include many other defects of this Complex that have also been reported. Once again, my role in this 20 year saga was more that of an interested pimp whose job it is to find the most interesting problems and farm them on to centres around the world that have developed n expertize in that particular area.

Why was this patient important?

This is the first patient to be described with her particular mutation in mtDNA. Mitochondria are present in every aerobic tissue and essential for aerobic respiration via the electron

transport chain. This system comprises a series of Complexes that are assembled from a mixture of subunits most of which are coded on normal DNA but 13 of which are derived from mitochondrial DNA. At the time this patient was diagnosed, mtDNA had just be sequenced, and the complexity of this system was known in general outline but little was known of the subunits or how they are assembled in the mitochondria. The study of mitochondropathies was just in its infancy, few clinicians were aware of the frequency and protean manifestations of this class of disorders that can indeed affect the functions of any aerobic tissue in the body. There were really no laboratories that could perform the complicated assays for teasing out which Complex of the electron chain was defective and moreover these studies had to be performed on fresh tissue thus requiring that the patient and the laboratory had to be in the same place. Dr Kennaway set out to develop the necessary skills to achieve this and in the end became an internationally recognized expert for such studies, continuing to be involved in this area still today.

It was a tour-de-force that unravelled the primary enzymatic defect but it took more than 20 years finally to prove that the real defect lay in the mtDNA and affected the gene for cytochrome b. In the mean time other laboratories were pursuing the whole field of mitochondrial bioenergetics and the molecular genetics of the electron chain has finally been revealed. Mitochondrial DNA is inherited exclusively from a mother and is now being used for studies of whole population migration and for mapping their travels from out of Africa since 200,000 years ago. Although the inheritance of mtDNA is matrilineal, the manifestations of even a well-diagnosed defect can be entirely different in different members of the same pedigree. This variation depends upon the post-fertilization distribution of mutant and normal mtDNA in every individual.

Since our early report on this patient, there are now dozens of syndromes attributed to mitochondrial problems, defects of every Complex have been reported and in many cases defects of individual subunits and even specific DNA mutations have been confirmed.

This patient was to play another role in this complicated story. The studies of P_{31} NMR spectroscopy performed in Philadelphia by Dr Britten Chance showed clearly that the intramuscular levels of creatine phosphate were low even at rest and that they depleted very rapidly on any exercise. Dr Chance's thought that an artificial quinone in the form of vitamin K and a reducing agent in the form of vitamin C might improve the flow of electrons along the chain. He was able to show this clearly by the NMR spectroscopy. This fueled a huge surge of attempts to improve mitochondrial function in patients with mitochondrial myopathies. Vitamin cocktails of all sorts of formulation have since been used for this purpose for over the past 20 years. There have been anecdotal reports that one vitamin or another has seemed to benefit some patients, but there has been no further publication s that actually prove their benefit.

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CHAPTER XXIII

Current research into quantitating infant sucking and feeding behaviours

The Project:

We have developed and patented a small device [The Orometer] that measures the negative pressures generated by sucking from a feeding bottle. The device depends upon a pressure transducer in a small flow chamber that is located between the feeding bottle and the nipple. Data from the pressure sensor is transmitted in real time to a data acquisition system with a computer from whence it can be downloaded to other systems for data analysis. Information upon suck frequency, strength, consistency between sucks and over time, periodicity, shape and other parameters are collated and analysed. Preliminary work, followed by a phase II and now a phase III studies have been funded by the NIH in the hope that a device with significant clinical potential might emerge.

Rationale:

The most complicated neurodevelopmental function infants face is the simultaneous coordination of sucking, swallowing and breathing, functions that are vital in successful transition from the uterus to independent life. Successful nutritive sucking demands sufficient maturation of the central nervous system, as well as adequate strength and oro-pharyngeal structures. Significant developmental maturation occurs between 34-42 weeks post-menstrual age [PMA] with continuing maturation over at least 6 months. As maturation progresses, characteristic patterns of rhythmicity, frequency and oral-motor function develop, while improved coordination of sucking, swallowing and respiration evolve. Permanent records of a test session are automatically obtained.

Over five percent of all births are premature, and at least two percent of all births have major birth defects. Many of these infants demonstrate feeding problems, with or without abnormal oral function, and these are particularly frequent in infants with prematurity, oropharngyeal dysfunction, or neurodevelopmental damage (that may manifest later as cerebral palsy). The more severe feeding difficulties can lead to poor growth, "failure-to-thrive" with recurrent hospitalization, and often present major diagnostic and management challenges. In fact, feeding problems are the most frequent topic raised by new mothers when seeing a paediatrician.

In addition, feeding problems are the commonest and earliest manifestation of serious brain damage that will only emerge after several months as the infant's brain develops. If it were possible to detect such cases soon after birth, diagnosis would be speeded up by many months and therapy could be started earlier than is usual at this time.

Current clinical assessment of feeding problems is largely observational. The more resistant difficulties require professional diagnosis and may require extensive examinations; but apart from radiologic or bariatric studies. A method that actually quantitates sucking behavior while bottle-feeding, offers the possibility of a new approach to these problems.

There is another, and possibly greater, use for this approach. It is well known that sucking behavior changes with maturing development. If the Orometer can reliably recognize feeding behaviours as a

developmental scale, it could also be used as a novel method to assess developmental maturation. This would surely be a worthwhile goal. No comparable instrument is currently in clinical use.

Results to date:
So far we have been able to develop certain characteristics of normal feeding behavior in normal infants and are now engaged in a large study of feeding in late pre-term infants – a group of infants with the highest rate of readmission for feeding problems. The hope is that we may be able to identify those infants that are the most likely to fail feeding at home and who therefore are most likely to be readmitted. Pre-discharge identification of these infants would lead to better feeding managements and less readmissions.

132. Lang WC, **Buist N.R.M**, Geary A, Buckley S, Adams E, Jones A, Gorsek S, Winter S, Tran H, Rogers B.R. (2011). Quantification of Intraoral Pressures during Nutritive Sucking: Methods with Normal Infants. Dysphagia. 26;139-144.

CHAPTER XXIV

DISCUSSION AND SUMMARY

In trying to describe the importance of the articles I have chosen to include in this thesis, I have tried to consider what seemed to be their importance at the time but also to consider what their longer-term relevance to Biochemical Genetics and to Medicine has been.

In the first place, there is a recurring theme that at the time that the disorders were recognized and the reports published, all of them, as well as others that I have chosen not to include were significant. If not to the larger world of clinical medicine, then to the small but growing band of people who considered themselves as experts in this specialty. During these early days each new disorder that was found, and at times there was a new one every week, was like prospecting for gold in a gold field! It was through simple "metabolic screening" [See Chapter VII], backed up by amino acid and gas chromatography as done in my laboratory, that most of the aminoacidopathies and organicacidopathies were found. As the field expanded, it became easier to recognize a new disorder or variant because a collective store of knowledge was gradually growing. Actually this process is still very much a part of clinical recognition of metabolic disorders in that the web site Metab-L receives almost daily questions regarding some problem that is unknown to the submitter who seeks for help. This service is of enormous value since it must be apparent that the very rarity of so many of these conditions means that only a few experts may ever encounter them over the course of their careers.

If we, as a group, had been satisfied by the finding of these disorders, we would have been negligent in not pursuing them further. It was for this reason that Biochemical Genetics was, at least in the US, considered primarily as a laboratory specialty. Indeed, as soon as it became possible to be trained and certified as a clinician in this specialty, it was essential to have done at least a year of basic work in a laboratory. Clearly with this bias, it was natural for the biochemistry and enzymology to fall into place. In spite of this however, it can be seen from most of my Chapters, that this evolution took many years.

The same phenomenon applies to the rapid way in which our specialty embraced DNA technology. The precise gene location, and often the function of most Inborn Errors of Metabolism have been determined. There are of course still great swathes of ignorance in some areas including the classes such as the peroxisomal, mitochondrial, sarcolemmal and Golgi disorders and it is certain that there will be other areas of human biochemistry that will yield important new knowledge about human physiology and disease. Membrane function, steadily being better understood, is a natural area ripe for further study; already several membranopathies are known but the mechanics of membrane function are still barely unraveled.

Quite often the fame goes to a worker who has spent their whole career in a narrow field rather than, like me being a "Jack of all trades and master of none". However, I would not have traded my way for theirs for an instant. With each new discovery, it has been richly rewarding to become an instant expert in each new area for a while. It would have been

boring to have only one arrow in my quiver so it was exciting to be able to progress to the next challenge without regrets.