

CONTRIBUTORS TO THIS VOLUME

Chester A. Alper, Robert J. Andina, Nicholas D. Carter, Barton Childs,
Ernest H. Y. Chu, Joan M. Finucci, Stanley M. Gartler, Sandra S. Powell,
Malcolm S. Preston, Ann E. Pulver, Fred S. Rosen, Richard E. Tashian

Edited by
H. Harris and
K. Hirschhorn

7

Advances in
HUMAN
GENETICS

ADVANCES IN HUMAN GENETICS

7

Edited by

Harry Harris

*Galton Professor of Human Genetics
University College London
London, England*

and

Kurt Hirschhorn

*Arthur J. and Nellie Z. Cohen Professor of Genetics and Pediatrics
Mount Sinai School of Medicine of The City University of New York*

PLENUM PRESS • NEW YORK AND LONDON

The Library of Congress cataloged the first volume of this title as follows:

Advances in human genetics. 1-
New York, Plenum Press, 1970-

v. illus. 24 cm.

Editors: v. 1- H. Harris and K. Hirschhorn.

1. Human genetics--Collected works. i. Harris, Harry, ed.
ii. Hirschhorn, Kurt, 1926- joint ed.

QH431.A1A32

573.2'1

77-84583

Library of Congress

70 [4]

© 1976 Plenum Press, New York
A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

**ADVANCES IN
HUMAN GENETICS**

7

CONTRIBUTORS TO THIS VOLUME

Chester A. Alper

*Center For Blood Research
and
Department of Pediatrics
Harvard Medical School
Boston, Massachusetts*

Robert J. Andina

*Departments of Medicine and Genetics
University of Washington
Seattle, Washington*

Nicholas D. Carter

*Department of Biochemistry
London Hospital Medical College
University of London
London, England*

Barton Childs

*Department of Pediatrics
Johns Hopkins University School
of Medicine
Baltimore, Maryland*

Ernest H. Y. Chu

*Department of Human Genetics
University of Michigan Medical School
Ann Arbor, Michigan*

Joan M. Finucci

*Department of Pediatrics
Johns Hopkins University School
of Medicine
Baltimore, Maryland*

Stanley M. Gartler

*Departments of Medicine and Genetics
University of Washington
Seattle, Washington*

Sandra S. Powell

*Department of Human Genetics
University of Michigan Medical School
Ann Arbor, Michigan*

Malcolm S. Preston

*Department of Pediatrics
Johns Hopkins University School
of Medicine
Baltimore, Maryland*

Ann E. Pulver

*Department of Pediatrics
Johns Hopkins University School
of Medicine
Baltimore, Maryland*

Fred S. Rosen

*Division of Immunology
Children's Hospital Medical Center
and
Department of Pediatrics
Harvard Medical School
Boston, Massachusetts*

Richard E. Tashian

*Department of Human Genetics
University of Michigan Medical School
Ann Arbor, Michigan*

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

ARTICLES PLANNED FOR FUTURE VOLUMES:

The Genetic Aspects of Facial Abnormalities • *Robert J. Gorlin and William S. Boggs*

Facts and Theories Relating to Chromosome Structure in Man and Other Animals • *John H. Evans*

The Human α -Amylases • *A. Donald Merritt and Robert C. Karn*

Genetics and Etiology of Human Cancer • *Alfred G. Knudson, Jr.*

Population Genetics Theory in Relation to the Neutralist—

Selectionist Controversy • *Warren J. Ewens*

Genetic Aspects of Folate Metabolism • *Richard W. Erbe*

Recent Advances in Hemoglobin Genetics • *Donald L. Rucknagel*

Chromosomes and Neoplasia • *David G. Harnden and A. M. R. Taylor*

Blood Group Antigens • *Winifred Watkins*

CONTENTS OF EARLIER VOLUMES:

VOLUME 1 (1970)

- Analysis of Pedigree Data • *J. H. Edwards*
Autoradiography in Human Cytogenetics • *Orlando J. Miller*
Genetics of Immunoglobulins • *H. Hugh Fudenberg and Noel E. Warner*
Human Genetics of Membrane Transport with Emphasis on Amino Acids
• *Charles R. Scriver and Peter Hechtman*
Genetics of Disorders of Intestinal Digestion and Absorption • *Jean Frézal*
and *Jean Rey*

VOLUME 2 (1971)

- Glucose-6-Phosphate Dehydrogenase • *Henry N. Kirkman*
Albinism • *Carl J. Witkop, Jr.*
Acatalasemia • *Hugo Aebi and Hedi Suter*
Chromosomes and Abortion • *D. H. Carr*
A Biochemical Genetic View of Human Cell Culture • *William J. Mellman*

VOLUME 3 (1972)

- Prenatal Detection of Genetic Disorders • *Henry L. Nadler*
Ganglioside Storage Diseases • *John S. O'Brien*
Induced Chromosomal Aberrations in Man • *Arthur D. Bloom*
Linkage Analysis Using Somatic Cell Hybrids • *Frank H. Ruddle*
The Structure and Function of Chromatin • *David E. Comings*

VOLUME 4 (1973)

- Genetic Screening • *Harvey L. Levy*
Human Population Structure • *Chris Cannings and L. Cavalli-Sforza*
Status and Prospects of Research in Hereditary Deafness • *Walter E. Nance*
and *Freeman E. McConnell*
Congenital Adrenal Hyperplasia • *Maria I. New and Lenore S. Levine*
Cytogenetic Aspects of Human Male Meiosis • *Maj Hultén and J. Lindsten*

VOLUME 5 (1975)

- The Chondrodystrophies • *David L. Rimoin*
New Techniques in the Study of Human Chromosomes: Methods and
Applications • *Bernard Dutrillaux and Jerome Lejeune*
The Thalassemias: Models for Analysis of Quantitative Gene Control • *David*
Kabat and Robert D. Koler
Spontaneous Mutation in Man • *Friedrich Vogel and Rüdiger Rathenberg*
Genetic Screening Legislation • *Philip Reilly*

VOLUME 6 (1976)

- Vitamin-Responsive Inherited Metabolic Disorders • *Leon E. Rosenberg*
Inherited Deficiency of Hypoxanthine-Guanine Phosphoribosyltransferase
in X-Linked Uric Aciduria • *J. Edwin Seegmiller*
Hereditary Hemolytic Anemia Due to Enzyme Defects of Glycolysis • *Sergio*
Piomelli and Laurence Corash
Population Structure of the Åland Islands, Finland • *James H. Mielke, Peter L.*
Workman, Johan Fellman, and Aldur W. Eriksson
Population Genetics and Health Care Delivery: The Quebec Experience
• *Claude Laberge*

Preface to Volume 1

During the last few years the science of human genetics has been expanding almost explosively. Original papers dealing with different aspects of the subject are appearing at an increasingly rapid rate in a very wide range of journals, and it becomes more and more difficult for the geneticist and virtually impossible for the nongeneticist to keep track of the developments. Furthermore, new observations and discoveries relevant to an overall understanding of the subject result from investigations using very diverse techniques and methodologies and originating in a variety of different disciplines. Thus, investigations in such various fields as enzymology, immunology, protein chemistry, cytology, pediatrics, neurology, internal medicine, anthropology, and mathematical and statistical genetics, to name but a few, have each contributed results and ideas of general significance to the study of human genetics. Not surprisingly it is often difficult for workers in one branch of the subject to assess and assimilate findings made in another. This can be a serious limiting factor on the rate of progress.

Thus, there appears to be a real need for critical review articles which summarize the positions reached in different areas, and it is hoped that "Advances in Human Genetics" will help to meet this requirement.

Each of the contributors has been asked to write an account of the position that has been reached in the investigations of a specific topic in one of the branches of human genetics. The reviews are intended to be critical and to deal with the topic in depth from the writer's own point of view. It is hoped that the articles will provide workers in other branches of the subject, and in related disciplines, with a detailed account of the results so far obtained in the particular area, and help them to assess the relevance of these discoveries to aspects of their own work, as well as to the science as a whole. The reviews are also intended to give the reader some idea of the nature of the technical and methodological problems involved, and to indicate new directions stemming from recent advances.

The contributors have not been restricted in the arrangement or organization of their material or in the manner of its presentation, so that the reader should be able to appreciate something of the individuality of approach which goes to make up the subject of human genetics, and which, indeed, gives it much of its fascination.

HARRY HARRIS

The Galton Laboratory

University College London

KURT HIRSCHHORN

Division of Medical Genetics

Department of Pediatrics

Mount Sinai School of Medicine

Contents

Chapter 1

Biochemical Genetics of Carbonic Anhydrase

Richard E. Tashian and Nicholas D. Carter

Introduction 1

The Isozymes: General Considerations 2

 Early Investigations 2

 Structure 4

 Secondary Isozymes 7

 Kinetic Properties 7

 Developmental Aspects 8

 Tissue Distribution 9

Nomenclature 9

Qualitative Genetic Variation 12

 Variants of Human Carbonic Anhydrase I 12

 Variants of Human Carbonic Anhydrase II 21

 Carbonic Anhydrase Variants in Nonhuman Primates 24

 Variants in Other Mammals 28

Quantitative Variation 29

 Nongenetic Factors 29

 Carbonic Anhydrase Levels in Human Variants 32

 Normal Individual Variation in Human Populations 32

 Regulation of Carbonic Anhydrase Levels in Primates 34

 Quantitative Deficiencies in Rodents 36

 Levels of Carbonic Anhydrase in Single Cells 36

 Correlation between CA I and CA II Levels in Erythrocytes 38

Linkage of CA I and CA II Loci	39
Additional Carbonic Anhydrase Loci	41
Clinical Aspects	42
Renal Tubular Acidosis	42
Respiratory-Distress Syndrome	44
Phylogenetic Variation	44
Comparative Activities	44
Rates of Evolution	45
Acknowledgments	47
Note Added in Proof	47
Bibliography	48

Chapter 2

Human Behavior Genetics

*Barton Childs, Joan M. Finucci, Malcolm S. Preston, and
Ann E. Pulver*

Introduction	57
Purposes of the Review	59
Definitions and Strategies	60
Behavior Genetics	60
Medical Genetics	61
Strategies for the Investigation of Behavioral Abnormality	61
The Genetic Analysis of Defined Phenotypes	63
Psychoses	63
Individual Variation in Response to Drugs Affecting Behavior	73
Tests of Psychological Functions	78
Behavioral Analysis of Defined Genotypes	87
Chromosome Abnormalities	87
Single Gene Differences	89
Conclusion	91
Individual Benefits	91
Social Benefits	91
Acknowledgment	92
Bibliography	92

Chapter 3

Mammalian X-Chromosome Inactivation

Stanley M. Gartler and Robert J. Andina

Introduction 99

Detection of X-Chromosome Inactivation 101

 Genetic Expression 101

 Chromosomal Nature of Inactivation 104

 Cytological Expression of X-Chromosome Inactivation 105

 Stability of X-Chromosome Inactivation 108

Embryonic Timing of X-Chromosome Inactivation 109

 Cytological Observations 110

 Genetic Expression 110

Utilization of Mosaic Cell Populations as Developmental
 Markers 114

X-Chromosome Aberrations and Inactivation 116

X-Chromosome Expression in Germ Cells 118

Control Systems 124

 Initiation of Inactivation 124

 Randomness 125

 Maintenance of X-Chromosome Inactivation 126

 Chromosomal Nature of the Inactivation Process 128

Evolutionary Aspects 129

Acknowledgments 132

Bibliography 133

Chapter 4

Genetics of the Complement System

Chester A. Alper and Fred S. Rosen

Introduction 141

Human Complement Genetics 144

 Structural Polymorphisms of Human Complement Proteins 144

 Genetic Defects in Human Complement 152

Animal Complement Genetics 165

 Structural Polymorphisms 165

 Complement Deficiencies in Experimental Animals 167

Linkage Relationships and Chromosomal Localization of Complement Genes	172
Addendum	173
Acknowledgments	176
Bibliography	176

Chapter 5

Selective Systems in Somatic Cell Genetics

Ernest H. Y. Chu and Sandra S. Powell

Introduction	189
Techniques for Clonal Isolation	190
Single-Cell Separation	191
Dilution Plating and Colony Picking	192
Microcultures	192
Isolation of Variants	193
Definitions	193
Spontaneous and Induced Mutations	194
Nonselective Techniques	194
Selective Techniques	199
Selection for Revertants	218
The "HAT" Selection	219
The "AA" Selection	222
The "HAM" Selection	223
The "dCR-dTR" Selection	224
Selection for Cell Hybrids	225
Selection against Unfused Parental Cells	225
Selection against One Parental Genome in Cell Hybrids	228
Selective Retention or Elimination of Specific Chromosomes	229
Intercellular Transfer of Genetic Materials	230
Gene Transfer via Purified DNA	231
Virus-Mediated DNA Transfer	233
Gene Transfer via Isolated Chromosomes	235
Gene Transfer via Intact Cell Fusion	237
Fusion between Intact Cells and Cell Fragments	239

Concluding Remarks	240
Acknowledgments	242
Bibliography	242
 Index	 259

Chapter 1

Biochemical Genetics of Carbonic Anhydrase

Richard E. Tashian

*Department of Human Genetics
University of Michigan Medical School
Ann Arbor, Michigan*

and

Nicholas D. Carter

*Department of Biochemistry
London Hospital Medical College
University of London
London, England*

INTRODUCTION

Carbonic anhydrase (EC 4.2.1.1. carbonate dehydratase) appears to be present in placental mammals as two distinct molecular forms, or isozymes, which are apparently under the control of two closely linked autosomal genes. Next to hemoglobin, carbonic anhydrase is the most abundant protein to be found in human erythrocytes. This feature, together with the easily definable electrophoretic phenotypes of the two isozymes, and the relative ease with which they can be purified from hemolysates, has made the carbonic anhydrase isozyme system a particularly attractive one for the study of genetic variation in humans at the molecular level.

There are a number of other advantages in working on the biochemical genetics of the human carbonic anhydrases: (1) Studies on the relationships of structure to function are now possible because the complete amino acid sequences and three-dimensional structures have

been determined for both human isozymes. (2) It is now feasible to study certain aspects of enzyme regulation, as the rates of synthesis for both isozymes are relatively easy to follow in reticulocyte systems. (3) The fact that antibodies specific for each isozyme can be produced has made it possible to determine accurately the level of each isozyme, not only in cellular lysates by a sensitive radioimmunoassay, but also in single cells by cytoimmunodiffusion and fluorescent-antibody techniques. (4) Qualitative and quantitative inherited variants, both rare and polymorphic, have been described for the two isozymes in human populations as well as in other mammalian species. (5) The activity of carbonic anhydrase toward various ester substrates has given us an additional tool with which to study mutant carbonic anhydrases.

Much remains to be learned about the physiological roles, distribution, and regulation of the carbonic anhydrases at the cellular level; nevertheless, few isozyme systems have been so well characterized with respect to their molecular structure, activity, genetic control, and evolution.

Although we will be primarily concerned here with genetic variation in the human carbonic anhydrases, genetic variability of the homologous forms of these enzymes in other species will be discussed when we feel that this information will be useful in understanding the genetic control of human carbonic anhydrase.

THE ISOZYMES: GENERAL CONSIDERATIONS

In the following brief accounts of the structure, activity, distribution, and development of the carbonic anhydrase isozymes, only those aspects are covered which serve as an introduction to the material covered in this review. The following reviews should be consulted for more detailed information: Maren⁹⁰ (chemistry and physiology); Edsall,³⁶ Tashian,¹³⁴ and Derrien and Laurent²⁷ (red-cell isozymes); Carter¹² (distribution and function of the isozymes); and Lindskog *et al.*⁸¹ (physical and chemical aspects).

Early Investigations

In 1932 and 1933, Meldrum and Roughton^{91,92} reported on the isolation of an enzyme from human red blood cells that rapidly catalyzed

the reversible hydration of CO_2 ($\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$), and named it carbonic anhydrase. Several important studies by Keilin and Mann^{63,89} were to follow which demonstrated that carbonic anhydrase contained zinc, and that the activity of the enzyme could be specifically inhibited by certain aromatic sulfonamides. However, it was not until about 30 years after its discovery that investigators in three laboratories independently demonstrated that carbonic anhydrase was present as two distinct molecular forms in human hemolysates.^{71,102,110} Using chromatographic separation procedures, they showed that the form present in lower concentrations had a higher specific CO_2 hydrase activity than the other form which was 5–6 times more abundant.

As early as 1956, Derrien and his associates in Marseilles described an electrophoretically distinct, nonheme protein from human red cells which they named X_1 ,²⁸ and a few years later, Laurent *et al.*⁷⁰ reported another nonheme protein which was designated Y. They were later to show that the X_1 and Y proteins represented the two major electrophoretic forms of red-cell carbonic anhydrase.⁷¹

In the early studies¹³¹ on the electrophoretically separated carboxyl esterase patterns of human hemolysates, a slowly migrating anodal band was designated A_8 ; later, a weakly staining, cathodally migrating esterase band was detected and designated A_9 . However, it soon became apparent that the A_8 and A_9 esterases behaved differently from the other classes of esterases (A, B, and C) when treated with certain activator and inhibitor compounds, and they were subsequently reclassified as D esterases and designated Da and Db , respectively. During the screening of red-cell esterases, Shaw *et al.*¹²⁰ discovered an electrophoretic variant of the Da esterase in a boy with Down's syndrome. This variant was also found in the normal father and normal paternal grandmother of the propositus, thereby indicating that the variant was under the control of an autosomal gene.

The findings that only the D esterases, but not the other red cell esterases, could be specifically inhibited by the specific carbonic anhydrase inhibitor, acetazolamide (Diamox), in addition to the fact that the D esterases could be visualized directly on a starch gel by a histochemical stain for carbonic anhydrase,¹²⁰ strongly suggested that the two esterases represented the two isozymes of carbonic anhydrase that had just been reported.^{71,102,110} The fact that esterase Db was not affected by this mutation also suggested that Da and Db were under the control of two separate loci. When electrophoretic patterns of Da and Db were