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Advances in HUMAN GENETICS

ADVANCES IN HUMAN GENETICS 7

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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]

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ARTICLES PLANNED FOR FUTURE VOLUMES:

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

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Chapter 1

Biochemical Genetics of Carbonic Anhydrase

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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: Maren[90] (chemistry and physiology); Edsall,[36] Tashian,[134] and Derrien and Laurent[27] (red-cell isozymes); Carter[12] (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 ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$), 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 studies[131] 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 D*a* and D*b*, respectively. During the screening of red-cell esterases, Shaw *et al.*[120] discovered an electrophoretic variant of the D*a* 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 D*b* was not affected by this mutation also suggested that D*a* and D*b* were under the control of two separate loci. When electrophoretic patterns of D*a* and D*b* were