# Biochemical Methods in Medical Genetics

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*Research Physician (Genetics)*
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This is a handbook of procedures, practiced and proven in the author's laboratory, for the analysis of specimens from patients with inborn errors and other heritable metabolic disorders. Designed primarily for the laboratory technician, the text features related facts for the instruction of physicians, genetic counselors, nurses and other health care personnel

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*By*

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# FOREWORD

MEDICINE is finding many new opportunities in the expanding field of heritable diseases where the combined attention of biochemists and physicians has yielded revolutionary advances. Doctor Kelly, who was highly competent in biochemistry before undertaking her medical studies, ideally expresses the advantages of this broad approach.

Her book includes those assays she has found useful in her direction of the biochemical laboratory of the Birth Defects Institute, New York State Department of Health. The value and reliability of the methods have been established by Doctor Kelly both in the study of patients and in the laboratory.

The compilation is also a step toward the standardization of laboratory techniques, a desirable sequel to the development of a diagnostic methodology. In this respect the work perpetuates the character of Wadsworth's *Standard Methods,** for many years the model in the public health laboratory field.

The book is highly recommended for its completeness and its trustworthiness.

Gilbert Dalldorf, M.D.

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*Wadsworth, Augustus B.: *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health,* 3rd ed. Baltimore, Williams & Wilkins Company, 1947.

# PREFACE

THE modern observer of heritable diseases faces unfamiliar territory, as the fresh breeze of genetics clears horizons and brightens the dark corners of medicine. Like other taxonomists, he gains confidence by thumbing his manual, relating the unknown to familiar species. May this slim handbook be such a tool!

The manual contains the procedures we use in providing physicians with laboratory data needed for the diagnosis, treatment, and control of heritable metabolic disease. Included are the diseases or groups of diseases in which the biochemical abnormalities are either pathognomonic or clearly associated; excluded are those in which the biochemical lesions are poorly defined, nonspecifically expressed or inaccessible. The procedures have been selected empirically, organized according to the metabolic pathway or structural protein affected, and the emphasis placed on assays which provide the biochemical facts necessary for the recognition of disease.

I hope that presentation of the details will encourage laboratory technicians to add to their armamentarium of diagnostic tests, that the brief clinical facts will alert medical workers to the laboratory's diagnostic potential, and that the compilation will serve as a sourcebook of methods for investigators who study other facets of biochemical medical genetics.

Many have helped me: Dr. Victor Tompkins, who introduced medical genetics into the concerns of the New York State Department of Health, invited me to contribute and directed me to the field; Dr. Ian Porter continued the leadership and gave me time to undertake the task; the editor encouraged its fruition by many years of friendly support. I also thank my laboratory staff for initiative and competence in providing the details, especially Lucille Desjardins, Edward Leikhim, Lewis Schedlbauer,

Rani Bakhru-Kishore, Alice Dagle, and James Seeger; Constance Christo for patiently typing the manuscript through many revisions; Dr. Marilyn Cowger for critically reading the manuscript; and Philomena Sculco for the drawings.

S.K.

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# Biochemical Methods in Medical Genetics

# BASIC CONCEPTS OF BIOCHEMICAL MEDICAL GENETICS

## *WHAT ARE INHERITED METABOLIC DISEASES?*

THE inborn errors and other heritable metabolic diseases excite both clinicians and biochemists. Indeed, their combined efforts developed the field to its present fertility. The two approaches help us to understand and control these diseases; the energies and skills of both disciplines bear jointly on the problems and find solutions together.

The clinician and biochemist cooperate intimately in managing these diseases. The clinician sees the disease — the biochemist identifies the underlying cause and means of control. Together they uncover the facade of overt disease in the patient and find a larger skeleton of biochemical disease hidden in the family.

## *GENES AND METABOLIC DISEASE*

Most heritable metabolic diseases appear only in one generation of a family. They are caused by mutant genes which express themselves as disease only when present in pairs. Thus, the patient usually has two mutant genes, one from each parent. Geneticists refer to this kind of disease as "recessively inherited." If the patient inherits a pair of mutant genes, he is homozygous for the gene and usually has clinical signs; if he inherits the gene from one parent only, he is heterozygous for, or a carrier of, the mutant gene. He has a corresponding normal gene for the trait and, consequently, rarely has the clinical signs. Both patient and carrier, however, have biochemical disease expressed in varying degrees.

Diseases which appear as the expression of single mutant genes, i.e. in heterozygotes, are considered dominantly inherited, are not associated with specific metabolic defects, and thus are not

referred to here, unless the clinical signs are similar to those of certain recessively inherited metabolic diseases.

Single mutant genes for recessively inherited diseases, not present as a pair, will cause disease under some circumstances, as, for example, those carried on the "female" or X-chromosome. Boys receive an X chromosome from their mothers; if it contains a mutant gene, the mutation is not balanced by a normal gene, because a boy's other sex chromosome is a Y chromosome received from his father, rather than another X chromosome. Only boys have the disease, and they receive the mutation only from their mothers. Such recessively-inherited diseases are considered X-linked.

The actions of mutant genes are at the core of inherited metabolic disease. The mutation triggers a chain of metabolic mistakes which lead to biochemical and, finally, clinical abnormalities.

The metabolic pathway through which the cells change nutrients into energy is often the target of mutant genes. The molecular changes proceed by steps, each step catalyzed by a specific enzyme, the production or regulation of which is governed by a gene. If the enzyme is absent, the step is blocked, and the metabolic pathway is interrupted. Immediate and precursor substrates accumulate, expected products do not form, abnormal derivatives appear, and alternative and less efficient pathways emerge. The primary result is deranged metabolism. Cells in the homozygous patient usually lack the enzyme entirely, so that the metabolic block is complete. The cells of carriers, however, form enough normal enzyme under normal gene direction to maintain the metabolic flow at relatively normal rates.

If, on the other hand, the mutation's target is cell structure, structural proteins, like hemoglobin, will be affected. The homozygous patient has none of the normal protein, e.g. hemoglobin A, but survives with a substitute and often less functional form. The heterozygote, however, forms both the substitute protein and enough normal protein for normal function.

How do genes regulate the production of structural and enzymic proteins? Molecular biologists theorize that genes direct the manufacture of both forms of cytoplasmic protein. Mutation interferes with this role by directing, instead, the manufacture of

changed and nonfunctional molecules. The direct effect of the mutation is a change in the primary structure of the protein which forms structural protein, e.g. hemoglobin, or enzyme protein, i.e. a change in the kind or sequence of amino acids which form the protein's "backbone" of polypeptide chains. Ordinarily, the manufacture of polypeptide chains in the cytoplasmic organelles is directed by normal genes. This theory of gene action is based on the Watson-Crick model of deoxyribonucleic acid (DNA), the major molecular component of nuclear chromatin, whereby a triplet of base pairs in the double-stranded helix functions as the gene, codes for an amino acid in the cytoplasm, and transfers the information by a messenger molecule of ribonucleic acid (RNA). The mechanism is clearly applicable to the manufacture of proteins like hemoglobin, whose structure is known, and is probably applicable to the enzymic proteins, whose primary structures are still mostly unknown.

## *NATURE OF "MARKERS"*

The clinical expression of a mutant gene for a recessively inherited disease usually appears only in the homozygote. The biochemical expression, however, may also appear in other members of the family — the carriers — and is thus a truer indication of the mutant gene's distribution in a family than is disease.

The biochemist searches for clues to the gene among the metabolic products of the patient's or carrier's cells, tissues, and body fluids. If biochemical abnormalities appear consistently in association with clinical signs, we accept them as a "marker" of the disease and, since the disease is caused by a mutation, a marker of the mutant gene.

The way or ways a mutant gene manifests itself to us is considered its phenotype. Thus, there are both clinical and biochemical phenotypes of the mutant genes for heritable metabolic diseases. Homozygous persons usually express both phenotypes. The heterozygous person usually expresses only the biochemical phenotype in a mild form. Conversely, the degree of expression is useful in predicting genotypes. When the expression is complete or severe, one predicts a homozygous genotype; if mild, one predicts the

heterozygous state. If absent, the family member may be homozygous for the normal gene.

"Markers" reflect the metabolic systems affected by the mutant gene. Fat storage is involved in Tay-Sachs disease, for example, carbohydrate degradation in galactosemia, and energy transfer in the red cell enzyme deficiency diseases. The particular process and step affected are characteristic of the individual gene and disease. The crux of the biochemist's problem is to identify the metabolic pathway involved and the step affected.

The biochemist chooses a laboratory test or battery of tests to expose the particular marker. He employs specific chemical or histochemical procedures for the suspected metabolite, measures enzyme function, or deciphers structure.

These tests implicate the metabolic system involved and may even identify the exact site of the biochemical error.

One also chooses the biological sample carefully, as gene-induced changes (and markers) are often found only at specific anatomic sites. Fortunately, many sites are accessible and pose no sampling problem. The body fluids and formed elements of the blood, for example, are obtained easily and contain a great many markers. Other sites are equally accessible, such as nail scrapings and hair bulbs, but contain only few or single markers. Fibroblasts, muscle, liver, and amniotic cells, on the other hand, are less accessible and samples must be obtained surgically. In certain tissues, furthermore, the markers are so weak that cells must be cultured before analysis, or, if lymphocytes, artificially stimulated to divide.

The structural proteins, the hemoglobins and myoglobins, for example, are reliable indicators of mutation because their formation and deviation from normal can be traced back to a single change in the DNA molecule. Indeed, the synthesis of the various polypeptide chains of the hemoglobins was the model for the current molecular theory of gene action! Furthermore, other phenotypic expressions of the mutant gene — a particular syndrome or constellation of clinical signs; clear-cut pedigree pattern including usually dominant inheritance of the marker itself; qualitative, "all-or-none" evidence for the cellular protein involved, based on demonstration of protein mass or its absence — are

consistently associated with abnormal structural protein.

Abnormality in enzyme protein, on the other hand, is a somewhat less reliable marker of mutation. The primary structures are for the most part unknown; therefore, changes are not directly traceable to mutational events. That the mutation results in a structural change in the immediate gene product is part of current molecular genetic theory. The uncertainty lies in where the errors are in the polypeptide chains. The association of enzymic protein markers with syndromes, pedigree patterns, etc., however, is consistent, like that of the structural protein markers. The test data, on the other hand, are valuable chiefly for their quantitative, rather than qualitative, feature. The quantitative data often consist of measures of enzyme activity. The enzyme proteins, furthermore, are more subject to variation and are found in a greater variety of anatomic sites than the structural proteins. Thus they are more variable markers of mutations.

The products of deranged metabolism which appear in response to the metabolic block form another group of markers — unused substrate, intermediary metabolites, storage products, and abnormal derivatives. They accompany the enzyme protein marker and, like the enzymes, are found in both cells and body fluids. Although their appearance is secondary to that of the enzyme protein markers and thus are less closely related to the mutation itself, they are useful when the defect is unknown, is detectable only in less accessible tissue or is more difficult to demonstrate.

These markers are subject to greater variability than the structural or enzymic proteins: Their molecular structures are by-products of the metabolic block rather than abnormalities deriving from a gene-directed amino acid substitution. They are nonspecific in that, as the product of deranged metabolism, they may arise by one of several metabolic routes, none of which is necessarily the result of a mutation. Since several synthetic and regulatory steps, furthermore, may intervene between the mutation and the appearance of the marker, nongenic influences may interfere with the mutant gene's expression.

Other dissimilarities to the structural or enzymic protein markers are their association with variable clinical signs and other

nongenic diseases. The biochemical pedigrees of families traced by this kind of marker are often incomplete, as the marker usually appears only in the homozygous patient. The data, furthermore, are primarily quantitative and can be interpreted variably. Galactosuria, for example, occurs in several diseases and conditions to varying degrees, and from several causes; it is a less certain marker of the mutant gene for galactosemia than is absence of the specific transferase protein.

## *HOW "MARKERS" ARE DETECTED*

The biochemist searches for markers among the products of cells, the formation of which is governed, directly or indirectly, by gene action. He recognizes the molecular changes mutant genes cause in structural protein by comparing the properties of the mutant protein with those of normal structural protein. The marker may have electrophoretic mobility, for example, or solubility or refraction properties which differ from those of the normal protein if the mutation results in the substitution of an amino acid with a change in net electric charge.

He recognizes the effects of mutant genes on enzymic protein through changes in kinetic rather than physical-chemical properties of enzymes. The marker is usually less or no activity. Factors which affect activity — substrate specificity and sensitivity, and pH optima or heat stability — are also useful markers, especially when searching for variant *forms* of disease. The discovery of isozymes or multiple forms of enzymes by electrophoresis reveals that enzyme protein, like structural protein, may be structurally heterogeneous. We infer, further, that the synthesis of enzyme protein, like that of structural protein, involves gene-directed changes in molecular structure.

The biochemist detects markers comprised of substrates, intermediates, and storage products by a variety of chemical or physical procedures, chosen either to demonstrate their presence or absence or to measure quantity by chromatography, colorimetry, turbidity, solubility, microbiological assay, or histochemistry.