INTERPRETATION OF ELECTROPHORETIC PATTERNS OF PROTEINS AND ISOENZYMES

A Clinical Pathologic Guide

PAUL L. WOLF, M.D.
JOHN C. GRIFFITHS, M.D.
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PAUL L. WOLF, M.D.
Clinical Professor of Pathology
University of California
San Diego, California
and Consultant in Laboratory Medicine
San Diego Naval Regional Medical Center
San Diego, California

JOHN C. GRIFFITHS, M.D.
Professor of Pathology
University of California
San Diego, California

JOHN W. KOETT, CDR MC USN
Department of Laboratory Medicine—
Clinical Chemistry
and Clinical Investigation Center
San Diego Naval Regional Medical Center
San Diego, California

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PREFACE

This book was written primarily to aid the practicing physician, resident physicians in clinical specialties, and medical students in the interpretation of electrophoretic patterns of serum proteins and isoenzymes. We have included electrophoretic patterns from patients with a diverse group of clinical conditions which we have encountered in the clinical laboratories of the Veteran's Administration Hospital, La Jolla, California, the Naval Regional Medical Center, San Diego, California, and Stanford University Medical Center. The abnormalities in the electrophoretic patterns are correlated with the patient's clinical data when it is pertinent. The elevated and decreased electrophoretic values are identified and are analyzed as they relate to the disease process. The important literature is cited relevant to the cause for the abnormality. Electrophoretic patterns which are analyzed include serum proteins, urine proteins, cerebrospinal fluid proteins, serum isoenzymes of lactate dehydrogenase, creatine kinase, alkaline phosphatase, and gamma glutamyl transferase.

Electrophoretic patterns are used throughout the world to provide important medical benefits. The most important benefit of electrophoresis is the procurement of diagnostic information which may not be apparent from the history, physical, chemistry, or hematology data or urinalysis. Certain abnormalities are especially valuable in identifying diseases which have not been detected from the usual diagnostic data. The electrophoretic technique has been refined in the last few years and the practical utilization in the clinical laboratory has become a relatively rapid, easy and low cost procedure. Abnormalities are identified quickly and there is the added benefit of rapid identification of abnormalities, institution of therapy, and decreased length of hospitalization.

Since the major goal of this book is to provide diagnostic information to the practicing physician for the optimal care of patients, a set of slides of 35 mm photographs will be available with a teaching cassette audio tape which may be used at teaching conferences.

We gratefully acknowledge the excellent secretarial work of Mrs. Patricia Lapiezo who typed the entire manuscript. We kindly acknowledge the reviewing assistance of William Orville Harrison, Capt., MC USN, Director, Clinical Investigation Center, San Diego Regional Medical Center. We also owe a great debt to Jeanne Rich and Alan Frankenfield for their support and advice during the preparation of this book.

DISCLAIMER

The opinions or assertions herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense or the Department of the Navy.

CONTENTS

THE SERUM PROTEINS

Over 100 serum proteins have now been identified in the serum. Some are in high concentrations and their measurement is simple, whereas others are in microgram quantities and elaborate immunological techniques are required for their determination. One of the easiest techniques of assessing abnormalities of the serum proteins is to perform serum protein electrophoresis. An easy technique utilizes a cellulose acetate membrane in conjunction with a pH 8.6 Tris-barbital buffer. A small quantity of sample, 0.25 micro-liters, is applied to one end of the membrane and an electrical current is applied across the membrane. After a determined time period the current is stopped, and the membrane is subsequently developed utilizing protein stains for visualization. It is then scanned with a density spectrophotometer and the areas under each fraction is calculated. The concentration of each serum protein component is next approximated.

The ability to separate proteins in an electrical field is based upon the amphoteric properties of the molecules. When utilizing cellulose acetate, the migrational properties of the individual proteins will be influenced by the surface charge which is dependent on the pH of the buffering system and component amino acids of the protein. With polyacrylamide gel the separation of the protein components is based not only upon the surface charge of the molecules but also on the molecular size due to the filtering action of the gel. The sequential location of the proteins remains the same when utilizing cellulose acetate or agarose gel systems, but not necessarily the polyacrylamide gels in that this latter system separates proteins on the basis of two parameters cited above.

Another technique which has been utilized in the past few years is isoelectric focusing. In this system a stabilized gradient of ampholytes with differing pHs is introduced into a cylindrical column. The sample is then placed into the column, and an electrical potential applied across the system. The individual proteins will migrate toward the anode or cathode until they reach the appropriate pH in which their net charge is zero, i.e. they have become Zwitterions at their isoelectric point.

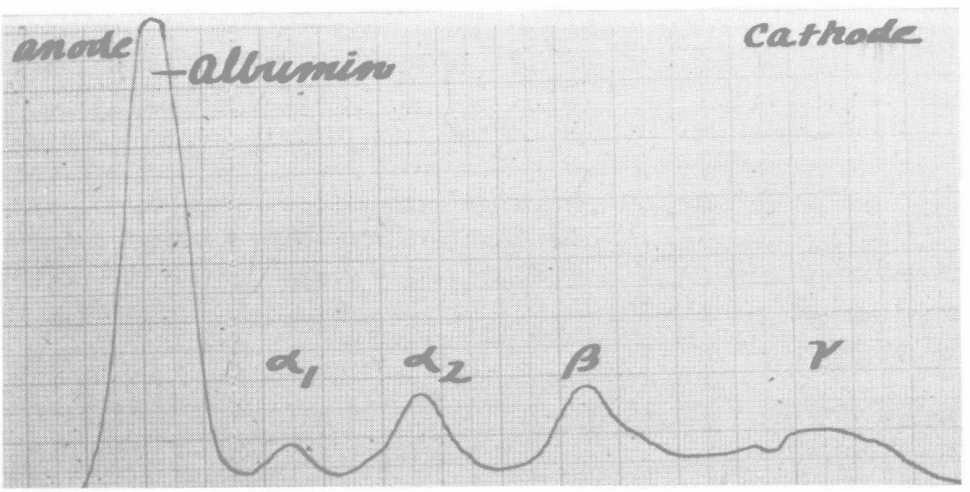
In the clinical laboratory methods have evolved by necessity that are easy to perform and adaptable to automated analysis. One of the simplest methods for measuring the concentration of protein is to measure the refractive index. As the concentration increases so does the refraction.

One of the most frequently used methods is the Biuret reaction. The chemical reaction involves the formation of a copper complex with the peptide bonds of the total protein in solution. The technique is rapid and simple in design which has made the method ideal for the clinical chemist. The formation of the blue-color complex on addition of alkaline copper sulfate is easily measured spectrophotometrically. The binding properties of specific proteins has been utilized to measure their concentration within the body fluids. For example, the binding of the dye Bromcresol green by albumin affords a reliable and specific method for its determination.

More selective methods employing immunological techniques to measure specific proteins have become useful in the past years and include radial immunodiffusion and laser nephelometry.

In summary, returning now to the more clinical aspects of protein analysis, the total protein is usually determined by the Biuret reaction, while the albumin is quantitated by the Bromcresol green reaction. The abnormalities of the individual serum proteins may be quickly identified by electrophoresis using cellulose acetate membranes. Patients with various abnormalities will now be presented with brief correlative clinical data, analysis of the serum, urine or cerebrospinal fluid and appropriate reference citations or suggested relevant reading. A normal electrophoretic pattern is present on the opposite page. The normal values are listed below the electrophoretic pattern.

NORMAL ELECTROPHORETIC PATTERN



Normals (gm.%)

T P	(6.0 - 8.0)
Albumin	(3.2 - 5.0)
Alpha 1	(0.1 - 0.4)
Alpha 2	(0.6 - 1.0)
Beta	(0.6 - 1.3)
Gamma	(0.7 - 1.5)

HISTORY

The patient, M.T., is a 46 year old white male with a marked degree of pain in the low back radiating down the right lower extremity. The diagnostic workup included a serum protein analysis, including the serum protein electrophoretic pattern on the opposite page. The patient's pain was found to be caused by a herniated lumbar intervertebral disc which was treated surgically.

ABNORMALITY

Bisalbuminemia, a serum albumin that is separated into two distinct bands.

ANALYSIS

This abnormal pattern is inherited and there is no disease associated.[1] The individuals are asymptomatic and the total levels of albumin and the other serum proteins are normal. Approximately ten electrophoretic forms of albumin have been reported. Five have traveled faster and five slower than normal albumin. The inheritance is autosomal codominant. The binding activity and stability of the variant albumin differs from normal albumin.[2] Normal albumin migration is increased with drugs such as aspirin and penicillin, and with hyperamylasemia and hyperbilirubinemia. Acquired bisalbuminemia may be induced by the therapeutic utilization of penicillin.[3] Increased bilirubin causes a shift of albumin mobility toward the anode.

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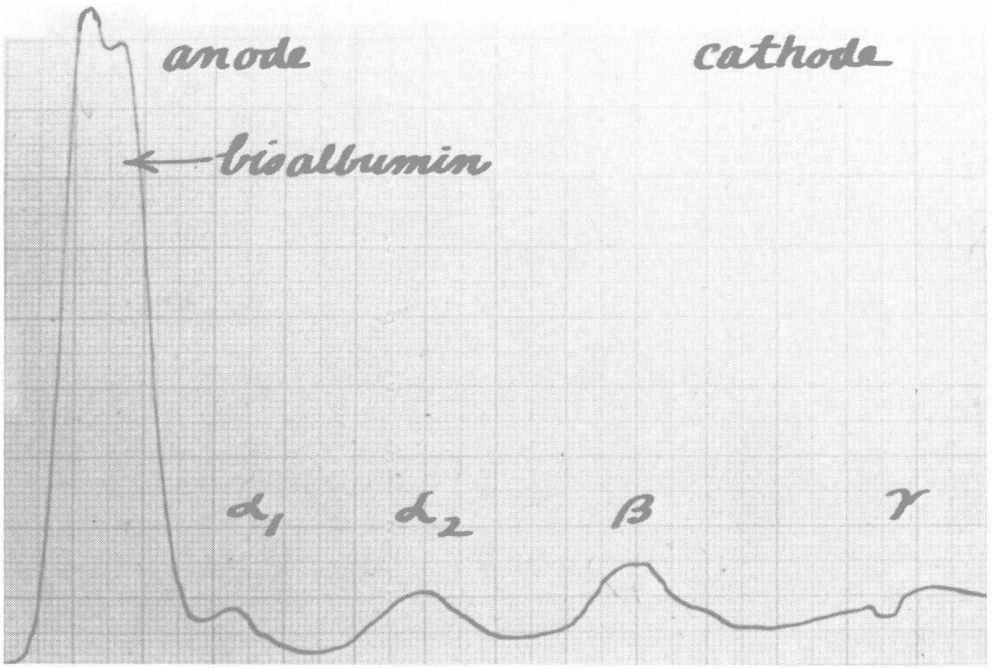

anode
cathode
bisalbumin
α1
α2
β
γ

PREALBUMIN

HISTORY

The patient, F.S., is a 29 year old white male who entered the hospital with a severe frontal headache. On examination, the neurological examination was negative except for anisocoria. A lumbar puncture was performed and the total protein was 40 mg/dl. The electrophoretic pattern of the cerebrospinal fluid is featured on the opposite page. The neurological workup revealed an aneurysm of the communicating branch of the anterior cerebral artery. This was treated surgically.

ABNORMALITY

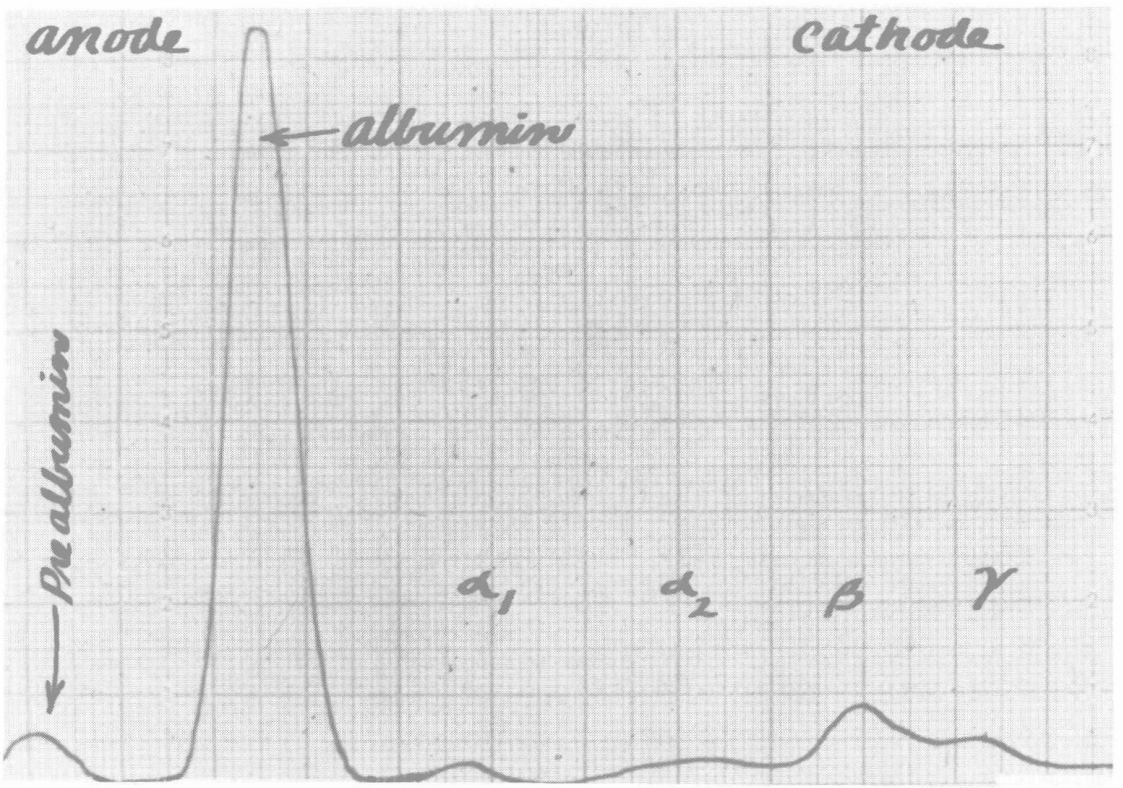
Presence of prealbumin which is a normal protein in the cerebrospinal fluid.

ANALYSIS

Notice the presence of a normal prealbumin band. The other protein components are normal including the normal most prominent beta globulin band. The normal percentages are:

Prealbumin	4%
Albumin	63%
Alpha-1-globulin	5%
Alpha-2-globulin	8%
Beta globulin	13%
Gamma globulin	7%

Prealbumin exists in the serum but is in such small quantity that it is not readily demonstrated by the clinical laboratory electrophoretic procedure.[1] It is synthesized in the liver and is a glycoprotein.[2] It is especially active in the binding of T3 and T4. It has a short half life of 1.9 days and this makes it a sensitive indicator of any changes affecting its synthesis and catabolism.[3] Serum prealbumin concentration is a good liver function test. Its normal concentration is 10 to 40 mg/dl by radioimmunodiffusion. The causes for a low serum prealbumin are severe liver disease, congestive heart failure, and burns.[4-8]


anode
cathode
albumin
Prealbumin
α_1
α_2
β
γ

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HISTORY

The patient, C.T., is a 7 year old white male who entered the hospital with a three-day history of swelling of the lower extremities, arms, and face. The patient complains of a poor appetite with nausea. The mother has noticed decreased physical activity. Several weeks prior to the onset of the generalized swelling the patient had a respiratory infection which was present in the patient's siblings. The physical examination revealed a pale child with generalized anasarca. The serum albumin was 0.6 gm/dl and the urine protein was 1220 mg per 24 hour specimen. Serum cholesterol was 465 mg/dl and serum triglyceride 505 mg/dl.

ABNORMALITY

See electrophoretic pattern on page 11. Decreased serum albumin, decreased Alpha-1-globulin, increased Alpha-2-globulin (Alpha-2-macroglobulin), increased beta globulin (beta lipoprotein (LDL), and decreased gamma globulin). The urine electrophoretic pattern next to it demonstrates the presence of albumin, Alpha-1, Alpha-2, beta and gamma globulin in urine.

ANALYSIS

The decreased serum albumin is related to the heavy proteinuria caused by the post-streptococcal glomerulonephritis and the nephrotic syndrome. The decreased Alpha-1-globulin and gamma globulin is also caused by the loss of these serum proteins into the urine due to the glomerular lesion. The prominent increase in the Alpha-2-globulin is related to the marked increased synthesis of Alpha-2-macroglobulin, and the elevated beta globulin is related to the increased beta lipoprotein caused by the disturbance in lipid metabolism in nephrosis.

In this discussion we will concentrate only on the decreased serum albumin. The normal range for serum albumin is 3.5 to 5 gm/dl with a mean of 4 gm/dl. Hypoalbuminemia is present when the albumin level is

below 3 gm/dl. The differential diagnosis of hypoalbuminemia includes increased loss as is found in heavy proteinuria in nephrosis, increased loss through the skin in severe burns or generalized exfoliative dermatitis, or increased loss into the stool due to protein wasting gastrointestinal diseases.[1-3] Other major causes for hypoalbuminemia are decreased synthesis due to severe liver disease since albumin is the major protein produced by the liver or malnutrition.[4-6] Decreased serum albumin also may result from increased degradation which is caused by increase in thyroid hormone (thyrotoxicosis) or cortisol (Cushing's syndrome). A rare cause for decreased or almost complete absence of serum albumin is congenital analbuminemia which is inherited as an autosomal recessive trait.

Frequently, hypoalbuminemia may be multifactorial in etiology. For example, it may be due to severe liver disease with decreased synthesis and malnutrition. Patients with severe liver disease cannot metabolize ADH and cortisol which results in plasma volume expansion (ADH excess), and pseudoCushing's syndrome (excess cortisol). Both of these disturbances in hormone metabolism contribute to hypoalbuminemia. Hypoalbuminemia results in decreased colloid osmotic pressure with consequent edema, decreased transport of hormones, drugs, fatty acids, and calcium.[7-8] In addition, hypoalbuminemia results in potential decreased endogenous source for amino acids.

Thus, the most common cause for a decrease in serum albumin is acute and chronic illnesses including the infectious diseases, congestive heart failure, trauma with tissue necrosis, postoperative state, collagen diseases, and malignancy.[9-11] Hypoalbuminemia is related to the acute phase reaction. An increased catabolism of albumin from increased serum cortisol and expansion of the intravascular space contribute to the lowered serum albumin.