High Resolution Protein Electrophoresis

A Clinical Overview with Case Studies

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There has never been a time in the history of medicine when knowing more about a patient's condition was detrimental. The major advantage of high resolution electrophoresis over conventional methods is that it gives us more information about the patient's condition.

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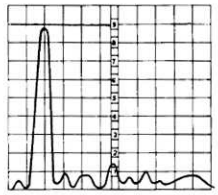
High Resolution Protein Electrophoresis –

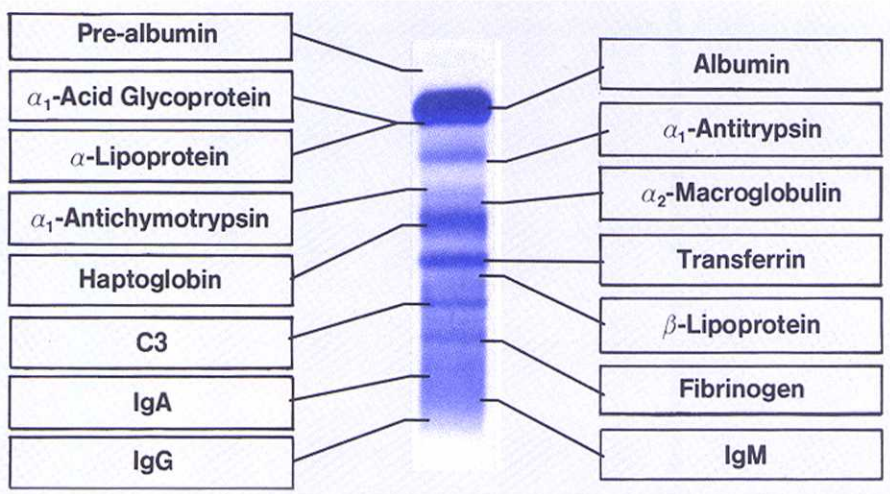


A Clinical Laboratory Test Whose Time Has Come

In 1972, Professor B.G. Johansson published a working method for agarose gel electrophoresis in the Scandinavian Journal of Clinical and Laboratory Investigation. This landmark paper included all the technical details needed at that time to perform high resolution, high sensitivity electrophoresis on body fluids in the clinical laboratory. The same issue of that journal contained an article by Professor C.B. Laurell which described in great detail the composition and variation of the agarose gel electrophoretic protein fractions of plasma, cerebrospinal fluid and urine. These papers were greeted with a collective yawn by most clinical laboratory professionals in the United States who were, for the most part, content with the "traditional" five-band patterns they were able to achieve by using cellulose acetate as a support medium for protein separations. In the intervening years, however, a quiet revolution has been taking place in the leading laboratories of this country and throughout the world. High resolution agarose gel electrophoresis has proven its value in providing valuable clinical information which cannot be obtained from any other analytical technique.

Fifteen Proteins Form Foundation of Clinical Knowledge

As recent studies with two-dimensional electrophoresis have amply shown, the human body is capable of synthesizing thousands of proteins. Most of these proteins have not even been characterized from a biochemical standpoint, and virtually nothing is known about their metabolic function or clinical significance. A small subset of this vast array of proteins, numbering perhaps 200, has been thoroughly studied and described in terms of physical constants such as molecular weight, isoelectric point and so forth, but the majority of this group are still enigmas in regard to their function. Our clinical knowledge of protein metabolism is limited to about 25-30 relatively high concentration components of plasma, cerebrospinal fluid, urine and other body fluids. Of these, 15 or so can be visualized by use of high resolution, high sensitivity agarose gel electrophoresis of serum. These fifteen proteins form the foundation of our clinical knowledge, since they have been extensively studied in a variety of disease states.



This agarose gel electrophoresis pattern of normal human plasma indicates fifteen major proteins.

Higher Resolution Gives More Information

The dilemma facing the laboratory director who has not yet changed over to the high resolution technique is often expressed by the comment, "What am I going to do with all those bands?" The problems inherent in moving from relatively simple to more complex technology were characterized in a recent Wall Street Journal cartoon by Schochet in which Sherlock Holmes is seen slumped down in his easy chair remarking to his colleague, "Things just aren't so elementary any more, Watson." This attitude is somewhat understandable in the general public, which feels hemmed in by advancing technology on all sides. But medicine, though still a great proportion art, depends on science for those incremental advances that eventually add up to a better overall health care. Indeed, there has never been a time in the history of medicine when knowing more about a patient's condition was detrimental. That is the major advantage of high resolution electrophoresis over conventional methods – it gives us more information about the patient's condition.

Interpretation of high resolution electrophoresis patterns is at once simpler and more complex than commenting on 5 band cellulose acetate results. It is a more complex exercise in that there are many more bands which can be detected and therefore demand some comment. This is a result of both higher resolution and greater sensitivity. The agarose used is superior in separating proteins with similar electrophoretic mobilities, and the large amounts of protein which can be applied to the agarose medium result in detection of some low-concentration proteins which are invisible on cellulose acetate. This more complicated pattern might lead to Sherlock's comment above, but actually the improved performance of the medium makes it much simpler for the laboratory director to make useful comments about the protein pattern. The proteins are present in the sample, whether they are adequately separated or not. With greater resolution we are not restricted to making obvious comments about only the most markedly abnormal patterns.

"To Scan Or Not To Scan"

"To scan or not to scan" has been a recurring question asked by laboratory directors contemplating the use of agarose gel. Some early papers took a hard line approach and strongly recommended against scanning high resolution gel patterns. These recommendations were based on the idea that visual examination of the patterns by experienced observers was by far superior to densitometric scanning for gaining an overview of a patient's protein status and for picking out subtle pathological patterns. It was further pointed out that even high resolution electrophoresis does not fully resolve the major proteins and that differences in dye binding affinities could lead to inaccuracies.

While all of these comments are true, there is still a place for good densitometric scanning of high resolution patterns if the limitations of the technique are fully understood. Densitometric scanning can provide useful quantitative information on albumin and M-components in the gamma region. Most densitometers are limited, however, in the intensity of staining they can accommodate, so high sensitivity patterns must be scanned carefully so as not to exceed the linear limit of the instrument. It is also impractical to expect a densitometer to provide "hard numbers" for the fifteen or so proteins which can be visualized on the agarose pattern of serum. This is due both to the limitations of resolution and protein staining mentioned above and to scanning limitations for most densitometers.

The overall clinical utility of the electrophoresis pattern, like beauty, must still be left to the eye of the beholder. The interpretive effort required for electrophoresis is similar to that provided by the radiologist on looking at an X-ray or a pathologist commenting on a biopsy specimen. In each case some judgement must be added to the basic test in order to utilize its full diagnostic potential.

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Clinical Significance Of The Plasma Proteins

Monoclonal Gammopathies
Genetic Deficiencies
The Inflammatory Response
Liver Diseases
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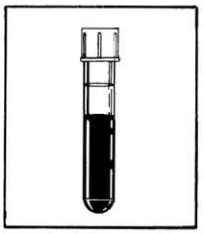
Case Studies

Section II

Clinical Significance Of The Plasma Proteins



Monoclonal Gammopathies

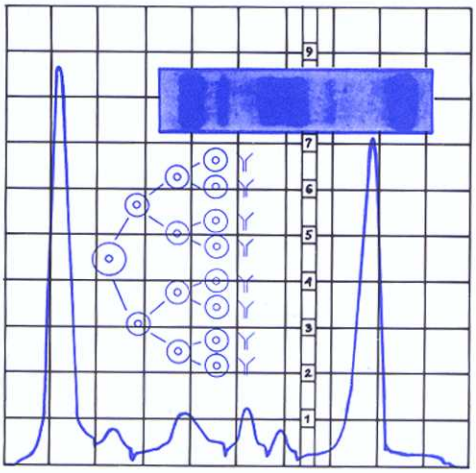
Monoclonal gammopathies are characterized by an uncontrolled proliferation of a single clone of plasma cells at the expense of other clones. This dysfunction often leads to the synthesis of large amounts of one homogeneous immunoglobulin or immunoglobulin subunit with decreased levels of normal immunoglobulins.

The stimulus for proliferation is unknown, but it is probably not antigenic. Some monoclonal immunoglobulins from human patients have reacted with selected antigens, but this evidence is less than compelling and may simply be a coincidental finding.

Since normal immunoglobulins are often suppressed in these disorders, there can be a functional immunodeficiency in association with increased gamma globulin levels. This immunosuppression can have life-threatening consequences in a patient whose condition is already compromised by the primary disease.

Electrophoretic patterns and immunoglobulin test results can be strikingly abnormal in patients with multiple myeloma and other B-cell-related neoplasms. As a result, protein analysis has been a valuable tool in the diagnosis and monitoring of these lymphoproliferative diseases.

Immunochemical methods such as nephelometry can quantitate the abnormal protein production that marks these disorders, but only electrophoresis can demonstrate its monoclonal nature. The gamma region of the electrophoretic strip can show a dense, highly restricted band from uncontrolled proliferation of one cell clone, with decreased background staining due to the shutdown of normal immunoglobulin synthesis.



Even though monoclonal gammopathies can have a dramatic pattern of protein change in some instances, their clinical interpretation can be difficult. It is important to consider that one-third of the patients with immunochemical evidence of monoclonal gammopathy are asymptomatic. These could be patients with benign or transient monoclonal proteins, especially in an older population. They could also represent an early or relatively dormant stage of the disease that might later accelerate.

There are some patients who show symptoms, but no abnormal protein pattern. This could be the result of a nonsecretory clone, or it might indicate production of immunoglobulin fragments or subunits that are excreted in the urine.

Other circumstances that can lead to difficulties in interpretation include production of more than one immunoglobulin subunit (such as complete molecules plus free light or heavy chains), polymerization of the monoclonal protein (as is sometimes the case with IgA), or depolymerization (as can happen with IgM). Each of these circumstances can result in multiple bands on electrophoresis, and can appear as diffuse increases. Suspected monoclonal gammopathies require careful interpretation and close consultation with the attending physician.

Suggested Protocol for Monoclonal Gammopathy Evaluation

Initial Work-up

1. Serum and urine high resolution electrophoresis (A 24-hour urine specimen is preferable, but a random specimen is adequate to characterize the monoclonal protein.)
2. Quantitative serum immunoglobulins
3. Serum and urine immunoelectrophoresis
4. Serum and urine immunofixation if conventional immunoelectrophoresis fails to identify anomalous band or bands.

Follow-Up Studies

1. Serum and urine high resolution electrophoresis (A 24-hour urine specimen is preferable, but a random specimen is adequate to characterize the monoclonal protein.)
2. Quantitative serum immunoglobulins
3. Immunoelectrophoresis or immunofixation may be helpful in follow-up studies for very rare cases in which the immunoglobulin class changes during the course of the disease.

Special Considerations for Light Chain Disease

Light chain disease is a monoclonal gammopathy in which only kappa or lambda monoclonal light chains, or Bence Jones proteins, are produced. Light chain disease comprises 10% to 15% of monoclonal gammopathies. It ranks behind IgG myeloma (about 60%) and IgA myeloma (about 15%) in incidence and occurs about as often as Waldenstrom's macroglobulinemia. Its diagnosis presents various difficulties not associated with other common monoclonal gammopathies and requires considerable clinical skill and sophisticated analytic techniques.

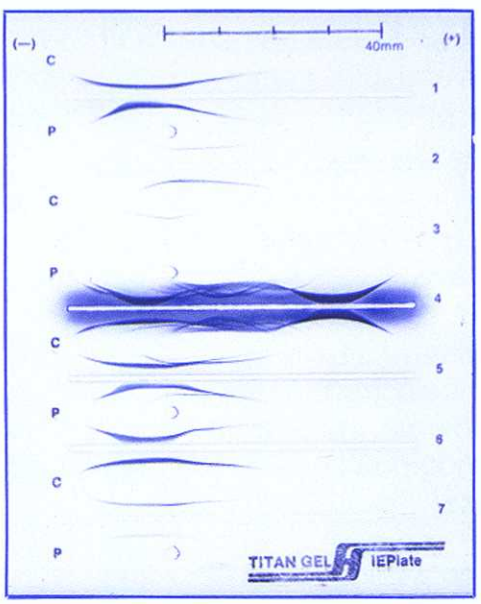
Patients with other B-cell malignancies may be asymptomatic until serum levels of monoclonal protein are very high. About 3 gm/dL is an accepted cutoff, above which patients are usually symptomatic. On the other hand, very small amounts of Bence Jones protein in serum can be associated with significant clinical problems, especially pathologic renal changes. Since free light chains filter through the glomerulus almost without obstruction because of their small molecular size, they accumulate in the tubules. Renal impairment can result from the toxicity of the light chains. Pathological changes can range from relatively benign tubular proteinuria to acute renal failure or amyloidosis.

High-quality electrophoretic analysis of serum and urinary proteins is a reliable tool for initial assessment of clone activity in patients with suspected light chain disease, as well as for monitoring treatment of confirmed disease. The method must combine sensitivity and high resolution to detect the small amounts of Bence Jones proteins in serum and urine and to separate them from proteins of similar electrophoretic mobility.

Agarose is the most appropriate support medium for electrophoretic studies of light chain disease, since as little as 50 mg/dL of the monoclonal protein can be detected visually. The resolution afforded by agarose also distinguishes small light chain bands from other proteins, especially when they migrate from the gamma region.

The laboratory workup of a patient with suspected light chain disease is similar to the workup for any lymphoproliferative disorder, but there are certain changes in approach due to low levels of paraprotein that can be involved. High resolution electrophoresis of serum and urine should be done to determine overall protein status. Even a urine specimen with a normal 24-hour protein excretion rate should be examined electrophoretically since almost all protein may be Bence-Jones protein. Densitometric scanning of electrophoretic patterns provides at least a semi-quantitative estimate of a monoclonal band if it is large enough, but small light chain bands often show no significant peak on a scan. Visual examination of the original pattern is thus an essential part of interpretation.

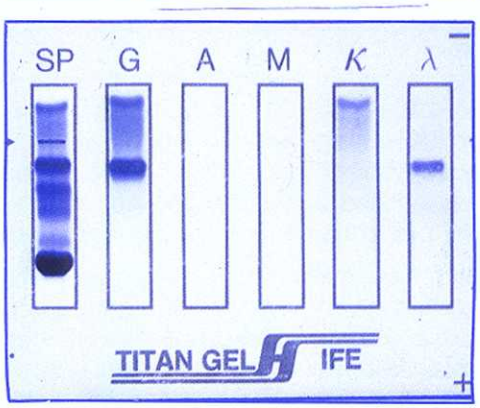
TITAN GEL IEPlate



Electrophoretic studies can be supplemented with quantitative immunochemical assays of immunoglobulins G, A and M to assess general humoral immunity and provide baseline values of Ig concentrations for follow-up.

As with other monoclonal gammopathies, immunoelectrophoresis is used in a light chain disease workup to identify suspicious serum or urinary electrophoretic bands. In addition to routine antisera for IgG, IgA, IgM, and light chains, antisera specific for IgD and IgE can help to differentiate those less common myelomas from light chain disease. Antisera specific for free light chains can confirm light chain disease, but their high cost means they should be used sparingly and only in double-immunodiffusion assays.

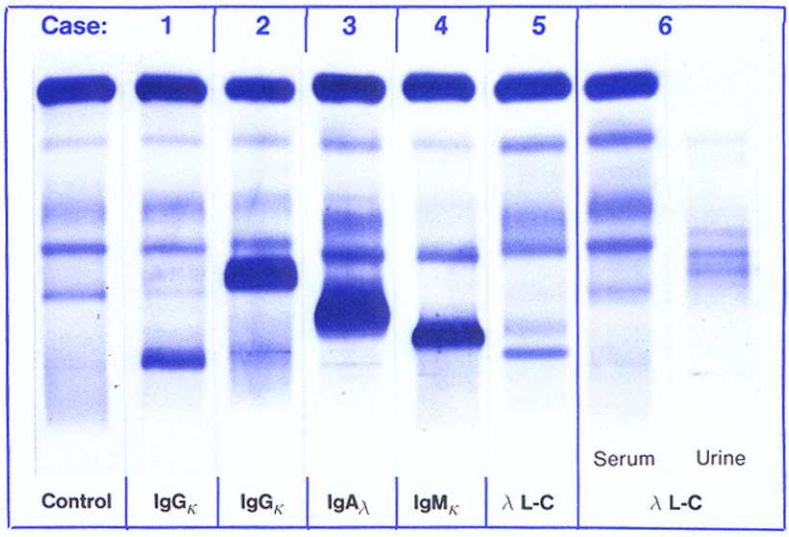
TITAN GEL ImmunoFix plate



When interpretation of immunoelectrophoretic patterns is difficult because of low levels of the monoclonal protein, immunofixation is the method of choice. It is highly sensitive, and its interpretation does not depend on small deviations in the shape of a precipitin arc, as is the case with immunoelectrophoresis.

Once the diagnosis of light chain disease has been confirmed, periodic evaluation of the patient by serum and urine electrophoresis and by quantitative Ig assay can help monitor therapy.

Case Studies: Monoclonal Gammopathies



Six cases of monoclonal gammopathies illustrating the differences in electrophoretic mobility and band appearance from case to case.

Case 1

IgG KAPPA MONOCLONAL GAMMOPATHY

Patient: 78 year-old male

History and Physical: The patient was admitted for evaluation of severe pain in his right leg. He has had pain for over a month in the distribution of the right sciatic nerve. The pain has gotten progressively worse.

Upon examination the patient showed intense pain and spasm over the right lumbar region. It was difficult to assess whether the patient had weakness in the right leg or that the limited movement was due to pain.

Laboratory Data: The patient had a slight elevation in LDH, borderline anemia and an elevated ESR. All other laboratory studies were normal.

SPE: A monoclonal band was detected in the gamma region, suggesting plasma cell dyscrasia or some other lymphoproliferative disorder.

IFE: Immunofixation identified the monoclonal band as IgG Kappa.

Hospital Course: CT scan of the lumbarsacral spine region showed some impingement of the L4-5 region on the right wth a combination of hypertrophy of the nerve root, facet hypertrophy and bulging of the disk. He was taken to surgery and the area was decompressed. Over the rest of his hospital stay he experienced marked, though not complete, decrease in pain.

The presence of a monoclonal gammopathy, suggesting a possible early plasma cell dyscrasia, was noted and a bone marrow biopsy was performed. It showed a non-diagnostic increase in plasma cells with some pleomorphism in the plasma cell series. The patient was referred to a hematologist/oncologist for follow-up and treatment.

Case 2

IgG KAPPA MONOCLONAL GAMMOPATHY

Patient: 78 year-old male

History and Physical: Three months prior to this hospital admission the patient was evaluated for various symptoms, including night sweats, weight loss, anemia and skin rash. He was found to have lymphadenopathy and was treated with chemotherapy with a very prompt response. Over the past few weeks, however, the patient has become increasingly weak, with a recurrence of his lymphadenopathy. He was admitted to the hospital for further treatment. Upon admission, he was weak. His skin was quite pale, but he had no rash.

Laboratory Data: Significant findings on the chemistry battery included decreased albumin (3.0 g/dL) and increased total protein (9.4 g/dL). The patient has severe anemia with marked decrease in RBC count (2.33 M), HGB (6.9 g/dL) and HCT (20.6%). Platelet count was slightly decreased.

SPE: Protein electrophoresis studies confirmed hypoalbuminemia and showed a large monoclonal component in the beta-2 region. Some protein precipitate was noted at the point of application, possibly due to immune complexes or other protein-protein complexes.

IEP: IEP identified the M-component as IgG Kappa. Monoclonal gammopathy is an atypical finding in immunoblastic lymphadenopathy. This disorder is usually associated with diffuse immunoglobulin increases. This finding led the patient's physicians to consider the possibility of another underlying disease. The patient did show the hypocomplementemia associated with immunoblastic lymphadenopathy, and the Raji cell test for immune complexes was markedly positive.

Hospital Course: The patient was treated with transfusions and plasmaphoresis. He showed rapid symptomatic improvement, and was discharged to be re-evaluated on an outpatient basis by his attending hematologist.

Case 3

IgA LAMBDA MONOCLONAL GAMMOPATHY

Patient: 74 year-old female

History and Physical: The patient was diagnosed as having myeloma about one month prior to this admission. She had been complaining of moderate rib and left shoulder pain, and she was found to be anemic. A bone marrow biopsy showed sheets of plasma cells and her serum electrophoresis showed a monoclonal protein. She was being treated with chemotherapy when she developed severe back pain radiating to the right flank. X-ray studies revealed a partial compression fracture of T-12. She was given analgesia and admitted to the hospital for radiation therapy.

Upon admission the patient had no palpable adenopathy in the cervical, supraclavicular, axillary or inguinal areas. She has pain in percussion in the lower thoracic and upper lumbar spine, in the right shoulder and the left anterior ribs. There are no palpable masses, petechiae, hemmorhages, organomegaly, or edema.

Laboratory Data: Chemistry battery was unremarkable except for borderline hypoalbuminemia and high normal total protein. WBC, RBC, HGB, and HCT all showed slight decreases.

SPE: The serum protein pattern showed a large monoclonal band in the beta-2 region, accounting for 47.8% of the total protein or 4.4 g/dL.

IEP: IEP identified the M-component as IgA Lambda. Quantitative immunoglobulins were: IgG 200 mg/dL (decreased), IgM 35 mg/dL (low normal), and IgA 4520 mg/dL (markedly increased).

Hospital Course: The patient was started on radiation therapy to the lower thoracic spine. During her hospitalization she had increasing pain in the lower back, the anterior chest over the sternum and the medial anterior left ribs. The radiation field was increased to include the sternum and anterior left ribs. The patient required pain medications and experienced nausea during the course of radiation therapy. She decided to continue treatment nearer her home, and arrangements were made to transfer her to another hospital. The discharge plan was for her local physician to continue chemotherapy and radiation therapy.

Case 4

IgM KAPPA MONOCLONAL GAMMOPATHY

Patient: 68 year-old male

History and Physical: The patient was admitted to the hospital for significant anemia and elevated IgM. Of interest in his past medical history is the finding of anemia 15 years ago. At that time, the patient had a minimally abnormal serum protein electrophoresis, but this was not diagnostic for any paraprotein and his immunoglobulins were within normal limits.

Upon admission the patient was extremely pale and appeared chronically ill. There was a trace of edema of the ankles, and he had several scattered areas of petechiae on his extremities.

Laboratory Data: The patient showed normal albumin, but a marked elevation in total protein (10.3 g/dL). He was anemic (RBC 2.24 M, HGB 6.8 g/dL, HCT 21.0%), and his blood smear showed rouleaux. His ESR was greater than 150 mm in ½ hour (normal: 0-20 mm/hr).

SPE: Serum protein studies showed a marked M-component in the beta-2 region. A large monoclonal band was also noted in the urine.

IEP: IEP identified monoclonal IgM Kappa in the serum and monoclonal free Kappa light chain in the urine. Quantitative immunoglobulins were: IgG 625 mg/dL (low normal), IgA 74 mg/dL (borderline decrease) and IgM 6515 mg/dL (profound increase).

Hospital Course: The patient underwent a bone marrow biopsy which was not diagnostic, but was suggestive of macroglobulinemia or possibly a lymphoproliferative disorder such as chronic lymphatic leukemia or malignant lymphoma. The patient was discharged to be treated and followed as an outpatient.

Case 5

LAMBDA LIGHT CHAIN MONOCLONAL GAMMOPATHY

Patient: 78 year-old male

History and Physical: The patient was admitted for dialysis after a routine anemia work-up revealed azotemia. His admitting physical examination was unremarkable.

Laboratory Data: The chemistry battery gave results consistent with renal failure; marked elevations in BUN, uric acid and creatine with decreased calcium. Total protein and albumin were decreased. The WBC count was slightly elevated with a shift to the left. RBC, platelet count, HGB and HCT were all decreased.

SPE: Routine electrophoresis revealed borderline acute inflammation (increased α_1AT and Hp with decreased pre-albumin, albumin and transferrin) and two small monoclonal components in the gamma region, one of which was slightly atypical.

Urine Electrophoresis: There was a slight elevation in urine total protein. Urine protein electrophoresis (not shown) suggested some glomerular-type proteinuria (increased albumin, α_1AT and transferrin), but the pattern was dominated by a large M-component in the gamma region plus a smaller cathodal component.

IEP: Serum and urine IEP revealed monocloncal Lambda light chain in both fluids and no monoclonal heavy chain, suggesting Lambda light chain disease.

Hospital Course: Results of the protein studies led to a bone marrow biopsy, which confirmed multiple myeloma. A renal biopsy was consistent with acute myeloma kidney. Hemodialysis, therapeutic plasma exchange and chemotherapy were begun with rapid symptomatic improvement. Renal function improved to the point that the patient was removed from dialysis.

Case 6

LAMBDA LIGHT CHAIN MONOCLONAL GAMMOPATHY

Patient: 82 year-old male

History and Physical: The patient was admitted to the hospital after he had fallen and fractured his left hip. He has a history of mild hypertension, but no history of heart disease or seizures. Upon admission he was awake and followed commands, but was unable to speak. He has right lower facial weakness and does not respond to a moving object in his right visual field. The diagnostic impression was that the patient had probably suffered a frontal lobe infarction.

Laboratory Data: The chemistry battery showed decreased calcium and total protein (5.1 g/L). Routine urinalysis was normal except for a trace of protein. WBC count was normal, but RBC count, HGB and HCT were decreased. The MCV was markedly elevated.

SPE: The protein pattern showed hypoalbuminemia, acute inflammation, and a relative decrease in haptoglobin which suggested in vivo hemolysis or increased RBC turnover. There was also mild hypogammaglobulinemia.

Urine Electrophoresis: Electrophoresis on a random specimen showed some albumin and other trace proteins with several small monoclonal bands present in the beta-region.

IEP: IEP identified monoclonal free Lambda Light Chain in the urine. No monoclonal proteins were detected in the serum.

Hospital Course: The diagnosis of an acute left cerebral infarction was confirmed. The patient's macrocytic anemia was found to be secondary to B-12 deficiency. The anemia was treated and the patient's condition stabilized enough for a left hip procedure to be done. The patient developed aspiration pneumonia postoperatively, however, and died of respiratory arrest in spite of antibiotic treatment. An autopsy was not performed.

CLINICAL PRESENTATION

MULTIPLE MYELOMA:

Clinical presentation in multiple myeloma is usually in one of three ways: (1) The most common presentation is that of bone pain, especially in the spine, pelvis or ribs. (2) Patients may present with renal failure of unknown etiology. This is often the case in patients with light chain disease. (3) Recurrent bacterial infections represent a third category of presentation.

Physical examination of these patients is usually not remarkable except for the presence of bone pain and possibly pallor if anemia is severe. Lymphadenopathy and hepatosplenomegaly are not present unless the patient has amyloid. Neurological examination may reveal signs of spinal cord compression. Pathological joint findings are uncommon.

MACROGLOBULINEMIA:

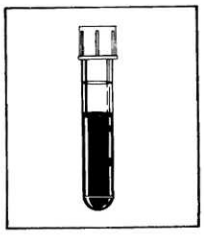
The most common presentation of Waldenstrom's Macroglobulinemia are those symptoms associated with hyperviscosity syndrome. They include fatigue, generalized weakness, skin and mucosal bleeding, visual disturbances, headache and several other neurological signs and symptoms. A history or evidence of Sjogren's Syndrome may be present. Some patients show cardiopulmonary abnormalities due to increased plasma volume and viscosity. If the monoclonal macroglobulin is a cryoglobulin or cold agglutinin, the patient will often have a history of cold sensitivity or Raynaud's phenomenon. As with multiple myeloma, some patients have recurrent bacterial infections.

Physical examination will frequently show purpura, some generalized lymphadenopathy and hepatosplenomegaly. The hyperviscosity syndrome may result in retinal venous engorgement and localized narrowing. Evidence of polyneuropathy is present in some patients.

FROM: Stone, Marvin J.: Monoclonal Gammopathies – Clinical Aspects. In *Protein Abnormalities Vol. 2 Pathology of Immunoglobulins*, Ritzmann, S.E., Ed., Alan R. Liss, New York, 161-236, 1982.

Section II

Clinical Significance Of The Plasma Proteins



Genetic Deficiencies

α1-Antitrypsin

Although α1-antitrypsin does inhibit trypsin, it is also a general inhibitor of serine proteases, including the neutral proteases of polymorphonuclear leukocytes and macrophages, as well as of trypsin, chymotrypsin, and pancreatic elastase. It is an acute-phase reactant, and its plasma levels increase in response to inflammation.

A genetically determined deficiency in α1-antitrypsin was discovered in 1963. The genetic transmission of this defect has since been thoroughly investigated, and a wide array of α1-antitrypsin variants has been documented. Most of the phenotypes of defective α1-antitrypsin are not known to be associated with disease. The link has been established between severe deficiency of this protease inhibitor and neonatal hepatitis and hepatic cirrhosis, while chronic obstructive pulmonary disease and hepatic cirrhosis are common findings in deficient adults. In rare instances, a number of other disorders can be associated with the deficiency.

The genetics of α1-antitrypsin deficiency, as established by studies of the families of affected individuals, show that a single autosomal gene locus encodes the production of this protein, with co-dominant expression producing the α1-antitrypsin phenotype in any given case. The locus of the gene that encodes α1-antitrypsin has been designated a Pi, and more than 40 alleles of the gene are now known. The alleles responsible for α1-antitrypsin deficiency include PiS (60% of normal levels), PiP (25% of normal levls), PiZ (15% of normal level, and Pi null (no synthesis of α1-antitrypsin).

Electrophoresis can be a useful tool in the initial evaluation of a patient with suspected α1-antitrypsin deficiency, but quantitative immunochemical assays and phenotyping studies are necessary to fully characterize a patient's status. In serum protein electrophoretic separations, α1-antitrypsin is the major protein found in the α1 region, and appears as a distinct band on either cellulose acetate or agarose gel electrophoresis plates. However, higher resolution techniques demonstrate a marked electrophoretic microheterogeneity in α1-antitrypsin, which can be exploited in phenotyping studies.

Another factor to consider in screening for α1-antitrypsin deficiency is that the α1 region of an electrophoretic separation of this protease inhibitor contains α1-lipoprotein as a diffuse background. For this reason, a densitometer scan of the α1 region may give values which are within the normal range, even though visual inspection of the pattern will reveal the absence or near absence of an α1-antitrypsin band.

Although the acute-phase elevation of α1-antitrypsin plasma levels during inflammation is a nonspecific phenomenon, it must be taken into account when screening for α1-antitrypsin deficiency. The increased stimulus for synthesis of this protein that is brought on during an inflammatory episode can mask borderline-low levels of α1-antitrypsin.

Estrogens and androgens also tend to cause increases in plasma levels of α1-antitrypsin. This effect should be considered when screening for deficiency.

Immunoglobulins

Deficiencies in the humoral antibody response can lead to decreased levels of one or more immunoglobulins. These can result in a wide variety of symptoms and clinical presentations, but they are often associated with recurrent infections. The most common immunodeficiency is a selective decrease in IgA, occurring in about 1 in 600 individuals. Although many immunodeficiency diseases result from known

defects and can be classified accordingly, the majority of cases are difficult to characterize and fall into a broad group known as common variable immunodeficiency.

Immunodeficiency Disorders with Abnormal Immunoglobulins

Isolated IgA deficiency
Isolated IgM deficiency
X-linked immunodeficiency with increased IgM
Wiskott-Aldrich Syndrome
Transient hypogammaglobulinemia of infancy
Ataxia Telangiectasia
Severe combined immunodeficiency (SKID)
Common variable immunodeficiency
- -pan hypogammaglobulinemia
- -IgG and IgA deficiency
- -isolated IgG deficiency

Complement Components

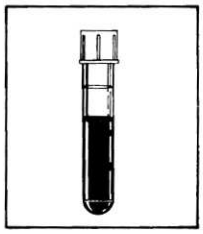
Deficiencies of most components of the complement sequence have been described. These conditions are not common and usually result in the total absence of a single component. Patients with complement deficiencies may present with increased susceptibility to infection or a wide range of other symptoms.

Other Proteins

Genetic deficiencies of other proteins are not common, but include albumin, haptoglobin, transferrin, cholinesterase, fibrinogen, coagulation factors, α_1-antichymotrypsin, α-lipoprotein, and β-lipoprotein. Decreased levels of ceruloplasmin are observed in Wilson's disease, but this is probably not due to a genetic inability to synthesize the protein.

Section II

Clinical Significance Of The Plasma Proteins



The Inflammatory Response

The inflammatory response is accompanied by characteristic plasma protein changes. These changes are known as the acute phase pattern and are the most common abnormalities seen in protein screening procedures such as electrophoresis. The acute phase pattern varies with the duration of the inflammatory process, and may be used to differentiate between acute, subacute and chronic pathological conditions. The intensity of the protein changes may in some cases reflect the extent of tissue damage, but little is known about the clinical significance of the acute phase response and the mechanisms which control it.

Although the acute phase pattern has been studied in a variety of diseases, it is not generally useful in establishing a differential diagnosis. This is due to the nonspecific nature of the inflammatory process itself. Detecting the presence of inflammation, however, and determining its progression is of clinical importance under many circumstances.

Most of the commonly measured plasma proteins are acute phase reactants. These proteins also have fundamental biological functions in addition to their roles as indicators of the inflammatory response. In interpreting protein abnormalities, both the primary biological role of the protein and the secondary inflammatory status of the patient must be taken into account.

Postsurgical Acute Phase Response

The postsurgical acute phase response has been well characterized and can serve as a general guide to protein changes seen in other inflammatory conditions.

Increases in C-reactive protein are seen within 6 to 8 hours after surgery, followed shortly by α_1-acid glycoprotein. These components reach maximum levels within 48 to 72 hours. By far the strongest response is seen with C-reactive protein, which can reach concentrations 10-fold higher than the presurgical level. C-reactive protein also shows a predictable decrease between the third and fourth postoperative days. These factors make sequential C-reactive protein determinations a sensitive indicator of postoperative infectious complications. Reactions by α_1-antitrypsin, haptoglobin and fibrinogen are observed at 24 hours. Prealbumin, albumin, α-lipoprotein and transferrin show decreases in the first few postoperative days. In the subacute phase, C3 and ceruloplasmin show moderate increases. Immunoglobulins are usually non-reactive in the absence of infection or immune stimulation.

Myocardial Infarction

The time of onset of myocardial infarction can be documented in most cases. Thus, MI also lends itself to studies that establish a typical acute phase reaction.

The general pattern in myocardial infarction consists of three phases. C-reactive protein, α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin and fibrinogen show rapid increases which maximize at about 5 days with partial return to normal levels by 3 weeks. Prealbumin, albumin, transferrin, α-lipoprotein and IgG show rapid decreases, reach minimum levels at day 5 and return to normal in 3 weeks. Ceruloplasmin and C3 show moderate increases which maximize during the second week. Some slight changes can be observed with C4, α_2-macroglobulin and IgM. This overall pattern is similar to that observed in the post-surgical period, even though the time course is slightly longer. It is useful in interpreting protein results of

a patient with suspected myocardial infarction, but its diagnostic value is limited by its nonspecific nature.

Clinical studies have found a quantitative relationship between infarct size, as estimated by serial enzyme measurements, and the response of C-reactive protein, α_1-acid glycoprotein, haptoglobin and fibrinogen. Humoral factors originating from the site of infarction are probably responsible for evoking increased synthesis of acute phase proteins by the liver.

Protein Changes In The Inflammatory Response

	ACUTE			SUBACUTE	CHRONIC
Pre-albumin		↓	↓↓	↓	↓
Albumin		↓	↓	↓	↓
ALPHA-1					
α-lipoprotein			↓	↓	↓
α_1-acid glycoprotein		↑	↑↑	↑	
α_1-antitrypsin		↑	↑↑	↑	
ALPHA-2					
Ceruloplasmin				↑	↑ or N
Haptoglobin		↑	↑↑	↑	
BETA-1					
Transferrin		↓	↓↓	↓	↓
BETA-2					
C_3				↑	↑ or N
Fibrinogen		↑	↑↑	↑	
IgA					↑
GAMMA					
IgM					↑
IgG					↑
CRP	↑	↑↑	↑↑↑	↑	

Immunoglobulins show diffuse increases when chronic inflammation is accompanied by infection or antigenic stimulation.

The absence of a symbol indicates normal levels.

Infectious Diseases

Changes in acute phase proteins have been characterized for some acute infectious diseases. α_1-Antitrypsin, haptoglobin, fibrinogen and C-reactive protein exhibit marked increases when compared to levels after recovery.

Studies have shown that the highest levels of α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin and C-reactive protein are observed with bacterial infections. Viral infections result in relatively small increases in C-reactive protein and α_1-acid glycoprotein with moderate elevations in α_1-antitrypsin and haptoglobin.

Rheumatoid Arthritis

The rheumatic diseases constitute a variety of disorders which are characterized by both acute and chronic inflammatory episodes. The inflammation usually involves connective tissues, but can be accompanied by systemic manifestations. Patients with rheumatic diseases frequently demonstrate plasma protein abnormalities. The most common abnormalities include those associated with the inflammatory response and those resulting from chronic stimulation of the immune system.

Rheumatoid arthritis is a chronic disease in which inflammation of the diarthtrodial joints is often combined with a variety of extra-articular symptoms. Though no distinct protein pattern is associated with rheumatoid arthritis, acute inflammatory patterns are commonly seen with α_1-acid glycoprotein, haptoglobin and C3 as the most consistently abnormal components.

Immunoglobulin increases are most often of the IgA class, with elevations in IgG and IgM occurring less often. No significant correlation has been shown between duration or stage of the disease and serum immunoglobulin levels.

Other Disorders

Concentrations of acute phase reactants have been shown to reflect the amount of disease activity in Crohn's disease and ulcerative colitis. Studies have shown increased serum concentrations of α_1-acid glycoprotein, α_1-antitrypsin and haptoglobin with decreased levels of prealbumin, albumin, ceruloplasmin, α_2-macroglobulin and transferrin in both diseases.

Tumors which cause tissue necrosis can bring about an atypical acute phase response. It can be characterized by low levels of C-reactive protein with marked elevations in α_1-acid glycoprotein and moderate increases in α_1-antitrypsin and haptoglobin.

Active systemic lupus erythematosus can result in a wide variety of protein abnormalities. The disease process can bring out a marked acute phase response, often with normal or decreased haptoglobin due to in vivo hemolysis. Rather than acting as subacute phase proteins, complement components C3 and C4 are often decreased in concentration due to activation of the complement system by circulating immune complexes. Immunoglobulins are commonly involved, with a cathodal increase in IgG being a characteristic, but not diagnostic, finding.

Section II

Clinical Significance Of The Plasma Proteins

Liver Diseases

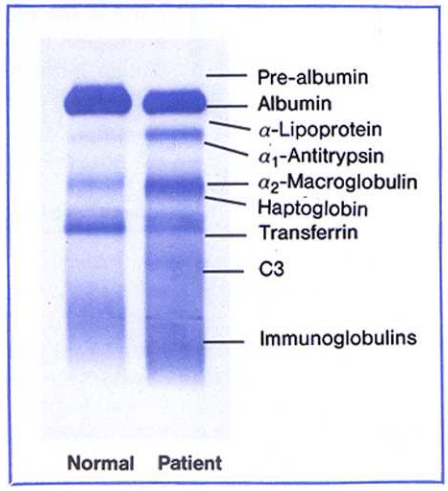
The liver is the primary organ for synthesis of all plasma proteins except the immunoglobulins. The liver plays a role in immunoglobulin production, however, by processing antigens from the gut before they are presented to the immune system. As a result of its dual function as the metabolic center for protein synthesis and a gateway to the immune system, the condition of the liver can greatly influence the plasma protein pattern. Advanced liver disease can lead to abnormalities in acute phase reactants, transport proteins, complement components and immunoglobulins.

Chronic Hepatocellular Disease and Cirrhosis

When damage to the liver is chronic and severe, both the ability of the liver to synthesize proteins and the effectiveness of Kuppfer cells to process antigens can be compromised. The most common pattern includes diffuse increases in IgG with proportionally greater increases in IgA and, less frequently, increases in IgM. Of the acute-phase reactants, α_1-antitrypsin is the most sensitive indicator for hepatocellular disease, while C-reactive protein and fibrinogen are usually normal or slightly increased. Levels of α_1-acid glycoprotein are normal or decreased. Haptoglobin is usually normal, but could be decreased as a result of hemolysis, increased red cell turnover, or reduced hepatic blood flow. Complement component C3 is usually normal, but advanced cirrhosis can lead to decreased synthesis and subnormal levels. Prealbumin, albumin, α-lipoprotein, and transferrin show characteristic decreases, with prealbumin the most sensitive monitor of hepatic function in cirrhosis. Concentrations of α_2-macroglobulin and ceruloplasmin are significantly elevated in cirrhosis, probably as a result of increased circulating estrogen in this disorder.

Case 7

Chronic Hepatocellular Disease



Patient: 39 year-old male

History and Physical: The patient has a long history of alcohol abuse. He was admitted through the emergency room because of a marked increase in his ascites, marked leg swelling over the last weeks, increasing shortness of breath and right side pain. There was marked enlargement of both liver and spleen. Chest exam revealed decreased breath sounds bilaterally. The legs showed 4+ pitting edema even up into the thigh region.

Laboratory Data: Chemistry battery showed elevations in LDH, alkaline phosphatase, ALT, AST, and γGT. The bilirubin was only slightly elevated. Decreases were seen in uric acid, cholesterol and calcium. Routine urinalysis showed 1+ occult blood and 2+ bile. The patient had an increased WBC count, was slightly anemic with an increased MCV, and a decreased platelet count and prolonged clotting studies.

SPE: The pattern showed hypoalbuminemia with normal electrophoretic migration. Prealbumin, alpha lipoprotein and transferrin were decreased – indicators of the chronic nature of the disease. There was a diffuse increase in background staining in the beta and gamma regions, most likely representing elevations in IgA and IgG.

Hospital Course: The patient's condition was stabilized, his ascites decreased and he

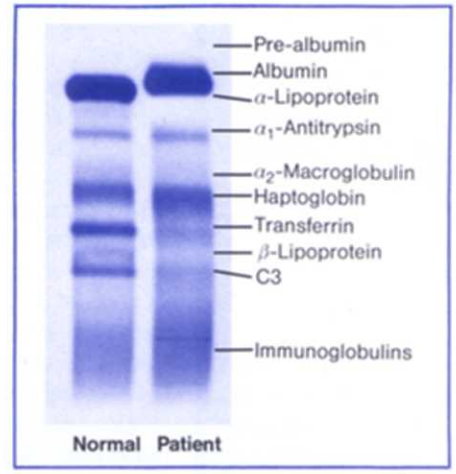
rapidly became less edematous. He was seen by a pulmonary specialist and treated for an abscess in his right lung. Liver function tests gradually returned to normal over a period of about 4 weeks. He was discharged in good condition.

Hepatitis

The various forms of hepatitis are often associated with the acute phase inflammatory response in the early stages and diffuse elevations in one or more of the immunoglobulins with chronic disease. Although these findings are consistent with the general pathophysiology of hepatitis, they are not useful in making a differential diagnosis.

Case 8

Cirrhosis



Patient: 54 year-old female

History and Physical: The patient has a history of chronic alcoholism and numerous previous admissions with alcoholic hepatitis, GI bleeding, cirrhosis and encephalopathy.

At the time of admission the patient was a deeply jaundiced female with a rapid pulse rate. There was hepatomegaly and splenomegaly.

Laboratory Data: The patient had multiple laboratory abnormalities. Total bilirubin and ammonia were markedly elevated. LDH, alkaline phosphatase, and AST were increased. Potassium was markedly decreased. Urinalysis showed 3+ occult blood and bile. RBC count, HGB and HCT were decreased with increased MCV. Coagulation studies were prolonged.

SPE: The pattern shows many of the classic signs of cirrhosis. Hypoalbuminemia is present with increased anodic mobility due to bilirubin binding. Pre-albumin, alpha lipoprotein and transferrin are all decreased as indicators of the chronic inflammatory nature of the disorder. Alpha lipoprotein is of particular interest since it frequently shows marked decreases in chronic liver disease. This can be seen as an almost complete lack of background staining in the region between albumin and alpha$_1$-antitrypsin. The cirrhotic process also produces diffuse increases in both IgG (cathodal to the point of application) and IgA (from the application point anodal to transferrin).

Hospital Course: The patient was rehydrated and stabilized over a period of several weeks in the hospital. She was discharged in good condition.

Section II

Clinical Significance Of The Plasma Proteins

Protein Losing Disorders

Selective Protein Loss

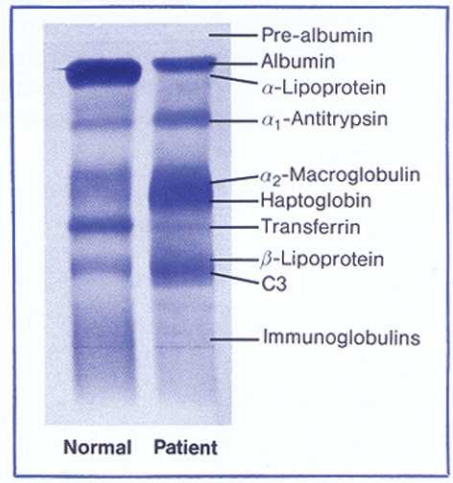
Nephrosis can result in loss of proteins in the urine in an inverse relationship to their hydrodynamic volume. This leads to elevations in the serum concentrations of large proteins with decreases in smaller components. The serum pattern thus shows increased levels of α_2-macroglobulin, β-lipoprotein, and polymeric forms of haptoglobin (Hp 2-1 and Hp 2-2), with decreases in such proteins as prealbumin, albumin, α_1-acid glycoprotein, α_1-antitrypsin, and transferrin. IgM is usually elevated in relation to the small proteins, and IgG is usually decreased.

Case 9

Selective Protein Loss
Acute Renal Failure
Patient: 57 year-old male

History and Physical: The patient noticed rapidly developing abdominal pain, enlarging abdomen, marked swelling of the feet and decreasing urine output. He consulted his physician who noted 4+ albumin and 100+ RBC in the urine. He was admitted for evaluation of acute nephrotic syndrome.

Upon admission he showed a blood pressure of 150/100 while lying down, but this dropped to 100/85 while sitting. His abdomen was distended and somewhat tender. There was marked pitting edema of the lower extremities. The diagnostic impression was probable acute glomerularnephritis presenting as nephrotic syndrome.



Laboratory Data: The chemistry battery showed decreased serum albumin, elevated cholesterol and creatinine. Urinalysis showed 4+ protein, 3+ occult blood with 100+ RBC. The patient was hemoconcentrated with a hematocrit of 57.9%.

SPE: The serum protein pattern showed a profound decrease in albumin (0.9 mg/dL) with elevated alpha$_2$-macroglobulin and beta lipoprotein. This pattern suggests a selective protein loss as is most commonly seen in glomerular-type proteinuria. Glomerular protein loss frequently presents as the nephrotic syndrome, as was the case with this patient.

Hospital Course: The patient was given large amounts of albumin in an effort to reestablish proper intravascular hydrostatic pressure. A kidney biopsy was done. Light electron microscopy revealed no glomerular pathology. Tubular necrosis was detected, and it was thought to be the result of some unknown toxin. These findings are somewhat in conflict with the electrophoresis results which suggest glomerular-type proteinuria and therefore imply some glomerular pathology. It is possible that the protein results reflect an acute physiological change which is not apparent in any histological change.

The patient was started on hemodialysis and showed gradual improvement of his renal function over a period of almost one month. He was discharged in much improved condition and will be followed as an outpatient with limited dialysis.

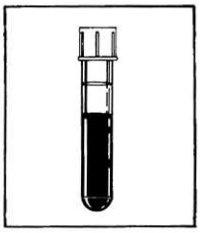
Final Diagnosis: Acute renal failure due to tubular necrosis which was possibly of a toxic nature.

Nonselective Protein Loss

Whole blood loss, congestive heart failure, liver failure, hemodilution and malnutrition can lead to diffuse hypoproteinemia in the plasma. This is also the case with protein-losing enteropathies, but the loss of lymphatic fluid into the GI tract results in relatively greater decreases in the immunoglobulins than the other plasma proteins.

Section II

Clinical Significance Of The Plasma Proteins



Pregnancy and Hyperestrogenism

Protein profiles have been studied in normal pregnancy and compared with values found in healthy non-pregnant women. Prealbumin, albumin, α_1-acid glycoprotein, and IgG are moderately decreased in pregnant women. Large relative increases are found for α_1-antitrypsin, ceruloplasmin, transferrin, and fibrinogen. Values for α-lipoprotein are increased to a moderate degree, while α_2-macroglobulin and hemopexin are slightly elevated in pregnant women. Haptoglobin and C_3 are essentially normal.

This pattern takes on added significance when considering that estrogen medication, including contraceptive pills, can produce hyperestrogenism. The pregnancy or "pseudo-pregnancy" pattern can be thus superimposed over pathological changes in young women taking contraceptive pills, others on estrogen medication, or patients with high levels of endogenous estrogen due to some disorder.

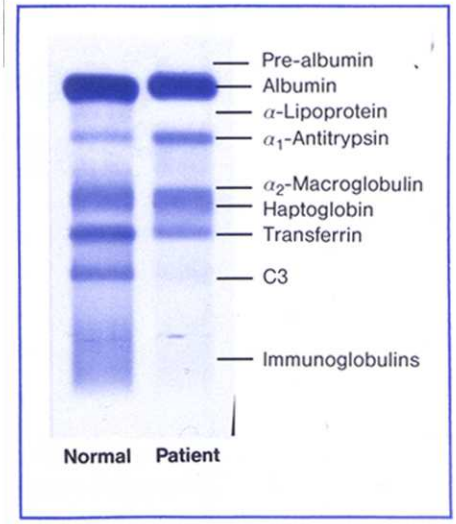
Case Studies: Plasma Proteins

Case 10

Hepatic Involvement in an Infant

Patient: 5 month old female

History and Physical: The patient is a previously healthy infant who was admitted to a community hospital with a fever of 104° of 24 hours duration, jaundice and hepatomegaly. The parents had noticed a yellow color in the eyes, somewhat yellowish skin and a golden color to the urine. The child has no prior history of jaundice or hepatomegaly, has been doing well, thriving, eating normally and gaining weight.



Admission laboratory work in the other hospital showed WBC count and differential, elevated bilirubin, AST, alkaline phosphatase and LDH. The patient was given a single dose of Tylenol and became afebrile. When the fever recurred the next morning, the child was transferred to this hospital.

Upon admission, the patient presented with fever and no obvious source of infection, although there was fairly marked hepatomegaly. A firm liver edge was palpated several cm below the level of the umbilicus. A small spleen tip was palpated.

Laboratory Data: Bilirubin was elevated on admission and hepatic enzymes were close to the normal range. WBC count was elevated with a shift to the left. Urinalysis was normal. Blood was drawn for culture.

SPE: The pattern showed hypoalbuminemia with increased anodic albumin mobility. The increased mobility in this case was most likely due to binding of bilirubin. Acute inflammation (increased α_1AT, and haptoglobin, with decreased pre-albumin, albumin α-lipoprotein and transferrin) was also noted with no apparent relative decrease in alpha$_1$-antitrypsin. The gamma region, though decreased relative to adult levels, was normal for age.

Hospital Course: The blood culture grew gram negative rods and antibiotics were started. The child continued to spike temperatures and antibiotics were changed. After several days the child became afebrile and remained so.

Ultrasound showed no masses in the liver and no dialated bile ducts. Sweat chloride, ceruloplasmin, alpha$_1$-antitrypsin and torch studies were all normal. Virus titers were negative. An open liver biopsy showed periportal fibrosis and early cirrhosis with bile duct proliferation. This was felt to be consistent with a post-infectious or congenital hepatic fibrosis complicated by cholangitis. The patient did well after the biopsy and was discharged in good condition. The parents were instructed to monitor the child's temperature closely and a follow-up visit was scheduled.

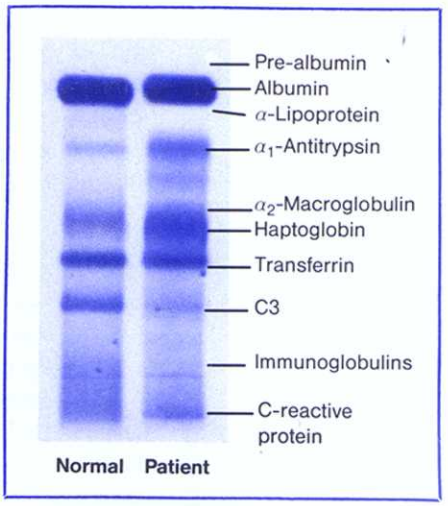
Case 11

Acute Renal Failure with Multiple Renal Abscesses and Acute Bacterial Endocarditis

Patient: 52 year-old male

History and Physical: The patient has a history of generalized weakness, progressive shortness of breath and tachycardia over a period of one month.

Upon admission he was severely anemic and azotemic. His blood pressure was normal, his heart rate was 115, and his temperature was 101.5°. Cardiac exam showed a grade III/IV systolic ejection murmur. There was mild jugular venous distension. Examination of the abdomen revealed mild hepatomegaly with no tenderness and no splenomegaly. The extremities showed 2+ edema.



Diagnostic Impression: Renal failure with no obvious reason for the renal disease such as diabetes or hypertension. The systolic ejection murmur was thought to be either a flow or valvular lesion with the possibility of subacute bacterial endocarditis. Several diagnostic possibilities were suggested for the anemia which included blood loss complicated by renal failure and bone marrow suppression secondary to malignancy or some other process.

Laboratory Data: The patient showed marked elevations in BUN (155 mg/dL), uric acid (11.3 mg/dL) and creatinine (15.1 mg/dL). Calcium was increased, phosphate was decreased, creatinine clearance was markedly decreased at 1.0 mL/min, as was serum iron at 7 ug/dL.

Urinalysis showed 3+ proteinuria and occult blood, with large numbers of RBC and WBC. Hematology showed a marked increase in white cell count with a shift to the left, and decreased HGB, HCT and RBC count. CSF showed a moderate increase in total protein with profound elevations in WBC and RBC counts.

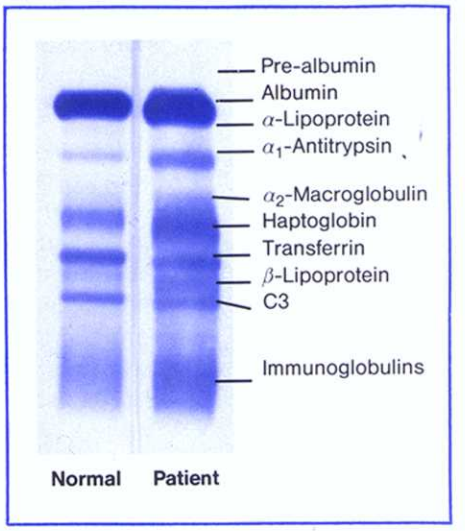
SPE: The pattern showed hypoalbuminemia and acute inflammation (increased α_1AT and Hp, decreased pre-albumin, albumin, α-lipoprotein and transferrin). There There was a low normal level of gamma globulin. A very faint band in the cathodal gamma region was probably C-reactive protein, since subsequent IEP showed no monoclonal immunoglobulin components.

Hospital Course: Blood cultures revealed Staphylococcus aureus septicemia. Although vigorous antibiotic therapy was instituted, the patient showed a progressive downhill course and died 5 days after admission.

Final Diagnosis: Acute renal failure secondary to multiple renal abscesses and acute bacterial endocarditis.

Case 12

Chronic Renal Failure With Acute Inflammation



Patient: 68 year-old male

History and Physical: The patient was admitted to the hospital with a long history of bladder outlet obstructive symptoms. He currently is suffering from abdominal pain, hematuria and probable bladder or urinary tract infection. The patient had already been started on antibiotic prior to his admission. His abdomen is non-tender and there is no hepatosplenomegaly or peripheral edema.

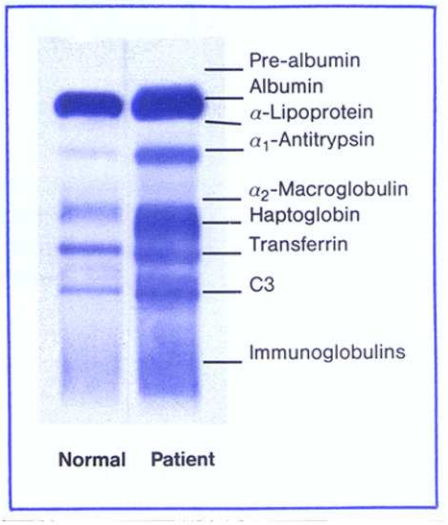
Laboratory Data: The chemistry panel showed severe azotemia with marked elevations in BUN, uric acid and creatinine. The creatinine clearance was 4.0 mL/min. Total protein, albumin and calcium were decreased, and phosphate was increased. Urinalysis showed 3+ proteinuria and 100+ RBC and WBC. Hematology data showed increased WBC count, and decreased RBC count, hemoglobin and hematocrit. Urine cultures were ordered.

SPE: The pattern showed hypoalbuminemia with increased anodal albumin mobility. Increased anodal mobility of albumin is frequently seen in patients with renal failure, and is perhaps due to binding of negatively charged endogenous substances. Acute inflammation was noted (increased α_1AT and increased Hp, decreased pre-albumin, albumin and transferrin). Electrophoresis on a random urine specimen showed a pattern consistent with a mixed glomerular-tubular proteinuria as is commonly seen with chronic renal failure.

Hospital Course: The patient was continued on antibiotics and treated with dialysis for his renal failure. The nephrologist noted that since very little renal parenchyma remained, minimal renal function would probably be regained after appropriate urinary drainage.

Case 13

Acute and Subacute Inflammation



Patient: 74 year-old male

History and Physical: The patient was in good general health prior to his present illness. He has been hospitalized for two weeks for treatment of pneumonia. In spite of aggressive antibiotic treatment, he developed additional chest infiltrates.

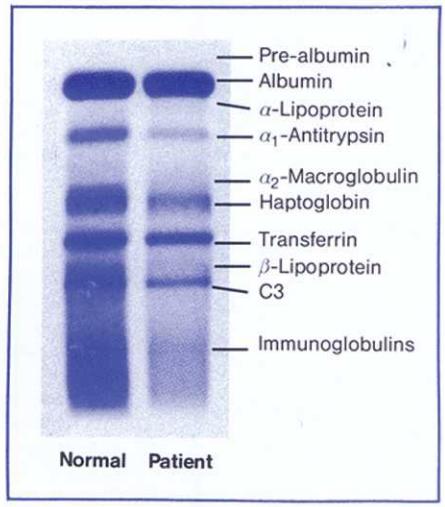
Laboratory Data: Chemistry battery showed decreased total protein, albumin, calcium and phosphate. Urinalysis was normal. Hematology showed increased WBC count, and normal RBC count, HGB and HCT. The ESR was elevated.

SPE: Hypoalbuminemia was detected and the albumin showed an increase in anodal mobility. This phenomenon is usually due to binding of some negatively charged drug or endogenous substance, and although of interest it has little clinical utility. The pattern also showed acute (increased α_1AT and Hp, decreased pre-albumin, albumin, α-lipoprotein and transferrin) and subacute (increased C3) inflammation.

Hospital Course: The patient improved rapidly on antibiotics and was discharged in good condition.

Case 14

Diffuse Hypergammaglobulinemia With Lymphoma



Patient: 63 year-old female

History and Physical: The patient experienced the onset of generalized pruritis, sweats and fatigue about 20 months ago. Three months after the initial onset, she had an episode of multiple subcutaneous nodules which were non-tender, occurring throughout her skin. She was hospitalized twice during this time with no clear diagnosis of her problem. She recently had another episode of multiple dermal nodules. Lymph node and skin biopsies showed poorly differentiated malignancy. She was admitted for further diagnostic evaluation.

Upon admission the patient presented with multiple tender dermal nodules. Some were close to the surface and were a deep red hue, but with no surrounding inflammation. There was right axillary adenopathy and bilateral inguinal adenopathy. There was no obvious organ involvement.

Laboratory Data: The chemistry battery was unremarkable except for an elevation in LDH (twice the upper limit of normal). The WBC count was elevated with a shift to the left. RBC, HGB and HCT were decreased. The ESR was increased.

SPE: The pattern showed diffuse increase in immunoglobulins, including the beta and gamma region, suggesting chronic inflammation, infection or antigenic stimulation. This is a non-specific finding, consistent with many diagnoses. Quantitative immunoglobulin results showed a mild increase in IgG, a marked increase in IgA and normal IgM.

Hospital Course: Biopsies of the left axilliary node, a subcutaneous nodule and the bone marrow were performed while the patient was in the hospital. The biopsy reports were consistent with lymphoma, although the type of cell involved could not be identified. The patient recovered well from the procedures and was discharged to be treated and followed on an outpatient basis.

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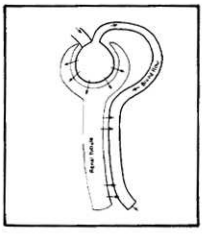
Section III

Urinary Proteins Of Plasma Origin

Introduction
Proteinuria In Renal Disease
Case Studies
Other Conditions with Increased Urinary Protein Excretion

Section III

Urinary Proteins Of Plasma Origin



Introduction

Filtration of plasma across the glomerular capillary membrane produces fluid containing a greatly decreased content of proteins with molecular masses> 40,000 daltons. Very small plasma proteins are normally filtered almost freely through the glomeruli and are subsequently reabsorbed and catabolized in renal tubules. Normal urinary protein excretion, therefore, is less than 150 mg/day. Two-thirds of this is made up of filtered plasma proteins – primarily albumin, low-Mr species, and immunoglobulin components. The remainder of the urine protein is derived from the urinary tract itself.

Immunochemical methods have allowed detection of numerous plasma proteins in normal urine. One extensive investigation showed a wide and uneven distribution of urine protein excretion in healthy individuals, characterized by many points clustered at low quantities, with asymmetrical "tailing" of data toward higher values. This study also found large physiologic day-to-day variations for excretion of several proteins in a healthy subject. This is in contrast to the small physiologic variations reported for proteins in plasma.

The lack of adequate reference intervals for specific urine protein components has limited most investigators to studies of clearcut proteinuria, or cases in which concentrations of individual proteins may be increased. This approach helps avoid distinctions between patients with normal renal function and those with only slight abnormalities.

Proteinuria in renal disease can be classified as resulting from either glomerular or tubular dysfunction. Glomerular proteinuria results from increased passage of proteins through the glomerulus and is characterized by the loss of plasma proteins the size of albumin or larger. Tubular proteinuria is caused by a decreased capacity of the tubules to reabsorb proteins and results in increased excretion of very small proteins such as β_2-microglobulin.

Certain systemic conditions can lead to increased urinary excretion of protein. These conditions include exercise proteinuria, postural proteinuria, proteinuria of pregnancy, and overflow proteinuria.

Electrophoresis of Urine Proteins

Electrophoresis is an excellent analytical technique to gain a broad overview of urine proteins when high resolution separations are developed with sensitive protein stains. Glomerular-type proteinuria, tubular-type proteinuria as well as mixed glomerular-tubular patterns and the various overflow states can be easily distinguished and characterized, thus providing useful information on specific functions within the nephron. This "biochemical biopsy" can be applied clinically in the differential diagnosis and monitoring of patients with renal dysfunction.

An electrophoretic pattern of normal urine will show a trace of albumin and sometimes transferrin.

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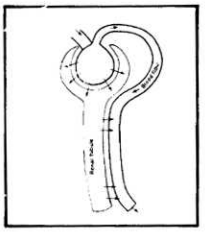
Urinary Proteins Of Plasma Origin



Proteinuria In Renal Disease

Glomerular Proteinuria

The renal glomeruli function as ultrafilters for macromolecules. The glomerular membrane is interrupted by pores of fixed dimensions which allow the passage of certain macromolecular species and retain others. Damage to the glomerulus which results in increased glomerular permeability leads to increased urinary excretion of proteins which are normally retained. These include albumin and other relatively high concentration plasma proteins of a similar size such as transferrin, α_1-antitrypsin and α_1-acid glycoprotein. These proteins have molecular weights ranging from 39,500 daltons (α_1-acid glycoprotein) to 76,500 daltons (transferrin). Very large portions such as α_1-macroglobulin (820,000 daltons) and β-lipoprotein (2,400,000 daltons) are not usually found in the urine in measurable amounts even with glomerular damage, since the glomerulus still maintains some selectivity. The very low molecular weight proteins that normally pass through the glomerulus such as β_2-microglobulin (11,800 daltons) are also absent from the urine in the early stage of glomerular disease before tubular reabsorptive capacity is compromised.

The urine pattern in glomerular proteinuria usually consists of strong bands for albumin, both α_1-acid glycoprotein and α_1-antitrypsin in a broad α_1-zone and transferrin (β_1). The serum pattern shows marked decreases in these proteins with increases in the large proteins which are retained by the glomerulus.

There are many pathological conditions, either primary glomerular diseases or disorders resulting in secondary glomerular damage, which can result in proteinuria. Severe proteinuria (greater than 3.5 grams of protein excreted per day) is a component of the nephrotic syndrome. In addition to proteinuria, this syndrome includes hypoalbuminemia, hyperlipidemia and massive edema. A list of disorders associated with the nephrotic syndrome is given in the following table.

Disorders Associated With The Nephrotic Syndrome

Glomerular Diseases

- Minimal change disease
- Focal and segmental glomerulosclerosis and hyalinosis
- Membranous glomerulopathy
- Proliferative glomerulonephritis
  - Membranoproliferative glomerulonephritis
  - Crescentic glomerulonephritis
  - Mesangial proliferative glomerulonephritis
  - Focal and segmental proliferative glomerulonephritis

Other Diseases

- Infections
  - Poststreptococcal glomerulonephritis
  - Infectious mononucleosis
- Drugs
  - Organic gold preparations
  - Mercury
  - Penicillamine
  - Antivenoms and antitoxins
  - Contrast media
- Neoplasia
  - Hodgkin's disease
  - Lymphomas
  - Leukemia
  - Carcinoma
  - Melanoma
  - Wilm's tumor
- Multisystem Diseases
  - Systemic lupus erythematosus
  - Schönlein-Henoch purpura
  - Vasculitis
  - Goodpasture's syndrome
  - Dermatomysitis
  - Amyloidosis
  - Sarcoidosis
  - Sjögren's syndrome
- Hereditary Disorders
  - Diabetes mellitus
  - Alport's syndrome
  - Sickle-cell disease
  - Fabry's disease
  - Congenitial nephrotic syndrome
- Miscellaneous
  - Preeclamptic toxemia
  - Renovascular hypertension
  - Chronic interstitial nephritis

(Adapted From: Glassock, R.J., and Brenner, B.M.: The Major Glomerulopathies. In Principles of Internal Medicine, Isselbacher, K.J., et al., Eds., McGraw-Hill, New York, 1311-1320, 1980.)

It should be clear from the table that an interpretation of "Glomerular-type proteinuria" is in no way diagnostic. It does, however, give information on the location of the renal lesion, and can therefore be useful in the differential diagnostic process.

Tubular Proteinuria

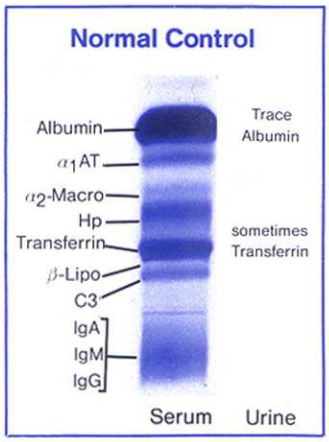
In the normal kidney the glomerulus acts as a molecular sieve to greatly decrease the concentrations of most large proteins while allowing almost free passage of proteins with molecular weights of less than 15,000 daltons. The tubules then reabsorb and catabolize 95 to 99 percent of the proteins that appear in the glomerular filtrate.

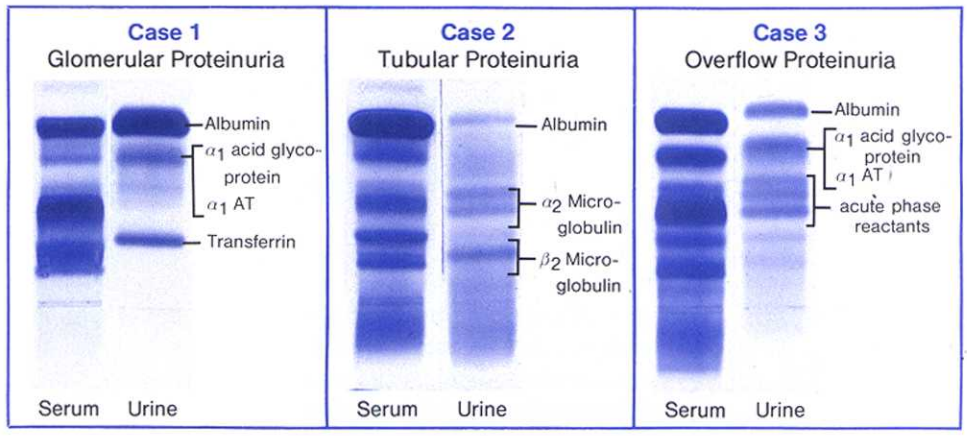
Tubular disease can cause a reduction in the capacity to reabsorb and catabolize these small proteins, thus leading to an increase in their urinary excretion. Tubular-type proteinuria is usually mild in comparison with glomerular proteinuria because these low molecular weight proteins are present in the plasma in very low levels. For this same reason, the serum protein pattern shows little or no effect of tubular proteinuria.

The urine pattern in tubular proteinuria usually consists of a faint albumin band, a double band in the α_2-region due to α_2-microglobulin (a protein about which we know very little), a strong band in the mid-beta region due to β_2-microglobulin, and sometimes diffuse background staining in the gamma region due to free light chains.

Damage to tubules leading to tubular-type proteinuria can result from a variety of causes. Tubular proteinuria was first discovered in workers chronically exposed to cadmium dust. Other metals, such as lead, mercury and gold can also cause tubular proteinuria. It is also seen with disorders as diverse as acute and chronic pyelonephritis, renal transplant rejection, toxicity due to aminoglycoside therapy, Fanconi syndrome, Wilson's disease, sarcoidosis, cystinosis, tubular acidosis, Balkan nephropathy and uremic medullary cystic disease. Classification of the type of proteinuria as "tubular" can be helpful in characterizing the renal lesion and ruling out non-tubular disorders.

Chronic renal disease or renal failure can lead to damage of both glomerulus and tubules. This results in a combined pattern with both "glomerular-type" and "tubular-type" proteins appearing in the urine.





Three cases illustrating the differences in protein electrophoresis patterns in glomerular, tubular and overflow proteinuria.

Case 1

Nephrotic Syndrome With Glomerular Proteinuria

Patient: 45 year-old white male

History: The patient has a long history of diabetes mellitus. He presented with nephrotic syndrome which is most likely secondary to diabetic glomerulosclerosis.

Laboratory Data: On admission the patient exhibited mild electrolyte imbalance, his serum total protein and albumin were decreased, his cholesterol was elevated, and he showed slight decreases in RBC count, HGB and HCT. Routine urinalysis revealed 1+ glucose, 3+ protein, and the microscopic examination showed some oval fat bodies and waxy casts. Quantitative urine protein excretion on a 24-hour specimen was 6.9 g/day.

SPE: SPE showed a marked decrease in pre-albumin, albumin and transferrin with increases in α_2-macroglobulin and β-lipoprotein, consistent with a selective renal protein loss.

Urine Electrophoresis: The urine pattern showed the presence of large amounts of albumin, α_1-antitrypsin and transferrin along with a trace of pre-albumin and some faint α_2-components. This pattern is also consistent with the sieveing glomerular-type protein loss seen in nephrotic syndrome.

Case 2

Heavy Metal Toxicity with Tubular Proteinuria

Patient: 52 year-old black male

History: The patient is a smelter worker suspected of having minimal heavy metal toxicity.

Laboratory Data: Routine laboratory tests were within normal limits.

SPE: SPE showed no significant abnormalities.

Urine Electrophoresis: Electrophoresis of the concentrated urine specimen revealed tubular-type proteinuria, with a trace of albumin and strong bands for α_2-microglobulin and β_2-microglobulin. The presence of these bands in the urine suggests compromise of tubular capacity to reabsorb and catabolize low molecular weight proteins.

Case 3

Septicemia with Overflow Proteinuria

Patient: 65 year-old white female

History: The patient began to experience high fever with intermittent chills and sweats several days ago. Her temperature was 102°F on admission, and she complained of aching muscles and joints.

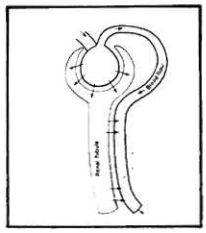
Laboratory Data: The admission laboratory workup showed many abnormal values including increased LDH, AST, WBC count (shift to the left) and a marked increase in ESR. HGB, HCT and RBC were decreased. Serum total protein was normal, albumin and transferrin were decreased, and both C3 and C4 showed mild increases. Microbiology studies detected E.coli in the blood and all other cultures were negative. The patient was treated with antibiotics.

SPE: SPE showed a marked acute/subacute inflammatory reaction with decreased pre-albumin, albumin, α-lipoprotein and transferrin; increased α_1-antitrypsin, haptoglobin, C3 and C-reactive protein.

Urine Electrophoresis: The pattern showed traces of albumin and transferrin with large amounts of α_1-acid glycoprotein and antitrypsin. Several fainter bands were detected in the region, suggesting the presence of other acute phase reactants such as antichymotrypsin and possibly Zn-α_2-glycoprotein. This pattern probably does not reflect true renal disease, but simply an overflow of elevated acute phase reactants from the plasma into the urine.

Section III

Urinary Proteins Of Plasma Origin



Other Conditions With Increased Urinary Protein Excretion

Exercise Proteinuria

Several physiologic conditions produce proteinuria in the absence of significant renal disease. Strenuous muscular exercise increases the urinary excretion of both high- and low-M_r proteins. Exercise proteinuria may be mainly the result of increased glomerular permeability combined with saturation or inhibition of tubular reabsorption capacity by some unknown mechanism.

Postural Proteinuria

Postural or orthostatic proteinuria is defined as a syndrome in which proteinuria is absent during recumbency but present when the patient is upright. This condition has long been regarded as benign and unassociated with renal disease, although some recent reports contradict this view. Total daily protein excretion is usually well below 1.5 g in this condition, with relatively large percentages of high-M_r proteins. Tentative mechanisms for postural proteinuria propose increased nonselective glomerular permeability on standing, but the underlying cause has not been characterized.

Pregnancy

Most commonly, the proteinuria seen in pregnant women is transitory and does not indicate renal disease in the usual sense of the term. Proteinuria of pregnancy can be classified as proteinuria associated with toxemia, proteinuria during delivery, proteinuria during renal infections, and proteinuria with no other clinical symptoms. In proteinuria with toxemia, a selective pattern is usually associated with a high rate of fetal mortality in utero. Proteinuria during delivery conforms with a glomerular pattern in most cases. Although most urinary tract infections in pregnancy do not cause proteinuria, those that do can show nonselective or mixed tubular patterns. Proteinuria without clinical symptoms usually shows a nonselective pattern.

Overflow Proteinuria

Some conditions can cause increased plasma concentrations of low-M_r proteins. These proteins will filter through the glomerulus in abnormal amounts, leading to overflow proteinuria. Bence Jones proteinuria is a classic example of this type, although myoglobin, hemoglobin and low-M_r acute phase reactants can also be excreted in this manner. Increased excretion of Bence Jones protein can cause inhibition of reabsorption of very-low-M_r proteins, resulting in a superimposed pattern of tubular proteinuria.

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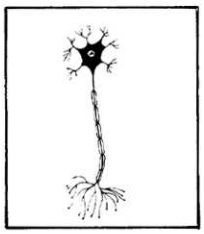
Section IV

Cerebrospinal Fluid Proteins

Introduction
Assessing the Permeability of the Blood-CSF Barrier
Assessing Abnormal Production of Protein Within the Central Nervous System
Laboratory Protocol to Rule Out Multiple Sclerosis
Case Studies

Section IV

Cerebrospinal Fluid Proteins



Introduction

Production of most of the cerebrospinal fluid (CSF) takes place by ultrafiltration and active transport of proteins, ions, water and other components through the vascular endothelium, basement membrane, and epithelium of the choroid plexuses. In addition to the fluid formed at this blood-CSF barrier, a small proportion is produced at other sites within the central nervous system.

Normal CSF Protein Composition

Much less protein is present in CSF than in plasma. Its protein composition, however, shows relative increases in some of the low molecular mass (M_r) species, owing to the fact that the blood-CSF barrier acts somewhat as a molecular sieve. The total amount of protein in CSF varies with the age of the individual and with the site of fluid removal. The accepted reference interval for fluid from the lumbar region in patients between the ages of 10 and 40 years is 150-450 mg/L, while infants and individuals older than 40 show higher concentrations. Fluids from the verticular and cisternal regions generally have a lower protein content than that drawn from the lumbar region.

An overview of CSF protein composition can be obtained by performing agarose gel electrophoresis on a sample which has been concentrated 80- to 100-fold (Figure 1). The pattern from a normal adult shows a prominent prealbumin fraction that migrates slightly faster than plasma prealbumin. Albumin is the major band on electrophoresis, comprising from 55 to 75% of the normal CSF protein. The α_1-band consists primarily of α_1-antitrypsin, the α-lipoprotein fraction being greatly decreased. The α_2-region is not a dominant fraction, as with plasma, owing to relative decreases in large proteins such as α_2-macroglobulin and the polymeric haptoglobin phenotypes. Transferrin is detected in the β_1-region, and the major β_2 protein is a carbohydrate-deficient "CSF-specific" transferrin. The γ-region, consisting almost exclusively of immunoglobulin G (IgG), can show some very faint banding in normal samples. The cathodal end of this zone often contains a low-M_r, nonimmunoglobulin protein, γ-trace, which is perhaps synthesized within the central nervous system, but has undetermined clinical significance.

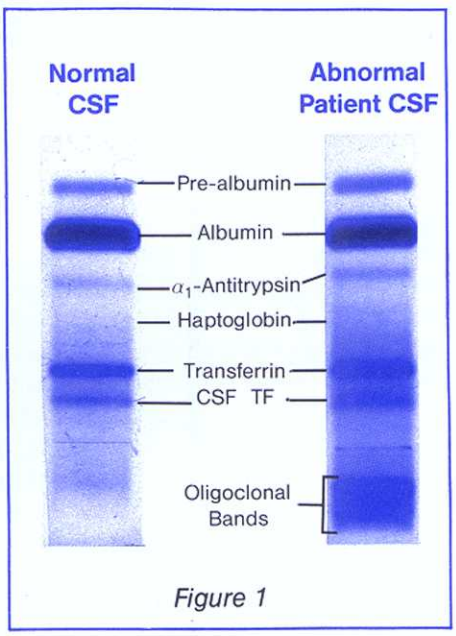
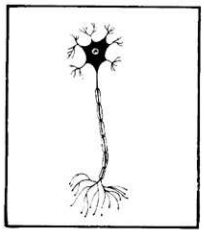


Figure 1

Section IV

Cerebrospinal Fluid Proteins



Assessing Permeability Of The Blood-CSF Barrier

Many disorders of the central nervous system can increase the permeability of the blood-CSF barrier, leading to increased concentrations of CSF proteins. These disorders include (a) bacterial, viral and other forms of meningitis; (b) neoplastic infiltration of the meninges; (c) spinal and cerebral tumors; (d) polyneuropathies; (e) disk herniations; and (f) cerebral infarctions. Even though demonstration of increased barrier permeability is usually not helpful in differentiating among these conditions, it can have diagnostic value when considered along with other laboratory data. It can also be useful in monitoring a patient's clinical condition. The convalescent stage of bacterial meningitis, for example, is usually accompanied by a progressive decrease in permeability of the blood-CSF barrier toward normal.

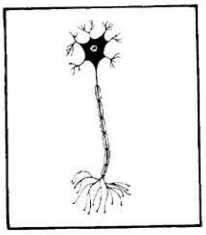
The integrity of the blood-CSF barrier is most commonly assessed through measurement of CSF total protein. Quantitation of a high Mr protein such as α_2-macroglobulin may be a more sensitive indicator of mild barrier disturbances, but large proteins are present in the CSF at very low concentrations and are difficult to measure. Until these difficulties are solved, CSF total protein will remain a valuable laboratory tool in some neurological diseases, particularly those of an inflammatory nature.

Protein ratios can also be used to estimate blood-CSF barrier permeability. Several groups have examined the CSF/serum ratios of albumin and IgG and compared them graphically. This multi-variate approach results in a two-dimensional reference area for normal individuals and produces a straight-line indicator of permeability increases. Albumin, not being synthesized to any extent within the central nervous system, serves as the reference protein for monitoring permeability. Thus, any increase in the CSF/serum albumin ratio indicates increased passage of that protein across the barrier. The second protein, IgG, is measured both because of its larger molecular size and the fact that its increased production within the central nervous system has important clinical implications. Extensive studies of protein filtration and secretion at human body fluid barriers have shown that the normal blood-CSF barrier is less selective than the normal renal glomerulus and other body fluid barriers. Pathological conditions involving increased permeability are usually not accompanied by substantial changes in the selectivity of the blood-CSF interface.

These newer approaches of measuring protein ratios and plotting the results have as their common aim a more quantitative and sensitive assessment of the permeability of the blood-CSF barrier. This is expected to be useful in detecting more subtle changes in disease than is currently possible. Even though these methods show promise, their clinical implications have not yet found acceptance for routine use except in ruling out increased permeability as the cause for an increased concentration of IgG in CSF. This aspect will be discussed in the next section.

Section IV

Cerebrospinal Fluid Proteins



Assessing Abnormal Production Of Protein Within The Central Nervous System

Demyelinating Diseases

Many studies have shown increased γ-globulin in the CSF of patients with multiple sclerosis. This increased gamma globulin is from synthesis of IgG within the central nervous system. The specific antigenic stimulant for this abnormal production of IgG has not been discovered, but mechanisms suggesting slow virus infection and autoimmune reaction have been proposed. Increased concentrations of CSF IgG are not unique to multiple sclerosis. A rare, demyelinating disease of childhood, subacute sclerosing panencephalitis, can also show IgG increases. Several other inflammatory and infectious disorders of the central nervous system and even some non-inflammatory CNS disorders can lead to increases of IgG. These other conditions, particularly those involving infection, can usually be distinguished from multiple sclerosis through consideration of clinical and other criteria. Increases of the remaining two major immunoglobulins, IgA and IgM, have been infrequently observed in demyelinating diseases and some other central nervous system disorders, but these findings have not proven to be of diagnostic or prognostic value.

IgG As a Percentage of Total Protein

Evaluation of CSF concentrations of IgG must take into consideration the condition of the blood-CSF barrier, to distinguish increases resulting from leakage of plasma proteins across the barrier from increased synthesis in the central nervous system. The first useful method of taking permeability into account was to express CSF IgG as a percentage of CSF total protein. In general, IgGs of 10% of total protein are considered suspicious; $>$ 13% suggests abnormal IgG production. These guidelines are useful not only for interpretation of quantitative IgG measurements, but also for evaluation of the γ-globulin fraction on CSF electrophoresis, because almost all of this fraction is IgG.

One limitation of expressing IgG as a ratio to total protein is that IgG itself contributes to the amount of protein. Several groups investigated use of the ratio IgG/albumin in CSF, and found it to be at least as discriminative for multiple sclerosis as the ratio for IgG/total protein in CSF and perhaps slightly better. Even though the substitution of albumin for total protein does improve sensitivity in detecting immunologic activity of the central nervous system in multiple sclerosis, the improvement is not dramatic.

CSF/Serum Protein Ratios

The diagnostic value of CSF protein measurements can be further enhanced if serum concentrations of protein are also taken into account. By measuring IgG and albumin in both fluids, any CSF abnormalities resulting from increases or decreases in the serum proteins can be normalized. A graphic presentation of these protein ratios allows for discrimination between patients without protein abnormalities and those with barrier disturbances, those with increased central nervous system production of IgG, and those with combined disorders.

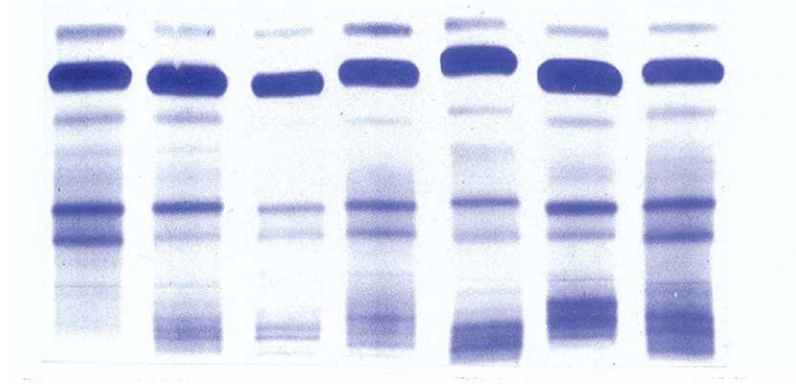
Various groups have used this dual-ratio approach, and some have published reference values for the coefficient of the two ratios in healthy adults. The mean values for the coefficient are about 0.5, and they show remarkable similarity, considering the fact that different analytical methods were used and reference populations from three separate countries were studied. The most extensive study found that 86% of patients with multiple sclerosis gave values above their reference range for the ratio coefficient.

Further manipulation of serum and CSF IgG and albumin data can be done by using a formula to estimate the rate of IgG synthesis within the central nervous system. This approach uses albumin as a quantitative marker for blood-CSF barrier permeability, and corrects for both IgG leakage into the central nervous system and serum concentrations of IgG by incorporating a series of constants into the equation, but adds nothing to the basic analytical data.

Oligoclonal Banding

In 1970, significant abnormalities were discovered in the electrophoretic morphology of the γ-globulin fraction in patients with multiple sclerosis and some other diseases of the nervous system. Multiple, restricted bands appeared in the γ-region on electrophoresis. This phenomenon, since then designated "oligoclonal banding," can be detected only by high resolution, high-sensitivity electrophoretic methods. It can occur in various forms, ranging from a few faint bands to many very intense bands. Oligoclonal banding patterns are present in a very high percentage of patients with multiple sclerosis. Reports from different groups vary slightly, but it is generally accepted that at least 90% of patients with multiple sclerosis will show oligoclonal banding at some time during the course of their disease. The banding phenomenon can appear very early in the disease, and generally persists even during subsequent remission and exacerbation cycles. Detection of oligoclonal banding, despite its major importance as a diagnostic tool, is not useful as a prognostic indicator, since the intensity of the pattern does not apparently correlate with the subsequent course of the disease.

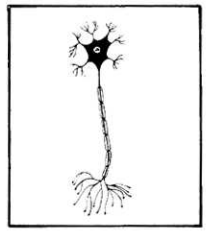
As with quantitative IgG measurements, the oligoclonal pattern is not pathognomonic for multiple sclerosis. The original report showed that inflammatory and infectious processes as well as some other noninflammatory central nervous system disorders could be associated with oligoclonal banding. Oligoclonal banding was detected in 100% of patients with subacute sclerosing panencephalitis, in 53% of cases of neurosyphilis, in 35% of cases of viral encephalitis or meningoencephalitis, and in 33% of cases of bacterial meningitis. It was also present with a low frequency (5% or less) in peripheral neuropathy, tumors, hydrocephaly, degenerative diseases, vascular disorders, and some miscellaneous other diseases. These findings should not be considered false positives, however, because they probably do indicate some increased immunologic activity of the central nervous system. It is important to note that CSF electrophoresis for oligoclonal banding should always be carried out in conjunction with electrophoresis of the patient's serum. Some systemic conditions, particularly the lymphoproliferative disorders, can cause oligoclonal banding in the serum. This pattern will then appear in the CSF and will result in a false positive unless the serum is examined.



It is now commonly accepted that oligoclonal banding is the best single laboratory test in the diagnosis of multiple sclerosis. This test should be performed first in ruling out multiple sclerosis as the basis for neurological dysfunction; with appropriate ratio measurements of the IgG and albumin in serum and CSF for questionable cases or those requiring further laboratory confirmation.

Section IV

Cerebrospinal Fluid Proteins



Laboratory Protocol To Rule Out Multiple Sclerosis

Although the diagnosis of multiple sclerosis must be made on clinical grounds, there are some laboratory tests which can provide the neurologist with objective data to evaluate the patient with neurological dysfunction suspected to be demyelinating in origin.

1. Draw both CSF and serum samples for analysis.
2. Perform routine tests on the spinal fluid samples including notation of color and appearance, determination of total protein, glucose, white cell count, differential and red cell count.
3. Perform high resolution electrophoresis with sensitive protein staining on the concentrated CSF sample to determine if oligoclonal banding exists.

   The interpretation of oligoclonal banding is not always a "positive" or "negative" exercise. In some cases, the laboratory director should be prepared to describe the findings and phrase the interpretation in such a way that the neurologists will understand the nature and probable significance of the results.
4. Perform high resolution electrophoresis on the serum sample to determine that no banding is present.

   Any banding in the serum, as seen with some lymphoproliferative conditions, will appear in the CSF but does not represent increased CNS immunologic activity.
5. Determine CSF and serum albumin and IgG ratios in borderline cases where oligoclonal banding is difficult to interpret.

   Since a calculation is being done on two ratios and any analytical errors will be magnified, only the most precise quantitative immunochemical techniques can be used for measuring the CSF and serum albumin and IgG. Once the ratio calculations are done, further manipulation of the data is unnecessary.

Laboratory Findings in Multiple Sclerosis

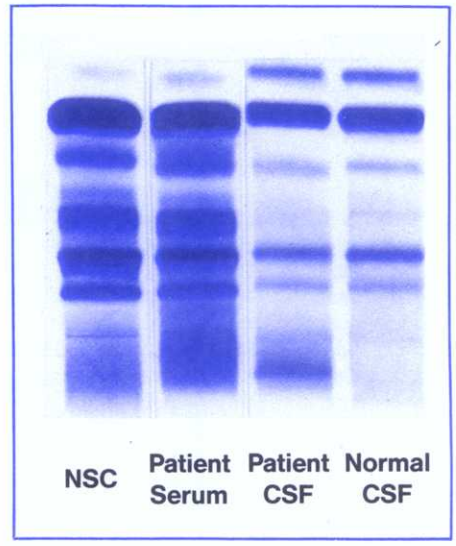
Cerebrospinal Fluid

Appearance	clear
Leukocytes	usually normal
Total protein	usually normal
Glucose	normal
Electrophoresis	oligoclonal banding in 90% of cases (with normal serum pattern)
IgG ratio	elevated in 90% of cases

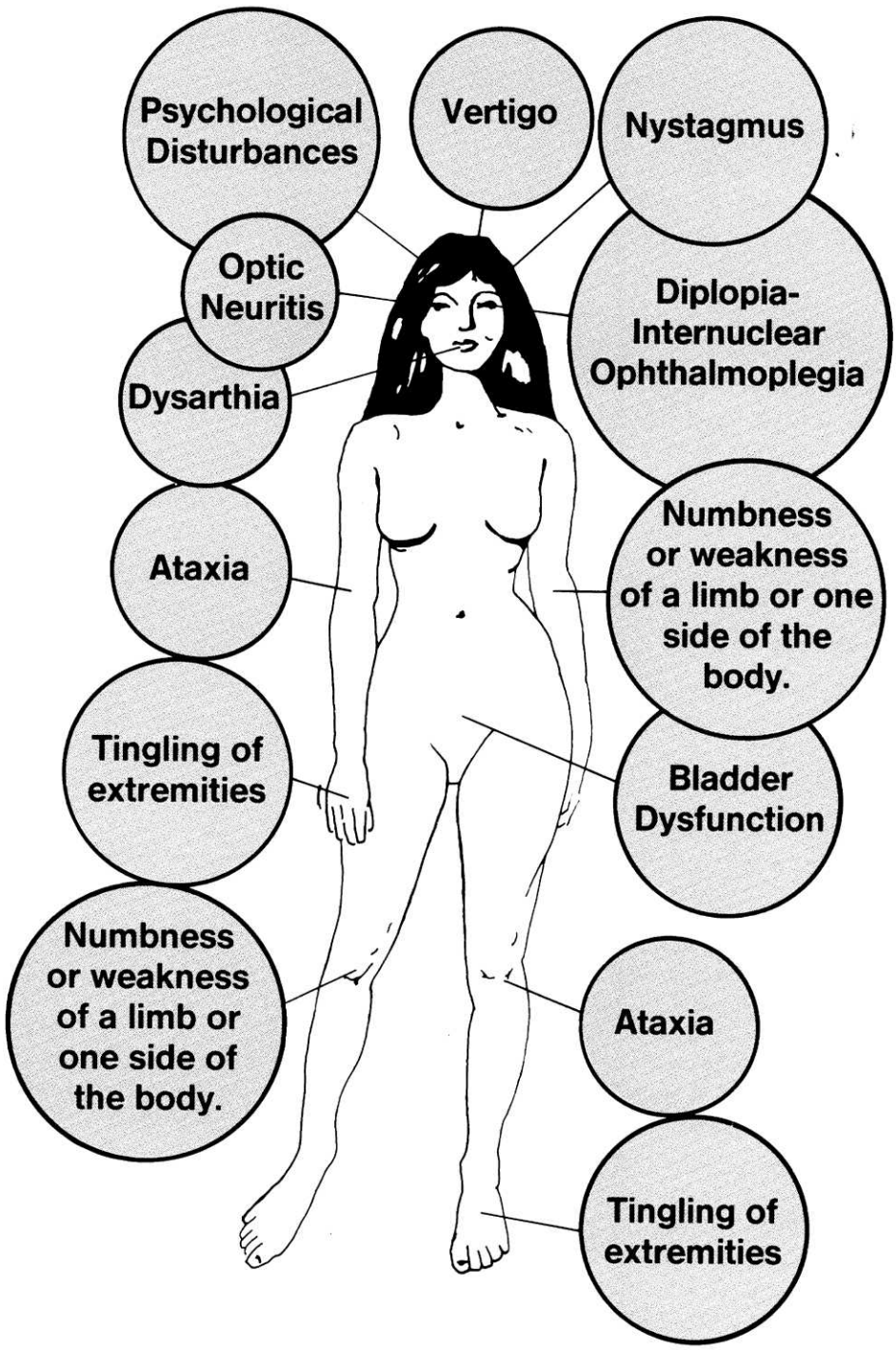
In general IgGs of 10% of total protein are considered suspicious. 13% or greater suggests abnormal IgG production.

$$\text{ratio} \quad \frac{\text{CSF/Serum IgG}}{\text{CSF/Serum albumin}}$$

The mean value for normal CSF (obtained by various groups) is 0.5



Clinical Manifestations of Multiple Sclerosis

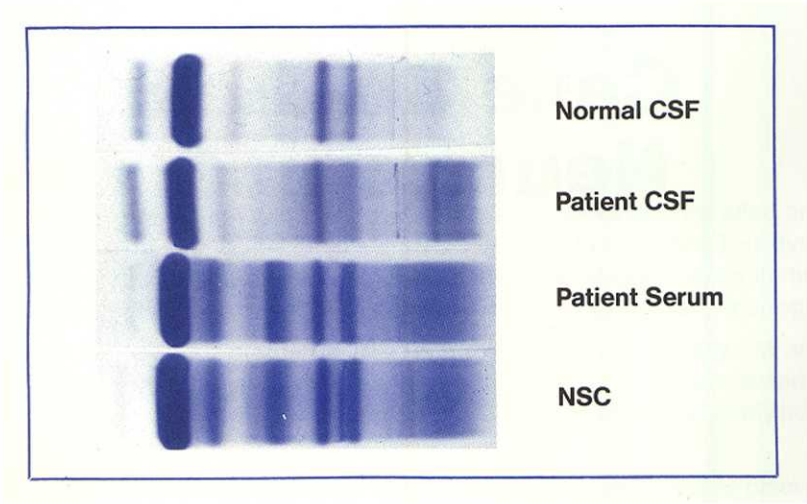


1. About 40 percent of patients with multiple sclerosis have an episode of optic neuritis as their initial symptom.
   The syndrome of optic neuritis has a rapid onset over a period of several days with partial loss of vision in one eye, often associated with pain on movement of the eye.
2. The remaining 60 to 70 percent of patients with multiple sclerosis will present with some evidence of a lesion of the spinal cord or brainstem.

*From Poskanzer, D.C., and Adams, R.D., Multiple sclerosis and other demyelinating diseases. In Principles of Internal Medicine. Isselbacher, K.J., et al. (eds.), 9th Edition, McGraw-Hill Book Company, New York, 1980.

Case Studies: Neurological Disease

Case 1 Multiple Sclerosis



Patient: 33 year-old female

History: The patient began to notice numbness on the left side of her body from the waist down about nine months ago. Three weeks after the first episode, she experienced numbness in the left lower extremity, some numbness from the mid-chest, down both sides and also severe dizziness. The dizziness resolved over a period of 2 to 3 weeks, and the numbness persisted for 4 more weeks. At that time routine laboratory screening tests were performed, and all were normal.
She remained in good health until about 2 weeks ago when she began to feel weak and unsteady. She also began to have trouble with intermittent "fuzzy" vision in her right eye, and numbness of her right lower cheek and jaw.

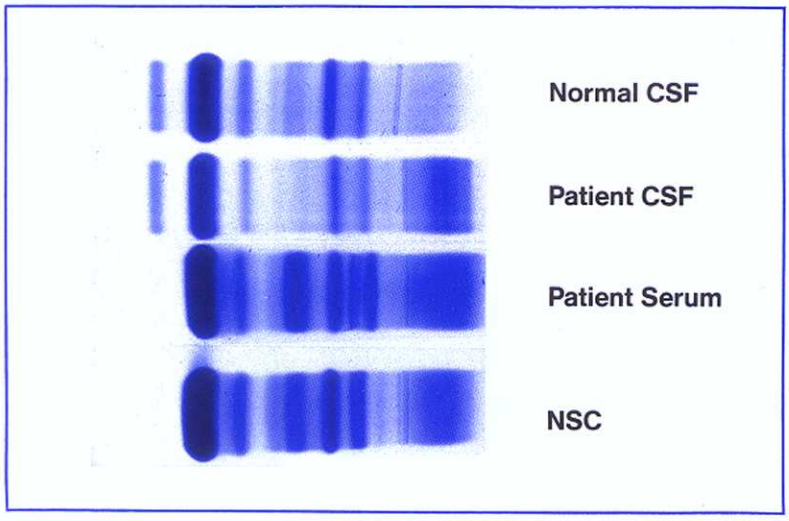
Physical: On admission the physical examination was normal and unremarkable except for the following neurological findings: The patient described a slight desaturation of color in the left eye compared to the right. The patient exhibited mild ocular dysmetria associated with some very slight rotary nystagmus on upward gaze and moderate lateral nystagmus on lateral gaze. The impression of the neurologist was a strong suspicion of demyelinating disease.

Laboratory Data: Chemistry screens, hematology studies and urinalysis results were all normal.

SPE: SPE was normal.

CSF: CSF was drawn and the specimen was clear and colorless with normal cell count, cytospin differential, glucose and total protein. CSF protein electrophoresis was performed on the concentrated specimen and revealed increased gamma globulin (19.0%) with a strong oligoclonal banding pattern, consistent with the neurological diagnosis of demyelinating disease.

Case 2 Multiple Sclerosis



Patient: 31 year-old male

History: The patient developed a decreasing ability to walk over the past 4 years. His difficulty is more pronounced on the left than the right, and is characterized by unsteadiness and a tendency to stumble or drag his feet. Two years ago he developed some numbness from the waist downward which fluctuated and resolved over a period of about a year. More recently his ability to concentrate has become impaired to the extent that he is not able to perform his work as an electrician adequately. He has demonstrated irritability and emotional outburst, has some tendency toward slurred speech, and has had some visual problems in the right eye. He has also developed urinary urgency. His symptoms have developed gradually and intermittently, with no true exacerbations or remissions, except for the leg numbness. He has experienced no headache, vomiting or loss of consciousness.

Physical: Physical examination on admission revealed gross nystagmus on lateral gaze with some reduction in adduction of the left eye. Vertical nystagmus was found on upward gaze but no nystagmus was detected on downward gaze. Speech was slightly slurred. Strength was good in upper and lower extremities, but there was a reduction in sensation to pin prick and temperature in the lower extremities. Moderate dysmetria was detected bilaterally on heel to knee to shin testing. His gait was clearly ataxic. The remainder of the physical examination was unremarkable. The clinical impression was that of diffuse neurological involvement, probably demyelinating in nature, with multiple sclerosis as the most likely diagnosis. Further testing was ordered to confirm the diagnosis and rule out arteritis, degenerative condition or toxicity as causes for the patient's neurological condition.

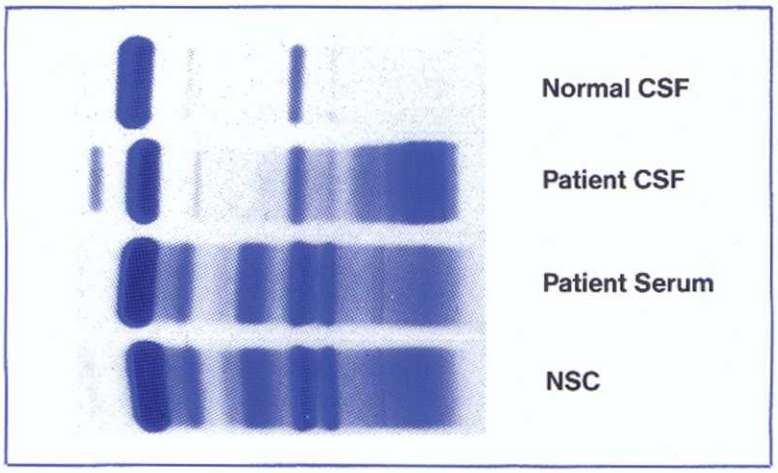
Laboratory Data: Chemistry screens, hematology studies and urinalysis results showed no significant abnormalities. Thyroid function tests were normal. ESR was normal, VDRL was negative, as were tests for ANF, lead, mercury and arsenic.

SPE: SPE was within normal limits.

CSF: The CSF was clear and colorless with normal glucose and 29 WBC/cmm (9% lymphocytes, 1% moncytes). The CSF total protein showed a borderline increase (55 mg/dL). CSF protein electrophoresis showed increased gamma globulin with oligoclonal banding, suggesting increased CNS immunologic activity, and supporting the preliminary diagnosis of multiple sclerosis.

Case 3

Multiple Sclerosis with Minimal Clinical Findings and Marked Laboratory Abnormalities



Patient: 36 year-old female

History: The patient developed numbness in her legs about one month ago. She has also recently experienced some urgency of urination, and noticed blurring of her vision for the past four months. She has been tiring easily, especially in the afternoon, for the past few months. She has not had diplopia or hearing changes, and she has not had any problems with coordination. Her memory is good, and she has had no change in her mood. She takes no medications and uses alcohol only rarely.

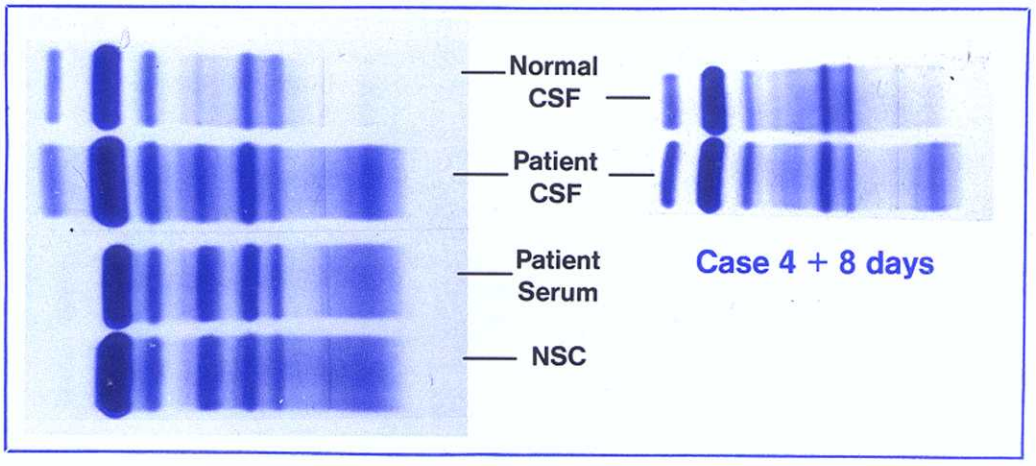
Physical: Physical and neurological examination revealed an unremarkable family history and past medical history except as noted above. Abnormal visual evoked potentials were detected, and nerve conduction studies in her legs were normal. Visual acuity in her right eye was slightly reduced compared with the left. No other objective abnormalities were detected. The impression of the neurologist was that the findings were consistent with multiple sclerosis, and protein studies were ordered to better establish the diagnosis.

SPE: The SPE was within normal limits.

CSF: The cerebrospinal fluid was clear and colorless with normal total protein, glucose and WBC count. The CSF protein electrophoresis revealed a profound elevation of the gamma globulin fraction (42.8% of total protein) with very strong oligoclonal banding. Quantitative immunochemical assays showed a remarkable elevation in CSF IgG (15.2 mg/dL, upper limit of normal = 9.0 mg/dL), and the ratio value of CSF/serum IgG to CSF/ serum albumin was 4.5 (upper limit of normal = 0.75). These laboratory results strongly suggest increased CNS immunologic activity and are consistent with the diagnosis of multiple sclerosis. They also emphasize the fact that disease activity in multiple sclerosis and clinical symptoms are not necessarily correlated with levels of IgG found in the CSF, since this patient had minimal clinical findings but marked laboratory abnormalities.

Case 4

Suspected Viral Meningoencephalitis



Patient: 25 year-old white female

History: The patient has had a progressive headache over the past 10 days with some distortion in mental status. She had contracted a flu-like illness about 4 weeks ago, but had been afebrile for the past 2 weeks. She became increasingly lethargic over the past few days and was admitted through the emergency room for further evaluation.

Physical: The patient was moaning, lethargic and generally unresponsive to verbal commands on admission. The physical examination was otherwise unremarkable.

Laboratory Data: A CT scan of the head was performed, and was normal. Chemistry battery and routine urinalysis were normal. Hematology data was normal except for increased white cell count. The differential showed 89% neutrophils, 5% bands, 5% lymphocytes and 1% monos.

SPE: The SPE was normal except for borderline hypoalbuminemia.

CSF: Lumbar puncture produced clear and colorless CSF with normal glucose, elevated total protein (83 mg/dL) and 158 WBC/cmm (100% monocytes). No organisms were detected in the CSF and VDRL was negative.
CSF protein electrophoresis showed increased gamma globulin (18.6%) with prominent oligoclonal banding. This pattern, in conjunction with the elevated total protein suggests increased permeability of the blood/CSF barrier with increased CNS immunologic activity. These findings suggest an infectious/inflammatory condition and are consistent with the working diagnosis of probable viral encephalitis.

Hospital Course: The patient improved remarkably on the second hospital day, showing no neurological symptoms and a marked decrease in her headache. She was discharged on Tylenol and was to be followed as an outpatient with careful monitoring for any deterioration.

Re-Admission: The patient did well at home for several days, but then redeveloped the headache without any disturbance in mental status. She was readmitted, and physical exam was unremarkable. She had a spinal tap which gave clear, colorless fluid with normal protein and glucose. No organisms were detected in the specimen. The CSF white cell count was elevated at 103/cmm with 90% lymphocytes and 10% neutrophils. CSF electrophoresis results were similar to the study 8 days prior, but showed a decreased amount of gamma globulin (14.5%) and decreased banding compared with that study. A transient increase in gamma globulin with the associated oligoclonal banding would be expected in an infectious/ inflammatory CNS disorder. The patient again improved rapidly. She was discharged on anti-inflammatory medication and was to be closely followed as an outpatient as before. No organism, viral or otherwise, was ever isolated from the CSF, and the patient had no further exacerbations.

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Subject Index

The Electrophoresis Patterns Show:

Case Studies Index

Special Presentations

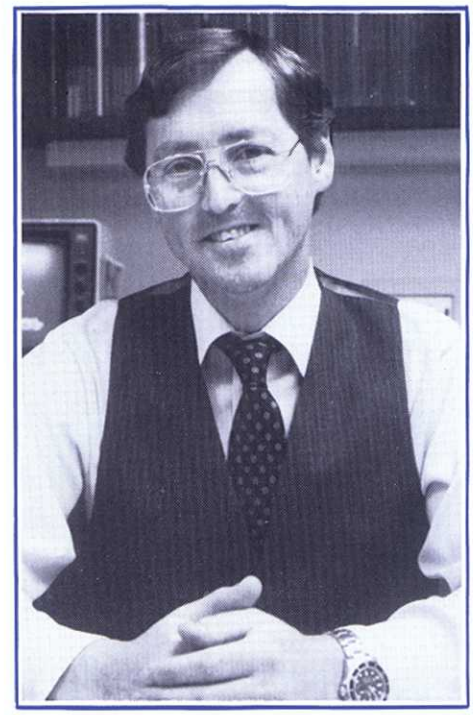
Abbreviations

α_1AT	α_1-antitrypsin
α_2-Macro	α_2-Macroglobulin
BUN	Blood urea nitrogen
HCT	Hematocrit
HGB	Hemoglobin
Hp	Haptoglobin
IEP	Immunoelectrophoresis
IFE	Immunofixation electrophoresis
Ig	Immunoglobulin
ESR	Erythrocyte Sedimentation Rate
TF	Transferrin

About The Author

Lawrence M. Killingsworth, Ph.D. has served as Director of the Clinical Chemistry and Immunology Laboratories in the Department of Laboratory Medicine at Sacred Heart Medical Center in Spokane, Washington since 1977. He also holds an appointment as Affiliate Assistant Professor in the Department of Laboratory Medicine at the University of Washington in Seattle.

He received his Bachelor of Science degree in Chemistry at Emory University in 1968. After spending two years in the U.S. Army, he attended the University of Florida where he was a National Institutes of Health Academic Pathology Fellow. He received his Ph.D. in Pathology (Clinical Chemistry) in 1973. From 1973 to 1977, Dr. Killingsworth was Associate Director of the Clinical Chemistry Laboratories at North Carolina Memorial Hospital and Assistant Professor of Medicine and Pathology at the University of North Carolina School of Medicine. During that time, and up until the present, he has continued his research into the application of electrophoretic and immunochemical techniques to problems in laboratory medicine and the study of protein physiology and pathophysiology.



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Dr. Killingsworth is a member of the American Association for Clinical Chemistry and serves as a member of the National Nominating Committee. He is certified as a Diplomate of the American Board of Clinical Chemistry. He is a fellow of the Association of Clinical Scientists and the National Academy of Clinical Biochemists, and is a member of the American Association for the Advancement of Science.

He served on the editorial board of Selected Methods in Clinical Chemistry from 1974 to 1983 and has been an invited reviewer of Clinical Chemistry since 1974. He serves as Chairman of the Board of Editors for Clinical Chemistry News, as associate editor of Diagnostic Immunology and as editorial advisor or reviewer for several other laboratory publications. He is a member of the Publications Board of the American Association for Clinical Chemistry. He organized and served as chairman of the AACC Specific Protein Laboratory Improvement program and served as a member of the Laboratory Improvement Program Management Group.

He lectures extensively on the subject of proteins, and he has published sixty-five papers, monographs and chapters. He is co-editor of the book "Proteins in Body Fluids, Amino Acids, and Tumor Markers." Although Dr. Killingsworth is best known for his contributions in the area of protein analysis and clinical interpretation, his professional interests cover the entire field of laboratory medicine. Dr. Killingsworth is an avid sailor in his spare time, and is editor/publisher of a regional boating newsletter called THE RED HERRING.

HIGH RESOLUTION PROTEIN ELECTROPHORESIS

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