Clinical Applications of Protein Determinations in Biological Fluids Other Than Blood

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Over the past decade, significant improvements in immunoochemical and electrophoretic techniques have enabled collection of heretofore unavailable data on proteins in biological fluids, greatly increasing our understanding of protein physiology in the various body compartments and providing the foundation for clinical use of protein analysis in body fluids. The most striking advance has been in the diagnosis of demyelinating disease through the use of serum/cerebrospinal fluid protein ratios and the morphological evaluation of immunoglobulin banding patterns. These laboratory tests are now considered obligatory for any patient in whom demyelinating disease is suspected as the cause of neurological dysfunction. Cerebrospinal fluid protein data can also be helpful in quantitating the permeability of the blood/cerebrospinal fluid barrier in many inflammatory or infectious central nervous system disorders. Assays of individual proteins in urine can help distinguish between different types of proteinuria, and can give quantitative data on the selectivity of the glomerulus and the reabsorbing capacity of the tubules. The protein content of saliva, synovial fluid, and milk has also been well characterized, and is clinically applicable to a wide range of disorders.


Cerebrospinal Fluid Proteins

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 (1). In addition to the fluid formed at this blood–CSF barrier, a small proportion is produced at other sites within the central nervous system (2).

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_2) species, owing to the fact that the blood–CSF barrier acts somewhat as a molecular sieve (3). 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 ventricular 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, sample N). 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_2, nonimmunoglobulin protein, γ-trace, which is perhaps synthesized within the central nervous system, but has undetermined clinical significance (4).

Most investigations of CSF proteins in neurological disease have concentrated on assessment of blood–CSF barrier permeability and abnormal protein production within the central nervous system (5). Many disorders with widely varying etiologies result in increases in permeability, and data on the

Fig. 1. Agarose gel electrophoresis patterns of cerebrospinal fluid

N: pattern from a patient without neurological disease; 1 through 6, patients with confirmed multiple sclerosis. The serum electrophoresis patterns of these latter patients showed no banding in the gamma region

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status of the blood–CSF barrier is usually pathophysiologic, rather than diagnostic (6). Detection of increased IgG synthesis in the central nervous system and the associated oligoclonal banding phenomenon, however, is valuable in the diagnosis of demyelinating disease (7–10). In addition, measurements of some proteins present in low concentrations in the cerebrospinal fluid show promise in monitoring the demyelinating process and in diagnosing central nervous system involvement in patients with leukemia and lymphoma (11, 12).

**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 bacterial, viral, and other forms of meningitis; neoplastic infiltration of the meninges; spinal and cerebral tumors; polyneuropathies; disk herniations; and cerebral infarctions (19–21). 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, but recent studies show that quantitation of a high-M₆ protein can be a more sensitive indicator of mild barrier disturbances (19). One drawback of this approach, however, is that large proteins—e.g., α₂-macroglobulin—are present in the CSF at very low concentrations and are therefore difficult to measure. Most quantitative immunochemical methods for specific proteins are also not easily adapted for emergency procedures. Until these methodological difficulties for specific proteins are overcome, CSF total protein will remain a valuable laboratory tool in some neurologic 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 (20–24). This multivariate 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. Calculations for the regression lines of ratio plots where \( y = \text{CSF/serum} \text{ IgG and } x = \text{CSF/serum} \text{ albumin} \) have been published by two groups of investigators. Britain et al. (21) reported \( y = 0.588x - 4.0 \times 10^{-4} \) with a standard error of estimate of \( 7.0 \times 10^{-4} \), and Reiber (24) published \( y = 0.414x + 1.4 \times 10^{-4} \) with a standard error of estimate of \( 5.0 \times 10^{-4} \). Data from other groups show similarities when presented in graphic form, but regression equations were not calculated (20, 22).

The most extensive studies on the nature of protein filtration and secretion at human body fluid barriers have been carried out by Felgenhauer et al. (3, 6, 25–27). They assessed permeability of the blood–CSF barrier by measuring several specific proteins in blood and CSF, and determined how the concentration ratios varied with the physical characteristics of the molecules. They found that hydrodynamic radius, i.e., the radius of the free diffusional volume, was the most important parameter in determining passage through the blood–CSF barrier. They further showed that for some high-M₆ proteins, the hydrodynamic radius did not directly correspond with molecular mass. They concluded that the barrier could be characterized by plotting the serum/CSF protein ratios on a log scale vs hydrodynamic volume. When this was done, the position of the “permeability line” on the ordinate provided information on the amount of protein passing through the barrier, and the slope of the line correlated with barrier selectivity. The normal blood–CSF barrier was found to have low selectivity when compared with the normal renal glomeruli and other body fluid barriers. They also showed that pathological conditions involving increased permeability were usually not accompanied by substantial changes in the selectivity of the blood–CSF interfaces (6, 27).

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.

**Abnormal Production of Protein within the Central Nervous System**

**Demyelinating diseases.** In 1948 Kabat et al. (28) measured proteins in CSF by the quantitative precipitin method and reported that eight of 14 patients with multiple sclerosis exhibited increased \( \gamma \)-globulin. This finding was not thought to be diagnostic of multiple sclerosis, because the study also showed 15 of 16 cases of neurosyphilis to have the same abnormality. These workers had, however, demonstrated increased immunologic activity in the central nervous system in demyelinating disease. Since then, numerous investigators have searched for the cause of this immunologic activity and for more sensitive and specific diagnostic tests for demyelinating diseases.

Quantitative immunochemical studies have shown that the increased \( \gamma \)-globulin content of CSF in multiple sclerosis is from synthesis of IgG within the central nervous system (13–15, 17, 29–34). The specific antigenic stimulant for this abnormal production of IgG has not been discovered, but mechanisms suggesting slow virus infection and autoimmunity reaction have been proposed (35). As with the early studies of Kabat et al., more recent investigations have found that 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 can lead to its increased immunologic activity and subsequent 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 (13–15, 17).

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 perme-
ability into account was to express CSF IgG as a percentage of CSF total protein (7, 13-15, 17, 30, 31, 33, 34). In one study, 88% of patients with multiple sclerosis gave “positive” results (17), while other investigators (7, 13, 15) reported less encouraging results, ranging from 56 to 75% of patients. One possible explanation for the discrepancy of results is that unanimity has not been reached on the upper limit of normal for the IgG/total protein ratio. In general, IgG 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. Tourtelotte et al. (29) investigated use of the ratio IgG/albumin in CSF, and found it to be as discriminative for multiple sclerosis as the ratio for IgG/total protein in CSF. Perry et al. (32, 36) reported that of 46 patients with a clinical diagnosis of multiple sclerosis, 74% had an abnormally high CSF IgG value relative to CSF albumin. Link and Tibbling (7) studied 59 patients with multiple sclerosis and found 80% of them to have an abnormal ratio for IgG/albumin in CSF. 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.

Ganrot and Laurell (20) showed that the diagnostic value of CSF protein measurements could be further enhanced if serum concentrations of protein were also taken into account. By measuring IgG and albumin in both fluids, any CSF abnormalities resulting from increases or decreases in the serum proteins could be accounted for. A graphic presentation of these protein ratios allows for discrimination between patients without protein abnormalities and those with barrier disturbances (as discussed in the previous section), those with increased central nervous system production of IgG, and those with combined disorders. Other groups have used this dual-ratio approach, and some have published reference values for the coefficient of the two ratios in healthy adults (7, 21-24, 37, 38). The mean values and ranges for this coefficient are given in Table 1. The mean values for the coefficient show remarkable similarity, considering the fact that different analytical methods were used and reference populations from three separate countries were studied. In the most extensive study, Link and Tibbling (7, 22, 23) found that 86% of patients with multiple sclerosis gave values above their reference range for the ratio coefficient. Brittain et al. (21) reported that eight of nine multiple sclerosis patients had increased ratio coefficients.

Tourtelotte (39) has proposed further manipulation of serum and CSF IgG and albumin data in a formula to estimate the rate of IgG synthesis within the central nervous system. His 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. The constants take into account such factors as the average normal rate of CSF formation, the average normal concentrations of IgG and albumin in serum and CSF, and the molecular mass ratio of albumin to IgG. The equation, in effect, factors out all sources of CSF IgG except de novo synthesis, and expresses the results as milligrams of IgG synthesized per day. Perhaps the major advantage of this approach is that results expressed this way are more easily understood than the dimensionless ratio coefficients published by others (27, 22, 37, 38). However, the analytical measurements are the same in both cases, and the synthetic rate expression is obtained simply by multiplying the basic data by several constants.

In 1970, Laterre et al. (40) discovered significant abnormalities in the electrophoretic morphology of the γ-globulin fraction in patients with multiple sclerosis and other diseases of the nervous system. They described the appearance of multiple, restricted bands in the γ-region on agar gel electrophoresis. This phenomenon, since then designated “oligoclonal banding,” can be detected only by high-resolution, high-sensitivity electrophoresis. It can appear in various forms, ranging from a few faint bands to many very intense bands (Figure 1, patients 1 through 6). Oligoclonal banding patterns are present in a very high percentage of patients with multiple sclerosis (8–10, 41–49). 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. Demonstration of oligoclonal banding, despite its importance as a diagnostic tool, is not useful as a prognostic indicator, the intensity of the pattern apparently not correlating with the subsequent course of the disease.

As with quantitative IgG measurements, the oligoclonal pattern is not pathognomonic for multiple sclerosis. The original report of Laterre et al. (40) showed that inflammatory and infectious processes as well as some other central nervous system disorders could be associated with oligoclonal banding. They detected oligoclonal banding in all their patients with subacute sclerosing panencephalitis, in 53% of their cases of neurosyphilis, in 35% of their cases of viral encephalitis or meningoencephalitis, and in 33% of their 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 increased immunologic activity of the central nervous system.

The limitations of diagnosing and monitoring demyelinating diseases through CSF immunoglobulin quantitation or electrophoresis prompted some investigators to search for a laboratory index of active demyelination (11, 49, 50). Given that myelin fragments were known to be present in the CSF of most patients with active multiple sclerosis, an assay was developed to measure one of its components, myelin basic protein. Cohen et al. (11) showed that patients with active demyelinating processes had high concentrations of this protein (17–100 μg/L), whereas patients with slowly progressive multiple sclerosis had intermediate values (6–16 μg/L), and those in remission had low values (<4 μg/L). They also showed that of 252 patients with nondemyelinating neurologic disease, only two were positive for myelin basic protein. The extremely low incidence of false-positive results

| Table 1. Coefficients * for CSF and Serum Protein Ratios in Healthy Adults |
|-----------------|-----------------|---------------|-----------------|
| Mean # | Range # | No. patients | Reference |
| 0.52 | 0.20–0.84 | 43 | 37, 38 |
| 0.50 | 0.34–0.66 | 47 | 21 |
| 0.46 | 0.34–0.58 | 93 | 22 |
| 0.51 | 0.25–0.80 | 36 | b |

* Coefficient = CSF IgG/serum IgG / CSF albumin/serum albumin

b Data from recent studies in the author’s laboratory on patients with no evidence of neurological disease.
indicates a high degree of clinical specificity for this test. The fact that myelin basic protein is positive only during periods of active demyelination, however, is both an asset and a deficiency. Even though the test might be used to monitor, and perhaps even quantitate, disease activity, its utility as a diagnostic tool is limited by the fact that a positive result can be expected only during an acute exacerbation. Immunoglobulin evaluation, though not useful in monitoring demyelination, would seem to be more appropriate in detecting a disease characterized by unpredictable periods of remission and exacerbation.

Leukemia and lymphoma of the central nervous system. Recently, β2-microglobulin has been studied as a tumor marker in myeloproliferative and lymphoproliferative disorders (51, 52). Increases of this protein are thought to reflect the rapid cellular turnover associated with these neoplastic conditions. Reports of its utility in monitoring cancerous and other disease states has led one group to study β2-microglobulin in CSF and serum as an indicator of central nervous system involvement in patients with acute leukemia and lymphoma (12). They showed that when serum and CSF were measured for β2-microglobulin simultaneously, patients with central nervous system involvement had a greater concentration of this protein in CSF than in serum. Their study also suggested that concentrations of β2-microglobulin in CSF could be used to follow the clinical course of central nervous system involvement in leukemia and lymphoma.

Urinary Proteins of Plasma Origin

Filtration of plasma across the glomerular capillary membrane, the initial event in the formation of urine, 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 by the renal tubules. Normal urinary protein excretion, therefore, is less than 150 mg/day (53-59). Two-thirds of this is made up of filtered plasma proteins—primarily albumin, low-Mr species, and immunoglobulin components. The remainder is derived from the urinary tract itself (60).

Immunochemical methods have allowed detection of numerous plasma proteins in normal urine (61-68). The extensive investigations of Hemmingsen and Skaerup (67) showed a wide and uneven distribution of urinary protein excretion in healthy individuals, characterized by many points clustered at low quantities, with much “tailing” of data toward higher values. They also found large physiologic day-to-day variations for excretion of several proteins in a healthy subject. This finding is in striking contrast to the small physiologic variations reported for proteins in plasma (69-71).

The lack of adequate reference intervals for specific urine protein components has limited most investigators to studies of clear-cut 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. Figure 2 gives a description of how proteins are normally handled by the kidney and illustrates two common mechanisms of proteinuria involving proteins of plasma origin.

Proteinuria in renal disease can be classified as resulting from either glomerular or tubular dysfunction. Glomerular proteinuria results from increased transcapillary passage of proteins through the glomerulus and is characterized by the loss of plasma proteins the size of albumin or larger (54, 56-58, 72-80). Tubular proteinuria is caused by a decreased capacity of the tubules to reabsorb proteins. With normal glomerular function, the glomerular filtrate contains high concentrations of low-Mr proteins to be reabsorbed by the tubules. Thus,
impaired tubular function causes increased excretion of these very small proteins (54, 56–58, 72, 76, 81–89).

Certain systemic conditions, some of which are considered benign, others pathologic, can lead to increased urinary excretion of protein. These conditions include exercise proteinuria, postural proteinuria, proteinuria of pregnancy, febrile proteinuria, and overflow proteinuria (54, 56–58, 64, 72, 80–94).

Glomerular Proteinuria

Several pathologic processes can cause glomerular injury resulting in proteinuria (57). Heavy urinary protein loss may be associated with immune complex diseases such as systemic lupus erythematosus, poststreptococcal glomerulonephritis, and membranous glomerulonephritis. The deposition of abnormal substances such as occurs in amyloidosis or diabetic glomerulosclerosis can increase glomerular permeability. Proteinuria is also observed in chronic pyelonephritis, some cardiovascular diseases, and some congenital renal abnormalities. Severe protein loss can occur with some renal diseases of unknown etiology, e.g., lipoid nephrosis and idiopathic nephrotic syndrome.

The renal glomeruli, which function as ultrafilters, are considered to be membranes interrupted by pores of fixed dimensions that allow passage of certain molecular species and retention of others (57, 72). Increased glomerular permeability, often associated with the nephrotic syndrome, results in increased urinary excretion of proteins such as albumin, transferrin, and the acute-phase reactants α1-antitrypsin and α1-acid glycoprotein (Figure 2). These proteins are present in relatively high concentrations in the plasma, but are normally retained by the glomerulus. Very large proteins such as α2-macroglobulin and β-lipoprotein are not found in the urine in appreciable amounts because the glomerulus maintains some selectivity. The very low-Mr proteins that pass through the normal glomerulus are also absent in the early stages of glomerular disease, before tubular reabsorptive capacity is compromised.

Quantitative assessment of glomerular function is possible through measurement of the renal clearance of proteins having different molecular sizes (73–75, 77, 79, 80). When relative protein clearance values are plotted vs molecular mass on a double-log scale, least-squares regression analysis produces a linear relationship, although there is often a considerable scatter of points. The slope of this line, designated the "selectivity index," has been used to characterize the selectivity of the glomerulus to protein excretion. Although these determinations have limited usefulness in the classification of disease entities, several studies have suggested that patients with selective nephrotic syndrome (slope >67°) generally have a better prognosis and response to steroid therapy than do patients with nonselective protein loss (73–75, 77).

The limitations of the selectivity index were pointed out by Hofer et al. (80), who reported little prognostic value for selectivity data in their studies of glomerulonephritis. More recent studies show that the amount of a given protein in urine is not directly proportional to its molecular mass alone (57, 68). Other factors potentially influencing protein passage through the glomerulus include molecular charge, hydrodynamic radius of the protein, viscous drag, and protein–protein binding, as well as the charge of the glomerular membrane and the glomerular filtration rate.

Even though the selectivity index is not helpful in all types of glomerular disease, Ellis and Buffone (95) showed that measurement of individual urinary proteins and selectivity determinations were useful in predicting the histopathology of the renal lesion and the prognosis in pediatric patients. They measured the renal excretion and clearance of albumin, transferrin, IgG, and α2-macroglobulin in children with idiopathic nephrotic syndrome. Statistical evaluation of the data demonstrated significant differences between patients with minimal change disease, focal glomerular sclerosis, and membranoproliferative glomerulonephritis.

Tubular Proteinuria

In 1950, Friberg (81) showed increased amounts of low-Mr proteins in the urine of workers exposed to cadmium dust for long periods. Butler and Flynn (82) reported in 1958 that the type of proteinuria found in patients with renal tubular dysfunction is markedly different from the proteinuria associated with glomerular damage. These findings have been confirmed in other studies, which have demonstrated that the urine proteins of patients with renal tubular diseases have low sedimentation coefficients and their electrophoretic mobilities are mainly in the α2- and β-regions (83–86). Revillard et al. (85) found a strong correlation between the presence of tubular-type proteinuria, as defined by its biochemical characteristics, and the presence of tubular or interstitial disorders, as defined histologically or by other biologic evidence.

As with glomerular disease, many renal disorders can result in tubular proteinuria. They include, in addition to cadmium toxicity, Fanconi syndrome, cystinosis, Wilson's disease, sarcoidosis, tubular acidosis, Balkan nephropathy, medullary cystic disease, pyelonephritis, renal transplantation, and aminoglycoside toxicity (57, 96, 97). There are two theories of the physiological mechanisms that result in the increased excretion of low-Mr proteins in tubular disease. One is based on the premise that low-Mr proteins are filtered by the normal glomerulus to a greater extent than proteins the size of albumin or greater. The low-Mr proteins are thus available for tubular reabsorption, by a nonselective competitive mechanism, in greater amounts than the larger proteins. Any decrease in the capacity of the tubules to reabsorb proteins, therefore, would be reflected to a greater extent in the increased excretion of the small proteins (98). The second approach (76) proposes that the smaller proteins are preferentially reabsorbed by the tubules and thus show larger urinary increases when tubular capacity is damaged. This theory is based on the observation that the urinary ratios of albumin to β2-microglobulin are much lower in tubular proteinuria than in normal urine. In addition, some patients with tubular proteinuria show marked increases in β2-microglobulin excretion with normal albumin excretion. The bottom section of Figure 2 depicts a typical pattern of tubular proteinuria.

The urine protein most often studied in tubular proteinuria is β2-microglobulin. This small protein (Mr 11,800), first described in 1968 by Berggård and Bearn (99), exhibits striking structural homogeneity with regions of the light and heavy chains of immunoglobulins. The urinary excretion rate of β2-microglobulin is about 100 μg/24 h; concentrations of β2-microglobulin in serum of adults range from 0.8 to 2.4 mg/L (100). The biologic function of β2-microglobulin is unknown, but its small size makes it suitable for studies of tubular reabsorptive function (76, 87–89, 96, 97).

Peterson et al. (76) suggested that quantitative determinations of urinary β2-microglobulin and urinary albumin could be useful in differentiating between glomerular and tubular proteinuria. They pointed out, however, that mixed glomerular/tubular types are observed in patients with chronic renal failure and chronic pyelonephritis. Wibell and Evrin (88) postulated a saturation point for tubular reabsorption of β2-microglobulin, when its concentration in serum is about 4.5 mg/L. They suggested that, at serum concentrations less than this, measurement of urinary β2-microglobulin is probably a good estimator of tubular reabsorption. Other investigators (88) have suggested that β2-microglobulin might also
be eliminated by an extraglomerular mechanism, possibly by direct uptake from peritubular vessels.

Ricanti and Hall (87) studied concentrations of \( \beta_2 \)-microglobulin in serum in the anuric phase after renal transplantation. They concluded that a progressive decrease in \( \beta_2 \)-microglobulin concentration in serum during this period suggested a viable graft, even in the absence of excretory renal function. Woo et al. (97) confirmed these findings recently, and suggested that measurement of \( \beta_2 \)-microglobulin in serum could be valuable in predicting acute rejection. They concluded that determination of daily clearances of both albumin and \( \beta_2 \)-microglobulin is an effective means of differentiating between glomerular and tubular dysfunction in patients recovering from renal allograft transplantation.

The tubular nephrotoxicity of aminoglycoside antibiotics is well known. The standard renal-function tests such as serum creatinine or creatinine clearance, however, do not directly assess tubular compromise. Schentag et al. (96) studied urinary \( \beta_2 \)-microglobulin excretion in patients who were being treated with aminoglycosides. They demonstrated increases above baseline values for all patients. Those patients who developed nephrotoxicity secondary to the treatment generally showed greater increases than patients without toxicity, and their excretion of \( \beta_2 \)-microglobulin increased five days before the serum creatinine did. Kaye et al. (101) reported similar results in a group of 18 patients receiving gentamicin therapy. These studies indicate that \( \beta_2 \)-microglobulin excretion is a more sensitive test for tubular damage than conventional kidney-function tests in aminoglycoside therapy, but leaves open the question of interpreting increased excretion rates in patients who have no other evidence of renal damage.

Other Conditions Associated with Increased Urinary Excretion of Proteins

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 (64, 90, 91). Poortmans (91) suggests that exercise proteinuria is mainly the result of increased glomerular permeability combined with saturation or inhibition of tubular reabsorption capacity by some unknown mechanism.

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 (92). 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.

Most commonly, the proteinuria seen in pregnant women is transitory and does not indicate renal disease in the usual sense of the term (93). 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.

Jensen and Henriksen (94) studied the urinary excretion of specific proteins in patients with various nonrenal infectious diseases. They found abnormal urine protein excretion in almost all patients and postulated that "febrile proteinuria" results from temporary immunologic injury to the glomerular basement membrane caused by deposition of antigen-antibody complexes.

Increased concentrations in plasma of low-\( M_r \) proteins will filter through the glomerulus in abnormal amounts, leading to a condition known as "overflow" proteinuria. Bence Jones proteinuria is a classic example of this type, although myoglobin and hemoglobin are also excreted in this manner (54, 56, 58). 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.

**Saliva**

Whole saliva contains a wide variety of proteins that function either in the digestive process or in maintaining the teeth and oral soft tissues. Some of these proteins are synthesized within the salivary glands, others originate in the plasma. There are substantial differences in the protein content and composition of saliva from the parotid, submandibular, and minor salivary glands, so care must be taken in collecting saliva and in interpreting the results of protein analysis (102, 103). The mean total protein content of parotid saliva is 2.5 g/L, while submandibular saliva contains less protein, about 1.5 g/L (103). Figure 3 shows a two-dimensional immunoelectrophoresis pattern of proteins in whole saliva from an adult.

Local diseases of the salivary glands can affect the concentration of some proteins in saliva. Extensive studies have been carried out on chronic recurrent sialadenitis, a disease which attacks primarily the parotid gland. An acute exacerbation of this disease causes increases in albumin, immunoglobulins, transferrin, lysozyme, and lactoferrin (104, 105). Most of this is from leakage of proteins from the plasma, but there also appears to be local synthesis of immunoglobulins and lactoferrin. In addition, significant amounts of lactoferrin and lysozyme are probably released by degranulation of the polymorphonuclear leukocytes associated with the local inflammation. As the inflammation resolves, the integrity of the blood–saliva permeability barrier returns toward normal and protein concentration in the saliva decreases dramatically. Concentrations of albumin and lactoferrin may remain slightly above normal for a long time, however, and can be used to monitor the chronic nature of the disease.

Systemic disorders affecting the salivary glands can lead to abnormalities in salivary proteins. Parotid saliva from patients with Sjögren's syndrome shows a slight increase in IgA, with more significant increases in \( \beta_2 \)-microglobulin and lactoferrin (105–110). The increase in IgA is thought to reflect the decreased salivary flow rate. The concentrations of \( \beta_2 \)-microglobulin correlate with the degree of lymphocytic infiltration, whereas the increased lactoferrin concentration is characteristic of the inflammatory process.

Parotid saliva has been examined in an effort to document salivary gland involvement in sarcoidosis (111–113). With

![Fig. 3. Two-dimensional immunoelectrophoresis pattern of proteins in whole saliva from an adult](https://example.com/figure3.png)

Electrophoresis was performed in the horizontal dimension at 25 V/cm for 45 min. In the vertical dimension, the electrophoresis was performed at 3 V/cm for 17 h into a gel containing goat antibodies to whole human serum, 100 ml/L.

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sarcoid involvement of the parotid glands, flow rate and amylase activity are markedly decreased. Albumin and lysozyme are increased, probably as a result of increased blood-saliva barrier permeability. Amylase and albumin return to normal with therapy, but lysozyme remains increased.

Saliva is a convenient sample for evaluating patients with possible immunodeficiency disorders involving the external secretory system (114–116). The presence or absence of secretory IgA can easily be determined on a sample of whole saliva, but precise quantitation should be done only on parotid saliva. Flow rate should also be determined in rigorous studies because it affects IgA concentration.

Other systemic conditions in which salivary proteins have been quantitated include cystic fibrosis, diabetes, pancreatitis, and cirrhosis. Changes in the concentration of some salivary proteins can be demonstrated in these disorders, but the clinical significance of the changes has not been firmly established.

Synovial Fluid

The high viscosity of synovial fluid, because of the presence of the polysaccharide joint-lubricant hyaluronic acid, has presented some analytical difficulties in studying its protein content. The hyaluronic acid can be depolymerized by treatment with hyaluronidase, thus allowing for routine protein analysis by electrophoretic and immunochemical techniques.

The total amount of protein in normal synovial fluid is approximately 20 g/L, and the electrophoretic pattern is similar to that of serum. The major proteins of clinical interest include the immunoglobulins and complement components, though many others have been identified and quantitated (117–119). Interpretation of abnormalities in synovial fluid proteins must take into account both the concentration of these proteins in serum and the permeability of the synovial membrane. Two common approaches to normalization for these factors are to calculate fluid/serum protein ratios or to express synovial fluid protein results as a percentage of the synovial fluid total protein (120–124).

Investigations of specific synovial fluid proteins have largely concentrated on inflammatory joint diseases. Attempts have been made to correlate protein concentrations with specific arthropathies, primarily rheumatoid arthritis. Patients with active rheumatoid arthritis have increased immunoglobulins and decreased complement in the synovial fluid of affected joints (121–123, 125–127). These findings do not, however, correlate with the stage or class of the disease or with the type of medication. Decreased concentrations of complement in synovial fluid are also seen in systemic lupus erythematosus and bacterial joint infections. Normal amounts of complement components are found in most other, nonrheumatoid forms of synovitis (120, 128).

Milk and Colostrum

Human colostrum and milk contain proteins that serve two major functions for the newborn. The caseins, α-lactalbumin, several enzymes, and carrier proteins provide necessary nutrients, while immunoglobulins, lactoferrin, and lysozyme contribute to the infant’s awakening immune-defense system (129–135).

Protein content is highest in colostrum (15–68 g/L) and decreases progressively with transitional milk (13–19 g/L) and mature milk (7–11 g/L) (129, 136). In addition to many proteins of plasma origin, human milk contains high concentrations of the various milk-specific proteins, thus making for significant differences between the electrophoretic patterns of milk and serum (137–139).

The casein fraction of human milk consists of a complex mixture of several polymorphic proteins whose major role is to provide nutrition (140, 141). The major whey protein, α-lactalbumin, is an important source of essential amino acids and also participates in lactose biosynthesis (142). Attempts to use concentrations of these two proteins in serum as tumor markers in breast cancer have not proved successful (143–147).

The most abundant immunoglobulin in human milk is secretory IgA, which functions to protect the infant from enteric infections and to prevent the passage of bacterial pathogens and other antigens from the gastrointestinal tract into the blood (148, 149). Nonspecific immunity is conferred by lactoferrin, which strongly binds iron and acts as a bacteriostatic agent, and lysozyme, which also exhibits antibacterial properties (150–153). (Ed. note: see also a review of this topic in our April 1982 special issue.)

Other Fluids

Other body fluids currently being studied include seminal plasma, vaginal secretions, lymph, intravascular fluid, pleural fluid, pericardial fluid, and ascites. Even though much progress has been made recently in characterizing the protein content of many of these fluids, clinical applications of the data have been minimal. Information on fluid protein metabolism may prove to be very useful, however, as we penetrate—diagnostically and therapeutically—all the body compartments.

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