ELSEVIER

Journal of Chromatography A, 698 (1995) 163–179

JOURNAL OF CHROMATOGRAPHY A

Review

# Clinical applications of two-dimensional electrophoresis

Donald S. Young[a,*], Russell P. Tracy[b]

[a]*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104-4283, USA*
[b]*Department of Pathology, University of Vermont, Colchester, VT 05446, USA*

**Abstract**

Two-dimensional electrophoresis is increasingly being used as an important tool for biological research although it continues to have few direct clinical applications. In the absence of simple systems to identify and quantify individual proteins or groups of proteins it is unlikely that clinical applications will increase. Measurement of some individual proteins, for example a single acute phase reactant, often yields as much clinically useful information as could be currently expected from quantitation of several proteins with the same physiological role. Cost-containment pressures within the clinical laboratory will prevent the technique from becoming widely used in the clinical laboratory until it can clearly demonstrate that it can produce clinically important and necessary information that can not be obtained by other means.

We continue to believe that the technique's greatest potential lies in identifying a protein or proteins whose concentration can be correlated with a disease and whose concentration varies with the progress of the disease. Antibodies to such proteins can then be produced and used to quantify the disease-associated proteins by a simple procedure, such as nephelometry. In spite of our belief of the likely clinical application of the technique there appears to be no systematic use of two-dimensional electrophoresis for this purpose. With clinical specimens a few investigators still run gels of serum or urine from patients with apparently unusual disorders and compare them visually with gels from healthy individuals. Nevertheless, the technique continues to have considerable unmet promise for clinical applications.

## Contents



* Corresponding author.

0021-9673/95/$29.00 
*SSDI* 0021-9673(94)00988-0

## 1. Introduction

This review is primarily concerned with papers published over the past three years which concentrated on the analysis of human-based samples by two-dimensional (2D) electrophoresis. Where pertinent, some earlier papers are also considered. Papers have been evaluated where the technique of 2D electrophoresis either has direct clinical application for diagnosis or management of human disease or where the technique has been used to identify or isolate proteins that may have such application, either as the proteins themselves or as a tool to develop antibodies that have clinical application.

We believe that 2D electrophoresis is, and will be in the near future, of greatest value in identifying proteins against which specific antibodies may be produced and which may be subsequently incorporated into simple enzyme-linked immunosorbent assays (ELISAs), allowing analyses to be performed rapidly and precisely in the clinical laboratory. However, while techniques have been published and a few examples are available in the literature this application remains remarkably underutilized.

The extraordinary resolution inherent in 2D electrophoresis not only allows us to focus on individual proteins, but also with the proper computer-assisted visualization instrumentation, enables us to observe patterns in the way cells and tissues respond to disease. However, little progress has been made in either cataloging or understanding the meaning of patterns. Therefore, the orientation of this review will be on individual gene products and their relationship to disease. In the future, it seems likely that as more and more sophisticated computerized scanning devices are developed, the analytical emphasis may shift from individual protein "spots" to coordinated patterns of protein changes, shedding light not only on the clinical picture, but also on the basic molecular pathobiology associated with each disease state.

This review is divided into sections dealing with the major body fluids and their components, followed by sections on solid tissues and cultures of individual cell systems. Trends in

technology are briefly summarized, as is the use of computers to develop pertinent databases for the interpretation of protein patterns.

## 2. Body fluids

2D Electrophoresis has been used to study individual components of many body fluids and solid tissues. Methods have been developed to prepare proteins for analysis in most body fluids and tissues. Selection of the appropriate fluid or tissue for analysis is typically determined by prior knowledge of association of proteins in it with disease.

### 2.1. Blood cells

#### 2.1.1. Erythrocytes

2D Electrophoresis has been used by Gaczynska [1] to study susceptibility of erythrocyte membrane proteins to proteolysis. He demonstrated that spectrin and band 3 protein from membranes from the youngest cells were significantly more slowly degraded than proteins from middle-aged cell and the oldest cell membranes. Connor et al. [2] have used 2D electrophoresis to show that while Rh-null erythrocytes are immunologically distinct, they possess an Rh-like protein that is structurally very similar to Rh(D) and Rh(c).

#### 2.1.2. Leukocytes

2D Electrophoresis has been used to demonstrate the presence of a cytosolic polypeptide, designated L4, in cells of patients with common acute lymphoblastic leukemia [3]. Only 4 of 64 patients with acute lymphoblastic leukemia did not show the presence of this protein. The L4 polypeptide was absent from normal peripheral B and T lymphocytes, lymphocytes isolated from thymus tissue, phytohemagglutin (PHA)-transformed T cells, Epstein Barr Virus (EBV)-transformed B cells, chronic lymphocytic leukemia and hairy cell leukemia. It was also absent from neutrophils, monocytes and platelets. Clancy et al. [4] have used 2D electrophoresis to demonstrate that nitric oxide stimulates ADP-ribosylation of two distinct substrates, one of which was actin, in polymorphonuclear cells.

Protein synthesis has been analyzed in patients with chronic lymphocytic leukemia (CLL) using 2D electrophoresis and $^{14}$C-labelled proteins [5]. The degree of expression of certain proteins appeared to correlate with the stage of the disease. There were marked differences between the leukemic cells and non-malignant cells.

#### 2.1.3. Monocytes/macrophages

Using 2D electrophoresis Kantengwa and Polla [6] were able to show that in human monocytes/macrophages the stress of exposure to *Staphylococcus aureus* caused a threefold increase in superoxide dismutase activity, selective and differentiation-induced induction of heat shock protein and de novo synthesis of heme oxygenase.

#### 2.1.4. Lymphocytes

Schick and Levy [7] used 2D gel electrophoresis to confirm that in B cells the transmembrane protein TAPA-1 is associated with the CD19 antigen and to establish an association with HLA-DR on the surface of B cells. Harada et al. [8] used 2D electrophoresis to analyze the surface glycoproteins of human natural killer (NK) cells to demonstrate a glycoprotein with a $M_r$ of 65 000 that is absent from T lymphocytes, although the significance of this has still to be determined. High-resolution 2D electrophoresis of the proteins from lymphocytes demonstrated an abnormality of actin in patients with either Alzheimer's disease or Down's syndrome [9]. The patients exhibited a double actin spot instead of the single spot characteristic of healthy controls. This finding supports the hypothesis that Alzheimer's disease is a systemic as well as a cerebral disease.

Analysis of the polypeptides of B cell lymphocytes has shown five distinct polypeptides in hairy cell leukemia which were absent from most chronic lymphocytic leukemia and normal B lymphocytes [10]. Two of the polypeptides were unique to hairy cell leukemia. Thus 2D electrophoresis of the proteins of B cells may be used to distinguish between hairy cell leukemia, chronic

lymphocytic leukemia and splenic lymphoma with villous lymphocytes.

*2.1.5. Platelets*

Hagmann and Burger [11] used 2D electrophoresis to show that when platelets attach and spread on a solid surface the $\alpha$ isoforms of the protein vinculin, a cytoskeletal protein believed to be involved in the linking of microfilaments to the cell membrane, become phosphorylated. This process is probably important in anchoring vinculin at sites of microfilament–membrane interaction. 2D Electrophoresis has been used to demonstrate an $M_r$ 28 000 polypeptide in most peripheral blood cells, including platelets, which appears to be useful as a genetic marker in gene mapping [12].

*2.2. Plasma and serum*

In 1991 Marshall and Williams [13] reviewed the applications of a simplified technique for performing 2D polyacrylamide electrophoresis in health and disease. Much of the work in this area has centered on the analysis of immunoglobulins. This is logical, since clonality issues are a major clinical concern, and the high resolution of 2D electrophoresis makes it ideal for this purpose. The technique has been used to detect and characterize the abnormal proteins observed in serum of patients with multiple myeloma and Waldenstrom's macroglobulinemia [14,15]. In both diseases the immunoglobulin heavy chains showed marked heterogeneity affecting both charge and molecular size. When non-denaturing isoelectric focusing was used less heterogeneity was observed. Goldfarb [16] has analyzed sera from 24 patients with monoclonal gammopathy in whom zone electrophoresis and immunofixation appeared to show a homogeneous immunoglobulin M component. Analysis by 2D electrophoresis showed that only five specimens had a true monoclonal pattern. When she studied patients with immunoglobulin G gammopathy she noticed that several had considerable amounts of $\gamma$ heavy chain at a reduced size ($M_r$ 34 000).

2D Electrophoresis has been used by Wiederkehr et al. [17] to study circulating immune complexes; they determined that it could be a valuable tool to determine the protein composition of the complex.

Tissot et al. [18] have recently reported on their 5-year experience of using 2D electrophoresis to assess the clonality of immunoglobulins. They noted that the majority of the monoclonal immunoglobulin light chains of monoclonal gammopathy detected by conventional electrophoresis appeared as a single large well-defined spot on 2D polyacrylamide gel electrophoresis (PAGE), with the remainder appearing as multiple spots. Oligoclonal immunoglobulins appeared as multiple spots on PAGE and several bands with immunofixation. This was observed in 5 of 26 patients after allogeneic bone marrow transplantation and in 5 patients with tumors. In most patients (77%) with hypergammaglobulinemia, light chains appeared as many small and discrete spots in 2D-PAGE. The authors suggest that oligoclonal Ig-secreting B cell clone expansion, and corresponding abnormalities are not detected by immunofixation. 2D Electrophoresis was able to detect oligoclonal immunoglobulin expansions in other patients after bone marrow transplantation and in 6 of 10 immunocompetent patients with acute severe infections when conventional electrophoresis failed to show any abnormality.

Harrison [19] surveyed the distribution and types of monoclonal light chain isoforms in 41 patients with monoclonal gammopathy. He showed that the light chain patterns were patient-specific and varied in both the type and number of isoforms present. Microheterogeneity was observed in 31 of 41 cases. Determination of light-chain heterogeneity has proved useful in resolving some diagnostic immunoglobulin typing questions and in the understanding of the molecular basis of neoplastic B-cell paraprotein metabolism. Harrison et al. [20] also used 2D electrophoresis to study B cell dyscrasias including the first reported case of POEMS (Takatsuki) syndrome comprising *p*olyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *m*yeloma or M-protein, and *s*kin changes.

Serum protein electrophoresis of specimens in patients 5–9 months after bone marrow trans-

plantation revealed L chain patterns in 8 of 19 patients that were similar to those of normal polyclonal immunoglobulins [21]. Sequential analysis of specimens from patients following bone marrow transplantation showed that imbalanced clonal reconstitution was transient, although clonal gammopathies are common after bone marrow transplantation.

In serum of patients suffering from alcohol abuse abnormal heterogeneity was observed in the pattern for $\alpha_1$-antitrypsin [22]. The concentrations of several unidentified proteins were augmented. Abstinence from alcohol caused the abnormal protein pattern to revert to normal.

2D Electrophoresis has recently been used to resolve apolipoprotein A-I-containing high-density lipoprotein (HDL) into 12 distinct subpopulations. These subpopulations were comprised of groups that had mobilities the same as albumin but also others that were faster and slower. These groups were described as pre-$\alpha$, $\alpha$ and pre-$\beta$. Nevertheless, ultracentrifugally isolated lipoproteins are different from the native apo A-I-containing subpopulations [23]. The major differences are in the loss of pre-$\beta_1$ and pre-$\beta_2$ particles from the density $< 1.21$ g/ml fractions to the density $> 1.21$ g/ml fractions. In patients with "fish-eye" disease, a rare familial dyslipoproteinemia associated with corneal opacities and poor vision, abnormal amounts of the apolipoprotein A-I precursor were present in serum but reduced amounts of the mature apolipoprotein A-I isoforms and apolipoprotein A-II were observed [24]. Miida et al. [25] used 2D electrophoresis to demonstrate that blood cells are incapable of maintaining plasma pre-$\beta_1$ HDL concentrations in the absence of peripheral cells. The reverse cholesterol transport is catalyzed by pre-$\beta_1$ HDL, with subsequent lecithin–cholesterol acyltransferase mediated cholesterol esterification, and is directly dependent upon the interaction of pre-$\beta_1$ HDL and competent peripheral blood cells.

In 4 of 16 patients with late stage diabetes mellitus, but in none of the 16 normal sera studied at the same time, a novel $M_r$ 30 000 protein, that appears to be related to chymotrypsinogen, was identified. This protein appeared to be an autoantigen in some late-stage insulin-dependent diabetes mellitus patients [26].

After a myocardial infarction the amount of several low-molecular-mass proteins was shown to increase in serum. These proteins appeared within 30 h of an infarction, peaked at about 50 h, and returned to normal within 5 days [27]. Co-electrophoresis of proteins extracted from human myocardium and serum from patients with myocardial infarcts suggested that one of the spots might be a myosin light chain from the heart and others of lower molecular mass were probably isoforms of apo-serum amyloid A protein, a non-specific acute phase reactant [28].

Guy-Crotte et al. [29] found that screening of serum to detect cystic fibrosis protein (CFP) was of little value, although in most sera from patients with the disease a protein of $M_r \approx 12\,000$ (P12) was quite characteristic. CFP was present in the sera of about 75% patients with cystic fibrosis and in 66% carriers of the disease, often in lesser amounts. It was present in trace amounts in about 20% sera from healthy individuals. The P12 protein was detected in 69% homozygotes, 65% heterozygotes and 33% healthy individuals. The CFP has been detected in granulocytes from which a monoclonal antibody has been developed, permitting a quantitative immunoassay to be devised with probably greater potential as a diagnostic tool for cystic fibrosis than 2D electrophoresis [30].

Miyano et al. [31] have studied complement components in the cord blood of 128 newborn infants. They observed that the concentrations of $C_3$, $C_4$, $C_{3d3}$, $CH_{50}$ and factor B were reduced in the 28 infants with respiratory distress syndrome compared with infants with other lung diseases or normal respiratory function at the same gestational age. The authors commented that lung surfactant is synthesized in alveolar type 2 cells, the site of synthesis of many complement components, and that common factors may regulate the synthesis of both complement components and surfactant.

Ultra-thin-layer polyacrylamide gel isoelectric focusing has been used to study isoforms of lactate dehydrogenase in human serum to determine their potential use as tumor markers

[32]. The enzyme yielded 15 bands in serum and 20 bands were detected in hemolysates of erythrocytes. The authors claimed that potential tumor markers were detected in the cytosols of different tumors. Abnormal bands were present in the cytosols of cells from hepatoma and intestinal cancers. These were not present in the cells from healthy organs or in the plasma or serum of patients with the malignant diseases. Whether increasing the sensitivity of the detection system will enable the abnormal proteins to be detected in serum has yet to be determined.

The clonality pattern of immunoglobulin light chains in the plasma of healthy newborns was shown to be similar to that of their mothers [33]. Analysis of specimens from infants aged 1 month to 5 years revealed discrete alterations of the clonality of immunoglobulin production. Between the second and fourth month of life several well-resolved immunoglobulin light chain spots appeared, but in infants older than 2 years the pattern evolved towards the normal adult polyclonal pattern which was evident at 5 years. Analysis of fetal blood plasma or serum after the 18th week of gestation showed the presence of many normal proteins, such as albumin, transferrin, factor B, antithrombin III, Gc-globulin, $\alpha_1$-antitrypsin, several apolipoproteins, retinol-binding protein, transthyretin and $\alpha$-fetoprotein [34]. The amount of $\alpha$-fetoprotein decreased towards birth but the number and size of other spots increased. In particular, the amount of heavy and light chains of IgG and $\alpha_1$-antichymotrypsin increased.

sCD27, a soluble form of the human T cell differentiation antigen CD27, has been found in the serum of healthy blood donors [35]. The authors suggested that its concentration may be quantified as a marker of T lymphocyte activation in vivo.

White et al. [36] have recently shown that a protein, hemonectin, in rabbits that binds granulocytes in a developmentally regulated manner is similar to fetuin ($\alpha_2$-HS-glycoprotein) in humans but whether the human protein has the same granulocyte binding properties has still to be determined.

### 2.3. Urine

Weber [37] has recently summarized previous electrophoretic analyses of urine.

Urine may be examined with the intent of evaluating abnormalities of the renal tract or to assess overall metabolism. 2D Electrophoresis provides a 100-fold increase in sensitivity over agarose electrophoresis for the examination of urinary proteins [38]. Grover and Resnick [39] have recently studied urine from healthy humans using a double stain. Through simplifying the processing procedure, they were able to identify the majority of acidic proteins that previously had only been captured with the ISO-DALT system and others that were precipitated during processing.

Several proteins in urine are identifiable as "kidney proteins". These are not present in plasma and do not usually occur in urine in proteinuria. These proteins are highly heterogeneous in both charge and size, most likely indicating extensive glycosylation. Absence of these proteins from urine in pathological proteinurias may be attributable to dilution causing masking of the small amounts of these proteins by the large amounts of plasma proteins. Many proteins of kidney origin have been identified. These include a brush border epithelial cell protein, adenosine deaminase binding protein (a proximal tubular protein), BE antigen (thermostable kidney antigen) and nephrocalcin, an inhibitor of crystal formation in the renal tubules. Tubular epithelial cells secrete Tamm–Horsfall protein and secretory IgG. Several tubular enzymes have been identified in urine. These include urokinase, kallikrein, N-acetylglucosaminidase, alanine aminopeptidase and $\gamma$-glutamyltransferase. Flynn [40] has recently summarized recent research on the use of monoclonal antibodies to analyze the urine of patients with renal disease. Whether any of the antigens that have been identified as being markers of tubular integrity are present as spots on 2D gels has still to be determined [38].

Gestosis, occurring as a complication of the last trimester of pregnancy is associated with

proteinuria of glomerular origin. Not only are the usual components of glomerular proteinuria identifiable, e.g. albumin, IgG, transferrin, and mid-range $\alpha_1$ and $\alpha_2$ proteins, a prominent spot overlapping transferrin is observed. This has been identified as pregnancy-specific $\beta_1$-glycoprotein but its role in the pathogenesis of gestosis-induced proteinuria has still to be determined [41].

Because of the mechanisms by which urine is formed, filtration of plasma in the glomerulus and reabsorption of small molecules in the tubules, it is usually possible to differentiate whether abnormalities are associated with a metabolic problem or a disorder of glomerular or tubular function in the kidney or a disorder of another part of the urinary tract. Generally glomerular proteins have a higher molecular mass than those of tubular origin. Glomerular proteinuria is thus characterized by several spots of proteins in plasma corresponding to albumin, IgG, transferrin and various $\alpha_2$ proteins. Many glomerular diseases are characterized by intraglomerular deposition of immunoglobulins and the complement components, $C_{1q}$, $C_3$ and $C_4$. In acute glomerular disease these complement components may be excreted into the urine [41]. If hematuria occurs, the low-molecular-mass monomer of hemoglobin may also be present [42]. The presence of fibrinogen and the tetramer of hemoglobin also indicate hematuria. Chronic glomerular disease is associated with increased urinary excretion of albumin, IgG and transferrin. This may be caused by increased capillary pressure in the intact glomeruli [41].

Tubular proteinuria is characterized by a cascade of $\alpha_1$-microglobulin, zinc $\alpha_2$-glycoprotein, Ig-light chains and $\beta_2$-microglobulin as well as albumin [41]. A glomerular proteinuria may be superimposed upon a tubular proteinuria. This may occur, for example, with renal transplant rejection. Tubular necrosis has a characteristic urinary protein pattern but may be associated with additional spots corresponding to necrotic or proteolytic products or leukocytic action. Tubular proteinuria may occur with ascending infections of the urinary tract involving the kidney. The proteinuria of pyelonephritis combines the tubular cascade with an exudative pattern of high-molecular-mass proteins such as IgM, IgG, $\alpha_2$-macroglobulin, fibrin products and hemoglobin. Albumin is not necessarily a prominent spot.

If inflammation is responsible for the renal damage, as with glomerulonephritis, $\alpha_1$-proteinase inhibitor and $\alpha_1$-antitrypsin may appear as prominently as albumin [42]. Inflammation of the lower renal tract shows the characteristic exudative pattern.

Tubular proteinuria is characterized by the presence of larger amounts of proteins of low molecular mass. The amount may vary with the extent of tubular damage. With mild damage, there typically will be increased excretion of the light chains of immunoglobulins, $\alpha_1$-microglobulin, zinc-$\alpha_2$-glycoprotein, retinol binding protein and $\beta_2$-microglobulin. With more severe damage the amounts of these proteins may be increased with the amount of albumin remaining the same [42].

Non-renal infection with fever may be associated with a mild proteinuria which includes an increased excretion of albumin and $\alpha_1$-acid glycoprotein (orosomucoid) and $\alpha_1$-microglobulin [42]. The increased $\alpha_1$-acid glycoprotein is claimed to be associated with leukocyte proliferation [42]. $\beta_2$-Microglobulin is a protein that is prominent in serum but its excretion is increased in both glomerular and tubular disorders [38].

Monoclonal gammopathies are associated with a tubular pattern of proteinuria together with proteins characteristic of the specific gammopathy [42].

### 2.4. Amniotic fluid

Proteins are typically present at such a high concentration in amniotic fluid that they may be detected by Coomassie Brilliant Blue. Nevertheless, silver staining allows trace fetal proteins of possible diagnostic significance to be detected. The overwhelming dominance of albumin may be eliminated by pretreatment with Sepharose or immobilized antibodies specific to maternal pro-

teins. Marshall and Williams [13] have summarized several studies in which $\alpha$-uterine protein, $\alpha$-fetoprotein, fibronectin and modified isoforms of $\alpha_1$-antitrypsin and apolipoprotein A-I have been identified in amniotic fluid.

Sun et al. [43] have used continuous monodimensional electrophoresis to study the urine of patients with mucopolysaccharidosis I or IV and have applied the same technique to study subsequent high-risk pregnancies in the previously affected mothers. Two affected fetuses were diagnosed and proved.

### 2.5. Cerebrospinal fluid

Proteins, even albumin which is present in the largest amount, are normally present at low concentrations in cerebrospinal fluid. The low concentration of proteins in normal cerebrospinal fluid necessitates the use of silver staining to obtain the necessary sensitivity [13]. Several studies have linked abnormalities in cerebrospinal fluid protein patterns to specific diseases. Marshall and Williams [13] have summarized the work of others demonstrating abnormal patterns in multiple sclerosis, Parkinson's disease, schizophrenia and Creutzfeldt–Jakob disease. In their study of 21 patients with Creutzfeldt–Jakob disease Harrington et al. [44] have shown that two protein spots were present in all patients with the disease and only present in five of 420 patients with other neurological diseases. Two other proteins were present in 14 of the 21 patients with Creutzfeldt–Jakob disease, although they were also present in 9 of 10 patients with herpes simplex encephalitis, 17 of 54 with schizophrenia, 3 of 26 with Parkinson's disease, 17 of 130 patients with multiple sclerosis, all four patients with Guillain–Barre disease and the one with Behcet's disease. None of the four abnormal proteins were detectable in the cerebrospinal fluid from healthy individuals. Using these four proteins in a blinded study, Harrington et al. [44] were able to differentiate patients with Creutzfeldt–Jakob disease from those with Alzheimer's disease, Huntington's disease, multi-infarct dementia, the Parkinsonian dementia of Guam or the specific dementia of acquired immunodeficiency syndrome (AIDS).

In about half of all schizophrenic patients two polypeptide proteins were detected in cerebrospinal fluid which were present much less frequently in other neurologic or psychiatric disorders [45]. These polypeptides were also present in many patients with affective disorders but were absent from cerebrospinal fluid of patients without neurological disorders. One of the two polypeptides has been confirmed as a fibrin fragment: while the identity of the other has not been established it probably also is a fibrin degradation product.

### 2.6. Synovial fluid

In the studies of Fritz et al. [46] there was an increase in the number of protein spots in the synovial fluid of patients with rheumatoid arthritis as compared with control patients. However, the number of protein spots was always less in synovial fluid than in either serum or synovial tissue. No rheumatoid arthritis-specific protein was identifiable in either serum, synovial tissue or fluid. The amount of IgG was greater in the serum, synovial tissue and synovial fluid of patients with rheumatoid arthritis compared with those of patients with other types of arthritis and healthy individuals, but there were no other consistent patterns [46].

### 2.7. Saliva

2D Electrophoresis has been used to demonstrate proline-rich proteins that stain pink with Coomassie Brilliant Blue (lumicarmines) [13].

### 2.8. Sweat

Marshall and Williams [13] have detected more than 400 polypeptides in non-dialyzed unconcentrated sweat. Lumicarmines, detectable in saliva, are also observed in sweat and tears [13]. Rubin and Penneys [47] have claimed the absence of an acidic protein ($M_r \approx 60\,000$) from the sweat of patients with cystic fibrosis.

### 2.9. Tears

Sack et al. [48] demonstrated a diurnal rhythm for proteins in tears with different compositions for "reflex", "open" and "closed eye" tears. The concentrations of total protein, secretory IgA and albumin increased in the order presented above. The concentrations of lysozyme, lactoferrin and tear-specific prealbumin were unaffected. Eye closure activated complement $C_3$. Sack et al. [48] speculated that eye closure induced a subclinical inflammation and that the increased albumin and secretory IgA concentrations may play a role in protecting the closed eye environment from pathogens.

In tears from women there was a larger amount of lactoferrin, and certain specific tear proteins than in tears from men. In the tears of patients with conjunctivitis haptoglobin and IgG were detected whereas they were absent from the tears of healthy individuals [49]. The proteins were more prominent in the tears of patients in whom the conjunctivitis was worst. However, in the tears of the patients with conjunctivitis secretory IgG, lactoferrin and five specific tear proteins were stained less than in healthy individuals.

Baguet et al. [50] showed that protein G, identified in tears is probably an in vitro artifact due to denaturing conditions with sodium dodecyl sulfate (SDS), arising from tear-specific prealbumin (transthyretin).

In aqueous humor from humans there are many polypeptides with molecular masses ranging from 14 000 to 170 000 that are similar to those identified in monkeys but the exact identity and role of these compounds has still to be determined [51].

### 2.10. Semen

In human sperm as many as 500 different proteins have been resolved with molecular masses ranging from 12 000 to 105 000 and isoelectric points from 5.0 to 8.5 [52]. Although previous authors attempted to correlate protein patterns with fertility, Kritsas et al. [52] did not attempt to do this. Frenette et al. [53] have shown that an $M_r$ 34 000 prostate-specific antigen (PSA) is the major protein binding diisopropylfluorophosphate (DFP) in seminal plasma. The concentration of this, and other DFP-binding proteins, is quite variable and is not correlated with any specific sperm characteristics. Most of these proteins have a prostatic origin, as does annexin 1 (lipocortin 1 and p35) which is secreted at a high concentration with a proteolytic cleavage product, des1-29-annexin 1, into seminal plasma [54].

Sutkowski et al. [55] have incubated human prostatic cells in culture with spermatocoele fluid and used 2D electrophoresis to demonstrate two separate growth factors responsible for the increased growth of both prostatic stromal and epithelial cells, whereas human serum had no effect.

### 2.11. Other fluids

In gliomal cyst fluid five immunoreactive bands were recognized with apparent molecular masses of 50 000–75 000. Isoelectric focusing suggested that these were due to a local immune response and might be involved in peritumoral events such as brain edema [56].

In developed countries about half the population has bile supersaturated with cholesterol, yet only about 10% of the population develops gallstones. In human bile a protein has been identified that inhibits cholesterol crystallization. It is a glycoprotein comprising two subunits of $M_r$ 63 000 and 58 000 which have comparable inhibitory activity [57]. The crystal growth-inhibiting activity of this protein may be important in preventing gallstone formation in healthy humans.

Signs of acute pancreatitis may occur after pancreas transplantation. The pancreatic juice is initially similar to that in normal pancreatic juice but high concentrations of albumin are typically present. Within two days of reperfusion of a grafted pancreas the amount of proteins with molecular masses of 17 000 to 20 000 increases substantially [58]. The increase is largely due to a new protein that comprises about 7.5% of the total secretory protein and reaches a maximum

five days after transplantation and thereafter gradually decreases but still is detectable 45 days after the transplant. The protein is believed to be associated with the inflammation that follows transplantation and is similar to a protein secreted in the rat under similar circumstances. It has been designated human pancreatitis-associated protein.

Studies of human perilymph have shown several proteins that are not present in plasma but their clinical pertinence still has to be determined [59].

## 3. Solid tissues

### 3.1. Heart

Human heart muscle proteins have been examined by 2D electrophoresis [60]. In patients with dilated cardiomyopathy 3 of 5 hearts showed an additional spot in the myocardial myosin light chain 1 area. The nature of this protein is unknown.

### 3.2. Brain

The brains from six patients with Alzheimer's disease have been examined by 2D electrophoresis [61]. A unique protein band, not observed in control brains, was shown to be a constituent of amyloid fibrils. Johnson et al. [62] have used 2D electrophoresis to identify elongation factor 2 (EF-2) in the brains of patients with Alzheimer's disease. Phosphorylation of EF-2 has been shown to inhibit protein synthesis and thus the increased phosphorylation of EF-2 in Alzheimer's disease is possibly important in the causation of the disease. Postmortem cortical tissue from the brains of patients with Alzheimer's disease has been shown to contain significantly greater amounts of the heat shock proteins hsp 72 and hsp 73 than control cortical tissues [63]. Induction of these proteins appears to be associated with the disease although the amount of the proteins is not increased in the cerebella of patients with Alzheimer's disease.

### 3.3. Thyroid

Although it had previously been postulated that differences from normal in the microsomal antigen might be involved in the development of autoimmune thyroid disease, Hamada et al. [64] have used 2D electrophoresis to show that the microsomal antigen in Graves' disease does not differ from that in normal thyroid. They concluded that the microsomal antibodies in autoimmune thyroid disease probably do not arise from differences in the antigen.

### 3.4. Muscle

Gel electrophoresis has shown that fetal and fast myosin were the predominant isoforms of myosin in children with congenital myotonic dystrophy, whereas the pattern of expression of myosin light chains, tropomyosin and troponin in X-linked myotubular myopathy was comparable to that of normal aged matched control muscle [65].

## 4. Malignant disease

Franzen et al. [66] have performed 2D electrophoresis of proteins in human lung cancers. They noted that there may be considerable intersample variability, even in tumors of the same histological type. Intrasample variability was typically much less. Nevertheless, local homogeneity and a constant amount of connective tissue are necessary if reproducible results are to be obtained.

SDS-PAGE has been used to study the cytokine profiles in solid human basal cell eptheliomas and the outer root sheath of the human hair follicle [67]. The cytokine profiles were almost identical which led the authors to speculate that the lower outer root sheath had a role in a cellular pool for the generation of basal cell epithelioma.

Hall et al. [68] have used 2D electrophoresis to confirm the identify of protein in melanoma cells as lipocortin I.

Aoyama et al. [69] have used 2D electro-

phoresis to show increased amounts of αB-crystallin expression in glial tumors such as astrocytoma, glioblastoma multiforme and oligodendroglioma. In these tumors αB-crystallin is predominantly unphosphorylated. The authors conclude that αB-crystallin may be a useful biochemical marker for studying the pathogenesis of various human brain tumors. Brunet et al. [70] have used 2D electrophoresis to characterize normal brain-reactive antibodies in glioma cyst fluid. In white matter extracts cyst fluids recognized five immunoreactive bands having apparent molecular masses of 50 000–75 000. Isofocusing experiments showed that these proteins were largely related to a local immune response.

2D Electrophoresis has been used to compare proteins in normal uterine smooth muscle and leiomyomas. A protein with $M_r \approx 27\,000$ was selectively expressed in normal uterine smooth muscle cells [71]. This protein is a low-molecular-mass variant of calponin but its function is not known.

Adult granulosa cell tumors have been shown to contain characteristic sets of proteins [72]. Twenty-three of 25 tumors showed strong positivity for vimentin; 9 of 25 contained desmin.

2D Electrophoresis of the proteins in embryonal carcinoma, endodermal sinus tumor, choriocarcinoma and teratoma was used to identify cytokeratins 8 and 18 in all tumors, whereas other cytokeratins were less constant. The majority of the germ cell tumors showed varied amounts of vimentin, often in conjunction with cytokeratins [73].

Williams et al. [74] have identified a novel cyclin-like protein in bone tumors in children differing from previously identified members of the cyclin D family of proteins. This protein, identified in Ewing's sarcomas and Wilms' tumors, is electrophoretically and chromatographically distinct from the previously identified p36cyclin D1 and p34cyclin D2 proteins.

Nuclear matrix proteins in normal prostate, benign prostatic hypertrophy and prostate cancer have been studied using 2D electrophoresis [21]. Fourteen different proteins from nuclear matrix preparations were common to prostate cancer specimens but were absent from normal prostate or prostates with benign hypertrophy.

Paradis et al. [75] observed a major $M_r$ 25 000 primary translation product in all renal cell carcinomas removed at surgery. The product appears to be a glycoprotein and has potential value as a marker for the tumor.

Bartek et al. [76] have used a series of new monoclonal antibodies to keratins to examine histochemically malignant tumors from humans. These were able to differentiate carcinomas from gliomas and lymphomas. They also observed decreased amounts of keratin number 7, including absence in some cases, in small subsets of breast cancers and induced expression of the keratin in some cases of stomach cancer.

## 5. Tissue culture

### 5.1. Endometrium

Culture of endometriosis and endometrial biopsy specimens has to be done to determine the differences in polypeptide synthesis between the two types of specimens [77]. Five major deviations were noted in protein synthesis and secretion. Endometriosis specimens showed two proteins not present in normal endometrial specimens which, in contrast, showed three proteins not present in the endometriosis specimens. The significance has yet to be determined, although the differences may allow the development of diagnostic markers.

### 5.2. Fibroblasts

2D Electrophoresis of human fibroblasts incubated with interferon showed that a common set of proteins were induced, some of which were also induced by dsDNA [78]. A greater number of polypeptides had their synthesis specifically induced by dsDNA. Guy et al. [79] have used 2D electrophoresis to analyze the signal transduction pathways of tumor necrosis factor (TNF-α) and interleukin-1 (IL-1α and β). They were able to show that the two cytokines activate many protein kinases. McCarthy et al. [80]

followed up on the known involvement by basic calcium phosphate crystals in synovial fluid in causing destructive arthropathies by incubating fibroblast cultures with basic calcium phosphate. Selective induction of two proteins consistent with collagenase occurred. Culard et al. [81] used SDS-PAGE of fibroblasts from normal skin to demonstrate annexins I, II and V and possibly VI and VII, also. This provides a basis from which the role and regulation of these proteins within the epidermis may be established.

Smith and Higgins [82] have shown that fibroblasts from the skin of the abdominal wall and lower leg exhibit different patterns of protein expression and that their response to γ-interferon is different. Higgins and Smith [83] have used 2D electrophoresis to demonstrate the regulatory influence of γ-interferon on the synthesis of many specific proteins in orbital fibroblasts. Bai et al. [84] have used 4-nitroquinoline 1-oxide to immortalize normal human fibroblasts and have used 2D electrophoresis to show that the number of disappearing cellular proteins was greater than the number of newly appearing proteins after the cells became immortalized.

Easty et al. [85] used 2D electrophoresis to study the response of two melanoma cell lines to cyclic adenosine monophosphate-stimulating agents. The more malignant cell line showed pronounced dendrification, decreased proliferation and a reduction in metastatic capacity whereas the less malignant cell line showed little effect. The stimulation of the more malignant cell line induced several proteins not previously present and were not present in the less malignant cell line either before or after treatment. The authors concluded that 2D electrophoresis could be a useful tool in the study of cellular differentiation.

### 5.3. Endothelial cells

Clarke and West [86] stimulated umbilical and capillary endothelial cells with simple mitogens and used 2D electrophoresis to study the induction of proliferation and tumor-related antigens on the surface of the cells. They subsequently used monoclonal antibodies to demonstrate that tumor-specific surface proteins are present on most tumor endothelium.

### 5.4. Bone

2D Electrophoresis of normal osteoblasts identified osteonectin, bone sialoprotein, the C-teleopeptide of collagen I as well as collagen I. Osteoblasts from patients with Paget's disease secreted an altered $M_r$ 30 000 C-teleopeptide of collagen demonstrating that osteoblasts from patients with Paget's disease are functionally abnormal [87]. Hankey et al. [88] have used 2D electrophoresis to investigate extracellular protein secretion by osteoblasts in vitro. They showed one protein chain with posttranslational modifications to be unique to osteoblasts, and also the absence of the N- and O-glycoforms of collagenase.

### 5.5. Lung

Mirski and Cole [89] used a small cell lung cancer cell line to demonstrate resistance-associated proteins which may be useful as potential markers in determining the mechanism for drug resistance. 2D Electrophoresis of lung cancer specimens showed that even with the same histological type there was significant intersample variation although the intrasample variation was low [90].

### 5.6. Head and neck

Ramsamooj et al. [91] used 2D electrophoresis to compare the protein profiles of three relatively radioresistant and three relatively radiosensitive head and neck squamous carcinoma cell lines. They were able to show that the radio-resistant cell lines preferentially expressed 14 proteins. Fifteen proteins were preferentially expressed in the radio-sensitive cell lines. The authors concluded that 2D electrophoresis could characterize the proteins that correlate with the radiation response-specific phenotype.

### 5.7. Brain

Muller et al. [92] studied nuclear proteins from human brain tumor cell lines and found that their composition was quite similar but that there also was a high degree of similarity between the proteins of glioblastomas and other human tumor cell lines. Nevertheless, they observed several proteins enriched in glioblastomas that were totally absent from low-grade astrocytomas and non-glial tumors.

## 6. Malignant cells

The growth and spread of tumors depends on the proliferation of the endothelial cells in their vasculature. Clarke and West [93] stimulated cultured human umbilical and capillary endothelial cells with mitogens and tumor-conditioned media. They used [$^{35}$S]methionine to demonstrate the induction of proliferation and tumor-related antigens on the surface of the endothelial cells. Specific monoclonal antibodies showed that tumor-specific proteins are present on most tumor endothelium.

Menzel and Unteregger [94] have used detergent-lysed nuclei from several human tumor cell lines to analyze the nuclear protein pattern. Certain proteins were common to all cell lines, but marked differences were also observed between cell lines when visualized with silver staining. Quantitative variations of the nuclear phosphoproteins 23/4 were detectable. This suggests a possible correlation between their synthesis and phosphorylation and the proliferation behavior of the tumor cells. They concluded that the distinct heterogeneity and tissue specificity may be of value in determining tumor-specific proteins.

## 7. Bacterial proteins

Norris [95] has used 2D electrophoresis to show that the protein profiles of the *Treponema pallidum* subspecies that cause syphilis, yaws and endemic syphilis are virtually indistinguishable from one another, but differ considerably from those of other treponemal species. Among the most abundant polypeptides are a group of lipoproteins of unknown function that appear to be important in the immune response during syphilitic infection. The amounts of 40 proteins increased in *Salmonella typhimurium* during their growth in eukaryotic cells, whereas the amounts of another 100 proteins decreased [96]. It is presumed that the intracellular environment imposed stress on the bacteria which accounted for these changes.

Abu Kwaik et al. [97] have shown that at least 35 proteins are induced in *Legionella pneumophilia* during infection of macrophages with selective repression of at least another 32 proteins. Wallis et al. [98] have identified a novel $M_r$ 58 000 protein of *Mycoplasma tuberculosis* which induces production of tumor necrosis factor by human monocytes. They postulate that the protein may have an important role in the immunopathogenesis of tuberculosis and in mycobacterial immunity.

Cultivation of *Campylobacter jejuni* with epithelial cells resulted in the new or enhanced synthesis of several proteins that did not occur in the absence of the epithelial cells, suggesting to Konkel et al. [99] that they may have a role in facilitating internalization.

## 8. Technology

Endler et al. [100] have summarized the milestones in the development of high-resolution 2D electrophoresis and presented their technique for the analysis of biological specimens.

Dunbar [101] has summarized how high-resolution 2D-PAGE can be combined with staining and immunochemical methods to provide exquisite tools to analyze and dissect complex biological systems.

Hochstrasser et al. [102] have modified the technique of 2D electrophoresis by increasing the pore size in isoelectric focusing and by replacing most of the Nonidet P-40, a non-ionic detergent, with a zwitterionic detergent. They also eliminated the equilibration step between

the first- and second-dimensional separation. By improving the cooling for the molecular mass separation faster migration could be obtained at a higher current. By using diacrylylpiperazine as a cross-linker protein separation was improved and detection with ammoniacal silver staining was enhanced [102]. Using these modifications and computer analysis of autoradiographs after the separation of identical liver cells the authors were able to increase the number of spots detected from 1100 to more than 1600. When they used silver staining the number of spots detected was increased from less than 300 to more than 950.

H'ansler et al. [103] have improved the detection of human pancreatic proteins by using double staining with Coomassie Brilliant Blue followed by silver stain. The technique appeared to be up to twenty times more sensitive than conventional Coomassie Brilliant Blue staining. The fluorophore, monobromobimane, was used by Urwin and Jackson [104] to label proteins containing a sulfhydryl group prior to the isoelectric focusing step of 2D electrophoresis. In a lymphoid cell line they were able to show an approximate 10% increase in the number of spots compared with conventional silver staining.

## 9. Databases

Celis et al. [105] have discussed how it is now possible to reveal phenotype-specific proteins, to microsequence them and to search for homology with previously identified proteins, and to clone the cDNAs, to assign partial protein sequences to genes for which the full DNA sequence and the chromosome location is known. This enables the regulatory properties and function of groups of proteins that are coordinately expressed in a given biological process to be determined. Human 2D electrophoretic protein databases are becoming increasingly important with the current attempts to map and sequence the entire human genome.

Anderson and Anderson [106] have published an updated 2D gel database of human plasma proteins. They provide a map together with a complete listing of the individual protein spots, with their locations, size and isoelectric points relative to internal charge standards [106]. A map of the proteins in human cerebrospinal fluid has also been published [107]. The authors provided maps of normal cerebrospinal fluid as well as separate maps of cerebrospinal fluid from patients with schizophrenia and Creutzfeldt–Jakob disease. Golaz et al. [108] have recently provided an updated map of polypeptides in erythrocytes. Twelve proteins or enzymes were localized on the map.

Grover and Resnick [39] have produced a map of proteins derived from unprocessed urine from 10 healthy men and 10 women. They observed more proteins that had been reported previously although the majority remain unidentified. They noted that women typically had more spots and a greater proportion of low-molecular-mass proteins than men. Tracy et al. [38] have also updated their own map of urinary proteins.

Hochstrasser et al. [109] have published a map of human liver proteins. They commented that the same map can be used to identify protein spots in other specimens such as rectal cancer biopsies.

Hanash et al. [110] have developed a database of lymphoid proteins detectable by 2D electrophoresis. The database comprises 2D electrophoretic patterns and information relating to polypeptide constituents of unstimulated and stimulated mature T cells and immature thymocytes, single-cell-derived T- and B-cell clones, leukemia cells and lymphoid cell lines. Celis et al. [111] have published a map of cellular proteins from transformed human epithelial amnion cells (AMA) and normal peripheral blood mononuclear cells. Madsen et al. [112] have documented the major proteins in normal human lymphocyte subpopulations.

Rasmussen et al. [113] have microsequenced 145 proteins in a master human keratinocyte 2D gel database. This database contains 2980 cellular proteins (2098 visualized by isoelectric focusing and 882 by non-equilibrium pH gradient electrophoresis). About 20% of the proteins have been identified [114].

Burggraf et al. [115] have constructed a 2D

database from five cell lines of different germ layers. The database was developed from the proteins in total cell lysate and the isolated and purified nuclei of each cell line. The database comprises the common proteins in the master protein patterns of each of the cell lines.

Ali et al. [116] have developed a sophisticated computerized laboratory processing system for 2D electrophoresis data. Appel et al. [117] have developed a set of computer programs which they entitled ELSIE/MELANIE to detect, quantify and compare protein spots on high-resolution 2D-PAGE. Their goal was to use the system to make automatic diagnoses from 2D gels. The diagnostic rules that they built into the system have enabled them to successfully diagnose cirrhosis of the liver and to determine a variety of types of cancer.

## References


[1] M. Gaczynska, *Cytobios*, 72 (1992) 197.
[2] J. Connor, M. Bar-Eli, K.D. Gillum and A.J. Schroit, *J. Biol. Chem.*, 267 (1992) 26 050.
[3] S.M. Hanash, R. Kuick, D. Nichols and L. Stoolman, *Dis. Mark.*, 6 (1988) 209.
[4] R.M. Clancy, J. Leszczynska-Piziak and S.B. Abramson, *Biochem. Biophys. Res. Commun.*, 191 (1993) 847.
[5] F.K. Saunders, R.M. Sharrard, D.A. Winfield, J. Lawry, J.R. Goepel, B.W. Hancock and M.H. Goyns, *Leuk. Res.*, 17 (1993) 223.
[6] S. Kantengwa and B.S. Polla, *Infect. Immun.*, 61 (1993) 1281.
[7] M.R. Schick and S. Levy, *J. Immunol.*, 151 (1993) 4090.
[8] K. Harada, H. Yamane, Y. Imai, T. Tsuji, S. Toyoshima and T. Osawa, *Glycoconj. J.*, 9 (1992) 198.
[9] W. Jabbour, A. Pouplard-Barthelaix, R. Houlgatte and J. Emile, *J. Neuroimmunol.*, 38 (1992) 199.
[10] S. Talebian-Ziai, Z. Azgui, F. Valensi, F. Sigaux and D. Charron, *Electrophoresis*, 13 (1992) 388.
[11] J. Hagmann and M.M. Burger, *J. Cell Biochem.*, 50 (1992) 237.
[12] S. Iwamoto, E. Kajii, T. Omi, S. Tsuchida and S. Ikemoto, *Hum. Hered.*, 42 (1992) 276.
[13] T. Marshall and K.M. Williams, *Electrophoresis*, 12 (1991) 461.
[14] A.L. Latner, T. Marshall and M. Gambie, *Clin. Chim. Acta*, 103 (1980) 51.
[15] A.L. Latner, T. Marshall and M. Gambie, *Electrophoresis*, 1 (1980) 82.
[16] M.F. Goldfarb, *Electrophoresis*, 13 (1992) 440.
[17] F. Wiederkehr, M.R. Bueler and D.J. Vonderschmitt, *J. Chromatogr.*, 566 (1991) 77.
[18] J.D. Tissot, P. Schneider, P. Hohlfeld, F. Spertini, D.F. Hochstrasser and M.A. Duchosal, *Electrophoresis*, 14 (1993) 1366.
[19] H.H. Harrison, *Clin. Biochem.*, 25 (1992) 235.
[20] H.H. Harrison, K.L. Miller, A. Abu-Alfa and S.J. Podlasek, *Am. J. Clin. Pathol.*, 100 (1993) 550.
[21] A.W. Partin, R.H. Getzenberg, M.J. Carmichael, D. Vindivich, J. Yoo, J.I. Epstein and D.S. Coffey, *Cancer Res.*, 53 (1993) 744.
[22] T. Marshall, K.M. Williams and O. Vesterberg, *Clin. Chem.*, 30 (1984) 2008.
[23] B.F. Asztalos, C.H. Sloop, L. Wong and P.S. Roheim, *Biochim. Biophys. Acta*, 1169 (1993) 291.
[24] T. Marshall, K.M. Williams, L. Holmquist, L.A. Carlson and O. Vesterberg, *Clin. Chem.*, 31 (1985) 2032.
[25] T. Miida, M. Kawano, C.J. Fielding and P.E. Fielding, *Biochemistry*, 31 (1992) 11112.
[26] Y.J. Kim, Z. Zhou, J. Hurtado, D.L. Wood, A.S. Choi, M.D. Pescovitz, K.A. Wartel, J. Vandagriff, J.K. Davis and B.S. Kwon, *Immunol. Invest.*, 22 (1993) 219.
[27] T. Marshall, J. Williams and K.M. Williams, *Electrophoresis*, 10 (1989) 584.
[28] A.F. Strachan, F.C. de Beer, D.C. van der Westhuyzen and G.A. Coetzge, *Biochem. J.*, 250 (1980) 203.
[29] O. Guy-Crotte, C. Barthe and C. Figarella, *Electrophoresis*, 10 (1989) 628.
[30] C. Hayward, S. Chung, D.J.H. Brock and V. Van Heyningen, *J. Immunol. Methods*, 91 (1986) 117.
[31] A. Miyano, H. Kitajima, F. Fujiwara, M. Nakayama, T. Fulita, M. Fujimura, T. Takeuchi and A. Shimizu, *Complement Inflamm.*, 8 (1991) 320.
[32] O. Romero-Saravia, E. Solem and M. Lorenz, *Electrophoresis*, 9 (1988) 816.
[33] J.D. Tissot, P. Hohlfeld, D.F. Hochstrasser, J.F. Tolsa, A, Calme and P. Schneider, *Electrophoresis*, 14 (1993) 245.
[34] J.D. Tissot, P. Hohlfeld, F. Forestier, J.F. Tolsa, D.F. Hochstrasser, A. Calme, E. Plouvier, H. Bossart and P. Schneider, *Appl. Theoret. Electrophor.*, 31 (1993) 183.
[35] R.Q. Hintzen, R. de Jong, C.E. Hack, M. Chamuleau, E.F. de Vries, I.J. ten Berge, J. Borst and R.A. van Lier, *J. Immunol.*, 147 (1991) 29.
[36] H. White, N. Totty and G. Panayotou, *Eur. J. Biochem.*, 213 (1993) 523.
[37] M. Weber, *J. Chromatogr.*, 429 (1988) 315.
[38] R.P. Tracy, D.S. Young, H.D. Hill, G.W. Cutsforth and D.M. Wilson, *Appl. Theoret. Electrophor.*, 3 (1992) 55.
[39] P.K. Grover and M.I. Resnick, *J. Urol.*, 150 1993) 1069.
[40] F. Flynn, *Clin. Biochem.*, 23 (1990) 49.

[41] A. Lapin and W. Feigl, *Electrophoresis*, 12 (1991) 472.
[42] A. Lapin, *J. Clin. Chem. Clin. Biochem.*, 27 (1989) 81.
[43] N.H. Sun, F.Y. Wang, Y.C. Wu and Y. Ning, *Chin. Med. J.*, 104 (1991) 1022.
[44] M.G. Harrington, C.R. Merril, D.M. Asher and D.C. Gajdusek, *N. Engl. J. Med.*, 315 (1986) 279.
[45] D.B. Wildenauer, D. Kurschenhauser, W. Hoechtlen, M. Ackenheil, M. Kehl and F. Lottspeich, *Electrophoresis*, 12 (1991) 487.
[46] P. Fritz, N. Arold, A. Mischlinski, H. Wisser, B. Oeffinger, V. Neuhoff, W. Laschner and G. Koenig, *Rheumatol. Int.*, 10 (1990) 177.
[47] R.W. Rubin and N.S. Penneys, *Anal. Biochem.*, 131 (1983) 520.
[48] R.A. Sack, K.O. Tan and A. Tan, *Invest. Ophthalmol. Vis. Sci.*, 33 (1992) 626.
[49] S. Mü, K. Nakamura, K. Takeo and S. Kurimoto, *Electrophoresis*, 13 (1992) 379.
[50] J. Baguet, V. Claudon-Eyl and A.M. Gachon, *Curr. Eye Res.*, 11 (1992) 1057.
[51] P. Russell and D.L. Epstein, *Curr. Eye Res.*, 11 (1992) 1239.
[52] J.J. Kritsas, W.M. Schopperle, W.C. DeWoolf and A. Morgentaler, *Electrophoresis*, 13 (1992) 445.
[53] G. Frenette, R.R. Trimblay and J.Y. Dub'e, *Int. J. Androl.*, 14 (1991) 186.
[54] P. Christmas, J. Callaway, J. Fallon, J. Jones and H.T. Haigler, *J. Biol. Chem.*, 266 (1991) 2499.
[55] D.M. Sutkowski, R.Z. Kasjanski, J.A. Sensibar, K.G. Ney, D.J. Lim, J.M. Kozlowski, C. Lee and J.T. Grayhack, *J. Androl.*, 14 (1993) 233.
[56] J.M. Brunet, F. Berger, T. Gustin, M. Laine and A.L. Benabid, *J. Neuroimmunol.*, 47 (1993) 63.
[57] T. Ohya, J. Schwarzendrube, N. Busch, S. Gresky, K. Chandler, A. Takabayshi, H. Igimi, K. Egami and R.T. Holzbach, *Gastroenterology*, 104 (1993) 527.
[58] V. Keim, J.L. Iovanna, B. Orelle, J.M. Verdier, M. Busing, U. Hopt and J.C. Dagorn, *Gastroenterology*, 103 (1992) 248.
[59] D.R. Paugh, S.A. Telian and M.J. Disher, *Otolaryngol. Head Neck Surg.*, 104 (1991) 517.
[60] L.I. Kovalyov, V.G. Naumov, H.V. Pulyayeva, A.M. Samko, M.N. Tsetkova, S.S. Shishkin and N.M. Mukharlyamov, *Electrophoresis*, 11 (1990) 333.
[61] G.G. Glenner and C.W. Wong, *Biochem. Biophys. Res. Comm.*, 120 (1984) 885.
[62] G. Johnson, J. Gotlib, V. Haroutunian, L. Bierer, A.C. Nairn, C. Merril and W. Wallace, *Brain Res. Mol. Brain Res.*, 15 (1992) 319.
[63] N. Perez, J. Sugar, S. Charya, G. Johnson, C. Merril, L. Bierer, D. Perl, V. Haroutunian and W. Wallace, *Brain Res. Mol. Brain Res.*, 11 (1991) 249.
[64] N. Hamada, L.J. Degroot, L. Portmann, J. Yamakawa, J. Noh, Y. Okamoto, M. Ohno, K. Ito and H. Morii, *Endocrinol. Jpn.*, 38 (1991) 471.
[65] N. Soussi-Yaicostas, M. Chevallay, C. Laurent-Winter, F.M. Tome, M. Fardeau and G.S. Butler-Browne, *Neuromuscul. Disord.*, 1 (1991) 103.
[66] B. Franzen, H. Iwabuchi, H. Kato, J. Lindholm and G. Auer, *Electrophoresis*, 12 (1991) 509.
[67] M. Asada, F.M. Schaart, H.L. de Alimeida, Jr. *Acta Derm. Venerol.*, 73 (1993) 286.
[68] S.C. Hall, D.M. Smith, F.R. Masiarz, V.W. Soo, H.M. Tran, L.B. Epstein and A.L. Burlingame, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (1993) 1927.
[69] A. Aoyama, R.H. Steiger, E. Frohli, R. Schafer, A. von Deimling, O.D. Wiestler and R. Klemenz, *Int. J. Cancer*, 55 (1993) 760.
[70] J.M. Brunet, F. Berger, T. Gustin, M. Laine and A.L. Benabid, *J. Neuroimmunol.*, 47 (1993) 63.
[71] A. Draeger, A.H. Graf, A. Staudach, A.J. North and J.V. Small, *Virchows Arch. B*, 64 (1993) 21.
[72] C.N. Otis, J.L. Powell, D. Barbuto and M.L. Carcangiu, *Am. J. Surg. Pathol.*, 16 (1992) 962.
[73] B. Lifschitz-Mercer, M. Fogel, R. Moll, N. Jacob, I. Kushnir, A. Livoff, R. Waldherr, W.W. Franke and B. Czernobilsky, *Differentiation*, 48 (1991) 191.
[74] R.T. Williams, L. Wu, D.A. Carbonaro-Hall, V.T. Tolo and F.L. Hall, *J. Biol. Chem.*, 268 (1993) 8871.
[75] G. Paradis, J. Gaudreau, G. Frenette, M. Thabet, R.R. Tremblay and J.W. Dub'e, *Biochem. Cell Biol.*, 69 (1991) 561.
[76] J. Bartek, B. Vojtesek, Z. Staskova, J. Bartkova, Z. Kerekes, A. Rejthar and J. Kovarik, *J. Pathol.*, 164 (1991) 215.
[77] K.L. Sharpe, R.L. Zimmer, W.T. Griffin and L.L. Penney, *Fertil. Steril.*, 60 (1993) 839.
[78] M.F. Bourgeade, C. Laurent-Winter, F. Besancon, M.N. Thang and S. Memet, *J. Interferon Res.*, 13 (1993) 175.
[79] G.R. Guy, S.P. Chua, N.S. Wong, S.B. Ng and Y.H. Tan, *J. Biol. Chem.*, 266 (1991) 14343.
[80] G.M. McCarthy, P.G. Mitchell and H.S. Cheung, *Arth. Rheum.*, 34 (1991) 1021.
[81] J.F. Culard, N. Basset-Seguin and B. Calas *J. Invest. Dermatol.*, 98 (1992) 436.
[82] T.J. Smith and P.J. Higgins, *Biochim. Biophys. Acta*, 1181 (1993) 300.
[83] P.J. Higgins and T.J. Smith, *Biochim. Biophys. Acta*, 1181 (1993) 23.
[84] L. Bai, K. Mihara, Y. Kondo, M. Honma and M. Namba, *Int. J. Cancer*, 53 (1993) 451.
[85] D. Easty, I.R. Hart, K. Patel, C. Seymour, M. Yacoub, A. Domscheit, S. Gunther, W. Postel, A. Gorg and M.J. Dunn, *Clin. Exp. Metastasis*, 9 (1991) 221.
[86] M.S. Clarke and D.C. West, *Electrophoresis*, 12 (1991) 500.
[87] D.P. Hankey, A.E. Hughes, R. Mollan and R.M. Nicholas, *Electrophoresis*, 14 (1993) 644.
[88] D.P. Hankey, R.M. Nicholas and A.E. Hughes, *Electrophoresis*, 13 (1992) 329.
[89] S.E. Mirski and S.P. Cole, *Br. J. Cancer*, 64 (1991) 15.
[90] B. Franzen, H. Iwabuchi, H. Kato, J. Lindholm and G. Auer, *Electrophoresis*, 12 (1991) 509.

[91] P. Ramsamooj, U. Kasid and A. Dritschilo, *J. Natl. Cancer Inst.*, 83 (1992) 622.
[92] A. Muller, W. Henn and G. Unteregger, *Electrophoresis*, 12 (1991) 515.
[93] M.S.F. Clarke and D.C. West, *Electrophoresis*, 12 (1991) 500.
[94] A. Menzel and G. Unteregger, *Electrophoresis*, 10 (1989) 554.
[95] S.J. Norris, *Microbiol. Rev.*, 57 (1993) 750.
[96] K.Z. Abshire and F.C. Neidhardt, *J. Bacteriol.*, 175 (1993) 3734.
[97] Y. Abu Kwaik, B.I. Eisenstein and N.C. Engleberg, *Infect. Immun.*, 61 (1993) 1320.
[98] R.S. Wallis, R. Paranjape and M. Phillips, *Infect. Immun.*, 61 (1993) 627.
[99] M.E. Konkel, D.J. Mead and W. Cieplak, Jr. *J. Infect. Dis.*, 168 (1993) 948.
[100] A.T. Endler, D.S. Young and R.P. Tracy, *Cancer Invest.*, 5 (1987) 127.
[101] B.S. Dunbar, *BioTechniques*, 5 (1987) 218.
[102] D.F. Hochstrasser, M.G. Harrington, A.C. Hochstrasser, M.J. Miller and C.R. Merril, *Anal. Biochem.*, 173 (1988) 424.
[103] M. H'ansler, G. Appelt and R. Rogos, *J. Chromatogr.*, 563 (1991) 63.
[104] V.E. Urwin and P. Jackson, *Anal. Biochem.*, 209 (1993) 5762.
[105] J.E. Celis, H.H. Rasmussen, H. Leffers, P. Madsen, B. Honore, B. Gesser, K. Dejgaard and J. Vandekerckhove, *FASEB J.*, 5 (1991) 2200.
[106] N.L. Anderson and N.G. Anderson, *Electrophoresis*, 12 (1991) 883.
[107] M. Yun, W. Wu, L. Hood and M. Harrington, *Electrophoresis*, 13 (1992) 1002.
[108] O.G. Golaz, C. Walzer, D. Hochstrasser, B. Bjellquist, H. Turler and L. Balant, *Appl. Theor. Electrophor.*, 3 (1992) 77.
[109] D.F. Hochstrasser, S. Frutiger, N. Paquet and A. Bairoch, *Electrophoresis*, 13 (1992) 992.
[110] S.M. Hanash, J.R. Strahler, Y. Chan, R. Kuick, D. Teichroew, J.V. Neel, N. Hailat, D.R. Keim, J. Gratiot-Deans and D. Ungar, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (1993) 3314.
[111] J.E. Celis, G.P. Ratz, A. Celis, P. Madsen, B. Gesser, S. Kwee, P.S. Madsen, H.V. Nielsen, H. Yde and J.B. Lauridsen, *Leukemia*, 2 (1988) 561.
[112] P.S. Madsen, M. Hokland, J. Ellegaard, P. Hokland, G.P. Ratz, A. Celis and J.E. Celis, *Leukemia*, 2 (1988) 602.
[113] H.H. Rasmussen, J. van Damme, M. Puype, B. Gesser, J.E. Celis and J. Vandekerckhove, *Electrophoresis*, 13 (1992) 960.
[114] J.E. Celis, H.H. Rasmussen, P. Madsden, H. Leffers, B. Honore, K. Dejgaard, B. Gesser, E. Olsen, P. Gromov and H.J. Hoffman, *Electrophoresis*, 13 (1992) 893.
[115] D. Burggraf, K. Andersson, C. Eckerskorn and F. Lottspeich, *Electrophoresis*, 13 (1992) 729.
[116] I. Ali, Y. Chan, R. Kuick, D. Teichroew and S.M. Hanash, *Electrophoresis*, 12 (1991) 746.
[117] R.D. Appel, D.F. Hochstrasser, M. Funk, J.R. Vargas, C. Pellegrini, A.F. Muller and J.R. Sherrer, *Electrophoresis*, 12 (1991) 722.