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# The Protein Disease Database of human body fluids: I. Rationale for the development of this database

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**We are developing a relational database to facilitate quantitative and qualitative comparisons of proteins in human body fluids in normal and disease states. For decades researchers and clinicians have been studying proteins in body fluids such as serum, plasma, cerebrospinal fluid and urine. Currently, most clinicians evaluate only a few specific proteins in a body fluid such as plasma when they suspect that a patient has a disease. Now, however, high resolution two-dimensional protein electrophoresis allows the simultaneous evaluation of 1,500 to 3,000 proteins in complex solutions, such as the body fluids. This and other high resolution methods have encouraged us to collect the clinical data for the body fluid proteins into an easily accessed database. For this reason, it has been constructed on the Internet *World Wide Web* (WWW) under the title *Protein Disease Database* (PDD). In addition, this database will provide a linkage between the disease-associated protein alterations and images of the appropriate proteins on high-resolution electrophoretic gels of the body fluids. This effort requires the normalization of data to account for variations in methods of measurement. Initial efforts in the establishment of the PDD have been concentrated on alterations in the acute-phase proteins in individuals with acute and chronic diseases. Even at this early stage in the development of our database, it has proven to be useful as we have found that there appear to be several common acute-phase protein alterations in the plasma and cerebrospinal fluid from patients with Alzheimer's disease, schizophrenia and major depression. Our goal is to provide access to the PDD so that systematic correlations and relationships between disease states can be examined and extended.**

**Keywords:** Electrophoresis; gel; two-dimensional; human; database; protein; disease; body fluids; electrophoretic; diagnostic; Alzheimer's disease; schizophrenia; acute phase proteins; serum; plasma; urine; cerebrospinal fluid.

## Introduction

The development of a disease-oriented relational database for proteins found in body fluids has been stimulated by the development of computers capable of handling large amounts of data and the development of high resolution methods for the quantitative analysis of proteins in body fluids. Before the advent of modern computer technology, the collation of clinical data required laborious, manual, literature searches. Now, once clinical data are present in a relational database, it is possible to utilize many complex search methods to find clinically relevant information.

The need for such databases became apparent soon after the development of high resolution two-dimensional protein electrophoresis (2-DE). In a study of lymphocyte proteins from Lesch-Nyhan patients, conducted soon after the introduction of 2-DE, it became apparent that numerous proteins might be affected even in diseases caused by a single gene mutation. As the Lesch-Nyhan syndrome is caused by a mutation in the gene for hypoxanthine phosphoribosyltransferase (HGPRTase), one might expect a change only in the protein spot representing HGPRTase. However, 2-DE demonstrated 11 additional proteins that were quantitatively affected by a factor of 2 or more in this syndrome (Merril *et al.*, 1981). In addition, early investigators studying this syndrome had found altered enzyme activities for 5 other enzymes involved with purine metabolism in tissues for Lesch-Nyhan patients. These studies suggested that the ability to quantitatively analyze and collate large numbers of protein changes in disease states could be useful diagnostically and could provide additional insights into the underlying pathophysiology. In addition, while more traditional linear literature search methods may have sufficed when proteins were first being identified, the enormous number of proteins now detectable by a wide variety of methods makes it critical to employ modern, networked computers for the analysis of such data.

From the first recognition of proteins by Gerardus J. Mulder in 1836, progress in resolving and identifying them has been growing at an exponential rate (Figure 1). By 1937, Tiselius was able to separate serum globulin into $\alpha$, $\beta$ and $\gamma$ components (Tiselius, 1937). By 1956, Smithies and Poulik were able to resolve 20 proteins (Smithies & Poulik, 1956) and in 1969, Freeman and Smith were able to resolve over 60 distinct serum proteins (Freeman & Smith, 1970). By 1977, Anderson and Anderson, using 2-DE, identified over 300 serum proteins

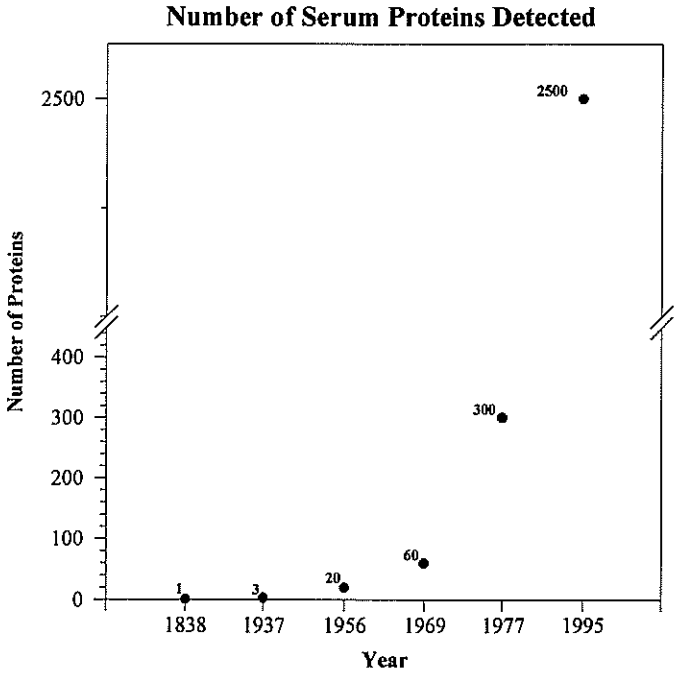


**Figure 1** Number of serum proteins detected since 1838. Advances in electrophoretic methodology since 1950, have resulted in an exponential growth in the number of proteins which can be detected in physiological fluids.

(Anderson & Anderson, 1977), and now Hochstrasser and his colleagues have increased the number of proteins visualized in plasma to 2,500 (Hochstrasser, 1995). In addition, over the same period of time research and clinical applications of analytical protein methods have generated a vast body of information in the biomedical literature pertaining to plasma, serum, CSF and urinary protein changes in disease states, including toxicant exposures. The protein content of these body fluids is not independent, in that proteins in urine and CSF are mainly the products of filtration of the plasma. While much of the data in the database described here were gathered by non-electrophoretic methods, we have adopted the model provided by high resolution 2-DE of arraying the proteins of the body fluid in two dimensions, one for the pI and the other for the molecular weight, with a third dimension to portray quantitative information. This format will, as the database is developed, allow graphic representation of quantitative protein changes in disease or toxicant-exposed states. The conversion and collation of information concerning these body fluids into a useful database will require the cooperative efforts of researchers throughout the biomedical community, including both those with computer expertise and those with clinical experience.

## Methods

### *Data collection*

Currently data are being gathered from peer reviewed journals. One major problem was to design uniform methods for data entry (as discussed in the accompanying paper by Lemkin *et al.*, 1995), and to develop methods to normalize and search the quantitative data. The need for normalization arises because many different methods have been employed to measure proteins. Historically, proteins have been detected by techniques such as the Kjeldahl, Folin, and Lowry methods which make use of the presence of elements or other reactive groups generally present in proteins. However, these methods could not distinguish one protein from another in complex mixtures such as the human body fluids. Today, many methods are employed to distinguish individual proteins.

### *Measurement of individual proteins present*

Of the numerous methods for detecting proteins some may be directly compared with others from independent studies, while other methods rely on different properties which may not be compared. In developing the database it was critical to be cognizant of the different methods utilized in each of the clinical studies and the basis for each of these methods. In addition, ranges for normal protein concentrations and/or activities need to be present in the database along with quantitative changes which occur during the course of a disease state.

### *Protein resolution by electrophoretic methods with quantitative detection by light or stains*

Once proteins are resolved by methods such as electrophoresis, some of these proteins, such as hemoglobin, may be detected by their absorption of visible light. However, most proteins do not absorb light in the visible range of the spectrum, and other methods have been required to detect their presence. Development of these protein detection methods has progressed in parallel with our ability to resolve proteins from tissues and body fluids. Although most proteins can be visualized by their absorption of ultraviolet light, greater sensitivity has been achieved with organic or inorganic stains and color-producing reactions. Currently the most commonly used stains are the organic stains, such as Coomassie Blue, and the silver stain, which depends on the reduction of ionic silver to metallic silver in the presence of proteins.

### *Protein detection by immunological techniques*

Body fluid proteins are often quantitatively analyzed with immunostains, employing both poly- and monoclonal antibodies. It has been possible to detect subnanogram quantities of a specific protein by reacting it with antibodies which are complexed with peroxidase. The antigen/antibody complex is visualized with a suitable peroxidase substrate. It is also possible to use antibodies which have been complexed with other enzymes, fluorescein, rhodamine, or gold. The last is enhanced with silver ion reduction. Some investigators have been able to detect as little as one picogram of a specific protein with these techniques. It has been difficult to directly analyze proteins separated in gels with antibodies, due mainly to the inability of the antibodies to diffuse into the gel matrix. However, antibody detection can be enhanced by transferring the proteins to a thinner matrix,

such as nitrocellulose paper, diazo-modified cellulose, cyanogen bromide-activated paper, nylon, or polyvinylidene difluoride membranes. This transfer is usually facilitated by electroblotting or Western blotting. By carefully selecting the transfer buffer and electrical parameters, it is possible to capture over 90% of the protein from the gel onto the membrane matrix. Many of the protein stains developed to visualize protein in gels can be applied to proteins bound to membranes. Additionally, India ink staining on membranes is almost as sensitive as silver-staining in the gel (Hancock and Tsang, 1983).

*Measurement of enzymatic activities*

Almost all of the enzymatic activity in the human body fluids are due to proteins. While some RNAs have also been shown to have enzymatic activity, these RNAs generally are located intracellularly (for a review, see Symons, 1992). The enzymatic properties of the body fluid proteins may be used as an aid in quantitatively detecting these proteins. A number of enzymatic reactions can be manipulated to produce a color. In some cases secondary reactions must be coupled to the initial enzymatic reaction. These color reactions can be used to detect the proteins in solution and in some cases directly in the electrophoretic support media. For example, acid phosphatase can catalyze the modification of phenolphthalein to produce a pink color, while amylase can be detected by placing a gel containing this enzyme in contact with a starch plate. Subsequent treatment of the starch plate with iodine will produce a purple color, except in regions which were in contact with amylase. These regions will appear as clear areas. A number of these assays use electron transfer dyes, such as methyl thiazolyl tetrazolium, to detect enzymes involved in electron transfer reactions. Over three hundred specific enzymes can now be detected by specific color reactions in a manner similar to those mentioned above (Manchenko, 1994). Electrophoretic parameters, buffers, and temperature must be optimized so that the proteins maintain their enzymatic activities for this type of detection to be successful. However, it should be noted that when pancreatic proteins were separated by the relatively harsh conditions of two-dimensional high resolution electrophoresis, which involve sulfhydryl reduction and denaturation by treatment with detergent, 15 pancreatic proteins still displayed enzymatic activity (Scheele, 1982).

*Normalization*

While methods which determine the amounts of specific proteins in independent studies may be compared with each other—provided details of the methods are recorded in the database, as a means to research possible variations between such studies—it is not possible to compare enzymatic activities with proteins detected by, for example, silver staining. While there may often be a correlation between enzymatic activity and the amount of protein detected on a gel by staining, one cannot count on such comparisons. In a study of the enzyme HGPRTase in samples from Lesch-Nyhan patients, significant amounts of the enzyme could be detected by immunoblotting and by staining, and yet, because of a point mutation in the codon for a single amino acid, very little enzyme activity could be detected in these samples (Merril *et al.*, 1981). Similarly, proteins measured by staining may or may not be comparable to those detected immunologically. The stains often depend on specific reactive sites in each protein. Coomassie Blue stains and silver stains depend to a large degree on the molar content of basic amino acids, while the antibodies depend on very specific regions in each protein for their recognition. Also secondary post-translational modification may specifically decrease or increase the sensitivity of some of these detection methods. For example, different quantities might be reported for a specific protein in plasma, if one study utilized an antibody detection method while another study relied on the stain intensity of that protein in an electrophoretic gel. Such differences could occur if the recognition site for antibody detection of that protein were lost, due to some post-translational modification resulting from the underlying pathophysiology, while the regions or sites needed for detection by staining were not affected.

Given the above problems, the database presented here normalizes data from independent studies by converting quantitative protein values into a dimensionless quantity, expressed as a concentration and/or activity *fold change*, in which the fold change for each protein is defined as the mean of the disease values/mean of the normal values. While this approach can be used to normalize data from independent studies, users of the database must be careful to assure that the methods utilized in the independent studies are equivalent with respect to a disease state (as noted above). In addition, the database tracks and displays the methods used in each study so that users of this database can judge for themselves as to whether the data from the independent studies under consideration can be compared in a meaningful manner. This normalization scheme is discussed in greater detail in the accompanying paper (Lemkin *et al.*, 1995).

*Querying the* Protein Disease Database

To ensure maximum availability to the scientific and clinical community and maximum interactivity to the individual researcher, our database is being constructed and maintained on the Internet *World Wide Web* (WWW) under the name *Protein Disease Database* (PDD). Access is achievable through the *World Wide Web Virtual Library* catalogue page for *Biosciences: Biochemistry, Molecular Biology, and Biophysics,* or through the httpd WWW server Internet address: ftp.ncsa.uiuc.edu. The PDD may be queried by protein name, disease name, literature reference, fold-change ranges, or clicking on protein spots in a 2-D electrophoretogram. Search results will allow the user further data analysis through additional queries of the PDD, or through hyperlinks to other WWW databases. (For details, see the accompanying paper by Lemkin *et al.*, 1995.) In the future, the user will be able to input research results into the PDD via a staging server located at the same address.

Future plans also include depicting the quantitative changes of all proteins associated with each disease visually on the appropriate electrophoretogram. This visual

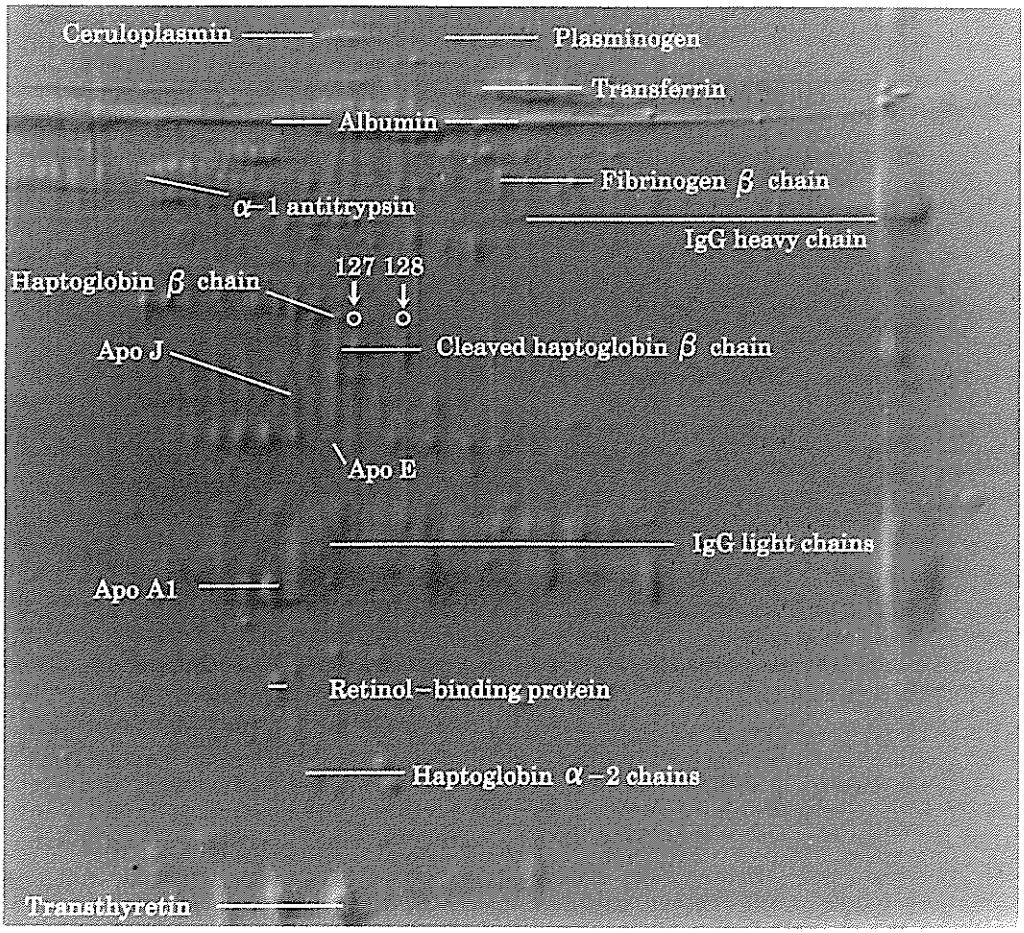


**Figure 2** Electrophoretogram of 300 μl of concentrated CSF. The locations of α-2-haptoglobin and two fibrin fragments (proteins 127 and 128), which increase in both schizophrenia and Alzheimer's disease, are identified, as well as, several other marker proteins. The electrophoretogram is presented in an embossed format to emphasize that the PDD contributes a third, quantitative dimension to the data being collected. Future development of the database will include 3-D visual cues in such an electrophoretogram, depicting the quantitative changes in all proteins associated with a given disease.

cue will be extended into the third dimension of the 2-D gel for each protein which increases with the pathology. A second query will result in the same third dimension cue for each protein which decreases with the pathology.

## Results and discussion

Although the PDD is in an initial state at this time, it has already proven to be helpful in studies of protein variations in the CSF of patients with diseases affecting the central nervous system. In these studies specific proteins, spots **127** and **128,** were observed to be increased in patients with schizophrenia and Alzheimer's disease (Harrington *et al.*, 1985; Johnson *et al.*, 1992) (Figure 2). These proteins have been identified to be fibrin which is derived from fibrinogen (Wildenauer *et al.*, 1991). In addition, α-2-FS haptoglobin has also been found to be increased in the CSF of patients with these diseases (Johnson *et al.*, 1992). As fibrinogen is associated with clotting in the blood, while haptoglobin is associated with heme metabolism, there would at first appear to be no common pathophysiological mechanism for an increase of both of these proteins in CSF. However, both of these proteins also belong to a class of proteins known as acute phase proteins. This class of proteins, first discovered in the laboratory of Avery in patients suffering from pneumococcal pneumonia (Abernathy & Avery, 1941), are generally defined as those whose concentrations increase or decrease by 25% or more in response to inflammation (Kushner, 1982; Kushner & Mackiewicz, 1993). The response of this class of plasma proteins has been well studied in tissue infarction, bacterial infections, bone fracture, neoplasms, surgery, general trauma and immunologically mediated inflammatory states (Kushner, 1982). As we began to gather data and to utilize the database it became apparent that as early as the 1950's, researchers had found acute phase protein alterations in mental illness (Figure 3). While many of these acute phase proteins were assayed by non-electrophoretic techniques, such as immune assays, sedimentation rates, or in some cases merely their interaction with specific substances, by using fold-change normalization, as described above, it was possible in some cases to directly compare these earlier results with our electrophoretic findings. When a direct comparison was not possible, the earlier results were still useful for the corroboration of the recent observations by merely indicating

Acute phase protein alterations in schizophrenia, major depression and Alzheimer's disease

| Illness | Protein | Variation | Reference(s) |
|---|---|---|---|
| schizophrenia | $\alpha_1$-acid glycoprotein | inc. | Bock et al. (1971) |
| | $\alpha_2$-HSglycoprotein | dec. | Bock et al. (1971) |
| | ceruloplasmin | inc./dec. | Schiemberg et al. (1957), Bock et al. (1971) |
| | haptoglobin | inc. | Bock et al. (1971), Gammack and Hector (1965) |
| | transferrin | dec. | Bock et al. (1971) |
| | transthyretin | inc. | Bock et al. (1971) |
| | $\alpha_2$-haptoglobin (CSF) | inc. | Johnson et al. (1992) |
| major depression | $\alpha_1$-acid glycoprotein | inc. | Bock et al. (1971) |
| | $\alpha_1$-antichymotrypsin | inc. | Joyce et al. (1992) |
| | $\alpha_1$-antitrypsin | inc. | Maes et al. (1992a), Bock et al. (1971) |
| | $\alpha_2$-HSglycoprotein | dec. | Bock et al. (1971) |
| | ceruloplasmin | inc./dec. | Maes et al. (1992a), Bock et al. (1971) |
| | haptoglobin | inc. | Joyce et al. (1992), Maes et al. (1992a,b) |
| | hemopexin | inc. | Bock et al. (1971) |
| | retinol binding protein | dec. | Maes et al. (1992a) |
| | transferrin | dec. | Maes et al. (1992b) |
| | transthyretin | inc. | Bock et al. (1971) |
| Alzheimer's disease | albumin | dec. | Behan and Feldman (1970) |
| | $\alpha_1$-antichymotrypsin | inc. | Matsubara et al. (1990), Brugge et al. (1992) |
| | $\alpha_1$-antitrypsin | inc. | Giometto et al. (1988), Behan and Feldman (1970) |
| | ceruloplasmin | inc. | Giometto et al. (1988) |
| | complement C3 | inc. | Giometto et al. (1988) |
| | complement C4 | inc. | Giometto et al. (1988) |
| | haptoglobin | inc. | Behan and Feldman (1970) |
| | $\alpha_2$-haptoglobin (CSF) | inc. | Johnson et al. (1992) |

**Figure 3** Fourteen acute phase proteins have been shown to be altered quantitatively in three mental health diseases. Note the qualitative and quantitative similarities in protein alterations found among the disease states.

whether the variations in protein concentrations in these independent studies are in the same direction.

The finding of common acute phase response protein variations in the serum and CSF proteins in patients with mental disorders such as schizophrenia, manic depressive disease, and Alzheimer's disease may provide some insight into some of the underlying pathophysiology of these diseases. For example, it is known that the acute phase response includes the activation of immunocompetent cells such as macrophages, monocytes and lymphocytes when these cells are exposed to antigens, toxins and products of cell injury (Dunn, 1991). These activated immunocompetent cells secrete cytokines (Dunn, 1991; Dinarello & Wolff, 1993) which initiate hepatic acute phase protein synthesis and secretion (Heinrich *et al.*, 1990; Koj *et al.*, 1993; Kushner & Mackiewicz, 1993). In addition, the elevation of these cytokine levels can have deleterious effects both directly and indirectly on neuronal cells and behavior (Figure 4).

While many of the complex correlations between diseases and proteins in the example described above were found by carefully reviewing the extensive literature of plasma, serum and CSF proteins, if a database such as the one which we are constructing were available, the task would have been much easier. In addition, since techniques such as high resolution two-dimensional electrophoresis permit researchers to quantitatively observe thousands of proteins in a single sample of a body fluid, it would be useful to know which diseases are associated with the protein variations observed in the electrophoretogram from the current patient of interest. Conversely, if one had reason to suspect a patient had a specific disease, the protein alterations observed could help to confirm the diagnosis. In addition, since the PDD is dynamically linked to other protein databases such as *SWISS-PROT*, once a protein is identified as of interest, it's possible to determine its structural and physiological functions, if they are known. This information may prove useful for an understanding of underlying pathophysiology.

It is our hope that the *Protein Disease Database* will prove useful for many applications. In this regard it should be noted that data currently being entered are being culled from the peer reviewed literature. In some cases the number of observations are limited and there are some which appear to be in contradiction. For these reasons the current database is intended only for research applications.

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Some effects of the inflammatory cytokines on neuronal cells and behavior

| Effect | Cytokine | Reference |
|---|---|---|
| oligodendrocyte cytotoxicity and demyelination | TNF-α | Selmaj et al. (1991) |
| increased neurite outgrowth and decreased neuronal cell survival | IL-6 | Alstiel and Sperber (1991) |
| stimulation of the synthesis of the β-amyloid precursor protein | IL-1, IL-6 | Goldgaber et al. (1989), Alstiel and Sperber (1991) |
| delusions, hallucinations, paranoia, agitation, anorexia, fatigue and severe cognitive changes | IL-2 | Denicoff et al. (1987) |
| astrocyte proliferation | TNF-α, IL-6 | Barna et al. (1990), Selmaj et al. (1990) |
| reduction of extracellular acetylcholine in the hippocampus | IL-1β | Rada et al. (1991) |
| increases the secretion of glucocorticoids via the HPA axis | IL-1, IL-2, IL-6,TNF-α | Hermus and Sweep (1990) |

**Figure 4** Effects of cytokines on neuronal cells and behavior. In addition, cytokine secretion initiates synthesis of acute phase proteins.

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