High-Resolution Protein Separation and Identification Methods Applicable to Virology**

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1 Introduction

The location, molecular structure, and stage of assembly of viruses and viral components in cells, tissues, and body fluids at various times after infection are important for the understanding of viral diseases. It is equally important to examine in detail the

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P. A. Bachmann (ed.), *New Developments in Diagnostic Virology*

morphological, structural, and molecular alterations occurring in native cell constituents after viral infection.

To explore virus-associated changes in detail, separation and analytical systems are required which allow tissues to be disaggregated into cells, the cells in turn to be fractionated into subcellular components, and the isolated components to be resolved into discrete molecular species.

Density gradient centrifugation, electro-optical cell sorting using fluorescent antibodies, and electrophoretic and affinity methods have partially solved the cell separation problem; while zonal centrifugation, preparative free-flow electrophoresis, and affinity techniques have facilitated subcellular fractionation.

The so-called s-ρ methods (*Anderson* et al. 1966), involving a two-dimensional separation based on sedimentation rate (s) in the first dimension and isopycnic banding density (ρ) in the second, led to the discovery of the so-called virus window, which is a region in the s-ρ plot where most virions appear, but which is empty in most plots of non-virus-infected cells and tissues. Fractionation of this type is capable of separating very small amounts of virus from tissues, and the application of the method resulted in the first isolation of hepatitis-associated particles from human plasma in 1964–1965 (*Harris* et al. 1966). Unfortunately, these methods have been used rarely in experimental virology, although they have great potential in the search for viruses that do not initially grow in standard cell lines under normal conditions. The general principles have been combined in continuous-flow-with-banding centrifuges now widely used for large-scale virus vaccine purification (*Anderson* et al. 1969).

The most difficult problem, however, has been to resolve the complex mixture of proteins found in virions, cells, and cell fractions. Hence, the problem has been first to develop, in a routinely usable form, the highest resolution protein separation methods available, and then to apply them in a systematic fashion to both normal and virus-infected cells. This work is in progress, and therefore this is a progress report.

2 Separations Based on Affinity Chromatography

Although affinity columns using antibodies have been widely used for the isolation of specific proteins, their use in resolving more complex mixtures has received rather less attention. Group separations may be performed using immobilized substances which bind to certain sets of proteins, for example, those binding certain nucleotide cofactors, heparin, heme, etc. Of greater interest are separations involving the use of sets of antibodies against sets of protein which either allow these proteins to be immobilized and isolated, or which facilitate the isolation of those not bound. Methods have been developed for systematically producing balanced columns to remove proteins normally present in a mixture (*Anderson* et al. 1975a, b), allowing proteins normally not present (and possibly of viral origin) to pass through. These methods are simple in operation but require lengthy development of suitable immobilized antibody mixtures. It would be extraordinarily useful to have commercially available balanced high-capacity columns for removing normal protein from plasma or tissues. This would facilitate the isolation of those proteins not normally present. Systems for rapid recycling of affinity columns have been developed which allow the same

columns to be used over again hundreds of times automatically (*Anderson* et al. 1975a). The possibility of using very small columns initially and then alternating the use of antibody and antigen columns to scale up gradually to a preparative level has not been fully explored.

3 High-Resolution Two-Dimensional Electrophoresis

The highest resolution analytical system currently available for proteins is two-dimensional electrophoresis in acrylamide gels using isoelectric focusing in the presence of urea and the detergent NP40 in the first dimension and electrophoresis in the presence of sodium dodecyl sulfate (SDS) in the second (*Scheele* 1975; *O'Farrell* 1975). These techniques separate on the basis of charge and mass, which are almost totally unrelated parameters. The resolution of each of these techniques using a suitable test mixture is well over 100 proteins, yielding a theoretical resolution for the two-dimensional system of approximately 10 000 proteins. One of the first problems has been to develop this method into an analytical system which allows large numbers of analyses to be run reproducibly (*Anderson N.G.* and *Anderson N.L.* 1979).

3.1 The ISO-DALT System

A system for casting and running 20–40 isoelectric focusing gels in parallel, and a matching system for casting 20 and running 20 or more slab gels in parallel have been developed (*Anderson N.G.* and *Anderson N.L.*, 1978; *Anderson N.L.* and *Anderson N.G.* 1978). These systems have allowed us to run over 50 000 analyses to date. Additional modifications to improve reproducibility and resolution are now in progress. The end objective is a totally automated analytical system for clinical use.

3.2 Image Analysis and Data Reduction

A very large amount of data is acquired in these analyses. Image data is gathered by scanning dye-stained or silver-stained gels or by scanning autoradiographs or fluorographs. After correcting for film response, or departure from Beer's law in the case of stained images, the pattern is reduced to an array of Gaussian spots, and overlapping spots are resolved (*Taylor* et al. 1980, 1981, 1982). Experimental patterns are stretched constellationally to match standard patterns, and the data is then stored as a spot list with sufficient information attached to each spot to allow the pattern to be reconstructed.

The end result in the TYCHO image analysis and data reduction system, which allows sets of analyses to be compared and differences to be presented as plots of amounts of protein in individual spots in two compared gels; or differences may be indicated by color changes on high-resolution color CRT displays (*Anderson* et al. 1981). A variety of other data reduction systems for two-dimensional gels have also been described (*Bossinger* et al. 1979; *Lemkin* et al. 1979; *Garrels* 1979; *Mariash* et al. 1982). It is not enough to be able to compare a small series of gels, however. One must be able to key into the increasing body of knowledge concerning individual proteins

and protein sets through the images themselves. This is now being done in several different ways.

First, each spot in a reference pattern of a cell or body fluid has a master spot number. By setting the cursor on a spot, that number appears together with (on a separate screen) a menu listing the types of information available (*Anderson* and *Anderson* 1982). Thus one may choose to have all known names for a particular protein, or its intracellular location, cells in which the protein is expressed, molecular mass, or a list of references relating to the protein indicated. This may be done not only for individual proteins, but also for sets of proteins (*Taylor* et al. 1982). For diagnostic purposes, one would want to know which proteins differ in a major way from those found in control preparations, and further, whether the sets of proteins which differ have anything in common. Have they been seen to vary together before? Are they all affected by a know drug? Are they indicative of a particular viral infection? To answer these questions, it is necessary to be able to confer interactively with a very large and constantly incrementing data base. From the point of view of this conference, it is important that the data base include information on virus-encoded proteins, and on cellular proteins altered in amount, distribution, or structure as a result of viral infection.

3.3 Standard Reference Maps

It is important to have reference maps of cells and tissues so that pre- and postinfection patterns can be compared. We have felt that it is important to identify as many proteins on these maps as possible and to determine spot coordinates as accurately as possible. The first problem has been to see if reproducible results can be obtained and whether the level of genetic variability seen in human cells and tissues is so high that identifications cannot be made. With systems for doing large numbers of analysis in parallel, and with careful attention to technique and to reagent quality, high reproducibility can be achieved. The problem of genetic variability has often been overestimated. In practice the level of heterozygosity for a given protein appears to vary greatly with its function, and proteins involved in structure formation appear to be under much greater constraints than those which are apparently free in solution, e.g. cell sap or plasma proteins (*McConkey* 1982). When lymphocytes from unrelated individuals are compared, approximately 1% of the spots on two-dimensional maps are not congruous (*Anderson N.L.*, unpublished data). Identifications may therefore be made with some certainty.

In this laboratory maps have been prepared of human plasma (*Anderson* and *Anderson* 1977, 1979; *Anderson N.L.* et al. 1982; *Anderson* 1981a), lymphocytes (*Anderson* and *Anderson* 1979; *Willard* and *Anderson* 1980, 1981; *Anderson* 1981b, c; *Willard* 1982a, b; *Anderson N.G.* et al. 1982; *Gemmell* and *Anderson* 1982; *Willard* et al. 1982; *Giometti* et al. 1982), muscle (*Giometti* et al. 1979, 1980a, b; *Giometti* and *Anderson* 1981b), hair follicles (*Anderson N.G.* and *Anderson N.L.* 1979), red-cell lysates (*Edwards* et al. 1979, 1981), fibroblasts (*Giometti* and *Anderson* 1981), urinary proteins (*Anderson* et al. 1979a, b; *Tollaksen* and *Anderson* 1980; *Edwards* et al. 1982), seminal plasma (*Edwards* et al. 1981), milk (*Anderson N.G.* et al. 1982), and saliva (*Giometti* and *Anderson* 1979).

3.4 Internal Standards

Two general types of internal standards are required. The first type aims at ultimately yielding numerical values for charge and SDS mass. We have prepared charge standards by sequentially carbamylating proteins such as creatine phosphokinase, carbonic anhydrase, or hemoglobulin subunits (*Anderson* and *Hickman* 1979; *Tollaksen* et al. 1981). When these are added to the initial sample mixture, an even series of spots, each differing by one charge, is produced across the gel, and the longitudinal position of other proteins may be compared with these reference spots in the standard charge train. Molecular mass standards have been prepared from muscle protein extracts (*Giometti* et al. 1980b) and may contain over 80 bands on SDS electrophoresis. The molecular mass standards are added in the agarose used to seal the first dimension gels into position between the second dimension plates.

The second type of internal standard is useful for studies on single proteins or very simple protein mixtures. The protein(s) of interest are run against a background of a known and characterized cell extract of which most proteins have been characterized for charge position (pI) and mass. Dr. Robert Stevenson, Director of the American Type Culture Collection, is arranging to prepare such standard cell preparations as a reference for general use.

4 Protein Identification

It is important to identify as many proteins in gel patterns as possible and to be able to reidentify them definitively in subsequent studies. Identification by position and spot numbers is obviously one method; however, it is insufficient for many of our purposes.

4.1 Identification by Activity

The obvious method for the assignment of an enzyme or other activity to a spot on a two-dimensional gel is to isolate the protein in question by classical methods and run it with and without samples of the starting mixture. (Alternatively, the purified protein may be labeled in a way which does not alter its charge.) If a number of different proteins in a complex mixture are to be identified, this is an extraordinarily laborious approach. In addition, many proteins are found to be partially degraded during isolation, especially if heating is used. Hence, other more convenient methods for identification are required.

When large numbers of gels may be run conveniently, and when enzyme assays may be run quickly and in large numbers, with a centrifugal fast analyzer (*Anderson* 1972) for example, then an alternative and more general approach may be taken. The starting mixture (e.g., an extract from virus-infected cells) is run through a series of separative procedures, and two-dimensional maps are made from all fractions. In parallel, enzyme activities of interest are measured in all fractions. From this data it can readily be determined which spots always correlate with a given activity (and several candidate spots for one activity may be found), and one can also determine

the most logical approach for the isolation of any enzyme (or any given spot). General methods have not previously been available for designing preparative procedures for the isolation of individual enzymes or antigens. This approach provides such a method. So far, it has been applied only to thermal denaturation (*Nance* et al. 1980), where it has proven to be quite useful. Substrates and cofactors may grossly alter the behavior or thermal denaturation temperature of specific enzymes during isolation and thus provide useful information both for identification and to guide isolation. This approach is useful when the objective is the identification of many different proteins in a protein mixture, as is the case with the Human Protein Index Project (*Anderson* and *Anderson* 1982).

4.2 Identification Using Antibodies

When antibodies against one or more proteins in a mixture are available, these may be used to assist in identifications and to solve the problem of whether spots seen in similar positions in different mixtures are indeed related or identical. Antibodies may be used in three general ways. If antibodies with known specificities are available, immunoprecipitates may be prepared or the antibody may be immobilized and used to isolate the antigen. The immunoprecipitates or the purified antigen may then be mapped. Alternatively, the antibodies may be used to remove one spot from the entire mixture, and that spot can then be identified on maps by difference. In the third method, entire two-dimensional patterns may be transferred electrophoretically to nitrocellulose or other immobilizing supports on which they renature sufficiently, in most instances, to bind polyvalent antibodies, which may then be detected in turn with suitably labeled counter antibodies (*Anderson* 1981a). Most monoclonal antibodies studied so far do not react with proteins on nitrocellulose transfers, however. It should be noted that polyvalent antibodies bound to spots on transfers may be used to immobilize an additional layer of nondenatured antigen which may be prelabeled, have been isolated in pure form, or which may be tested for enzymatic activity on the transfer using cytochemical techniques. In addition, very small amounts of specific antibody can be eluted from individual spots on transfers and used for cytological localization studies or for the isolation of small amounts of antigen using micro versions of the CYCLUM system (*Anderson* et al. 1975a).

4.3 Genetic Methods for Identification

Cells or viruses known to lack a particular enzyme, activity, or antigen may be mapped and compared with wild types to find which proteins are missing or altered. In many instances more than one protein is affected by a mutation or deletion, and a clear identification may not be possible. However, candidate spots can be located for further study.

4.4 Peptide Mapping

Single spots from two-dimensional gels may be conveniently cut out, reelectrophoresed through a band of an SDS-resistant protease, and the resulting peptide bands compar-

ed (*Giometti* and *Anderson* 1981a). Although pattern similarity does not prove that the compared proteins are identical, nonmatching patterns rule out identity.

4.5 Amino Acid Sequencing

Several laboratories are developing microsequenators which allow partial sequencing of a protein eluted from a single spot on a two-dimensional gel. This in turn allows the synthesis of a DNA probe with which to isolate the relevant gene. This completes the development of the minimum techniques required to interrelate high-resolution protein mapping and genetic engineering, and it allows, in theory, proteins identified on protein maps to be produced in large amounts for research purposes, for the development of clinical tests, or for replacement therapy.

5 Protein Modification and Processing

Many proteins synthesized for export (for example, cell surface or plasma proteins), many viral proteins, and a fraction of intracellular proteins are modified or processed post-translationally. It is useful to identify the primary translation product, and this may be conveniently done by translating isolated mRNA. Careful pulse-labeling studies using protease inhibitors in the solubilizing media, the use of inhibitors of glycosylation, and neuraminidase digestion of sialated proteins allow the relationships between the primary product and the processed products to be worked out. In addition, labeled primary products may be added to cell extracts and the reactions followed in time. Two-dimensional mapping using both isoelectric focusing to equilibrium and non-equilibrium pH gradient electrophoresis (so-called BASO gels) allow nearly all the components of a mixture to be seen and followed in time (*Willard* et al. 1979), facilitating the identification of intermediates and stages in processing.

6 Organization in Structures

To determine how specific proteins are arranged to form structures, it is essential to determine which proteins are nearest or contact neighbors. By cross-linking neighboring proteins with reagents which can be subsequently dissociated, dimers and multimers may be isolated, dissociated, and then mapped, as has been done with the Rauscher murine leukemia virus envelope glycoprotein (*Zarling* et al. 1980), for example. In addition, the subunits of multimeric proteins may be identified by electrophoresing protein mixtures in one direction in undissociated form, and in the second direction, after dissociation into subunits (*Nagai* et al. 1978). All proteins which were initially monomeric fall on a diagonal in such an analysis; those initially multimeric do not. Hence the term "diagonal electrophoresis".

7 Preparative Scale Counterparts

To make full use of high-resolution protein mapping, preparative methods of equal resolution are required. In some instances, proteins from spots recovered from the

analytical gels can serve for preparative purposes, and sufficient protein recovered to stimulate antibody production. However, there is still a requirement for isolating proteins from minor spots which may not be very antigenic. Intense efforts are now underway in several laboratories to provide high-resolution preparative counterparts of analytical two-dimensional gels (*Edwards* and *Anderson* 1981).

8 Variations of Two-Dimensional Electrophoresis

The two highest resolution analytical methods currently available for polypeptide separation are isoelectric focusing in the presence of urea and a nonionic detergent, and electrophoresis in the presence of SDS. Hence, the combination of these methods yields the highest resolution two-dimensional method and is referred to here as high-resolution two-dimensional gel electrophoresis. Any two other electrophoretic methods may also be combined, and nearly all have been for one purpose or another. For example, a nondenaturing two-dimensional separation may be carried out using nondenaturing isoelectric focusing in one dimension and nondenaturing gradipore electrophoresis in the second. Alternatively, standard gel electrophoresis, which depends on charge-to-mass ratio and to a lesser extent on sieving, may be used in one dimension; or, conventional electrophoresis at two different pHs may be combined. For studies on nearest-neighbor identifications, neighboring proteins may be cross-linked by dissociable reagents and separated in one dimension in cross-linked form by mass using SDS electrophoresis. They may then be separated in the second dimension in SDS after dissociation, as mentioned above. This allows identification of the cross-linked species.

9 Applications of Two-Dimensional Electrophoresis in Virology

The number of proteins encoded by the DNA or RNA of viruses is very small relative to the number of proteins encoded by mammalian host cell DNA. Using postinfection pulse-labeling studies, it is quite simple to sort out virus-encoded proteins from those of the host cell. For many studies on virion protein, especially those of small viruses, the high-resolution systems described here are not essential. Two-dimensional electrophoresis has been applied, however, to the analysis of a number of viral proteins as shown in Table 1 (Note that several different types of two-dimensional electrophoresis are listed).

Clinically, it is of interest to use high-resolution two-dimensional electrophoresis to search for proteins related, either indirectly or directly, to viral diseases in samples which may be available from patients. These may be either of viral origin or may result secondarily from infection (e.g.) by oncogenic viruses which affect host gene expression). Further more, one may be confronted with a previously unknown virus and hence have little idea what to expect, either by way of new physical particles to be seen in s-ρ plots or new spots on two-dimensional gels. In such instances, purely empirical approaches are useful.

Recently, new proteins have been described in the circulating lymphocytes of patients with mononucleosis (*Willard* 1982b), as shown in Fig. 1. In addition, charac-

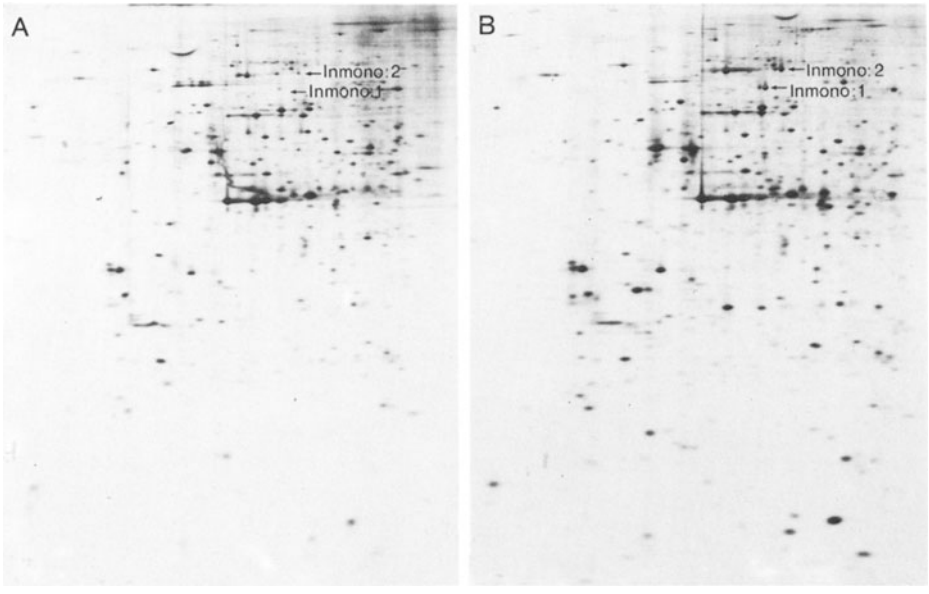
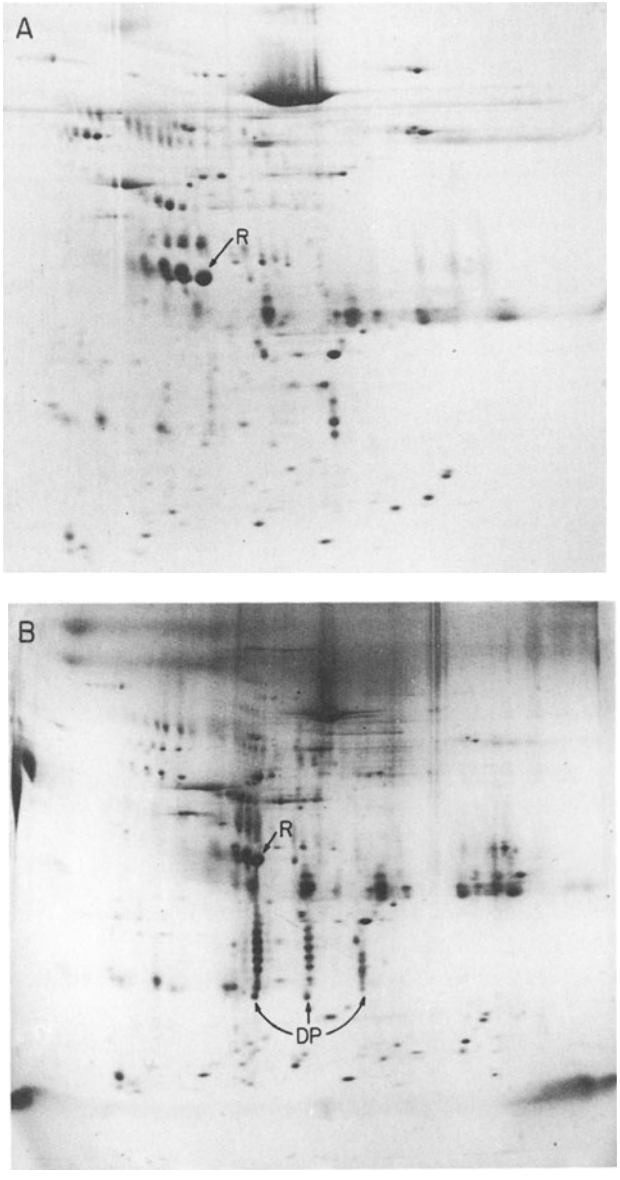


Fig. 1A, B. High-resolution two-dimensional electrophoretic analysis of leukocyte protein pattern changes observed in infectious mononucleosis. Sections of autoradiographs are shown from (A) a healthy 20-year-old female without mononucleosis, and (B) a 20-year-old female mononucleosis patient who was Monospot positive. The *Inmono* (infectious mononucleosis-specific) proteins are identified by number (*Willard* 1982a). The first dimension gels were run with pH 3.5–10 ampholytes. Slab gels were linear gradients of 10%–20% polyacrylamide from the top to the bottom of the gel. The gels are oriented with the basic end of the isoelectric-focusing gel to the right and the acidic end to the left

teristic changes in lymphocyte protein patterns have been observed in maps of lymphocytes from patients with rheumatoid arthritis (*Willard* et al. 1982) (not thought to be of viral origin). Further detailed mapping of human cells and tissues will doubtless turn up many new disease-associated alterations. Most will probably be due to changes in gene expression; however, a few will in all likelihood be due to viral infections. Once a disease-associated alteration is observed, the protein(s) involved may be isolated, antibodies prepared, and epidemiological studies initiated.

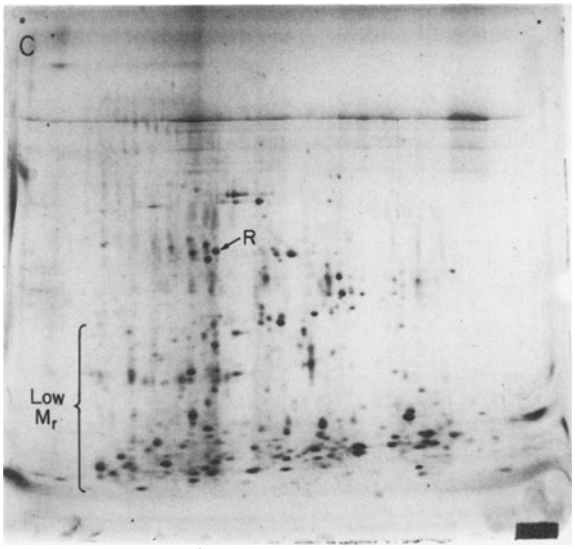
The use of nitrocellulose transfers opens up an additional interesting diagnostic possibility. By comparing patterns stained with pre- and postinfection sera, one may discover which protein in a cell extract is the antigen(s) being reacted to. In previous studies it has been shown that several hundred proteins could be resolved by high-resolution two-dimensional electrophoresis of concentrated human urinary protein samples. The human glomerulus passes proteins below about 50–60 000 Daltons hence, nearly all urinary proteins are below this cutoff point, and antibodies against early proteins of viral infection occur (*Griffiths* et al. 1980). Considering the mass of infected tissue in an average 70-kg man, the fact that infected cells leak viral proteins that some of these proteins are efficiently removed from the circulating blood, and that urine normally contains very little protein, it can be expected that proteins of early virus infection which do reach the circulation (*Griffiths* et al. 1980) will be found in the urine. The first question is simply, is there an increase in low molecular



weight proteins during infection? We have only begun to examine this question and have run urine samples from staff members with fevers of presumed viral origin. The results are shown in Fig. 2 and suggest that a systematic study of urinary proteins in viral infections in which the infective agent is identified would be rewarding. Some of the viral proteins may react on transfers with convalescent sera.

10 Nomenclature of Viral Proteins

If specific viral protein identification is to become more widely used diagnostically, a systematic method for identifying and describing the proteins, analogous to the



▲

◀ Fig. 2. A–C. High-resolution two-dimensional electrophoretic analysis of concentrated human urinary proteins. A) Representative pattern from normal adult male. B) Pattern observed during first day of fever of unidentified cause in an otherwise normal adult male. (a pattern very similar to that in (A) was obtained from this donor before and after illness.) Vertical spot trains marked *DP* are believed to be degradation products of proteins at the top of the train. C) Pattern observed during febrile illness of normal adult female. Note overwhelming increase in low molecular weight (*Mr*) peptides. (Normal patterns were also observed for this donor before and after illness.) Protein marked *R* is a characteristic urinary protein which serves as a landmark for comparing gels A, B and C

system required for the Human Protein Index, will be required. The simplest method would be the virus name, strain, and protein number, with the numbers assigned either in mass order or in chromosome position order. Such combined alphanumeric designators could then be used to interrogate a data base containing sequence and other analytical data as well as information on the source and specificity of both polyvalent and monoclonal antibodies.

11 Conclusions

High-resolution methods for protein separation and analysis allow virus-encoded proteins to be separated and identified and alterations in cellular proteins which may result from virus infection, to be detected. This allows not only a fuller understanding of all steps in virus infection, but the identification and isolation of markers which may be diagnostically useful. As new antiviral drugs become available, and interest increases in *not* using antibodies except when actually needed, it will become important to distinguish bacterial from viral infections early and to provide rapid and specific identifications for each. Tests based on specific viral or bacterial proteins using sensitive immunoassays may solve these problems. The first step is to identify potential indicators, and the techniques described make such identification possible.

Table 1. Two-dimensional electrophoretic analysis of virus-encoded proteins and proteins from virus-infected and virus-transformed cells

Virus	Type of analysis	Method used[a]	Cell or tissue	Reference
Adenoviridae				
Adenovirus 5	CR	B	CV-1 (Green monkey)	*Bosselman* et al. 1978
Adenovirus 5	CR	A (R)	BHK and hamster embryo	*Tuszynski* et al. 1979
Adenovirus 2	VC, CFT	A	KB cells	*Brackmann* et al. 1980
Adenovirus 2	VC	A	KB cells	*Symington* et al. 1981
Adenovirus 12	VCFT	A	Hamster cell lines, rat brain tumor	*Esche* and *Siegman* 1982, *Esche* 1982
Tupaia Virus	VI	A	Isolated virus	*Faissner* et al. 1980
Herpetoviridae				
Epstein-Barr	VTC	A	Burkitt lymphoma, EB^+ lymphoblastoid	*Bachvaroff* et al. 1981
Epstein-Barr	VTC	A	EB-transformed T and B cells	*Altevogt* et al. 1980
Epstein-Barr	VC	A	P3HR-1 Burkitt lymphoma cells	*Roubal* et al. 1981
Herpes type 1	VC	A	KB and BHK cells	*Cohen* et al. 1980
Herpes type 1	VC	A	Various	*Little* et al. 1981
Herpes type 1	VC	A	BHK cells	*Haarr* and *Marsden* 1981
Herpes types 1 and 2	VC	A	Vero cells	*Heilman* et al. 1981
Frog virus 3	VC	A	BHK	*Elliott* et al. 1980
Tupaia herpes	VI	A	Tree shrew tissues	*Faissner* et al. 1982
Papoviridae				
SV40	CR	B	GMK, CV-1	*Bosselman* et al. 1978
SV40, polyoma	VI, VC	A	Mouse embryo, GMK-CV-1	*Ponder* et al. 1977
SV40	VI, VC	A	CV-1	*Milavetz* et al. 1980
SV40, polyoma	VC	A	3T3B-SV40, BHK21 (polyoma)	*Bravo* and *Celis* 1980
SV40	VC	B (M)	GMK CV-1	*Bakayev* and *Nedospasov* 1981
SV40	VC	A	Monkey, mouse, human	*Fanning* et al. 1981
SV40	VC	A	BALB/3T3	*Strand* and *August* 1977

Virus	Type of analysis	Method used[a]	Cell or tissue	Reference
Poxviridae				
Vaccinia	VC, CR	A	Mouseblast carcinoma, EAT mouse L cells	*Beaud* and *Dru* 1980
Vaccinia	VI	A	Mouse L cells	*Tschannen* and *Schafer* 1980
Vaccinia	VC	A, C	KB cells	*Ichihashi* 1981
Monkey poxvirus	VC	A	Cynomolgus monkey kidney cells	*Kilpatrick* and *Rouhandeh* 1981
Coronaviridae				
JHMV and A59V	VC	A	Mouse 17CL-1 and DBT cells	*Bond* et al. 1979
Orthomyxoviridae				
Influenza A	VI	A	Calf kidney cells	*Horisberger* 1980
Influenza A	I	A	Embryonated eggs	*Leavitt* et al. 1979
Influenza A	VI	A	–	*Ulmanen* et al. 1981
Influenza	VI, VC	A	Madin-Darby canine kidney cells	*Privalsky* and *Penhoet* 1978
Influenza	VC	A	Chick embryo fibroblast	*Petri* and *Dimmock* 1981
Paramyxoviridae				
Newcastle disease virus	VI	C	–	*Nagai* et al. 1978
Newcastle disease virus	VI, VC	C	Chick eggs	*Samson* et al. 1980
Newcastle disease virus	VI, VC	A	Chick embryo fibroblasts, chick eggs	*Chambers* and *Samson* 1980
Newcastle disease virus	VC	A	Chick embryo fibroblasts	*Chambers* and *Samson* 1982
Newcastle disease virus	VI	A	Chick eggs	*Smith* and *Hightower* 1981
Newcastle disease virus	VC	A	Chick embryos	*Samson* et al. 1981
Picornaviridae				
Encephalomyocarditis virus	VI	A	Hela cells	*Churchill* and *Radloff* 1981
Poliovirus	VI	A	–	*Hamann* et al. 1977
Poliovirus	VC	A	Hela cells	*Van Dyke* and *Flanegan* 1980
Poliovirus	VC	A	Hela cells	*Wiegers* and *Dernick* 1981
Foot and mouth virus	VC	A	–	*McCahon* 1981
Foot and mouth virus	VI, VC	A	BHK cells	*King*, this volume, p 219

Table 1. (continued)

Virus	Type of analysis	Method used[a]	Cell or tissue	Reference
Rhabdoviridae				
Rabies	CR	A	BHK cells	*Naito* and *Matsumoto* 1978
Vesicular stomatitis	CR	B	Mouse L cells	*Marvaldi* and *Lucas-Lenard* 1977
Vesicular stomatitis	VI	C	BHK-21 cells	*Dubovi* and *Wagner* 1977
Vesicular stomatitis	CR	B	CV-1 cells	*Bosselman* et al. 1978
Vesicular stomatitis	VI	C	BHK-21 cells	*Muds* and *Swanson* 1978
Vesicular stomatitis	CR	B	Mouse L cells	*Jaye* et al. 1980
Vesicular stomatitis	VI	A	–	*Kingsford* et al. 1980
Vesicular stomatitis	VI, CR	C	BHK	*Thimmig* et al. 1980
Vesicular stomatitis	CR	A	Human fibroblasts	*Weil* et al. 1980
Vesicular stomatitis	VC	A	Chinese hamster lung V-79	*Maack* and *Penhoet* 1980
Vesicular stomatitis	VI	A	–	*Hsu* and *Kingsbury* 1982
Retroviridae				
Friend Leukemia	CR	A	Friend erythroleukemic cells (FrC18)	*Reeves* and *Cserjesi* 1979
Friend Leukemia	VI	C	Eveline cells	*Schneider* et al. 1980
B-tropic WN1802B	CR	A	Mouse embryo 10T 1/2-cl 8 cells	*Yoshikura* et al. 1982
Maloney murine sarcoma and murine leukemic virus	VI	A	BALB/3T3 fibroblasts	*Forchhammer* and *Turnock* 1978
Murine leukemia virus	VI	C	Mouse 60A lymphoblastic cell line	*Takemoto* et al. 1978
Murine leukemia virus	VC, CR	A	Maloney mouse lymphoma	*Fox* and *Weissman* 1979
Mouse mammary tumor	VI	A	Mm 5 mt/cl cells	*Nusse* et al. 1980
Mouse mammary tumor	VI	C	Mm 5 mt/cl cells	*Racevskis* and *Sarkar* 1980
Kirsten murine sarcoma, Maloney sarcoma virus	C, CR	A	NIH/3T3 and BALB/3T3 cells	*Strand* and *August* 1977, 1978
Gross murine leukemia	VC	A	C57 BL/6, AKR, MOPC–21	*Ledbetter* 1979
Radiation-induced leukemia virus	CR	A	Mouse tumor cells	*Meruelo* et al. 1978
Rauscher leukemia	VC, CI	A	JLS-V16 mouse embryo and JLS-V5 spleen	*Karshin* et al. 1977

Virus	Type of analysis	Method used[a]	Cell or tissue	Reference
Retroviridae				
Rous sarcoma virus	CFT	A	Chick embryo fibroblasts	*Kamine* et al. 1978
Rauscher leukemia	CR	A	Mouse JLSV5	*DeLey* et al. 1979
Rauscher leukemia	VC, CR	C	C57BL/6 mouse leukemia cells	*Zarling* et al. 1980
Avian Rous sarcoma	CR	A	Chick embryo fibroblasts	*Isaka* et al. 1978
Rous sarcoma	CR	A	Chick embryo fibroblasts	*Shiu* and *Pastan* 1979
Rous sarcoma	CR	A	Chick embryo fibroblasts	*Radke* and *Martin* 1979, *Radke* et al. 1980
Rous sarcoma	CR	A	Rat myoblast-mouse fibroblast hybrids	*Brzeski* et al. 1980
Avian sarcoma and murine sarcoma	CR	A	Indian muntjac cells	*Yuasa* et al. 1980
Rous sarcoma	CR	A	Chick embryo fibroblasts	*Laszlo* et al. 1981
Rous sarcoma	CR	A	Chick embryo fibroblasts	*Kobayashi* et al. 1981
Rous sarcoma	CR	A	Chick embryo fibroblasts	*Hendricks* and *Weintraub* 1981
Rous sarcoma	CR, VC	A	Chick embryo fibroblasts	*Gilmore* et al. 1982
Togaviridae				
Sindbis virus	CR	A	Aedes albopictus cells	*Eaton* 1982
Semliki Forest	VI, VC	C	BHK-21	*Richardson* and *Vance* 1978

Abbreviations: VI, isolated virions analyzed; VC, virus-encoded proteins identified in cell extracts; VCFT, analysis of peptides produced by cell-free translation of viral messenger RNA; VTC, virus-transformed cells mapped; and CR, cellular response to virus infection examined, e.g., effect of synthesis of cell proteins
A, isoelectric focusing in urea and NP-40 (either equilibrium or nonequilibrium) in the first dimension and electrophoresis in SDS in the second, according to *O'Farrell* (1975) and *Scheele* (1975); B, electrophoresis in acid urea in the first dimension and SDS in the second for ribosomal subunit analysis; and C, electrophoresis in SDS under nonreducing conditions in the first dimension and in SDS under reducing conditions in the second dimension to study cross-linking of nearest neighbors. R indicates dimensions are reversed; M indicates method has been modified

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