STUDY OF ISOTACHOPHORESIS CONDITION OF SERUM PROTEIN ANALYSIS

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The analytical conditions of serum protein using a capillary isotachophoresis were studied.

A Shimadzu isotachophoretic analyzer IP-2A equipped with two migration tubes in serial conection,UV detector and PG detector was used.Two migration tubes were of 1.0 mm I.D.,100 mm length and of 0.5 mm I.D.,300 mm length,respectively.The migration current was 75 μA.Serum was mixed with a carrier ampholytes as a spacer ion.

The experiments were carried out by using several viscous agents such as triton X-100,HEC,HPMC and PVA, with leading ion of chloride.The concentration of each agent was changed from 0.05% to 0.2% every 0.05 %.This resulted in the specific separation of serum protein at the concentration of 0.1% HPMC and 0.2% PVA.As HPMC showed a smaller noise level than PVA, HPMC was added to the leading electrolyte as a viscous agent.

The influence of several leading ions such as chloride ion,lactate ion and MES ion on separation of larger mobility component ions than alubmine was studied. 4.5 mM each leading ion with added 0.1% HPMC was ajusted at pH 9.1 by ammendiol.The useage of MES resulted in the exclusion of the component ions and the highest sensitivity of protein.

The effect of various pH range of the carrier ampholytes for the serum protein separation was investigated,which resulted in satisfactory separation over the pH range of 3.5 to 11.

Human serum,pI marker,alubmine,transfferin and γ-globulin were analyzed under optimum conditions.

Memo:

A NEW PREPARATIVE ISOELECTRIC FOCUSING APPARATUS

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The problem of large scale preparative focusing has been solved by our development of the recycling isoelectric focusing apparatus, RIEF (Electrofocus/78, Elsevier). In the RIEF, the flow of the recirculating liquid to be focused is stabilized by means of parallel filter elements. These elements, actually monofilament screens of fine porosity, avoid the problems of electroosmosis inherent in other continuous-flow electrophoretic instruments. Resolution achievable is of the order of 0.1 pH units, processing volumes being in the range of 200 to 10,000 ml.

To be able to handle smaller sample volumes, a new apparatus has been developed, embodying some of the same principles. Separation is achieved within an annulus between two concentric tubes, with coextensive electrodes at both ends. A series of parallel monofilament screens subdivide this annular space into twenty subcompartments of 2 ml. capacity, each. The outer plastic cylinder contains inlet and outlet ports for each subcompartment, while the inside glass tube is cooled by means of recirculating brine. The whole assembly is made to rotate around its horizontal axis. Hjerten was the first to show the effectiveness of rotation in overcoming gravitationally induced convection, but his apparatus was limited to narrow bore tubes.

The total volume to be fractionated, 40 ml. in the present configuration, is loaded into the apparatus, thereafter commencing rotation and current flow. After focusing for 2 to 8 hrs, all twenty fractions are collected simultaneously by simple means. The resolution of the apparatus is comparable to that of the RIEF or LKB density stabilized columns. Its main virtues are simplicity and speed of operation, the separation being achieved without the need of forming beds of supporting materials or density gradients.

Supported in part by NASA Contract NAS8-32950.

Memo:

IMPROVEMENTS IN PREPARATIVE ISOELECTRIC FOCUSING IN AGAROSE GELS.

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The use of agarose as a supporting medium for preparative IEF is improved by the addition of non-crosslinked polyacrylamide. Electroendomosis is significantly reduced and resolution of proteins improved but recovery of proteins is not affected.

Gels are composed of 1% IEF grade agarose, 1% non-crosslinked polyacrylamide and 2% ampholytes. IEF conditions are 0.03 Watt/cm^2 surface area for 50 V.hr/cm gel length. Resolution of proteins depends only on the final volt-hour integral - it is not influenced by varying the final field strength. Loading capacity depends on the amount of protein of interest in each homogeneous band and is approximately 40 µg protein/band/mL gel. Proteins which differ by as little as 0.01 - 0.05 pH units can be purified easily and with recoveries of greater than 80%.

Preparative IEF in 6.5M urea is often difficult in agarose gels. However, incorporation of non-crosslinked polyacrylamide reduces electroendosmosis and enables the gel to be focused for sufficient volt-hours to achieve optimal resolution. Urea gels require focusing for 120 V.hr/cm gel length at a constant power of 0.03 Watt/cm^2 surface area.

Preparative IEF is used routinely to purify intact human immunoglobulins and their fragments in the clinical investigation of lymphoproliferative disorders.

Memo:

ISOELECTRIC FOCUSING WITHOUT CARRIER AMPHOLYTES

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Steady-state rheoelectrophoresis, or isoelectric focusing without carrier ampholytes, using monovalent weak acid and weak base was discussed theoretically by Rilbe (Electrophoresis, 2:268-272, 1981).

A system using acetic acid as a weak acid, trisaminomethane, imidazole or 2-amino-2-methyl-1,3-propandiol as a weak base and AgaroseIEF gel as a supporting medium was found to work in practice without the complicated pumping device proposed by Rilbe (Electrophoresis, 2:261-267, 1981).

Main features of the procedure are formation of linear concentration gradients of weak acid and weak base in an agarose gel plate by combination of two wedge-shaped gel pieces containing acidic and basic buffer solutions, using large amounts of concentrated electrode buffer solutions to prevent pH change caused by electrolysis, and prevention of evaporation from and dew formation on the gel surface during electrolysis at low temperature (10°C) by covering with a polythene film.

Combination of reverse linear concentration gradients of one of the bases and acetic acid at concentrations between 0.006M and 0.038M, 0.006M and 0.031M and 0.006M and 0.025M formed on gel plates 10cm in width, 11cm in length and 1mm in thickness were tested.

Serum protein patterns obtained by electrolysis at current density of 1.25mA/cm gel width for 1.5 to 2 hours followed by Coomassie blue staining showed that the procedure worked approximately as an isoelectric focusing without carrier ampholytes.

Memo:

ISOELECTRIC FOCUSING WITH IMMOBILIZED PH GRADIENTS FOR THE ANALYSIS OF HUMAN GENETIC SERUM PROTEIN POLYMORPHISMS

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Stable immobilized pH gradients can be designed for very narrow pH ranges and are, therefore, suitable for the analysis of subtle inherited structural differences of proteins. Analyzed were the systems Pi, Gc, and Tf. For each serum protein system the appropriate pH gradients were determined. The common subtypes as well as the rare genetic variants of these systems can be readily classified. Improved resolution of very small IEP differences was accomplished.

Memo:

SMALL SCALE PROTEIN FRACTIONATION IN IMMOBILIZED pH GRADIENTS

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With the advent of immobilized pH gradients (Bjellqvist *et al.* J. Biochem. Biophys. Methods 6, 1982, 317-339), new possibilities arise for protein fractionation. Since the buffering groups responsible for the pH gradient are covalently fixed into the polyacrylamide matrix, no contaminant carrier ampholytes are co-eluted with the protein fractions, thus eliminating the need for an additional purification step. High purity is also obtained since the resolution needed to resolve two proteins with close pI's can easily be obtained by specially designing a narrow pH gradient, with full control of the experimental parameters, like buffering capacity, ionic strength and pH gradient.

We have developed a two-step technique based on IEF in narrow immobilized pH gradients in the first dimension (1-D), followed by an electrophoretic elution in an agarose gel containing an intermediate hydroxyapatite (HA) granulated gel layer (2-D). For the proteins we have investigated (hemoglobin, myoglobin, bovine serum albumin, carbonic anhydrase, ovalbumin and transferrin), when using a gel with dimensions of 250 x 110 x 0.5 mm and with a buffering capacity of 5-6 mEquiv./liter, the maximum loading capacity is in the range of 25-30 mg protein/ml gel. Above this limit, overloading effects can occur, such as droplet formation: this phenomenon, though, is only linked to the protein structure and behaviour, since the pH gradient, being grafted to the matrix, is totally unsensitive to such disturbances. After IEF, the Immobiline gel strips were transferred to the 2-D gel and the protein zones electrophoretically removed from the polyacrylamide matrix into the zone containing the HA-Ultrogel, whereafter the proteins were eluted by using phosphate buffer 0.5M, pH 6.8. By this method, the total protein recovery for the investigated samples is in the range 75-98%. (Supported in part by grants from Consiglio Nazionale delle Ricerche, Roma).

Memo:

EVALUATION OF STAINED AND UNSTAINED ELECTROPHEROGRAMS BY MEANS OF PHOTOACOUSTIC SPECTROSCOPY

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It is demonstrated that photoacoustic spectroscopy is a superior tool in the evaluation of electropherograms.

The local distribution of proteins in ultra-thin layers of polyacrylamide gels used for isoelectric focusing (PAGE-IEF substrates) is mapped with a resolution equal or better than with conventional densitometers. Even unstained samples of colored proteins can be mapped with excellent sensitivity and resolution due to the fact that the photoacoustic effect is caused only by the absorbed fraction of the incident light; scattered light therefore does not cause a signal. Photoacoustic spectra of previously localized zones allow the identification of the prosthetic groups of these proteins.

Memo:

This abstract is also presented in the Fee Communication session.

AN INTEGRATED APPROACH TO THE ANALYSIS OF HUMAN HAEMOGLOBIN VARIANTS BY COMBINING IEF, FPLC AND ELECTROPHORETIC TITRATION CURVE ANALYSIS.

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Electrophoretic titration curve analysis has been used to optimise the separations of human haemoglobins. By using chromatofocusing in the pH range 6.7 to 8.2 with a Mono P column on FPLC (Fast protein liquid chromatography), high resolution separations of haemoglobin variants, including A, F, A_2 and E were obtained. It was possible to resolve closely related variants (A_2 and E) using a narrow range pH gradient together with the zwitterionic agents taurine and betain. Peaks were identified by IEF.

Baseline separations of haemoglobins A and A_{1c} were obtained within 10 minutes using cation exchange chromatography on Mono S at pH 5.7. Peaks were eluted with a LiCl gradient. These conditions were chosen with the aid of information obtained from the titration curve analyses.

A combination of these three techniques (FPLC, IEF and titration curves) enabled high resolution separations of haemoglobins to be obtained rapidely and efficiently, both on an analytical and preparative scale. This is a methodological approach which can usefully be applied to the analysis of most protein mixtures.

Memo:

ISOELECTRIC FOCUSING OF PLASMA LIPOPROTEINS IN THE DIAGNOSIS AND PROGNOSIS OF LIVER DISEASE

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Recently we described a new high-resolution method of isoelectric fractionation of plasma lipoproteins prestained with tetrazolium blue, shown to be rapid and reproducible[1]. Here we report on the results of its application in the diagnosis and follow-up of liver damage.

In obstructive jaundice we observed the disappearance of VLDL and HDL fractions and an altered profile of LDL with one or more additional bands appearing in this region. There is also a clear quantifiable bilirubin band indicative of jaundiced serum. During recovery, after removal of the cause of obstruction, the isoelectric pattern returns to normal.

Patients with viral hepatitis (hepatocellular liver damage) show during the acute phase disappearance of the VLDL fraction and reduced or absent HDL and additional bands in the LDL region. It is interesting to note that during recovery VLDL slowly reappears, and so does HDL, only after an interim phase of multiple intermediary bands extending on either side of the LDL region, until normal band pattern and complete recovery are reached.

The isolation and identification of the specific bands of the LDL region during hepatocellular damage can provide substantial information on LpX, as well as other lipoprotein subgroups characterizing various types of liver disfunction.

1. D. Stathakos, G. Rekoumis, A. Avgerinos, N. Kalantzis, T. Kanaghinis in "Electrophoresis'82" (Walter de Gruyter & Co, Berlin, New York 1983) pp 665-674.

Memo:

DETERMINATION OF CYSTATHIONINE AND ITS DERIVATIVES USING ISOTACHOPHORETIC ANALYZER

H. Kodama, H. Mikasa and T. Ageta

A method for measurement of cystathionine and its derivatives in the biological samples has been developed by using an isotachophoretic analyzer. The determination of these compounds was carried out by measuring a zone length on a chart in isotachophoresis.

The amount of cystathionine in brains of normal rats determined by using this method was 0.084 ± 0.023 μmoles /g. This value agreed well with earlier reports.

The amount of cystathionine in several tissues of rats with experimental cystathioninuria was also determined. The results determined by using this method for the determination of cystathionine in the rat tissues agreed well with the results obtained by using an amino acid analyzer.

The determination of perhydro-1,4-thiazepine-3,5-dicarboxylic acid was also devised by this method.

Memo:

AGAROSE ISOELECTRIC FOCUSING - A PREPARATIVE TECHNIQUE FOR STUDYING THE IMMUNOSUPPRESSIVE PROPERTIES OF α_2-MACROGLOBULIN

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Over the past few years increasing attention has been given to the presence of immunosuppressive factors in the plasma of normal individuals and patients with different pathological conditions. Many studies have demonstrated the presence of immunosuppressive factors in plasma of patients with malignancy by variety of in vivo and in vitro techniques.

In this study, fractionation of the plasma from both normal subjects and patients with cancer by preparative isoelectro-focusing (IEF) with the Multiphor System has shown a major peak of inhibitory activity within the α_2-Macroglobulin region. The immunosuppressive activity of fractions was measured by their ability to prevent autologous or allogeneic lymphocyte reaction to purified protein derivative (PPD) in the Tanned Erythrocyte Electrophoretic Mobility (TEEM) test. Both the method by which the blood is collected and the storage of samples have been found to influence suppressive activity and the fractionation pattern on IEF.

The method of agarose IEF used in these studies has given excellent conditions for immunofixation, crossed immuno-isoelectric focusing and detection of immunoregulatory proteins in our assay system.

Pure α_2M was prepared by the method of Virca[1] and its isoelectric focusing pattern has been studied according to its concentration.

1. Virca G.D., Travis J., Hall P.K., Roberts R.C. Analyt.Bioc.1978;89:274.

Memo:

MEASUREMENT OF GLYCOSYLATED HEMOGLOBIN (HbA_1) ON CELLULOSE ACETATE MEMBRANES IN A MOBILE AFFINITY ELECTROPHORESIS (MAE) SYSTEM

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Affinity electrophoresis (AE) utilizes electrophoretic medium containing ligand capable of specific interactions with components of the mixture to be separated; the ligand is entrapped in the matrix of supporting medium. In MAE described, the ligand is in the mobile phase (buffer); standard cellulosic membranes are used.

The concept of MAE was applied to measurement of glycosylated hemoglobin (HbA_1) using dextran sulfate (DS) as active ligand. DS, an anionic substance, has affinity for non-glycosylated hemoglobins, presumably via the free terminal valine amino group, and thus increases their anodic mobility relative to HbA_1. DS is component of the electrophoretic, pH 6.5, citrate-EDTA buffer (Glyco-Phore Buffer, Gelman).

Washed red cells are hemolysed with saponin solution and electrophoresed on a mylar supported cellulosic membrane (Super Sepraphore, Gelman), for 45 min. at 150 V. The separations are stained with Ponceau S, cleared to transparency and scanned at 525 nm. The normal HbA_1 (%) values established for this procedure: 6.1, mean; 4.6-7.9, 5-95th percentile range; 5.4-6.7, 25-75th p. range. The test is independent of temperature between 4° and 30°C. Hemglobin variants do not interfere; HbC and HbS virtually comigrate with HbA and their glycosylated forms with HbA_1. HbF comigrates with HbA_1. Overall precision (CV) 3-5%. The results correlate well with Corning agar electrophoresis, and Isolab and Bio Rad mini-column procedures. Up to 24 samples can be run simultaneously in one chamber. It is a simple and economical test for monitoring long-term control in diabetic patients.

Memo:

REEVALUATION OF THE PREALBUMIN ZONE IN MURINE PLASMA : DISCRIMINATION BETWEEN CORTICOSTEROID BINDING GLOBULIN (CBG) AND THYROXIN BINDING PREALBUMIN (TBPA) BY CROSS IMMUNOELECTROPHORESIS (C.I.E.) AND AUTORADIOGRAPHY.

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The electrophoretic behaviour of a protein depends on the electrophoresis conditions. Therefore, the characterization of a protein solely by its migration in an electric field, relative to albumin may lead to major errors of interpretation. In particular the widely used name of prealbumin is confusing.

We present results in rat and mouse, showing that by using C.I.E. and subsequent autoradiography it is possible to locate unambiguously the TBPA and the CBG : two important hormone-binding proteins, which play major physiological roles, especially during development.

When the first dimension of the electrophoretic run is carried out in 7 % acrylamide (disc-electrophoresis), both proteins migrate as prealbumins.However, when it is carried out in gelose (conventional electrophoresis), the CBG remains a prealbumin but the TBPA becomes a post-albumin. Buffer parameters do not seem to have essential effects.

We have thus studied the concentrations of the two proteins during inflammation and ontogenesis in the rat. The comparison of our results to those of other authors show that these, having studied without discrimination the " prealbumin " zone, characterize in fact the patterns of CBG.

Our studies stress the necessity of defining a protein at least by one intrinsic property, such as a binding function.

Memo:

Rh_o(D) ACTIVITY IN BAND 3 OF RED CELL MEMBRANE

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The isolation of Rh blood type antigen from human red cell membrane has been frustrated due to its weak activity and integral membrane nature. We have suggested that Rh-Hr blood type activities were detected in only Band 3 of red cell membrane. We intend to confirm the suggestion by electrophoresis (EP) in this report. Stroma from D positive red cell was solubilized by 1% deoxycholic acid (DOC) and was applied onto Sepharose 4B and 6B and eluted with Tris-HCl buffer containing 0.2% DOC, followed by fractionation with activated thiol Sepharose 4B. By this method Band 3 which was confirmed to contain no carbohydrate was prepared. The Band 3 (2 mg/ml) was incubated with half volume of human anti-D (Ortho Co.) for 30 min to 3 hr at 37°C and then immune complex which was soluble in 0.2% DOC was produced. This complex was separated by Sepharose 4B gel filtration. The complex was not produced with red cell components other than Band 3 and with Band 3 from D negative red cell. The complex inhibited the reaction of anti-D and D positive red cells. Molecular weight of the complex formed by 1 hr incubation was estimated to be 500,000 to 700,000 by the gel filtration method. The molecular weight increased according to the time of incubation. The complex gave a precipitation line in counter immunoEP, a single peak in crossed immunoEP against anti-Band 3,and a line at -globulin region in immunoEP against anti-human serum. SDS-polyacrylamide gel EP of the complex showed the pattern consisting of Band 3 and fragments of IgG which were not stained with PAS reagent. These results support our results that Rh-Hr blood type activities located in Band 3 almost free of carbohydrate.

Memo:

THE BINDING SITES OF SEMINAL ACID PHOSPHATASE TO CANAVALIA GLADIATA DC LECTIN

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Our previous work has pointed out that a lectin (Canavalia gladiata,Can G) reacted specifically with acid phosphatase (AcP) of seminal plasma (SP). We investigate contineously the binding sites of the lectin to AcP by crossed immuno-affinoelectrophoresis(IAEP).Can G produced a precipitation line possessing AcP activity in Ouchterlony plate against SP, indicating that AcP of the complex with Can G remained its activity. The precipitate of AcP with Can G in a test tube was solubilized with α-methyl-D-mannoside and was fractionated with Sephadex G-200. Two protein fractions with AcP activity were eluted and its elution pattern was similar to that of fractionation of SP, suggesting that the reaction of Can G and AcP was reversible.

For IAEP with anti-SP three types of intermediate gels were prepared; reference gel, gel containing free Can G or agarose-bound Can G at a concentration of 0.1 mg/cm^2. AcP bound to anti-SP or to Can G was visualized with the azo-dye method. In IAEP a glycoprotein molecule possessing one binding site to Can G will be retained only in the intermediate gel containing immobilized Can G. In this experiment AcP was bound to immobilized Can G as well as to free one. It meant that AcP had two or more binding sites per molecule to Can G.To ascertain binding sites of AcP to Can G,AcP activity of frozen sections of the prostate were examined before or after treatment with Can G. AcP activity of the sections was inhibited with tartaric acid but not with Can G. However, Can G bound to AcP of the sections could be detected with FITC-labeled anti-Can G. It showed close agree with the result of IAEP. From these results it is concluded that AcP in SP has two or more binding sites per molecule to Can G which have different property to the substrate.

Memo:

HETEROGENEITY OF RABBIT ANTI-DNP ANTIBODY STUDIED BY TWO-DIMENSIONAL AFFINITY ELECTROPHORESIS

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Rabbit anti-Dnp antibody was separated two-dimensionally in the first direction by isoelectric focusing(IEF) and in the second direction by affinity electrophoresis(AEP).

Rabbits were immunized with Dnp-human-γg-globulin. The anti Dnp antibodies were purified by ammonium sulfate fractionation followed by affinity chromatography with a Dnp-lysyl-Sepharose column. The obtained antibody preparations were composed of almost only IgG species in tests by SDS-disc electrophoresis, Ouchterlony procedure, and immunoelectrophoresis. IEF was carried out in 5% polyacrylamide gel containing 6 M urea, 10% sucrose and carrier ampholyte pH 4.0 - 9.0. AEP was carried out with the buffer system of Reisfeld et al. in the presence of Dnp- or Tnp-polyacrylamide conjugate.

Anti-Dnp-antibodies were separated into over 50 distinct IgG-spots. All the spots appeared to be homogeneous. They were divided into 8 - 10 groups according to the extent of their affinity to Dnp- and Tnp-haptens. While the affinity of every group to Dnp-hapten was different from those to the heterologous Tnp-hapten, the affinity of every spots belonging to each group changed at the identical rate. This indicated each group may be composed of a monoclonal antibody. These antibody groups have 2 to 7 times stronger affinity to Dnp-hapten than to Tnp-hapten except one group, which has 1.6 times stronger affinity to Tnp-hapten. Dissociation constants calculated by the original affinity equation amounted to 2×10^{-6} M to 1×10^{-4} M for Dnp-hapten and 1.4×10^{-5} to 1×10^{-4} M for Tnp-hapten.

Memo:

This abstract is also presented in the Free Communication session.

EFFECT OF METAL IONS ON THE INTERACTION BETWEEN CONCANAVALIN A AND CARBOHYDRATE STUDIED BY AFFINITY ELECTROPHORESIS

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Concanvalin A (Con A) exists electrophoretically in the three molecular forms, type I, native and full-active form, type II, partially active form, and type III, apo-Con A or inactive form. Dissociation constant of type I and II for dextran calculated from the affinity equation at pH 4.3 at 20°C amounted to 0.29 and 1.1 mM, respectively. Type III had no affinity for dextran. Interconversion of the three forms of Con A was reversible.

In the presence of 2.5 mM Ca ion at pH 4.3, type III was not converted to type I or II. Its electrophoretic mobility was not changed. When the 0.01 mM Mn ion was present together with 2.5 mM Ca ion, type III converted to type II and I. In the presence of 1 mM Mn ion, Con A converted fully to type I. Co ion has a stronger effect and Ni ion a weaker effect than Mn ion has. In the presence of 2.5 mM Ca ion and 0.01 mM Co ion, type III converted exclusively to type I, while in the presence of 0.01 mM Ni ion, type III is still observed together with type II and I. In the presence of 1 mM Ni ion, type III converted exclusively to type I. On the other hand, Mg ion had weak effect. In the presence of 2.5 mM Ca ion and 1 mM Mg ion, three molecular forms of Con A are still observed.

Mobility of three types of Con A are different. Type II migrated 10% faster than type III and type I 10% faster than type II. Those enhancement of mobility appears to be due to binding of metal ions. From the results, it can be postulated that type III is a metal free form, while type II binds one molecule of metal ion, and type I two molecules of metal ions per subunit.

Memo:

IMMUNOELECTROPHORETIC ANALYSIS OF ACTIVATION OF COMPLEMENT BY CHORIONIC VILLI

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The aim of study : The mechanism of induction of contraction of uterus (labor pain) is not yet unknown. Aging of chorionic villi (placenta)will induce the contraction of uterus, as the results resection of chorionic villi will occur. This will be induction of labor pain.

Methods : Activation of complement by chorionic villi is determined by immunoelectrophoresis(Ph 8.5 veronal buffer including 0.01 mol EDTA. Heparin and zymosan and other acid polysaccharides are used as control. Chorionic villi of each trimester was used. CH 50, A1-CH50 were measured by hemolysis. C3, C3-activator, and C4 are measured by single radial immunodiffusion. Siliac acid in chorionic villi of each trimester was measured.

Results : In immunoelectrophoresis of the conversion of C3, the precipitate with anti-C3 appears in the zone of β-globulin and α-globulin and C3a(anaphylatoxin) was produced by necrotic or aged chorionic villi in which siliac acid decreased. In case of threatend abortion wit necrotic chorionic villi, CH50, A1-CH50, C3 and C4 and S3-activator in serum come down. By frish row chorionic villi in which siliac acid is includet,,the complement was not activated and in immunoelectrophoresis with anti-C3 the conversion did not appear.

Conclusion : The aged and necrotic villi activated C3, as the results C3a and C3b produced and C3a is anaphylatoxin.
These mechanism will become trigger of onset of labor.

Memo:

SENSITIVE AND SIMPLE IDENTIFICATION OF MONOCLONAL PROTEINS IN SERUM AND URINE BY IMMUNOFIXATION ELECTROPHORESIS (IFE)

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In IFE, the electrophoretically separated proteins of a single antigenic species can be anchored in situ via immunoprecipitation with monospecific antibodies (Ab). Following electrophoresis (EP) on Mylar supported cellulosic membrane (CM) (Super Sepraphore, Gelman), a 1 x 2 cm antiserum transfer strip (ATS) soaked with 25 ul monospecific antiserum, is applied over the electrophoretic track containing the monoclonal protein (MP) to be identified. The ATS/CM sandwich is incubated for 2 min. during which time the Ab molecules diffuse into the CM and form immune complex with the respective antigen (Ag) at the site to which it has electrophoretically migrated. Given the titre of heavy and light chains antisera supplied with the Ig-Fix Kit (Gelman) and to satisfy the Ag/Ab equivalence conditions, concentration of MP in the sample has to be adjusted by diluting to 100 mg/dL prior to EP. Following the incubation, all unreacted proteins are washed out in saline leaving only the immunoprecipitate in the CM. The immuno complex is visualized by staining with amido black.

The results are easy to interpret. The identity of MP is determined by the antiserum which gives positive reaction. The "fixation" band typically appears as a narrow, sharp band corresponding in position to the MP band in the reference track.

IFE is more sensitive and easier to interpret than immunoelectrophoresis (IEP). Particularly at low levels of MP relative to the corresponding polyclonal immuno globulin, IEP often fails to identify the MP (umbrella effect). The described IFE is as sensitive as IFE on agarose, but due to porous structure of CM it is much faster. The entire procedure takes less than 2 hours on CM vs. 1 to 1-1/2 days on agarose. IEF is applicable to identification of other proteins in EP patterns.

Memo:

IDENTIFICATION OF CELL-ATTACHMENT PROMOTING FACTORS FROM PARTIALLY HEPATECTOMIZED RAT PLASMA BY BLOTTING ELECTROPHORESIS

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Calf Fetal Serum(CFS) is needed in culture medium composition to facilitate cell attachment to plastic culture dishes. Rat serum can promote, like other animal sera, rat hepatocyte attachment with the same efficiency as CFS. This activity is due to plasmatic fibronectin. The plasma of partially hepatectomized (PH) rats allowed the adhesion of hepatocytes to culture dishes with much higher efficiency than normal rat serum. This activity sharply increased during the first four hours and remained high during the hyperplasic phase of liver regeneration (1). In order to identify the protein responsible for this biological action, the following technique was used. We first enriched the activity from PH rat plasma by ammonium sulphate precipitation and DEAE chromatography . The active fraction containing a complex mixture of proteins was electrophoresed in non denaturing conditions in a linear gradient (20-5% T, 2.5% C) polyacrylamide gel until the dye front left the gel. The cover glases were carefully removed and the gel was applied onto a nitrate/acetate mixed ester cellulose paper and electrophoretically transfered at a low voltage (2-3 V/cm) for 3 hours. Half of the paper was stained for proteins to asses the transfer into the paper. To identify the biological activity, hepatocytes obtained by collagenase perfusion were allowed to interact with the electrophoretic blotting in a cell incubator for 60 minutes. The paper was then washed with warm PBS and inmediately fixed with absolute ethanol, and stained with 2% eosine. Cells that were attached to protein bands gave a deep red band. A mjor active band was observed in the plasma of PH rats. The physicochemical and immunological properties suggest this protein is plasmatic fibronectin.

(1) J. Castell et al. 17th Meeting of the European Association for the study of the Liver. Abst. 123, Gotteborg, Sweden 1982

Memo:

FUNCTIONAL HETEROGENEITY OF RAT LIVER ISOFERRITIN DEPENDENT ON A SINGLE DOSE OF IRON

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Alterations of ferritin contents, H/L subunit ratio of ferritin and isoferritin pattern in the livers were studied by the rats at 3, 24 and 72 hr after a single dose of iron and/or by iron deficient rats. The following results were observed.

1) The concentration of liver ferritin protein at 3 hr after the iron dose was 3 times more than the control, but it decreased to the level of twice the control at 72 hr.

2) The H/L subunit ratio of newly synthesized ferritin was at 3 hr H-subunit excess (H/L:57/43) compared with the control (33/67). The ratio of iron deficient ferritin was 47/53.

3) The rat liver ferritin was fractionated to 6 isomers in the range from pI 5.04 to 5.72 by preparative isoelectric focusing. The most acidic isoferritin of pI 5.04 was H-subunit dominant (H/L:9/1). The ratio decreased gradually with the shift of pI to the basic side, and the most basic isomer became L-subunit dominant, and L-subunit consisted 77% of the composition.

4) The isoferritin patterns detected by the protein were altered after the iron administration. The pattern became the acidic ferritin dominant at 3 hr, then the intermediate type at 24 hr, and finally reverted to the level of the control at 72 hr after the iron dose.

Memo:

Hb F DETERMINATION BY THE AGAROSE GEL ELECTROPHORESIS

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Hb F determinations were carried out by the agarose gel electrophoresis, tris-EDTA-borate buffer, PH 6.5 - 8.5, and agar gel conc. 1.0 - 2.5 %. We found the preferred separation as followings;
1. Hb F separation was clear and definite in the conc of 2% agar gel and tris-EDTA-Borate buffer, pH 7.0.
2. Hb F conc in the newborn infants was 92-93.3 by this method and 56.8% by alkali denaturation. And no interrelationships between agarose gel and alkali denaturation were found.

Memo:

SIGNIFICANCE OF ELECTROPHORESIS IN OUR NEUROLOGICAL STUDY

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Authors are one of group which began silver stain in electrophoresis in Japan. Therefore we study the nervous system disease with electrophoresis. We want to present some cases.

Case 1 is leukoencephalopathy. She has headahe and nystagmus and symptom of the brain hyper-tension. CK-Mb band is dominant in her CK of CSF. Case 2 is lekoencephalopathy too.

Her disease is to bite her lip. CK-BB band is dominant in CK of liquor. Casel and Case 2 have some schape in CT. Case 3 is the baby of Hydrocephalus. CK-BB is dominant in his CSF.

We have the case of Syndrome Malin and Shizophrenie. Syndrom Malin has fever and muscular rigidity and high volume of serum CK-MM. But CK-BB is dominant in CSF. Two dimensional electrophoresis of serum CK and normal are same. It is known that Shizophrenie of psychotic signs like Halluzinose etc. has the rising CK. But Shizophrenie has not polypeptids in Anode side than prealbmin of CSF on silver stain. There are polypeptids in organic disease-brain tumor, meningitis in our study.

Lastly there is not change of subband of CK-MM on Myotonic dystropy discussed in the 33 Japanese circular.

Memo:

ELECTROPHORETIC ANALYSIS OF HOLSTEIN BEEF CATTLE SERA AFFECTED WITH LIVER ABCESS

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We studied about the changes of serum protein occured when fattening steers suffered liver abcess and following results are gained.

In the serum of liver abcessed fattening steers,the amount of total protein, α-globulin and γ-globulin increased and the decrease of alubumin and A/G ratio were seen. Cellurose acetate membrane electrophoretic patterns were devided into 5 types based on the shapes of γ-globulin area, i.e. AA-type : remarkable increase of γ_1- and γ_2-globulin were seen, A-type : comperatibly the increase of γ_1-globulin was remarkable, B-type : the increase of γ_2-globulin was comperatibly remarkable, C-type : the increase of γ_3-globulin was comperatibly remarkable, D-type : no remarkable increase of γ-globulin was seen. A-type were devided into 12 subtypes and named A-1 type～A-12 type. Samely B-type were devided into 11 subtypes and C-type into 4. Among these types C-types were considered to be the specific patterns of the liver abcessed fattening steeres.

Memo:

LATEX AGGULUTINATION TEST FOR ANALYSIS OF IMMUNOGLOBULINS IN CSF

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Determination of immunoglobulins in CSF has been rather difficult in routine analysis because of their low concentration. But recently its direct analysis became capable by Latex aggulutination reaction and also a new automatic method was developed. LA SYSTEM (AIC Co.) was used in the current study to measure immunoglobulins in CSF by the initial change in absorbance due to the turbidity created by the antigen-antibody reaction. Latex emulsion coated with purified anti-human immunoglobulin taken from rabbit serum, following Fritz et al's method, was obtained from Eiken Chemical Co. Delta absorbance was measured at 585nm between 35 and 135 seconds after mixing of sample (10-100µl) and reagent (400µl) at 37°C. Following the recommendation by WHO, a standard material with known concentration was used to obtain a standard curve (cubic equation). The levels of sensitivity when 100µl of sample was used were IgA 0.1 mg/l, IgG 0.1 mg/l, and IgM 0.5 mg/l, and upper limits in this situation were IgA 130 mg/l, IgG 180 mg/l, and IgM 150 mg/l. The samples with higher concentration were also analyzed by Single Radial Immunodiffusion method (LC Partigen, Hoechst Co.). The values obtained by two different methods had a higher positive correlation each other. The distribution of the data is shown in the Table.

		Range	Highest Distribution
Greater concentration was observed in the patients with brain tumor, mengitis, subarachinoidal hemorrhage, and subdural hemorrhage.	IgA	0.5-400mg/l	0.3 - 6 mg/l
	IgG	0.5-260	10 - 50
	IgM	0 -180	0 - 6

It was suggested that the method used in the current study could be useful in the laboratory because the precise analysis can be performed quickly using a small amount of sample.

Memo:

UNIQUE PROPERTIES OF BOVINE UBIQUITIN: ESTERASE ACTIVITY AND ACETYLATION AS JUDGED BY ELECTROPHORESIS

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Ubiquitin was isolated from bovine erythrocytes by a relatively simple procedure involving extraction with chloroform and ethanol, chromatographies on DEAE cellulose and gel filtration.

Ubiquitin exhibits esterase activity to p-nitrophenylesters but not to other esterase substrates.

It had a turnover number, 116 mmoles for p-nitophenol at pH 7.7 at 30°C and this activity was relatively stable to heat treatment. Electrophoretic studies indicated the ubiquitin was sequentially acetylated by p-nitrophenyl acetate as judged by the appearance of more anodically migrating components. Ubiquitin also has CO_2 hydration activity and can be localized by the CO_2-bromothymol blue method. The strong inhibitor of carbonic anhydrase, acetazolamide, is also effective against ubiquitin. An antibody against this protein did not precipitate with bovine carbonic anhydrase. These facts indicate that the ubiquitin has some of the biological activities of carbonic anhydrase. (This work was supported by the Grant-in Aid for scientific res. from Ministry of Education, Science and Culture, Japan and the Naito Foundation Res. Grant for 1982)

Memo:

AFFINOPHORESIS OF TRYPSINS. ELECTROPHORESIS OF TRYPSINS IN THE PRESENCE OF A SOLUBLE POLYELECTROLYTE BEARING AFFINITY LIGAND.

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We devised a new separation technique for the protein, "affinophoresis", which is based on its specific affinity and utilizes electrophoresis. This technique requires a carrier molecule, "affinophore", which contains both an affinity ligand for a certain protein and many charges, either positive or negative, in order to migrate rapidly in an electric field. When a mixture of proteins is electrophoresed in the presence of the affinophore, the protein having an affinity with the ligand will form a complex with the affinophore. This results in a change in the apparent electrophoretic mobility. If the protein is sufficiently accelerated, we can separate it from other materials.

A cationic affinophore for trypsin was prepared. Soluble dextran (MW 10,000) was coupled with a DEAE-group and m-aminobenzamidine, a competitive inhibitor of trypsins. Electrophoresis of trypsins from several origins on agarose gel plates in the presence of the affinophore showed that affinophoresis actually occurred. The electrophoretic mobilities of trypsins increased towards the cathode, the same direction as the affinophore movement. The presence of leupeptin and treatment of the trypsins with TLCK suppressed the effect of the affinophore. Streptomyces griseus trypsin, contained in Pronase, was easily separated and detected.

This procedure is distinct from affinity chromatography and so-called affinity electrophresis in that the support of the affinity ligand moves, and has advantages especially for analytical purposes: for example, the detection of specific molecules regardless of their isoelectric points.

Memo:

This abstract is also presented in the Free Communication session.