RELATION BETWEEN AGGLUTINATION AND ELECTROPHORETIC MOBILITY OF SHEEP ERYTHROCYTES

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It was recently shown that agglutination of lymphocytes was induced by particulate materials such as histone (Sabolovic, et al., 1975) and synthetic peptide (Bauer and Ax, 1977) in the malignancies. The purpose of this study was to measure change of surface charge of the sheep erythrocytes incubated with histone and poly-L-lysine, and ability to agglutinate with these materials.

Fresh and tanned and sulphosalicylic acid-stabilized sheep erythrocytes (EIC, supplied by Behringwerhe) were used. Electrophoretic mobility was measured by an analytical cell microelectrophoresis apparatus (Sugiura Lab. Co., Tokyo). For 5×10^{-5} mg of poly-L-lysine electrophoretic mobility was not changed in fresh erythrocytes, but reduced in EIC. Agglutination score was +2 in addition of fresh erythrocytes with 5×10^{-4} mg of poly-L-lysine, and electrophoresis could not be measured due to strong agglutination. However, even for 5×10^{-2} mg of poly-L-lysine, agglutination score was +1 and electrophoretic mobility was 0.536 μ/sec/V/cm in EIC. For histone isolated from calf thymus, the same experiment was performed. In 1 μg of histone, electrophoretic mobility of fresh erythrocytes was not changed, but that of EIC was reduced. Agglutination with 1 μg of histone showed score +1, and that with 5 μg of histone was score +4 in fresh erythrocytes. In EIC agglutination with 5 μg of histone showed score +2. These results suggested that decrese of zeta potential in cell surface was not correlated with strength of agglutination.

Memo:

A FUNCTIONAL TEST OF THE CHORIONIC VILLI

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OBJECTIVES: If intrauterine fetal death occurs in pregnancy, chorionic cells seem to be rejected by cellular immunoresponse. What is the mechanism of rejection? We studied this mechanism by means of the Tanned Sheep Erythrocyte Electrophoretic Mobility (TEEM) test. Because this method is simple and easy and we can get constant data.

METHOD: As antigen, chorionic protein was extracted according to Field & Caspary (1970). Human lymphocytes were isolated by the Ficol Conrey method or human lymphocytes in heparin blood supernatant was used. Chorionic protein (100ug) and lymphocytes (1×10^6/ml) were mixed in RPMI and incubated for 60 min. at 37°C. This incubation mixture was then centrifuged and to this supernatant tanned sheep erythrocytes (4×10^7/ml) were added and incubated for 60 min. at 37°C according to Stavitsky's method. The slowing time of electrophoretic mobility of erythrocytes was measured.

RESULTS: In cases of normal pregnancy slowing time did not change significantly, but in cases of abnormal pregnancy, especially threatened abortion and intrauterine fetal death slowing time was remarkably increased in the TEEM test. Lymphocytes of pregnant women release lymphokines, so-called macrophage slowing factor (M.S.F.) after incubation with chorionic protein. The M.S.F. is able to change the migration of mactophages and change the mobility of indicator cells in a defined electrical field. In our study we used tanned sheep erythrocytes as indicator cells instead of macrophages.

CONSLUSION: This immunological method is useful as a chorionic function test.

Memo:

DIFFERENT DISTRIBUTION OF THE ELECTROPHORETIC MOBILITY OF PERIPHERAL BLOOD LYMPHOCYTES IN 3 BABIES WITH DI GEORGE SYNDROME

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The distribution and the mean value of the electrophoretic mobility of lymphocytes isolated from the blood of 3 babies with the rare Di George syndrome were compared and found to be essentially different: the distribution was bimodal in 2 patients but nearly gaussian with an abnormal mean value in the third case.

Cell electrophoresis method, slightly modified for the management of small quantities of cell suspension, may also be developed as a test of clinical relevance in neonatalogy.

Memo:

MEASUREMENT OF THE CELL POPULATION IN HETEROGENEOUS SUSPENSION BY THE AUTOMATED CELL ELECTROPHORETIC INSTRUMENT

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Although some kinds of automated cell electrophoretic instruments have recently been developed, it seems impossible to measure the cell population in heterogeneous cell suspension by the instrument using the scattering light principle, especially the mixture of cells with different size. We tried to measure the cell population using the automated cell electrophoretic instrument (Parmoquant) that could measure the each individual cell mobility by the same principle as manual instrument. This instrument consists of the microscope, TV camera, image processing and computor.

At first, the mixture of sheep (SRBC) and rabbit (RRBC) erythrocytes was measured by changing the ratio of SRBC to RRBC. The mean mobilities of SRBC and RRBC were 0.42 and 1.05 μm/sec/V/cm, respectively, and the histograms of high (HMC) and low (LMC) mobility cell population were separated completely. The ratio of SRBC to RRBC and the ratio of HMC to LMC were quite coincident ($Y=1.006X + 0.016$, $R=0.998$). And the mean mobility of whole cells changed linearly according to the ratio of SRBC to RRBC.

In the case of peripheral lymphocytes of normal donors, there observed two prominent peaks in the histogram of electrophoretic mobility, and cells were divided into two types; lower mobility cell (LMC) than 0.95 and higher mobility cell (HMC) than 0.95 μm/sec/V/cm. T cells, passed through the nylon wool column, revealed to be the HMC, and B cells, adherent to it, to be the LMC. And correlation coefficients between HMC and T cell (assayed by E rosette formation) as well as LMC and B cell (by EAC) were both 0.99. These observations suggest that it is possible to measure the mobility and population of the heterogeneously mixed cells by the automated cell electrophoretic instrument using the opto-electronic principle.

Memo:

SEPARATION OF RAT LIVER CELL ORGANELLES RESPONSIBLE TO HEMOGLOBIN-HAPTOGLOBIN METABOLISM BY MEANS OF CARRIER-FREE ELECTROPHORESIS

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Intracellular site of the incorporation and degradation of heme and globin moiety of hemoglobin-haptoglobin (Hb-Hp) in rat liver cells was investigated.

The Hb-Hp formed by intravascular hemolysis is removed by the liver parenchymal cells through the receptor specific for the molecule (K. Kino, H.Tsunoo, Y.Higa, M. Takami, H. Hamaguchi, and H.Nakajima, J. Biol. Chem., 255, 9616-9620 (1980)). Intracellular distribution of the Hb-Hp has been investigated by Percoll and discontinuous sucrose density gradient centrifugation as well as combined differential centrifugation and carrier-free electrophoresis after intravenous administration of (^{3}H-heme, ^{14}C-globin)Hb-Hp.

The Hb-Hp was incorporated first in organelles of low anodic mobility and low density (density range, 1.05-1.07 g/ml) recovered in Golgi subfractions of the liver cells in a substantially intact form, and then these organelles progressively acquired a higher density as well as a higher anodic mobility presumably through fusion with other organelles. In the resulting organelles of a high anodic mobility and a high density (1.07-1.15 g/ml), the Hb-Hp is dissociated symmetrically into two 82,000-dalton subunits at an early stage of degradation. Thereafter, the heme and protein moiety of the subunits would be converted to bilirubin and amino acids, respectively, via several intermediates in vivo

Memo:

The effect of phytohemagglutinin on the electrophoretic mobility of purple membrane particles

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The binding of phytohemagglutinin to purple membrane particles was investigated using the cell-electrophoretic technique. The mobility of purple membrane fragments was studied in order to measure the changes in the electric surface charge density. The effect of different concentrations of phytohemagglutinin on the electrophoretic mobility of purple membrane particles was examined.

The binding behaviour of phytohemagglutinin on the purple membrane surface was discussed.

Memo:

A rapid *in vitro* assay of lymphocyte dose response to immunodepressant agents

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Increasing interest is being shown in the clinical application of *in vitro* assays of patient immune response in assessing susceptibility to immunodepressant drugs.[1] The application of these assays is limited by the fact that they tend to be time consuming. We have had experience of a rapid (3 h) cytophereometric test of lymphocyte function - the Tanned Erythrocyte Electrophoretic Mobility Test (TEEM) which has proved of value in renal allograft prognosis.[2]

We have found that the test can be used to demonstrate a significant and reproducible dose response for normal lymphocytes to corticosteroid and Cyclosporin A and that this compares favourably with suppression both in our own transformation studies and published reports. We have used the test to demonstrate significant ($p<0.005$) and reproducible differences in lymphocyte response between normal and uraemic subjects. The described test may provide a convenient and safe means of testing patient immune response to a variety of medications.

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Memo:

A POSSIBLE MECHANISM OF IMMUNE REGULATION PRODUCED BY α_2-MACRO-GLOBULIN. A.H. Alomran, B.K. Shenton, D.M.A. Francis, P.K. Donnelly, G. Proud, R.M.R. Taylor, Department of Surgery, University of Newcastle upon Tyne, England.

Plasma samples from a group of 25 patients with malignant diseases, 20 patients with benign disease and 11 normal subjects were fractionated on an ACA22 Ultragel column. Fractions were tested for their suppressive effect on lymphocyte reactivity to PPD in the Tanned Erythrocyte Electrophoresis Mobility (TEEM) test.[1] Most of the plasma suppressive activity was associated with a_2-Macroglobulin (α_2M) in both normal subjects and cancer patients. α_2M binding to physiological levels of proteases was associated with an increase in the ability to suppress lymphocyte reactivity in the TEEM test. α_2M binding to protease releases a peptide which is a component of the α_2M. This peptide was shown to suppress lymphocyte reactivity to mitogenic, antigenic and allogeneic stimuli in the TEEM test and in the lymphocyte transformation assay. Physically and biologically similar peptides have been found in plasma of cancer patients,[2] patients with thermal burns,[3] uraemia,[4] acute pancreatitis, and rheumatoid arthritis.

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Memo:

DEVELOPMENT OF 3 ISOENZYME PROCEDURES FOR CHARACTERIZATION OF CELL CULTURE SPECIES BY AGAROSE ELECTROPHORESIS

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Contamination of continuously grown cell culture lines by other species of cells has been shown to be a widespread problem. Various methods to detect contamination exist but can be difficult to use and interpret. Electrophoretic methods exist but are difficult to put into general practice. We have optimized and developed three colorimetric reagents to easily distinguish between specific cell lines using thin-layer agarose gel electrophoresis. Reagents for purine nucleoside phosphorylase (EC 2.4.2.1, NP) malate dehydrogenase (EC 1.1.1.37, MD) and asparate aminotransferase (EC 2.6.1.1, AST) were formulated. Reagent for NP consists of 50 mM inosine, 250 mM potassium phosphate, $1x10^3$ units/L xanthine oxidase (EC 1.2.3.2), 0.2 mM phenazine methosulfate (PMS), and 17 mM malate, 30 mM NAD, 0.012 mM EDTA, 0.18 mM PMS, and 12 mM MTT. The novel AST reagent consists of 10 mM ∝-Ketoglutarate, 150 mM L-asparate, 125 mM NAD, 5 mM ADP, 0.04 mM pyridoxal-5-phosphate, $1.5x10^5$ units/L glutamate dehydrogenase (EC 1.4.1.3), 0.18 mM PMS and 12 mM MTT. Each of these reagents makes use of the purple formazan formed in the presence of the respective isoenzymes. The reagents are each prepared in 130 mM Veronal buffer pH 8.6. Separation is performed at 23 V/cm for 25 minutes on thin-layer agarose plates, maintained at 4-10°C, containing 65 mM Veronal, 0.035% EDTA and 5% sucrose at pH 8.6. One ml of reagent is then spread upon the gel and incubated at 37°C, washed in dH_2O and dried at 60°C. The duration of each step is 20 minutes.[2]

Separations are clear, well resolved and easily readable. As low as 25 IU/L for each isoenzyme is detectable with 1 ul of sample. The upper limit of linearity is at least 1000 IU/L per zone. Reproducibility of migration is better than ±2mm under these conditions.

Memo:

USE OF THREE ISOENZYMES FOR CHARACTERIZATION OF CELL CULTURE SPECIES BY AGAROSE ELECTROPHORESIS.

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Reports of cross contamination of tissue cultures have appeared frequently in recent years. The problem is quite common, with an estimated 25% of cultures having incorrectly designated identities, and occurs in laboratories and cell banks in several countries. We have studied the use of isoenzyme electrophoresis for routine monitoring of cell culture identity. In this method a combination of several isoenzymes from distinct alleles is used to describe the uniqueness of a given cell line. We describe the use of three isoenzymes to help characterize cultures.

This method separates the cytoplasmic enzymes purine nucleoside phosphorylase (NP), malate dehyrogenase (MD) and asparate aminotransferase (AST) by electrophoresis in a thin-layer agarose gel for 25 minutes at 23 V/cm. The locations of the enzymes are marked by an insoluble formazan produced after reaction in situ with an enzyme-specific reagent. The migration distances of enzymes vary in different species. These differences can be used to identify the cell species.

The reproducibility of migrations is most affected by the time, voltage and temperature of electrophoresis and must be controlled. A standard is used to correct for minor differences in experimental conditions. Interlaboratory results are repeatable to 2mm(1SD). The standard chosen is an extract of a mouse lymphoma line. A control is used to help verify that separations have occurred properly and to serve as a marker for the most commonly encountered contaminant, e.g. HeLa.

This system has allowed us to distinguish among thirteen cell lines derived from different species with at least 95% confidence. The result is unaffected by the tissue of origin or by strains within the species. With proper calibration the system can be used to assign species identities to unknown cell lines.

Memo:

ISOZYMES OF BETA-LACTAMASES FROM AEROMONAS HYDROPHILA

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Beta-lactamases hydrolyse the ciclic amide bond in the beta-lactam ring of penicillins, cephalosporins and related compounds. Isoelectric focusing has been used in the classification of beta-lactamases.

We wish report here the separation of beta-lactamases from two strains of Aeromonas hydrophila isolated in our laboratory from a clinical specimens. These strains shown different resistances to the beta-lactam antibiotics.

Cell-free extracts were prepared by ultra-sound treatment. Cell debris were removed by centrifugation at 48,000 xg, 45 min. in a refrigerated centrifuge. Supernatants are used as crude extracts. Beta-lactamases have been separated using a flatbed of polyacrylamide gel electro focusing, pH: 3.5-9.5. These enzymes have been visualized on the gel using a chromogenic substrate (nitrocefin). Beta-lactamases hydrolyse the amide bond in the beta-lactam ring of nitrocefin. This reaction results in a distinctive colour change from yellow to red.

We found two different types of beta-lactamases isozymes patterns from Aeromonas hydrophila:
TypeI: only one isozyme with a pI: 7.20.
TypeII: two isozymes with pI's: 7.20 and 8.70.

Memo:

ELECTROPHORESIS AND ISOELECTRIC FOCUSING OF NON-SPECIFIC ESTERASES IN BULL SEMINAL PLASMA

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Studies were carried out on the molecular pattern of bull seminal plasma non-specific esterases.

Disc electrophoresis analysis revealed no more than 3 isoenzymes of the non-specific esterases. The latter were separated by isoelectric focusing into 12 isoenzymes distributed in the pH range of 5.0-8.0.The isoenzymes of the non-specific esterases according to their activity manifested three degrees of staining:strongly,moderately and weakly.

Memo:

SEPARATION OF MOLECULAR FORMS OF RAT BRAIN SOLUBLE ACETYLCHOLINESTERASE (AChE) BY POLYACRYLAMIDE GEL ELECTROPHORESIS FOR THE STUDY OF THE MODIFICATIONS DURING INTOXICATION BY DFP.

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A rapid and simple method for the separation and quantitative evaluation of molecular forms of rat brain soluble AChE, using cylindrical gels and enzymatic detection with acetylthiocholine(AcThCh), was deviced. The three bands separated by electrophoresis corresponded to the peaks of enzymatic activity obtained by gel filtration on Sephacryl S-300 and by ultracentrifugation on sucrose gradient. Within the range of 1-5 mU (nanomoles of AcThCh hydrolyzed/min) the reaction on polyacrylamide gel showed satisfactory linearity and reproducibility. The molecular weights of the three forms, estimated according to Hedrick and Smith (Archs Biochem. Biophys, 126, 155, 1968) were of over 700,000, about 340,00 and 115,000.

A single administration of DFP-1.1 mg/kg s.c.- to weanling and adult male Wistar rats caused a comparable maximum inhibition of AChE (of about 80%) However, the complete recovery of enzymatic activity was considerably faster in weanling (2-3 days) than in adult rats (14-18 days). A transient increase of medium molecular weight forms during the initial phase of recovery was constantly observed both in weanling and adult rats. A long-lasting increase of medium forms was observed during repeated treatments of adult rats (subsequent doses of DFP 0.7 mg/kg on alternate days). These increases may be due to their accumulation as precursors of heavy forms in the biosynthesis of enzyme molecules or to their higher turnover numbers which would indicate their different metabolic activity and different physiological significance.

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Memo:

DETECTION OF ALKALINE PHOSPHATASE-LINKED IMMUNOGLOBULIN A IN HUMAN SERUM

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Alkaline phosphatase(ALP)-linked immunoglobulin(Ig) was found in the sera from 33 out of 10,000 patients by immuno-electrosyneresis(IES) on agar-agarose gel. In the sera from 5 of them, ALP-linked IgA was demonstrated. In this study, electrophoretic mobility and column chromatographic pattern of ALP-linked IgA were investigated in order to find the difference between ALP-linked IgG and -linked IgA.

The existence of ALP-linked IgA was confirmed by immuno-electrophoresis(IEP), immunofixation electrophoresis(IFE) and IES. By column chromatography on Sephadex G-200, high molecule ALP was demonstrated in both fractions of M and G-M in two patients, and only in G-M fraction in others. ALP activity of G-M fraction was found after IES or IFE with antiserum to IgA. G fraction containing normal molecule ALP and M fraction did not show ALP activity after IES or IFE. Therefore ALP-linked IgA seemed to be eluted in G-M fraction.

Only in one patient, electrophoretic pattern on polyacrylamide gel showed a slow moving ALP, so called ALP VI, besides liver isoenzyme. This ALP VI band was caused by the presence of ALP-linked IgG. A slow moving ALP band was not observed in 4 other patients. In one of them, only liver isoenzyme was shown. In two others, both isoenzymes of liver and bile origin were observed. Main ALP activity was present in the bone isoenzyme position in other child.

In conclusion, for the detection of ALP-linked IgA, immunological methods will be necessary and more efficient. Because ALP-linked IgA can not be observed by isoenzyme analysis like ALP-linked IgG is observed as ALP VI band.

Memo:

ELECTROPHORETIC ANALYSIS OF α-GLYCEROPHOSPHATE DEHYDROGENASE AND ITS ROLE OF METABOLIC REGULATION

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A glycolysis in the muscle of a patient with lactate dehydrogenase(LDH) M subunit deficiency is markedly retarded at the position of glyceraldehyde 3-phosphate dehydrogenase(GAPD) and glycogen or glucose-derived triose phosphates are partially trapped to glycerol and α-glycerophosphate due to the participation of cytosol α-glycerophosphate dehydrogenase(αGPD). (1,2) In this study, heterogeneity of αGPD is electrophoretically analyzed.

α-Glycerophosphate dehydrogenase is dimeric protein composed of different subunit H (heart type) and L (liver type). Thus, three forms are observed in various tissues using Cellogel and polyacrylamide gel electrophoresis. HH form is main isoenzyme in RBC and heart muscle and LL is found in liver, skeletal muscle, lung and kidney. Hybridized type of the enzyme, HL, is observed in almost all of tissues analyzed. Moreover, kinetic property of these three enzymes seems to be a little different. Then, not only the contents of the cytosol enzyme but also the kinetical difference could regulate the ratio between NAD and NADH in tissues. In contrast, GAPD in RBC and in skeletal muscle is kinetically and physically proved to be identical.(2) The data obtained from a patient with LDH M subunit deficiency suggested that GAPD might be regulated by the levels and the ratio of NAD and NADH. Then, the ratio and the contents of these three dehydrogenases are compared in several tissues and the regulatory role of cytosol αGPD is discussed.

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Memo:

ELECTROPHORETIC SEPARATION OF TRIPEPTIDE AMINOPEPTIDASE AND ITS DISTRIBUTION IN HUMAN TISSUES

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Tripeptide aminopeptidase (EC 3.4.11.4) catalyses the hydrolysis of tripeptides at their N-terminal peptide bond. Electrophoretic separation of tripeptide aminopeptidase from other aminopeptidases such as leucinaminopeptidase and arylamidase was performed using discontinuous buffer system described by Kohn (1,2). Moreover, quantitative activity staining method using leucine dehydrogenase as coupling enzyme revealed the heterogeneity of leucyl-peptide splitting enzyme activities. Then, substrate specificity and kinetic properties of electrophoretically separated aminopeptidases were easily analysed by the substitutions of substrate, leucyl-peptides, and by the addition of inhibitors in the activity staining reagent (3).

Employing this separation technique, we studied distribution and subcellular localization of the tripeptide aminopeptidase in human embryonal and adult tissues. In this study, L-leucyl-glycyl-glycine was used for the substrate of the enzyme. Most of the tripeptide aminopeptidase activities were recovered in the supernatant fraction of 105,000 x g and the cytosol enzyme activities were measured in several tissues obtained from surgical procedures. High activity of the enzyme was observed in liver, spleen, pancreas, intestine, kidney and lympho nodus. In contrast, the activity of the enzyme was relatively low in skeletal and cardiac muscles. Finally, clinical significance of the enzyme elevated in patient serum is studied and discussed.

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Memo:

LIVER MITOCHONDRIAL SPECIES OF CREATINE KINASE

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Four forms of creatine kinase (CK) from liver mitochondria were separated by agar gel electrophoresis; 2 bands at the cathodal side of CK-MM (CKmL1 and CKmL2 from the cathodal side), a band at the MM position (CKmL3) and the other between CK-MM and CK-MB (CKmLT). CKmL1 and CKmL2 were obtained directly from mitochondria of normal liver, and CKmLT was from that of metastatic tumor liver. CKmL3 was obtained after 2M/1 urea treatment of the above forms at 26°C for 30 min. The all forms are not adenylate kinases because they were invisible when creatine phosphate was omitted from the reaction mixture. The relative molecular masses of CKs were estimated to be approximately 320,000 to 350,000 for CKmL1 and CKmLT, and 80,000 for CKmL2 and CKmL3. The all forms reacted with anti-mitochondrial CK antibodies but not with anti-CK-M and -CK-B subunit antibodies. Therefore the antigenicities of the CKs were found similar to that of mitochondrial CK but not to those of cytoplasmic ones. The residual CK activities of CKmL1 and CKmLT after heating at 56°C for 4 min were 79% and 75% respectively, so that they are heat stable. CKmL2 was almost completely inactivated by heating at 56°C for 1 min. Electrophoretic mobilities of the CKs were unstable. After storage at -40°C for 3 years, CKmLT migrated to CKmL1 and CKmL3 positions. CKmL1, CKmL2 and CKmLT were also migrated at CKmL3 position after urea treatment. From the results, we conclude that CKmLT was a modified form of CKmL1 with negative charge, CKmL1 was an oligomeric form of CKmL2, and CKmL3 was the final product of the 3 forms. The negative charge of CKmLT is not due to carbohydrates or phosphoric acids because the CK had no affinity to concanavalin A and no sensitivity to neuraminidase or phosphorylase.

Memo:

LDH ISOENZYME OF RABBIT GRANULOCYTE

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LDH isoenzyme of rabbit granulocyte obtained from periferal blood and peritoneal exduate was studied.

Sample: Cells were collected from periferal blood or peritoneal exduate of rabbit by gravity-layer centrifugation. The sample (granulocyte) was obtained from one of cell layer. The sample was used after freezing-melting.

Electrophoresis and LDH: The method of thin-layer polyacrylamid-gel electrophoresis was used. After electrophoresis (200V, 20mA, 3hrs.) of sample, LDH staining was performed.

LDH isoenzyme of granulocyte of periferal blood was the next: LDH1 23.2, LDH2 23.2, LDH3 23.2, LDH4 18.5, LDH5 12.0(%). LDH isoenzyme of granulocyte of peritoneal exduate was the next: LDH1 10.1, LDH2 18.6, LDH3 23.7, LDH4 23.7, LDH5 23.7(%).

As seen in the results, composition of LDH isoenzyme was different in between granulocyte of periferal blood and granulocyte of peritoneal exduate.

Granulocyte of periferal blood was B dominant and granulocyte of peritoneal exduate was A dominant in LDH isoenzyme.

It is indicated that composition of LDH isoenzyme of granulocyte is able to change in it's condition.

Memo:

PROPERTIES OF NEWLY DISCOVERED LDH-X USING ISOELECTRIC FOCUSING

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We clarified the possibility of futher separation of sperm-specific human LDH-X by IEF.

We studied the properties of each LDH-X fractions and the relationship between the number of the spermatozoa and the motility.

We used the methodology of the optimum conditions of detection previously reported in the paper*1 and found as follows :

1) We could separate 5 fractions of sperm-specific LDH-X in spermatozoa by isoelectric focusing (from anode LDH-Xa, Xb, Xc, Xd, Xe).
2) The total LDH-X is lower in the group II, III having low sperm count, than in the normal group, and LDH-Xb fraction in the group II, III is slightly lower than in the normal group.
3) The percentage of LDH-Xc+ LDH-Xd in total LDH-X decreases in the group I, III having low motility compared with the group having higher motility.
4) The thermal stability of LDH-X is stronger in the low motility group compared with other groups.

We also have been investigating the amount and the composition of carbohydrate in LDH-X fractions.

*1 M. Yoshida, T. Imai, et al : J. Clin.Chem.Clin.Biochem. 19, 883, 1981

Memo:

STUDIES ON A SHIFT OF LDH-ISOZYME DURING HYPOXIA

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An increase in the proportion of M-subunit of LDH-isozyme has been reported in cardiac muscles of patients with coronary arterial disease. We induced a relative hypoxic state by the administration of thyroxine and an absolute hypoxemia by the low oxygen loading in rats, and studied the alteration of LDH-isozyme in their heart muscles and liver using electrophoresis.

There was a decrease of LDH-1 and an increase of LDH-2 in the heart muscles in the case of hyperthyroidism. While in addition to the same changes of LDH-1 and LDH-2, further marked increases of LDH-3 were observed in myocardial LDH-isozyme in the case of absolute hypoxemia. Namely a decrease of H-monomer and a increase of hybrid in LDH-isozyme of the hearts were recognized in hypoxic states. On the other hand there were no changes in LDH-isozyme of the liver during hypoxia. We also studied the myocardial LDH-isozyme of mice under the same hypoxic conditions. Mice died in an earlier stage than rats because of the different resistance to hypoxia. In their myocardial LDH-isozyme, there were more apparent findings of decreasing H-monomer and increasing hybrid during hypoxia.

To summarize our results, a shift toward an anaerobic isozyme distribution was observed in the myocardial LDH-isozyme during acute hypoxia.

Memo:

CHANGES OF PROTEASE INHIBITORS IN PATIENTS WITH DISSEMINATED INTRAVASCULAR COAGULATION

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Malignant tumors are the most common of diseases underlying DIC. We determined plasma levels of protease inhibitors and plasminogen and investigated the interrelations between plasma levels of protease inhibitors in patients with DIC underlain by malignant tumors. This paper reports the results thus obtained.

Materials & Methods: From serial admissions to the ward of the second Department of Internal Medicine of our hospital those patients who had presumed clinical evidence of intercurrent DIC were chosen for the study.

The primary diseases in these patients included leukemias, malignant lymphoma, multiple myeloma, stomach cancer and liver diseases. From each patients blood was taken at fasting early in the morning and the plasma separated therefrom was stored at -80°C until used for assay.

Seven protease inhibitors, i.e. α_1AT, α_2M, IαI, α_1X, AT III, C$_1$INA and α_2-plasmin inhibitor, and plasminogen in blood plasma were determined by single radial immunodiffusion method.

Plasma levels of protease inhibitors in DIC were thus demonstrated to undergo marked changes with the progression of underlying pathology, hence in good agreement with clinical course, and are considered to be of help in establishing an early diagnosis of DIC prior to its actual development.

Memo:

ELECTROPHORETIC ANALYSIS OF ESTERASE ISOZYMES IN ORGANOPHOSPHATE RESISTANT MOSQUITOES, CULEX PIPIENS

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Esterase isozymes of individual mosquitoes, Culex pipiens, were analyzed by thin layer agar gel electrophoresis. Changes in esterase isozyme patterns during developmental stages were studied. In egg, a pattern showing only one band was revealed. During larval stages many bands were gradually formed, and the most anodal bands were lost as the stages progressed to the pupa. These larva-specific esterases may be controlled by three or more co-dominant alleles. In the adult, many bands were revealed that were probably controlled by at least seven loci. Est-2 may be controlled by co-dominant alleles and by a recessive silent gene.

Esterase isozyme patterns were compared between organophosphorus insecticide- resistant and -susceptible strains. Resistant strains had very active esterase bands and the differences in pattern were correlated with the kinds of organophosphorus insecticides to which each strain showed resistance.

Memo: