dextran fatty

the highest sensitivities, general, depending procedure specificity (85.0%), EU/L.

necessary diagnostic sample testing that were lower than for serial testing.

A test for MI should have a high efficiency, a false negative being as undesirable as a false positive (9). The Embria-CK IRMA method produced comparable efficiencies for both hospital-admission-samples and serial testing, 85.0

87.5% respectively. Cellulose acetate electrophoresis is about as efficient as the IRMA method (82.5%), but less sensitive. The Du Pont CK-MB method, a relatively rapid procedure that may be used in an urgent situation, has the lowest diagnostic efficacy of the three methods studied for admission sample testing.

I conclude that, although not ideal, IRMA provides good diagnostic efficacy for the detection of MI 0 to 10 h after the onset of chest pain. Nonetheless, serial testing at 12-h intervals, beginning when the patient is admitted, remains necessary to confirm the diagnosis.

References


Cell Membrane Abnormality Detected in Erythrocytes from Patients with Multiple Sclerosis by Partition in Two-Polymer Aqueous-Phase Systems

James M. Van Alstine1 and Donald E. Brooks1,2

Erythrocytes from multiple sclerosis patients differ significantly (p < 0.005) from those from controls with regard to hydrophobic affinity partition in two-polymer aqueous-phase systems containing dextran, poly(ethylene glycol) and poly (ethylene glycol)-fatty acid esters. The most likely source of the abnormality is the cell membrane.

Additional Keyphrases: polyethylene glycol · dextran · fatty acid esters

Among the abnormalities reported for erythrocytes from patients with multiple sclerosis (MS) are increased cell diameter, enhanced osmotic fragility, altered linoleic and arachidonic acid composition in the membranes (1 and references therein) and the effect of exposure to polyunsaturated fatty acids (80 µg/mL) on the electrophoretic mobilities of both fresh and glutaraldehyde-fixed erythrocytes (1–3). Alterations in cell function or membrane lipid composition have also been reported for platelets (4, 5) and lymphocytes (6–8) from MS patients. These blood cell abnormalities may be related to changes in plasma lipids or other plasma components (1, 5, 8–10).

Cell partition in two-polymer aqueous-phase systems is an analytical and preparative technique that is sensitive to cell-membrane properties. When the neutral polymers dextran and poly(ethylene glycol) (PEG) are mixed in aqueous solution above certain concentrations, two phases form, with PEG predominating in the upper phase and dextran in the lower.

These systems can be rendered isotonic and buffered to physiological pH by the addition of various salts. Cells can be selectively partitioned into the upper phase in appropriately buffered systems on the basis of several cell-surface properties, including affinity for PEG molecules esterified to various saturated and unsaturated long-chain fatty acids (11–14). In the latter case the fatty acyl tails appear to intercalate into the lipid bilayer and (or) bind to other hydrophobic sites on the cell surface, coating the cell with PEG molecules. The PEG-coated cells exhibit dramatically increased affinity for the upper, PEG-rich phase.

In recent experiments we have attempted to differentiate between normal and MS erythrocytes with respect to their hydrophobic surface properties on the basis of their partition in two-phase systems containing micromolar concentrations of PEG esters of stearic (C18:0) acid and linoleic (C18:2) acid.

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Materials and Methods

Two-phase systems consisting of 50 g of dextran T500 (\(M_w\), the wt.-av. molecular mass, = 481 000, lot 7830; Pharmacia Fine Chemicals, Uppsala, Sweden), and 40 g of PEG 6000 (\(M_w\), = 6650, lot B-59-9104; Union Carbide, New York, NY) per kilogram of phosphate-buffered saline (150 mmol/L NaCl (ACS grade), 7.2 mmol/L \(\text{Na}_2\text{HPO}_4\) (ACS grade) and 2.3 mmol/L \(\text{NaH}_2\text{PO}_4\) (ACS grade), pH 7.2 at 20°C) were prepared as described previously (12, 13). The systems were allowed to equilibrate overnight in a separatory funnel and the upper and lower phases were isolated. Erythrocytes from MS patients or control volunteers were isolated by centrifugation \((500 \times g\) for 10 min) from citrated fresh blood and washed three times in phosphate-buffered saline. Erythrocytes from MS patients or normal subjects were added to an aliquot of the upper phase to produce a final concentration of approximately 12 \(\times\) 10^6 cells/mL. The cell-containing upper phase was recombined with an equal portion of lower phase in a 13 \(\times\) 100 mm glass tube. Either PEG 6000-linoleate or PEG 6000-stearate (custom synthesized by Chem Service Chemicals Inc., West Chester, PA), purified and analyzed as described previously (15), was then added at final concentrations of 2.00 and 0.25 μmol/L, respectively (sufficient to induce maximal erythrocyte partition into the upper phase). The tube was capped and its contents were mixed by inversion 20 times. The tube was then placed upright for 30 min until the phases had reseparated. The cell concentration in the upper phase was then determined and expressed as a fraction of the total number of cells added to the system. By chi-square analysis the probability that our normal subjects’ erythrocyte partition values were normally distributed was \(p = 0.990\) (16). On the average, 77.1% (SD 8.5%) of the normal cells added to an ester-containing system partitioned into the upper phase, compared with 4% in ester-free systems. Experiments were conducted blind, at the same time, on coded paired normal and MS or normal and control-disease samples of blood collected at the same time. MS and other control-disease subjects were age- and sex-matched as closely as possible to their paired normal subjects. Such pairing eliminated the influence of day to day variations in the experimental conditions such as room temperature. Individuals with various types of MS included seven with chronic progressive MS, 14 who had a history of relapse and remission, and two whose diagnoses were probable and only later well established. Control-disease samples were taken from six patients with other neurological disorders (hysterical paralysis, quadriplegia, paraplegia, meningitis, cerebral injury, and Guillain–Barré polyneuritis) and nine patients with acute non-neurological disorders (sepsis, staphylococcus septicemia, zoster uveitis, myocardial infarction, atrial fibrillation, undiagnosed chest pain, undiagnosed jaundice, hemoptysis, and malignant melanoma).

Results and Discussion

The MS and normal control results are shown in Figure 1, the partition behavior of the MS erythrocytes being expressed as a percentage of the value obtained for the normal sample of each pair. On the average, MS samples partitioned 7% less than normals into the upper phase \((p < 0.005,\) Wilcoxon rank sum test (16)) with either ester. A higher concentration of the unsaturated linoleate ester was required to achieve maximal partition, presumably because of the decreased hydrophobicity of this ester and the relative difficulty of inserting unsaturated chains into the lipid bilayer of the membrane (14). There appeared to be no correlation between the results and the MS patients’ age, sex, or stage of disease, although the sample populations are as yet too small to support definite conclusions on this. The factors responsible for this partition behavior and the degree to which it is specific to MS (17, 18) have also been preliminarily investigated. The partition behavior of the "other disease" control samples averaged 102.2 ± 7.7% of the normal cell upper phase partition \((p > 0.10,\) Wilcoxon rank sum test). The possibility of these results being a result of serum lipid alterations induced by drugs taken by the MS patients (19) seems remote, in that many of the control-disease patients were undergoing such treatment whereas many of the MS patients were not. Using radiolabeled PEG esters prepared as described previously (15), we have found that normal erythrocyte partition in these two-phase systems is directly related to the amount of PEO ester bound to the cell (20). Presumably MS erythrocyte membranes bind both saturated and unsaturated fatty acid esters differently from the way normal cells do (1-10). Although most of the ester must be bound to the lipid bilayer, this binding could well be modified by the presence of membrane protein and carbohydrate (13, 14), and abnormalities in any of these components could produce the observed results. Our observations therefore suggest that erythrocyte membrane alterations do occur in MS and are consistent with the hypothesis that lipids in blood-cell membranes are altered in this disease.

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Direct Analysis for Urinary Protein with Biuret Reagent, With Use of Urine Ultrafiltrate Blanking: Comparison with a Manual Biuret Method Involving Trichloroacetic Acid Precipitation

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We describe a method for measuring urinary protein with a centrifugal analyzer. Biuret reagent is used, and blanking with an ultrafiltrate of urine eliminates interferences from the nonprotein, biuret-positive chromogens in urine. We compare results by this new method with those by a manual method in which trichloroacetic acid precipitation and biuret reagent are used. The new method shows good precision and excellent correlation (r = 0.997) with the manual method. The ease and convenience of this assay should make this a useful method for the routine clinical laboratory.

Additional Keyphrase: centrifugal analyzer

Not all hyperproteinuria is clinically significant, but persistently high concentrations of protein in the urine may indicate renal or urinary-tract disease. Thus, detection and quantification of urinary proteins are important laboratory procedures (1), for which many methods have been devised.

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