Electrophoretic Behaviour of Some Human Blood Cells

Cell electrophoresis is a convenient method for obtaining information about cell surfaces. The electrophoretic mobility of a mammalian cell is due to the presence of fixed charged (ionogenic) groups and also of adsorbed ions at the surface. At physiological pH all cells so far studied are negatively charged[1,2]. The main reason for this charge is the presence of the terminal carboxyl group of n-acetyl-neuraminic acid, as demonstrated by neuraminidase treatment[3].

The measurement of electrophoretic mobility, reflecting the electrical state of that aspect of the cell in direct contact with its environment has been used, for example, to try to explain the adhesive behaviour[4] and the aggregation[5] of some cell types. However, while the electrophoretic mobility of red cells has been shown to be different in different species[6], it must always be borne in mind that the mobility can change in cells in mitosis[7] and in immature cells[3].

It is the purpose of this communication to report a study on fresh human peripheral blood cells.

Materials and methods. Blood was obtained from healthy donors of different blood groups using heparin (Liquemine Roche), 500 U per 20 ml blood, as the anticoagulant. All glassware was siliconized.

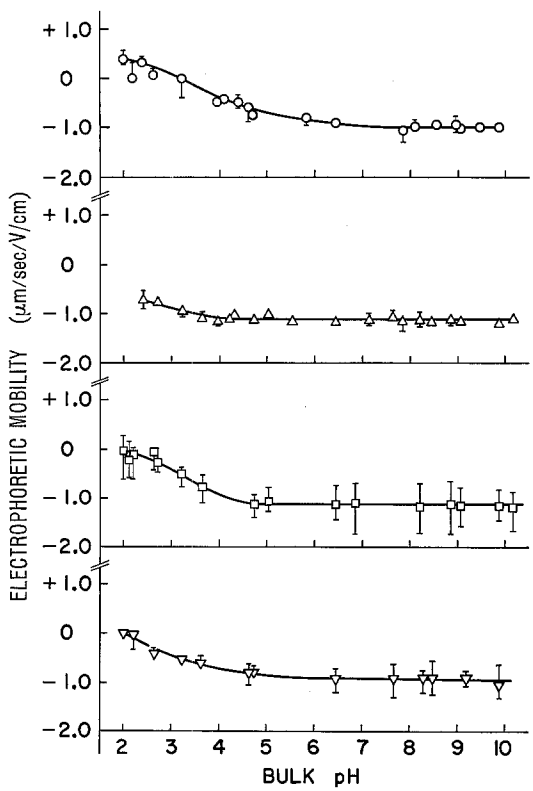
Lymphocytes or granulocytes were isolated by the technique of Böyum[8] using a Ficoll-Isopaque density gradient. After Dextran (T 500 Ph. F. Chemic,) sedimentation, the contaminating erythrocytes were removed by hypotonic lysis. Platelets were obtained from the leucocyte-platelet rich supernate after Dextran sedimentation of blood and centrifuging it at 100 *g* for 12 min. The plasma phase was used as a source of platelets. Contamination with other cells is not so important in the case of platelets as they are well recognized, by their small size, in the course of electrophoresis experiments. Red cells were obtained by centrifugation of blood at 400 *g* for 10 min, followed by washing with saline.

Each of the cell suspensions was washed 3 times with saline before being suspended in Michaelis buffer, for the electrophoretic mobility measurements. Suspensions of leucocytes were checked for their purity after May Grunwald staining of a dried smear, from 200 to 500 cells being counted. The isolated lymphocytes were of 95 ± 3% purity (6 experiments) and the granulocytes 95 ± 4% (5 experiments). Eosinophils were present from 2.5 to 7.8% among the purified granulocyte fractions.

Microelectrophoresis. Electrophoresis measurements were carried out at 25°C in a cylindrical cell apparatus with platinized platinum electrodes (Rank Bros., Cambridge), with the microscope linked to a closed-circuit television. Michaelis buffer at constant ionic strength was used in all the experiments. For each pH value the time to travel 56 μm (28 in each direction) was measured for 20–40 cells. At low (< 5) and at high (> 8) pH, all the measurements were carried out as rapidly as possible in order to avoid excessive cell damage.

Results and discussion. The Figure shows the electrophoretic mobility as a function of pH for platelets, red cells, lymphocytes and granulocytes. Each point represents the average electrophoretic mobility of 20 to 40 values. The range of the smallest and the greatest values of electrophoretic mobility is also shown on the Figure. There are some differences in the results presented, with respect to those of Bangham et al.[2], who studied the electrophoretic mobility of some sheep cells from blood, lymph nodes and the peritoneal cavity, mainly concerning the electrophoretic mobility of lymphocytes and platelets. These differences, especially at low pH, imply differences due to animal species or in the source of cells used.

Platelets have a very low positive electrophoretic mobility at low pH, having an isoelectric point at pH 3.25. Lymphocytes do not have a true isoelectric point, although at pH 2 some cells have a positive mobility, even though the mean mobility is zero. This behaviour is very similar to that found by others[9] for peripheral lymphocytes from a leukaemic patient. The curve in the Figure for red cells, showing a mobility of –1.1 μm/sec/v/cm at pH 7.4, is very close tothe value of –1.07 μm/sec/v/cm reported by Bangham et a l. for sheep[2]. Furthermore no differences in mobility were seen with respect to the age, blood groups or sex of the donor. Granulocytes show zero electrophoretic mobility at pH 2. The scatter in the values for lymphocytes and granulocytes and the fact that some of the lymphocytes at pH 2 have a positive charge, denotes the heterogeneity in these two populations, that



Electrophoretic mobility of various blood cells as a function of the bulk pH. ○, Platelets; △, Erythrocytes; □, Lymphocytes; ▽, Granulocytes.

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is not correlated to their morphology. That is to say, the distribution of electrophoretic mobilities within such a population of lymphocytes or granulocytes does not reflect the fact that large, medium and small lymphocytes are present, neither does it reflect the ratio of eosinophils: basophils : neutrophils in the granulocyte population.

It has been shown[3] that for different cells the relation between the electrophoretic mobility and the sialic acid released is by no means constant. In fact, granulocytes having more sialic acid per μm^2 of surface, have less charge than other cells[3]. These data and the curves reported here suggest the presence of other ionogenic groups, that in the case of platelets and of some lymphocytes become cationic at low pH, such as amino groups.

Riassunto. Viene riportato uno studio sui gruppi carichi alla superficie di alcune cellule del sangue, usando la tecnica della microelettroforesi. Differenze in mobilità elettroforetica, in funzione del pH, per piastrine, eritrociti, linfociti e granulociti sono presentate.

A. ZERIAL and D. J. WILKINS[10]

Battelle Research Center, 7, route de Drize, CH-1227 Carouge-Genève (Switzerland), 21 June 1972.

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Effect of Vasopressin on Hepatocytic and Ductal Bile Formation in the Dog

According to the current view[1], the secretion of bile by the hepatocytes is primarily dependent upon bile salt excretion. This 'canalicular bile' may subsequently be modified in bile ductules and ducts by the net addition of water and electrolytes. The latter mechanism is exemplified by the effects of the gastrointestinal hormone secretin[2].

A variety of other hormones have been shown to affect hepatic function and in particular bile formation. Except for hydrocortisone[3], their mechanism of action is as yet ill-defined. Furthermore, disparate results have been reported following administration of the pituitary hormone vasopressin[4,5]. They may reflect differences in methods of bile collection, the interference of anesthetic drugs and the lack of replacing the interrupted enterohepatic circulation with continuous infusions of bile salts.

To resolve these difficulties, the present studies were undertaken in the non-anaesthetized dog. The results clearly demonstrate a choleretic effect of vasopressin in the dose range employed.

Material and methods. The experiments were performed in 8 adult unanaesthetized female dogs (body weight 15–25 kg) at least 1 month previously cholecystectomized and equipped with a THOMAS[6] duodenal cannula. Prior to each study, the animals were fasted for 16 h. The common bile duct was catheterized under direct vision with a ureteral catheter (No. 6, french size) and quantitative collection of the biliary output was obtained by gravity drainage. To compensate for the interrupted enterohepatic circulation of bile salts, a continuous i.v. infusion of pure sodium taurocholate[7] (approximately 12 μEq per min) was administered by way of a polyethylene catheter throughout the study. Suppression of endogenous secretin release was achieved by i.v. injection of the anticholinergic pipenzolate methylbromide[8]. This procedure was shown previously to result in a relative steady state of bile formation[2]. Following a control period of at least 60 min, lysine-8-vasopressin[9] was given as slow single i.v. injections (during 10 min) in a dose of 0.5 U/kg body weight.

In 2 experiments erythritol-^{14}C[10] was administered at a rate of about 0.04 μCi/min after an initial priming dose of approximately 3 μCi[11,12]. An equilibration period of at least 60 min. was followed by the control and experimental periods. In these studies, sodium taurocholate was administered at a rate of 24 μEq/min. Carbon dioxide content in bile was measured with a Natelson microgasometer, and bicarbonate concentrations were calculated assuming a P_{CO_2} of 40 mm Hg. Chloride content was assessed with the Cotlove titrimeter, sodium and potassium concentrations with flame-photometry.

Bile acid concentrations were calculated as the difference between $\Sigma[Na^+ + K^+]$ and $\Sigma[Cl^- + HCO_3^-]$[2]. This procedure was shown to yield results in excellent agreement with direct determination of bile salts using a purified hydroxysteroid dehydrogenase[13]. Radioactivity in plasma and bile samples was measured with a Packard Tri-Carb model 3380 liquid scintillation spectrometer.

Results. An increase in bile flow was noted in all 8 studies within minutes following the start of the vasopressin injection. The increment in flow varied from 10% to 190% and was on the average 55% in excess of control values.

A typical study is depicted in the Figure. During this choleresis, in general, the concentrations of Na^+ and K^+ in bile fell slightly, whereas HCO_3^- and Cl^- concentrations rose. Consequently, the calculated bile acid content decreased in every instance.

Except for the output of bile salts, which remained relatively unchanged during the vasopressin-induced choleresis, the excretion rate of all electrolytes increased significantly in a given experiment. The mean increment amounted to 17.6 μEq/min of Na^-, 0.6 μEq/min of K, 10.1 μEq/min of Cl^- and 7.4 μEq/min of HCO_3^- (Table). The composition of this increment, calculated as the ratio of the increment in output of each constituent and the increment in flow, was characterized by a high bicarbonate (average 66.5 mEq/l) and chloride (average 98.6 mEq/l) concentration, similar to that observed following administration of the hormone secretin[2].

The clearance of erythritol-^{14}C was measured before and after administration of vasopressin in 2 experiments. The average clearance during the control phase ranged

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