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Red Cell Charge as Affected by Low Viscosity Dextran.* (31354)

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Electrical charge as a factor in the mutual repulsion of red blood cells was first suggested by Jevons in 1870(1). After the introduction of a microcataphoretic cell by Northrop and Kunitz(2), actual measurements became possible. Improvements in the technique were developed by Abramson(3) and by Moyer(4). Bernstein *et al* measured the cellular charge as affected by *in vitro* mixing of blood and low molecular weight dextran(5). The following study includes determinations of the effects of trauma and of low viscosity dextran† (LVD) on the surface charge of erythrocytes by means of a previously developed electrometric method (6,7).

Experimental procedure. Electrometric studies were made on the erythrocytes of 41 patients from the surgical wards of Cook County Hospital. Eleven untreated control patients who were awaiting elective cosmetic surgery were considered to be normal from the point of view of their circulation. Five other patients who had recently undergone elective herniorrhaphy were also studied. They were infused with one liter of physiologic saline at the rate of 5 ml per min before and after which electrometric determinations were made.

Thirty patients received intravenous infusions of LVD in the dose of 1.4 g per kg body weight at the rate of 5 ml per minute and the cellular charge was measured on samples obtained 30 minutes after termination of the infusion. Eleven of the patients were normal controls. Fourteen patients who had undergone either abdominal or head and neck surgery were infused 2 to 4 hours after operation in a similar manner. In addition, the electrical charge of erythrocytes of 11 patients were either septic or hypovolemic shock were also studied electrometrically. LVD then was administered to 5 patients of this group and electrical charge again was measured.

Electrometric Method. Ten ml blood samles were obtained by antecubital venipuncture. Heparin (10 units) was added to each sample. The samples were collected in plastic test tubes and were immediately centrifuged in a refrigerated centrifuge at 2000 *g* for 20 minutes. A small perforation was made in the bottom of the tube, and about 2 ml of packed cells were drained by gravity into a second test tube containing 1 ml of 0.15 M NaCl buffered at pH 7.4. This sample was centrifuged under the same conditions.

The electrometric method, as previously described for connective tissues(8), epidermis (9) and ascites cells(10 was adapted to the measurement of red cell charge.

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† Low viscosity dextran, Rheomacrodex®, was kindly supplied as a 10% solution in isotonic saline (Batch No. 4847), by the Pharmacia Laboratories, Uppsala, Sweden. Low viscosity dextran has an average molecular weight of 41,000 with an intrinsic viscosity of 0.19. For simplicity, low viscosity dextran is referred to as LVD.

$$Hg \mid Hg_2Cl_2 \mid KCl_{(sat.)} \parallel NaCl_{0.15M} \mid \text{Packed red cells} \mid NaCl_{(0.15M \text{ or } 0.015M)} \parallel KCl_{(sat.)} \mid Hg_2Cl_2 \mid Hg$$

Half cell I (Inner solution) (Outer solution) Half cell II

Diagram of electrical circuit

The half cells I and II were connected respectively to the inner and outer solutions by means of saturated KCl salt bridges, as indicated by the double vertical lines. Each junction was made with saturated KCl in solidified agar. This was contained in glass tubing drawn to a fine tip. Before measurement of the EMF, a 21 gauge perforation was made in the bottom of the tube containing the centrifuged cells. Electrical contact was made by immersing the bottom of the tube into a beaker containing the outer isotonic NaCl solution buffered at pH 7.4 (total phosphate, 0.015 Eq per liter; chloride, 0.135 Eq per liter). The circuit was completed by introducing the KCl-agar junctions into inner and outer solutions.

All electrometric determinations were made in duplicate. The first measurement of electromotive force, E_1, was made with buffered NaCl (solution 1) as the outer solution. After stabilization, this was replaced by solution 2, which was unbuffered 0.015 M NaCl. Stable potentials were obtained within 10-15 seconds, provided the surface had previously been immersed into another dilute 0.015 M NaCl solution to remove adhering drops of isotonic saline. After determination of a stable value of the displaced potential, E_2, the surface was reequilibrated with 0.15 M NaCl as the outer solution, and E_1 was redetermined, usually agreeing with the first determination to $\pm$ 0.2 mV. The dilution potential, E_d, is defined as the difference $(E_2\text{-}E_1)$, where E_1 is the mean of the initial and final reading. Thus:

$$E_d = E_2 - E_1 \qquad (1)$$

It has been previously shown(6-10) that the dilution potential, E_d, is determined by the density of immobile negative charge (x) of the colloidal surface, where x is expressed as equivalents per kg water. For values less than about 0.1 Eq, the relation is approximately linear:

$$E_d = -11.8 + 197\ x \qquad (2)$$

The constant —11.8 mV is the theoretical value of E_d at 25°C for a boundary at which the density of charge, x, is zero. The value of the factor 197 depends on standard values for the mobilities of sodium and chloride ions; it is also proportional to the absolute temperature. At low values of x (less than 0.059 Eq), the values of E_d are negative; at high values of x, they become positive. Negative values indicate that chloride and other ions transport more than half the current. Positive values imply that sodium conducts more than half the current, and this indicates a rather high value of x, the density of immobile negative charge, which is neutralized by sodium ions.

Results. A summary of the dilution potentials, E_d, and the negative colloidal charge, x, is presented in Table I, which includes mean values for normal patients, shock patients, and patients undergoing major surgery. It also includes mean values for each group of

TABLE I.

	No. patients	Avg age	E_d mV observed	(×) equiv calculated
Normal patients untreated	11	31	—2.5 ± .4	.046 ± .002
Major surgery patients untreated	14	42	+1.8 ± .8	.068 ± .004
Shock patients untreated	11	52	+4.4 ± .5	.081 ± .003
Normal patients treated with saline	5	27	—2.3 ± .3	.047 ± .002
Normal patients treated with LVD	11	31	+ .3 ± .5	.060 ± .002
Major surgery patients treated with LVD	14	42	+4.5 ± .5	.081 ± .003
Shock patients treated with LVD	5	49	+5.1 ± .7	.084 ± .003

patients, as observed following infusion of LVD. Untreated normal patients showed the lowest values of x (0.046 ± 0.002 Eq). After treatment with LVD, the same group of 11 normal patients showed about a 30% increase in the value of x (0.060 ± 0.002 Eq). A group of 11 untreated shock patients showed very high values of x (0.081 ± 0.003 Eq). After treatment with LVD, 5 of these subjects showed only small increases of x (0.084 ± 0.003 Eq). A group of 14 major surgery patients (untreated) showed moderately high values of x (0.068 ± 0.004 Eq). When treated with LVD, this group showed a considerable increase of x (0.081 ± 0.003 Eq). In 5 normal patients, saline infusion resulted in no significant change of the colloidal surface charge.

Discussion. The phenomenon of sludging is most commonly found in shock patients (11). It is this group which, in the untreated state, shows the highest density of negative charge (0.081 Eq). All of the 11 shock patients showed microcirculatory derangements associated with the high negative charge of the erythrocyte surface. On the other hand, all of the 11 untreated normal patients were free of circulatory derangements, and had low values of x (0.046 Eq). Therefore, in these 2 groups of patients, there is negative correlation between the density of colloidal charge and the tendency toward red cell aggregation.

In 3 groups of patients there is an increase of colloidal charge density following administration of LVD. This amounted to about 30% in the normal group, 20% in the group of surgical patients, and less than 5% in the group of shock patients. All patients in the surgical and shock groups showed marked improvement in the circulatory status after LVD infusion. This improvement, however, does not appear to be necessarily correlated with the increase of negative colloidal charge, which is very small in the group of shock patients. The group of normal patients showed the greatest increase of x (30%) after LVD infusion. Since no patient in this group had any circulatory disorder, the increase is not correlated with clinical change.

It has been found experimentally by Rothman *et al*(12) that LVD forms a film at the red cell surface (2.5 to 15 × 10^3 dextran molecules per cell). This film, according to the foregoing results, is characterized by a high density of negative charge when compared to that of the normal cell surface. However, it is not necessarily highly negative as compared with the surface charge of red cells in untreated pathological states. Nevertheless the LVD film on the surface of red cells seems always to inhibit the formation of cellular aggregations or "sludging" and to improve the microcirculation in pathological states.

Summary. The effects of trauma and LVD on red cell charge were studied electrometrically using normal subjects, post-surgical and shock patients. The highest levels of negative colloidal charge were found in a group of 11 shock patients, all characterized by cellular aggregation and microcirculatory derangements. The administration of LVD resulted in increases of negative colloidal charge in all patients. The greatest increases (30%) were found in normal patients. LVD infusion produced a marked improvement in the circulatory status of shock patients, but the negative colloidal charge was increased only by about 5%. The results show that the effect of LVD on agglutination of erythrocytes depends not only on the magnitude of the surface charge, but also on the physicochemical nature of the surface which is formed.

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Studies on Induction of Virus from Adenovirus and SV_{40} Hamster Tumors 1. Chemical and Physical Agents.* (31355)

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Cells of various animal species rendered neoplastic *in vivo* or *in vitro* by viruses commonly exhibit properties which are presently believed to be indicative of continued presence in the cells of viral genetic material even though the presence of infectious virus is no longer demonstrable. These indicators of viral presence include a new transplantation antigen and "T" antigen (sometimes referred to as neo or ICFA antigen) which is demonstrable either by fluorescence microscopy or by complement-fixation using sera from tumor-bearing animals as the source of antibody (1-13). That these antigens are virus-specific rather than host species-specific is taken as support for the concept of continued presence in the cells of part or all of the viral genome.

The retention of viral genetic markers in neoplastic cells of animals is of interest in relation to pathogenesis of tumor and raises the possibility of retention of viral genetic material in human tumor for which viral etiology is presently unproved. Development of methods for inducing retained viral genome in animal tumor to release infectious virus might find ready application in attempts to induce virus from human neoplastic tissues.

Several workers(5,9,13-18) have recorded attempts by a variety of methods to induce or detect occasional infectious virus release from animal tumors initiated by DNA viruses, mostly with negative results. Gerber(19,20), however, claimed to have induced infectious virus from "virus-free" but "virogenic" SV_{40} tumor using chemical or physical procedures or by copropagation *in vitro* with susceptible cells. Additionally, Sabin and Koch(21,22) have presented evidence to indicate spontaneous release, on rare occasions, of infectious virus from SV_{40} tumors and have demonstrated increased release of virus by prolonged cultivation of tumor cells *in vitro*, by X-irradiation or by copropagation with indicator cells in culture.

Extensive studies carried out in our laboratories during the past 2 years have sought to ascertain, in a definitive way, whether infectious virus could be induced from virus-free SV_{40} or adenovirus hamster tumor cells propagated *in vitro*. The methods included exposure to chemical agents known to have metabolic significance, to X-ray, to increased oxygen, and to potential helper viruses with assay for virus by subculture into susceptible cells and by copropagation with susceptible indicator cells. Tumor cell cultures proved free of infectious virus were chosen for study since these seem to bear the closest analogy to human tumors, which, to the present, have not yielded infectious virus(1,23-25) of proved etiologic significance. The present report summarizes the work relating to attempts to induce infectious virus from tumor cells or from "superinfected" tumor cells employing chemical and physical procedures. A sec-

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