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*ELECTROCHEMICAL PRECIPITATION OF HUMAN BLOOD CELLS AND ITS POSSIBLE RELATION TO INTRAVASCULAR THROMBOSIS**

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Communicated January 27, 1964

Several recent experiments have suggested that surface chemical phenomena are involved in both the prevention and production of intravascular thrombosis.[1-4] These experiments indicated that polarization of the blood vessel wall resulted in the formation of an intravascular thrombus on the positively polarized wall. However, the precise mechanism is not known.

We have carried out simple experiments designed to measure the precipitation potential of the various cellular elements of blood on metal electrodes. It was found that human erythrocytes and leucocytes deposit semireversibly on both platinum and gold at a reproducible potential. This potential is the same for both metals, and at a pH of about 7.4 is approximately + 0.33 volt ± 0.02 volt with respect to the reversible hydrogen electrode.

The precipitation potential is demonstrably independent of concentration of red blood cells over a range of three decades (10^3–10^6 RBC/ml) but depends on the pH of solution, changing approximately 85 mv ± 15 mv per unit of pH change.

These findings show that the cellular elements of blood undergo some change in contact with a surface polarized to a potential more positive than a critical value. This would seem to be due to a reduction in the density of negative charge[5] on the blood cell wall with a consequent reduction in the stability conferred by mutual repulsion of the particles. We are not suggesting that a simple process of this type is necessarily all that is involved in such a complex process as the clotting of blood, but rather that since thrombosis may be induced in such a simple and controllable way, useful information may be gained from a study of this phenomenon.

By implication at least, this would suggest that the blood vessel wall exists in a state which ordinarily prevents cellular precipitation on the wall by the mechanism described here. An unproved corollary of this general observation would suggest that the protein elements in the plasma also have a charge similar to that of red cells. This possibility is now being investigated.

Materials and Methods.—Precipitation chamber: The precipitation chamber consisted of a Lucite box with optical glass sides. A thin platinum rod was inserted through the side of the box close to the anterior optical glass surface through which it was observed. This platinum rod served as the precipitation electrode. Two 5-mm holes were drilled into the top surface of the chamber in the upper cor-

ners so that agar bridges could be inserted into the erythrocyte-containing solution, thus making connection to saline or Krebs solution in a beaker on either side of the chamber (Fig. 1).

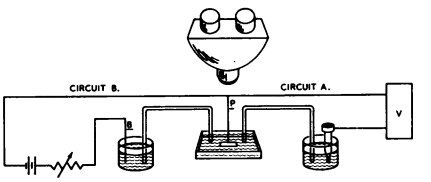

FIG. 1.—Schematic diagram. Platinum precipitation electrode, *P*, in center chamber is connected to calomel electrode through voltmeter, *V*, and agar bridge in Circuit A. Bias Circuit B is completed from precipitation electrode, battery, resistance, and subsidiary platinum bias electrode, *B*, to agar bridge, which completes the circuit to solution in the precipitation chamber.

Optics: A standard Bausch and Lomb binocular microscope, Model PVB-5B, was used to observe the blood cells in the precipitation chamber, which was placed on the stage of the microscope. Light filtered through either a green or neutral filter was used to illuminate the chamber. Total magnification of the microscope as it was used in the experimental arrangement was 100–200 ×.

Circuitry.—A Keithley Model 502 potentiometer was placed in series with the calomel electrode and the precipitation electrode to measure electrode potential. An agar bridge connecting the saline surrounding the calomel electrode to the erythrocyte-containing solution in the precipitation chamber completed the primary circuit (Fig. 1, Circuit A). The precipitation electrode was, in addition, connected to a subsidiary circuit (Circuit B) containing a 1.5–6 volt battery in series with a 0.1–15 megohm variable resistance. Circuit B was completed by an agar bridge from the second beaker to the solution in the chamber containing the precipitation electrode.

The secondary circuit was used to change the potential difference between the erythrocyte-containing saline and the precipitation electrode (measured with reference to the calomel electrode). The precipitation potential difference of erythrocytes was converted to the reversible hydrogen scale since the potential difference between the two couples is known.

Human Erythrocyte and Leucocyte Preparations.—After considerable experimentation it was decided that the most satisfactory source of erythrocytes was a punctured fingertip of one of the experimenters. Thus, the fingertip was stabbed with a sterile stylet, and the resulting droplet of blood was rapidly introduced into solution by swirling the fingertip in the recipient Krebs saline serum substitute at known pH. Blood placed in Krebs solution using this technique showed a much sharper precipitation potential than blood collected in syringes or bottles containing anticoagulant. Collected blood displayed an increasingly diffuse precipitation potential when stored for increasingly long periods.

It was necessary to collect leucocytes by a different technique. Approximately 35–50 ml of blood was drawn into a 50-ml syringe containing 10 mg of heparin anticoagulant. The blood was placed in a vessel, and the erythrocytes were allowed to sediment into the bottom of the solution leaving plasma containing leucocytes and platelets. This plasma was pipetted in either $^1/_2$ or 1-ml amounts into the precipitation chamber, following which the plasma was diluted with Krebs saline serum substitute of known pH and the heparin in the total solution neutralized by an appropriate amount of protamine.

Technique.—Following the suspension of a small drop of blood from the stabbed finger or leucocyte-containing plasma in the Krebs solution, the solution was mixed

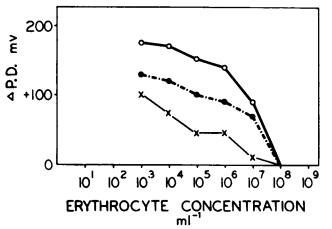

FIG. 2.—Effect of erythrocyte concentration on spontaneous p.d. in Circuit A.

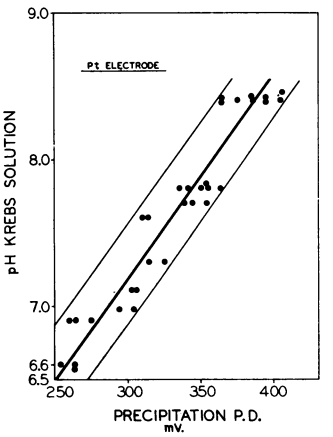

FIG. 3.—Precipitation potential of erythrocytes (concentration $\sim 10^4$/ml) on platinum.

carefully so as to minimize trauma to the cells. The dilution of the sample by the Krebs solution generally tended to prevent coagulation. The chamber was placed on the stage of the microscope, and Circuit A was completed. The spontaneous potential difference generated between the precipitation electrode surrounded by the solution, and the calomel cell was recorded (Fig. 2).

Next, Circuit B was completed, and the voltage on the potentiometer was slowly increased until visible precipitation of cells on the precipitation electrode occurred. At this point, the potential difference on the Keithley meter was recorded. After a considerable period of precipitation the voltage was again changed in a more negative direction in an attempt to separate the cells from the electrode. This was always possible with the exception of a few cells which persistently stuck. The separation potential was also recorded.

Changes in pH were obtained by bubbling gas mixtures containing varying amounts of oxygen and carbon dioxide through the solutions. Pure oxygen provided a pH of approximately 9.2. Fifty per cent carbon dioxide, fifty per cent oxygen yielded a pH of approximately 6.3 in our system, once the solution was transferred to the precipitation chamber. By this technique, the effect of pH on the precipitation potential was determined.

Results.—As can be seen from Figures 3, 4, and 5, human erythrocytes and leucocytes precipitate at + 0.33 ± 0.02 volt at a pH close to 7.4. The precipitation potentials change approximately 85 + 15 mv per unit of pH change over the range of 6.5–9.0, depending somewhat on the electrode material (Pt or Au).

After being precipitated and then redispersed into the solution several times by reversal of the potential, the cells exhibited an increasingly diffuse precipitation potential.

Discussion.—It has been determined that human erythrocytes and leucocytes may be electrochemically precipitated at a reproducible potential (+ 0.33 volt with respect to a reversible hydrogen electrode). This would seem to be of importance if it can also be determined or demonstrated that the protein elements of blood have similar properties leading to an hypothesis that elements of suitable electrochemical character at the pH of blood would not favor the precipitation of protein and cellular elements on the surfaces of such an intravascular material in the form of, for instance, a vascular prosthesis.

It has been established from electrophoretic behavior of erythrocytes that they carry a negative surface charge in the pH range of our experiments[5] and that the charge density decreases as the solution becomes more acid. The simplest interpretation of our data is that sufficient positive polarization of the electrode leads to

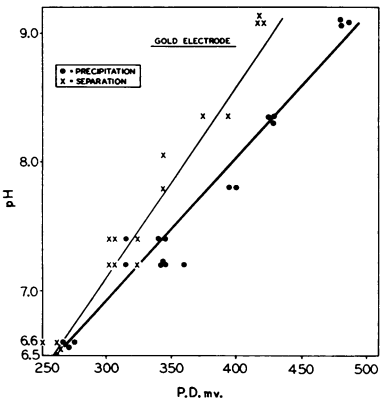

FIG. 4.—Precipitation and separation potentials of erythrocytes (PNS) on gold. The apparent scatter at intermediate pH is a consistent change occurring in the course of several precipitations and separations.

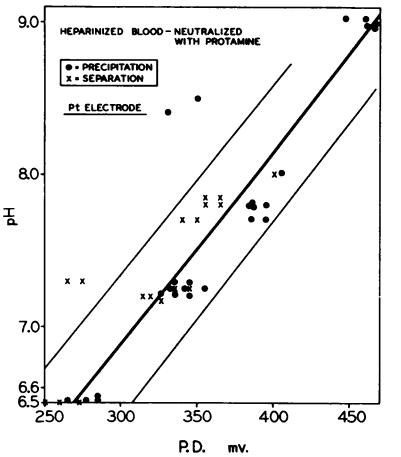

FIG. 5.—Precipitation and separation of human leucocytes on platinum.

a reduction in the surface charge density of the cells below the point of stability for the suspension. Two possible mechanisms suggest themselves: (1) transfer of electrons from the cell surface to the electrode (i.e., electrochemical oxidation), or (2) change in pH close to the electrode surface (i.e., more acid for positive polarization), leading to adsorption of hydrogen ion on the cell surface. By means of mechanism (2), the cells may experience a reduction of the negative surface charge, leading to aggregation when the forces of mutual repulsion are sufficiently small. We feel that this mechanism may be rejected for at least two reasons:

(1) The currents involved in polarizing the electrode to the precipitation potential are very small and should have insignificant effects on the pH of the solutions, which are buffered with bicarbonate.

(2) The precipitation is reversible on reversal of the polarization. Erythrocytes coagulated in acid are not redispersed simply by increasing the pH.

For these reasons we favor the first mechanism described above. In the absence of detailed knowledge of the chemical configuration of the cell surface, it is possible only to write the reaction in the generalized form:

$$\text{Normal cells} \underset{+\text{electrons}}{\overset{-\text{electrons}}{\rightleftharpoons}} \text{oxidized (precipitated) cells.}$$

The observed precipitation potential is not the reversible potential of the above reaction as determined by the free energy change involved. It includes also an overvoltage necessary to cause the reaction to proceed from left to right at the arbitrary rate that an observer judges to be the onset of precipitation. This, of course, is a subjective judgment and could account for some of the scatter in our data.

The observation that in more alkaline solutions a more positive potential is required to cause precipitation is consistent with the fact that the negative surface charge (and hence the stability due to mutual repulsion) increases with increase in pH. Since the formation of precipitated cells seems to involve transfer of electrons

to the metal, it follows that, granting this mechanism, erythrocytes should be more stable in the presence of metals of low work function (least electron affinity). Currently, experiments are being carried out *in vivo* to check this hypothesis.

The authors are indebted to Mr. J. Bruno and Miss Jeanette Ogoniak for technical assistance.

* Supported in part by grants-in-aid HE-07371-01 and -02 from the National Heart Institute, National Institutes of Health, U.S. Public Health Service.

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PRODUCTION OF TYPE-SPECIFIC C ANTIGEN IN VIRUS-FREE HAMSTER TUMOR CELLS INDUCED BY ADENOVIRUS TYPE 12

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Communicated January 6, 1964

Previous reports in these PROCEEDINGS presented evidence that virus-free cancer cells induced by adenovirus 12[1-3] produced antigens which in hamsters and rats resulted in complement-fixing antibody responses to viral antigens associated with the growth of adenovirus type 12 in tissue culture systems. These antibodies seemed largely type-specific, occurring earlier and reaching higher titers with the homologous viral antigen than they did with the heterologous, but related, adenovirus 18 serotype. Similarly, antigen preparations from hamster tumors and tissue culture-grown cells derived from them reacted with serums of hamsters carrying adenovirus type 12- or type 18-induced tumors, but not with a pool of human serums used in our laboratory as a standard representative of adenovirus group-reactive antibody.[1, 2] In addition, serums of hamsters containing high-level antibodies to adenovirus 12 and/or type 18 viral antigens revealed little or no antibody response to the group-reactive antigen of other adenoviruses, the latter an important characteristic of all adenoviruses. These data led to the hypothesis that the virus-free but adenovirus-induced tumor cells probably replicated the type-specific C (or E) antigen, but not the group-reactive A (or L) antigen described for various adenoviruses by Klemperer and Pereira,[4, 5] and Wilcox and Ginsberg.[6]

This communication describes the fractionation of type 12 adenovirus into its