Influence of pH, pCO_2 and pO_2 on Erythrocytic Adsorption of Fibrinogen.* (31141)

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Erythrocytic surfaces are capable of adsorbing fibrinogen from plasma; the amount adsorbed varies with the plasma concentration and inversely with the temperature(1). Calculations, based on studies *in vitro,* suggest that approximately 11% of all the fibrinogen in the blood vascular compartment is bound onto erythrocyte surfaces(1); hence, this storage site, potentially, is important in the maintenance of plasma fibrinogen concentration.

Physiological variables, pH, pCO_2, pO_2, were used to evaluate their influence on erythrocyte capacity for fibrinogen binding. These variables may be important factors which, *in situ,* regulate the amount of fibrinogen circulating in the plasma compartment.

Materials and methods. Canine erythrocytes were washed with imidazole-saline, pH 7.25, 3 times prior to their use(1). Fibrinogen concentration was determined by colorimetric analysis of fibrin hydrolysis(2). A Beckman expanded scale pH meter and Beckman electrodes (Series 39024-5) were utilized to measure the pH. Two Beckman Model 160 Physiological Gas Analyzers and appropriate electrodes were used to measure pCO_2 (Beckman 39028) and pO_2 (Beckman 325814). The electrodes and analyzers were calibrated with a liquid system prior to use(3).

Experimental procedure and results. Influence of respiratory gas tensions. Approximately 220 ml of blood were removed from anesthetized dogs and mixed with 25 ml 4% sodium citrate. The citrated blood was introduced into the beaker depicted in Fig. 1 and stirred constantly with a magnetic stirrer. The cover was placed snugly over the circular beaker rim. Previously calibrated electrodes (pH, pCO_2, pO_2) and a thermometer were inserted into appropriate openings; each sensor tip was immersed approximately 20 mm below the liquid surface.

Five gases were successively introduced through the inlet and passed over the blood surface (Fig. 1). These gases included: A) 20% O_2-80% N_2, B) 12% CO_2-20% O_2-68% N_2, C) 6% CO_2-20% O_2-74% N_2, D) 6% CO_2- 94% O_2, and E) 6% CO_2-94% N_2. These analyzed gases were bubbled through a water trap and saturated before passing into the assembly inlet. Although each gas passed over the blood surface for over 2 hours, the blood did not completely equilibrate with the gas phase; hence, the need for measuring the various gas tensions in blood. The gases were administered in a different sequence on each experimental run to decrease any errors which were a reflection of time or the sequence used.

Blood aliquots were withdrawn from the assembly by use of oil-coated syringes. Upon centrifugation (3,000 rcf × 20 minutes × 20°C), the plasma was removed and its fibrinogen concentration was determined. A total of 6 separate aliquots were analyzed for each gas tension used. This was repeated on the blood of 6 different dogs.

When gas C (6% CO_2-20% O_2-74% N_2) was used, the pH, pCO_2 and pO_2 of blood in the assembly approximated that present in arterial blood (Fig. 2). Accordingly, the results obtained with the other 4 gas mixtures were compared to that obtained with gas C (control).

The changes in fibrinogen concentration as a result of different pCO_2 and pH, the pO_2 remaining essentially unchanged, is depicted in Fig. 2. It will be noted that when the pCO_2 was greater (Gas B) than under control conditions (Gas C), there was a signifi-

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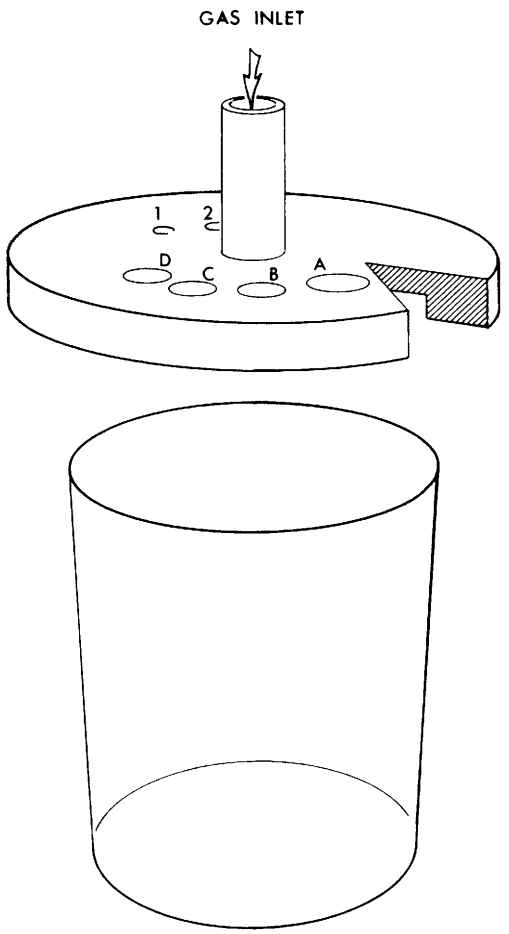

FIG. 1. The above assembly was used to measure pH, pO_2 and pCO_2 of blood in the beaker. Openings in cover were for: A. pCO_2 electrode; B. pO_2 electrode; C. and D. pH electrodes; 1. thermometer; 2. sampling and gas escape port.

cantly increased amount of fibrinogen in the plasma compartment ($0.02>p>0.01$). When the pCO_2 was reduced (Gas A), then less fibrinogen remained in the plasma compartment than was present under control conditions ($0.02>p>0.01$). The pH varied inversely with the pCO_2 in this series; hence, also may have been contributory to the changes described.

Changes in fibrinogen concentration were unremarkable when blood was subjected to different oxygen tensions, the pH and pCO_2 remaining fairly constant (Gases C, D, E). The amount of fibrinogen removed from the plasma compartment with pO_2 tensions of 411 mm Hg and 67 mm Hg could not be proven statistically to be different from the amount removed under a pO_2 tension of 114 mm Hg ($p>0.5$).

Influence of pH. Approximately 40 ml of blood were removed from a dog and were placed in a plastic tube containing 4% sodium citrate. After centrifugation (3,500 rcf $\times$ 20 min), the plasma was removed and the erythrocytes were washed.

Aliquots (2.50 ml) of the citrated plasma were placed into four 5 ml beakers. One milliliter of washed erythrocytes was added to 2 of the beakers. The pH was adjusted to pH 7.00 in one, to pH 7.40 in the second, by the addition of either 0.20 M monobasic or 0.20 M dibasic sodium phosphate. The volumes of these 2 test samples were increased to 4.00 ml with 0.9% sodium chloride and the pH of each sample was re-read to insure its stability. The plasmas in the 2 remaining beakers were handled in the same manner except that no erythrocytes were added (con-

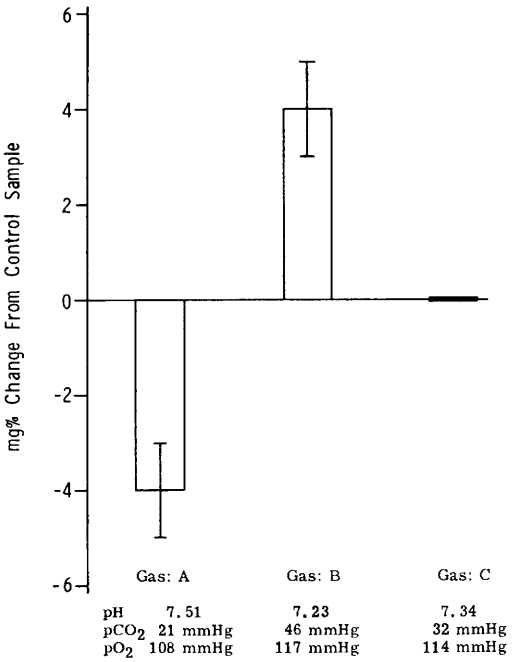

FIG. 2. The difference between the mg% of plasma fibrinogen after blood pCO_2 and pH are altered (A, B) and that present in control plasma (C) is plotted on the ordinate. Blood pH, pCO_2 and pO_2 are listed under appropriate column. Bars represent mean difference $\pm$ S.E.M. Positive numbers represent desorption from erythrocytes; negative numbers, adsorption.

TABLE I. Effect of pH on Erythrocytic Binding Affinity for Fibrinogen.

pH	mg fibrinogen adsorbed/ml erythrocytes (mean ± s.e.m.)
7.00	.18 ± .15
7.40	.39 ± .18
	(.025 > p > .01)

trol samples). After the pH was checked, the content of each beaker was decanted into separate conical centrifuge tubes and incubated at 37°C × 30 minutes with stirring at 10-minute intervals. Following incubation, the tubes were centrifuged at 3,500 rcf × 20 min × 20°C.

The fibrinogen concentration in the supernatant phase of each tube was determined. The mg fibrinogen adsorbed by one ml erythrocytes at a given pH was calculated by subtracting the fibrinogen concentration in the test sample (originally incubated with erythrocytes) from that in the control sample and multiplying the difference by 0.03. The above procedure was performed 5 times on the blood of a different dog. Five dogs were used in this study. Assuming linearity in the relationship between the fibrinogen adsorbed and pH, an additional 0.05 mg fibrinogen was adsorbed onto one ml of red cells for each 0.10 pH unit increment.

Discussion. In each of the studies cited above, the amount of fibrinogen present in the container used was fixed. A decrease in fibrinogen concentration in the plasma compartment is attributed to an increased adsorption of the protein by the erythrocytes. Previous studies with EACA(1) tend to negate the possibility of fibrinogenolytic activity as a source of fibrinogen loss. The amount of fibrinogen adsorbed onto beaker surface was internally controlled in the above studies.

An increase in the amount of fibrinogen remaining in the plasma compartment is attributed to a decreased affinity for the protein on the erythrocyte surface. In the gas study, some fibrinogen may have been desorbed from erythrocyte storage sites as well.

Alterations in oxygen tension had little or no effect on the amount of fibrinogen binding by erythrocytes. Since the range of tensions used encompasses that normally encountered *in situ*, the influence of pO_2 on fibrinogen binding is interpreted to be negligible.

Alteration of carbon dioxide tension, on the other hand, significantly affected the amount of fibrinogen removed by erythrocytic adsorption. More fibrinogen was removed at a low pCO_2 than at higher tensions. Since the change in pCO_2 also resulted in an inverse alteration in pH, it was not ascertained whether the alteration in pCO_2 directly or indirectly influenced the amount of fibrinogen binding.

In the second study, pH was altered by buffers instead of carbon dioxide. In this system, the pCO_2 was minimally affected yet the amount of fibrinogen removed from the plasma compartment varied directly with the pH. Thus, it is suggested that the influence of carbon dioxide tension on erythrocyte binding capacity is through its influence on pH.

The mechanism by which pH can alter erythrocyte binding capacity for fibrinogen is unknown. Studies by other investigators lead to the concept that pH, by altering the negatively charged sites on the erythrocyte surface, may influence the binding of fibrinogen onto these sites. The reasoning is as follows: a) Erythrocytes(4), have an overall negative charge on their surface. The negativity of the erythrocytic charge is reduced more profoundly in plasma than in serum(5); also reduced in acid medium(6). b) Although fibrinogen also has an overall negative charge (7) it is adsorbed onto substances having a negative charge(8). This is explicable if some of the adsorptive sites on the fibrinogen molecule are positively charged prosthetic groups which have a high degree of affinity for negatively charged groups. Equally valid an explanation is the possibility of a common diffuse layer of cations between the negatively charged binding sites of fibrinogen and those of its adsorbent. c) A reduction in pH and a concomitant reduction in the negativity of the erythrocyte surface would decrease the binding affinity for fibrinogen either by decreasing the attraction for the postulated positively charged binding sites on fibrinogen or by reducing the concentration of the cations in the diffuse layer around the erythrocytes.

Another possibility exists that lowering the

pH alters the charge density of fibrinogen as the isoelectric point is approached. Results of preliminary studies in which red cells were suspended in a chelating medium (EDTA), however, tend to be supportive of the diffuse cationic layer concept presented above.

Summary. The amount of fibrinogen adsorbed from plasma by erythrocytes varies with the pH and inversely with the pCO_2. The influence of pCO_2 is probably mediated through its alteration of blood pH. The influence of pO_2 on erythrocytic adsorption of fibrinogen is negligible. Physiological alterations in pH may affect the amount of fibrinogen stored on erythrocytic surfaces or released to the plasma compartment.

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Effect of Age on Surface Properties of Excised Lungs.* (31142)

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It has been reported that excised lungs subjected to numerous cycles of inflation and deflation in rapid succession lose their normal stability of expansion(1). This was readily confirmed in adult rats, but infant lungs failed to show the change. The present study was undertaken in order to examine the effect of repeated inflations on stability of expansion in relation to age.

Material. All lungs from cats, dogs and rabbits were apparently normal. The numbers and ages are indicated in Fig. 1. Of the 7 adult rats examined, 4 had an initial stability within the normal range, and were used in arriving at the data in Fig. 1. The remaining 3 rats had an abnormally low initial stability. This is known to occur especially among rats more than 6 months old and has tentatively been ascribed to early chronic murine pneumonia(2). The human material, derived from autopsies on infants, is quite heterogeneous (Fig. 2). Among neonates, both above and below a body weight limit of 1000 g, a large proportion were clustered in a high-stability area, and a similar number had very low stability. Only the high-stability cases were used to construct the line for newborns in Fig. 1. The low-stability cases as well as the majority of an intermediate group (Fig. 2b) may be assumed to have had the respiratory distress syndrome. All those who survived more than 2 hours after birth, had hyaline membranes and the others would presumably have developed them had they survived longer(3). One infant with high stability and hyaline membranes was presumably recovering from the pulmonary manifesations of the disease when it died at 2 days of age(3). There is a lack of older infants in the present study, and therefore initial stability data obtained by a few, slow inflation cycles, are added to Fig. 2c and denoted by circles.

Method. Specimens from infants, usually the left lung, were obtained at autopsy and inflated with the apparatus previously described. This is based on the method of Gribetz, Frank and Avery(4) in which the amount of air displaced from a reservoir into the lung is measured with a burette. The desired pressure is produced by raising the burette, and read on a water manometer. Separate lobes of lungs of small adult dogs

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