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Effect of Metabolic Acidosis and Alkalosis on Human Platelet Aggregation Induced by Epinephrine and ADP[1] (37887)

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(Introduced by Roger M. Des Prez)

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Despite a large volume of literature concerning platelet aggregation, very few attempts have been made to control the acid–base environments of platelet-rich plasma (PRP) during such studies (1, 3–6). A method to control the pH of PRP using CO_2 gas was described from this laboratory recently (2). In this study it was demonstrated that platelet aggregation in response to a threshold challenge with epinephrine was greatly enhanced by alkalosis and inhibited by acidosis within clinical ranges of pH change. Since the pH was controlled by varying pCO_2 in these experiments, the question remained as to whether this was a property of pH change per se or of changes in the pCO_2. Accordingly, in the present studies, platelet aggregation in response to threshold challenge with epinephrine, and with ADP, was measured in samples of PRP in which the pH was altered while the pCO_2 was maintained constant. The results indicate that the important determinant in platelet response is pH rather than pCO_2.

Materials and Methods. Preparation of platelet-rich plasma. Whole blood was collected in plastic syringes from fasting healthy human volunteers by venipuncture and transferred immediately to siliconized conical centrifuge tubes containing 3.8% sodium citrate in sufficient quantity to provide a final sodium citrate concentration of 0.38%. Platelet-rich plasma was prepared by centrifugation at 280*g* for 10 min at 20°. The upper layer of PRP was removed by aspiration and respun at 325*g* for 3 min to remove any remaining leukocytes and erythrocytes. The remainder of the initial sample was centrifuged at 2100*g* for 12 min to provide PPP (platelet-poor plasma). The PRP was adjusted to 12% transmittance at a wavelength of 600 μ (Beckman DU Spectrophotometer) using autologous PPP as diluent and blank. At 12% transmittance, the PRP sample contained approximately 2×10^8 platelets/ml.

Stabilization of plasma pH. Three-milliliter aliquots of PRP were transferred to siliconized 25-ml Erlenmeyer flasks, and CO_2 (5%) in compressed air (95%) from a tank was delivered through small rubber tubing to each flask for 3 min. The flasks were then sealed with parafilm and allowed to equilibrate for at least 5 min prior to use. Each time the PRP samples were transferred to aggregation cuvettes, re-equilibration was done in the same manner with the 5% CO_2. The temperature of samples was maintained at 22° (room temperature) be-

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fore aggregation.

Aggregation studies. Epinephrine (aqueous epinephrine hydrochloride 1:1000, Parke, Davis and Co.) was diluted with normal saline immediately before use. Equine muscle ADP (Sigma Chemical Co.) was kept frozen in 20 m*M* stock solution and was diluted 1:1000 with normal saline immediately before use. Various stock concentrations of HCl and NaOH were also prepared to provide the final plasma pH. Aggregation studies were performed using a Chronolog Aggregometer (Model No. S201, Chrono-Log Corp.) The recorder was adjusted so that the photocell signaled less than 10 with unaltered PRP and greater than 90 with PPP (total theoretical aggregation). The temperature of the samples was maintained at 37° during aggregation and the sample magnetically stirred with a 2-mm siliconized metal flea (used once and discarded) at 1000 rpm.

The 1-ml aliquots of PRP equilibrated with 5% CO_2 were transferred to siliconized aggregometer cuvettes containing 0.1 ml of a specified concentration of HCl, NaOH (0.1–0.03 *N*), or a saline control. The samples were then re-equilibrated with 5% CO_2 and sealed with parafilm. The pH was recorded immediately after aggregation using a Radiometer pH meter (Model 26 Copenhagen) calibrated frequently using a commercial standard (Thomas Buffer Solution, pH 7.000). Each sample challenged by injection through the parafilm of 0.1 ml and the diluted epinephrine concentration in the PRP was 10 or 50 μM. The final ADP concentration was 2 μM. Both concentrations of epinephrine consistently gave a clear-cut biphasic aggregation response. All aggregation studies were performed within 3 hr of venipuncture.

Results. Figure 1 exhibits the pH dependency of platelet aggregation in response to 10 μM epinephrine challenge. Aggregation became maximal with increasing pH (curves A and B, Fig. 1). It can be seen that in comparison to curve C (pH 7.43), aggregation at more alkalotic pH values is associated with a greater magnitude of aggregation that is reached earlier. Acidotic

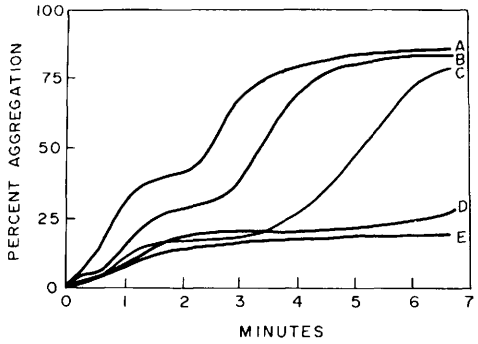

FIG. 1. Epinephrine 10 μM. Aggregometry tracings obtained with citrated human PRP after challenge with epinephrine, final concentration 10 μM. Each sample was equilibrated with pCO_2 of 40 mm Hg, and varying concentrations of HCl, NaOH, or a saline blank (curve C) were added prior to aggregation. The pH values were measured in each sample immediately after aggregation and were as follows: (A) 7.82, (B) 7.57, (C) 7.43, (D) 7.29, (E) 7.16.

pH values (curves D and E, Fig. 1) were associated with failure of biphasic aggregation and the initial slope was depressed also.

Figure 2 demonstrates a similar response with 50 μM epinephrine. In general, the slope of the initial wave of aggregation was depressed, total aggregation decreased, and biphasic aggregation inhibited at acidic pH values.

Figure 3 shows the response to pH differences when ADP was used as the challenge. Over a pH range of 7.43–7.92 (curves A–C, Fig. 3) the initial wave of aggregation was similar and monophasic. Maximal aggregation was obtained at the more alkalotic pH levels achieved in this range; as the pH decreased to 7.34 (curve D, Fig. 3), a significant decrease in maximal aggregation can be seen. At more acidotic values (curves E and F, Fig. 3), the slope and extent of initial aggregation decreased and disaggregation occurred.

pCO_2 values in equilibrated samples (after aggregation) varied from 33.6 to 36.3 with a mean of 35.03 mm Hg. Two of the PRP samples that were allowed to equilibrate with room air had a pCO_2 of 5.0 and 6.1 mm Hg, respectively. The osmolality of 5 samples of varying pH were

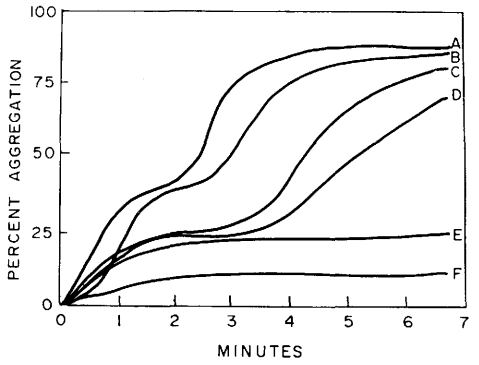

FIG. 2. Epinephrine 50 μM. Aggregometry tracings under the same conditions as Fig. 1 except final epinephrine concentration was 50 μM. The pH values after aggregation were as follows: (A) 7.78, (B) 7.67, (C) 7.44, (D) 7.34, (E) 7.24, (F) 7.17.

measured. The values ranged from 287 to 293 mOsm/liter.

Repeated experiments have shown that these findings are easily reproducible (providing pCO_2 remains constant in the PRP samples). The results depicted graphically are a composite of 6 separate experiments.

Discussion. The present studies confirm the observations of Rogers that platelet aggregation in response to epinephrine is enhanced by alkalosis and inhibited by acidosis (2). They further establish that the important variable is pH per se rather than pCO_2. Accordingly, any relevance to human acid–base disorders would appear to pertain to metabolic as well as respiratory acidosis and alkalosis.

The method employed to control pCO_2 was quite simple and fairly satisfactory. However, the necessity of re-equilibrating samples when they were moved from one container to another introduced an awkward manipulation. If, as would seem to be indicated, rigorous control of pH is to become an important factor in other studies of platelet aggregation, new methodology allowing easier control of both pH and pCO_2 would be advantageous. Attempts to develop such methods are now in process in this laboratory.

As previously noted (2), the basic mechanism whereby pH modifies platelet aggregation remains to be elucidated. However, pH-dependent functions are characteristic of most biologic systems, and the modification of platelet behavior by pH changes is not surprising. Since platelet aggregates are found in the pulmonary vasculature in some conditions characterized by alkalosis and abnormal capillary permeability (7, 8), this phenomenon may have clinical relevance.

An inhibitory effect of acid pH on ADP-induced aggregation has been observed by Skoza *et al.* (9) and McLean and Veloso (10). These studies did not address themselves to epinephrine-induced aggregation, however.

Summary. Platelet-rich plasma undergoes pH drift in the alkaline direction when exposed to air. A technique for maintaining pH *in vitro* using 5% CO_2 from a constant source is described. This technique is used further to elucidate the effect of metabolic (exogenous) pH change on *in vitro* platelet aggregation. It is seen that platelet aggregation in response to epinephrine or ADP challenge is inhibited by acidosis and enhanced by alkalosis produced by pH changes while maintaining a constant pCO_2.

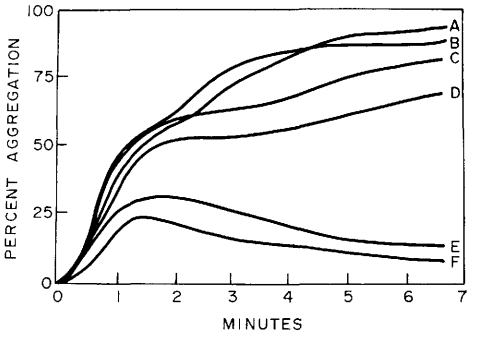

FIG. 3. ADP. Aggregometry tracings were obtained under conditions identical to Fig. 1 except ADP (final concentration 2 μM) was used as challenge. The pH values measured just after aggregation were as follows: (A) 7.92, (B) 7.64, (C) 7.43, (D) 7.34, (E) 7.24, (F) 7.18. As in Figs. 1 and 2, the sample depicted by curve C represents the pH of unaltered citrated PRP (no HCl or NaOH added) aggregated while maintaining pCO_2 constant at values approximating *in vivo* conditions.

1. Aledort, L. M., *in* "The Circulating Plate-

let" (S. A. Johnson, ed.), Academic Press, New York (1971).

2. Rogers, A. B., Proc. Soc. Exp. Biol. Med. **139**, 1100 (1972).

3. Rabiner, S. F., and Molinas, F., Amer. J. Med. **49**, 346 (1970).

4. Horowitz, H. I., Arch. Intern. Med. **126**, 823 (1970).

5. Ballard, M. S., and Marcus, A. J., Scand. J. Haematol. **9**, 198 (1972).

6. Horowitz, H. I., Stein, I. M., and Cohen, B. D., Amer. J. Med. **49**, 336 (1970).

7. Berman, I. R., Gutierrez, V. S., Burran, E. L., and Boatright, R. D., Surg. Forum **20**, 14 (1969).

8. Wilson, R. F., Gibson, D., Percinel, A. K., Ali, M. A., Baker, G. B., LeBlanc, L. P., and Lucas, C., Arch. Surg. **105**, 197 (1972).

9. Skoza, L., Zucker, Marjorie B., Jerushalmy, Z., and Grant, R., Thrombos. Diathes. Haemorrh. **18**, 713 (1967).

10. McLean, J. R., and Veloso, H., Life Sci. **6**, 1983 (1967).

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