The Effect of pH on Human Platelet Aggregation Induced by Epinephrine and ADP[1] (36307)

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(Introduced by R. M. Des Prez)
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During the course of some experiments concerning the effects of dextrans on platelet aggregation, it was noted that shifts in plasma pH due to diffusion of CO_2 greatly modified platelet aggregation in response to epinephrine. Careful control of this variable has not in general been attempted in studies of platelet aggregation. Since acceptable methods to control pH during the course of experiments on platelet rich plasma (PRP) were not available from the literature, it was considered worthwhile to develop such a method and further evaluate the effects of pH on platelet aggregation.

Materials and Methods. Preparation of PRP. Whole blood was collected from healthy human volunteers into plastic syringes and delivered directly into siliconized conical centrifuge tubes containing 3.8% sodium citrate in quantities sufficient to provide a final citrate concentration of 0.38%. PRP was prepared by slow speed (300*g*) centrifugation at 4° for 11 min. The PRP (in the upper one-third of the tube) was removed by aspiration, and the remainder of the sample was centrifuged at high speed (2000*g*) to provide platelet poor plasma (PRP). PRP was adjusted to 12% transmittance at a wavelength setting of 600 μm (Beckman DU Spectrophotometer) using autologous PPP as the diluent and as the blank. This preparation contained approximately 2×10^8 platelets per ml.

Aggregation studies. Epinephrine (aqueous epinephrine hydrochloride 1:1000, Parke, Davis and Company) was diluted with normal saline immediately before use. Equine muscle ADP (Sigma Chemical Company) was kept frozen in a 20 m*M* stock solution and diluted 1:1000 with normal saline immediately before use. The stock solution maintained its potency for at least three weeks and was discarded after that period.

Aggregation studies were performed using a Chronolog Aggregometer, a device which measures increases in light transmission through a curvette of stirred PRP as an index of platelet aggregation. Changes in light transmittance were directly recorded, with upward deflection of the recorder pen indicating increased transmittance. The recorder was adjusted so that the photocell signal with unaltered PRP in the cuvette produced a tracing at approximately zero and the photocell signal with PPP in the cuvette (theoretical total aggregation) was still within the span of the recorder paper. The settings of the aggregometer were not altered throughout the course of the experiments. Temperature in the sample cuvette was controlled at 37° and the sample was magnetically stirred with a small siliconized metal flea at 1000 rpm.

Stabilization of plasma pH. Whereas blood immediately after phlebotomy demonstrated a pH in the 7.45–7.55 range, it was noted that PRP consistently demonstrated a pH of 7.6 or higher, and that the pH tended to drift to 8.0 in samples maintained at room temperature for any period. Initial attempts to

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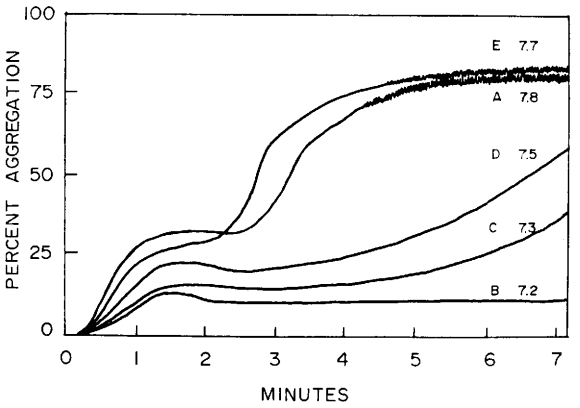

FIG. 1. Aggregometry trace (A) was obtained with unaltered human citrated PRP challenged with epinephrine, final concentration 10 μM. This challenge dose, selected because it produced a clearcut biphasic response, was employed after equilibration of the same PRP with three different concentrations of CO_2 (curves B, C, and D) and after a portion of the sample with which curve (B) was obtained had been re-equilibrated with air (curve E). The pH is indicated on each curve.

prevent pH shifts with various buffers were unsatisfactory. Dilute buffer concentrations were ineffective, and much stronger solutions were unacceptable. Bubbling various concentrations of CO_2 mixed with air through PRP samples provided good control of pH but resulted in spontaneous platelet aggregation at the higher pH range. A satisfactory and simple method was developed which involved simply replacing the atmosphere over the PRP sample with various mixtures of CO_2 and air and then sealing the receptacle with parafilm. Pure CO_2 and compressed air were delivered one after the other into a 60 ml syringe. The system between the pressurized gas and the syringe nozzle was sealed so that various mixtures of CO_2 and air could be prepared with fair accuracy by observing the displacement of the syringe plunger using the calibrations on the syringe barrel. The mixtures utilized herein were approximately 16%, 33%, and 50% CO_2 in air. The syringe containing the desired concentration of CO_2 was inverted over a 4 ml sample of PRP and the gas expelled gently so as to displace the air over the sample. The tubes were then sealed with parafilm and allowed to equilibrate at room temperature for at least 5 min. The pH of samples treated in this fashion ranged from 7.0 to 7.6 depending on the gas mixture used and remained constant for the duration of the experiments. For aggregation studies, a 1 ml sample of the desired equilibrated PRP was removed and quickly delivered into an aggregometer cuvette. The atmosphere in the equilibrating tube and the aggregometer cuvette was then replaced with the desired mixture of CO_2 and air and each was then sealed with parafilm. The challenge materials used to produce aggregation (epinephrine or ADP) were injected through the parafilm cover using a 1 ml tuberculin syringe with a #26 needle. Challenges were in general introduced in a 0.1 ml volume per ml of PRP. The pH of the sample studied in the aggregation cuvette remained the same as that in the incubation mixture from which the sample was removed. pH was measured with an Orion pH meter (Orion Ionanalyzer, Model 8010) equipped with a small tipped electrode and calibrated frequently using a commercial standard (Thomas Buffer Solution, pH 7.000).

Results. Figure 1 demonstrates that platelet aggregation by epinephrine was pH dependent. At a pH range of 6.9 to 7.2, aggregation in response to epinephrine challenge was either very slight or nil. This lack of aggregation was observed with challenge dosage much larger than those used in the experiments illustrated. At pH range 7.3 to 7.4 a biphasic aggregation trace was produced. However, both the slope of the first wave of aggregation and the total degree of aggrega-

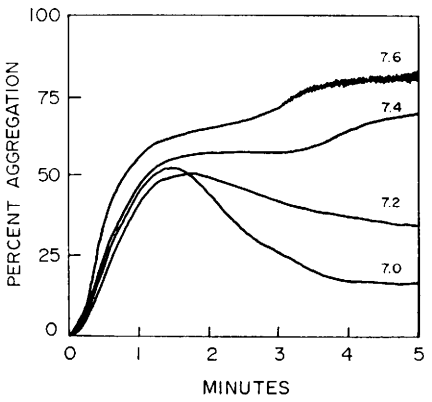

FIG. 2. Aggregometry traces obtained by challenge with ADP, final concentration 2×10^{-6} *M*, in PRP equilibrated with different concentrations of CO_2 so as to produce the indicated pH values.

tion were depressed, and the latent period between the first and second wave of aggregation was prolonged in comparison with the results obtained at higher pH values. It is of note that these effects were completely reversible. The sample initially equilibrated with high CO_2 gases so as to produce low pH values and inhibition of aggregation (curve B, Fig. 1) promptly re-equilibrated to higher pH values when re-exposed to air and again demonstrated enhanced aggregation in response to epinephrine (curve E, Fig. 1).

Figure 2 demonstrates a similar series of experiments using ADP as the challenge material. In contrast to the results obtained with epinephrine, the promptness of onset and initial slope of aggregation were not pH dependent, but the extent of aggregation and, particularly, the rate and degree of disaggregation, were sharply pH dependent. Aggregation was least and disaggregation most prompt and complete at acidic pH values.

Discussion. The present studies demonstrate that platelet aggregation in response to epinephrine is inhibited at acidic pH values and enhanced at basic pH values produced by controlling the plasma pCO_2. The range of pH tested is not greatly beyond that observed *in vivo* suggesting that the results may have relevance to clinical acute acid–base disorders of respiratory origin. Hypercapnic acidosis not only blunted the rapidity and extent of epinephrine-induced platelet aggregation but also inhibited the second wave of aggregation. This second wave is generally thought to be caused by release of endogenous ADP from platelets (1), suggesting that not only aggregation but also platelet-release reactions may be altered by pH changes within the physiologic range. The results obtained with ADP indicate that aggregation produced by this agent is not very pH dependent, but disaggregation is enhanced by hypercapnic acidosis and inhibited by hypocapnic alkalosis. Whether this effect is due to changes in the platelet membrane or in the rapidity of ADP catabolism in plasma is not known (2).

The method utilized herein to control the partial pressure of CO_2 in plasma was simple, reliable, and required no complex equipment. It seems reasonable to suggest its applicability to many studies of platelet aggregation since this important variable has not, in general, been controlled (3). For reasons of time, only epinephrine and ADP were investigated systematically, but preliminary studies suggest that pH changes may also affect other aggregation-producing systems and platelet release reactions as well.

The mechanism or mechanisms whereby pH changes alter platelet aggregation remain to be determined. Such pH dependent phenomena as net surface charge and platelet cyclic-AMP metabolism may be involved. Carrier *et al.* have reported inhibition of epinephrine-induced contraction of vascular smooth muscle by hypercapnic acidosis (4). The parallel findings with regard to the platelet response to epinephrine reported herein accordingly extend the analogy between platelet contraction and smooth muscle contraction suggested by several authors (5, 6).

Summary. Platelet rich plasma exposed to air undergoes pH drift in the alkaline direction due to diffusion of CO_2. A simple technique has been developed to control this variable in aggregation studies by layering CO_2 enriched air over the samples. Studies using this technique demonstrate inhibition of epinephrine-induced aggregation by acidosis and enhanced aggregation by alkalosis within

the physiologic range. ADP-induced aggregation was less pH dependent, but the rate and extent of disaggregation were much greater at acidic pH values.

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