

Comparison of the effect of heparin and citrate on platelet aggregation

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SYNOPSIS When whole blood was passed through a column of glass beads, heparinized and native blood gave similar results. With citrated blood far fewer platelets were lost on the column. Thus citrate inhibits this test and heparin has no effect.

Platelet-rich plasma (PRP) was prepared from citrated and heparinized blood and the responses in a number of aggregation tests were compared. With added adenosine diphosphate and adrenaline (first wave), the response in heparin PRP was greater than that in citrate PRP and was proportional to the amount of free calcium ions. With added collagen and adrenaline (second wave) the response was worse in heparin than in citrate platelet-rich plasma. It is concluded that heparin has no effect on the adhesive forces but inhibits the release mechanism. This may be of therapeutic importance.

Clotting must usually be prevented before any platelet aggregation studies can be carried out. Citrate and heparin are often used. Spaet and Zucker (1964) found a more vigorous response in heparinized platelet-rich plasma. Skoza, Zucker, Jerushalmy, and Grant (1967) show that citrate decreases aggregation by removing calcium ions. When O'Brien (1968) studied the inhibitory effect of aspirin on the release mechanism, the results in heparin PRP were less clear cut than in citrate platelet-rich plasma. Accordingly, it seemed appropriate to compare the effect of these two anticoagulants in a number of situations and especially in those involving the release mechanism.

METHODS

For the glass bead column experiments the method of O'Brien and Heywood (1967) was used. Blood was collected into siliconed tubes containing saline or the appropriate concentration of anticoagulant, mixed, and then sucked into the plastic tube for attachment to the column of glass beads. A fast speed was used (transit time 3 sec). The order of testing these mixtures was varied and the experiments were always finished within 10 minutes.

The basic design for aggregation experiments consisted of collecting blood from a single donor into sample tubes containing heparin or citrate of varying concentrations. Platelet-rich plasma was prepared from each sample and the response of all the samples to a variety of aggregating agents was compared. The technique and

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the methods of measuring the degree of aggregation have already been detailed (O'Brien, Heywood, and Heady, 1966). Essentially, PRP is stirred at 37°C and the aggregating agent added. Changes in light transmission are recorded graphically and the various maximal 'slopes' are measured. These indicate the rate of aggregation and hence the number of successful collisions; thus the slopes reflect the 'stickiness' of the platelet membrane. The number of platelets in each sample was counted and the results adjusted accordingly. Usually the counts in all samples were similar so no adjustment was necessary.

The final concentrations of citrate were 0.32% and 0.64% and that of heparin was 3.6 units and 91 units per ml (Pularin, Evans Medical). The strength of the aggregating agents was chosen according to the plasma used, to give a moderate response (eg, a 'slope' of 60) in the 0.32% citrate sample. The slopes of the various samples were measured, and for comparison were expressed as a percentage of the response in 0.32% citrate. For each aggregating agent, four to eight independent experiments were made and the results were averaged. In almost all experiments there was close agreement.

To study the possible inhibitory effect of excess heparin, PRP was prepared, using heparin 3.6 units per ml, and this was compared with the same PRP to which more heparin had been added to give a final concentration of 104 units per millilitre.

The aggregating agents and the range of final concentrations were as follows: ADP (Sigma) $5M \times 10^{-7}$ to $2.5M \times 10^{-6}$; serotonin (Sigma) $5M \times 10^{-6}$; adrenaline hydrogen tartrate $5M \times 10^{-9}$ to $5M \times 10^{-4}$ (BDH). A suspension of collagen was prepared by grinding the dried material (Sigma) by hand in saline. Coarse

particles were removed by centrifugation and the strength was adjusted by dilution to give a moderate response.

RESULTS

When citrated (0.32%) blood was passed through the glass bead column very few platelets (7%) were retained (Table I), so weaker citrate concentrations were also studied. In native blood 33% of the platelets were retained. The addition of heparin had no effect, but the addition of citrate even in weak concentration (0.10%) prevented the retention of most of the platelets (14% retained).

TABLE I

EFFECT OF CITRATE, HEPARIN, AND NO ANTICOAGULANT ON THE RETENTION OF PLATELETS IN A COLUMN OF GLASS BEADS

Final Concentration of Anticoagulant	Percentage of Platelets Retained
Citrate 0.32%	7
Citrate 0.21%	11
Citrate 0.10%	14
Native blood	33
Heparin 3.6 units per ml	35

In the aggregation experiments (Table II) 0.64% citrate with ADP gave a very poor response. When the citrate was weaker (0.32%) a better response occurred. In heparin PRP (3.6 units per ml) the response was greater still. Increasing the concentration of heparin to 91 units per ml did not alter the results. The first wave of aggregation with adrenaline

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF CITRATE AND HEPARIN ON THE RESPONSE OF PLATELETS TO DIFFERENT AGGREGATING AGENTS

Final Concentration of Anticoagulant	Aggregating Agent			
	(Slope Expressed as a Percentage of that Obtained with Citrate 0.32%)			
	ADP	Adrenaline	Collagen	5 HT
		1st Slope	2nd Slope	
Citrate 0.64%	18	47	65	3
Citrate 0.32%	100	100	100	100
Heparin 3.6 units per ml	167	150	37	43
Heparin 91 units per ml	176	160	61	29

was influenced by these various concentrations of both anticoagulants in a parallel manner. The second wave of aggregation with adrenaline (O'Brien, 1963) and the response to collagen gave a quite different pattern of results. Again 0.64% citrate gave a poor response and 0.32% a better response but the response in the presence of heparin was much

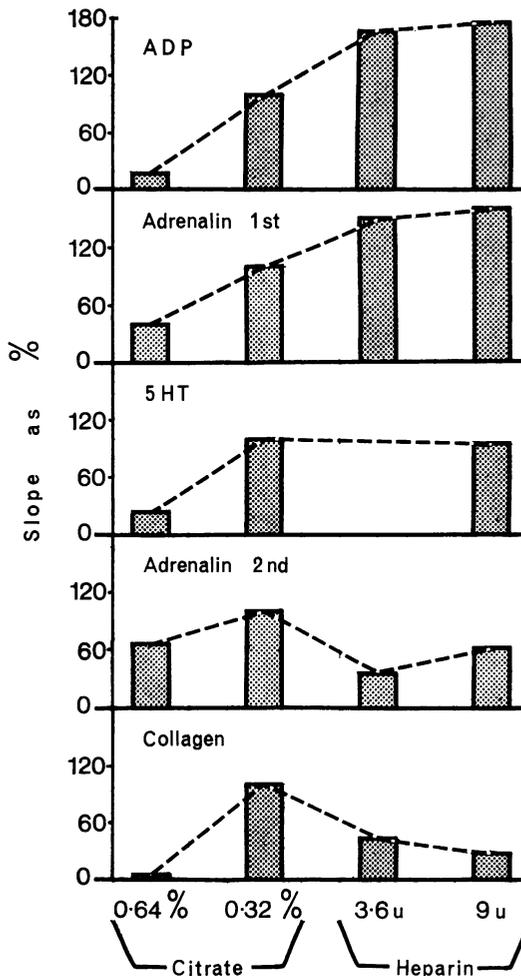


FIG. 1. Diagram of the results.

worse than that in citrate 0.32%; the results in strong heparin may have differed from those in weaker heparin but were still far worse than those in the citrate platelet-rich plasma. The pattern of response to 5 HT was intermediate; the result in heparin was similar to that in 0.32% citrate platelet-rich plasma. These results are represented semi-graphically in Figure 1. It will be seen that the position on the abscissa of citrate 0.32% has arbitrarily been put equidistant from 0.64% citrate and heparin 3.6 units per ml. Thus the position of 3.6 units per ml heparin corresponds to the hypothetical position of 0.0% citrate.

The poor results in heparin PRP with collagen and adrenaline (second slope) might suggest that heparin had an inhibitory effect. Accordingly,

heparin PRP (3.6 units per ml) was prepared. To half of this PRP strong heparin was added (final concentration 104 units per ml) and saline to the other half. These two subsamples were compared using all the aggregating agents (Table III). It will be seen that the further addition of heparin had no effect on aggregation induced by ADP, adrenaline first and second slopes, or 5 HT, but strong heparin markedly decreased the response to collagen.

TABLE III

EFFECT OF VARYING THE CONCENTRATION OF HEPARIN ON PLATELET AGGREGATION

Final Concentration of Heparin (units/ml)	Aggregating Agent				
	ADP	Adrenaline		Collagen 5 HT	
		1st Slope	2nd Slope		
3.6	143	88	41	71	97
104	137	98	46	26	94
104	Result with 104 units per ml expressed as a percentage of the result with 3.6 units per ml				
	96%	111%	112%	37%	97%

DISCUSSION

In the glass bead column test, native blood and heparinized blood gave similar results; it is concluded that heparin has no inhibitory effect. The presence of even weak citrate results in very few platelets being retained so it may be concluded that this situation is very sensitive to the concentration of calcium ions.

In all the aggregation tests, increasing the concentration of citrate from 0.32% to 0.64% always resulted in a decreased response. These results are in line with those of Skoza *et al* (1967) and suggest that all these aggregation phenomena are calcium dependent. This suggestion gains strong support from Figure 1. It will be seen that with ADP and the adrenaline (first slope) the result using heparin PRP almost coincides with the hypothetical point where the citrate concentration is nil. Thus the apparent increase in response in heparin may be fully explained by the absence of citrate. There is no evidence that heparin itself enhances or inhibits these responses since the addition of strong heparin (Table III) or the collection of blood into strong heparin did not alter the result. Clayton and Cross (1963) and Rowsell, Glynn, Mustard, and Murphy (1967) also produced evidence that heparin itself has no inhibitory or enhancing effect on ADP-induced aggregation.

It is common knowledge that PRP prepared in heparin is more likely when handled to undergo spontaneous aggregation than citrate platelet-rich plasma. A possible interpretation was that heparin was a weak aggregating agent. However, no evidence to support this has been found, and thus this

difference is presumably due to the relative inhibitory effect of citrate. Accordingly, although heparin PRP is more difficult to work with, its instability probably represents more accurately the state of platelets *in vivo*.

Hovig (1963) reported that strong heparin added to citrated rabbit PRP decreased the response to a tendon extract. The present results are in agreement with this. Why are the collagen and adrenaline second slope results in heparinized PRP so much less than in citrated PRP, when it has already been shown that in other situations citrate decreases the response by removing calcium and heparin has no inhibitory effect? It is now established that in both these situations, namely, collagen-induced aggregation and the adrenaline second wave, aggregation is induced by the liberation of intrinsic ADP from platelets as a result of the release mechanism (Grette, 1962). Thus heparin may have some inhibitory effect on the release mechanism. Increasing the concentration of the heparin further decreases the result with collagen (Tables II and III) thus supporting the suggestion that heparin inhibits release. Heparin could have a specific effect on collagen itself, but this seems less probable because the adrenaline second slope is inhibited similarly and it depends entirely on release due to propinquity (O'Brien, and Woodhouse, 1968). *In vitro*, heparin could not be added to citrate PRP without sometimes producing aggregation. However, Rowsell *et al* (1967) showed that heparin given to dogs decreases the response of citrated PRP to collagen. They comment that there may be a more subtle interaction of platelets with the endothelium. It can now be suggested that the interaction was the effect of heparin on the release reaction.

These findings *in vitro*, suggesting that heparin inhibits the release reaction, may be of more than academic interest. Certainly most effects of heparin *in vivo* are related to its antithrombotic and its lipoprotein lipase-stimulating activity. The release phenomenon probably is of physiological importance and a recent study of the effect of aspirin (O'Brien, 1968) would support this suggestion; thus a further action of heparin *in vivo* may be its inhibition of the release mechanism of platelets. This action may contribute to its effectiveness as an antithrombotic agent and be involved in some curious findings, for example, the wave-like bleeding time when high concentrations of heparin are given (Hjort, Borchgrevink, Iverson, and Stormorken, 1960).

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