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Inhibition of Human Platelet–Collagen Adhesion Reaction by Amitriptyline and Imipramine[1] (37962)

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Adhesion of blood platelets to collagen or other vascular wall components is presumably the essential early event in the process of hemostasis (1–3). Platelets also have been shown to adhere spontaneously to various collagen preparations *in vitro* (4). This initial adhesion of platelets to collagen is followed by release of ADP, serotonin, and other components from the adherent cells, and these agents in turn are thought to cause aggregation of nearby nonadherent platelets (5–7).

Many drugs, including antihistamines, tranquilizers, and antidepressants, have been shown to influence platelet behavior *in vitro* (7). It is known that imipramine and related tricyclic antidepressants, when used in appropriate concentrations, inhibit ADP-induced platelet aggregation (8). Mills and Roberts (9) demonstrated that desmethylimipramine and amitriptyline in fact prevent the secondary but not the primary wave of aggregation, suggesting that these drugs likely inhibit only the release reaction. They also noted that these drugs prolong the lag phase of collagen-induced aggregation. Evidence is presented here to show that imipramine hydrochloride and amitriptyline hydrochloride, at appropriate concentrations, effectively inhibit not only platelet aggregation induced by collagen, a reaction probably mediated by release of ADP from adherent platelets, but also the adhesion of platelets to collagen.

Materials and Methods. Blood from healthy human donors who denied having received any medication during the preceding week was drawn using as anticoagulant either 3.2% sodium citrate or 1% EDTA (1 vol anticoagulant + 8 vol of blood). Platelet-rich plasma (PRP) was prepared by centrifugation of anticoagulated blood at 350*g* for 10 min at 23°. Platelets in PRP were counted by the method of Brecher and Cronkite (10) and the count was adjusted to 300,000 platelets/mm^3 with platelet-poor plasma (PPP) from the same donor.

Collagen suspension was prepared by grinding 4 g of human Achilles tendon following the method described by Hovig (4). Tendon was obtained from nondiseased extremities of cadavers. Effects of collagen suspension prepared from tendons obtained from 6 cadavers were studied. It is realized that these are crude preparations which likely contain elastin and other components in addition to collagen.

Platelet adhesion and aggregation were studied by use of a Chronolog aggregometer (Chrono-log Corp., Broomall, PA). Four-tenths of a milliliter of PRP was mixed with 0.05 ml of 0.154 *M* NaCl (saline) (controls) or solutions of inhibitor (tests) and allowed to stand at 23° for 4 min. The reaction mixture was then transferred to a small tube and placed in an aggregometer thermostatically controlled at 37°. After 1 min, 0.05 ml collagen suspension or ADP solution was added. The output from the aggregometer was received by a Bausch and Lomb VOM 5 recorder.

Platelet–collagen adhesion was quantitated indirectly by performance of platelet counts on stirred and unstirred mixtures as

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follows: to 0.4 ml of PRP in a silicone-coated glass tube 0.05 ml of saline (control) or saline containing imipramine (2×10^{-4} *M*) or amitriptyline (2×10^{-4} *M*) was added. These mixtures were prepared in duplicate. After allowing one of these duplicate mixtures to stand for 5 min to permit inhibitors to react with platelets, 0.05 ml collagen suspension was added. This tube was centrifuged immediately at 100*g* for 1 min and an aliquot removed for the baseline platelet count. Five-hundredths of a milliliter of collagen suspension was added to the second tube and the mixture was stirred slowly (300 rpm) for 5 min, then centrifuged at 100*g* for 1 min and the platelet count of the supernatant determined. The difference in the platelet counts before and after stirring with collagen in the presence of EDTA and in the presence and absence of the drugs under study was taken as the number of platelets adhering to collagen. EDTA inhibits platelet aggregation but not adhesion of platelets to collagen.

Inhibitory agents were imipramine HCl (Geigy Pharmaceuticals, Ardsley, NY), amitriptyline HCl (Merck, Sharp and Dohme, Rahway, NJ), chlorpromazine HCl (Smith, Kline and French, Philadelphia, PA), promethazine HCl (Wyeth Labs, Philadelphia, PA), and glucosamine (Sigma Chemical Co., St. Louis, MO). Each inhibitor was dissolved in saline. The pH of the PRP containing these inhibitors was found in each case to be between 7.2 and 7.5.

All other chemicals used were of analytical grade. All glassware with which platelets came in contact was silicone coated with G.E. Drifilm SC87 (10% in toluene) except the uncoated aggregometer tubes. Experiments were performed after 1 hr and before 3 hr of the time blood was drawn. All operations were carried out at 23° except the platelet aggregation or adhesion reaction which was performed at 37°.

Results. Studies with citrated PRP; Inhibition of adhesion and aggregation. In citrated human PRP, normal platelet aggregation was observed upon addition of saline extracts of human Achilles tendon. The various collagen suspensions obtained from different cadavers induced comparable platelet aggregation in citrated PRP. When PRP was preincubated with 2×10^{-4} *M* imipramine or amitriptyline, subsequent addition of collagen produced no appreciable increase in the transmitted light, suggesting that these compounds inhibited collagen-induced platelet aggregation (Fig. 1, curves 4 and 5). A closer examination of the collagen particles by means of phase-contrast microscopy revealed that these compounds also had inhibited markedly, if not completely, the adhesion of platelets to collagen (Fig. 2). Addition of either drug to citrated or EDTA PRP altered the platelet count only slightly in control tests. Likewise, platelet counts did not change greatly even after addition of collagen to PRP which contained either imipramine or amitriptyline (Table I). Lower concentrations of these drugs (10^{-4} *M*) inhibited platelet aggregation induced by collagen only moderately (Fig. 1, curve 3) whereas still lower concentrations (10^{-5} or less) had no appreciable effect (Fig. 1, curve 2).

Studies with EDTA PRP; inhibition of adhesion. Platelets adhered to collagen particles in the presence of EDTA but failed to

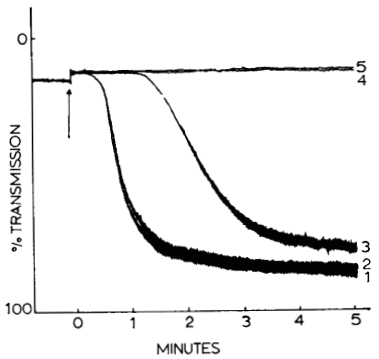

FIG. 1. The effect of imipramine or amitriptyline on collagen-induced platelet aggregation in citrated human PRP preincubated at 23° for 5 min with (1) saline, (2), (3), and (4) 10^{-5}, 10^{-4}, and 2×10^{-4} *M* imipramine, respectively, and (5) 2×10^{-4} *M* amitriptyline. Results with lower concentrations of amitriptyline were same as shown with imipramine. 93.5 μg (dry weight) collagen was added to each mixture at the point indicated by arrow.

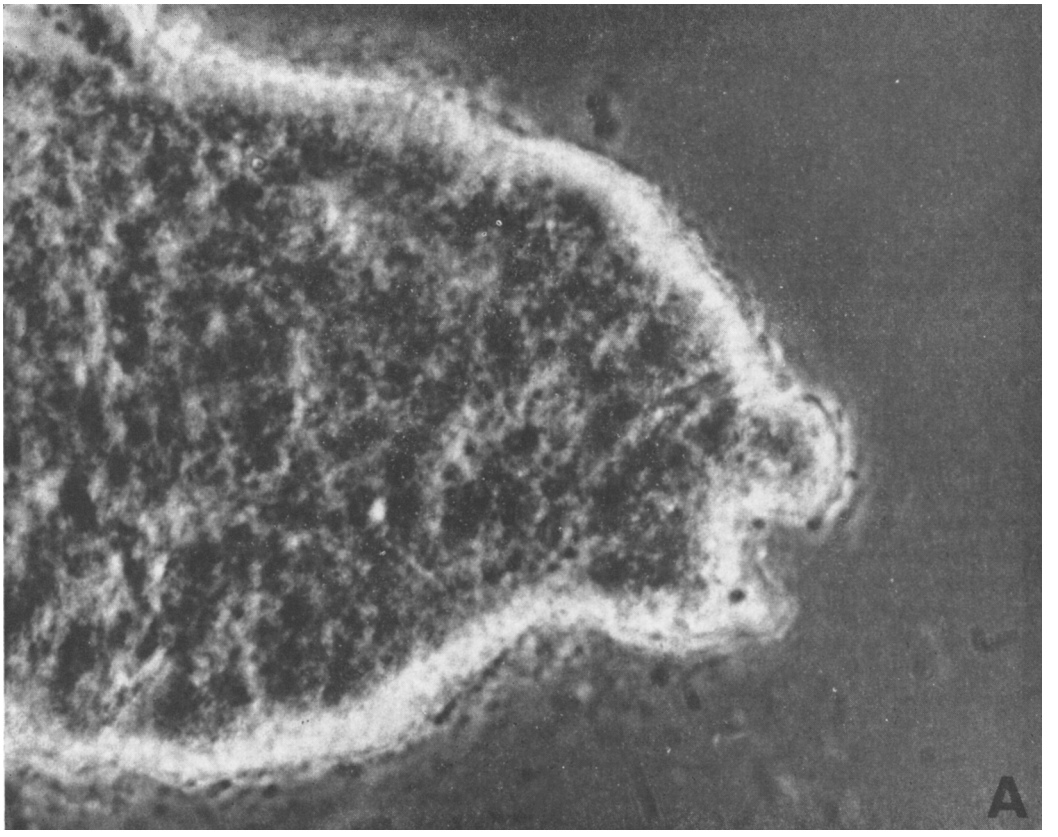

FIG. 2. Inhibition of adhesion of platelets to collagen and of collagen-induced aggregation of platelets in citrated human plasma containing imipramine or amitriptyline. PRP was preincubated with (A) saline or (B) $2 \times 10^{-4} M$ imiprimine at 23° for 5 min before 93.5 μg (dry weight) of collagen was added and stirred gently for 5 min. In (A) platelets have normal aggregates in the presence of collagen. In (B) only a few platelets appear to adhere to collagen.

aggregate. In order to observe adhesion of platelets to collagen photometrically, the amount of collagen added to test mixtures was increased approximately 6 times over that used in aggregation studies. As shown in Fig. 3 (curve 1), when EDTA PRP was stirred with a collagen suspension, an increase in the transmitted light was recorded after a lag phase of about 1 min. That this increase in the transmitted light was due to platelets adhering to collagen was further confirmed by: (i) determination of platelet count both before and after the PRP–collagen mixture was stirred; this showed a marked decrease in platelet count after the PRP was stirred with the collagen suspension (Table I) and (ii) microscopic examination of PRP–collagen mixtures; platelets were seen adhering extensively to collagen particles (Fig. 4A). Addition of $2 \times 10^{-4} M$ imipramine or amitriptyline to this test system markedly inhibited this adhesion of platelets to collagen. In the presence of either of these two drugs, changes in transmitted light did not occur when collagen–platelet mixtures were stirred (Fig. 3, curves 2 and 3). Microscopic examination (Fig. 4B) as well as platelet counts (Table I) also confirmed inhibition of the platelet–collagen adhesion reaction in the presence of these drugs. Although the extent of inhibition varied somewhat, a $2 \times 10^{-4} M$ concentration of either of the drugs was found effective in almost all cases and generally

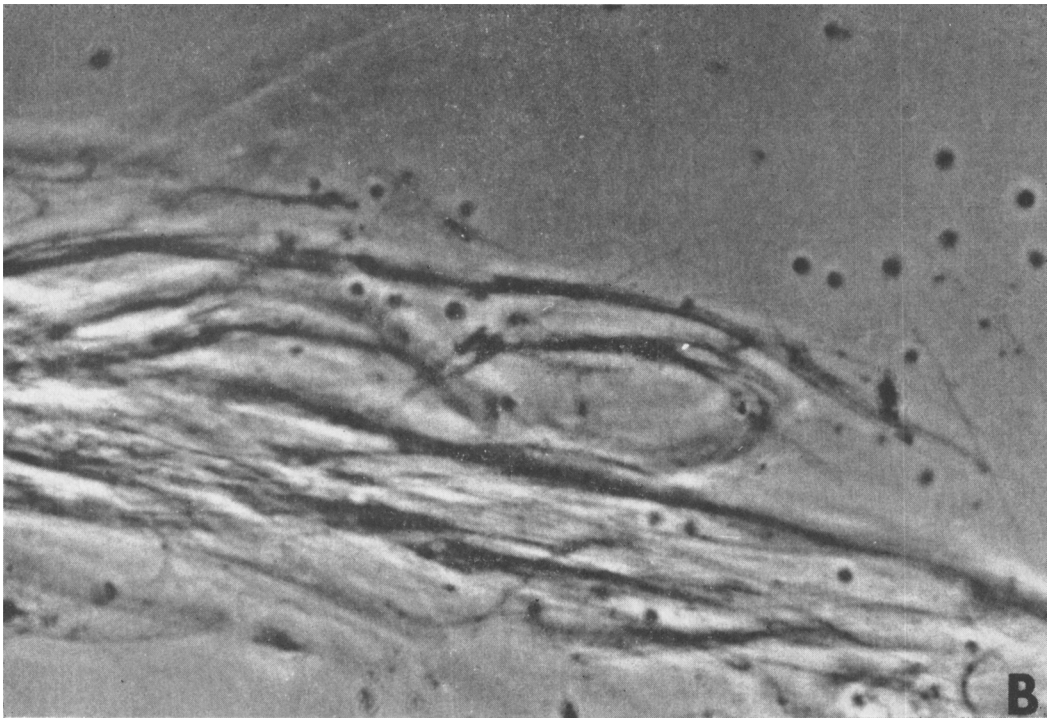

FIG. 2 (*Continued*).

produced marked inhibition of platelet adhesion to collagen.

In EDTA PRP, chlorpromazine ($2 \times 10^{-4} M$) or promethazine ($2 \times 10^{-4} M$) also inhibited platelet–collagen adhesion considerably (Table I), but these drugs were found to be less effective as inhibitors of adhesion than were imipramine or amitriptyline when tested in citrated PRP. Similarly, $10^{-3} M$ glucosamine inhibited markedly platelet adhesion to collagen in EDTA PRP (Table I), but it failed to inhibit either adhesion or aggregation in citrated PRP even at $2 \times 10^{-3} M$ concentration.

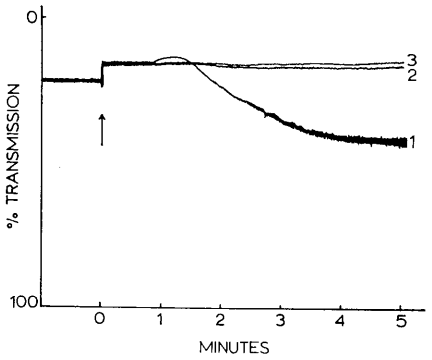

FIG. 3. The effect of imipramine or amitriptyline on platelet–collagen adhesion in EDTA PRP. PRP was incubated at 23° for 5 min with: (1) saline, (2) $2 \times 10^{-4} M$ imipramine, or (3) $2 \times 10^{-4} M$ amitriptyline. In each experiment 0.56 mg (dry weight) collagen was added at the point indicated by arrow.

Inhibition of aggregation induced by ADP. Both imipramine and amitriptyline inhibited platelet aggregation induced by addition of ADP to citrated PRP at $2 \times 10^{-4} M$ (Fig. 5, curve 5). At lower concentrations, these compounds inhibited only the secondary wave of ADP-induced aggregation (Fig. 5, curves 3 and 4) and failed to inhibit markedly either collagen-induced adhesion or aggregation (Fig. 1, curves 2 and 3). The lowest concentration of either drug tested had only slight effect on aggregation (Fig. 5, curve 2).

Discussion. Although imipramine and re-

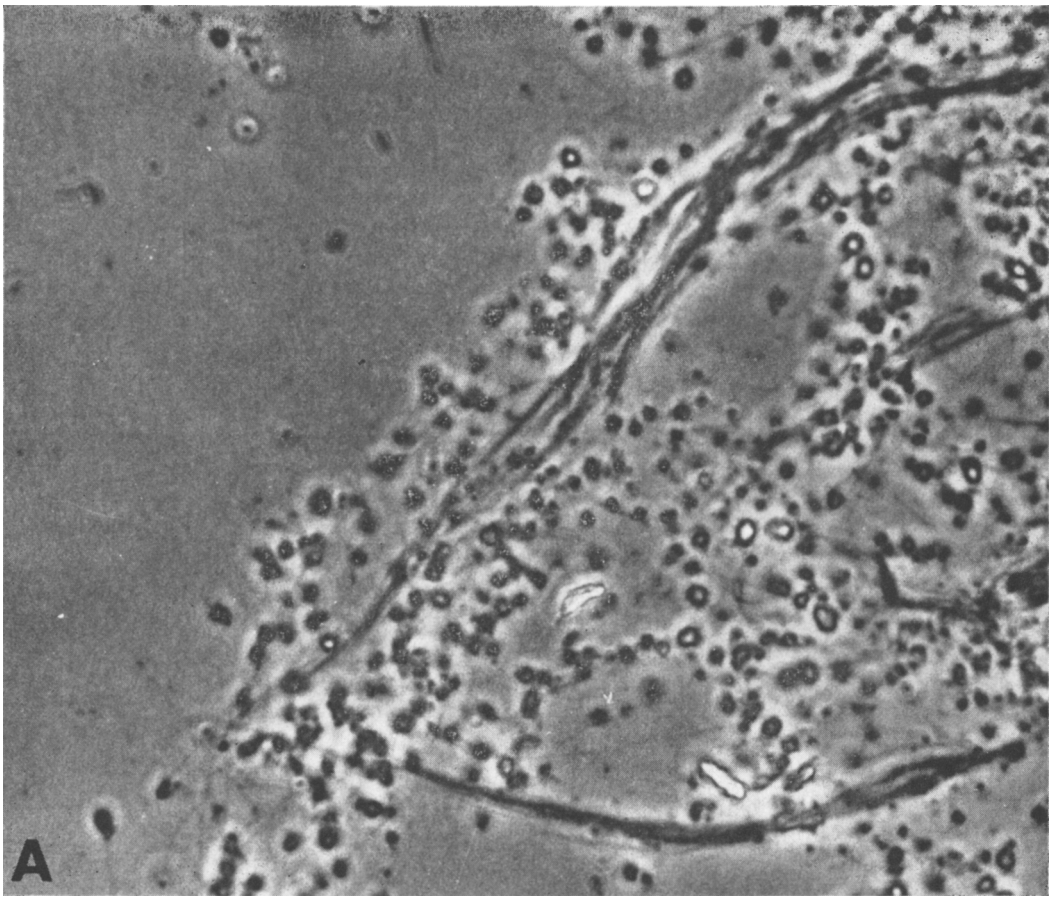

FIG. 4. Inhibition of platelet–collagen adhesion reaction in EDTA PRP. PRP was incubated at 23° for 5 min with: (A) saline or (B) $2 \times 10^{-4} M$ amitriptyline. 0.56 mg (dry weight) collagen was added in each experiment.

lated drugs are known to inhibit platelet aggregation induced by ADP or serotonin (7, 9), their effect on the platelet–collagen adhesion reaction has received scant attention. Enhancement of the lag phase of collagen-induced aggregation in the presence of low concentrations of desmethylimipramine, nortriptyline, and amitriptyline was noted by Mills and Roberts (9). However, effect of these drugs on the platelet–collagen adhesion reaction was not studied.

Imipramine and amitriptyline inhibited platelet–collagen adhesion in citrated or EDTA PRP in the present studies. Since the concentrations of these drugs which inhibited platelet–collagen adhesion in EDTA PRP also inhibited platelet aggregation induced by addition of ADP to citrated PRP, the presence of EDTA itself in the first reaction mixture was not necessary for prevention of aggregation. It must, however, be emphasized that, whereas platelet adhesion to collagen was significantly inhibited by these drugs in EDTA or citrated PRP, a few platelets were occasionally seen adhering to collagen even though the drug was present. Also, the extent of inhibition varied slightly with different donors; however, at $2 \times 10^{-4} M$ concentration, these drugs significantly inhibit the platelet–collagen adhesion reaction in PRP from nearly all donors. This concentration of imipramine or amitriptyline is lower than that at which sulfhydryl group inhibitors (*N*-ethyl maleimide, $5 \times 10^{-4} M$; *p*-hydroxymercuribenzoate, $10^{-3} M$) have been shown to inhibit

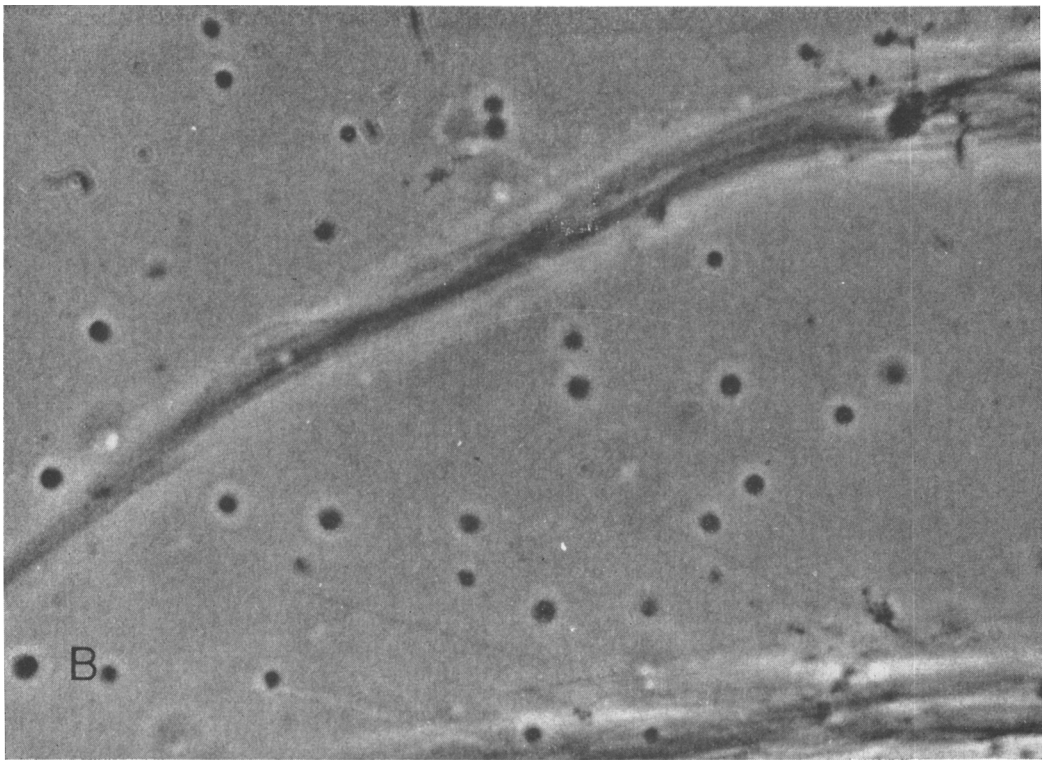

FIG. 4 (*Continued*).

TABLE I. Inhibition of Platelet–Collagen Adhesion Reaction in Human PRP.

Reaction mixture	Platelet count × 10^3 [a]	
	Before addition of collagen suspension	After addition of collagen suspension and stirring for 5 min[b]
A. Citrated PRP[c]		
1. Control	295.0 (305.0) [d]	25.0
2. 2×10^{-4} *M* imipramine HCl	295.0 (308.3)	253.3
3. 2×10^{-4} *M* amitriptyline HCl	288.3 (300.0)	253.3
B. EDTA PRP[c]		
1. Control	280.0 (302.5)	68.3
2. 2×10^{-4} *M* imipramine HCl	290.0 (295.0)	261.6
3. 2×10^{-4} *M* amitriptyline HCl	293.3 (305.0)	266.6
4. 2×10^{-4} *M* chlorpromazine HCl	270.0 (286.6)	223.3
5. 2×10^{-4} *M* promethazine HCl	285.0 (291.6)	210.0
6. 10^{-3} *M* glucosamine	281.6 (288.3)	223.3

[a] Mean of 3 experiments from the same donor. This is representative of similar experiments performed on blood from 15 different donors.

[b] Platelet counts performed on supernatant of test mixtures following centrifugation at 100*g* for 1 min to remove aggregates and collagen fibers.

[c] PRP was diluted with PPP to give a count of 300,000 platelets/mm^3 in the reaction mixture.

[d] Figures in parentheses show the platelet counts before the reaction mixture was stirred for 5 min at 23° in the absence of collagen suspension.

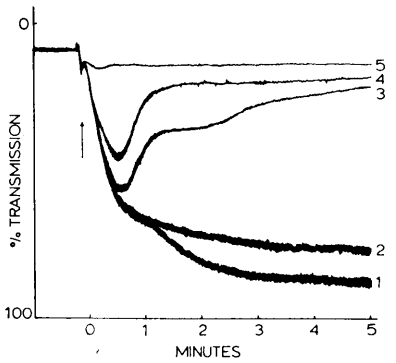

FIG. 5. The effect of various concentrations of imipramine on ADP-induced platelet aggregation in human citrated plasma. PRP was incubated at 23° for 5 min with: (1) saline, (2), (3), (4), and (5) $10^{-5}M$, $5 \times 10^{-5}M$, $10^{-4}M$, and $2 \times 10^{-4}M$ imipramine, respectively. $1.5 \times 10^{-6}M$ ADP was added in each experiment at the point indicated by arrow. Similar results were obtained with amitriptyline.

platelet adhesion to collagen (11). However, it is difficult to assess the effective concentrations of various inhibitors in plasma due to protein binding effects, and this is particularly true for sulfhydryl inhibitors. Hence a comparison of results based on the amounts of various inhibitors added may be inappropriate. As for other known inhibitors of platelet–collagen adhesion reaction, chlorpromazine and promethazine ($2 \times 10^{-4}M$ or greater) were found effective in EDTA PRP, but both were less effective when tested in citrated PRP. Although glucosamine at $10^{-3}M$ inhibited adhesion of platelets to collagen in EDTA PRP, it did not inhibit adhesion or aggregation in citrated PRP even at concentrations of up to $2 \times 10^{-3}M$.

That the concentration of imipramine or amitriptyline which inhibits platelet adhesion to collagen both in citrated and EDTA PRP also inhibits platelet aggregation induced by addition of ADP to citrated PRP is of great interest. It seems probable that there may be a common mechanism for platelet–platelet (aggregation) and platelet–collagen (adhesion) reactions, and that in the presence of appropriate concentrations of these inhibitory drugs, platelets would fail to adhere to collagen or to aggregate with one another. An apparent similarity between platelet aggregation and adhesion reactions has also been pointed out by Mason *et al.* (12).

Imipramine and related drugs are known to influence platelet behavior *in vitro* (7–9, 13–15). Observations of Jamieson *et al.* (16) suggest the formation of a complex between glucosyltransferases on platelets and a carbohydrate moiety on collagen. These investigators have also observed a parallel between the inhibition by aspirin ($10^{-3}M$), chlorpromazine ($4 \times 10^{-4}M$), and glucosamine ($10^{-3}M$) of glucosyltransferase activity and the inhibition of platelet–collagen adhesion. Penicillin G also inhibits the adhesion of platelets to collagen (17). In addition, a plasma component when absorbed onto collagen is known to inhibit the aggregation-inducing effect of collagen (18). The mode of action of any of these inhibitory agents is not understood clearly at present although all but glucosamine and Penicillin G are said to be membrane stabilizing compounds. It remains to be seen if inhibition of platelet–collagen adhesion in every case would also mean inhibition of platelet aggregation. The observations reported here, however, suggest the similarity between the mechanism of platelet aggregation and platelet–collagen adhesion. The observations could also form the basis for further investigations on the possible role of imipramine and related drugs in the prevention of thrombotic conditions.

Summary. The capability of human blood platelets to adhere spontaneously to collagen, both in citrated and EDTA platelet-rich plasma, was shown to be inhibited markedly in the presence of imipramine or amitriptyline. Adhesion of platelets to collagen was quantitated by a method specific for adhesion.

A possible similarity between the mechanism of platelet aggregation and that of platelet–collagen adhesion is suggested.

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