Long-Term Inhibition of Platelet Functions by Aspirin[1] (34683)

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Acetylsalicylic acid (aspirin) has marked effects on platelet function in animals (1) and man (2–5). Aspirin ingestion suppresses the second phase of aggregation which is induced by epinephrine (4, 5) or a critical concentration of adenosine diphosphate (ADP) (2, 5) at 37° in stirred, citrated, platelet-rich plasma (PRP). It also inhibits the development of platelet factor-3 (phospholipid-like) activity induced by a critical concentration of ADP, and the release of platelet-bound ^{14}C-serotonin caused by this or a higher concentration (2). In addition, it decreases platelet aggregation and release of ADP and ^{14}C-serotonin induced by suspensions of connective tissue particles (CT) (2, 3, 5). The effects of a single oral dose may persist up to 7 days (3, 4). Although aspirin may markedly prolong the bleeding time of patients with a pre-existing hemorrhagic diathesis (6, 7), it prolongs the bleeding time of normal subjects only slightly, without producing a clinical hemorrhagic tendency (3, 6, 8, 9).

Since aspirin may prove useful as an antithrombotic agent, experiments were carried out to ascertain whether the drug's effects on platelets persist during long-term administration. The subjects were ambulatory patients with rheumatoid arthritis or rheumatic fever who attended the Irvington House Outpatient Clinic. One group of 10 patients, 9 to 15 years of age, had taken 0.6 to 5.3 g of aspirin daily for 3 weeks to 2 years. The control group of 9 patients, 14 to 27 years of age, had not taken aspirin for at least 3 weeks.

Materials and Methods. Twenty-seven ml of blood were placed in a plastic tube containing 3 ml of 3.2% sodium citrate and 0.03 ml of ^{14}C-serotonin (10). PRP prepared by slow centrifugation and kept at 37°, was used within 90 min of blood collection. Platelet aggregation was induced at 37° by stirring PRP with epinephrine (Adrenalin chloride, 1:1000, Parke, Davis) at a final concentration of 50 μM, ADP (disodium salt, Sigma Chemical) at final concentrations of 2 and 10 μM, or a suspension of washed CT (10). It was recorded as previously described (2); the degree of aggregation was expressed as the maximum decrease in optical density (OD) noted within 4 min. At that time, platelet factor-3 activity was estimated by measuring the recalcified clotting time of part of the sample with Russell's viper venom (RVV) (Stypven, Burroughs Wellcome) (2). The remainder of the sample was centrifuged and the plasma used to determine the percentage of platelet-bound ^{14}C-serotonin released (2, 10). Isotonic saline solution was substituted for an aggregating agent in the control samples.

Results. The platelets in the control PRP of both patient groups failed to aggregate when stirred for 4 min with isotonic saline and contained 79–96% of the added ^{14}C-serotonin. The RVV clotting time was 30–40 sec, depending on the strength of the RVV and the particular PRP, and did not change during stirring. Results with aggregating agents are summarized in Fig. 1. With 50 μM epinephrine, a small amount of aggregation (primary)

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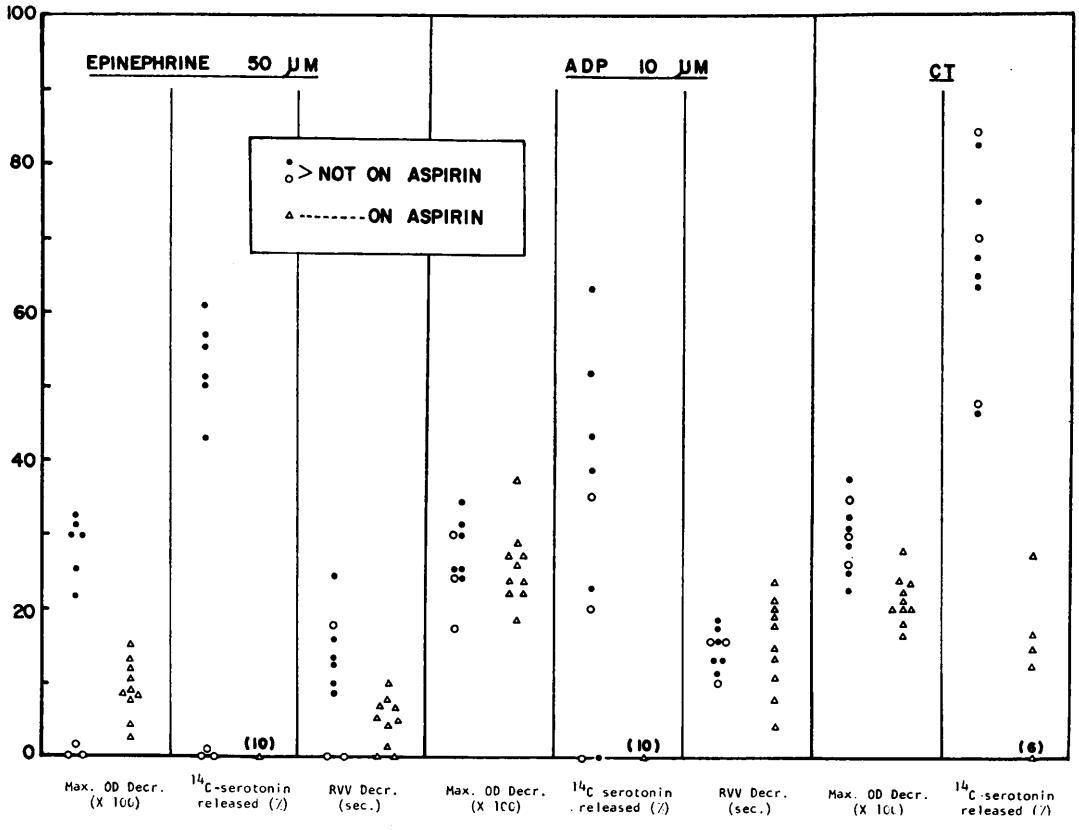

FIG. 1. The effect of epinephrine, ADP, and CT on platelet aggregation, release of platelet-bound ^{14}C-serotonin and shortening of the Russell's viper venom clotting time in control patients (●, ○), and in patients receiving aspirin (△); (○), control patients who failed to show a second phase of aggregation with 50 μM epinephrine. Numbers in parentheses show the subjects whose platelets failed to release ^{14}C-serotonin.

occurred within a few seconds. In 6 of 9 control patients, as in many normal individuals (4), more marked (secondary) aggregation followed in about 1 min, causing the OD to decrease more than 0.20, and 40% of the platelet-bound ^{14}C-serotonin was released. Secondary aggregation and ^{14}C-serotonin release were not observed in the aspirin-treated patients or the other 3 control patients. In the subjects with the secondary aggregation, the RVV clotting became considerably shorter than in the control samples, indicating development of platelet factor-3 activity. It was also shortened in some of the subjects whose PRP did not show secondary aggregation, and there was some overlap between the two groups. With 10 μM ADP, primary and secondary aggregation cannot be differentiated, and the OD decrease in the two groups was the same. The RVV clotting time shortened to the same extent in both groups. Platelets of 7 of 9 control subjects released over 30% of their ^{14}C-serotonin, whereas none was liberated from the platelets of the aspirin-treated subjects. With 2 μM ADP (not illustrated), aggregation and platelet factor-3 activity were also the same in both groups and ^{14}C-serotonin release failed to occur in 2 additional control subjects. CT caused considerably less aggregation and much less release of ^{14}C-serotonin in aspirin-treated patients than in untreated patients. Its effect on the development of platelet factor-3 activity could not be studied since CT itself shortened the RVV clotting time.

Discussion. The platelet function tests and

response to aspirin were the same in patients with rheumatic fever or arthritis as in normal subjects. The effect of aspirin on secondary aggregation is more readily apparent with epinephrine than with ADP since the latter produces secondary aggregation only with use of a critical concentration—usually about 1 μM, though it varies in different PRP samples. Aspirin causes striking inhibition of ^{14}C-serotonin release induced by epinephrine, ADP, or CT. It inhibits the development of platelet factor-3 activity caused by the critical concentration of ADP (2), but not by the 2 or 10 μM concentrations used in the present study. This finding suggests that the development of platelet factor-3 activity depends on the degree of aggregation, whether primary or secondary, and does not parallel the release of ^{14}C-serotonin or endogenous ADP (11). A similar conclusion was reached by Sixma and Nijessen (submitted for publication).

Summary and Conclusions. The inhibitory effects of aspirin on platelet function persist during long-term administration. This drug would therefore appear to hold promise as an antithrombotic agent.

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