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Inhibition of human platelet aggregation by aspirin *in vitro* and *ex vivo*

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Aspirin (acetylsalicylic acid; ASA) has been tested clinically as an antithrombotic, but with disappointing results (M.R.C. Steering Committee, 1973). The rationale for clinical trial was the previous observation that platelet aggregation was inhibited by ASA *in vitro* and *ex vivo* (i.e. *in vitro* testing after oral administration) (Weiss, Aledort & Kochwa, 1968). Using pig platelets, we recently showed that the inhibitory potency of ASA was much greater in citrated platelet-rich plasma (CPRP) than in heparinized platelet-rich plasma (HPRP) (Gordon & MacIntyre, 1974). We have now extended these studies to human platelets.

Collagen-induced platelet aggregation was measured photometrically (Born, 1962; Gordon & Drummond, 1974) in CPRP (3.8 mg/ml trisodium citrate), in HPRP (5 iu/ml sodium heparin), and in washed platelet suspensions (Walsh, 1972). Inhibition by ASA was calculated as described previously (Gordon & MacIntyre, 1974).

Collagen was 2-3 times more potent as an aggregating agent in CPRP than in HPRP. The IC_{50} value for ASA *in vitro* was 17.6 ± 2.5 μg/ml in CPRP and 144.1 ± 13.2 μg/ml in HPRP (mean ± s.e.; $n = 18$). This difference was not due to the higher collagen concentration used in HPRP. We previously reported in studies with pig platelets a similar difference in the inhibitory potency of ASA, although collagen was a more potent aggregating agent in pig HPRP than in CPRP. The IC_{50} in suspensions of washed human platelets was 21.0 ± 3.5 s.e. μg/ml—similar to that in CPRP. The inhibitory potency of ASA was increased by preincubation in PRP, and the time-course was similar in CPRP and HPRP.

After ingestion of 900 mg ASA, aggregation responses in CPRP, HPRP and washed platelet suspensions were similarly inhibited. This was unexpected and we therefore repeated the studies *in vitro* in CPRP with added heparin. The effectiveness of ASA was greatly reduced by the prior addition of heparin, but heparin added after ASA had only a slight antagonistic effect. Control aggregation responses were not significantly reduced by adding heparin to CPRP.

Our earlier results (Gordon & MacIntyre, 1974) indicated that the antithrombotic value of ASA might have been over-estimated because the potency of ASA in CPRP was exaggerated compared with HPRP. The present experiments show, however, that the potency of ASA is in fact reduced by prior addition of heparin, and no discrepancy between HPRP and CPRP exists in tests *ex vivo*. If platelet aggregation is mediated by activation of coagulation factors at the platelet membrane (Ardlie & Han, 1974) ASA could act by acetylating one or more of these factors. Heparin might interfere with this process by masking the site of acetylation. The present study does not explain the discrepancy between the effectiveness of ASA *in vitro* and *in vivo*, but provides a possible clue to the site of action of ASA on platelets.

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