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Inhibition of collagen-induced platelet aggregation by aspirin

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Ingestion of acetylsalicylic acid (A.S.A.) inhibits platelet aggregation *in vitro*, and the release of platelet constituents induced by collagen (Zucker & Peterson, 1970), but A.S.A. has little or no antithrombotic activity in clinical trials (M.R.C. Steering Committee, 1972). The present study suggests reasons for this discrepancy.

Fresh pig blood was obtained from an abattoir and anticoagulated with 6% v/v acid-citrate-dextrose (Aster & Jandl, 1964) or 10% v/v sodium heparin (50 I.U./ml). Platelet rich plasma (P.R.P.) was prepared by centrifuging the blood for 15 min at 180 g. Aggregation induced by human subcutaneous collagen (H.S.C.) (Zucker & Borrelli, 1962) was measured photometrically (Born, 1962). The maximum rate of aggregation was measured by drawing a straight line through the steepest part of the recording, and expressed as light transmission units (L.T.U.) per minute. The potency of the H.S.C. was expressed as that amount of collagen protein (Hydroxyproline x 7) needed to give a half-maximal rate of aggregation (the EC_{50}).

Collagen was more potent in heparinized than in citrated plasma. Mean EC_{50} values in 13 plasmas were 0.51 μg/ml in citrate and 0.20 μg/ml in heparin. H.S.C.-induced aggregation was inhibited by A.S.A., preincubated for 2 min at 37°C in the P.R.P. Using EC_{50} collagen, the A.S.A. concentration which inhibited aggregation by 50% (the IC_{50}) was found.

The IC_{50} was always greater in heparin than in citrate, despite the fact that less collagen was used as the aggregating stimulus in heparinized plasma. Mean IC_{50} values for A.S.A. versus EC_{50} collagen were 23 μg/ml in citrated P.R.P. and 233 μg/ml in heparinized P.R.P. When collagen concentrations greater than EC_{50} were used, the inhibitory potency of A.S.A. declined.

After oral ingestion of A.S.A., plasma levels of total salicylate in man may reach 0.5 mg/ml or more, but rapid hydrolysis keeps maximum levels of A.S.A. itself around 20 μg/ml, and only the acetylated moiety has any significant effect on platelet aggregation or release (Zucker & Peterson, 1970). *In vitro*, 20 μg/ml A.S.A. inhibits collagen-induced aggregation in citrated plasma, provided a submaximal collagen concentration is used, but has no effect in heparinized plasma. These results suggest that the antithrombotic potential of A.S.A. may have been overestimated because of results of previous studies performed in citrated plasmas using suboptimal collagen concentrations as the aggregating stimulus.

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