oestrogen alone and the extent of this reduction appears to be related to the ratio of oestrogen to progesterone.

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Potentiation by prostaglandin E_1 and arachidonic acid of oedema in the rat paw induced by various phlogogenic agents

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Although prostaglandins have been implicated as mediators of inflammation (Vane, 1970) the nature of their involvement remains to be elucidated. In light of the findings of Ferreira (1972) that exogenous prostaglandins can potentiate the pain-producing effect of exogenous bradykinin and histamine in humans, it was of interest to determine if the ability of various phlogogenic agents to induce inflammation in the rat paw could be potentiated by prostaglandin E_1 (PGE_1) and arachidonic acid (A.A.).

Male Wistar (CE/CFHB) rats weighing 80-100 g were divided into three groups, each containing 10 animals. Groups of the animals received either: (a) a submaximal dose of the phlogogenic agent, (b) PGE_1 (100 ng) or A.A. (100 μg), or (c) a combination of the phlogogenic agent and either PGE_1 (100 ng) or A.A. (100 μg). Injections were via the subplantar route into the right hind paw. The contralateral paw received an equal volume of vehicle (0.05-0.1 ml). The mean difference between right and left paw volumes for each group, as measured by mercury displacement plethysmometry, was determined at the time of maximal oedema, namely 0.5 h for PGE_1 treated groups and 1.5 h for A.A. treated groups. To quantify the degree of potentiation, the inflammations ensuing after separately administered phlogogenic agent and either PGE_1 or A.A. were summated. This value was subtracted from the oedema observed when the agent and either PGE_1 or A.A. were administered concomitantly. The difference was expressed as a percentage of the summated oedema produced by the agent and either PGE_1 or A.A. given separately.

PGE_1 did not potentiate the summated responses to serotonin (1 μg), histamine (10 μg), compound 48/80 (1 μg) or dextran (6 μg). However, PGE_1 did enhance the summated response to carrageenan (1 mg) by 83 ± 17%, kaolin (10 mg) by 80 ± 16%, bradykinin (4.5 μg) by 110 ± 25% and trypsin (50 μg) by 38 ± 8%. A.A. potentiated the summated response to carrageenan (1 mg) by 25 ± 5% and kaolin (10 mg) by 69 ± 11% but did not enhance the action of serotonin (1 μg), histamine (10 μg), compound 48/80 (1 μg), dextran (6 μg), bradykinin (4.5 μg) or trypsin (50 μg).

Leucocytes have been implicated in carrageenan- (Di Rosa, Papadimitriou & Willoughby, 1971), but not bradykinin-induced oedema (Ward, 1972). Since leucocytes constitute a source of prostaglandin synthetase (Higgs & Youlten, 1972), the inability of A.A. to potentiate the response to bradykinin may result from inadequate conversion of A.A. to prostaglandins. Inflammation induced by carrageenan and kaolin, but not by serotonin, histamine, dextran or compound 48/80, is potentiated by exogenous PGE_1 and A.A. Non-steroidal anti-inflammatory drugs, such as indomethacin, are potent inhibitors of carrageenan-induced oedema whilst being less effective inhibitors of inflammation induced by serotonin or dextran (Winter, 1964). Should inhibition of prostaglandin synthetase constitute the mechanism of action of non-steroidal anti-inflammatory drugs (Vane, 1970), prostaglandins would not appear to be involved in the mediation of serotonin and dextran oedema. Hence, the results of this study suggest a correlation between the role of prostaglandins in experimentally induced oedemas and the ability of PGE_1 and A.A. to potentiate such oedemas.

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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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