platelet-rich plasma *in vitro* was induced by acoustical energy of very low intensity. The aggregation of platelets was probably caused by the release from platelets of adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT) and this release was similar to the release of nucleotides and amines occurring during the second phase of platelet aggregation.

The platelet-rich plasma was prepared from citrated blood of man or sheep. Samples of platelet-rich plasma were stirred in the Born aggregometer and high frequency (20,000 Hz) acoustical power was delivered to the samples through a microprobe attached to a piezoelectric transducer. The intensity and the duration of the stimulus was accurately controlled at very low levels (2–100 J) which did not cause obvious platelet disruption. Following the measured application of the acoustical energy, platelets aggregated. The initial rate and extent of aggregation depended on the total amount of energy delivered into the sample; the temperature did not increase significantly. Aggregation was reversible and the platelets could be aggregated again after disaggregation.

Some drugs which have a stabilizing effect on cell membranes (Seeman & Weinstein, 1966) prevented or reduced aggregation caused by low intensity acoustical energy. Chlorpromazine (1×10^{-6}M), amitriptyline (1×10^{-7}M) or imipramine (5×10^{-7}M) inhibited the effect in concentrations which normally are ineffective in preventing aggregation caused by ADP. Bromolysergic acid diethylamide and lysergic acid diethylamide inhibited the effect in concentrations (1×10^{-8}M) which normally inhibit platelet aggregation caused by 5-HT, but the inhibition was not complete. The effect was inhibited also by adenosine (1×10^{-5}M) and 2-chloroadenosine (5×10^{-6}M). The specific inhibitor of ADP, 2-methylthioadenosine-5′-phosphate (Michal, Maguire & Gough, 1969) caused only a partial inhibition of sonic aggregation (1×10^{-4}M).

These observations indicate that the platelets release material including ADP and 5-HT when subjected to the low intensity acoustical vibration. Membrane stabilizing drugs probably prevent the release from occurring and the specific antagonists of ADP or 5-HT prevent the subsequent action of the released substances on the platelets.

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Formation and release of prostaglandins by platelets in response to thrombin

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When thrombin acts on resuspended platelets of man, pig, rabbit and guinea-pig there is a release of substances which increase vascular permeability (Packham, Nishizawa & Mustard, 1968). In rabbits, this permeability effect is abolished by antihistamines. Prostaglandins E_1 and E_2 have been shown to increase vascular permeability in rats (Crunkhorn & Willis, 1969) and this effect is also blocked by antihistamines. It was, therefore, worthwhile to find out whether thrombin causes

platelets to form and release prostaglandins, particularly as they are known to affect platelet function (Kloeze, 1966). We now show that human platelets can form and release at least two prostaglandins, namely PGE_2 and $PGF_{2\alpha}$, under the influence of thrombin.

Platelets were resuspended in 5–10 ml of a buffered saline solution (134 mM NaCl; 5 mM D-glucose; 15 mM Tris-HCl, pH 7·4) as previously described (Born & Smith, 1970). Suspensions containing $7–75 \times 10^8$ platelets/ml were shaken at 37° C for 5 min with bovine thrombin (1 N.I.H. unit/ml) or with buffered saline. For determining release, platelets were sedimented by centrifugation and the supernatant was decanted for subsequent extraction. Samples of whole suspensions, supernatants, and platelets were cooled to 4° C, adjusted to pH 3 and extracted twice with ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness and partitioned between 67% aqueous ethanol and petroleum ether to remove non-hydroxylated fatty acids. After drying, the ethanol extracts were dissolved in Krebs solution and assayed by their effects on the rat stomach strip (Vane, 1957), rat colon (Regoli & Vane, 1964) and chick rectum (Mann & West, 1950), superfused in cascade at 4 ml/min with Krebs solution containing a mixture of antagonists as already described (Willis, 1969). Portions of the reconstituted extracts were acidified and re-extracted with ethyl acetate. The extracts were dried, dissolved in ethanol and submitted to thin layer chromatography in the AI, AII (Green & Samuelsson, 1964) and CII (Ramwell & Daniels, 1969) solvent systems, modified as described at this meeting (Willis, 1970).

Recovery of PGE_2 (20 ng) added to a platelet suspension was 75%. No prostaglandin-like activity could be extracted from the thrombin. In five subjects, control suspensions contained little or no PG-like activity (less than 0·1 ng/10^8 platelets estimated as PGE_2 on the rat stomach strip) whereas suspensions treated with thrombin all contained activity (0·25–2·0 ng/10^8 platelets). About 90% of the activity of the thrombin-treated samples was found in the suspending medium. Extracts chromatographed in the AI system showed a distribution of activity consistent with both E and F types of prostaglandins. In the AII and CII systems, activity was almost entirely located in zones corresponding to PGE_2 and $PGF_{2\alpha}$. It now seems that some permeability effects may be mediated by the release of prostaglandins from platelets.

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