Specific binding of [^{3}H]-5-HT was essentially instantaneous and was directly proportional to the number of platelets. Binding to the highest affinity site (Ka^{-1} = 10 nM) was prevented by 5-HT antagonists such as methergoline (IC_{50} = 0.7 nM). There was good correlation between inhibition of this [^{3}H]-5-HT binding and inhibition of the 5-HT-induced shape change (Table 1). Inhibitors of 5-HT uptake also affected shape change and binding to the highest affinity site, but only at micromolar concentrations. This provides direct evidence in support of the observation that inhibitors of 5-HT uptake can also act as 5-HT antagonists (Domenjoz & Theobald, 1959).

Binding of [^{3}H]-5-HT to the middle affinity site (Ka^{-1} = 0.1 μM) was insensitive to 5-HT antagonists but was blocked by 5-HT uptake inhibitors. There was good correlation between inhibition of 5-HT uptake and inhibition of binding to the middle affinity site for chlorimipramine (IC_{50} value against uptake, 0.2 μM), Lilly 103947 (0.25 μM), Lilly 110140 (0.5 μM), imipramine (0.7 μM), amitriptyline (1.2 μM) and desmethylimipramine (7.5 μM). Our results suggest that the binding of [^{3}H]-5-HT to the highest affinity site is involved in the production of the platelet shape change and that the middle affinity site may be related to the carrier for the active transport of 5-HT.

A.H.D. is an M.R.C. scholar.

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A study of the binding of drugs of blood constituents

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This communication reports some preliminary results obtained in the study of the nuclear magnetic resonance spectroscopy (n.m.r.) of compounds that inhibit platelet aggregation.

Platelet suspensions were prepared from citrated rabbit and human blood by a modified procedure of Ardlie & Han (1974). To aid the resuspension of platelets after centrifugation, 1 vol of a 10% sucrose solution was added to 5 vol of platelet rich plasma (PRP). Submaximal aggregation was produced by adenosine diphosphate (ADP) 2 μM and the aggregation was recorded by the turbidimetric method of Born & Cross (1963). The n.m.r. spectra were obtained using a 60 or 100 MHz spectrometer with D_2O as a solvent.

ADP-induced platelet aggregation occurred to the same degree in platelet suspensions made up in water or D_2O. This enabled the NMR spectra of compounds to be studied in washed platelet suspensions without any impairment of the ability of platelets to aggregate.

Dipyridamole, its analogues 2,6-bis(diethanol-amino)-4-piperidinopyrimido-[5,4-d] pyrimidine (RA 233), 4-morpholino-2-piperazine-thiopheno-[3,2-b] pyrimidine (VK 774) and 2-[(2-amino-ethyl)amino] -4-morpholinothiopheno- [3, 2-b] pyrimidine (VK 744) were studied together with AG19417 (CIS 1, 2, 3, 4, 4a 10b-hexahydro-8, 9-dimethoxy-2-methyl-6-phenylbenzo [c] [1,6]-naphthyridine bis hydrogen maleate) (Ott & Smith, 1971) and its 4-acetoaminophenyl analogue (AH21132).

Preliminary binding studies were performed using four or five concentrations of bovine serum albumin (BSA). Dipyridamole and its analogues gave spectra that were unsuitable for quantitative study but the addition of increasing concentrations of albumin did cause the spectral peaks of dipyridamole and its analogues to broaden.

The spectra of AG19417 exhibited single isolated peaks which allowed calculations of relaxation rates to be made. An increase in BSA concentration resulted in a linear increase in the relaxation rates of the three main peaks. The increase for the phenyl group was significantly greater ($P > 0.95$) than the increase for either the two methoxy groups or the *N*-methyl group suggesting that the phenyl group is specifically involved in the binding process.

In washed platelet suspensions (4×10^6 platelets/mm^3) the peaks of both AG19417 and

AH21132 gave the same relative increase in relaxation rates. The relaxation rate of the relaxation of maleate ion also increased in platelet suspensions but to a lesser degree. The greater increase in the relaxation rates of drug molecules indicates a specific binding to platelets.

The results obtained indicate that if suitable preparations of platelets can be prepared, information of binding characteristics of drugs which exhibit single peak spectra can be obtained.

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Effects of prostaglandins E_1, E_2 and D_2 on platelet aggregation: variation with animal species and ionized calcium concentration

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Prostaglandin E_1 (PGE_1) is a potent inhibitor of platelet aggregation in all animal species (Ki $\sim$ 20 nM). Prostaglandin D_2 (PGD_2) is even more effective than PGE_1 at inhibiting the aggregation of human platelets but is apparently much less potent when tested on platelets from some other species (Smith, Silver, Ingerman & Kocsis, 1974). Prostaglandin E_2 (PGE_2) is inhibitory at micromolar concentrations and there is some controversy as to whether lower concentrations enhance aggregation (Bruno, Taylor & Droller, 1974). These studies were performed using platelets suspended in media containing sub-physiological concentrations of ionized calcium. The effect of PGE_1 varies with the ionized calcium concentration (Vigdahl, Marquis & Tavormina, 1969) and platelet aggregation is also calcium-dependent to a variable extent in different animal species (Mürer, 1972). We have investigated the effects of PGE_1, PGE_2 and PGD_2 on collagen-induced aggregation of human, pig and rat platelets in platelet-rich plasma (PRP) anticoagulated with citrate (which chelates free calcium) or heparin (Gordon & MacIntyre, 1974, Gordon and Drummond, 1974).

Results are shown in Table 1. PGE_1 was most potent in man and least potent in the pig. PGD_2 was more potent than PGE_1 in man, less potent than PGE_1 in the pig and was inactive in the rat. PGE_2 was much less potent than PGE_1 in all species. In man and rat, all three prostaglandins were more potent inhibitors in citrate PRP than in heparinized PRP, but in the pig the reverse was true, and PGE_2 induced aggregation directly in pig heparinized PRP. PGE_2 never induced aggregation in human or rat PRP, although with concentrations around 0.3 μM, collagen-induced aggregation was enhanced in heparinized PRP but not in citrated PRP. Platelet aggregation induced by PGE_2 in pig heparinized PRP was inhibited by citrate, EDTA, PGE_1 and PGD_2.

It has been previously shown that platelet aggregation can be induced by endoperoxide intermediates in the PGE_2 biosynthetic pathway (Willis, 1974) and by synthetic derivatives of PGE_2 (Fenichel, Stokes & Alburn, 1975) but the results of the present study are the first demonstration of platelet aggregation induced by a stable, naturally-occurring prostaglandin.

Table 1 Effect of prostaglandins on collagen-induced platelet aggregation IC_{50} values (μM)

Prostaglandin	*Man*		*Rat*		*Pig*	
	Citrate	*Heparin*	*Citrate*	*Heparin*	*Citrate*	*Heparin*
PGE_1	0.015	0.054	0.06	0.09	0.27	0.12
PGD_2	0.008	0.015	> 200.0	> 200.0	1.4	0.15
PGE_2	6.0	22.0	75.0	135.0	67.0	*

* Induced aggregation directly.