Br. J. Pharmac. (1971), **43**, 580–592.

Comparison of the effects of drugs on the aggregation of hamster platelets *in vivo* and *in vitro*

NICOLA A. BEGENT AND G. V. R. BORN

Department of Pharmacology, Royal College of Surgeons of England, London, WC2

Summary

1. A method is described for measuring the inhibitory effectiveness of drugs on the aggregation by ADP of hamster platelets *in vivo*.

2. The method was used to compare the effects of several drugs, *viz.* adenosine, imipramine, desmethylimipramine and aspirin, on platelet aggregation *in vivo* with their *in vitro* effects measured photometrically.

3. The concentrations of adenosine and imipramine present in the cheek pouch after 10 min infusions were measured using radioactively labelled drugs.

4. The results show that adenosine (0·4 μM) inhibited platelet aggregation *in vivo* by 43%, whereas several times this concentration was required to produce the same inhibition *in vitro*.

5. Imipramine and desmethylimipramine (0·4 μM) did not inhibit platelet aggregation *in vivo* ; *in vitro*, however, desmethylimipramine caused up to 34% inhibition at concentrations as low at 0·25 μM.

6. Aspirin (estimated 0·2 mM) inhibited platelet aggregation *in vivo* by 37% whereas similar inhibition *in vitro* required about 1 mM aspirin. Sodium salicylate was several times less potent than aspirin *in vivo*.

Introduction

The aggregation of mammalian platelets can be inhibited in different ways by different substances. The most potent inhibitors known so far are prostaglandin E_1 ; adenosine and some of its derivatives ; some phenothiazines and imipramine ; and non-steroidal anti-inflammatory drugs, particularly aspirin (for reviews see Born, 1970 ; Michal & Firkin, 1969 ; and Mustard & Packham, 1970). The different modes of action of these inhibitors have been intensively investigated *in vitro* and some are becoming clear (Mills & Smith, 1971 ; Smith & Willis, 1970 ; Born, 1971).

Much less is known about the effectiveness of inhibitors *in vivo* where the adhesion and aggregation of platelets in injured or diseased blood vessels are responsible for haemostasis and some types of thrombosis. The main reason why so little is known is the lack of a reproducible method for quantitating aggregation *in vivo*. Injury methods have shown that some of the substances active *in vitro* are also active *in vivo*. Thus, platelet thrombi formed in mechanically injured vessels on the cerebral cortex of anaesthetized animals can be inhibited by infusions of adenosine or 2-chloroadenosine (Born, Honour & Mitchell, 1964). Vascular injuries produced by other means, for example electrical heating or a laser beam, result in the formation of platelet thrombi which are preventable or reversible by

adenosine or AMP (Berman, 1967) or by dextran (Arfors, Hint, McKenzie, Matheson & Svensjö, 1970). Both quantitation and reproduceability are limited by irreversible alterations at each injury site so that successive experimental runs have to be made at different sites. To determine the factors that initiate the adhesion of platelets to vessel walls *in vivo*, a technique is required that is more delicate but equally reproducible.

Recently, we described such a method (Begent & Born, 1970a), in which microiontophoresis is used to apply the thrombogenic agent adenosine diphosphate (ADP) to the outside of uninjured small venules in the hamster cheek pouch. By this means it is possible to estimate the amount of thrombogenic agent applied, at least to the outer wall of the vessel (Begent & Born, 1970b). Furthermore, platelet thrombi are formed in the vessel for only as long as the iontophoretic current is passed. The behaviour of the site remains almost constant during successive application of the currents, which are so small (10–300 nA) that electronmicrographs show no evidence of abnormality at the endothelial surfaces.

This paper describes the use of the technique for evaluating the inhibitory effectiveness on platelet thrombogenesis *in vivo* of three drugs which inhibit platelet aggregation *in vitro*. By using radioactively labelled drugs we have tried to correlate their inhibitory potencies *in vitro* and *in vivo*.

Methods

Measurement of drug effects in vivo

The method of preparing the hamster cheek pouch for microscopic observation, the iontophoretic application of ADP to induce platelet thrombi and the filming and measuring of the growth of thrombi have been described (Begent & Born, 1970a).

The right jugular vein of each hamster was cannulated with fine polythene tubing (PP10) which was attached to a fine needle (25G) and filled with saline. Before the infusion of any drug a white body was induced in the selected venule and filmed to give a control value for the growth rate constant. The cannula was then connected to a slow infusion pump and the drug under investigation infused at 12·5 μl/min for a measured time immediately after which a white body was induced at the same site and filmed. The infusion was stopped and further white bodies were induced at various times after the end of the infusion.

Estimation of drug concentrations in blood and cheek pouch

Imipramine

^{14}C-Imipramine (8·05 mCi/mM) (1 mM) was infused at 12·5 μl/min for 10 min into the jugular vein. The pouch was then clamped and cut off, blotted and weighed. The tissue was cut into small pieces and homogenized in 0·5 ml distilled water. The proteins of 0·5 ml of homogenate were precipitated with 40 μl of 13 M perchloric acid and the supernatant was neutralized with 80 μl of 2 M K_2CO_3. A quantity (0·5 ml) of the neutralized supernatant was taken for counting radioactivity. Two dilutions of the original ^{14}C-imipramine were also counted as standards.

Estimation of blood volume in cheek pouch

The amount of blood in the cheek pouch was determined using an acid haematin method in the following way. The supernatant of cheek pouch homogenate was diluted 1 in 100 with 0·1 N HCl. The acid haematin colour was developed for 40 min and read at 580 nm. From the readings for blood a standard curve was drawn and from this the volume of blood was determined which produced the colour measured in the pouch homogenate. The concentration of imipramine radioactivity in the cheek pouch was calculated from the radioactive counts in the test and standard samples and from the estimated blood volume. For these calculations we assumed that during the period of infusion the imipramine was not metabolized and was still present in the blood.

Adenosine

^{3}H-Adenosine (1·4 mM and 0·36 Ci/mM) was infused into the jugular vein at 12·5 μl/min for 10 minutes. The cheek pouch was clamped and cut off, blotted and weighed. The pouch was homogenized in 100 μl distilled water containing inosine, hypoxanthine and adenosine, each at a concentration of 10 mM, to act as 'carriers' for subsequent paper chromatography. The proteins were precipitated with 20 μl of 13 M perchloric acid and the extract was neutralized with 40 μl of 2 M K_2CO_3 and centrifuged. Supernatant (50 μl) was taken for radioactive counting and 50 μl were spotted on paper and run for about 4 h with inosine, hypoxanthine and adenosine as standards using the Schwartz solvent system 30 (butanol: water 86: 14). The chromatogram was dried, the fluorescent spots were identified under ultraviolet light and the area of paper over which the test sample had run was cut into even strips. Each strip was cut into small pieces which were dropped into a counting vial; the radioactivity was eluted with the scintillation fluid and counted. In one experiment 50 μl of cheek pouch extract which contained added nucleotides and nucleosides each at a concentration of 10 mM to act as 'carriers' and was diluted with 1 ml H_2O, passed through a column of Dowex cation exchange resin and eluted with 1 ml water. The radioactivity in the eluate was determined. Another 50 μl sample of the cheek pouch extract was also spotted on to Whatman 541 paper with ATP, ADP, AMP, inosine, adenosine and hypoxanthine as standards and run for 4 h in the solvent system of Randerath & Struck (1961) (butanol: acetone: 5% v/v NH_3: water: 0·1 M EDTA, 45: 15: 10: 19: 1). The fluorescent spots were identified under ultraviolet light and marked. The paper was cut up and counted as before. A sample of blood was treated in exactly the same way as the cheek pouch. The percentage of radioactivity still present as adenosine and the percentage that has been converted to nucleotides or to other nucleosides were calculated from the results of the chromatography. The concentration of adenosine in the cheek pouch and in the blood could then be calculated from the percentage of adenosine in the total radioactive counts in the test samples and from the cheek pouch weight or the volume of the blood sample. For the calculation of concentration in the cheek pouch it was assumed that during the infusion adenosine diffused throughout the whole tissue.

Differential radioactive count on hamster blood

In one experiment after the infusion of ^{3}H-adenosine for 10 min the hamster was

bled by cardiac puncture. The blood was mixed with 0·1 vol. 3·8% trisodium citrate as anticoagulant and centrifuged at 200 *g* for 10 minutes. The supernatant platelet-rich plasma was pipetted off and centrifuged at 1,000 *g* for 10 min to obtain platelet-free plasma and a platelet pellet. The red cells were washed with normal saline and spun at 1,000 *g* for 10 minutes. The proteins of 100 μl each of whole blood, platelet-rich plasma, platelet-free plasma and red cells were precipitated with 40 μl of 13 M perchloric acid ; the extracts were neutralized with 80 μl of 2 M K_2CO_3 and centrifuged. Samples of 50 μl of each supernatant were counted. Dilutions of the original ^{3}H-adenosine were also counted as standards. The haematocrit was estimated as 40% and from this and the radioactive counts the percentage of radioactivity in each fraction of blood was calculated.

Measurement of drug effects in vitro

Anaesthetized hamsters were bled by cardiac puncture into sodium citrate. The blood was pooled, centrifuged at 200 *g* for 10 min and the platelet-rich plasma was pipetted off ; plastic tubes were used throughout. The platelets were counted using the method of Brecher & Cronkite (1950). The platelet-rich plasma was diluted 1 in 4 with Tris-saline. Samples of 3 ml of the diluted plasma were incubated at 37° C without and with each of several drugs, namely, adenosine or 2-chloroadenosine, desmethylimipramine and aspirin, which inhibit platelet aggregation in different ways. The aggregation induced by ADP (final concentration of 10^{-6}M) was measured using the turbidimetric method (Born, 1962) after incubation for 1, 5 and 10 min at 37° C without and with the inhibitory drugs. The slope of the optical density trace was used as a measure of the velocity of platelet aggregation. From the slopes for the control plasma and the plasmas plus inhibitor, the percentage inhibition brought about by each drug was calculated.

Materials

Adenosine 5′-diphosphate (sodium salt) and adenosine from Sigma Chemical Company. Imipramine (Tofranil) and desmethylimipramine (Pertofran) from Geigy (U.K.) Ltd. Aspirin was made up from acetylsalicylic acid and sodium hydrogen carbonate, both obtained from B.D.H. Ouabain B.P. (Strophanthin G) from Burroughs Wellcome & Co. Imipramine-^{14}-C[N-(3-Dimethylaminopropyl) iminodi(benzyl-methyline-^{14}C)hydrochloride] was obtained from the Radiochemical Centre, Amersham. Adenosine-^{3}H-(adenosine-2,8-^{3}H(N)) was obtained from New England Nuclear Corporation. 2-Chloroadenosine was kindly given by Dr. Frank Michal. All drugs were made up to the required concentration in 0·154 M saline, except the ADP (10^{-2}M) for microiontophoresis which was made up in distilled water.

Results

Adenosine

Adenosine (10^{-3}M) infused at a rate of 12·5 μl/min for 10 min inhibited the growth rate by 43% (Fig. 1). The inhibition was reversible, the growth rate returning to the control value 10 min after the end of the infusion. This concentration of adenosine did not cause any change in the velocity of blood flow. Infusion of adenosine at the same rate but at a concentration 10 times higher (10^{-2}M) for 10 min caused complete inhibition of white body formation which was

accompanied by a fall in blood flow velocity, presumably due to widespread vasodilatation; these effects were also reversible.

The concentration of adenosine present in the cheek pouch was determined after infusing $1{\cdot}4 \times 10^{-3}$M ^{3}H-adenosine (specific activity 0·36 Ci/mmol for 10 min (Table 1). Of the total radioactivity in the cheek pouch, only 29% was intact adenosine; 11% was inosine while 60% had been converted to adenine nucleotide. The concentration of adenosine itself was estimated from the amount recovered after chromatography (see **Methods**) and on the assumption that it had reached diffusion equilibrium between blood plasma and extracellular water in the cheek pouch. A correction was also made for recoveries of adenosine from homogenized cheek pouch, which in two experiments was 72% and 58%, mean 65%.

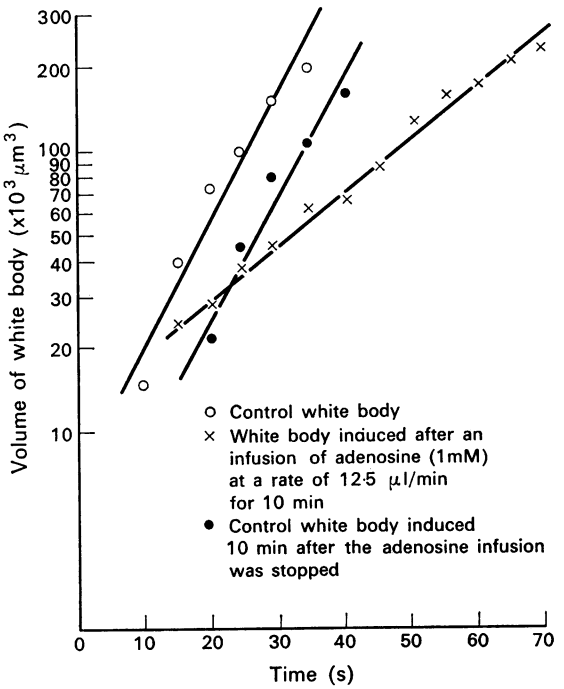

FIG. 1. Volume ($\times 10^3$ μm^3) of white bodies induced by iontophoresis of ADP plotted semi-logarithmically against time (s).

TABLE 1. *Distribution of ^{3}H-radioactivity in nucleosides and nucleotides in mixed cardiac blood and in whole cheek pouch after intravenous infusion of ^{3}H-adenosine for* 10 *minutes*

Extraction technique	Paper chromatography solvent (Randerath & Struck, 1961)		Cation exchange resin		Paper chromatography solvent (butanol:water 86:14)	
Experiment no.	1		2		3	4
Tissue	Blood	Cheek pouch	Blood	Cheek pouch	Cheek pouch	Cheek pouch
ATP	84·0	46·0	92·0	66·0	58·0	53·0
ADP	10·0	20·0				
AMP	0·7	6·0				
cAMP	0·4	2·0				
Inosine	0·9	7·0			15·0	12·0
Hypoxanthine, Adenine, Adenosine	4·0	19·0	8·0	34·0	27·0	35·0

The concentration of adenosine was determined in mixed cardiac blood taken at the same time. Intact adenosine accounted for even less, that is 6% of the total radioactivity. In two experiments recoveries from whole blood were 96% and 89% mean 93%. The concentration of adenosine in both cheek pouch and blood was

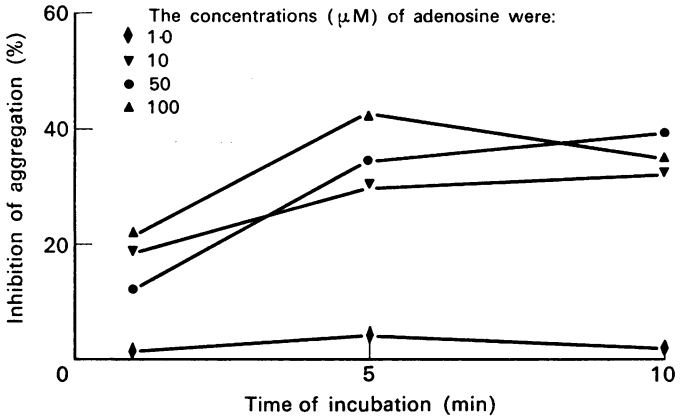

FIG. 2. Inhibition of platelet aggregation in hamster plasma brought about by adenosine which was added 1, 5 or 10 min before ADP (1 μM). Abscissa: time (min) of incubation of the plasma with the drug before addition of ADP. Ordinate: percentage inhibition of velocity of aggregation by ADP.

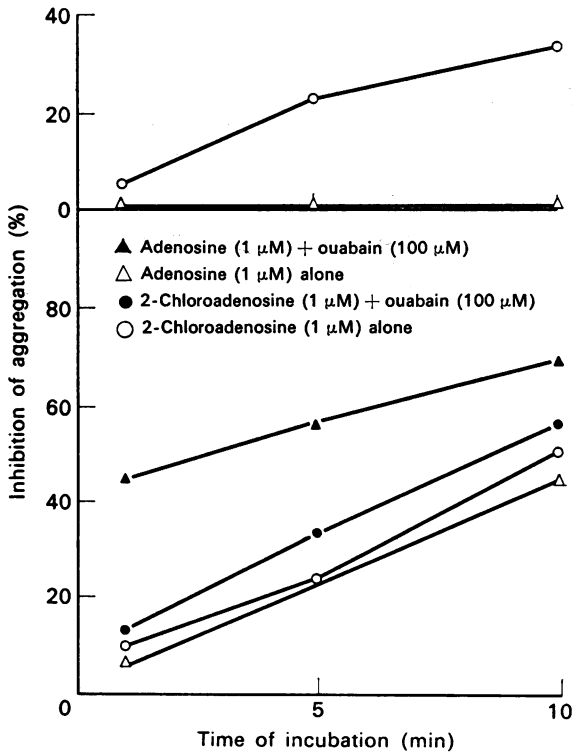

FIG. 3. Inhibition of platelet aggregation in hamster plasma brought about by adenosine (1 μM) or 2-chloroadenosine (1 μM) with or without ouabain (100 μM) which was added 1 min before the adenosine or 2-chloroadenosine. These drugs were added 1, 5 or 10 min before the ADP (1 μM). Abscissa and ordinate as in Fig. 2.

about 0·4 μM. By far the greater part of the radioactivity, that is 94% was recovered as AMP, ADP and ATP (also as a trace of cyclic AMP), in proportions similar to those in erythrocytes (Table 1). The radioactivity in the hamster blood was distributed so that about 96% was in the red cells and about 4% in the plasma, whether platelet-rich or platelet-poor, therefore there was no detectable radioactivity associated with the platelets. When human erythrocytes are incubated with adenosine it is rapidly converted inside the cells to adenine nucleotides (Overgaard-Hansen, Jørgensen & Praetorius, 1957). If the same happens with hamster erythrocytes, their radioactivity was presumably due to adenine nucleotides. Furthermore, as the whole blood contained very little inosine and even smaller amounts of hypoxanthine, most of the plasma radioactivity must have been adenosine.

The higher proportion of adenosine in cheek pouch than in blood could be explained by assuming that intact adenosine passed from the blood into the tissues where it was no longer exposed to uptake and phosphorylation by the red cells. The results show that the concentration of adenosine which *in vivo* inhibited white body growth rate by about 40% was less than 0·5 μM.

In vitro, when adenosine at 1 μM was incubated in hamster platelet-rich plasma for 10 min, aggregation by ADP was not significantly inhibited except in one out of three experiments when there was 45% inhibition (Figs. 2 and 3). One possible reason for the absence of inhibition was the rapid inactivation of adenosine by adenosine deaminase. This enzyme is inhibited by ouabain (Michal & Thorp, 1966). When ouabain (100 μM) was added 1 min before adenosine its inhibitory effectiveness after incubation for 10 min increased by 53% (Fig. 3). In three experiments, at least 10 μM adenosine was needed to produce inhibition similar to that produced by about 0·5 μM *in vivo*.

When hamster platelet-rich plasma was incubated *in vitro* with adenosine its inhibitory effectiveness increased with time, as in other mammalian species (Born, 1964). The increase was greater in the first 5 min than during the second 5 min,

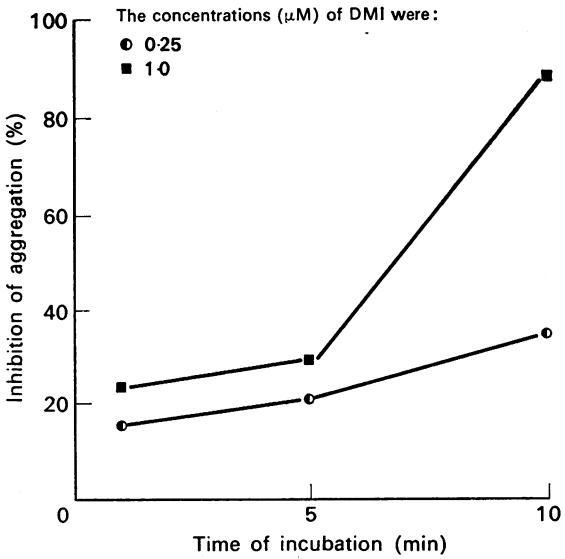

FIG. 4. Inhibition of platelet aggregation in hamster plasma brought about by desmethylimipramine which was added 1, 5 or 10 min before the ADP (1 μM). Abscissa and ordinate as in Fig. 2.

presumably because of increasing deamination (Fig. 2). This conclusion was supported by comparing inhibition by adenosine with that by 2-chloroadenosine, both at 1 μM (Fig. 3). The 2-chloroadenosine which, unlike adenosine, is not a substrate of adenosine deaminase inhibited similarly to adenosine after incubation for 1 min but considerably more than adenosine after incubation for 10 minutes.

Imipramine and desmethylimipramine

In vivo, imipramine (4 mM) or desmethylimipramine (DMI) (up to 40 mM)

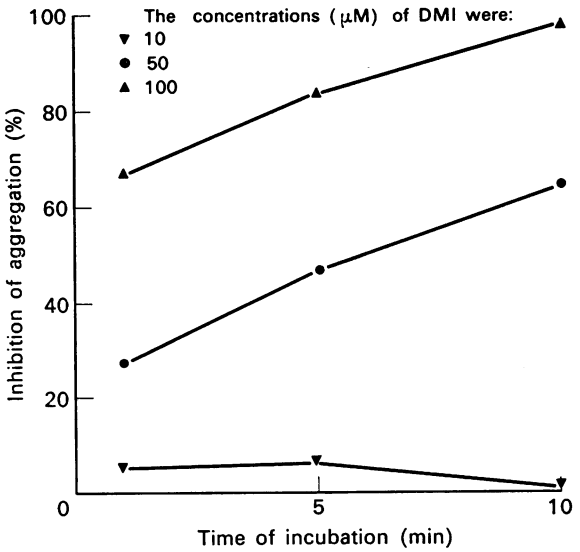

FIG. 5. Inhibition of platelet aggregation in hamster plasma brought about by desmethylimipramine which was added 1, 5 or 10 min before the ADP (1 μM). Abscissa and ordinate as in Fig. 2.

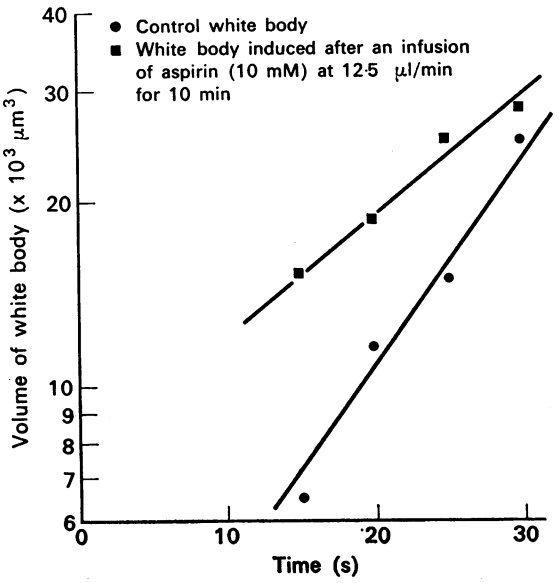

FIG. 6. Volume ($\times 10^3$ μm^3) of white bodies induced by ADP plotted semi-logarithmically against time (s).

infused at 12·5 μl/min for up to 30 min did not alter the growth rate of white bodies induced by ADP. The concentration of ^{14}C-imipramine in the cheek pouch after infusion for 10 min at 12·5 μl/min was 0·2 μM and, assuming a recovery similar to that of adenosine, the true concentration was probably no higher than 0·4 μM.

In vitro, DMI inhibited platelet aggregation down to a concentration of 0·25 μM (Fig. 4) in one experiment, while 50 and 100 μM DMI were even more potent than the same concentrations of adenosine. The inhibition brought about by DMI in-

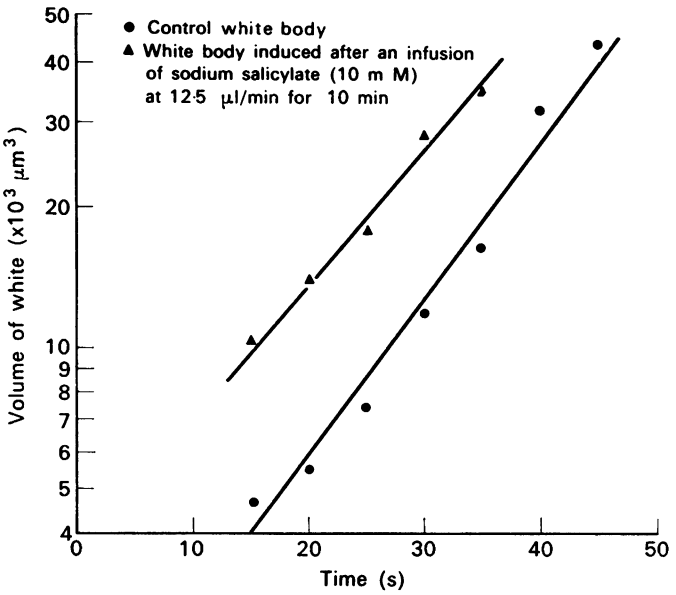

FIG. 7. Volume (×10³ μm³) of white bodies induced by ADP plotted semi-logarithmically against time (s).

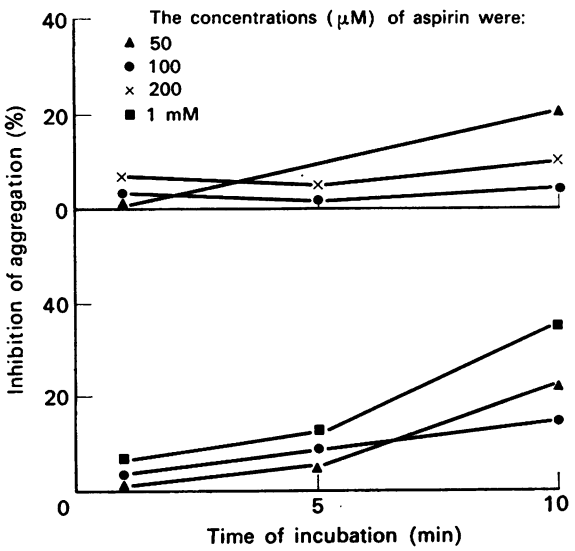

FIG. 8. Inhibition of platelet aggregation in hamster plasma brought about by aspirin which was added 1, 5 or 10 min before the ADP (1 μM). Abscissa and ordinate as in Fig. 2.

creased considerably when the drug and platelet-rich plasma were preincubated for up to 10 min before the addition of ADP (Fig. 5).

Sodium acetylsalicylate and sodium salicylate in vivo

Sodium acetylsalicylate (aspirin) (10 mM) infused at 12·5 μl/min for 10 min, decreased the growth rate constant in two experiments by 33% and 40%, mean 37% (Fig. 6). In three other hamsters aspirin at this concentration or less (5 mM) infused for up to 20 min inhibited white body formation to such an extent that the growth of the platelet mass could not be quantitated. In no experiment did aspirin affect the blood flow velocity. The growth rate remained diminished for at least 30 min after the end of the infusion except in one experiment in which the control growth rate had been exceptionally high, perhaps because the vessel had been damaged.

Sodium salicylate (10 mM) infused at the same rate for the same time decreased the growth rate constant in two experiments by 9% and 11%, mean 10% (Fig. 7) and this effect was reversible within 10 minutes.

The concentration of aspirin in the cheek pouch was not measured directly but can be estimated indirectly as follows. An infusion of aspirin (10 mM) at 12·5 μl/min for 10 min into blood volume of about 7 ml in a hamster weighing about 100 g should result in a whole blood concentration of about 0·2 mM on the assumption that only negligible amounts have left the circulation in that time.

In vitro the inhibitory effect of aspirin was rather variable but at least 1 mM aspirin was needed to inhibit aggregation by ADP by more than 35% (Fig. 8). The inhibition by aspirin increased with increasing periods of incubation of the drug in the plasma before addition of ADP.

Discussion

These results show that the microiontophoretic techniques which we recently introduced for the quantitation of platelet adhesion and aggregation *in vivo* can be used for demonstrating and quantitating effects of drugs on these processes. This is a welcome advance because, up to now, the various techniques by which antiplatelet agents could be tested *in vivo* all depended on the production of some type of vascular injury. Much effort has been expended in the search for injury techniques which could be standardized well enough to make measurements of thrombus formation and drug effects sufficiently reproducible. The best of these methods were: pinching exposed vessels on the cerebral cortex of rat or rabbit with fine ophthalmic forceps (Honour & Ross-Russell, 1962; Honour & Mitchell, 1963; Born *et al.*, 1964) or damaging very limited areas of endothelium by means of a laser beam (Arfors *et al.*, 1970). All injury techniques have several disadvantages, namely (1) it is virtually impossible to make lesions of any kind identical; (2) a site that has been damaged cannot be used more than once; (3) the mechanism which causes platelets to adhere and to aggregate is likely to involve several different factors, both known and unknown. The laser technique has the advantage when used with the rabbit ear chamber that the animal can be conscious and that the same animal can be used again and again to serve as its own control.

In contrast, the production of platelet aggregates in a normal blood vessel to which ADP is applied by microiontophoresis allows the same site to be used many

times so that internal comparisons are possible. Furthermore, the method allows platelet thrombi or white bodies to form on a site in which the only signs of abnormality are a decrease in electron density in the cytoplasm of the vascular endothelial cells after staining with osmium tetroxide and, possibly, some increase in the tendency of granulocytes to emigrate (Begent & Born, 1970a); these points are still under investigation (Born & Dawson, 1971). We have established that white bodies form on a site only as long as the iontophoretic current is passed and that the response remains qualitatively and quantitatively very similar as long as the preparation remains in good condition, as assessed by the absence of oedema or gross inflammation and by the constancy of blood flow velocities in the vessels; this can be for as long as 3 hours.

On the basis of these criteria we found that some drugs which inhibit platelet aggregation *in vitro* (see Mustard & Packham, 1970) inhibited the formation of white bodies in normal venules of the hamster cheek pouch and that this inhibition could be quantitated, whereas some other drugs which inhibit *in vitro* were ineffective.

Adenosine was effective on hamster platelets both *in vitro* and *in vivo*. However, on the assumption that infused adenosine diffused uniformly throughout the tissues, the concentration which inhibited *in vivo* was many times smaller than that which produced similar inhibition *in vitro*. Such a difference has already been observed with rat platelets (Cuthbertson & Mills, 1963; Born & Philp, 1965; Philp, 1970). Why adenosine should be more inhibitory *in vivo* than *in vitro* in these species has not yet been explained.

Several processes favour the rapid disappearance of adenosine from blood plasma. First, adenosine is highly diffusible and moves rapidly into the tissues. Second, it is taken up by cells; in our experiments most of the infused adenosine radioactivity was recovered as adenine nucleotides in the red cells. Third, plasma contains a deaminase which converts adenosine to inosine. Therefore, the demonstration of the inhibitory effect of adenosine on platelet aggregation depended on its continuous infusion.

2-Chloroadenosine was a more potent inhibitor than adenosine *in vitro*. One reason for this is that 2-chloroadenosine is not a substrate for adenosine deaminase. No such comparison was made *in vivo* because 2-chloroadenosine is much more toxic than adenosine (Clarke, Davoll, Philips & Brown, 1952).

The inactivation of adenosine by the plasma deaminase can be inhibited by ouabain (Michal & Thorp, 1966). No *in vivo* experiments were made with ouabain because the concentrations (about 10^{-4}M) required to inhibit the deaminase are greater than those known to have toxic effects on the heart in living animals.

In vitro, inhibition by adenosine increased more steeply in the first minute than afterwards; this had already been observed with rabbit and human platelets (Born, 1964). The reason is not known. Recent results (Mills & Smith, 1971; Born, 1971) have provided evidence that the inhibitory effect of adenosine and 2-chloroadenosine on platelet aggregation depends on an increase of intracellular cyclic AMP. Apparently the nucleosides stimulate adenyl cyclase in platelets. Platelet cyclic AMP can be increased more by combinations of adenosine or 2-chloroadenosine with drugs such as papaverine, dipyridamole or its analogue compound RA 233 which are inhibitors of phosphodiesterase. These *in vitro* observations suggest that

the potency of adenosine *in vivo* might be greatly increased by the simultaneous administration of a phosphodiesterase inhibitor.

Imipramine and desmethylimipramine inhibit the second phase of aggregation of human platelets in concentrations (5–50 μM) which have no effect on the first phase of aggregation (Mills & Roberts, 1967). At the same time the release of 5-hydroxytryptamine and of adenine nucleotides from storage granules is inhibited. With hamsters the optical method (Born, 1962) provides no evidence of a separation of the aggregation of platelets into first and second phases. However, desmethylimipramine caused considerable inhibition of aggregation of hamster platelets *in vitro*; the inhibition, like that produced by adenosine, increased rapidly during the first minute and continued to increase more slowly for at least 10 minutes. Thus, after preincubation with DMI (0·25 μM) for 10 min aggregation by ADP was inhibited by 34%. *In vivo,* no inhibition by imipramine or DMI was detected, either with an infusion which resulted in a concentration of about 0·4 μM or with an infusion of imipramine at a 10 times higher concentration. The *in vivo* measurements were made after continuous infusions of the drugs for 10 min so that the absence of any detectable inhibition was probably not due to their inactivation through protein binding or metabolic degradation. However, our estimate of the value for the drug concentrations *in vivo* depends on the assumption that DMI and imipramine are not inactivated very rapidly, that is within a minute or two. It is still possible that the concentrations of the drugs produced by the infusions *in vivo* were too low to cause inhibition. Therefore, the absence of *in vivo* inhibition cannot yet be explained and requires further investigation.

Aspirin inhibits platelet aggregation in man (Evans, Nishizawa, Packham & Mustard, 1967) and in many other species (see Mustard & Packham, 1970) and, like imipramine and DMI, inhibits the second phase of aggregation induced by ADP and other agents (Zucker & Peterson, 1968) as well as the release of platelet constituents. Although two-phase aggregation by ADP could not be demonstrated *in vitro* with hamster platelets we found that aspirin did inhibit aggregation of hamster platelets both *in vitro* and *in vivo*. The inhibition increased during 10 min preincubation of platelet-rich plasma with aspirin. This brings to mind the observation (Zucker & Peterson, 1970) that preincubation with aspirin for up to 15 min increases its inhibitory effect on the release of constituents from human platelets by connective tissue particles.

In vivo, infusion of aspirin giving an estimated concentration in the blood of 200 μM (see **Results**) was several times more inhibitory than a similar concentration *in vitro*. There has been some controversy about the relative inhibitory potencies of aspirin and sodium salicylate (see Mustard & Packham, 1970) but in the hamster *in vivo* aspirin was several times more potent than sodium salicylate.

REFERENCES

ARFORS, K. E., HINT, H. C., MCKENZIE, F. N., MATHESON, N. A. & SVENSJÖ, E. (1970). The effect of dextran and heparin on platelet reactivity *in vivo*. *Proceedings of VI Conference on Microcirculation*. Aalborg, Denmark: European Society for Microcirculation (in the Press).

BEGENT, N. & BORN, G. V. R. (1970a). Growth rate *in vivo* of platelet thrombi, produced by iontophoresis of ADP as a function of mean blood flow velocity. *Nature, Lond.*, **227**, 926–930.

BEGENT, N. A. & BORN, G. V. R. (1970b). Determination of the iontophoretic release of adenosine diphosphate from micropipettes. *Br. J. Pharmac.*, **40**, 592*P*.

BERMAN, H. J. (1967). Suppression of *in vivo* injury-induced platelet thrombosis by inhibitors of ADP thrombotic activity. *Bibl. Anat.*, **9**, 92–97.

BORN, G. V. R. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature, Lond.*, **194**, 927–929.

BORN, G. V. R., HONOUR, A. J. & MITCHELL, J. R. A. (1964). Inhibition by adenosine and by 2-chloroadenosine of the formation and embolisation of platelet thrombi. *Nature, Lond.*, **202**, 761–765.

BORN, G. V. R. (1964). Strong inhibition by 2-chloroadenosine of the aggregation of blood platelets by adenosine diphosphate. *Nature, Lond.*, **202**, 95–96.

BORN, G. V. R. & PHILP, R. B. (1965). Effects of adenosine analogues and of heparin on platelet thrombi in non-lipaemic and lipaemic rats. *Br. J. exp. Path.*, **46**, 569–576.

BORN, G. V. R. (1970). Platelet pharmacology in relation to thrombosis. Thrombosis and coronary heart disease. First Paavo Nurmi Symposium, Porvoo, Finland, 1969. *Adv. Cardiol.*, **4**, 161–174.

BORN, G. V. R. (1971). Platelet aggregation and cyclic AMP. In: *Effects of Drugs on Cellular Control Mechanisms*. Macmillan: London, in the Press.

BORN, G. V. R. & DAWSON, H. A. (1971). Quantitative investigations of the adhesiveness of circulating leucocytes to blood vessel walls. *J. Physiol., Lond.*, in the Press.

BRECHER, G. & CRONKITE, E. P. (1950). Morphology and enumeration of human blood platelets. *J. appl. Physiol.*, **3**, 365–377.

CLARKE, D. A., DAVOLL, J., PHILIPS, F. S. & BROWN, G. B. (1952). Enzymatic deamination and vasodepressor effects of adenosine analysis. *J. Pharmac. exp. Ther.*, **106**, 291–302.

CUTHBERTSON, W. F. J. & MILLS, D. C. B. (1963). Some factors influencing platelet clumping. *J. Physiol., Lond.*, **169**, 9*P*.

EVANS, G., NISHIZAWA, E. E., PACKHAM, M. A. & MUSTARD, J. F. (1967). The effect of acetylsalicylic acid (aspirin) on platelet function. *Blood*, **30**, 550.

HONOUR, A. J. & ROSS-RUSSELL, R. W. (1962). Experimental platelet embolism. *Br. J. exp. Path.*, **43**, 350–362.

HONOUR, A. J. & MITCHELL, J. R. A. (1963). Platelet clumping *in vivo*. *Nature, Lond.*, **197**, 1019–1020.

MICHAL, F. & FIRKIN, B. G. (1969). Physiological and pharmacological aspects of the platelet. *Annual Review of Pharmacology*, **9**, ed. Elliott, H. W., Cutting, W. C. & Dreisbach, R. H., pp. 95–118. Palo Alto, California. Annual Reviews Inc.

MICHAL, F. & THORP, R. H. (1966). Enhanced adenosine inhibition of platelet aggregation in the presence of cardiac glycosides. *Nature, Lond.*, **209**, 208–209.

MILLS, D. C. B. & ROBERTS, G. C. K. (1967). Membrane active drugs and the aggregation of human blood platelets. *Nature, Lond.*, **213**, 35–38.

MILLS, D. C. B. & SMITH, J. B. (1971). The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3′-5′-cyclic monophosphate in platelets. *Biochem. J.*, **121**, 185–196.

MUSTARD, J. F. & PACKHAM, M. A. (1970). Factors influencing platelet function, adhesion, release and aggregation. *Pharm. Rev.*, **22**, 97–187.

OVERGAARD-HANSEN, K., JØRGENSEN, S. & PRAETORIUS, E. (1957). Rephosphorylation produced by inosine and adenosine of adenosine monophosphate and adenosine diphosphate in human erythrocytes. *Nature, Lond.*, **179**, 152–153.

PHILP, R. B. (1970). Species-dependent inhibition of platelet aggregation by adenosine and dipyridamole: Paradoxical potentiation in the rat. *Thromb. diath. haemorrh.*, **23**, 129–139.

RANDERATH, K. & STRUCK, H. (1961). Dunnschichtchromatographishes trennung von nucleosaure derivaten on cellulose schichten. *J. Chromat.*, **6**, 365.

SMITH, J. B. & WILLIS, A. L. (1970). Formation and release of prostaglandins by platelets in response to thrombin. *Br. J. Pharmac.*, **40**, 545*P*.

ZUCKER, M. B. & PETERSON, J. (1968). Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicyclic acid ingestion. *Proc. Soc. exp. Biol. Med.*, **127**, 547–551.

ZUCKER, M. B. & PETERSON, J. (1970). Effect of acetylsalicylic acid, other nonsteroidal anti-inflammatory agents and dipyridamole on human blood platelets. *J. Lab. clin. med.*, **76**, 66–75.

(*Received June* 16, 1971)