INFLAMMATORY MEDIATORS: THE INVOLVEMENT OF HISTAMINE, SEROTONIN AND PLATELET ACTIVATING FACTOR IN ANAPHYLACTOID-TYPE REACTIONS.

A thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of

Doctor of Philosophy

in

The Department of Clinical and Experimental Pharmacology, University of Adelaide,

by

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DECLARATION

I declare this thesis to be based on original data obtained while I was enrolled as a Ph.D. candidate in the department of Clinical and Experimental Pharmacology at the University of Adelaide. To the best of my knowledge this thesis contains no material which has been previously accepted for the award of any degree or diploma in any university, nor any material previously published by any person, except where due reference is cited in the text.

Data from this thesis have been presented to Meetings of the Australian Society of Clinical and Experimental Pharmacologists in Brisbane (1985) and Sydney (1989) and a Meeting of the British Pharmacological Society in London (1988).

Caroline Herd

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LIST OF PUBLICATIONS IN SUPPORT OF THIS THESIS

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SUMMARY

The effects of four different radiographic contrast media (RCM) (the ionic, high osmolality Urovison; the ionic, low osmolality Hexabrix; and the non-ionic, low osmolality Iopamiro and Omnipaque) were examined with respect to histamine release, cardiovascular effects and adverse drug reactions (ADR) in a group of 200 patients undergoing intravenous urography (IVU). Urovison produced the greatest number of ADR; Iopamiro caused the least. However, despite the fact that Urovison caused the greatest and Iopamiro the least change in plasma histamine level one minute post-injection, no significant correlation between the magnitude of the change in histamine level and the production of a particular ADR could be demonstrated. Heart rate increased significantly following the administration of Urovison, Hexabrix and Iopamiro in the absence of any appreciable change in blood pressure. These results indicate that the use of low-osmolality contrast media minimise ADRs, histamine release and patient discomfort.

The effect on platelet function of Urovison and Iopamiro was then examined in a further 20 patients undergoing IVU. It was noted that Urovison caused significant inhibition of platelet aggregation in response to collagen *ex vivo* but not in response to ADP, adrenaline or arachidonate. No significant difference in aggregation induced by any of the foregoing platelet stimuli was evident following Iopamiro injection. Neither RCM significantly altered thromboxane B_2 (TXB_2) production. In a complementary *in vitro* study, both RCM caused a dose-dependent inhibition of platelet aggregation in response to ADP and collagen, the effects of Urovison occurring at lower concentrations than for Iopamiro.

A substantial component of the work presented in this thesis is devoted to the modification and improvement of a method for studying platelet function *in vivo*. Homologous platelets labelled with ^{111}Indium were injected intravenously into anaesthetised experimental animals (guinea-pigs and rabbits) and these circulating labelled platelets continuously monitored in the thoracic region using a microcomputer-based system. Dose-effect relationships were obtained for known platelet agonists (*eg*. ADP,

collagen, PAF and thrombin) in guinea-pigs and rabbits. The response to PAF was further characterised with the use of various drugs in an attempt to elucidate mechanisms of the PAF-induced *in vivo* platelet response. It appeared that PAF induced platelet accumulation was under the modulatory influence of the adenylate cyclase-cAMP system in the two species. ^{111}Indium-labelled platelet studies were also conducted in rabbits using the RCM Urovison and Iopamiro, when it was shown that neither contrast agent administered in a dose comparable to that used in the clinical situation in man had any effect on platelet accumulation.

The concluding experimental chapter in this thesis examines relevant aspects of the toxicity of dextran. It is well recognised that the intravenous infusion of this agent in patients is associated with a low incidence of serious and even fatal anaphylactoid reactions. A study was therefore undertaken to examine the mechanism by which dextran sulphate causes anaphylactoid reactions in rabbits. As a rule, intravenous dextran sulphate in rabbits causes a decrease in heart rate, mean arterial blood pressure and dynamic pulmonary compliance with a concomitant increase in the respiration rate and airways resistance. These effects were significantly reduced, but not completely abolished, by vagotomy or by prior treatment with the 5-HT_3 receptor antagonist, ICS 205-930. Dextran sulphate was shown to elicit substantial intrathoracic ^{111}In-labelled platelet accumulation which was resistant to inhibition by drugs. The foregoing results indicate that dextran sulphate elicits cardiopulmonary effects largely by releasing 5-HT from platelets lodged in the pulmonary circulation. Once released, this 5-HT stimulates 5-HT_3 receptors located on chemoafferent nerve endings resulting in a series of complex reflex events.

ABBREVIATIONS USED IN THIS THESIS

AA	Arachidonic acid
AC	Adenylate cyclase
ADP	Adenosine diphosphate
ADR	Adverse drug reaction
AGEPC	1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine
AIMS	Automated isotope monitoring system
ASA	Acetyl salicylic acid (Aspirin)
ATP	Adenosine triphosphate
AUC	Area under the curve
beta-TG	*Beta*-thromboglobulin
BSA	Bovine serum albumin
^{o}C	Degrees celcius
cAMP	Cyclic adenosine monophosphate
C_{dyn}	Dynamic pulmonary compliance
CFTP	Calcium-free Tyrode's buffer containing PGE_1
cGMP	Cyclic guanine monophosphate
Ci	Curie
cm	Centimetre
cpm	Counts *per* minute
DAG	Diacylglycerol
DP	Diastolic pressure
DSCG	Disodium cromoglycate
ECP	Eosinophil cationic protein
EDRF	Endothelium-derived relaxing factor
FPL 55712	Sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate
g	Gram

HETE	Hydroxyeicosatetraenoic acid
HHT	Hydroxyheptadecatrienoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HR	Heart rate
HSA	Human serum albumin
IC_{50}	Concentration required to inhibit maximal platelet aggregation by 50%
ICS 205-930	3*alpha*-tropanyl-1H-indole-3-carboxylic acid ester
IgE	Immunoglobulin E
IgG	Immunoglobulin G
i.m.	Intramuscular
i.p.	Intraperitoneal
IP_3	Inositol 1,4,5 triphoshate
I.U.	International units
i.v.	Intravenous
IVU	Intravenous urography
kg	Kilogram
L	Litre
LT	Leukotriene
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
LTE_4	Leukotriene E_4
M	Moles *per* litre
MAP	Mean arterial pressure
MBP	Major basic protein
MBq	Megabequerel
MDA	Malondialdehyde
MeV	Mega electron volts
mg	Milligram

MHz	Megahertz
min	Minutes
ml	Millilitre
mm	Millimetre
mM	Millimoles *per* litre
MR_{50}	Concentration of stimulus required to elicit 50% maximal platelet aggregation
MX	Methylxanthine
*n*g	Nanogram
NSAID	Non-steroidal anti-inflammatory drug
oxine	8-hydroxyquinoline
PAF	Platelet activating factor (PAF-acether; AGEPC)
PAF-acether	Platelet activating factor (PAF; AGEPC)
PDE	Phosphodiesterase
PDGF	Platelet-derived growth factor
PF4	Platelet factor 4
PFP	Platelet-free plasma
*p*g	Picogram
PG	Prostaglandin
PGD_2	Prostaglandin D_2
PGE_1	Prostaglandin E_1
PGE_2	Prostaglandin E_2
PGF_{2alpha}	Prostaglandin F_{2alpha}
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
PGI_2	Prostacyclin (Prostaglandin I_2)
PL	Phospholipid
PLA_2	Phospholipase A_2
PMN	Polymorphonuclear leucocytes
PPP	Platelet-poor plasma

PRP	Platelet-rich plasma
RBC	Red blood cell
RCM	Radiographic contrast medium/media
R_L	Airways resistance
sec	Seconds
sem	Standard error of the mean
SRI 63-441	*cis* (±)-1-[2-[hydroxy [tetrahydro-5 [(octadecylaminocarbonyl)oxyl] methyl]furan-2-yl]methoxy-phosphynyloxy]ethyl]-quinolinium hydroxide inner salt
SRS-A	Slow reacting substance of anaphylaxis
TGF-*beta*	Transforming growth factor *beta*
TPP	Transpulmonary pressure
TXA_2	Thromboxane A_2
TXB_2	Thromboxane B_2
*u*g	Microgram
*u*l	Microlitre
v/v	Volume *per* volume
w/v	Weight *per* volume
$\bar{x}$	Mean
^{51}Cr	^{51}Chromium
^{111}In	^{111}Indium
^{125}I	^{125}Iodine
5-HT	5-hydroxy tryptamine (serotonin)
6-keto-PGF_{1alpha}	6-keto-prostaglandin F_{1alpha}

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CHAPTER I : GENERAL INTRODUCTION

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CHAPTER III : THE EFFECT OF RADIOGRAPHIC CONTRAST MEDIA (RCM) ON PLATELET FUNCTION *EX VIVO*, *IN VITRO* AND *IN VIVO*.

CHAPTER I

GENERAL INTRODUCTION

I.1 RADIOGRAPHIC CONTRAST MEDIA

By definition, positive radiographic contrast media (RCM) are compounds that have a density greater than the tissues of the body (soft tissue and bone) and absorb X-rays to a greater extent[1]. RCM are used extensively in diagnostic radiology to enhance the information provided by imaging systems.

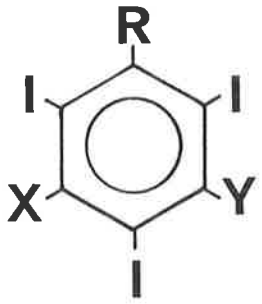
I.1.1 *Chemistry*

Density is related to the molecular weight of a substance and the contrast effect of an agent increases (providing better radiographic visualisation) approximately with the fourth power of its atomic number (Schinz *et al.*, 1968). All contrast media available for intravascular injection are water soluble. Those in common use are fully saturated, ionic, monomeric, tri-iodinated benzoic acid derivatives (Figure I.1.1 (a)). The ratio of iodine atoms to dissolved particles in solution for these agents is 1.5 since they are ionic and dissociate into two particles in solution, with the anion containing three iodine atoms and a cationic moiety that is most commonly sodium, an organic cation, such as methylglucamine (meglumine), or a mixture of both. Iodine is universally used to confer the necessary radiologic contrast because of its high density and low toxicity. It is readily attached to organic compounds that are filtered easily and not absorbed by the glomeruli and renal tubules (Goldberg, 1984).

Most commonly used contrast media are hypertonic in solution with osmolalities ranging from 1200 to over 2000 mOsmol/kg H_2O relative to human blood at 300

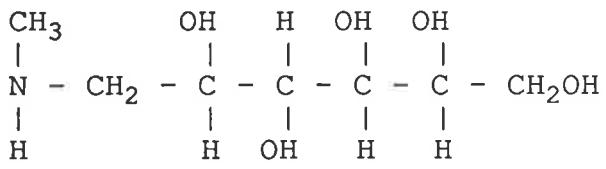
[1]Negative contrast media, such as air and helium, are less dense than soft tissue and bone, and absorb X-rays to a lesser extent. They have a different spectrum of toxicity to intravascular contrast media and will not be discussed in this thesis.

Figure I.1.1 (a)
Generalised Anion Structure



Cation Structures

Na^+; Ca^{2+}; Mg^{2+}



Methylglucamine (or Meglumine)

Figure I.1.1 (b) Diatrizoate

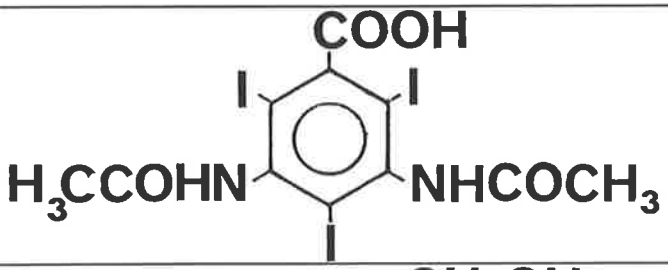


Figure I.1.1 (c) Iopamidol

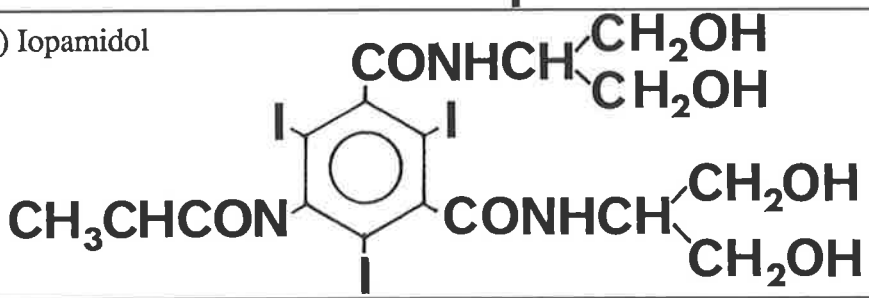


Figure I.1.1 (c) Iohexol

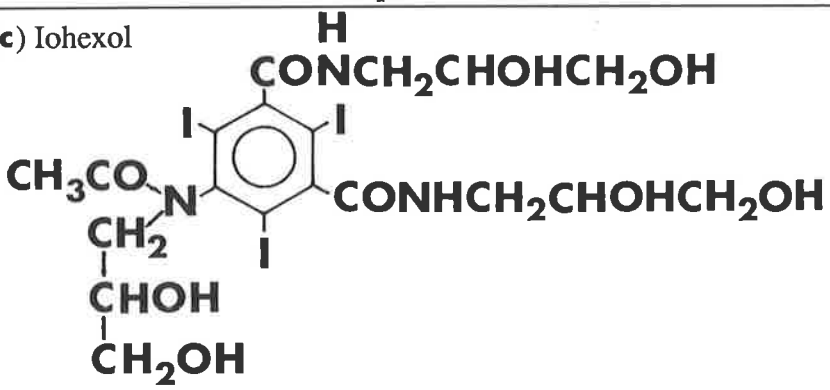


Figure I.1.1 (d) Ioxaglate

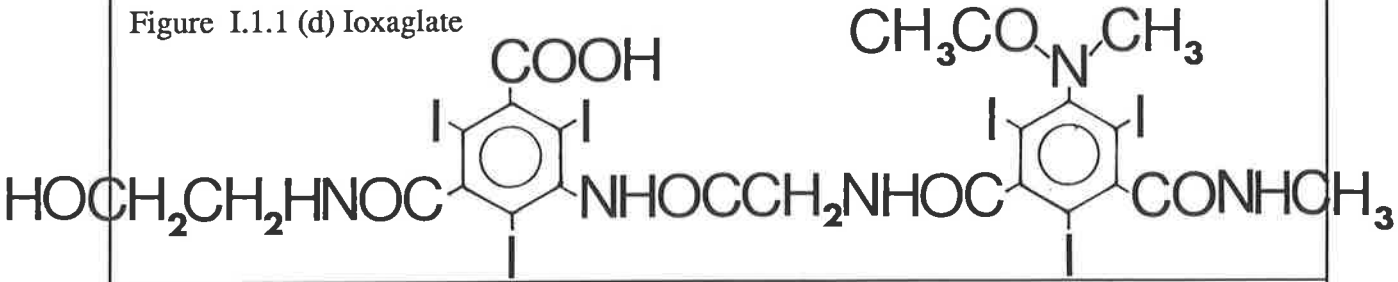
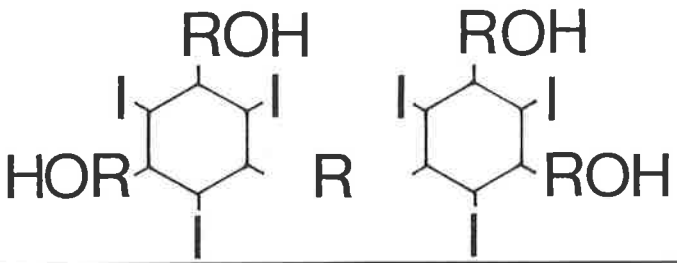


Figure I.1.1 (e) Acidic non-ionic dimer: Future media?



mOsmol/kg H_2O, *eg*. Urovison 58% (sodium and meglumine diatrizoate, Figure I.1.1 (b)), 1650 mOsmol/kg H_2O; Conray (methylglucamine iothalamate), 1217 mOsmol/kg H_2O; Conray 400 (sodium iothalamate), 1965 mOsmol/kg H_2O; Isopaque 280 (methylglucamine and calcium metrizoate), 1500 mOsmol/kg H_2O. These contrast agents provide good radiopacification, are generally well tolerated by patients and are relatively safe. However, hyperosmolality is held responsible for many of the haemodynamic, cardiac and non-specific side-effects produced by current contrast media. Therefore, recent developments have aimed at reducing the osmolality by altering the chemical structure *ie*. by changing the substituted side groups (Amundsen *et al.*, 1978). In order to decrease the osmolality without changing the iodine concentration of a contrast medium, the ratio between the number of iodine atoms and the number of dissolved particles must be increased. The ratio is increased to 3 by producing a non-ionic monomer, *eg*. Iopamiro 370 (iopamidol, Figure I.1.1 (c)), 870 mOsmol/kg H_2O; Amipaque (metrizamide), 450 mOsmol/kg H_2O; Omnipaque 300 (iohexol), 690 mOsmol/kg H_2O. A mono-acidic dimer, Hexabrix 320 (sodium and meglumine ioxaglate) is also a ratio-3 contrast medium with low osmolality (600 mOsmol/kg H_2O), but still has an ionised anion in solution (Figure I.1.1 (d)). A non-ionic dimer ratio-6 contrast medium has been developed that reduces the osmolality even further to 300 mOsmol/kg H_2O, isotonic with blood (Figure I.1.1 (e)).

I.1.2 *Adverse Reactions*

The overall incidence of untoward reactions to the administration of RCM has been reported to range from approximately 5% (Shehadi and Toniolo, 1980) to approximately 8% (Ansell, 1970; Witten *et al.*, 1973). Most contrast reactions are mild (a sensation of warmth, nausea, vomiting, minor urticaria or itching), quick in onset (usually within 2 minutes of contrast injection), of short duration and require no therapy. A more serious manifestation of contrast reactions is a decrease in systemic vascular resistance, with resultant hypotension and compensatory tachycardia. Life-threatening reactions such as laryngeal and facial oedema, serious cardiac arrhythmias, severe bronchospasm,

pulmonary oedema and cardiovascular collapse, have been detected in from one in 3,000 to one in 14,000 patients undergoing contrast studies. However, 96% of patients with these potentially fatal reactions can be resuscitated with aggressive treatment (Ansell *et al.*, 1980). Death from contrast reactions is rare, ranging from approximately one in 10,000 (Shehadi, 1975) to one in 70,000 procedures (Hartman *et al.*, 1982).

Several factors may be associated with an increased risk of a contrast reaction. Reactions occur during both intravenous and intra-arterial injection, but there appears to be a higher incidence for intravenous procedures (Shehadi and Toniolo, 1980). Ansell and colleagues (1980) reported a higher frequency of reactions when the total iodine dose exceeded 20g. Age appears to be a factor as the peak incidence of reactions occurs in patients 20 to 50 years of age (Shehadi, 1975; Ansell *et al.*, 1980; Kalimo *et al.*, 1980; Shehadi and Toniolo, 1980; Shehadi, 1982). Sex, weight (Ansell *et al.*, 1980; Shehadi and Toniolo, 1980), race or geographical location (Shehadi, 1982) or time of the day (Cunningham, 1977) do not significantly alter the incidence of adverse reactions to RCM. The species of cation present in a particular RCM compound is unimportant in determining the likelihood of a reaction (Dahl *et al.*, 1976). Patients with electrolyte disturbances, particularly hyperkalaemia, are at risk for developing serious arrhythmias following contrast infusion (Cohan and Dunnick, 1987).

Contrast reactions have been classified as idiosyncratic or non-idiosyncratic. Idiosyncratic reactions are considered to be "allergic-type" reactions and range from hives and itching to facial and laryngeal oedema, bronchospasm and circulatory collapse. Non-idiosyncratic reactions are thought to result from direct toxic effects of contrast material and/or contrast hyperosmolality, and include nausea, vomiting, cardiac arrhythmias, pulmonary oedema and cardiovascular collapse (Cohan and Dunnick, 1987).

In most prospective studies, the presence of an "allergy" appears to enhance the incidence of a reaction in patients from 2 to 4 times that of "non-allergic" patients (Witten *et al.*, 1973; Shehadi, 1975; Brasch and Caldwell, 1976; Littner *et al.*, 1977; Ansell *et al.*, 1980; Kalimo *et al.*, 1980; Shehadi and Toniolo, 1980). In these studies however, no tests were performed to confirm a history of allergic disease, hence the term "allergy" has been

used to refer to diverse reactions including idiosyncratic, non-atopic drug reactions, unclassified food intolerances and general (unspecified) allergy. Subjects suffering from atopic respiratory disease may also demonstrate an increased *in vitro* sensitivity to RCM as evidenced by histamine release from human basophils (Arroyave, 1980). Furthermore, although most subjects receiving RCM develop asymptomatic obstruction to airflow (Littner *et al.*, 1977; Rosenfield *et al.*, 1977), the incidence of clinically apparent bronchospasm and wheeze appears to be significantly higher in known asthmatic subjects (Shehadi, 1982).

Patients who have experienced a previous anaphylactoid reaction to RCM are at increased risk of developing another reaction on the repeat administration of RCM (Peters *et al.*, 1966; Gates and Ceccarelli, 1972; Schatz *et al.*, 1975). The incidence of occurrence ranges from a low of 15%-16% to a high of 30% (Peters *et al.*, 1966; Gates and Ceccarelli, 1972; Schatz *et al.*, 1975; Shehadi, 1975; Shehadi, 1982; Greenberger, 1984).

Haemodynamic changes occur following the intravascular administration of contrast material (Friesinger *et al.*, 1965; Hilal, 1966; Fischer, 1968; Lindgren, 1970; Chahine and Raizner, 1976; Fischer and Thomson, 1978). These changes include transient systemic hypotension, an increase in pulmonary artery pressure, tachycardia and peripheral vasodilatation. Low osmolality RCM have been shown to cause less marked overall cardiovascular alterations than high osmolality RCM (Higgins *et al.*, 1982; Svenson, 1984; Bettman and Higgins, 1985). However, haemodynamic changes similar to those observed following the intravascular injection of contrast agents have been shown following the infusion of other hyperosmotic solutions (such as mannitol 25%, glucose 50% and saline 1.5 M) in humans, rabbits and rats (Cote *et al.*, 1979; Garcia-Estan *et al.*, 1989).

Low osmolality contrast media may induce fewer adverse reactions than their traditional high osmolality counterparts, but the indications for their use have been a point of intense debate because of their much greater cost. Kinnison *et al.* (1989) undertook a comprehensive examination of the results from randomised controlled trials (RCTs) conducted in humans (published between 1980 and 1987) to compare the safety and efficacy of intravascularly administered low osmolality media (LOM) with that of high

osmolality media (HOM). In summary, RCTs comparing intravascular injection of LOM and HOM in humans suggest that (a) the image quality afforded by LOM is at least as good as, or better than, that provided by HOM; (b) the sensations of heat and pain occur less frequently with LOM and (c) LOM produce fewer cardiovascular changes. RCTs have not demonstrated significant differences between HOM and LOM in terms of the frequency of nephrotoxicity, laboratory test abnormalities, neurotoxicity, life-threatening reactions or death.

In view of their high cost, one strategy for the use of LOM has been to selectively employ LOM in patients thought to be at greater risk for an adverse reaction. However, RCTs comparing LOM and HOM have not focused on high-risk patients and have failed to define clearly the types of patients studied or to analyse patients according to risk factors for adverse reactions. Therefore, RCTs have not clarified whether LOM decrease adverse reactions in specific subsets of patients thought to be at increased risk (Kinnison *et al.*, 1989). Those authors suggest that the failure of published RCTs to demonstrate differences between LOM and HOM should not be taken to mean that such differences do not exist (Kinnison *et al.*, 1989). In addition to this, because trials have tended to enrol relatively small numbers of patients, RCTs have limited statistical power to detect possible differences between LOM and HOM (Powe *et al.*, 1989).

I.1.3 *Pathogenesis of Adverse Reactions to Contrast Media*

The aetiology of contrast media chemotoxicity remains unclear. The diversity of the reactions have led to the formulation of many different proposals of pathogenic mechanisms, including (a) antigen-antibody reactions, (b) cellular release of mediators (*eg.* histamine), (c) involvement of acute activation systems (complement, coagulation, kinin, fibrinolytic) and (d) psychogenic factors. These major headings are not necessarily mutually exclusive. Some reactions may involve one, all, or any combination of these mechanisms.

Evidence exists for an antigen-antibody involvement in the pathogenesis of adverse

reactions to RCM. The clinical spectrum of contrast reactions include many that are known manifestations of allergy (*eg*. hives, itching, urticaria, laryngeal oedema, bronchospasm and shock) suggesting immune hypersensitivity or anaphylaxis as possible causes. Like allergic reactions, RCM reactions are not dose related and intense acute anaphylaxis has been reported after even very small doses of RCM, suggesting a role for IgE in some patients (Brasch, 1980)[2]. As mentioned previously, large population studies of RCM reactivity reveal an increased reaction rate among patients with known allergy, particularly asthmatics (Ansell, 1970). Lymphocyte transformation, a necessary precursor to antibody formation, has been demonstrated to occur after *in vitro* exposure of lymphocytes to contrast media (Halpern *et al.*, 1967; Lasser, 1968). Contrast media are of low molecular weight and can function as haptens (Lang *et al.*, 1974; Till *et al.*, 1978) and considering their binding properties to proteins (Lasser and Lang, 1970), may develop full antigenicity. Globulin binding studies have shown greater binding by a contrast medium for reactor sera than for control sera (Brasch and Caldwell, 1976; Sweeney and Klotz, 1983), providing evidence for the presence of circulating antibodies. It has recently been demonstrated that contrast media can be converted to antigenic iodoproteins (Nilsson *et al.*, 1987). Anti-RCM antibodies have been identified in individual patients (Kleinknecht *et al.*, 1974; Wakkers-Garritsen *et al.*, 1976) and have been induced in rabbits (Brasch *et al.*, 1976; Siegle *et al.*, 1988) and mice (Siegle *et al.*, 1988) immunised with RCM covalently coupled to protein carriers.

There is however, evidence against the immunological theory. Attempts to raise antibodies to contrast media in rats (Carr and Walker, 1984*a*) and dogs (Lasser *et al.*, 1976), and to elicit RCM hypersensitivity in guinea-pigs (Dunn *et al.*, 1975) were unsuccessful. Attempts to correlate the potential for reactions with standard tests for IgE-mediated anaphylaxis, the measurement of histamine and hypersensitivity skin testing, have failed to show a consistent relationship (Lieberman *et al.*, 1978). Many groups have

[2]Sensitivity against iodide could also account for some contrast reactions. Although the iodine atoms substituted in the benzene ring of the CM molecules are extremely stable, free iodide will be found in each contrast vial as a consequence of the manufacturing process.

failed to detect circulating antigen-antibody complexes or isolate anti-contrast antibodies in blood sampled from patients during acute reactions (Lasser, 1968; Siegle and Lieberman, 1976; Arroyave *et al.*, 1979; Walker and Carr, 1986). Generally therefore, contrast reactions are considered to be "anaphylactoid" (pseudo-allergic) rather than "anaphylactic".

The similarity between the symptoms of contrast media reactions and those produced by a histamine-releasing agent was first recognised in the early 1960's (Mann, 1961); (1) blood pressure decreases after a delay of 20-30 seconds following injection of both histamine and RCM, (2) histamine-releasing agents and RCM produce the triple response of flush, flare and wheal when injected intradermally in humans, (3) effects produced by histamine administered intravenously are similar to those adveres reactions associated with the intravascular administration of RCM, (4) the histamine-like effects of RCM are dose-dependent, (5) patients with allergy appear more predisposed to the development of adverse reactions to RCM when compared with non-atopic patients, and finally (6) the structural configuration of RCM fulfils criteria which may confer histamine-releasing activity on them.

The first experimental confirmation of a relationship between contrast media and histamine release was provided by harvested rat peritoneal mast cells incubated *in vitro* with the sodium salts of iodipamide, acetrizoate, diatrizoate and iothalamate (Rockoff *et al.*, 1970). Further work by this group expanded the number of contrast media known to elicit histamine release *in vitro*, and determined that certain RCM such as meglumine iodipamide, were more potent histamine releasers than others tested (Rockoff *et al.*, 1971).

Radiographic contrast media have also been shown to induce release of histamine from human basophil leucocytes *in vitro* (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Assem *et al.*, 1983) and from perfused histamine-rich animal organs (lung and liver) (Lasser *et al.*, 1974).

In vitro histamine release occurs in a dose-dependent manner (Ring *et al.*, 1978*a*; Rice *et al.*, 1983; Younger *et al.*, 1986), and is probably greater with meglumine salts than with sodium salts (Rockoff *et al.*, 1972). Basophils from atopic or allergic individuals

appear more sensitive to histamine release by RCM *in vitro* than non-atopics (Siegle *et al.*, 1980), perhaps as an expression of increased "releasability" of histamine (Findlay and Lichtenstein, 1980). Similarly, basophils of previous reactor patients are more sensitive than those who have received contrast media in the absence of a reaction (Ring *et al.*, 1978*a*; Rice *et al.*, 1983).

In vivo plasma histamine release has been demonstrated in experimental animal models (Rockoff and Brasch, 1971; Ring *et al.*, 1978*b*) and man during both intravenous (Brasch *et al.*, 1970; Seidel *et al.*, 1974; Simon *et al.*, 1979; Robertson *et al.*, 1985; Herd *et al.*, 1988) and intra-arterial (Rockoff and Aker, 1972; Cogen *et al.*, 1979) administration of RCM. Elevated histamine levels have also been shown in urine sampled from patients undergoing intravenous urography (Kaliner *et al.*, 1984; Keyzer *et al.*, 1984).

No direct correlation between the extent of *in vivo* histamine release and the induction and/or severity of pathophysiologic events has been demonstrated. However, there does appear to be some correlation between the occurrence of reactions and the potential of RCM to induce histamine release *in vitro* (Arroyave, 1980).

The mechanism by which histamine release is produced has not been elucidated. It is not dependent on the presence of iodine since non-iodinated RCM analogues can elicit histamine release (Rice *et al.*, 1983). Evidence exists in favour of the hypothesis that release is due to the interaction between RCM and a cell membrane receptor. Firstly, *in vitro* release may be specific for the RCM producing a reaction in a given individual (Miller *et al.*, 1975; Ring *et al.*, 1978*a*), thus implying a role for the prosthetic group in the induction of release and suggesting the existence of a subtle stoichiometric relationship between RCM and a cell surface receptor. Secondly, histamine release occurs when washed basophils are stimulated by RCM *in vitro* (thus serum complement is not necessary for degranulation) (Miller *et al.*, 1975; Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*), and resembles calcium ionophore-induced release (Ring *et al.*, 1978*a*; Younger *et al.*, 1986). Thirdly, RCM binds to the surface of human leucocytes but not to human red blood cells (Siegle *et al.*, 1980). However, an attempt to define a definite structure-function relationship between RCM and a proposed basophil receptor has been unsuccessful (Rice

et al., 1983).

Evidence for the role of complement in the degranulation process is limited to the observation that *in vitro* histamine release by RCM is augmented in the presence of normal human serum and that this enhancement is eliminated when serum is first depleted of complement (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*). Nonetheless, *in vitro* release occurs in the absence of complement (Miller *et al.*, 1975; Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Rice *et al.*, 1983).

Hyperosmolar substances have been shown to directly incite histamine liberation from human basophils *in vitro via* non-cytotoxic mechanisms (Findlay *et al.*, 1981) and hyperosmolality enhances the *in vitro* release of histamine induced by ragweed and IgE (Hook and Siraganian, 1981). Furthermore, low osmolality contrast media have been shown to release less histamine from human basophils *in vitro* than their high osmolality counterparts (Assem *et al.*, 1983; Salem *et al.*, 1986). Isotonic contrast agents probably produce reactions less frequently than hyperosmolar agents, and have been administered to previous reactors without the elicitation of recurrent anaphylactoid episodes (Rapoport *et al.*, 1982). However, the time course of histamine release produced by other hypertonic agents (Findlay *et al.*, 1981) differs significantly from that described for RCM-induced release (Ring *et al.*, 1978*a*), and other hypertonic agents infused intravenously into rabbits failed to produce the same effects (histamine release, complement consumption and microvascular changes) as RCM of equal hypertonicity (Ring *et al.*, 1978*b*). Metrizamide, an isotonic RCM, is capable of inducing histamine release from washed human basophils in amounts equal to that released by diatrizoate, a hypertonic agent (Rice *et al.*, 1983). In general, most studies indicate that hypertonicity does not fully account for the histamine-releasing process with contrast media *in vitro* (Rockoff *et al.*, 1970; Lasser *et al.*, 1974; Rice *et al.*, 1983) or *in vivo* (Lasser *et al.*, 1971; Rockoff and Brasch, 1971; Seidel *et al.*, 1974). Furthermore, the intravenous infusion of the hyperosmolar RCM diatrizoate, failed to significantly elevate general serum osmolality (Freyria *et al.*, 1982). Thus, the hyperosmolality of contrast agents alone is insufficient to explain the degranulation process. Concentrations of RCM used clinically may not significantly alter plasma

osmolality at sites other than the area of injection.

The complement system of serum proteins is a complex set of biochemical mediators activated *via* immunogenic, haemolytic, fibrinolytic and possibly osmotic interactions with other plasma components. Activation of the complement cascade can induce degranulation of basophils and mast cells (with release of histamine), increased vascular permeability, release of the physiologically active peptides C_{3a} and C_{5a} anaphylatoxins and chemotactic activators, cell membrane disruption and thrombin activation and enhanced phagocytosis by binding to cell membranes (Goldberg, 1984). Thus, contrast media-induced activation of complement may have relevance to the production of clinical symptoms.

Contrast media have been shown to activate the complement system *in vitro* (Lang *et al.*, 1976; Arroyave and Tan, 1977; Kolb *et al.*, 1978; Till *et al.*, 1978; Arroyave *et al.*, 1979; Lasser *et al.*, 1979*a*; Dawson *et al.*, 1983*b*; Westaby *et al.*, 1985), *in vivo* in dogs (Lang *et al.*, 1976; Lasser *et al.*, 1976), but it is uncertain whether it occurs *via* the classical or alternate pathways (Arroyave and Tan, 1977), other pathways (Kolb *et al.*, 1978; Till *et al.*, 1978), the activation of some unidentified plasma enzyme (Arroyave *et al.*, 1979) or *via* the fibrinolytic system (Neoh *et al.*, 1981). Significant falls in complement occur in human subjects receiving RCM (Arroyave *et al.*, 1976; Arroyave *et al.*, 1979; Lieberman and Siegle, 1979; Lasser *et al.*, 1979*a*; Gonsette and Delmotte 1980; Freyria *et al.*, 1982; Parvez and Moncada, 1985). Both high and low osmolality RCM have been shown to activate complement *in vitro* (Dawson *et al.*, 1983*b*; Westaby *et al.*, 1985) and *in vivo* (Gonsette and Delmotte, 1980; Freyria *et al.*, 1982). However, evidence exists that low osmolality contrast media do not activate the serum complement system (Parvez *et al.*, 1983; Parvez and Patel, 1988).

There appears however, to be no correlation between adverse contrast reactions and decreases in complement level (Simon *et al.*, 1979; Arroyave *et al.*, 1979; Gonsette and Delmotte, 1980; Freyria *et al.*, 1982), *ie.* non-reactors cannot consistently be distinguished from reactors on the basis of decreases in complement. In contrast, baseline levels of serum complement (CH50) were found to be reduced in a group of reactors when levels were compared with controls (Lieberman and Siegle, 1979; Lasser *et al.*, 1979*a*). Reactors

were also found to have depressed C_1 esterase inhibitor function (an enzyme which exerts an inhibitory action on the complement, kinin and plasmin systems) in both baseline and post-infusion serum samples (Lasser *et al.*, 1979*a*). On the basis of these findings, it was postulated that reactions occur in patients with clinical conditions producing a "preceding sustained activity of either plasmin, kallikrein, the intrinsic coagulation system, or the classic complement pathway" resulting in a "gradual consumption of the inhibitor of the first component of complement and of total complement" (Lasser *et al.*, 1979*a*). However, a prospective analysis of subjects experiencing reactions did not support the contention that patients with diseases characterised by ongoing complement activation would be predisposed to anaphylactoid reactions to RCM (Shehadi, 1982).

RCM have been shown to damage vascular endothelial surfaces (Laerum, 1983), and it has been suggested this may result in activation of factor XII, the initiating protein of the intrinsic coagulation and contact systems (Lasser *et al.*, 1979*a*; Lasser *et al.*, 1981*a*). Activation then continues through the factor XII-induced cleavage of pre-kallikrein to kallikrein, and terminates in the kallikrein-modulated cleavage of high molecular weight kininogens to kinin (Lasser, 1985). The generation of bradykinin in normal citrated whole blood has been demonstrated in response to RCM *in vitro* (Fareed *et al.*, 1984*b*). Furthermore, elevated plasma bradykinin levels have been noted following intravenous contrast injections in non-reacting patients (Fareed *et al.*, 1984*b*). Bradykinin can elicit the same target-tissue responses as histamine and can mobilise arachidonic acid thus providing a substrate for the production of leukotrienes and vasoactive prostaglandins (Wasserman, 1983).

Plasma from patients experiencing reactions to contrast material have an increased capacity to convert pre-kallikrein to kallikrein compared with nonreactors (Lasser *et al.*, 1981*a*; Carr and Walker, 1984*b*), potentiating the rate of bradykinin production. As mentioned previously, C_1 esterase inhibitor depresses complement activation, kinin formation and plasmin activity, and it has been suggested that some individuals are predisposed to react to RCM because of pre-existing low levels of this enzyme (Lasser *et al.*, 1979*a*). Furthermore, contrast molecules in concentrations that can occur in the

pulmonary circulation following intravenous injections, have the potential to inhibit the major pulmonary vascular-based enzyme responsible for the hydrolysis of bradykinin (angiotensin-converting enzyme) (Lasser, 1988).

Lasser (1988) proposed that non-immunological phenomena such as contrast reactions (and potentially other forms of non-immunological anaphylaxis) occur with increasing frequency in individuals with true allergies because ongoing contact system activity in their plasmas primes them for accelerated contact system-engendered bradykinin release. The primed system will be activated when a substance such as a contrast molecule, capable of acutely mobilising soluble contact-activity-negative surfaces is introduced into the bloodstream. Three factors have been identified in the plasmas of allergic individuals that will accelerate newly initiated contact activation, and it has been demonstrated *in vitro* that the exposure of their plasmas to exogenous contact activators will significantly accelerate their contact system activation in comparison with controls (Lasser, 1988).

The significant clinical protection offered against adverse RCM events by corticosteroid pre-treatment may be due to a down-regulation of ongoing low-level contact system activation (Lasser *et al.*, 1987). In animals it has been demonstrated that corticosteroids produce a plasma elevation of factor XII and of C_1-esterase inhibitor, two factors that would otherwise be partially consumed in ongoing contact activation (Lasser *et al.*, 1981*b*). In patients, corticosteroid pre-treatment produces a significant fall in the concentration of the cryptic soluble negative surfaces that are capable of contact activation when present in sufficient concentrations (Lasser, 1988).

Dose-dependent anticoagulant properties of contrast media have been described *in vitro* by using standard tests (*ie.* both global and monospecific functional coagulation tests) on human plasma (Bernstein and Gans, 1966; Parvez *et al.*, 1983; Fareed *et al.*, 1984*a*; Belleville *et al.*, 1985; Dawson *et al.*, 1986; Stormorken *et al.*, 1986; Andes, 1988; Parvez and Patel, 1988; Corot *et al.*, 1989). Similar observations have been noted following the administration of RCM to patients (Parvez *et al.*, 1984). The inhibitory effect of contrast media on clotting appears less marked with non-ionic compared with ionic agents (Parvez

et al., 1983; Belleville *et al.*, 1985; Dawson *et al.*, 1986; Stormorken *et al.*, 1986; Andes, 1988; Parvez *et al.*, 1988; Parvez and Patel, 1988; Corot *et al.*, 1989). In a study of patients undergoing cerebral angiography, results showed a negligible influence of ionic and non-ionic contrast media on systemic haemostatic parameters *ex vivo* (Stormorken *et al.*, 1986). Those authors suggest that concentrations of contrast media attained in systemic blood were not sufficient to reflect the differences found in *in vitro* experiments.

The clinical relevance of these findings for haemostasis in clinical diagnostic radiology is debatable. McAfee (1957) and Bernstein and Gans (1966) stressed the clinical disadvantages of adverse effects on haemostasis. In a survey of 13,000 aortographies, 13 cases of post-procedure bleeding severe enough to result in clinical shock were reported (McAfee, 1957). It has been suggested that the properties of contrast agents may eliminate the need for anticoagulant therapy in vascular studies and thereby be advantageous (Katzen, 1980). However, this theoretical advantage is probably usually offset by the greater propensity of the conventional agents to damage vascular endothelium and hence stimulate platelet aggregation and coagulation (Dawson *et al.*, 1986). Local thrombosis is a well recognised complication of intravascular contrast media administration (Laerum and Holm, 1981).

Disseminated intravascular coagulation (DIC) has occurred in patients undergoing reactions to RCM (Zeman, 1977; Lasser *et al.*, 1980), and fibrin-split products have been found in the sera of both reactors and non-reactors receiving RCM (Simon *et al.*, 1979). Mortality in rabbits receiving lethal doses of RCM can be related to the activation of the clotting cascade (Lasser *et al.*, 1979*b*). In addition to the prolongation of coagulation time, RCM can inhibit fibrin polymerisation and generate fibrin-split products *in vitro* (Schulze, 1980; Andes, 1988).

RCM may bind non-specifically to clotting factors (Schulze, 1980) and other serum proteins (Lang and Lasser, 1967; Lasser and Lang, 1970; Lasser, 1971). Binding of contrast media to proteins, or to platelet or erythrocyte membranes may alter protease activity, leading to activation of the complement and coagulation/fibrinolytic systems (Goldberg, 1984).

Serotonin, a potent mediator of immediate hypersensitivity in rodents (Saxon, 1981), is stored in the dense granules of human platelets and is released during platelet aggregation. This autacoid may cause bronchoconstriction (Baldini and Ebbe, 1974) and affect vascular permeability. Upon stimulation, platelets can also synthesize the metabolites of arachidonic acid *ie.* thromboxane A_2, and various prostaglandins and hydroxy-acids which play an important role in the pathogenesis of anaphylactic and allergic reactions in the body (Goetzl, 1980; Yen and Morris, 1981).

Studies of contrast agents on platelet function have yielded conflicting results. Both high (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Ring and Sovak, 1981) and low osmolality contrast media (Ring and Sovak, 1981) have been shown to release ^{3}H-serotonin from washed human platelets *in vitro* in a dose-dependent manner. Elevated serotonin levels have been demonstrated in human whole blood following incubation with high osmolality contrast agents *in vitro* (Parvez *et al.*, 1982; Fareed *et al.*, 1984*b*). In contrast, neither high nor low osmolality RCM induced the release of ^{14}C-serotonin from platelet-rich plasma (PRP) *in vitro* (Ring *et al.*, 1978*a*; Rao *et al.*, 1985). Furthermore, RCM have been shown to inhibit ^{14}C-serotonin release from PRP in response to ADP, adrenaline and collagen *in vitro* (Rao *et al.*, 1985) or *ex vivo* (Zir *et al.*, 1974).

Platelet aggregates surrounding basophilic granules have been observed with the use of electron microscopy following the incubation of washed platelets and normal human sera with RCM *in vitro* (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*). It has been suggested that this phenomenon is due to the release of platelet activating factor (PAF) (Ring *et al.*, 1978*a*).

RCM have been found *in vitro* to dose-dependently inhibit platelet aggregation induced by a range of exogenous stimuli (Zir *et al.*, 1974; Shapiro *et al.*, 1977; Gafter *et al.*, 1979; Belleville *et al.*, 1982; Paajanen *et al.*, 1984; Parvez *et al.*, 1984). Similarly, following the intravascular administration of contrast agents in patients, inhibition of platelet aggregation has been reported (Zir *et al.*, 1974; Shapiro *et al.*, 1977; Gafter *et al.*, 1979; Parvez *et al.*, 1984; Stormorken *et al.*, 1986). Generally, the effects of low osmolality agents on platelet aggregation are less marked than those observed with high

osmolality RCM (Parvez *et al.*, 1983; Dawson *et al.*, 1986; Stormorken *et al.*, 1986).

Contrast media have been shown to cause marked decreases in thromboxane B_2 generation (the stable metabolite of TXA_2) when incubated with human whole blood (Fareed *et al.*, 1984*b*). Only slight inhibition of TXB_2 formation was noted following the incubation of RCM with platelet-rich plasma *in vitro* (Paajanen *et al.*, 1984). However, plasma TXB_2 concentrations were unchanged in patients following the intravascular injection of high and low osmolality RCM (Parvez *et al.*, 1988).

The overall findings of studies investigating the effects of RCM on haemostatic parameters suggest that contrast media are anticoagulant rather than pro-thrombotic. However, if endothelial damage occurs, secondary passage of plasma and platelets over the injection site may lead to platelet activation, adherence and degranulation along with thrombin generation, possibly leading to permanent vessel wall damage and/or thrombus formation (Dawson *et al.*, 1986).

The intravascular injection of ionic contrast agents has been shown to retard blood flow (Margolis *et al.*, 1959; Read *et al.*, 1959; Sobin *et al.*, 1959), possibly due to the influence of RCM on the rheological properties of human blood. Many contrast agents deform erythrocytes, rendering them more rigid *in vitro* (Aspelin, 1978; Aspelin, 1979; Aspelin *et al.*, 1980*c*). An *in vivo* consequence of this may be a reduced perfusion of the microcirculation, which is reflected as an increase in peripheral vascular resistance and may be a major factor in the transient pulmonary hypertension induced in pulmonary angiography (Almen *et al.*, 1980) and in contrast-induced impairment of renal function (Golman and Holtas, 1980).

A relationship between osmolality of contrast media in solution and the degree to which they impair red cell filterability *in vitro* has been demonstrated (Dawson *et al.*, 1983*a*). The effect of hyperosmolar agents on red cells is thought to be due to a loss of internal water from the cell with a consequent increase in its internal viscosity (Bollman *et al.*, 1979). High osmolar media cause a more marked reduction of haematocrit, presumably due to osmotic cell shrinkage (Dawson *et al.*, 1983*a*). Morphological changes in red cells have been detected with exposure to high (Aspelin *et al.* 1980*b*; Dawson *et al.*, 1983*a*;

Kimball *et al.*, 1988; Miyazawa *et al.*, 1989), low and iso-osmolar solutions of contrast agent (Aspelin *et al.*, 1980*b*; Dawson *et al.*, 1983*a*), possibly as a result of protein binding and inhibition of the enzyme glucose-6-phosphate dehydrogenase (Lasser, 1971; Dawson *et al.*, 1983*a*). *In vitro* evidence also exists for haemolysis of red blood cells after contact with high (Robertson, 1987; Aspelin *et al.*, 1988) and low osmolality (Robertson, 1987) RCM.

Non-ionic contrast media and isotonic glucose have been shown to produce more marked red cell aggregation than ionic agents (Aspelin and Schmid-Schonbein, 1978; Aspelin *et al.*, 1980*a*; Raininko and Ylinen, 1987; Kimball *et al.*, 1988). The formation of erythrocyte aggregates by non-ionic media is unrelated to blood coagulation because red cell aggregates form rapidly even in heparinised blood, do not contain fibrin, and disappear when they are added to plasma or other ionic media (Zucker and Mauss, 1988). Red call aggregates were not observed with washed erythrocytes rather than whole blood, presumably because of the absence of plasma proteins (Zucker and Mauss, 1988). Aspelin *et al.* (1988) demonstrated that the increased red cell aggregation caused by non-ionic media dispersed after being submitted to shear force and concluded that the phenomenon is associated with high concentrations of contrast media in non-flowing blood and therefore the high shear rate in arteries and arterioles make it unimportant in the *in vivo* situation. In addition, within highly concentrated contrast media solutions, blood clot formation was not inducible (Aspelin *et al.*, 1988; Kimball *et al.*, 1988).

Contrast media inactivate the enzyme cholinesterase leading to the reduced hydrolysis of acetylcholine (Lasser and Lang, 1966) and hence the potentiation of parasympathetic activity. It has been suggested that pathological changes induced by RCM, such as peripheral vasodilatation and increased blood flow (Coel and Lasser, 1971), bronchospasm (Ansell, 1970), urticaria (Ansell, 1970), disturbances of cardiac rhythm (Pfister and Hutter, 1980) and convulsions could be explained on the basis of exaggerated cholinergic activity. The finding that the administration of atropine reverses the manifestation of bradycardia associated with hypotension in patients following RCM administration has lead to the proposal that such reactions are due to the inhibition of

acetylcholinesterase by RCM (Stanley and Pfister, 1976). Non-ionic media have been shown to inhibit cholinesterase to a lesser degree than ionic media (Dawson and Edgerton, 1983). A recent study however, provides evidence for poor interaction between contrast agents and acetylcholinesterase (Guidollet *et al.*, 1988).

Contrast media also depress the specific activity of several other enzymes, such as B-glucuronidase, glucose-6-phosphate dehydrogenase, lysozyme, alcohol dehydrogenase, adenosine triphosphatase and carbonic acid anhydrase (Lasser and Lang, 1970; Lang and Lasser, 1975). Enzyme inhibition by contrast agents is largely a function of their protein binding capacity (Lasser and Lang, 1970).

The central nervous system has been implicated in adverse reactions to radiographic contrast media. Hypnotic suggestion is effective in reducing the reactions of nausea and vomiting (Lalli, 1974). A theory based on central nervous system control has been formulated (Lalli, 1980). According to this hypothesis, the brain is initially primed by anxiety, causing the limbic portion to act upon the hypothalamus by means of the direct connections between them. Contrast media cross the blood-brain barrier (Lampe *et al.*, 1970) and stimulate the hypothalamus. This can generate responses influencing the respiratory and vasomotor centres and (*via* the reticular formation of the medulla) the autonomic nervous system. By this means, but principally through the sympathetic side of the autonomic nervous system, all reactions, ranging from urticaria to pulmonary oedema, can be accounted for (Lalli and Greenstreet, 1981).

Heart rate often increases in response to intravenous contrast media, in part because of the baroreceptor reflex to decreased blood pressure (Lawton *et al.*, 1982; Spataro *et al.*, 1987). Decreases in heart rate have also been noted, even in the presence of decreased blood pressure, in patients receiving both high (Andrews, 1976; Stanley and Pfister, 1976) and low (Poulsen *et al.*, 1987) osmolality contrast agents. It has been suggested that these reactions are vaso-vagal in origin (Andrews, 1976; Stanley and Pfister, 1976), as the administration of atropine reverses the reaction.

In man, hypertonic RCM are generally thought to produce hypotension, at least transiently (Friesinger *et al.*, 1965; Hilal, 1966; Lindgren, 1970; Chahine and Raizner,

1976). However, there are reports of elevated blood pressure, particularly if measurements are made some minutes after RCM administration (Fischer *et al.*, 1984; McClennan *et al.*, 1986). The intravenous infusion of diatrizoate, but not equiosmotic mannitol, has been shown to significantly increase blood pressure in rabbits (Harnish *et al.*, 1987*a*). Similarly, diatrizoate, but not iohexol, significantly increased blood pressure in rats and was prevented by pretreatment with naloxone (Harnish *et al.*, 1987*b*). As diatrizoate has been shown to stimulate the release of the endogenous opioid, *beta* endorphin from cultured hypothalamic neurons (Harnish *et al.*, 1988), endogenous opioid release by RCM has been implicated as a cause of contrast media-induced blood pressure alterations (Harnish *et al.*, 1987*b*).

It has become obvious that adverse reactions to radiographic contrast media cannot be attributed to a single cause, but rather to a composite of immunological, cardiovascular and neurological perturbations. RCM have been shown to interfere with virtually every physiological system, given sufficient time and dose. Behind each of the altered physiological parameters measured is a long chain of causes and effects, intertwined and retrolinked by feedback loops and amplification systems. Many segments of this labyrinth have been researched and many connections identified, but what appears to be the initiating factor of the adverse reactions is the capacity of RCM to damage cells mechanically by the osmotic strength of their solution and/or, at the molecular level, to perturb the functions of the biomacromolecules.

I.1.4 *Premedication*

Patients who have experienced a previous anaphylactoid reaction to RCM are at increased risk of developing another reaction on the repeat administration of RCM (Peters *et al.*, 1966; Gates and Ceccarelli, 1972; Schatz *et al.*, 1975).

Mixing an antihistamine with the RCM prior to administration (Olsson, 1951), and pretreating patients with an H_1-histamine antagonist before the RCM procedure (Peters *et al.*, 1966; Gates and Ceccarelli, 1972; Small *et al.*, 1982) resulted in a decreased number of

reactions. Pretreating high-risk patients with corticosteroids alone (Zweiman *et al.*, 1975; Lasser *et al.*, 1987) or in combination with H_1-antagonists (Kelly *et al.*, 1978; Greenberger *et al.*, 1980), has been shown to greatly reduce the number of repeat reactions. A pretreatment regimen evaluating the combination of the H_1 antagonist clemastine plus the H_2 antagonist cimetidine was found to significantly reduce the number of patients who experienced RCM-induced side-effects when compared with patients who had received clemastine or prednisolone alone (Ring *et al.*, 1985). Furthermore, anaphylactoid reactions to plasma substitutes have been effectively inhibited by pretreatment with a combination of H_1 and H_2 receptor antagonists (Schoning *et al.*, 1982).

I.2 PLATELETS

It has been known for almost a century that blood platelets are essential elements in haemostasis and that abnormal platelet function may contribute to a variety of pathophysiological conditions including thrombosis (Born, 1985), atherosclerosis (Woolf, 1981), myocardial infarction (Moore, 1976) and pulmonary embolism (Malik, 1983). More recently, it has been recognised that platelets have non-haemostatic functions (Page, 1988), having been implicated in inflammatory conditions (*eg.* rheumatoid arthritis) (Nachman and Weksler, 1980) and allergic diseases (*eg.* bronchial asthma) (Gresele *et al.*, 1982).

I.2.1 *Platelet Physiology*

Blood platelets are small, anucleate cells, derived from megakaryocytes in the bone marrow by the process of fragmentation. Normally they constitute a small fraction of the circulating elements, the platelet count in healthy human blood ranges from 1.3-4.0 $x10^5$ platelets/*u*l.

The plasma membrane represents the site of platelet interactions with the external environment and is ultimately involved in the control or generation of the many specialised functional properties of platelets. Loss of cell surface glycoproteins appears to be a

primary mechanism of platelet senescence *in vivo* (Greenberg *et al.*, 1979). A number of platelet receptors for ligands of biological or pharmacological significance have been identified, the functions of which are probably largely mediated by membrane proteins.

The most numerous organelles contained within the platelet cytoplasm are the platelet granules. Dense granules contain ADP and ATP, serotonin and Ca^{2+}. The more numerous *alpha* granules contain a variety of proteins, some platelet specific. These include the anti-heparinoid platelet factor 4 (PF4), platelet derived growth factor (PDGF), *beta*-thromboglobulin (*beta*-TG), transforming growth factor *beta* (TGF-*beta*), fibrinogen and clotting factor V.

Platelets are capable only of limited protein synthesis. Mitochondria are few in number, but contribute significantly to energy metabolism of the cell by providing ATP for the cytoplasmic metabolic pool. Lysosomes, glycogen granules and peroxisomes are randomly distributed throughout the cytoplasm.

Just beneath the cell membrane a bundle of microtubules travels the entire circumference of the cell. In the resting state this band is a flexible cytoskeleton exerting tension outward, maintaining both the discoid shape of the platelet and geographical integrity of the organelles. In close configuration with the microtubule band is a microfilament matrix which provides contractile force for the secretion of cell constituents during the platelet release reaction.

Two membrane systems weave throughout the cell interior, effectively increasing the platelet surface area. The open canalicular system, a random series of invaginations of the plasma membrane, contains channels which are continuous with the extracellular space and hence serve as a conduit through which endogenous substances pass to the cell exterior. The dense tubular system, derived from megakaryocyte endoplasmic reticulum, is associated with the circumferential microtubule band. This system is implicated as a major site of calcium sequestration, used for the initiation of platelet activation processes.

The platelet lifespan has been estimated at 8-12 days by a variety of radioisotopic labelling techniques (Aas and Gardner, 1958; Najean and Ardaillou, 1969). Destruction of effete platelets is accomplished by macrophages of the reticulo-endothelial system in the

spleen, liver and bone marrow.

I.2.2 *Haemostasis*

Platelets play a central role in the prevention of excessive blood loss. The dual contribution of platelets and coagulation pathways to haemostasis is briefly summarised in Figure I.2.1. Intact blood vessels are lined by haemostatically inert endothelial cells and as a consequence, sub-endothelial structures do not normally come into contact with flowing blood. Spontaneous or traumatic interruption of vascular continuity is the stimulus required to initiate a series of complex and interdependent reactions. Platelet surfaces will adhere to the exposed collagen fibres causing the cells to change shape from discoid to a more spherical form. This shape change is mediated by the contractile microtubular system and is characterised morphologically by the extension of dendritic pseudopodia. A secretory process ensues, whereby substances stored in platelet granules are extruded from the platelet *ie.* the platelet release reaction. Adenosine diphosphate (ADP), discharged from the dense granules and thromboxane A_2 (TXA_2), generated by the activation of platelet membrane phospholipase, influence the recruitment of additional circulating platelets to clump on those already adhered to the injured site. If the flow conditions are sufficiently disturbed, platelet aggregates form on the vessel wall and serve as a focus for the acceleration of coagulation reactions *via* platelet factor 3 (a phospholipid). Contact of blood with the sub-endothelium and release of the tissue factor thromboplastin from the damaged vessels, initiates a cascade of proteolytic reactions in the intrinsic coagulation pathway, culminating in the formation of thrombin. This newly formed thrombin acts synergistically with ADP and TXA_2 to promote further aggregation of platelets to form an enlarging platelet mass as the haemostatic plug (Zucker, 1980). Thrombin converts fibrinogen, present in plasma and released from platelets, into fibrin monomers which polymerise to stabilise and reinforce the platelet plug. The fibrin meshwork contains platelets and some red and white blood cells. Platelet contractile proteins thrombosthenin and actomysin, are stimulated by thrombin and clot retraction is initiated (Zucker, 1980).

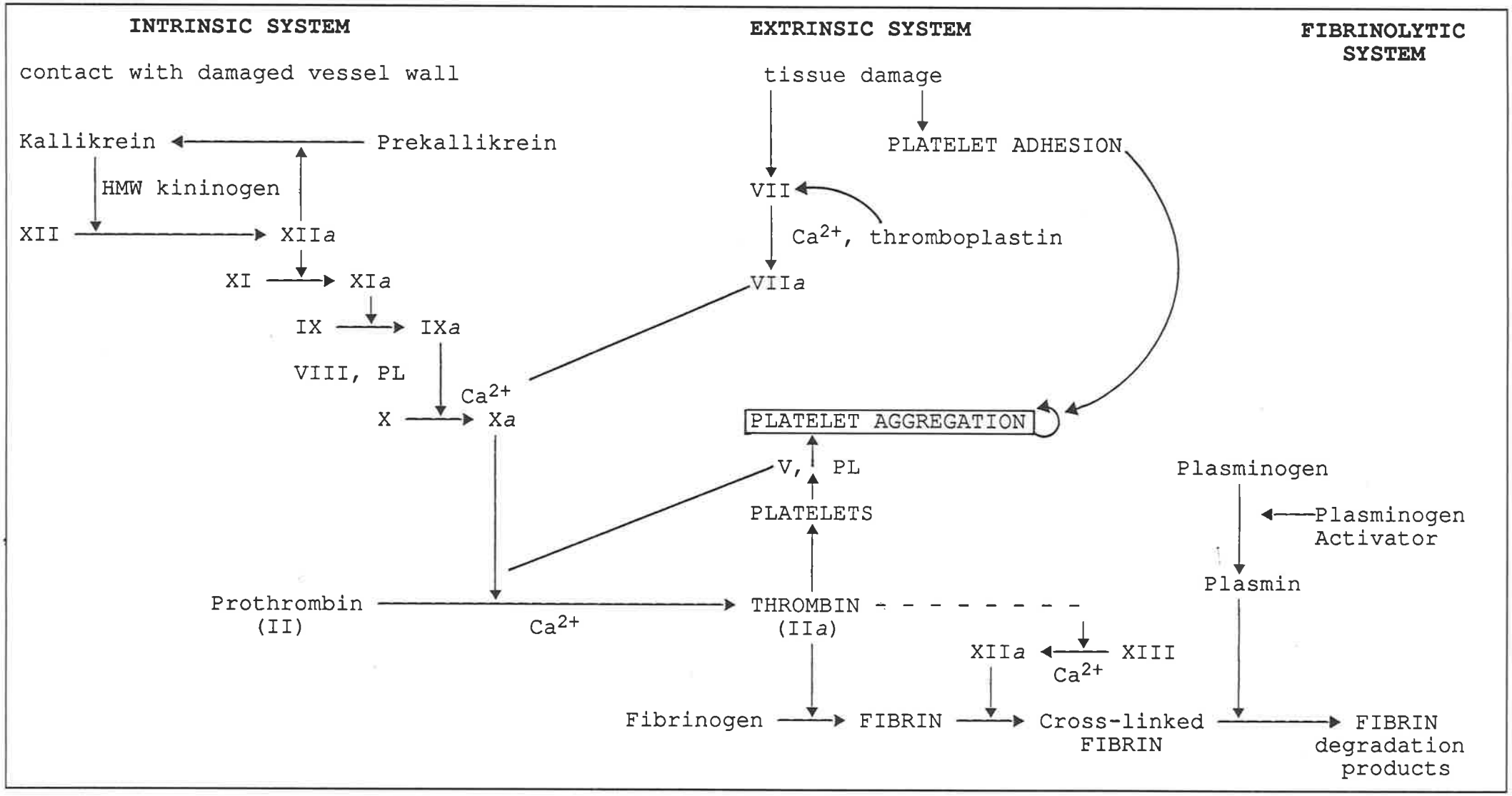


Figure I.2.1 Pathways in the haemostatic mechanism. PL, phospholipid; HMW, high molecular weight.

Subsequently, plasmin is cleaved from its plasminogen precursor and by its lytic action on fibrin causes the slow dissolution of the clot (Figure I.2.1).

I.2.3 *Thrombosis and Atherosclerosis*

Thrombosis may be considered an abnormal product of the normal haemostatic mechanism. A thrombus can be defined as a mass or deposit formed from blood constituents on the surface lining of blood vessels anywhere in the circulation and has a characteristic structure comprising platelets, red cells and fibrin strands. Mural thrombi adhere only to one side of a vessel and blood continues to flow past the free borders. Emboli that dissociate from the wall are transported to other sites in the vasculature and may form occlusive thrombi occupying the entire vessel lumen. Blood flow in this case becomes obstructed and ischaemia may result.

Coagulation is the major factor implicated in the development of venous (red) thrombi, which tends to occur in valve pockets and at points of maximum stasis along relatively stagnant channels. In comparison, arterial (white) thrombi which have a larger platelet component, mainly form in areas of disturbed flow (Mustard, 1981).

Most commonly, arterial thrombi develop in areas of atherosclerotic lesions which provide abnormal vessel walls on which the endothelium may be less stable compared with the normal case. However, there is considerable debate as to the precise aetiological relationship between these two conditions (Mitchell, 1981). Atherosclerosis follows when platelets deposit in the vicinity of damaged endothelium, smooth muscle cells proliferate and invade the vessel intimal layer. Lipids and cholesterol accumulate and the plaque is subsequently overgrown by the endothelium. The mitogenic stimulus for smooth muscle cell proliferation may be PDGF released from the *alpha* granules of aggregated platelets (Zucker, 1980).

I.2.4 *Non-Thrombotic Processes*

Despite being devoid of a nucleus, platelets possess many of the features of classical inflammatory cells such as polymorphonuclear leucocytes (PMNL). They are capable of undertaking phagocytosis and chemotaxis and release a variety of mediators that can augment inflammatory cell recruitment (Nachman and Weksler, 1980).

Platelet aggregation can be induced by a number of bacterial pathogens which can become sequestered in clumps of platelets (Clawson, 1971). As a result of the subsequent platelet release reaction (and possibly also as a result of the production by the aggregated platelets of chemotactic metabolites of arachidonate), the platelet-bacterial aggregates become chemotactic for PMNL and for monocytes (Clawson, 1974). Therefore evidence exists for the involvement of platelets in non-allergic defense mechanisms such as the removal of bacterial infections. Furtheremore, platelets are known to release bacteriocidal products such as *beta*-lysin (Hirsh, 1960). Platelet activation is also a recognised feature of clinical and experimental malignancy (Hilgard, 1982).

Platelets possess both IgG and IgE membrane receptors. The IgE receptors are of low affinity when compared with those found on mast cell or basophil surfaces, but are of comparable affinity to IgE receptors on other inflammatory cell types such as alveolar macrophages and eosinophils (Capron *et al.*, 1986). The physiological relevance of the platelet IgE receptor may be associated with a mechanism for aiding the removal of parasitic infections (Page, 1988). It has been shown that IgE-activated platelets release cytotoxic free radicals in sufficient concentrations to kill parasites (Ameisen *et al.*, 1985). The ability of platelets to generate free radicals is not accompanied by classical platelet aggregation and may thus represent a distinct mechanism of platelet activation.

Accumulating evidence suggests that platelet activation is a feature of diseases where there is activation of the allergic response (such as asthma and urticaria), although such diseases are not normally associated with thrombosis (Storck *et al.*, 1955; Knauer *et al.*, 1981; Gresele *et al.*, 1982; Traietti *et al.*, 1984; Wasserman and Ginsberg, 1984; Page *et al.*, 1985; Johnson *et al.*, 1986; Szczecklik *et al.*, 1986; Taytard *et al.*, 1986; Gresele *et al.*, 1987; Martin *et al.*, 1987; Taytard *et al.*, 1987). In certain clinical (Rao and Walsh, 1983) and experimental (Henson and Pinckard, 1977) conditions where there is known to be

excessive platelet activation in the circulation, platelets become partially refractory to subsequent stimulation *in vitro*. In particular, the second phase of platelet aggregation *in vitro* is often unresponsive to physiological stimuli. A number of studies have reported that platelets from asthmatics behave abnormally *in vitro*, lacking the second wave of aggregation (Fishel and Zwemer, 1970; Solinger *et al.*, 1973; Maccia *et al.*, 1977; Thompson *et al.*, 1984) and thus possessing an abnormality *in vitro* suggestive of overstimulation *in vivo* (Page *et al.*, 1985; Gresele *et al.*, 1987).

Thrombocytopoenia was first reported to accompany asthmatic attacks in 1955 (Storck *et al.*). This observation of platelet activation *in vivo* during provoked or spontaneous asthmatic attacks has also been shown by the detection of circulating platelet aggregates (Gresele *et al.*, 1982) or the morphological characterisation of activated platelets in the circulation (Traietti *et al.*, 1984). Furthermore, a number of studies have demonstrated the release of two platelet-specific markers, platelet factor 4 (PF4) and *beta*-thromboglobulin (*beta*-TG), into the circulation associated with bronchoconstriction induced by antigen or exercise (Knauer *et al.*, 1981; Gresele *et al.*, 1982; Gresele *et al.*, 1985; Metzger *et al.*, 1985; Page *et al.*, 1985; Johnson *et al.*, 1986). The release of these markers is indicative of *in vivo* platelet activation (Messmore *et al.*, 1984) and in the study of Knauer and colleagues (1981), the increased plasma levels of platelet-derived markers occurred in parallel with the bronchoconstriction induced by antigen provocation of allergic asthmatics. Release of PF4 and *beta*-TG was not observed following comparable bronchoconstriction induced by methacholine suggesting that the platelet-derived markers were released as a consequence of the allergic reaction rather than of the bronchoconstriction.

Release of platelet-derived factors have not been consistently observed leading some investigators to reject the role of platelets in asthma (Greer *et al.*, 1984; Durham *et al.*, 1985; Shephard *et al.*, 1985). However, other clinical observations support the concept that platelets may be involved in this disorder. Platelet survival time in atopic asthmatics is severely shortened, suggesting continuous activation of this blood element (Taytard *et al.*, 1986). Treatment of these asthmatics with anti-asthma drugs such as glucocorticoids and

ketotifen corrects this shortened platelet survival (Taytard *et al.*, 1987). Shortened platelet regeneration time, an index of *in vivo* platelet activation associated with accelerated platelet consumption (Harker, 1978), has been reported in asthmatics undergoing acute asthma attacks (Gresele *et al.*, 1987). Increased bleeding time has been observed in a group of atopic asthmatics (Szczecklik *et al.*, 1986). At autopsy, lungs from asthmatics dying from *status asthmaticus* have abnormal megakaryocytes present in abundance (Martin *et al.*, 1987).

Studies suggest that platelets are involved in the pathogenesis of airway hyperresponsiveness, the characteristic feature of asthma (Boushey *et al.*, 1980), and that some mechanism other than platelet aggregation within the pulmonary vasculature contributes to this phenomenon, possibly the extravascular diapedesis of platelets (Robertson and Page, 1987). The fate of platelets in the circulation of asthmatics is unknown although overt trapping in the pulmonary vasculature is not a feature of either stable asthmatics or those undergoing bronchoconstriction (Gresele *et al.*, 1987). However, platelets have been observed to undergo diapedesis into the extravascular tissue of the lungs of guinea-pigs following antigen challenge or treatment with the phospholipid platelet activating factor (PAF), known to be released from a number of inflammatory cells in the lung (Page *et al.*, 1985). The extravasated platelets have been observed in close proximity to bronchial smooth muscle and to infiltrating eosinophils. However, treatment of experimental animals with other platelet agonists such as ADP, whilst inducing platelet aggregation in the pulmonary vasculature, does not elicit extravascular diapedesis of platelets and eosinophils (Lellouch-Tubiana *et al.*, 1985), suggesting a possible link between extravascular platelets and eosinophils. Platelets have also been reported in bronchoalveolar lavage (BAL) fluid obtained from both allergic rabbits and allergic asthmatics undergoing late-onset airways obstruction following antigen challenge (Metzger *et al.*, 1987). The extravascular platelets in this clinical situation have again been observed in close association with other inflammatory cells such as the eosinophil (Metzger *et al.*, 1987).

In guinea-pigs, PAF-induced bronchial hyperreactivity is platelet dependent since it

can be abrogated by rendering animals selectively thrombocytopoenic (Mazzoni *et al.*, 1985*a*). Platelet depletion has been reported to reduce PAF and antigen-induced eosinophil infiltration into the lungs of normal and allergic animals repectively (Lellouch-Tubiana *et al.*, 1988), suggesting a central role for platelets in the induction of eosinophil accumulation which both facilitates the removal of parasitic infection and contributes to the bronchial hyperreactivity observed in asthma. It is conceivable that platelets release factors chemotactic for eosinophils as they are known to release materials chemotactic for neutrophils (Nachman and Weksler, 1980). The PAF antagonist BN 52021 has recently been demonstrated to inhibit eosinophil infiltration associated with the late-onset response following antigen challenge and the subsequent increase in bronchial hyperreactivity (Coyle *et al.*, 1987). This finding suggests that antigen-induced release of PAF may play a central role in the platelet activation necessary to initiate the eosinophil infiltration into the airways that contributes to bronchial hyperreactivity.

Platelets have been described as a source of mitogenic factors such as platelet-derived growth factor (PDGF) which participate in wound repair after tissue injury. PDGF release at sites of continuous vessel wall injury has been suggested to contribute to the vascular smooth muscle hypertrophy which characterises cardiovascular diseases such as atherosclerosis. Similarly, bronchial smooth muscle hypertrophy is a feature of the asthmatic lung at autopsy and it remains plausible that continuous platelet recruitment and extravascular diapedesis into the airways with consequent release of mitogens could also contribute to this feature of asthma.

I.2.5 *Platelet Aggregation and Release*

Platelet aggregation has been extensively studied *in vitro* in an aggregometer, by methodology based on that originally described by Born (1962). With the addition of most of the biologically important aggregating agents such as ADP, thrombin and collagen fibrils to platelet-rich plasma (PRP), aggregation is preceeded by a change in the shape of the platelet, as evidenced by a decrease in light transmission through a stirred suspension

of PRP. Adrenaline differs in this respect as it does not elicit the characteristic change prior to the aggregatory response. As platelets aggregate, a rapid increase in light transmission follows.

The addition of collagen to PRP induces a single wave of aggregation. The hypothesis that collagen initiates ADP release from platelets, and that this extracellular ADP is subsequently responsible for the observed aggregation (Hovig, 1963) has gained widespread acceptance. However, Nunn (1979) reported that human platelets can aggregate in response to collagen under circumstances in which they cannot respond to ADP. Thus it seems that secreted ADP is mainly, although not essentially, responsible for aggregation induced by collagen. The mechanisms of the collagen-platelet surface interaction have not been completely elucidated.

At least 3 steps are involved in the early stages of ADP-induced aggregation *ie.* (1) interaction of ADP on surface receptor(s), (2) internal changes in the platelet resulting in the availability of fibrinogen receptors on the platelet membrane and (3) interaction of fibrinogen with its receptor. Fibrinogen is required to support ADP-induced aggregation and dissociates from platelet surfaces when they disaggregate (Mustard *et al.*, 1978). Not only is fibrinogen present in plasma but also in platelet *alpha* granules, thus may facilitate further binding upon release (Zucker, 1980).

The link between thromboxane A_2 (TXA_2) and platelets developed from the observation that collagen-induced aggregation was inhibited by aspirin (Weiss *et al.*, 1968). Once it was established that aspirin could inhibit prostaglandin biosynthesis it became apparent that aggregation induced by collagen was mediated by the formation of an aggregatory prostanoid. Arachidonic acid (AA), a substrate for platelet prostaglandin synthesis, becomes available when a stimulus such as collagen or thrombin acts on the platelet surface, liberating the unsaturated fatty acid from membrane phospholipids *via* the enzyme phospholipase A_2 (Figure I.2.2). Following release, AA is converted by the enzyme cyclo-oxygenase to two labile cyclic endoperoxides, PGG_2 and PGH_2, both active pro-aggregatory substances. Alternatively, AA can be converted by the enzyme lipoxygenase to HPETE (12-hydroperoxyarachidonic acid) and HETE (12-

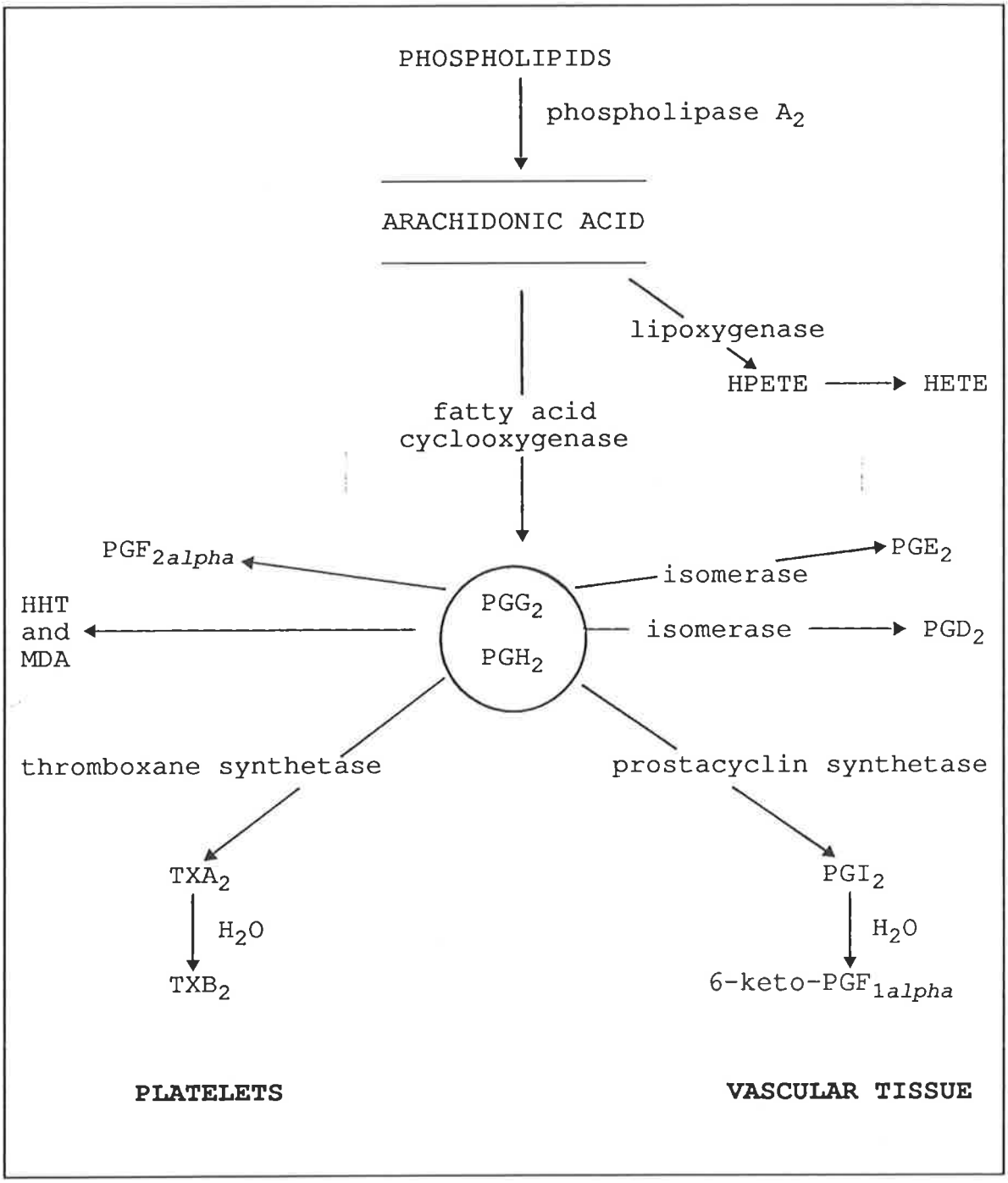


Figure I.2.2 Metabolic pathway of arachidonic acid in platelets and vascular tissue. Arachidonic acid is produced from both arterial and platelet membrane phospholipid and gives rise to the prostaglandins (PG) G_2 and H_2. Thromboxane synthetase in platelet microsomes produces the aggregant TXA_2, while PGI_2 synthetase produces prostacyclin in endothelial cells. Minor metabolites: HPETE/HETE, hydroperoxy/ hydroxyeicosatetraenoic acid; HHT, hydroxyheptadecatrienoic acid; MDA, malondialdehyde.

converted to TXA_2 which is a potent aggregating agent and stimulus for secretion. TXA_2 is highly unstable and is degraded to thromboxane B_2 (TXB_2) (Figure I.2.2) which is devoid of functional activity (Hamburg *et al.*, 1975). TXA_2 is thought to mediate its pro-aggregatory effect by inhibiting platelet cyclic AMP production (Miller *et al.*, 1977) and mobilising Ca^{2+} stores from within platelet granules (Figure I.2.3).

TXA_2 plays a key role in the regulation of aggregation and release although its relative importance is dependent upon the platelet stimulus. When TXA_2 is inhibited by aspirin, release can be elicited by high doses of thrombin (Smith and Willis, 1971) or collagen (Charo *et al.*, 1977). In contrast to this, arachidonic acid induces aggregation which is completely abolished by aspirin (Silver *et al.*, 1973). ADP may cause primary wave aggregation in the absence of TXA_2, while second wave aggregation is dependent on TXA_2 production (Smith *et al.*, 1973). Thus, platelet aggregation and release can be induced by TXA_2-dependent and TXA_2-independent mechanisms. Figure I.2.3 diagramatically summarises mechanisms of platelet release. At present, details of alternative pathways involved in aggregation and secretion are unknown and their relative *in vivo* significance not fully understood.

In vascular tissue endoperoxide precursors were found not to be converted to TXA_2 as in the platelet, but to a labile substance that relaxes coeliac and mesenteric arteries of rabbits (Bunting *et al.*, 1976). This substance was referred to as PGX and was evaluated as a platelet aggregation inhibitor where it was found to be 30 times more active than PGE_1 (Moncada *et al.*, 1976). PGX relaxed vascular strips *in vitro*, caused vasodilatation, was the most potent inhibitor of platelet aggregation discovered and possessed antithrombotic properties. Furthermore, it was the major metabolite of arachidonic acid in vascular tissue (Moncada and Vane, 1979). PGX was shown to be the unstable metabolic intermediate in the formation of 6-keto-PGF_{1alpha} (Pace-Aschiak, 1976) which is functionally inactive (Tateson *et al.*, 1977) (Figure I.2.2). The structure of PGX was elucidated and renamed prostacyclin (PGI_2) (Johnson *et al.*, 1976). PGI_2 is thought to inhibit platelet aggregation by modulating cellular adenylate cyclase, leading to an increase in platelet cyclic AMP levels (Gorman *et al.*, 1977; Tateson *et al.*, 1977) (Figure I.2.3).

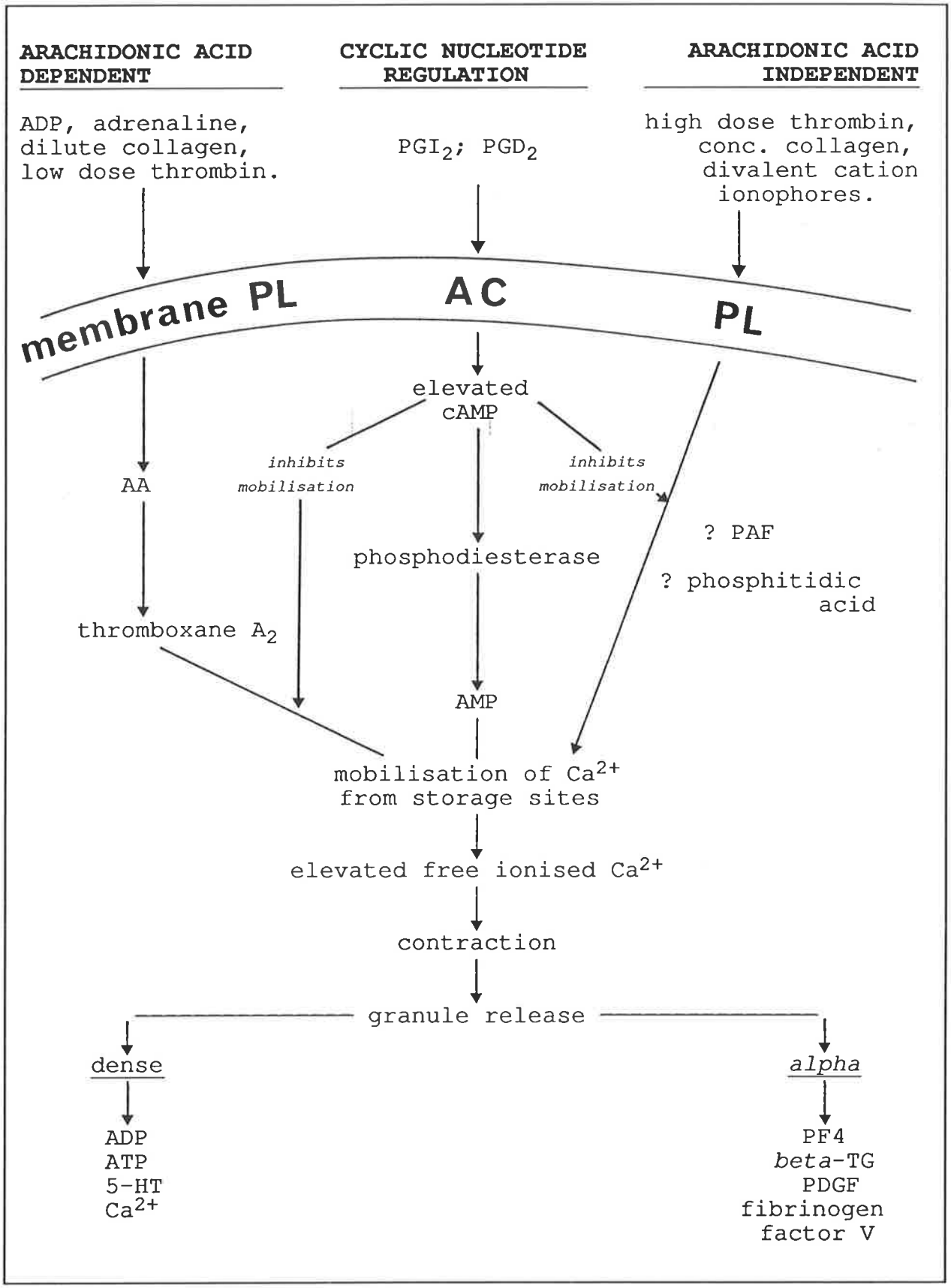


Figure I.2.3 Postulated scheme relating mechanisms of platelet release. AA, arachidonic acid; AC, adenylate cyclase; PL, phospholipid.

levels (Gorman *et al.*, 1977; Tateson *et al.*, 1977) (Figure I.2.3).

Moncada and Vane (1978*b*) concluded from many studies that the vessel wall can synthesise PGI_2 not only from its own endogenous precursors but also from prostaglandin endoperoxides released by platelets, thus suggesting a biochemical co-operation between the platelet and vessel wall. This hypothesis was challenged by Needleman and colleagues (1978) who claimed that some degree of vascular damage was necessary for endoperoxides to be utilised by PGI_2 synthetase in the endothelium.

I.2.6 *In Vitro Methods of Monitoring Platelet Function*

Platelets can readily be isolated from whole blood for study *in vitro*. Born's method of aggregometry (1962) has allowed the detailed analysis of platelet aggregation *in vitro* and has facilitated delineation of many underlying biochemical pathways. At least three independent pathways of platelet aggregation have been identified *ie.* adenosine diphosphate (ADP)-dependent, thromboxane A_2 (TXA_2)-dependent and platelet activating factor (PAF)-dependent (Vargaftig *et al.*, 1981*a*). Additionally, a plethora of pharmacological agents have been found to exert inhibitory activity on platelet aggregation in vitro, raising the possibility that such substances may have potential as anti-thrombotic agents (Didisheim and Fuster, 1978; Owen, 1979). However, the majority of substances that inhibit platelet aggregation *in vitro* fail to prevent, or even retard, thrombosis in intact animals (Didisheim and Fuster, 1978; Owen, 1979) suggesting the participation of other blood elements in the induction and development of thrombosis. The Born aggregometer functions only with translucent cell suspensions, thus solely permitting the study of platelet-platelet interactions. A whole blood aggregometer has since been developed (Cardinal and Flower, 1980) and studies of platelets in this experimental system may provide an assessment of platelet behaviour *in vitro* that is more predictive of the behaviour of platelets in their natural environment. However, this method, which measures changes in electronic impedance between two electrodes, requires the presence of the anticoagulant citrate which, by nature of its mode of action, reduces calcium availability.

Since calcium ions are necessary for platelet activation (Ardlie, 1982), the *in vitro* system is non-physiological. Moreover, small changes in citrate concentration have dramatic effects upon the resulting platelet aggregation *in vitro* (Ts'ao *et al.*, 1976). The greater the amount of anticoagulant, the slower the aggregation and the lesser the extent of aggregation in response to ADP, adrenaline and collagen (Ts'ao *et al.*, 1976).

Therefore, an obvious need exists for predictive *in vivo* techniques for the evaluation of platelet function.

I.2.7 *In Vivo Methods of Monitoring Platelet Function*

There have been numerous, often ingenious experimental systems developed to study platelet function *in vivo* (Born and Cross, 1963; Hornstra, 1970; Smith and Frueler, 1973; Bourgain and Six, 1974; Wu and Hoak, 1974; Baumgartner *et al.*, 1976; Kohler *et al.*, 1976; Buchanan and Hirsch, 1978; Gryglewski *et al.*, 1978; Butler *et al.*, 1979; Shishido and Katori, 1981; Garcia Rafanell and Form, 1982; Badimon *et al.*, 1983; Pinon, 1984; Hanson and Harker, 1987). The majority of experimental systems used to date are highly invasive and cumbersome and do not allow repeated observations to be made in a single animal, making them expensive in terms of both time and money. However, Smith and Freuler (1973) developed a method for the continuous extracorporeal counting of animal platelets in blood removed at a constant rate by a special "double cannula". This system employs a Technicon Autocounter, a fully automated system that effectively separates and optically counts the platelets (red cells are lysed) and provides a continuous recording on precalibrated chart paper. The major criticism of this technique is that it requires the removal of 6 ml of blood per hour. Other *in vivo* systems require systemic anticoagulants, thus precluding the analysis of the function of platelets in their physiological environment.

The retention of platelets within the pulmonary vasculature, assessed histologically, has provided a quantitative index of platelet activation *in vivo* (Pinckard *et al.*, 1977; Butler *et al.*, 1979). In addition, ^{51}Cr-labelled platelets present in organs (*eg.* lung tissue) and in blood samples following antigen challenge were assessed using a gamma

scintillation counter. Whilst this test system has been used productively for the evaluation of aggregatory stimuli and of inhibitors of platelet function *in vitro* (Butler *et al*, 1979), it has the disadvantage of being both time consuming and expensive. The sacrifice of a relatively large number of animals is required to define the time course of a response, or to provide dose-response relationships.

I.2.8 *Automated Isotope Monitoring System (AIMS)*

Armed with the knowledge that platelet agonists induce the entrapment of platelet aggregates within the pulmonary vasculature (Pinckard *et al.*, 1977; Butler *et al.*, 1979) a technique for automating the detection of the fate of radiolabelled platelets in the circulation has been devised (Davies *et al.*, 1981). This system has been adopted from the clinical procedure whereby blood elements radiolabelled with gamma-emitting isotopes can be externally imaged with gamma camera devices.

Use of the isotope ^{51}Chromium as a label for blood platelets was described in 1958 (Aas and Gardner, 1958). ^{51}Cr-labelled platelets have been shown to be evenly distributed within the platelet population (Busch and Olson, 1973), to function normally *in vitro* (Butler *et al.*, 1979) and have the same survival time as unlabelled platelets *in vivo* (Thakur *et al.*, 1976). The use of ^{51}Cr has been largely superceded by ^{111}Indium complexes such as oxine (8-hydroxyquinoline) or tropolone (2-hydroxy,2,4,6,-cyclo-heptatienone) (Thakur *et al.*, 1976; Goodwin *et al.*, 1978; Joist *et al.*, 1978; Heaton *et al.*, 1979; Grimley *et al.*, 1981; Thakur *et al.*, 1981). ^{111}In has advantages over ^{51}Cr as it has higher labelling efficiency, a shorter radioactive half-life and higher energy gamma photon emissions. Furthermore, a substantially smaller volume of blood is required to label platelets with ^{111}In. Platelets may be labelled using ^{111}In-oxine without a change in physiological function and have a half-life similar to their unlabelled counterparts within the circulation of man and experimental animals (Thakur *et al.*, 1976; Goodwin *et al.*, 1978; Grimley *et al.*, 1981). ^{111}Indium chelated with oxine forms a lipid soluble compound and greater than 90% accumulates in the cytosol of most mammalian platelets and is not released when the

platelets are activated *in vitro* or *in vivo* (Thakur *et al.*, 1976). As ^{111}In-oxine binds avidly to proteins and other cells, radiolabelling of platelets with this isotope must be carried out in a plasma-free environment (Thakur *et al*, 1976). ^{111}In-oxine is a relatively weak complex and in the presence of plasma translocates ^{111}In to transferrin which will not diffuse into the platelet (Thakur *et al*, 1976).

The intravenous administration of a various aggregatory stimuli (*eg*. ADP, collagen, platelet activating factor (PAF) and antigen) have been shown to evoke increases in thoracic radioactive counts of experimental animals pre-loaded with ^{111}In-labelled platelets. Such increases can be attributed to the retention of platelets within the pulmonary vasculature, as confirmed by histological evidence of platelet aggregates throughout lungs from animals sacrificed when thoracic counts are elevated (Butler *et al.*, 1979; Dewar *et al.*, 1984). In addition to this, there is a fall in the count rate detected by the abdominal probe which for ADP parallels the time course of thrombocytopoenia (Barrett *et al.*, 1984). The time course of thrombocytopoenia is similar to that observed with the method of Smith and Freuler (1973).

To ensure that the responses are not reflecting changes in blood flow, red blood cells are labelled with ^{111}In-oxine and monitored as for platelets. For example, following inhalation of endotoxin there is a progressive intrathoracic accumulation of platelets over 2 hours, a phenomenon which is not observed when red blood cells are radiolabelled and monitored (Beijer *et al*, 1987).

In vivo platelet accumulation studies have produced some anomalous results. For example, 5-HT has no aggregatory effect on rat platelets *in vitro*, yet induces dose-related accumulation of platelets in the pulmonary vasculature of rats *in vivo* following i.v. administration. Similarly, adrenaline has no direct action on rat platelets *in vitro*, although it can be shown to act synergistically with ADP to potentiate aggregation (Yu and Latour, 1977). In contrast to this, adrenaline infused into intact rats *in vivo* produces a dose-dependent inhibition of ADP-induced aggregation (Oyekan and Botting, 1986). This inhibitory effect of adrenaline can be prevented by prior treatment with *alpha*-, but not *beta*-, adrenoceptor antagonists (Botting and Oyekan, 1986).

The endothelium has recently been shown to liberate the 21 amino acid peptide endothelin (Yanagisawa *et al.*, 1988), which not only constricts vascular smooth muscle, but also inhibits *ex vivo* platelet aggregation (Thiemermann *et al.*, 1988). In addition, endothelial cells produce substances that relax vascular smooth muscle, including endothelium-derived relaxing factor (EDRF) (Furchgott and Zawadski, 1980). The relaxation produced is similar to that produced by nitrovasodilators and recent evidence suggests that EDRF is nitric oxide (Palmer *et al.*, 1987). Like the nitrovasodilators, EDRF mediates its effects on vascular smooth muscle by stimulating guanylate cyclase and elevating cytosolic cyclic GMP levels (Ignarro *et al.*, 1981; Rapoport and Murad, 1983; Busse *et al.*, 1985). EDRF has been shown to inhibit platelet aggregation both *in vitro* (Azuma *et al.*, 1986; Furlong *et al.*, 1987; Radomski *et al.*, 1987*a*) and *ex vivo* (Hogan *et al.*, 1988) and to inhibit platelet adhesion (Radomski *et al.*, 1987*b*; 1987*c*). These findings suggest a role of this substance in the control of platelet activation.

Recently, the effect of exogenous EDRF on platelet function *in vivo* has been studied using ^{111}In-labelled platelet accumulation (Bhardwaj and Moore, 1988). The ability of carbachol to stimulate the release of EDRF in the whole animal (a process inhibited by methylene blue or haemoglobin) (Bhardwaj and Moore, 1987), has been utilised to investigate the inhibitory effect of endogenous EDRF on *in vivo* platelet aggregation by monitoring changes in pulmonary platelet accumulation (Bhardwaj and Moore, 1988).

I.3 PLATELET ACTIVATING FACTOR (PAF)

I.3.1 *Historical Background*

The release of histamine into rabbit plasma during the acute allergic reaction was reported by Barbaro and Zvaifler (1966). This finding posed a paradox since blood histamine in this species is stored almost exclusively within platelets (Humphrey and Jaques, 1954), yet allergic hypersensitivty is dependent upon interaction of allergen with immunoglobulin E (IgE) upon the surface of basophils in this, and in other species (Siraganian and Osler, 1969). Subsequently, rabbit basophils were found to respond to

allergen stimulation by releasing a substance that was capable of activating platelets (Benveniste *et al.*, 1972). This material was found to be actively released from the leucocytes by a calcium- and temperature-dependent process (Benveniste *et al.*, 1972).

A method for preparing this basophil product, which was referred to as platelet activating factor (PAF), was described by Benveniste and colleagues in 1972. Benveniste's group initiated the characterisation of the biologically active constituent of PAF responsible for platelet activation (Benveniste, 1974; Benveniste *et al.*, 1977) and on the basis of physicochemical properties concluded that it was a phospholipase A_2 (PLA_2)-sensitive phospholipid (Benveniste *et al.*, 1977; 1979; 1982). The chemical structure of PAF was identified in 1979 by three independent groups as 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Benveniste *et al.*, 1979; Blank *et al.*, 1979; Demopoulos *et al.*, 1979) (Figure I.3.1). Partial synthesis of PAF was reported in 1979 by Benveniste *et al.* and in 1980 total synthesis of the molecule was achieved (Godfroid *et al.*, 1980).

A consequence of the chemical characterisation of PAF has been an expanded nomenclature, whereby various synonyms are used to refer to the active material *ie.* PAF-acether (*ace* referring to acetate and *ether* for the ether bond) (Benveniste, 1974), acetylglycerylether-phosphorylcholine (AGEPC) (Demopoulos *et al.*, 1979) or antihypertensive renomedullary lipid (APRL) (Blank *et al.*, 1979).

I.3.2 *Synthesis and Metabolism*

Cell damage or disruption does not yield substantial amounts of PAF, which suggests that this material is neither pre-formed nor stored (Tence *et al.*, 1980). In several cell types there is coincident formation of PAF and a non-acetylated compound termed lyso-PAF (Mencia-Huerta *et al.*, 1981; Benveniste *et al.*, 1982). Lyso-PAF has similar physicochemical properties to PAF but is essentially devoid of biological activity. Lyso-PAF has been shown to be released *in vitro* by platelets (Benveniste *et al.*, 1982), neutrophils (Jouvin-Marche *et al.*, 1984) and macrophages (Mencia-Huerta *et al.*, 1981) in response to activation by a variety of agents and the formation of lyso-PAF can be

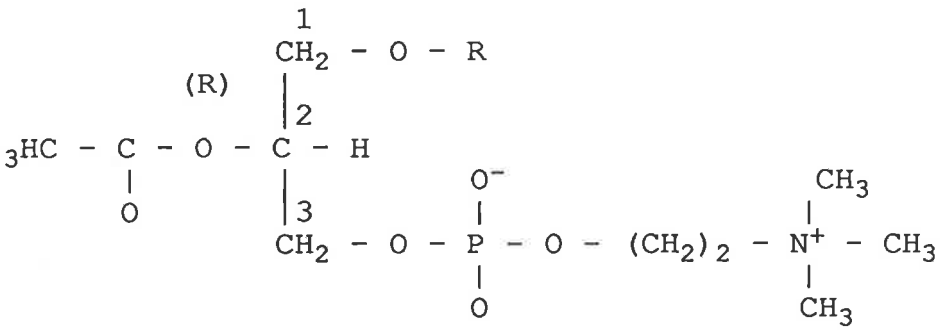


Figure I.3.1 Chemical structure of PAF; R = $C_{16}H_{35}$ or $C_{18}H_{37}$

abolished by inhibitors of phopholipase A_2, indicating the involvement of this enzyme in the synthesis of PAF (Vargaftig *et al.*, 1981*b*).

Evidence exists to support the concept that lyso-PAF is a precursor of PAF. Addition of synthetic lyso-PAF and acetyl-CoA to rat macrophages yeilds PAF (Ninio *et al.*, 1982) and platelets incorporate radiolabelled acetate into PAF (Chap *et al.*, 1981). It is now recognised that acetylation of lyso-PAF to PAF can be achieved by an acetyl transferase enzyme that has been described in a number of cell types (Mencia-Huerta and Benveniste, 1981; Lee *et al.*, 1984; Pirotzky *et al.*, 1984*a*) and acts as a rate-limiting enzyme for PAF synthesis (Snyder, 1985) (Figure I.3.2).

More recently, a second pathway for PAF synthesis has been described involving the transfer of phosphorylcholine from ether-linked phospholipids (plasmalogens) (Renooij and Snyder, 1981; Snyder, 1985). This is a single step reaction catalysed by the enzyme choline phosphotransferase (Renooij and Snyder, 1981; Snyder, 1985). The extent to which these enzymes participate in the production of PAF *in vivo* is not fully understood, but it appears that in inflammatory cells the two step synthetic pathway involving the rate-limiting acetyl transferase enzyme predominates (Snyder, 1987), while in cell types such as renal cells, where PAF may be produced continuously and serve as a physiological hormone, the phosphocholine transferase enzyme appears active (Snyder, 1987).

The lability of PAF *in vivo* is due to the widespread distribution of a cytosolic acetyl hydrolase enzyme (Farr *et al.*, 1980) (phosphatide-2-acetyl-hydrolase) which is able to cleave the acetate moiety at the 2-n position to leave lyso-PAF. Lyso-PAF is further metabolised by the removal of the *o*-alkyl group by an enzyme that is either similar to, or identical with, the well characterised tetrahydropteridine-dependent alkyl mono-oxygenase enzyme isolated from rat liver (Lee *et al.*, 1981). This process generates a fatty aldehyde and the hydrosoluble glyceryl-3-phosphorylcholine.

The total synthesis of PAF has led to the synthesis of a wide range of PAF analogues, thus permitting the examination of structure-activity relationships to determine the optimal requirements for the biological activity of PAF. This has been extensively reviewed recently (Braquet and Godfroid, 1986; Braquet *et al.*, 1987), but in particular it is

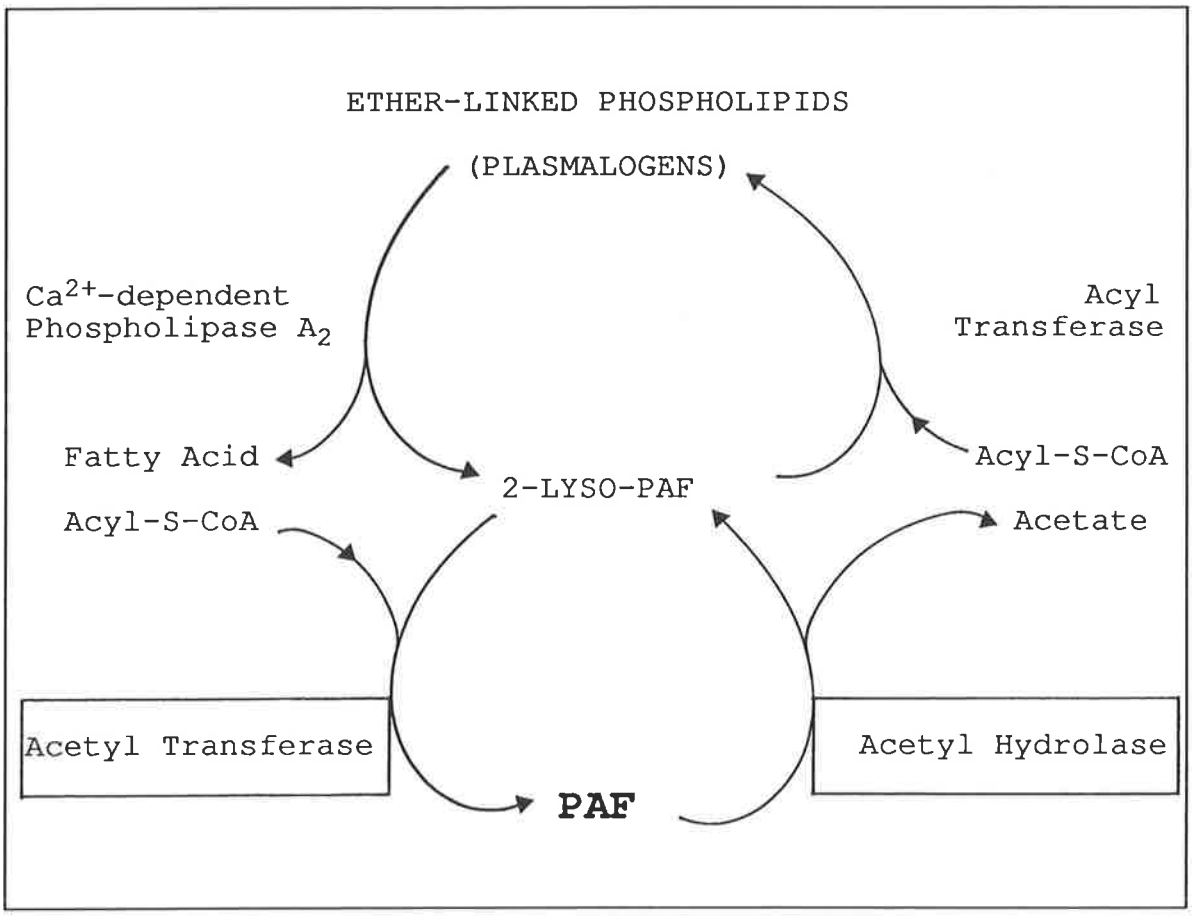


Figure I.3.2 PAF is generated from ether-linked phospholipids by a 2-step enzymatic process; (1) removal of a fatty acid to leave lyso-PAF (biologically inactive precursor of PAF) and (2) acetylation of lyso-PAF by a rate-limiting acetyl transferase enzyme. PAF is rapidly metabolised in plasma by an acetyl hydrolase to leave the inactive lyso-PAF.

known that the presence of the ether linkage and the length of the alkyl side-chain at position 2 of the molecule is less critical. PAF derived from biological origin, including human skin, is a mixture of mainly C_{16} and C_{18} types (Mallet *et al.*, 1985) and the biological activities of C_{16} PAF and C_{18} PAF do not appear to be qualitatively or quantitatively different (Archer *et al.*, 1986). Structure-activity studies have also permitted the description of a putative structure of the PAF binding site for PAF (Braquet and Godfroid, 1986).

A number of groups have reported that high-affinity binding sites exist for PAF and these have been demonstrated in platelets (Valone *et al.*, 1982; Hwang *et al.*, 1983; Kloprogge and Akkerman, 1984), neutrophils (Valone and Goetzl, 1982), macrophages (Lambrecht and Parnham, 1986) and lung tissue (Hwang *et al.*, 1985*b*). Valone (1984) isolated a PAF binding site from human platelets which appears to be a protein. With the availability of PAF antagonists, Parnham and colleagues have suggested there may actually be two distinct binding sites, the anatomical distribution of these binding sites being selective, since macrophages and platelets have different affinities for the PAF antagonist kadsurenone. On the basis of these observations, PAF receptor subtypes 1 and 2 have been proposed (Lambrecht and Parnham, 1986).

Following binding of PAF to its receptor, there is a subsequent internalisation of the PAF receptor complex (Kloprogge and Akkerman, 1984), which may explain the rapid desensitisation of PAF-induced responses in a variety of tissues (Henson, 1976; Page *et al.*, 1983). Several biochemical changes follows PAF receptor occupation. Phospholipase C activation triggers the degradation of phosphoinositides to inositol triphosphate (IP_3) and diacylglycerol (DAG). Both these substances are known to act as second messengers and initiate a variety of intracellular events (Lapetina, 1982; Shukla and Hanahan, 1983). For example, DAG is capable of activating protein kinase C, leading to the phosphorylation of specific intracellular proteins involved in physiological processes such as secretion or contraction (Ieyasu *et al.*, 1982). IP_3 may release intracellular Ca^{2+} from internal stores (Berridge, 1984) which in turn regulates other intracellular events such as Ca^{2+}-dependent K^+ channels (Garay and Braquet, 1986). In some cells, including human platelets, PAF

activation will inhibit the formation of cyclic AMP by other endogenous agents *eg.* prostaglandins (Haslam and Vanderwel, 1982).

I.3.3 *Cellular Origins of PAF*

PAF was originally described as a product of IgE-sensitised basophils in the rabbit (Benveniste *et al.*, 1972). Whether PAF is a product of human basophils is unclear, since a number of investigations have failed to demonstrate the formation of PAF by human basophilic leukaemic cell lines (Betz *et al.*, 1980; Sanchez-Crespo *et al.*, 1980). There is also species variation in the ability of mast cells to form PAF. Bone marrow-derived mast cells from the mouse (Mencia-Huerta *et al.*, 1983) and dog (Elias *et al.*, 1985) mastocytoma cell lines have been shown to release PAF, but PAF appears not to be released by human lung mast cells in response to either antigenic or non-antigenic stimulation (Lichtenstein *et al.*, 1984).

However, human alveolar macrophages (Arnoux *et al.*, 1983) and eosinophils (Lee *et al.*, 1984) are rich sources of PAF and are capable of releasing large amounts in response to activation by IgE-dependent mechanisms. These cell types are present in the airways of asthmatics and are activated following antigen provocation (Metzger *et al.*, 1987; Beasley *et al.*, 1989). Eosinophils obtained from patients with eosinophilia (including asthmatics) have a much enhanced capacity to generate PAF which seems to reflect an underlying defect in the normally rate-limiting acetyl-transferase enzyme (Lee *et al.*, 1984; Snyder, 1985). Since the eosinophil is probably a central cell in the pathophysiology of asthma (Frigas and Gleich, 1986), this observation has implication for the detection of "pre" asthmatics and ultimately for the understanding and treatment of the disorder.

A number of other cell types have been shown to release PAF, including neutrophils (Jouvin-Marche *et al.*, 1984), platelets (Benveniste *et al.*, 1982) and vascular endothelial cells (Camussi *et al.*, 1983*a*), which may all play a role in the pathophysiology of asthma. Platelets also possess IgE-binding sites and antigen stimulation of platelets leads to PAF release (Khalife *et al.*, 1985). Like eosinophils and alveolar macrophages, platelets have

been observed extravascularly in the lungs of asthmatics (Metzger *et al.*, 1987) and these platelets undergo activation following antigen provocation of sensitised asthmatics. The activation of human lung (Lichtenstein *et al.*, 1984) or skin mast cells (Phillips *et al.*, 1983; Ting *et al.*, 1983) is not inhibited by known anti-asthma drugs such as disodium cromoglycate (DSCG). By contrast, IgE-dependent activation of alveolar macrophages (Joseph *et al.*, 1981) and platelets (Capron *et al.*, 1985) can be inhibited by DSCG.

All cell types capable of releasing PAF can be activated by non-antigenic stimuli specific to that particular cell type but not in response to non-specific cell damage. For example, neutrophils readily release PAF in response to phagocytosis of foreign particles (Jouvin-Marche *et al.*, 1983) and alveolar macrophages synthesise PAF following stimulation with foreign materials such as endotoxin (Beijer and Rylander, 1983).

I.3.4 *PAF and Asthma*

The involvement of PAF in IgE-dependent anaphylaxis in the rabbit was first reported by Henson and Pinckard (1977) who demonstrated that platelets, which are specifically desensitised to PAF after systemic anaphylaxis, play a crucial role for antigen- and PAF-induced bronchopulmonary alterations (Halonen *et al.*, 1985).

A general scheme has been presented that might account for the exacerbation and perpetuation of asthma by mechanisms in which PAF is a central agent (Morley *et al.*, 1984) (Figure I.3.3).

I.3.4.1 *Acute Bronchospasm*

PAF induces acute reversible bronchoconstriction in all species examined *in vivo* except the rat (Halonen *et al.*, 1980; Vargaftig *et al.*, 1980; Denjean *et al.*, 1981; Chung *et al.*, 1986). This includes man where, both following inhalation by normal (Cuss *et al.*, 1986; Rubin *et al.*, 1987; Chung *et al.*, 1989) and asthmatic (Rubin *et al.*, 1987) volunteers and intratracheal administration in individuals with brainstem injury (Gateau *et al.*, 1984),

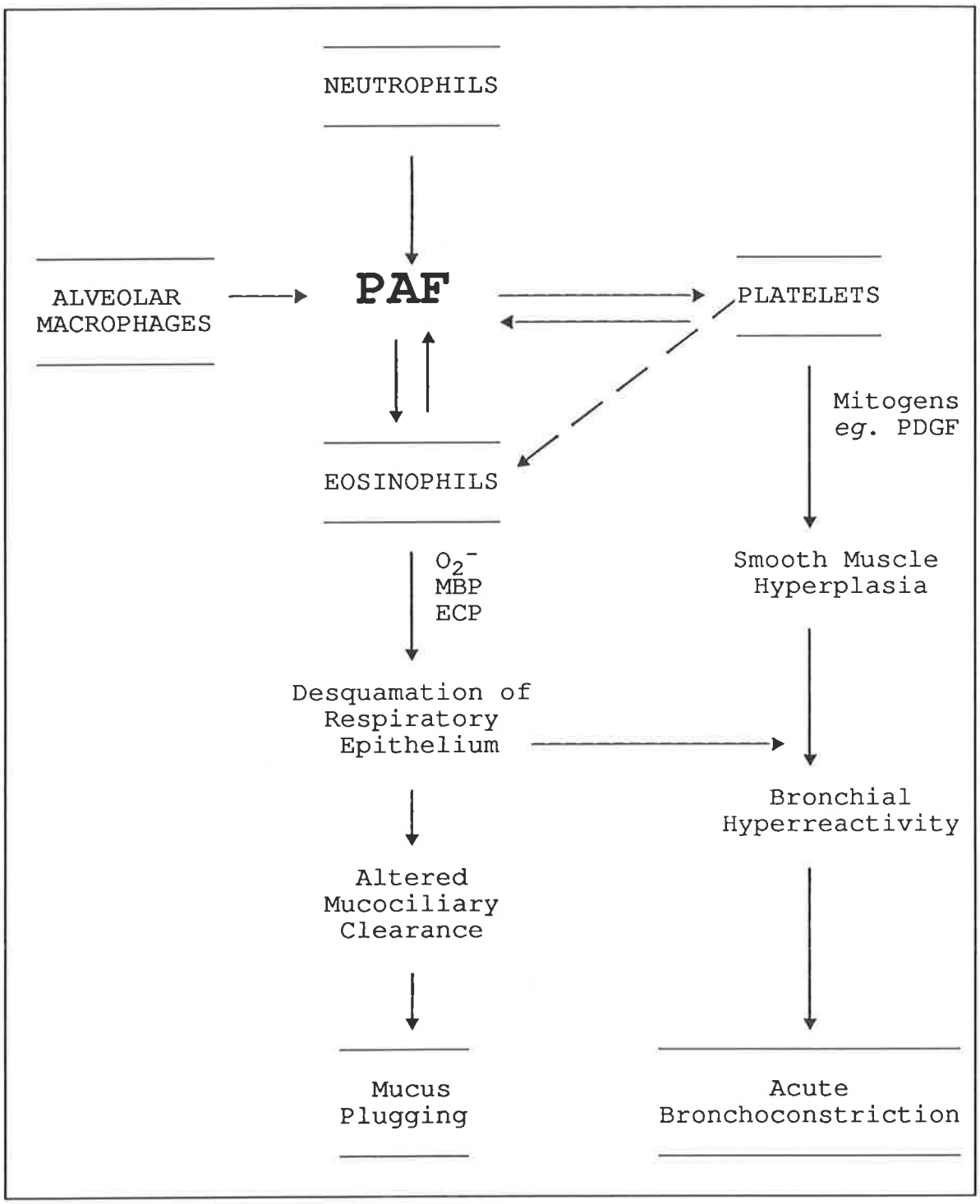


Figure I.3.3 A schematic repesentation of the possible relationship between PAF release from inflammatory cell populations within the lung and the subsequent pathological events in asthma. PDGF, platelet-derived growth factor; MBP, major basic protein; ECP, eosinophil cationic protein.

there is a rapidly reversed bronchoconstriction. PAF is one of the most potent spasmogens yet identified in both experimental animals and man, being approximately 1,000 times more active than histamine, peptidoleukotrienes (Patterson and Harris, 1983) or acetylcholine (Morley *et al.*, 1989*b*).

PAF is unable to induce bronchoconstriction in rats and rat platelets are totally insensitive to PAF and lack PAF binding sites (Vargaftig *et al.*, 1981*a*; Inarrea *et al.*, 1984). In other experimental animals such as the rabbit and the guinea-pig, PAF-induced bronchoconstriction is accompanied by platelet activation within the circulation which is evident as thrombocytopoenia (Halonen *et al.*, 1980; Vargaftig *et al.*, 1981*b*) and subsequent accumulation of platelets within the pulmonary vasculature (Page *et al.*, 1984; Lellouch-Tubiana *et al.*, 1985). The latter has been observed isotopically using ^{111}Indium-labelled platelets (Page *et al.*, 1984) and histologically, where platelets have been observed in close proximity to neutrophils within the pulmonary vasculature (Dewar *et al.*, 1984).

The bronchospasm that follows the intravenous administration of PAF is platelet-dependent, since depletion of platelets by prior infusion of a lytic anti-platelet antiserum abolishes the response (Vargaftig *et al.*, 1980). The intravenous injection of other platelet stimuli (*eg.* ADP) induces bronchoconstriction, hence it has been suggested that the response is of reflex origin since there is evidence that embolised materials induce reflex bronchoconstriction (Mills *et al.*, 1969). However, peak changes in lung function largely (>90%) precede detectable accumulation of isotopically-labelled platelets in the pulmonary vasculature. Thus, intravascular embolisation *per se* cannot be the central determinant of the bronchoconstriction (Page *et al.*, 1984). Furthermore, section of the vagus nerve does not diminish the magnitude of PAF-induced bronchospasm (Vargaftig *et al.*, 1980).

The mechanism of PAF-induced bronchospasm has not been fully elucidated. It appears to depend in part upon generation of arachidonic acid metabolites of platelets and neutrophils, since thromboxane A_2 is a potent spasmogen for airway smooth muscle and the magnitude of the acute constrictor response to PAF is reduced by pretreatment with the thromboxane synthetase inhibitor OKY-046 (Chung *et al.*, 1986). Furthermore, it has been reported that PAF-induced bronchospasm is inhibited by a mixture of histamine (H_1) and

serotonin antagonists when aspirin or salicylate is also present (Vargaftig *et al.*, 1982).

A number of drug categories can effect substantial inhibition of the acute bronchospasm that follows the intravenous injection of PAF in guinea-pigs. These include PAF antagonists, theophylline, ketotifen, FPL 55712 and certain benzodiazepines (Morley *et al.*, 1985*b*; Alabaster and Keir, 1987; Casals-Stenzel, 1987*b*; Takizawa *et al.*, 1988). In contrast, histamine (H_1) or serotonin antagonists, mast cell stabilising drugs, lipoxygenase inhibitors, DSCG, glucocorticoids (Morley and Aoki, 1986) and cyclo-oxygenase inhibitors (Vargaftig *et al.*, 1980; 1982) have proved ineffective.

The release of as yet unidentified spasmogens following the interaction between PAF and specific binding sites is the most likely basis of PAF-induced bronchoconstriction (Chignard *et al.*, 1982; Vargaftig *et al.*, 1982). It is also possible that metabolism of PAF by platelets yields spasmogenic substances.

PAF has not been shown to induce contraction of airway smooth muscle, except when platelets are added to the bathing fluid of tracheal smooth muscle in an isolated preparation and hence are external to the vascular system (Schellenberg *et al.*, 1983). The ability of platelet depletion to fully abrogate the bronchoconstriction due to intravenous PAF argues against the direct effects of PAF upon airway smooth muscle, if modest doses are used. The bronchospasm induced when larger doses of PAF are administered by inhalation is unaffected by platelet depletion (Lefort *et al.*, 1984). Based on studies using parenchymal strips it has been proposed that higher concentrations of PAF may have a direct effect upon smooth muscle (Stimler and O'Flaherty, 1983).

Evidence with anti-neutrophil antiserum suggests that neutrophils may also participate in PAF-induced bronchoconstriction (Saunders *et al.*, 1983) and the close association between these two cell types observed histologically (Dewar *et al.*, 1984) is suggestive of a possible cellular cooperation in bronchoconstriction induced by PAF. In other circumstances platelets and neutrophils interact synergistically to produce increased amounts of PAF (Coeffier *et al.*, 1984) and other biologically active materials (Marcus *et al.*, 1982; 1984). It is possible that platelets and neutrophils interact to produce an as yet unidentified spasmogen.

I.3.4.2 *Increased Vascular Permeability*

Increased vascular permeability is a prominent component of the pulmonary response to PAF and changes in vascular permeability can be inferred from measurement of the extravasation of plasma protein into pulmonary tissues using isotope labelling or vital dye.

PAF is capable of producing oedema in all species examined, including man (Wedmore and Williams, 1981; Humphrey *et al.*, 1982; Morley *et al.*, 1983; Archer *et al.*, 1984*a*; 1984*b*; Basran *et al.*, 1984; Humphrey *et al.*, 1984; Paul *et al.*, 1984; Pirotzky *et al.*, 1984*b*; Archer *et al.*, 1985*a*). PAF is 1,000 times more potent than classical mediators such as histamine in inducing increased vascular permeability in human skin (Archer *et al.*, 1984*b*). Unlike bronchoconstriction, this biological property of PAF is independent of platelet (Morley *et al.*, 1983; Paul *et al.*, 1984; Pirotzky *et al.*, 1984*b*) or neutrophil (Wedmore and Williams, 1981; Pirotzky *et al.*, 1984*b*) activation and is most likely the result of a direct effect of PAF on vascular endothelial cells (Humphrey *et al.*, 1984). Thus, depletion of platelets or neutrophils is without effect on PAF-induced vascular permeability changes in the skin of experimental animals (Wedmore and Williams, 1981; Paul *et al.*, 1984; Pirotzky *et al.*, 1984*b*). In human skin, PAF induces an acute wheal and flare response that can be augmented by concomitant administration of vasodilator prostaglandins (*eg.* PGE_1) and analogues of prostacyclin (PGI_2), known inhibitors of platelet function (Archer *et al.*, 1984*a*). This suggests that in man, PAF-induced vascular permeability is probably also platelet-independent. Oedema formation in response to local administration of PAF is independent of cyclo-oxygenase products of arachidonic acid metabolism or histamine, as it cannot be reduced by indomethacin or the histamine H_1 antagonist mepyramine (Archer *et al.*, 1985*a*). However, prior treatment with glucocorticoids significantly inhibits PAF-induced oedema formation in human skin (Pinckard *et al.*, 1984).

PAF is capable of producing oedema in the pulmonary (Heffner *et al.*, 1983; Hamasaki *et al.*, 1984) and bronchial (Evans *et al.*, 1987) circulation of several animal

species, a process which is essentially platelet-independent (Hamasaki *et al.*, 1984; Evans *et al.*, 1987). This response however, may be amplified by products liberated by activated platelets when higher concentrations of PAF are used (Heffner *et al.*, 1983).

I.3.4.3 *Inflammatory Cell Activation and Recruitment*

A wide range of inflammatory cells can be activated by PAF *in vitro*, including platelets (Vargaftig *et al.*, 1981*b*), neutrophils (O'Flaherty *et al.*, 1981), monocytes (Yasaka *et al.*, 1982), macrophages (Hartung *et al.*, 1983) and eosinophils (MacDonald *et al.*, 1986). In particular, PAF is a potent inducer of neutrophil (O'Flaherty *et al.*, 1981) and eosinophil (Wardlaw *et al.*, 1986) chemotaxis. Additionally, PAF can release a variety of other mediators from these inflammatory cells, such as lipoxygenase products of arachidonic acid from neutrophils (O'Flaherty *et al.*, 1981).

In vivo, PAF-induced cellular activation is reflected by the recruitment of various inflammatory cells into tissues following PAF administration. PAF induces a sustained inflammatory response (lasting up to one month) in rabbit lungs following local administration and is associated with the accumulation of inflammatory cells and epithelial cell damage (Camussi *et al.*, 1983*b*). An eosinophil-rich infiltration into the lungs has been demonstrated following local (Arnoux *et al.*, 1988) and systemic (McManus and Hass, 1986; Lellouch-Tubiana *et al.*, 1988; Takizawa et al., 1988) administration of PAF in experimental animals. In addition to this, a transient but pronounced neutropoenia has been reported following PAF administration in experimental animals (Halonen *et al.*, 1980; Desquand *et al.*, 1986) and in normal (Chung *et al.*, 1989) and asthmatic subjects (Chung and Barnes, 1989), which probably reflects sequestration of these cells into the pulmonary vascular bed.

Four to six hours following the local administration of PAF to the skin of normal human volunteers, an infiltration of inflammatory cells, notably neutrophils, is evident and a mixed cellular infiltrate comprising neutrophils and mononuclear cells at 24 hr (Archer *et al.*, 1985*b*). By contrast, local administration of PAF to the skin of atopic individuals

results in the selective infiltration of eosinophils similar to that observed following the administration of antigen to the skin of the same subjects (Henocq and Vargaftig, 1986).

PAF has been observed to cause an extravascular recruitment of platelets into the lung parenchyma of guinea-pigs, followed by the positioning of these platelets close to bronchial smooth muscle (Lellouch-Tubiana *et al.*, 1985). The platelet-derived smooth muscle mitogen platelet-derived growth factor (PDGF) is known to contribute to vascular smooth muscle hyperplasia in diseases such as atherosclerosis. Since bronchial smooth muscle hyperplasia is a prominent feature of asthma, the observation of Lellouch-Tubiana and colleagues may have significance in the chronic changes associated with this disease. In addition to PDGF, platelets contain transforming growth factor *beta* (TGF-*beta*) (Assoian *et al.*, 1983), which may recruit and activate monocytes during inflammation and vascular injury (Wahl *et al.*, 1987). Platelets have been noted in bronchoalveolar lavage fluid obtained from asthmatic subjects (Metzger *et al.*, 1987) and platelet aggregates have been found adherent to the vascular endothelium of asthmatic lung at biopsy (Beasley *et al.*, 1989).

I.3.4.4 *Respiratory Epithelium*

PAF has been shown to increase mucus output and to alter the physical properties of the mucus produced (Lang *et al.*, 1987). Topical (Camussi *et al.*, 1983*b*) or intravenous (Takizawa *et al.*, 1988) administration of PAF to experimental animals causes the damage and loss of bronchial ciliated epithelial cells which may result in the impairment of mucociliary clearance. It is not known if the changes induced in the respiratory epithelium are a direct cytotoxic effect of PAF or secondary to the release of cytotoxins such as major basic protein (MBP) released by infiltrating eosinophils (Frigas and Gleich, 1986). Epithelial dysfunction is associated with the pathogenesis of asthma and infection of the airways, suggesting that damage to the airway epithelium may play an important role in causing bronchial hyperreactivity, because of a decreased ability of the respiratory epithelium to release epithelium-derived relaxing factor(s) (Vanhoutte, 1987).

I.3.4.5 *Airway Hyperreactivity*

In the guinea-pig, repeated intraperitoneal injection of PAF induces hyperplasia of smooth muscle cells within the abdominal aorta (Handley *et al.*, 1983). More recently, infusion of PAF from an implanted resevoir has been shown to cause generalised enlargement of vascular smooth muscle, especially within the pulmonary vasculature (Ohar *et al.*, 1987). It might be presumed that such effects of PAF are secondary to platelet activation since platelets contain and release PDGF, a stimulus of smooth muscle hyperplasia (Ross *et al.*, 1985). The capacity of PAF to induce morphological changes in smooth muscle prompted consideration of this substance as a mediator of airway hyperreactivity (Morley *et al.*, 1984), the central underlying feature of asthma (Boushey *et al.*, 1980) (Figure I.3.3).

The bronchial hyperreactivity that results from PAF exposure is non-selective, since enhanced responses are evident whether the test spasmogen is histamine, substance P, prostaglandin F_{2alpha} or bombesin, in addition to PAF itself (Morley *et al.*, 1989*b*). The PAF-induced change in airway sensitivity persists for days and hence is long-lasting by comparison with the transient hyperreactivity that follows exposure of the airways to peptidoleukotrienes (Fennessy *et al.*, 1986). In the guinea-pig, hyperreactivity can be detected 24 hr after PAF inhalation and in the rabbit it is evident 7 days after a single intravenous infusion of PAF (Mazzoni *et al.*, 1985*a*). Following a single inhalation of PAF in normal subjects, increased reactivity to methacholine has been shown to persist for 14 days and can require up to 28 days to return to pre-treatment levels (Cuss *et al.*, 1986). The time course and magnitude of PAF-induced bronchial hyperreactivity resembles the reaction to inhalation of small doses of allergen by atopic asthmatic patients (Cockcroft *et al.*, 1977). Interestingly, PAF-induced airway hyperresponsiveness has not been found in asthmatic volunteers (Rubin *et al.*, 1987; Chung and Barnes, 1989).

The mechanism whereby PAF can modify the response of airway smooth muscle to spasmogens remains obscure. Modest changes might be interpreted as a manifestation of reduced cross sectional area of the airways due to oedema or increased mucus secretion

(Moreno *et al.*, 1986). However, in thrombocytopoenic animals exposure to PAF causes no change in airway sensitivity to spasmogens (Morley *et al.*, 1985*a*), even though PAF-induced increased vascular permeability is largely unaffected by such treatment (Morley *et al.*, 1983). In addition, the infusion of histamine, in doses known to induce oedema, does not cause hyperreactivity. Similarly, substance P and carbachol can produce substantial increases in mucus secretion without inducing persistent change in airway reactivity (Morley *et al.*, 1989*b*). The inability of spasmogens such as substance P and histamine to induce hyperreactivity indicates that this phenomenon is not a consequence of sustained bronchospasm. Nor can altered vagal tone be considered the primary determinant of bronchial hyperreactivity, since vagal section does not influence the expression of hyperreactivity following an infusion of PAF in the guinea-pig (Mazzoni *et al.*, 1985*a*).

Selective elimination of platelets by lytic antisera fully suppresses acute airway hyperreactivity in the guinea-pig (Morley *et al.*, 1985a). Neutrophils accumulate in the lung tissue in parallel with platelets in response to PAF (Camussi *et al.*, 1983*b*; McManus, 1987). However, this phenomenon does not appear to contribute to the development of increased hyperreactivity, since other stimuli of neutrophil activation (LTB_4, C_{5a} and C_{3a}) do not induce hyperreactivity. Eosinophil-mediated damage to airways is well documented (Frigas and Gleich, 1986) and might intensify bronchospasm either by reflex mechanisms (Barnes, 1986) or by loss of an endogenous dilator substance (Flavahan *et al.*, 1985). These findings suggest a link between the activation of inflammatory cells and bronchial hyperreactivity. Furthermore, animals rendered hyperreactive to exogenous spasmogens by pretreatment with PAF have a reduced sensitivity to *beta*-adrenergic agonists such as isoprenaline, which cannot be attributed to loss of *beta*-receptor numbers or affinity in lung tissue (Barnes *et al.*, 1987*b*). Such observations are indirect evidence for a non-spasmogenic component contributing to the induction of bronchial hyperreactivity following PAF treatment which may involve oedema formation, cellular infiltration or epithelial cell damage.

Mechanisms whereby platelets may modify the function of airway smooth muscle have not been established. Platelets can rapidly convert PAF to other lipids (Snyder, 1987)

which have yet to be evaluated in airway tissues. The endogenous platelet spasmogen, which is presumed to cause contraction of bronchial smooth muscle in response to intravenous PAF appears unlikely to account for hyperreactivity, since drugs such as indomethacin and the lipoxygenase inhibitor QA 208-199 can diminish acute bronchospasm without affecting the induction of hyperreactivity. Furthermore, DSCG and steroids can inhibit the development of hyperreactivity without influencing the acute bronchospasm that results from PAF exposure (Morley *et al.*, 1985*b*). Changes in airway reactivity induced by PAF may be sustained by the migration of platelets into the airway tissues (Lellouch-Tubiana *et al.*, 1985), as secretion of growth factors such as PDGF could serve to change the volume and hence contractile properties of airway smooth muscle.

Activation of platelets by PAF must differ from activation by other agonists, since infusion of ADP or thrombin in amounts sufficient to cause comparable accumulation *in vivo* does not induce airway hyperreactivity as does PAF (Smith *et al.*, 1989*b*). Since different platelet stimuli elevate internal calcium by distinct processes (Sage and Rink, 1986*a*; 1986*b*) it is possible that the calcium influx that follows PAF exposure includes a unique component.

A factor released from platelets has recently been reported to induce airway hyperreactivity (Sanjar *et al.*, 1989). The intravenous injection of PAF into thrombocytopoenic guinea-pigs neither provoked an increase in airways resistance nor enhanced airway responsiveness to PGF_{2alpha}. However, in platelet-depleted animals, the supernatant from guinea-pig PRP incubated with PAF, induced airway hyperreactivity (Sanjar *et al.*, 1989). The generation of this platelet-derived factor was inhibited by incubation of PRP with the stable prostacyclin- mimetic iloprost prior to activation with PAF. The secretion or formation of this mediator of hyperreactivity appears PAF-specific as neither platelet disruption nor activation of platelets with ADP induced its production. The chemical nature of this material remains as yet unidentified. Ketotifen and prednisolone have been shown to inhibit the airway hyperreactivity induced by PAF-stimulated platelet supernatants, whereas cromoglycate and aminophylline were without effect (Morley *et al.*, 1989*a*). Similarly, when ketotifen or prednisolone were incubated

with PRP prior to the addition of PAF, the injection of supernatants into thrombocytopoenic guinea-pigs resulted in reduced airway hyperreactivity (Morley *et al.*, 1989*a*).

In the guinea-pig, prior treatment with prophylactic anti-asthma drugs (cromoglycate, ketotifen, glucocorticoids and theophylline) (Mazzoni *et al.*, 1985*b*) impairs the development of PAF-induced hyperreactivity with a potency comparable to the existing PAF antagonists (Deeming *et al.*, 1985). Other drug catagories including histamine (H_1) antagonists (mepyramine, oxatomide, clemastine, astemizole, azelastine), SRS-A antagonists (FPL 55712, AA-673), mast cell stabilising drugs (oxatomide, tranilast, azelatine, zaprinast), cyclooxygenase (indomethacin) or lipoxygenase (QA 208-198, AA-861) inhibitors or a *beta*-adrenoreceptor agonist (isoprenaline) did not produce significant inhibition (Morley and Aoki, 1986). Thus, the pharmacological sensitivity of PAF-induced bronchial hyperreactivity in the guinea-pig appears to be predictive of the agents known to be clinically effective as prophylactic anti-asthma drugs. Furthermore, such animal experiments predict that *beta*-agonists should not be prophylactic in asthma, which is supported by clinical evidence that the *beta*-agonist terbutaline does not reduce bronchial hyperreactivity in situations where glucocorticoids are effective (Kraan *et al.*, 1985). The evidence obtained with PAF-induced hyperreactivity suggests that methylxanthines differ from *beta*-agonists in their therapeutic action, even though traditionally both are considered as symptomatic bronchodilators. It now appears from clinical studies (Magnussen *et al.*, 1985; Pauwels *et al.*, 1985) that methylxanthines have additional pharmacological properties similar to the conventional prophylactic agents such as DSCG *ie.* the ability to inhibit late-onset airway obstruction following antigen challenge (Pauwels *et al.*, 1985). Furthermore, lodoxamide or other anti-allergic drugs specifically developed as mast cell-stabilising agents, do not inhibit PAF-induced hyperreactivity in the guinea-pig (Mazzoni *et al.*, 1985*b*), which correlates with their failure as novel therapeutic advances in the treatment of asthma.

I.3.4.6 *PAF Antagonists*

The first PAF antagonist to be described was CV-3988, an analogue of PAF (Terashita *et al.*, 1983), which has led to the development of a range of PAF analogues having antagonistic properties, including ONO-6240, SRI 63-073 and Ro-19-3704 (Braquet and Godfroid, 1986; Braquet *et al.*, 1987). Useful as experimental tools, these types of PAF antagonists have a limited clinical future as they are phospholipids and active only following systemic administration. A number of natural products have been described as having PAF antagonistic properties, including kadsurenone (isolated from the Chinese herb *Piper futokadsura*) (Shen *et al.*, 1985). Based on this original structure, synthetic neolignans which have improved potency and oral activity have been developed (L-652-731 and L-653-150) (Hwang *et al.*, 1985*a*). The ginkgolides (isolated from the leaves of the *Ginkgo biloba* tree) comprise another class of naturally-derived PAF antagonists (Braquet *et al.*, 1985*b*). A diverse range of other chemical entities have since been demonstrated to act as PAF antagonists, including triazolobenzodiazepines (*eg.* alprazolam, triazolam and brotizolam) (Kornecki *et al.*, 1984; Casals-Stenzel, 1987*b*), certain calcium channel blocking agents (*eg.* gallopamil and diltiazem) (Tuffin and Wade, 1985) and thiazole derivatives (RP 48740) (Mondot *et al.*, 1989).

All of the PAF antagonists are, to varying extents, capable of inhibiting the wide array of PAF-induced pathological effects both *in vitro* and *in vivo*. This includes the ability of some of the PAF antagonists to inhibit PAF-induced hyperreactivity in guinea-pigs (Deeming *et al.*, 1985). Several PAF antagonists also inhibit allergic bronchospasm in experimental animals. In particular, BN 52021 (Braquet *et al.*, 1985*a*), kadsurenone (Darius *et al.*, 1986) and WEB 2086 (Casals-Stenzel, 1987*a*) have all been observed to inhibit the bronchoconstriction accompanying active or passive anaphylactic shock in sensitised guinea-pigs. L-652-731 has been shown to inhibit Arthus responses in the skin, suggesting the involvement of PAF in this type of hypersensitivity reaction (Hellewell and Williams, 1986).

A ginkgolide preparation (BN 52063) (containing a mixture of the PAF antagonists BN 52020, BN 52021 and BN 52022) has been studied in normal healthy volunteers. Oral pretreatment of these subjects with BN 52063 selectively inhibited PAF-induced wheal

and flare responses in the skin and PAF-induced platelet aggregation *ex vivo* (Barnes *et al.*, 1987*a*). BN 52063 is without effect on histamine-induced wheal and flare responses or ADP-induced platelet aggregation. These findings provide evidence that BN 52063 is a selective PAF antagonist in man.

PAF is able to mimic the features of asthma more closely than any other mediator so far described. In particular, this naturally occurring phospholipid is able to evoke eosinophil activation and recruitment in asthmatics and to induce a long-lasting, non-specific increase in bronchial hyperreactivity (the diagnostic feature of asthma). It is anticipated that the availability of a selection of PAF antagonists for use in man in the near future will allow a detailed investigation into the precise role of PAF in health and disease and possibly provide a novel approach to the treatment of bronchial asthma.

I.4 AIMS OF THE PRESENT STUDY

1. To determine the role of the inflammatory mediator histamine and the involvement of platelets in the development of the anaphylactoid response to radiographic contrast media in human subjects.

2. To develop a sensitive and reproducible method for the *in vivo* monitoring of isotopically-labelled platelets in both the guinea-pig and the rabbit.

3. To characterise *in vivo* platelet responses in the guinea-pig and the rabbit.

4. To investigate platelet responses to the ether-linked phospholipid platelet activating factor (PAF) in the guinea-pig and the rabbit and to assess the susceptibility of this response to modification by drugs.

5. To investigate the role of platelets and serotonin in the development of the anaphylactoid response to dextransulphate in the rabbit.

CHAPTER II

THE ROLE OF HISTAMINE IN ADVERSE REACTIONS TO RADIOGRAPHIC CONTRAST MEDIA (RCM)

II.1 Introduction

Radiographic contrast media (RCM) are used extensively for image enhancement during diagnostic X-ray procedures. The immunopharmacological action of these drugs is at best unpredictable and may result in idiosyncratic reactions in some patients. The overall incidence of adverse reactions to intravascularly administered contrast media has been reported to range from 5% to 8.5% (Ansell, 1970; Shehadi and Toniolo, 1980).

Histamine, an important mediator of acute anaphylaxis, was proposed by Mann in 1961 to be responsible for anaphylactoid reactions to contrast agents. Many components of contrast reactions mimic the effects of systemic histamine release by other drugs or mechanisms, including vasodilatation and flushing, urticaria or other rashes, rhinitis, facial oedema, bronchospasm, and systemic hypotension.

Early studies however, failed to substantiate the relationship between RCM and histamine release. A consistent elevation of histamine levels in peripheral venous plasma of man following intravenous administration of contrast agents was not demonstrated (Peters *et al.*, 1966; Brasch *et al.*, 1970) and Lasser and colleagues (1970) could find no evidence of any histamine release in plasma following contrast material administration in dogs. These negative findings may have been the result of inherent methodological inadequacies in the histamine assay technique, or blood samples being obtained at a time subsequent to the peak histamine rise.

Experimental confirmation of a relationship between contrast media and histamine release was provided by the incubation of various RCM with harvested rat peritoneal mast cells *in vitro* (Rockoff *et al.*, 1970; Rockoff *et al.*, 1971). Subsequently, the rise and fall of plasma histamine in subjects receiving clinical doses of different contrast media was

demonstrated (Seidel *et al.*, 1974), in the absence however of any correlation between histamine liberation and the occurrence of side-effects. Similarly, Simon and colleagues (1979) reported elevated plasma histamine levels in 40% of patients undergoing intravenous pyelography which did not correlate with immediate generalised reactions, history of allergy, or tests for immediate cutaneous reactivity to RCM. They concluded that other modulating factors must play a significant role in the development of an adverse reaction to these agents.

Substantial increases in plasma histamine levels have been reported in association with profound haemodynamic changes following the intra-arterial injection of RCM in dogs (Rockoff and Brasch, 1971). In man however, a consistent relationship between arterial histamine increase and cardiovascular response was not observed (Rockoff and Aker, 1972).

New, low osmolality contrast media have been examined *in vitro* and have been shown to release less histamine from human basophils than their high osmolality counterparts (Assem *et al.*, 1983; Salem *et al.*, 1986). Furthermore, low osmolality agents probably produce reactions less frequently than do conventional high osmolality agents (Kinnison *et al*, 1989).

The present study focusses on patients undergoing intravenous urography and examines the histamine releasing potential and cardiovascular effects of both high and low osmolality contrast media administration on these parameters during this procedure.

II.2 **Methods**

II.2.1 *Contrast Agents*

Four different contrast media were assessed *ie.* one conventional high osmolality ionic contrast medium, sodium and meglumine diatrizoate (Urovison 58%, Schering), and three newer, low osmolality media, the ionic sodium and meglumine ioxaglate (Hexabrix 320, May & Baker), the more recently introduced non-ionic iopamidol (Iopamiro 370,

Schering), and iohexol (Omnipaque 300, Winthrop). The characteristics of these compounds are outlined in Table II.1.

II.2.2 *Subjects*

This study was carried out with the co-operation of a total of 200 patients, who were all undergoing routine intravenous urography at the Royal Adelaide Hospital. Fifty patients were randomly assigned to receive each of the four contrast media under investigation. Each subject gave informed consent to participate in the study. Prior to contrast administration, details pertaining to age, sex, underlying medical conditions, present drug therapy, and a personal history of asthma, drug and/or food allergies were obtained. In addition, it was determined whether any of the patients had been previously exposed to radiographic contrast media, and if so, if any adverse reactions were experienced. The system of classification of adverse reactions to the contrast agents used in this study was adopted from Robertson *et al.* (1985) (Table II.2).

II.2.3 *Contrast Media Injection*

The contrast medium was injected *via* a 19 or 21 gauge butterfly needle (connected to a 20 cm flexible polythene tube) inserted into an antecubital vein by the attending radiologist and its rate of administration standardised to 100 ml/min. The average volumes (dose) of contrast medium injected are listed in Table II.1. These volumes were selected in an attempt to standardise the dose of iodine administered to each subject.

II.2.4 *Blood Sampling*

Five ml blood samples were obtained prior to and at 1, 2, 5, 10 and 20 min following contrast medium injection from a separate antecubital vein. Blood was drawn into sterile plastic syringes (Terumo) and then transferred into heparinised polypropylene tubes

	Urovison 58%	**Hexabrix** 320	**Iopamiro** 370	**Omnipaque** 300
Composition/ml RCM	0.4g sodium diatrizoate 0.18g meglumine diatrizoate	0.2g sodium ioxaglate 0.39g meglumine ioxaglate	0.755g iopamidol	0.647g iohexol
Iodine concentration (mg/ml)	325	320	370	300
Average dose injected (ml)	100	100	90	100
Iodine content (g/dose)	32.5	32	33.3	30
Osmolality at 37^{o}C (mOsm/kg H_2O)	1650	600	870	690

Table II.1 Characteristics of the intravenous radiograghic contrast media.

Reaction Grade	Description
0	Nil.
I	Feeling of warmth, flushing and/or nausea. No treatment required.
II	Vomiting or dry retching, lightheadedness, slight dyspnoea, rash over part or whole body and/or mild urticaria. No treatment required.
III	Urticaria, facial oedema, bronchospasm, larangeal oedema and/or transient hypotension. Treatment and complete recovery whilst in the radiology suite.
IV	Prolonged hypotension, circulatory collapse, chest pain, angina, myocardial infarction, ventricular fibrillation, convulsions, coma or paralysis. Hospitalisation required.
V	Death

Table II.2 Classification of adverse reactions to RCM.

(containing 125 I.U. heparin/10 ml) which were placed immediately on ice. As soon as possible (normally within 2 hours), the samples were returned to the laboratory where the plasma was separated by centrifugation (1000 *g*, 5 min, followed by 3000 *g*, 10 min). This gradual rise in centrifugation speed causes minimal basophil degranulation (Frewin *et al.*, 1984). Centrifugation was carried out at 0-4^{O}C to avoid temperature related histamine release (Lorenz *et al.*, 1972). Only the upper portion of the plasma layer was removed to avoid basophil contamination. Plasma samples were stored at -20^{O}C until assayed.

II.2.5 *Blood Pressure and Heart Rate Measurements*

Blood pressure and heart rate were determined in 179 of the patients studied, prior to contrast medium administration and at 3 and 20 min following the injection. These measurements were obtained with the use of a Copal Digital Sphygmomanometer (UA-271) attached to the arm not being used for blood sampling. Blood pressure was analysed in terms of both diastolic pressure (DP) and mean arterial pressure (MAP). MAP was calculated according to the following equation:

$$MAP = diastolic\ pressure + 1/3\ pulse\ pressure.$$

II.2.6 *Histamine Assay*

Plasma histamine levels were determined using a single isotopic enzymatic assay for histamine as described by Shaff and Beaven (1979) with modifications by Head and Jonsson (personal communications).

The assay involves incubating the plasma sample with an isotopic methyl donor, S-adenosyl-L-methionine (^{3}H-SAMe), and the enzyme histamine-N-methyltransferase (HNMT) to convert histamine to ^{3}H-labelled N-methylhistamine. This is then separated from the remaining ^{3}H-SAMe by solvent extraction into chloroform/ether (1:1), rather than chloroform alone, which was used in the original method. This permits separation of the organic and aqueous phases by the less tedious snap-freezing technique, rather than by

aspiration. The aqueous phase is air-dried and assessed for radioactivity.

This technique is highly specific, sensitive and reproducible to a concentration of 0.05 *ng*/ml (Frewin *et al.*, 1984; 1986). A full description of the assay and modifications appears in Appendix I.

II.2.7 *Data Analysis*

A two-way analysis of variance was performed on the plasma histamine data. Subsequently a Tukey's HSD test for the multiple comparison of means was applied. To assess the statistical significance of the cardiovascular data, Student's *t*-tests for paired observations were employed. A p value of < 0.05 was accepted as the lowest level for the expression of significance.

II.3 **Results**

II.3.1 *Reactions*

Of the 200 patients studied, 104 (52%) experienced some reaction (albeit mild and transient) following the intravenous administration of the radiographic contrast agents. The distribution of reactions for each contrast medium is shown in Table II.3. Urovison produced the most adverse reactions, with 80% of the patients receiving this high osmolality agent experiencing some associated discomfort. Reactions of a more severe nature (*ie.* Grades II and III) were experienced only by patients who received either of the ionic agents *ie.* four with Urovison and one with Hexabrix.

Information regarding the relationship between adverse reactions to RCM and a patient history of allergy and/or asthma is also shown in Table II.3. Of all the patients studied, 41 (20.5%) reported a positive history of allergy and 13 (6.5%) suffered from asthma. All patients who received either Urovison or Omnipaque, and who were known to suffer allergic reactions, experienced adverse effects. Allergy-prone subjects who received

Contrast Medium	Grade of Reaction	Allergy	Asthma	Allergy and Asthma	No Allergy or Asthma	Totals
Urovison	0	0	1	0	9	10
	I	8	3	0	25	36
	II	2	2	0	0	4
Total		10 (100%)	6 (83%)	0	34 (74%)	50 (80%)
Hexabrix	0	7	1	1	22	30
	I	5	2	1	12	19
	III	0	0	0	1	1
Total		12 (42%)	3 (67%)	2 (50%)	35 (37%)	50 (40%)
Iopamiro	0	7	0	0	25	32
	I	2	0	0	16	18
Total		9 (22%)	0	0	41 (39%)	50 (36%)
Omnipaque	0	0	1	0	23	24
	1	10	3	0	13	26
Total		10 (100%)	4 (75%)	0	36 (36%)	50 (52%)
Totals		41	13	2	146	200

Table II.3 Relationship between grade of reaction and history of allergy/asthma in the patients.

The figures in parentheses represent the percentage of patients in each of the catagories who experienced a reaction to the contrast medium.

Hexabrix or Iopamiro did not differ markedly from the non-allergic subjects in their incidence of adverse reactions to the RCM. There were no subjects with a history of asthma in the Iopamiro group and the numbers of asthmatics in the other groups are too small to draw any conclusions. The percentage incidence of reactions relative to previous exposure and previous adverse reactions to RCM is shown in Table II.4. Urovison figured prominently in causing reactions, irrespective of whether the patients had been previously exposed or not.

II.3.2 *Plasma Histamine Data*

The mean baseline histamine concentration for all patients studied was 0.32 ± 0.02 ng/ml (mean $\pm$ sem; n=200), which is in agreement with previous findings (Robertson *et al.*, 1985). The changes in histamine levels following the injection of the four different RCM are presented in Table II.5 and graphically in Figure II.1. Mean plasma histamine levels rose in each case at the one min post-injection mark, with Urovison causing the greatest increase and Iopamiro the least. The plasma histamine elevation was short-lived in each case, with Hexabrix causing the most sustained levels. The changes in plasma histamine were not statistically significant ($p > 0.05$). There were also no statistically significant differences found between the maximum change in plasma histamine levels (H_{max}) following injection of the various agents (regardless of sampling time) ($p > 0.05$). These data are shown in Table II.6.

No significant differences were found between the baseline histamine levels of reported allergy/asthma sufferers and non-allergy/asthma sufferers ($p > 0.05$). There were also no observable differences between H_{max} of allergy/asthma and non-allergy/asthma sufferers following the administration of any of the RCM evaluated ($p > 0.05$).

The H_{max} of patients who had previously been exposed to RCM (undefined) did not differ significantly from the H_{max} determined for those patients who received the agent for the first time ($p > 0.05$).

No age or sex differences were found in the H_{max} in any of the groups studied ($p >$

	Urovison	Hexabrix	Iopamiro	Omnipaque	Mean
No previous exposure	81 (n=37)	31 (n=32)	27 (n=33)	43 (n=35)	46 (n=137)
One previous exposure only	70 (n=10)	53 (n=17)	53 (n=17)	67 (n=9)	61 (n=53)
More than one previous exposure	100 (n=3)	100 (n=1)	-	83 (n=6)	94 (n=10)
Previous adverse reaction	60 (n=5)	64 (n=11)	46 (n=11)	75 (n=12)	62 (n=39)

Table II.4 Percentage incidence of reactions to RCM relative to previous exposure and previous adverse reactions to these agents.

	Urovison (n=50)	**Hexabrix** (n=50)	**Iopamiro** (n=50)	**Omnipaque** (n=50)
Pre-RCM	0.39 ± 0.05	0.29 ± 0.04	0.32 ± 0.03	0.29 ± 0.03
1'	0.54 ± 0.10	0.42 ± 0.05	0.35 ± 0.04	0.37 ± 0.05
2'	0.41 ± 0.07	0.41 ± 0.07	0.37 ± 0.03	0.27 ± 0.03
5'	0.39 ± 0.06	0.39 ± 0.06	0.29 ± 0.03	0.33 ± 0.03
10'	0.36 ± 0.04	0.29 ± 0.03	0.30 ± 0.03	0.25 ± 0.03
20'	0.30 ± 0.03	0.33 ± 0.03	0.33 ± 0.05	0.34 ± 0.04

Table II.5 Plasma histamine levels (*n*g/ml) prior to and 1, 2, 5, 10 and 20 min following intravenous injection of RCM (mean ± sem).

	Urovison (n=50)	**Hexabrix** (n=50)	**Iopamiro** (n=50)	**Omnipaque** (n=50)
H_{max}	0.37 ± 0.09	0.42 ± 0.07	0.27 ± 0.04	0.33 ± 0.05

Table II.6 Maximum change in plasma histamine levels (H_{max}) (*n*g/ml) following RCM injection (mean ± sem).

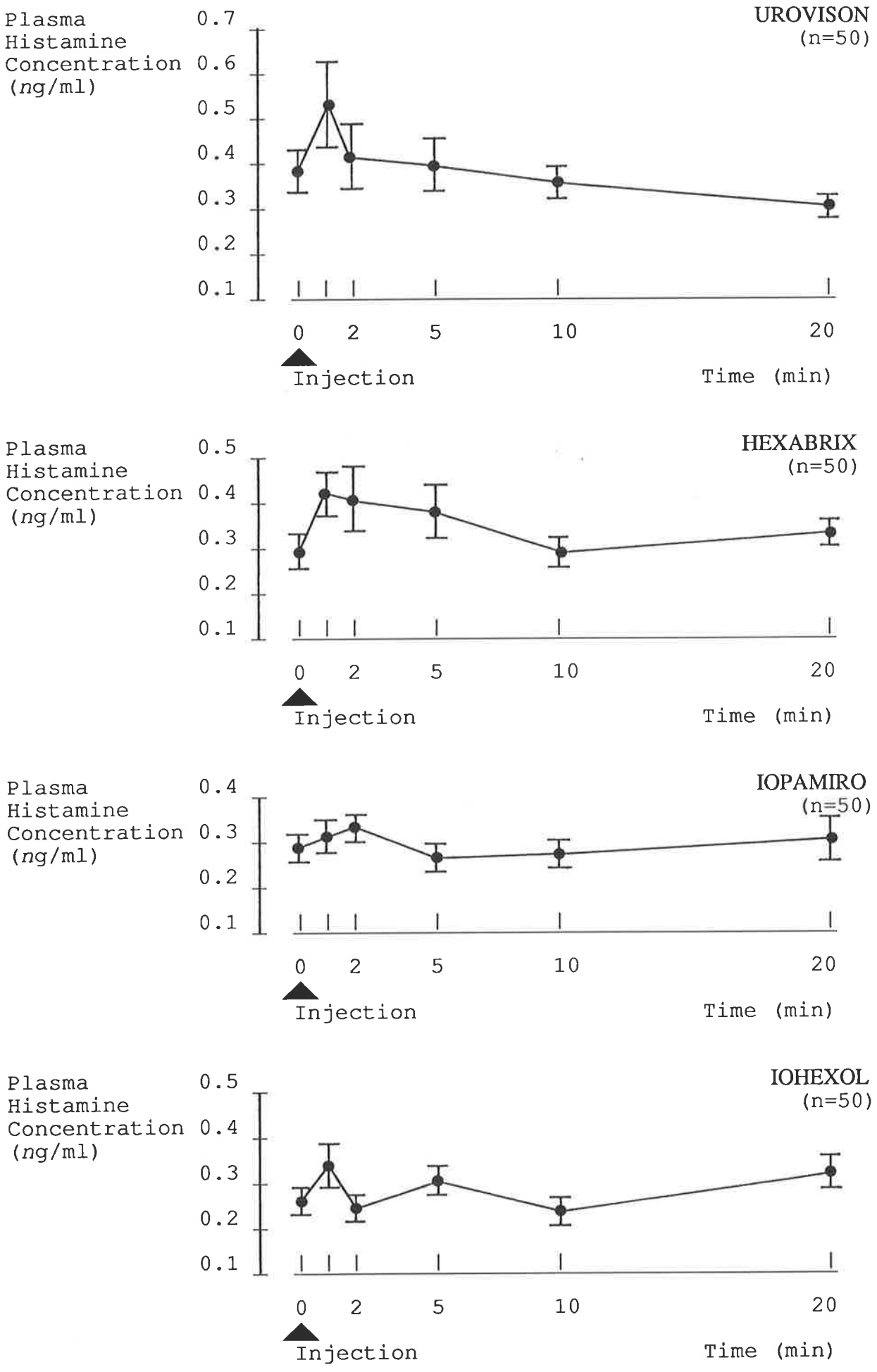


Figure II.1 Plasma histamine concentrations (mean ± sem) prior to and 1, 2, 5, 10 and 20 min following the injection of the four different radiographic contrast media.

0.05).

II.3.3 *Correlation Between Plasma Histamine and Adverse Reactions*

The magnitude of the change in plasma histamine levels did not correlate with the grades of reactions experienced by patients receiving any of the different radiographic contrast media. The only exception to this was one patient who, after receiving Hexabrix, experienced a reaction of grade III severity with a corresponding increase of 2.54 ng histamine/ml. It was the case however, that the greatest overall change in plasma histamine levels and the greatest number of adverse reactions were observed when Urovison was the diagnostic agent. The pre-injection histamine levels were similar in the patients who experienced Grade I, II or III reactions, and were not significantly different from those seen in the patients who had no reactions at all ($p > 0.05$).

II.3.4 *Cardiovascular Data*

The data obtained for both blood pressure (MAP) and heart rate (HR) are shown in Table II.7 and Figure II.2. No significant changes in blood pressure (MAP or DP) were evident following the injection of any of the different media ($p > 0.05$). No significant differences existed between the data from the four agents at any of the time points ($p > 0.05$). However, significant increases in heart rate occurred with the three compounds Urovison, Hexabrix and Iopamiro ($p < 0.05$). By the 20th min following the injection of Omnipaque, a statistically significant decrease in heart rate had occurred compared with the pre-injection value ($p < 0.05$).

II.4 Discussion

All procedures utilizing the injection of radiographic contrast media carry a definite risk of an adverse reaction. While most reactions are minor, life-threatening and fatal

		Urovison (n=41)	Hexabrix (n=45)	Iopamiro (n=46)	Omnipaque (n=47)
MAP	Pre	97.0 ± 3.1	94.8 ± 2.8	96.4 ± 2.6	94.1 ± 2.3
(mmHg)	3'	96.8 ± 2.7	96.8 ± 2.8	96.1 ± 2.5	91.2 ± 2.5
	20'	93.1 ± 2.4	95.6 ± 2.9	96.8 ± 2.6	91.0 ± 2.3
DP	Pre	72.5 ± 2.5	72.9 ± 2.6	75.6 ± 2.1	73.3 ± 2.0
(mmHg)	3'	71.0 ± 2.8	74.2 ± 2.6	75.5 ± 2.4	69.7 ± 2.4
	20'	71.9 ± 2.4	75.6 ± 2.7	78.0 ± 2.2	70.4 ± 2.1
HR	Pre	74.0 ± 2.3	74.6 ± 2.2	70.1 ± 2.2	77.3 ± 2.4
(beats	3'	82.8 ± 2.2[a]	83.0 ± 3.7[a]	74.4 ± 2.5[b]	76.4 ± 2.2
/min)	20'	72.1 ± 2.6	74.2 ± 2.7	67.4 ± 2.3	69.9 ± 2.1[a]
n		41	45	46	47

a: $p < 0.005$ }
b: $p < 0.05$ } comparison with pre-injection values

Table II.7 Blood pressure (mean arterial pressure (MAP) and diastolic pressure (DP) (mmHg)) and heart rate (HR) (beats/min) measurements prior to, and 3 and 20 min following RCM injection (mean ± sem).

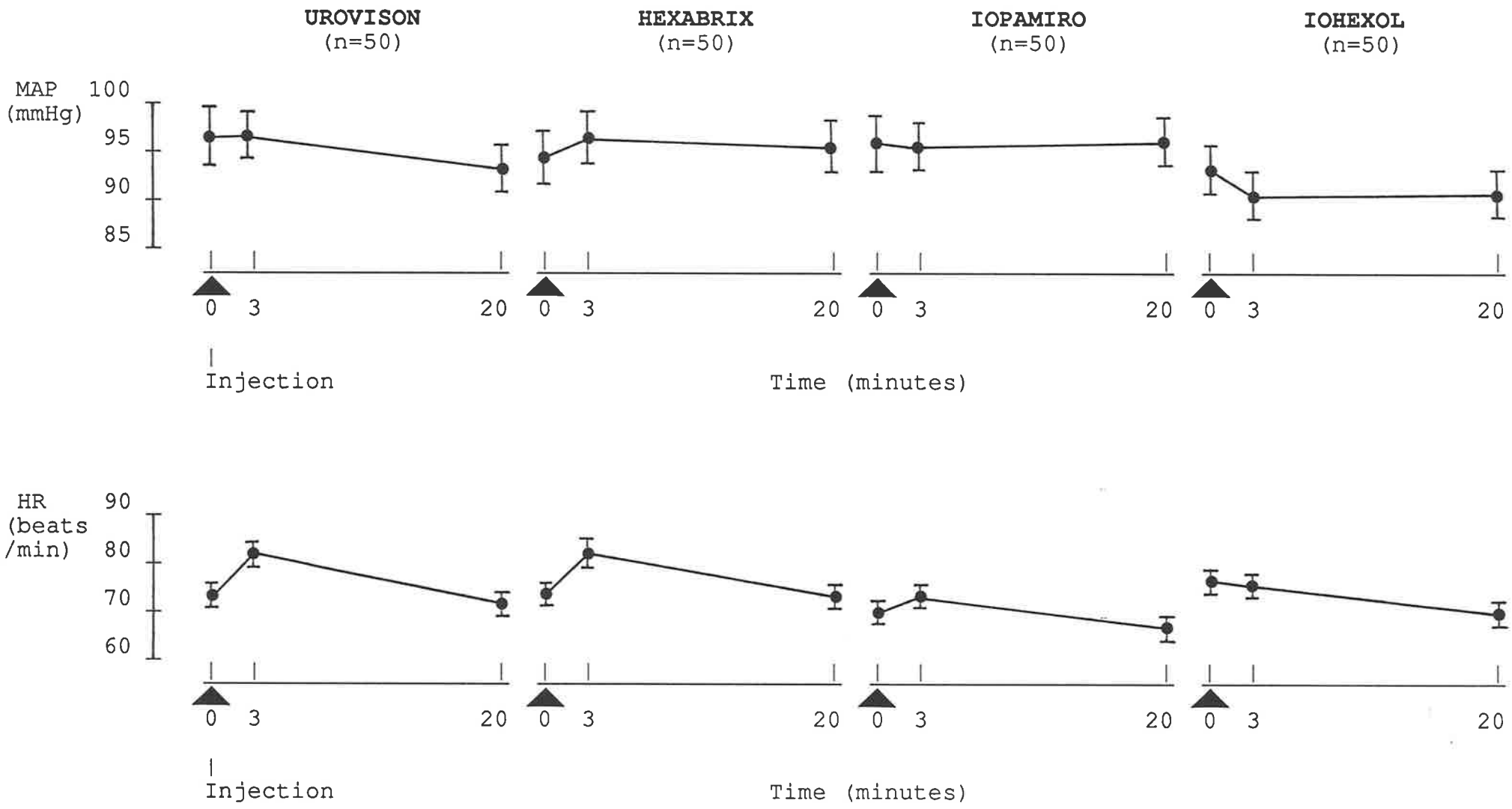


Figure II.2 Mean arterial blood pressure (MAP) and heart rate (HR) prior to and 3 and 20 min following the injection of the four radiographic contrast media (mean ± sem).

reactions may occur without warning. No fatal reactions occurred in the present study of 200 patients undergoing intravenous urography. However, 49.5% experienced mild side-effects, such as local pain, heat, flushing and nausea, and reactions of a more severe nature were manifest in 3% of all patients evaluated.

Current drug development in the field of radiographic contrast media has been aimed at the production of new agents that reduce toxicity and unpleasant side-effects without sacrificing diagnostic quality. In this study, 80% of patients injected with the conventional high osmolality contrast medium Urovison experienced an adverse reaction. A significantly lower proportion of patients experienced adverse effects to the newer low osmolality agents Hexabrix, Iopamiro and Omnipaque (40%, 36% and 52%, respectively).

The diversity of reactions and their apparent idiosyncratic nature have led to the formulation of many different proposals regarding pathogenic mechanisms. Anaphylactoid-type reactions to contrast media appear to parallel the effects of systemic histamine release (Mann, 1961). The failure of previous studies to establish a significant correlation between elevated plasma histamine levels and adverse reactions following the administration of contrast media (Seidel *et al.*, 1974; Simon *et al.*, 1979) may have been the result of insensitive assay tecniques used to measure the histamine concentrations. The radioenzymatic method utilised in this study is sensitive to a level of 0.05 ng/ml histamine. It was noted that plasma histamine concentrations rose within the 20 minutes following RCM injection in 80% of all patients studied. This finding is similar to a previous report (Robertson *et al.*, 1985). Again, the trend was the return to pre-injection plasma histamine levels within 10 minutes of the rise.

The majority of adverse reactions were observed to commence during or immediately following RCM injection and all occurred within the first 10 minutes. If histamine is to be implicated in such reactions, the primary event would have to be the interaction of RCM with blood basophils, resulting in the liberation of stored autacoid. This increased systemic histamine may induce a variety of symptoms including a feeling of warmth, flushing, nausea, vomiting, circulatory collapse and cardiac anomalies. Circulating histamine is rapidly metabolised, accounting for the short duration of effects.

Some circulating histamine, together with small quantities of contrast agent may infiltrate into the tissue compartment, leading to the stimulation of mast cell histamine release. Tissue release of histamine may cause warmth, flushing, itching, urticaria and the more serious effects of bronchospasm and laryngeal oedema.

In this study of 200 patients receiving one of four different contrast media, no significant correlation could be demonstrated between the magnitude of the change in plasma histamine following RCM administration and the manifestation of a particular adverse reaction. Individual patient data did, however, reveal great variability *eg.* some patients reported no side-effects in the presence of increased plasma histamine levels following RCM and some patients experienced a variety of minor reactions in the absence of any concomitant histamine release. There was one exception. The only reaction classified as grade III severity was associated with a comparatively large increase in plasma histamine level over the monitored period (2.54 ng histamine/ml; *ie.* an increase of over 20 times the resting value). This result, together with previous findings (Robertson *et al.*, 1985) may indicate that histamine is of greater significance clinically in the development of more severe reactions to RCM. However, in two patients who received Urovison and experienced a grade I reaction only, plasma histamine increases of this magnitude were also detected.

In accordance with previous findings (Robertson *et al.*, 1985), the present study casts doubt on the hypothesis that a threshold level of plasma histamine exists (*ie.* 0.8-1.0 ng/ml) (Ind *et al.*, 1983), above which certain haemodynamic disturbances may occur *ie.* increased heart rate and skin temperature and decreased diastolic blood pressure. The maximum plasma histamine levels achieved following any of the contrast agents associated with Grade 0, I and II reactions were 0.57 ng/ml, 0.67 ng/ml and 0.68 ng/ml respectively. However, the maximum histamine level detected in the plasma of the one patient who experienced a reaction of Grade III severity was 2.66 ng/ml.

It is difficult to determine a clear relationship in this study between adverse reactions to RCM and plasma histamine levels because of both patient variation and the absence of more serious reactions. The variability encountered in patient responses may implicate the

existence of differential histamine or tissue sensitivity between individuals. As pre-RCM plasma histamine concentrations of patients who experienced no side-effects were not significantly different from those of patients who did experience adverse effects, it appears that the circulating basal levels of histamine are not a predisposing factor in the production of adverse reactions to RCM.

Many factors influence the risk of a reaction to RCM. It has been reported that a history of allergy or atopic disease can significantly increase the risk of a reaction (Ansell *et al.*, 1980; Greenberger *et al.*, 1980). In the present study, all patients with a history of allergy who received either Urovison or Omnipaque experienced reactions of a grade I or II severity. This is a greater incidence than that encountered in the "non-allergic" group (74% and 36% for Urovison and Omnipaque respectively). This predisposition (*ie.* related to a history of allergy) was not observed when the other two agents were used; that is, there was no marked difference in the incidence of adverse reactions observed between allergy sufferers and patients without a history of allergy. Pre-injection histamine measurements were statistically similar in both groups, as was the extent of histamine liberation following contrast injection. It is also claimed that patients with asthma have an increased incidence of adverse reactions to RCM (Shehadi, 1975). The number of asthmatics studied was small and it is therefore difficult to comment on associated risk factors in this group. Plasma histamine data did not appear different to that found in other groups.

It is generally accepted that patients who have experienced a previous reaction to RCM are at increased risk (by comparison with the general population) of having another reaction during a repeat study (Witten *et al.*, 1973). With the exception of Urovison, where patient numbers are small, the percentage incidence of reactions to RCM is greater in the groups of subjects who reported a positive history of adverse reaction to previous administration of RCM. These observations may be complicated by the accuracy of the patient's recollection of the event (when hospital records were unavailable). An even greater percentage of patients experienced side-effects to RCM if they had been previously exposed to these agents on more than one occasion (Table II.4). It may be that these

previous exposures had induced a hypersensitivity phenomenon. However, the number of cases in this group remains too small for appropriate statistical evaluation of the data to be undertaken.

Ansell *et al.*, (1980) found that the dose of iodinated RCM is an important risk factor. It is for this reason that in the present study the average dose was adjusted for each of the four agents so as to standardise the quantity of iodine injected (Table II.1). In one previous report, the dose of RCM administered was relative to body weight, state of hydration and blood urea nitrogen levels (Brasch *et al.*, 1970).

The rate of dye injection may also influence the risk. Intravenous urography performed by rapid single bolus injection (less than 2 min) is accompanied by a lower incidence of reactions than when performed by slower infusion (Shehadi, 1975). In the present study, the rate of contrast injection was standardised to 100 ml/min, a factor not considered in many previous reports.

Haemodynamic changes have been shown to occur following the intravenous administration of contrast material (Fischer, 1968; Fischer and Thomson, 1978). These changes include transient systemic hypotension, an increase in pulmonary artery pressure, tachycardia and peripheral vasodilatation. Iohexol has been shown to have significantly less effect on these parameters than diatrizoate after intravenous injection (Higgins *et al.*, 1982). In the present study, no significant changes in blood pressure were evident with any of the different media used. This may be due to the transient nature of the hypotensive response (Lindgren, 1970; Chahine and Raizner, 1976) which was not detected in an adequate number of patients at the predetermined time of measurement. The time chosen was 3 min post-contrast administration, since it was the earliest time available to physically take the measurements without interrupting the rigorous early sequence of the X-ray schedule. By this 3 min mark it may have been possible that the reported lowered peripheral resistance induced by a generalised histamine release with concomitant peripheral capillary dilatation had already been compensated for. Statistically significant increases in heart rate were found at 3 min post-injection with Urovison, Hexabrix and Iopamiro. Twenty min following the injection of Omnipaque, a statistically significant

decrease in heart rate was evident. Heart rate often increases in response to intravenous contrast media, in part because of the baroreceptor reflex to decreased blood pressure (Lawton *et al.*, 1982; Spataro *et al.*, 1987). Decreases in heart rate have also been reported, even in the presence of decreased blood pressure, with both high (Andrews, 1976; Stanley and Pfister, 1976) and low (iohexol) (Poulsen *et al.*, 1987) osmolality contrast agents. It has been suggested that these reactions are vaso-vagal in origin (Andrews, 1976; Stanley and Pfister, 1976) or caused by the inhibition of acetylcholinesterase (Stanley and Pfister, 1976), supported by the finding that the administration of atropine reverses the reaction.

One disadvantage of including hospital inpatients in a study of this nature is their concurrent drug therapy. The combination of drugs employed to combat a wide range of illnesses was quite substantial. Thus, the probability of a particular patient being administered drugs which affect histamine release is considerable. Despite the foregoing, there did not appear to be a relationship (positive or negative) between drugs known to affect histamine release (*eg.* opiates, histamine antagonists, $beta_2$ agonists, and other agents such as theophylline and nifedipine) and adverse reactions to RCM. The picture may be clouded by interactions between the several drugs taken by some individuals.

This study has therefore confirmed that clinical advantages exist in using the low osmolality contrast media, Hexabrix, Iopamiro and Omnipaque, in intravenous urography. These agents would appear to be most beneficial in patients with known risk factors. From the results of the present study, histamine liberation cannot be implicated as the sole cause of the production of adverse reactions to radiographic contrast media.

CHAPTER III

THE EFFECT OF RADIOGRAPHIC CONTRAST MEDIA (RCM) ON PLATELET FUNCTION *EX VIVO* AND *IN VITRO*

III.1 **Introduction**

Radiographic contrast media (RCM) have been reported to initiate a range of biological and biochemical effects on a variety of physiological systems (Fareed *et al.*, 1984*b*), including blood coagulation, the fibrinolytic system, platelet function and the vascular endothelium (Stormorken, 1988). The combined effects on platelets and the coagulation cascade are held responsible for adverse haemostatic effects observed in angiographic procedures (McAfee, 1957; Bernstein and Gans, 1966).

Serotonin (5-HT), a potent mediator of immediate hypersensitivity in rodents (Saxón, 1981) is stored in platelet dense granules and released during platelet aggregation. If platelets are activated while in the pulmonary circulation, the released 5-HT may cause bronchoconstriction (Baldini and Ebbe, 1974) and affect vascular permeability. In addition to serotonin release, platelets can synthesize the metabolites of arachidonic acid upon stimulation *ie.* thromboxane and various prostaglandins and hydroxy-acids, which are thought to play an important role in the pathogenesis of anaphylactic and other allergic reactions in the body (Goetzl, 1980; Yen and Morris 1981).

Studies investigating the effects of contrast media on platelet function have yielded conflicting results. Both *in vitro* and *ex vivo* RCM have been shown to inhibit platelet aggregation induced by various agonists (Zir *et al.*, 1974; Shapiro *et al*, 1977; Gafter *et al.*, 1979; Belleville *et al.*, 1982; Parvez *et al.*, 1983; Paajanen *et al.*, 1984; Parvez *et al.*, 1984; Rao *et al.*, 1985; Stormorken *et al.*, 1986; Parvez and Patel, 1988). Paradoxically, RCM are reported to stimulate the release of serotonin from platelets (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Ring and Sovak, 1981) and whole blood (Parvez *et al.*, 1982; Fareed *et al.*, 1984*b*) *in vitro.*

To investigate this problem further, the effects of a high osmolality, ionic contrast medium (Urovison) and a low osmolality, non-ionic agent (Iopamiro) on platelet aggregation and thromboxane A_2 production both *ex vivo* and *in vitro* in humans were examined.

III.2 Methods

III.2.1 Human *Ex Vivo* Study

III.2.1.1 *Subjects*

Twenty patients undergoing routine intravenous urography at the Royal Adelaide hospital took part in this study. Informed consent was obtained from all subjects and relevant information pertaining to current drug therapy, previous exposure and/or reaction to RCM and history of allergy or atopic disease was obtained. Patients receiving drugs known to affect platelet function were excluded from the study.

III.2.1.2 *Protocol*

Patients involved in the study were randomly assigned to receive one of the two contrast agents under evaluation. Ten patients received Urovison 58% (Schering), a conventional high osmolality ionic contrast medium (sodium and meglumine diatrizoate) and another 10 patients received the more recently introduced Iopamiro 370 (Schering), which is low osmolality and non-ionic (iopamidol). The dose administered to each patient was standardised with respect to iodine concentration *ie.* 100 ml Urovison 58% (325 mgI/ml) and 90 ml Iopamiro 370 (370 mgI/ml). Contrast media were injected into a forearm antecubital vein by the attending radiologist *via* a 19- or 21-gauge butterfly needle (connected to a 20 cm flexible polythene tube) and the rate of adminstration was standardised to 100 ml/min.

Blood samples from a different antecubital vein were collected from all subjects prior to and at 10 min following the administration of the RCM, for platelet function studies and complete blood examination (CBE) analysis.

III.2.1.3 *Platelet Aggregometry*

Blood (30 ml) was collected from a forearm antecubital vein using a 19- gauge siliconised butterfly needle (connected to a flexible polythene tube) and drawn into polypropylene syringes. The tourniquet was immediately released to minimise forearm ischemia and additional prostaglandin formation. The blood was dispensed into polypropylene tubes containing 0.1 M tri-sodium citrate (10% v/v), mixed gently by inversion and centrifuged as soon as possible.

To obtain platelet rich plasma (PRP) blood was centrifuged at 200 *g* for 10 min at room temperature. The PRP supernatant was removed and the platelet count determined using a Coulter counter (model S plus). For each patient the platelet count in the PRP obtained before RCM administration was standardised to that obtained in the PRP 10 min after the RCM administration. This was achieved by appropriate dilutions with autologous platelet free plasma (PFP) obtained by the centrifugation of platelet poor plasma (PPP) (1000 *g*, 5 min). The resultant test plasma (PRP') was transferred to an airfree polypropylene syringe and incubated in a water bath at 37^{O}C to maintain a constant pH (Watts *et al.*, 1983; Watts *et al.*, 1985). PRP' was incubated at 37^{O}C for at least 20 min before aggregation tests were begun.

Platelet aggregation was measured at 37^{O}C as a change in light transmittance in a dual channel platelet aggregometer (Payton, Ontario, Canada), based on the methodology described by Born (1962). Each channel was calibrated with PRP' (0% light transmittance) and PFP (100% light transmittance).

Aliquots (270 *u*l) of PRP' were placed in siliconised aggregation cuvettes containing siliconised metal stir bars and transferred to the aggregometer where the platelet suspension was magnetically stirred at 500 r.p.m. PRP' was incubated at 37^{O}C in the

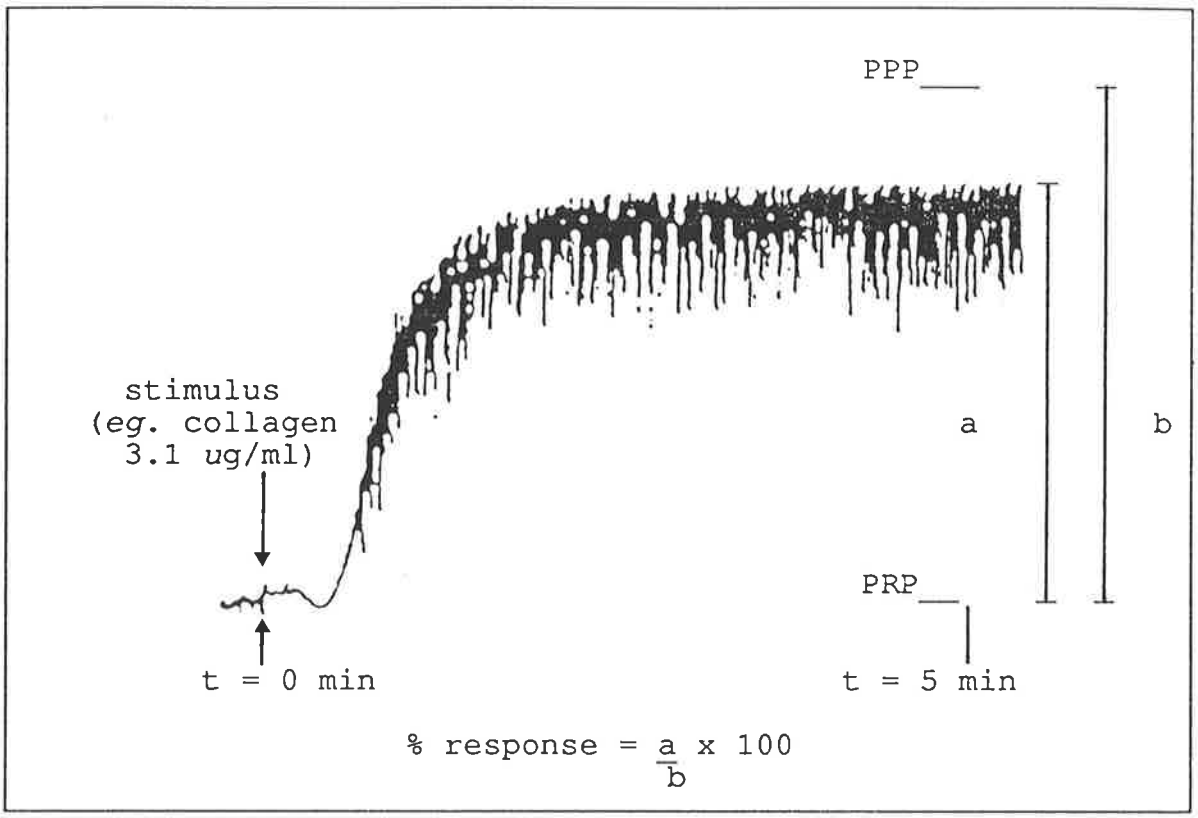


Figure III.1 Calculation of percentage maximal platelet aggregation.

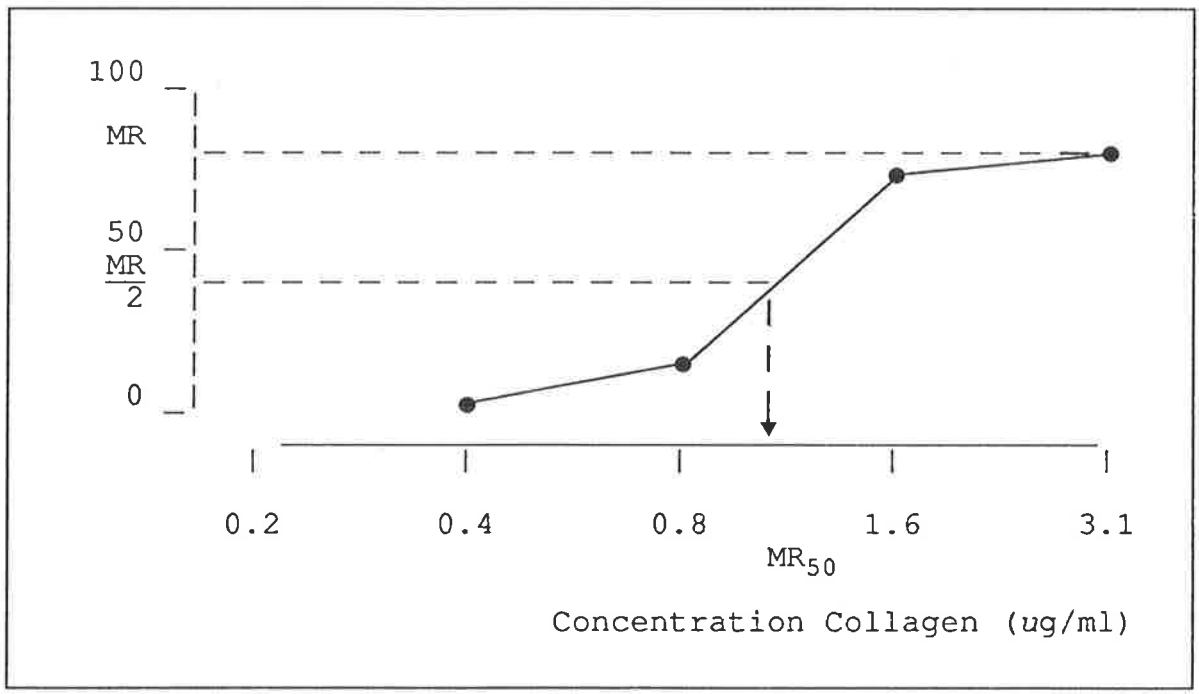


Figure III.2 Calculation of MR_{50} value (concentration of aggregating agent *eg.* collagen) required to elicit 50% maximal response.

aggregometer for one min prior to the addition of the aggregating agent (30 *u*l). Changes in light transmittance were measured for 5 min and the extent of platelet aggregation was calculated as the percentage change in light transmittance between PRP' and PFP (Figure III.1).

Dose-response curves were constructed for platelet aggregation in response to a range of concentrations of ADP (1.25 $x10^{-7}$M - 8.0 $x10^{-6}$M), collagen (0.2 *u*g/ml - 6.3 *u*g/ml), adrenaline (1.0 $x10^{-6}$M - 1.0 $x10^{-5}$M) and arachidonic acid (0.10 mM - 1.65 mM). The concentrations are expressed as the final concentration in the aggregation cuvette. The concentration of aggregating agonist was plotted on a logarithmic scale against the percent aggregatory response for each specific stimulus and a MR_{50} value determined by visual inspection of the graph. MR_{50} is defined as the concentration of platelet stimulus required to elicit 50% of maximum aggregation (Figure III.2).

III.2.1.4 *Reagents*

Trisodium citrate (Ajax) was dissolved in distilled water, the pH was adjusted to 7.35 and the resultant 0.1 M solution stored at $4^{O}C$. Modified Tyrode's buffer, containing no calcium or magnesium, was prepared by dissolving NaCl (0.8 g), KCl (20 mg), $NaHCO_3$ (0.1 g) and $NaH_2PO_4.2H_2O$ (6.5 mg) in 100 ml distilled water. After titration of the pH to 7.35 the solution was stored at $4^{O}C$. Collagen (insoluble type I bovine Achilles tendon) (Sigma) was dissolved in acetic acid as described by Cazenave *et al.* (1973) and the resultant stock solution stored at $4^{O}C$ until diluted in normal saline (0.9% w/v) upon demand. Adenosine diphosphate (ADP) disodium salt (Sigma) was dissolved in distilled water to a concentration of 1.0 $x10^{-2}$ M and then further diluted to 1.0 $x10^{-3}$ M in modified Tyrode's buffer. 0.5 ml aliquots were stored at $-70^{O}C$ and appropriately diluted on the day of use in modified Tyrode's buffer. Adrenaline injection BP (David Bull Laboratories) 1 in 1,000, was stored at $5^{O}C$ and diluted in modified Tyrode's buffer to 1.0 $x10^{-5}$ M as required. Arachidonic acid (lyophilised sodium arachidonate) (Biodata) was reconstituted with distilled water and stored at $4^{O}C$ after flushing with nitrogen. All glassware was

siliconised using a 5% water soluble silicone solution (Prosil-28, PCR Research Chemicals Inc., Fla., USA).

III.2.1.5 *Complete Blood Examination*

An additional 5 ml of venous blood was collected from each patient into dipotassium EDTA for complete blood examination. Analyses were performed in the Haematology Laboratories of the Institute of Medical and Veterinary Science, Adelaide, using a verified automated method.

III.2.1.6 *Thromboxane B_2 Measurements*

Thromboxane A_2 (TXA_2) production was measured as its stable metabolite (hydrolysis product) thromboxane B_2 (TXB_2) by radioimmunoassay (Fitzpatrick, 1982). The lower limit of sensitivity of the assay was 0.2 *n*g/ml. Tritiated TXB_2 was supplied by Amersham, UK, unlabelled TXB_2 by Caymen chemicals, Denver, USA, and antibody to TXB_2 by Seragen, Boston, USA.

The generation of TXB_2 was measured in platelet-rich plasma in response to a critical concentration of collagen (*ie.* lowest concentration of collagen required to elicit > 50% maximal aggregation concentration), in the presence and absence of contrast agent. Aggregation was recorded for 5 min, after which time the reaction was quenched by the addition of 540 *u*l cold ethanol. All samples were then stored at -70^{O}C until required for assay. Samples were thawed, centrifuged and the precipitate removed. The supernatant was evaporated to dryness at 37^{O}C under nitrogen and the residue resuspended in 1.56 mM sodium bicarbonate. TXB_2 was then measured by radioimmunoassay.

Controls and collected samples were diluted to within the range of the standard curve with gelatin-Tris buffer (pH 7.6) and incubated overnight at 4^{O}C with TXB_2 antisera and radiolabelled thromboxane B_2 (^{3}H-TXB_2). Dextran coated charcoal was then added to sequestrate ^{3}H-TXB_2 which was not antibody-bound. Following centrifugation (1600 *g*, 30

min at $4^{O}C$) supernatants were transferred into scintillation vials to which scintillation fluid was added. Radioactivity within the supernatant (containing ^{3}H-TXB_2 which remained bound to the antibody) was assessed with a Beckman scintillation counter.

All standards and samples were assayed in duplicate. To determine the percentage of ^{3}H-TXB_2 bound, duplicate counts were averaged, blank counts subtracted and the resultant counts divided by the zero control.

The TXB_2 standard *pg*/100 *ul* incubation mixture was plotted against the percentage of ^{3}H-TXB_2 bound (using a four parameter logistic curve fitting programme on a Canon BXI computer). From the percentage bound, the amount of TXB_2 in each sample was calculated from the standard curve, corrected for sample dilution and platelet count, and expressed as the amount of TXB_2 (*pg*) *per* 10^5 platelets. A complete description of the thromboxane B_2 assay can be found in Appendix II.

III.2.2 Human *In Vitro* Study

III.2.2.1 *Subjects*

Blood was obtained from 6 healthy volunteers (as previously described) on 2 separate occasions, following (i) at least 10 days of abstinence from any medication known to affect platelet function and (ii) ingestion of 600 mg soluble aspirin (Aspro-Clear, Nicholas, Australia) 12 hours previously. Studies were performed in duplicate *ie.* blood was taken from each subject on 2 separate days, and at the same time each day, to account for possible diurnal variation (Tofler *et al.*, 1987; Brezinski *et al.*, 1988).

III.2.2.2 *Platelet Aggregometry*

Platelet rich plasma (PRP') was prepared as outlined in the previous section. PRP' was incubated (5 min, $37^{O}C$) with saline, Urovison 58% (0.501 mgI/ml - 32.4 mgI/ml) or Iopamiro 370 (4.07 mgI/ml - 37.0 mgI/ml) prior to the addition of one of the aggregating

stimuli. Aggregation was tested in response to 1, 2 and 4 times the critical concentrations of ADP or collagen (*ie.* lowest concentration required to elicit greater than 50% maximal platelet aggregation). Concentrations represent final concentrations in the aggregation cuvette. Dose-response curves were constructed and IC_{50} values determined for both Urovison and Iopamiro (IC_{50} = the concentration of RCM required to inhibit platelet aggregation by 50%).

III.2.3 *Data Analysis*

Student's *t*-tests for paired observations were performed on MR_{50} values, TXB_2 measurements, platelet counts and IC_{50} values obtained in the presence and absence of RCM. In all cases a p value of < 0.05 was accepted as the lowest level for the expression of statistical significance.

III.3 **Results**

III.3.1 Human *Ex Vivo* Study

III.3.1.1 *Platelet Aggregation*

Urovison caused a statistically significant inhibition of platelet aggregation in response to collagen (Table III.1 and Figure III.3 (a)) ($p < 0.05$). There was no significant difference in platelet aggregation induced by ADP, adrenaline or arachidonate following Urovison administration ($p > 0.05$) (Table III.1 and Figure III.3 (b)-(d)). When Iopamiro was the contrast agent under investigation, platelet aggregation in response to any of the four platelet agonists was not significantly altered ($p > 0.05$) (Table III.2 and Figure III.3 (a) -(d)).

III.3.1.2 *Thromboxane B_2*

Platelet Agonist	n	MR_{50} values (mean ± sem) Pre-Urovison	Post-Urovison	p
Collagen (ug/ml)	10	1.09 ± 0.23	1.28 ± 0.22	0.0449
ADP ($x10^{-6}$ M)	10	0.88 ± 0.07	1.03 ± 0.10	0.1346
Adren. ($x10^{-5}$ M)	9	0.89 ± 0.29	0.87 ± 0.29	0.8330
Arachidonate (mM)	6	0.46 ± 0.07	0.39 ± 0.08	0.2427

Table III.1 MR_{50} values obtained for collagen, ADP, adrenaline and arachidonic acid prior to and 10 min following the injection of Urovison in patients (mean ± sem). MR_{50} represents the concentration of agonist required to elicit 50% maximum platelet aggregation.

Platelet Agonist	n	MR_{50} values (mean ± sem) Pre-Iopamiro	Post-Iopamiro	p
Collagen (ug/ml)	10	1.04 ± 0.11	0.99 ± 0.17	0.6625
ADP ($x10^{-6}$ M)	10	1.33 ± 0.19	1.50 ± 0.31	0.3972
Adren. ($x10^{-5}$ M)	8	1.53 ± 0.33	1.44 ± 0.31	0.6746
Arachidonate (mM)	8	0.68 ± 0.12	0.61 ± 0.12	0.1753

Table III.2 MR_{50} values obtained for collagen, ADP, adrenaline and arachidonic acid prior to and 10 min following the injection of Iopamiro in patients (mean ± sem). MR_{50} represents the concentration of agonist required to elicit 50% maximum platelet aggregation.

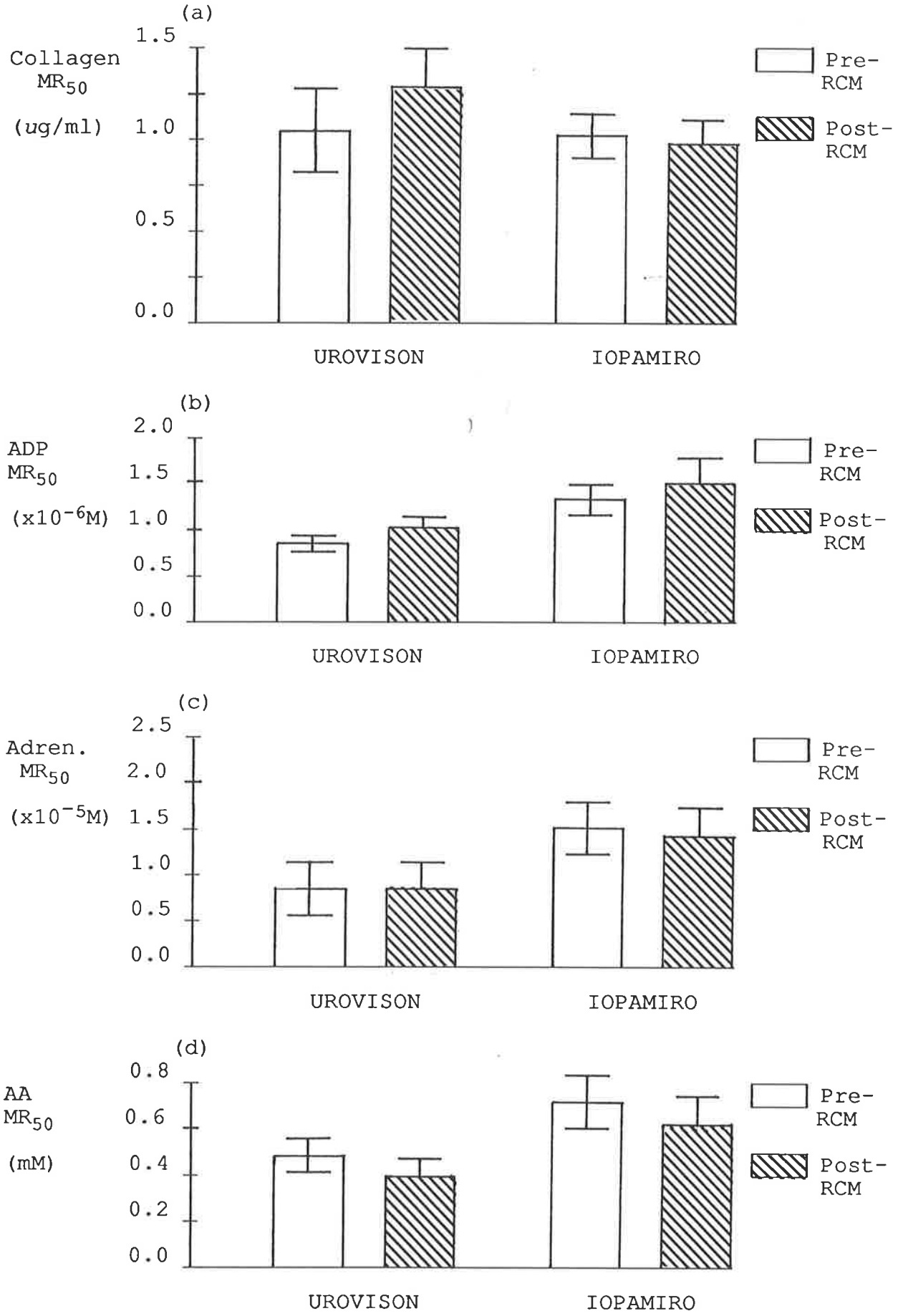


Figure III.3 MR_{50} values (mean ± sem) obtained for (a) collagen, (b) ADP, (c) adrenaline and (d) arachidonic acid (AA) obtained prior to and 10 min following the injection of Urovison and Iopamiro in patients (n=10).

TXB_2 generation in response to a critical concentration of collagen (*ie.* lowest concentration of collagen required to elicit > 50% maximal aggregation concentration) prior to the administration of either Urovison or Iopamiro in patients was not significantly different to that following RCM ($p < 0.05$) (Table III.3).

III.3.1.3 *Platelet Counts*

A statistically significant reduction in the whole blood platelet count was evident 10 min after both Urovison and Iopamiro administration ($p < 0.05$) (Table III.4).

III.3.2 Human *In Vitro* study

III.3.2.1 *Platelet Aggregation*

Both contrast media caused a dose-dependent inhibition of platelet aggregation in response to 1, 2 and 4 times the critical concentration (*ie.* lowest concentration required to elicit > 50% maximal aggregation concentration) of the 2 aggregating agents, collagen (Figure III.4 and Table III.5) and ADP (Figure III.5 and Table III.5). The effects of Urovison were elicited at significantly lower concentrations than for Iopamiro ($p < 0.05$) (Table III.5).

The critical concentration of both collagen and ADP was increased following the ingestion of 600 mg aspirin *in vivo* (*ie.* a higher concentration of collagen and ADP was required to elicit > 50% maximal platelet aggregation). The inhibition of aggregation of aspirinised platelets in response to 1, 2 and 4 times the critical concentration of collagen (Figure III.4 and Table III.7) and ADP (Figure III.5 and Table III.8) by Urovison was dose-related. A dose-relationship for Iopamiro was not established as the concentration required to inhibit aggregation induced by a critical concentration of collagen (Table III.7) or ADP (Table III.8) exceeded the maximal concentration utilised in this study. However, the effects of Urovison were elicited at lower concentrations than for Iopamiro (Table III.7

	TXB_2 ($pg/10^5$ platelets)		
	Pre-RCM (mean ± sem)	Post-RCM (mean ± sem)	*p*
Urovison (n=10)	666.7 ± 121.4	888.5 ± 182.4	0.2056
Iopamiro (n=7)	978.3 ± 177.9	983.0 ± 208.6	0.9228

Table III.3 TXB_2 ($pg/10^5$ platelets) production in response to a critical concentration of collagen (*ie.* concentration of collagen required to elicit 50% maximum platelet aggregation) prior to and 10 min following the administration of Urovison and Iopamiro in patients (mean ± sem).

	Whole Blood Platelet Counts (x $10^3/ul$)		
	Pre-RCM (mean ± sem)	Post-RCM (mean ± sem)	*p*
Urovison (n=10)	285.8 ± 27.65	270.0 ± 27.48	0.0004
Iopamiro (n=10)	246.0 ± 24.21	226.1 ± 22.39	0.0011

Table III.4 Platelet counts in whole blood (x $10^3/ul$) prior to and 10 min following the administration of Urovison and Iopamiro in patients (mean ± sem).

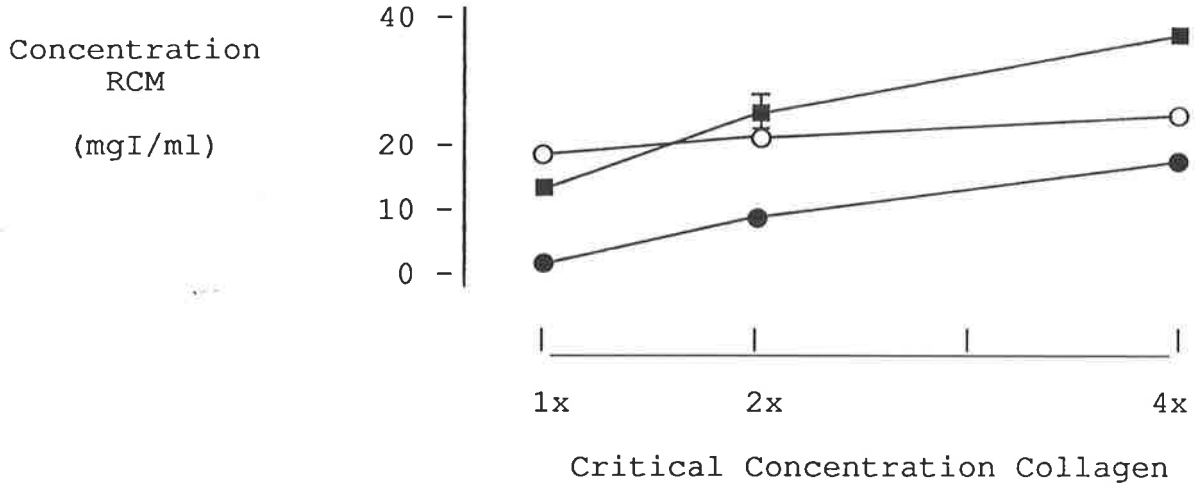


Figure III.4 IC_{50} values (mgI/ml) determined for collagen (concentration of RCM required to inhibit the maximum platelet aggregation induced by collagen by 50%) for both Urovison (●) and Iopamiro (■) (mean ± sem). Critical concentration = lowest concentration of collagen required to induce > 50% platelet aggregation. IC_{50} values in aspirinised platelets for Urovison is also shown (○) (n=5-6).

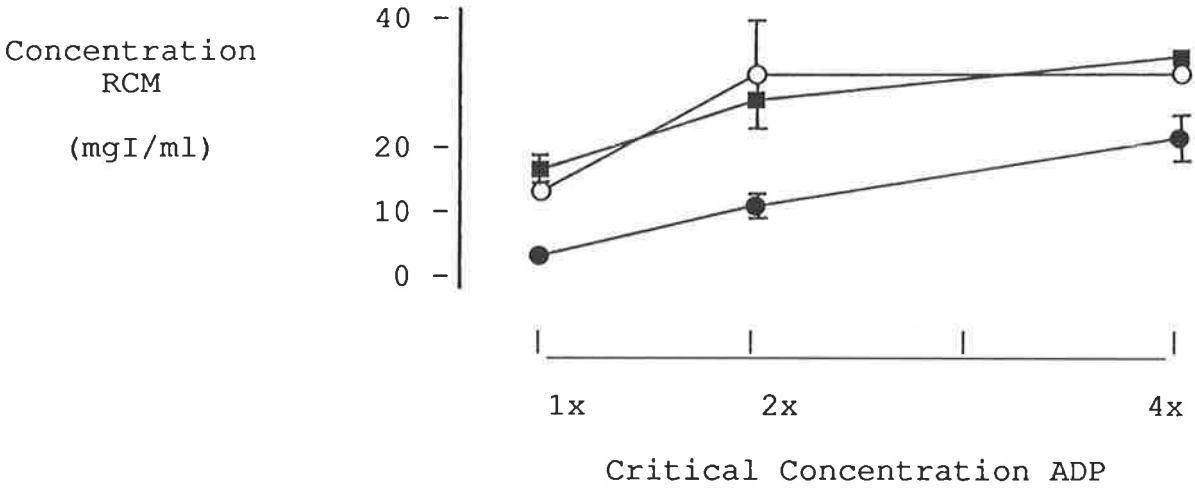


Figure III.5 IC_{50} values (mgI/ml) determined for ADP (concentration of RCM required to inhibit the maximum aggregation induced by ADP by 50%) for both Urovison (●) and Iopamiro (■) (mean ± sem). Critical concentration = lowest concentration of ADP required to elicit > 50% platelet aggregation. IC_{50} values in aspirinised platelets for Urovison are also shown (○) (n=6).

Collagen	Urovison	n	Iopamiro	n	p
1x critical conc.	2.44 ± 0.44	5	13.88 ± 1.43	5	0.0047
2x critical conc.	8.97 ± 1.59	6	26.04 ± 2.92	4	0.0020
4x critical conc.	17.53 ± 1.59	6	> 37	5	

Table III.5 IC_{50} values (mgI/ml) determined for collagen (concentration of RCM required to inhibit the maximum platelet aggregation induced by collagen by 50%) for both Urovison and Iopamiro (mean ± sem). Critical concentration = lowest concentration of collagen required to induce > 50% platelet aggregation.

ADP	Urovison	n	Iopamiro	n	p
1x critical conc.	4.57 ± 0.81	6	16.38 ± 2.49	6	0.0011
2x crtitical conc.	11.63 ± 2.06	6	28.60 ± 4.56	6	0.0069
4x critical conc.	21.19 ± 3.23	6	35.78 ± 1.53	6	0.0022

Table III.6 IC_{50} values (mgI/ml) determined for ADP (concentration of RCM required to inhibit the maximum aggregation induced by ADP by 50%) for both Urovison and Iopamiro (mean ± sem). Critical concentration = lowest concentration of ADP required to elicit > 50% platelet aggregation.

Collagen	Urovison	n	Iopamiro	n
1x critical concentration	17.84 ± 0.89	6	> 37	6
2x critical concentration	21.37 ± 0.71	6	> 37	6
4x critical concentration	25.38 ± 1.22	6	> 37	6

Table III.7 IC_{50} values (mgI/ml) determined for collagen in aspirinised platelets for both Urovison and Iopamiro (mean ± sem). IC_{50} = concentration of RCM required to inhibit the maximum platelet aggregation induced by collagen by 50%. Critical concentration = lowest concentration of collagen required to induce > 50% platelet aggregation.

ADP	Urovison	n	Iopamiro	n
1x critical concentration	13.01 ± 1.80	6	> 37	6
2x crtitical concentration	31.28 ± 8.66	6	> 37	6
4x critical concentration	> 32.5	4	> 37	6

Table III.8 IC_{50} values (mgI/ml) determined for ADP in aspirinised platelets for both Urovison and Iopamiro (mean ± sem). IC_{50} = concentration of RCM required to inhibit the maximum platelet aggregation induced by collagen by 50%. Critical concentration = lowest concentration of collagen required to induce > 50% platelet aggregation.

and Table III.8).

Therefore, trends observed in aspirinised platelets parrallel those demonstrated in normal platelets *in vitro*.

III.4 Discussion

Accumulating evidence suggests that platelet activation is a feature of clinical disorders in which there is substantial activation of the allergic response (*eg*. asthma and urticaria), although such diseases are not normally associated with thrombosis (Storck *et al*., 1955; Knauer *et al*., 1981; Gresele *et al*., 1982; Traietti *et al*., 1984; Wasserman and Ginsberg, 1984; Page *et al*., 1985; Johnson *et al*., 1986; Szczecklik *et al*., 1986; Taytard *et al*., 1986; Gresele *et al*., 1987; Martin *et al*., 1987; Taytard *et al*., 1987). In certain clinical situations where excessive platelet activation in the circulation is recognised, abnormal platelet activation is often observed *in vitro*. In particular, the second phase of platelet aggregation *in vitro* is often unresponsive to known platelet stimuli (Rao and Walsh, 1983). A number of studies have reported that platelets from asthmatics behave abnormally *in vitro*, lacking the second wave of aggregation (Fishel and Zwemer, 1970; Solinger *et al*., 1973; Maccia *et al*., 1977; Thompson *et al*., 1984), suggestive of overstimulation *in vivo* (Page *et al*., 1985; Gresele *et al*., 1987).

It is possible this is the phenomenon manifest in the *ex vivo* study *ie*. inhibition of *in vitro* platelet aggregation by contrast media due to overstimulation of platelets *in vivo* (in patients). A few cases of acute, severe thrombocytopenia have been reported in patients following the intravenous injection of diatrizoate (Wein *et al*., 1982; Shojania, 1985; Lacy *et al*., 1986; Chang *et al*., 1989). The mechanism for this phenomenon is essentially unknown, but the possibilities include immune-mediation, *in vivo* aggregation resulting in platelet consumption or direct chemical destruction. In the present study, mild thrombocytopoenia was evident following administration of both diatrizoate and iopamidol. This may be the result of haemodilution or platelet lysis. On the other hand, the reduced platelet count following contrast administration may indicate a degree of *in vivo*

platelet aggregation. Stormorken and colleagues (1986) reported a similar reduction of platelet counts in venous plasma following cerebral angiography, but did not suggest the finding to represent *in vivo* platelet activation.

The results of the present study indicate that radiographic contrast media inhibit platelet aggregation when studied *in vitro*. Differences in the platelet inhibitory capacity of the two representative contrast media tested were evident. Urovison (ionic, high osmolality RCM) was approximately 4 times more potent than Iopamiro (non-ionic, low osmolality RCM) in its ability to inhibit platelet aggregation induced by a critical concentration of collagen and ADP by 50%.

This finding conflicts with other reports that various high and low osmolality contrast agents activated platelets and induced dense granule secretion *in vitro*, as evidenced by the release of ^{3}H-serotonin (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Ring and Sovak, 1981). However, the results of the present study are in accordance with those of Rao and colleagues (1985), who explain the discrepancy in terms of methodological differences *ie.* experiments were performed using washed platelets, therefore, platelets removed from their plasma enviroment and suspended in a buffer devoid of plasma proteins. Since contrast media are known to bind avidly to plasma proteins (Lasser and Lang, 1970), the latter's presence or absence would considerably affect the results. This criticism is substantiated by the finding that the addition of plasma proteins to platelet suspensions inhibited contrast media-induced platelet activation (Ring *et al.*, 1978*a*). The findings of the present study reinforce those of other groups (Zir *et al.*, 1974; Shapiro *et al.*, 1977; Gafter *et al.*, 1979; Belleville *et al.*, 1982; Parvez *et al.*, 1984) indicating that to varying degrees, contrast agents inhibit rather than activate platelets when incubated *in vitro*.

In the present study, approximate *in vivo* plasma concentrations of RCM achieved after intravenous administration in man were: (i) Urovison: 100 ml of 325 mg iodine/ml in 5 L (estimated volume of distribution) = 32500 mgI/5 L = 6.5 mgI/ml and (ii) Iopamiro: 90 ml of 370 mg iodine/ml in 5 L (estimated volume of distribution) = 33300 mgI/5 L = 6.66 mgI/ml. Comparable concentrations of Urovison were achieved *in vivo* to those required to

cause an inhibitory effect *in vitro*. However, while platelet aggregation in response to collagen and ADP was reduced by Urovison in the *ex vivo* study, only the effect on the collagen-induced response reached statistical significance. From the results of the *in vitro* study, concentrations of Iopamiro attained following i.v. administration in patients were not sufficient to cause significant inhibition, which could explain the lack of effect of this agent on platelet aggregation in the *ex vivo* study.

The mechanism(s) by which radiographic contrast media inhibit platelet aggregation *in vitro* remain to be elucidated. The hypertonicity of these agents may be a factor as osmolality has been reported to have a profound effect on platelet function. Once again, previous studies yield conflicting results. Incubation of platelets in solutions (*eg*. glucose, mannitol and saline) equiosmolar with RCM did not reveal platelet inhibition (Ring *et al.*, 1978*a*; Belleville *et al.*, 1982; Parvez *et al.*, 1984). In other studies however, hypertonicity has been shown to inhibit ADP- but not arachidonate-induced aggregation (Paajanen *et al.*, 1984) and sucrose has been shown to be more inhibitory than sodium chloride at similar osmolality (Stormorken *et al.*, 1986). Dawson *et al.*, (1986) reported that high osmolality contrast agents caused greater inhibition of platelet aggregation than low osmolality RCM. However, the ranking of the magnitudes of effects did not strictly parallel their osmolalities.

Several components of contrast media have been shown to bind Ca^{2+} (Morris *et al.*, 1982). The myocardial depression induced by contrast media injected into the coronary circulation has been attributed, at least in part, to the Ca^{2+}-lowering effect of these agents (Fischer and Thomson, 1978). As calcium is essential for platelet aggregation and secretion during stimulation of platelet rich plasma (Ardlie, 1982), chelation of Ca^{2+} may possibly account for inhibition of platelet function by contrast agents. However, the addition of exogenous Ca^{2+} to platelets did not consistently ameliorate the inhibitory effect of the contrast agents (Rao *et al.*, 1985). The addition of Ca^{2+} to platelets incubated with the ionic contrast medium iothalamate had a beneficial effect on the primary wave of ADP-induced aggregation, without significant effect on the impaired ^{14}C-serotonin release (Rao *et al.*, 1985). This inference is supported by findings of other groups (Parvez *et al.*,

1984; Belleville *et al.*, 1982). Furthermore, the non-ionic, low osmolar iopamidol, does not decrease ionic calcium *in vitro* (Morris *et al.*, 1982). Therefore, it appears from that Ca^{2+} chelation is not the major mechanism of platelet inhibition by radiographic contrast media.

Contrast agents impair platelet aggregation and activation without causing rupture of the cytoplasmic membranes, as evidenced by the measurement of lactate dehydrogenase (LDH) levels following RCM exposure (Paajanen *et al.*, 1984). LDH is a cytoplasmic enzyme known to be liberated when platelets are lysed (Ring *et al.*, 1978*a*). LDH is not detected (Ring and Sovak, 1981) or elevated (Paajanen *et al.*, 1984) following RCM challenge to platelets *in vitro*. These results indicate that release of stored aggregatory substances within the platelet by RCM is an active secretory process.

Thromboxane A_2 (TXA_2) is a potent vasoconstrictor and pro-aggregatory substance derived from platelet membrane arachidonic acid (AA) *via* the enzyme cyclo-oxygenase. Rapid synthesis of TXA_2 is the main mechanism of exogenous AA-induced platelet aggregation (Hamberg *et al.*, 1975). Ioxaglate has been shown to slightly inhibit TXB_2 production in response to AA *in vitro* (Paajanen *et al.*, 1984) and diatrizoate has been shown to inhibit TXB_2 formation in whole blood *in vitro* in a dose-dependent manner (Fareed *et al.*, 1984*b*). As the cyclo-oxygenase pathway is calcium dependent, those authors suggest the effect is due to the Ca^{2+} chelating capacity of RCM. However, increased TXB_2 levels have not been demonstrated following administration of high or low osmolality RCM to patients (De Maeyer *et al.*, 1985; Parvez *et al.*, 1988).

To determine whether the effects of radiographic contrast agents on human platelet aggregation were due to alterations in TXA_2 synthesis, the stable metabolite TXB_2 was measured in PRP in response to platelet stimulation by collagen *in vitro*. No significant differences were demonstrated between TXB_2 levels prior to and following RCM administration in patients *in vivo*. Furthermore, neither Urovison nor Iopamiro exerted any significant effect on platelet aggregation induced by AA *ex vivo*. These results indicate that RCM do not impair TXA_2 generation and that inhibition of *ex vivo* collagen-induced platelet aggregation by Urovison *in vivo* is not conferred *via* a direct action on TXA_2 synthesis.

Aspirin, by inactivating the platelet cyclo-oxygenase (Roth and Siok 1978), is a potent inhibitor of TXA_2 production and TXA_2-dependent platelet aggregation (Smith and Willis, 1971). A dose of 600 mg in normal volunteers has been found to elicit maximum inhibition of both platelet aggregation and TXA_2 formation *ex vivo* (Herd *et al.*, 1987). In particular, this dose of aspirin *in vivo* completely abolished AA-induced TXA_2 production and platelet aggregation (Herd *et al.*, 1987). Therefore, it can be assumed that *in vivo* TXA_2 synthesis in the present study was totally inhibited by the oral administration of this dose of aspirin to healthy subjects. The resultant platelet aggregation induced by collagen and ADP was inhibited by both RCM, the effects of Urovison being exerted at lower concentrations than for Iopamiro. The higher concentrations of RCM required to induce inhibition of aspirinised platelets as compared with non-aspirinised platelets, is a reflection of the TXA_2-dependent nature of the collagen and ADP platelet aggregation. A greater concentration of collagen and ADP was required to elicit at least 50% maximal aggregation in the presence of aspirin (*ie.* the critical concentration was increased), therefore, higher concentrations of RCM were necessary to cause the same degree of inhibition. The ability of both RCM (*ie.* Urovison and Iopamiro) to inhibit TXA_2-independent platelet aggregation is further evidence that RCM convey their inhibitory effect *via* a mechanism(s) distinct from the interference of TXA_2 formation.

The binding of these highly iodinated compounds to platelet surface membrane proteins may contribute to their inhibitory capacity. It is probable that radiographic contrast media inhibit platelet function *via* multiple mechanisms, with differences in the effects of various agents induced by inherent physicochemical heterogeneity (*eg.* ionicity and osmolality).

The findings of the present studies suggest that radiographic contrast media do not stimulate platelets but actually inhibit platelet function when this is studied *in vitro*. Moreover, it is apparent that non-ionic, low osmolality agents such as Iopamiro (iopamidol) suppress platelet activation to a lesser degree than ionic, high osmolality media such as Urovison (sodium and meglumine diatrizoate).

CHAPTER IV

A METHOD FOR THE ASSESSMENT OF PLATELET ACCUMULATION *IN VIVO*

IV.1 Introduction

The techniques of platelet (Born, 1962) and whole blood (Cardinal and Flower, 1980) aggregometry have permitted the detailed analysis of platelet aggregation *in vitro* and have facilitated the delineation of many of the underlying biochemical events. Cellular mechanisms underlying platelet aggregation *in vitro* are not necessarily predictive of *in vivo* manifestations of platelet activation (de Gaetano *et al.*, 1987). Discrepancies between *in vitro* and *in vivo* observations may arise because the use of cell suspensions cannot make allowances for any interaction between intact vascular endothelium and developing thrombi. The endothelium may influence thrombus formation by affecting ADP uptake or by liberating autacoids such as prostacyclin (PGI_2) (Bunting *et al.*, 1976), endothelium-derived relaxing factor (EDRF) (Furchgott and Zawadzki, 1980) and the recently described peptide endothelin (Yanagisawa *et al.*, 1988), which not only influence vascular smooth muscle tone, but also affect platelet activation (Radomski *et al.*, 1987*a*; Thiemermann *et al.*, 1988). Additionally, when endothelial cells interact with platelets or neutrophils, an altered pattern of arachidonate metabolism (Marcus *et al.*, 1981; 1982) and enhanced phospholipid metabolism (Oda *et al.*, 1986) may result. It follows, therefore, that a need exists to complement *in vitro* aggregometry with an *in vivo* method that is sensitive, reproducible and operationally simple.

As outlined in the opening chapter, various methods have been proposed to achieve this goal (Born and Cross, 1963; Hornstra, 1970; Smith and Frueler, 1973; Bourgain and Six, 1974; Wu and Hoak, 1974; Baumgartner *et al.*, 1976; Kohler *et al.*, 1976; Buchanan and Hirsch, 1978; Gryglewski *et al.*, 1978; Butler *et al.*, 1979; Shishido and Katori, 1981; Garcia Rafanell and Forn, 1982; Badimon *et al.*, 1983; Pinon, 1984; Hanson and Harker,

1987). However, none of these experimental systems is free from major limitations. The majority are highly invasive and cumbersome and do not allow repeated observations to be conducted in a single animal, thus making them expensive in terms of both time and money. Consequently, *in vivo* aggregometry has yet to become established as a routine test for screening inhibitors of platelet activation. Monitoring the intrathoracic accumulation of isotopically-labelled platelets (Davies *et al.*, 1981) has been proposed previously and a preliminary evaluation was reported (Page *et al.*, 1982*b*). Subsequently, both the recording equipment and the experimental procedures have been considerably refined, so as to provide a technique that is more sensitive and reproducible than previously described.

IV.2 **Methods**

IV.2.1 *Animals*

Male Dunkin-Hartley guinea-pigs, 400-600 g body weight were used throughout this study. Test animals were anaesthetised with urethane (1.4 g/kg i.p.) and an external jugular or dorsal foot vein cannulated for the administration of isotopically-labelled materials and drugs or agents causing platelet activation. Intravenous cannulae with a dead space volume of at least 0.2 ml were used.

IV.2.2 *Reagents*

Adenosine diphosphate (ADP) disodium salt (Sigma), SRI 63-441 (*cis* (±)-1-[2-[hydroxy [tetrahydro-5 [(octadecylamino-carbonyl)oxyl] methyl]furan-2-yl]methoxy-phosphynyloxy]ethyl]-quinolinium hydroxide inner salt) (Sandoz, Basel, Switzerland) and heparin (5 I.U./ml) (Hoffmann LaRoche, Basel, Switzerland) were diluted in physiological saline (0.9% w/v). Collagen (Hormon-Chemie, Munich, FRG) was diluted in isotonic glucose buffer (pH 2.7). Platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (Bacham, Bubensdorf, FRG) was dissolved in ethanol and kept as a

stock solution (1 mg/kg), which was diluted in a solution of bovine serum albumin (BSA) (0.25% w/v) (Sigma) in saline (0.9% w/v) as required. Citrate buffer, for separation and labelling of platelets or erythrocytes, contained trisodium citrate (2.8%), sodium dihydrogen phosphate (0.015%) and glucose (0.2%) (all reagent grade) and was autoclaved at 15 lb/inch^2 for 30 min (Mollison, 1967). Acetyl salicylic acid (ASA, aspirin) (Sandoz) was dissolved in 200 *u*l $NaHCO_3$ and diluted with tris buffer (10% w/v). ^{111}Indium oxine (^{111}In, 37 MBq/ml) and ^{125}Iodine-labelled fibrinogen (^{125}I-fibrinogen, 37 MBq/ml) were obtained from Amersham International (Amersham, England).

IV.2.3 *Preparation of ^{111}Indium-Labelled Platelets*

Platelets were isolated from blood removed from a separate donor animal. Blood (20 ml) was collected *via* cardiac puncture from an ether-anaesthetised guinea-pig into citrate buffer (10% v/v) and placed in polystyrene tubes. After centrifugation (200 *g*, 10 min), platelet-rich plasma (PRP) was removed and mixed with an equal volume of citrate buffer containing PGE_1 (300 *ng*/ml) to prevent spontaneous aggregation (Shio and Ramwell, 1972). Following a second centrifugation (1000 *g*, 10 min), the supernatant containing plasma proteins was decanted and the remaining platelet pellet washed and resuspended in 1 ml of citrate buffer. The platelet density was determined by an automated platelet counter (Cell-dyn 100) (Sequoia-Turner, California). ^{111}In oxine (1.5 MBq) was added to the platelet suspension, incubated for 5 min at room temperature and centrifuged (1000 *g*, 10 min). Unbound ^{111}In oxine in the supernatant was discarded and the surface of the platelet pellet was washed twice with citrate buffer to remove unbound ^{111}Indium associated with the meniscus. Platelets were resuspended (5.0 - 9.0 x 10^8/ml) in 1 ml of citrate buffer and 0.3 ml aliquots of the platelet suspension were injected intravenously *via* a jugular vein or a dorsal foot vein, followed by 1 ml of heparinised saline to maintain patency of the cannula. ^{111}In-labelled platelets that were injected into recipient animals, comprised between 2 and 8% of the circulating platelet population. The capacity of ^{111}In-labelled platelets to aggregate in response to PAF or ADP was similar to unlabelled

platelets *in vitro*, hence, aggregometry studies were not undertaken routinely to assess platelet function.

IV.2.4 *Preparation of ^{111}Indium-Labelled Erythrocytes*

Blood was collected as for the preparation of platelets. After centrifugation (200 *g*, 10 min), PRP was decanted and a 1 ml aliquot of packed erythrocytes was incubated with 1.5 MBq of ^{111}In oxine for 5 min at room temperature. After centrifugation (1000 *g*, 10 min), the supernatant was discarded and erythrocytes were resuspended in 1 ml of citrate buffer. Aliquots of 0.3 ml were injected intravenously as described for platelet injection.

IV.2.5 *Monitoring of ^{111}Indium-Labelled Blood Cells*

Anaesthetised, spontaneously breathing guinea-pigs were placed supine. Sodium iodide crystal scintillation detectors (1 inch crystal, DM1-1, Nuclear Enterprises) were housed in lead collimators (18 mm thick) with a circular aperture (33 mm) at the crystal face (Figure IV.1). One detector was placed above the thorax such that the collimator edge overlaid the xiphisternum to avoid acceptance of signals from the spleen or liver. A second detector was placed adjacent to the first detector over the abdomen (Figure IV.1). NIMS series amplifier/analysers (Nuclear Enterprise 4697) were used to amplify and select signals from each detector. Pulses from the NE 4697 modules were logged at up to 5 MHz by a 6 channel co-processor within a host computer (IBM AT3) and count rates were displayed graphically in real time. Prior to commencing experimental observations, this system was used to provide a ratemeter for each of the 6 spectrometers, thereby allowing selection of an optimal voltage threshold for the isotope. ^{111}Indium, a gamma emitting isotope with a half-life of 2.8 days, emits radiation with peaks at 0.171 MeV and 0.245 MeV on the electromagnetic spectrum. The voltage threshold of the Ne 4697 was adjusted to a level between 900 and 1050 volts in order to detect the major peak. Count rates in the region of 10,000/sec over the thorax and 3,000/sec over the abdomen were obtained when ^{111}In-labelled blood elements were administered.

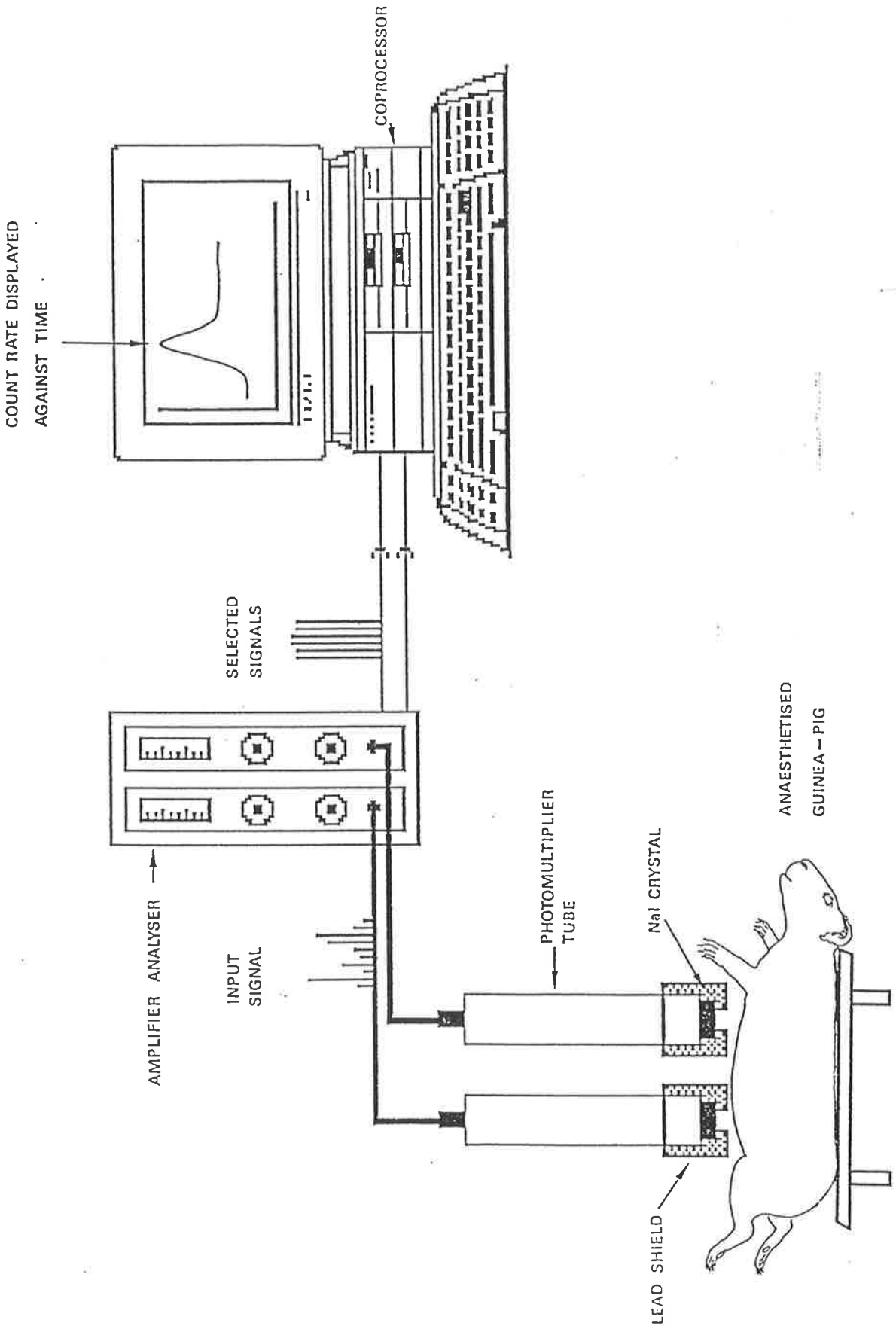


Figure IV.1 Diagrammatic representation of the system used for monitoring radioisotopes *in vivo*.

Immediately following injection of ^{111}In-labelled platelets or erythrocytes, count rates over the thorax were stable. Nevertheless, a period of 5-10 min was allowed to elapse before commencing an experiment. Recording commenced 50 sec before injection of platelet aggregatory stimuli and count rates were recorded over 30 successive 10 sec periods. At the end of an observation period, data was archived automatically to allow for subsequent examination or for inclusion within group data. The Automated Isotope Monitoring System (AIMSplus, Mumed Ltd. London) allowed three experiments to be undertaken concurrently and independently of one another.

Platelet stimulants were injected intravenously in a volume of 0.2 ml so as to occupy the dead space in the intravenous cannula, followed by an infusion of saline (0.8 ml over 10 sec) at a constant rate (4.7 ml/min) from an infusion pump (B. Braun, Melsengun). Injections of ADP, collagen and PAF were given at 30 min intervals. For dose-effect studies, 3 doses of aggregatory stimuli selected randomly were used in each animal and responses recorded in groups of 6-8 animals. When testing for inhibition of platelet accumulation, drugs or putative inhibitors were injected intravenously 1 min prior to injection of the platelet stimulant. At the end of an experiment, animals were sacrificed by a lethal i.v. injection of sodium pentobarbitone.

IV.2.6 *Monitoring of ^{125}Iodine-Labelled Fibrinogen*

^{125}I emits radiation with a major peak at 0.035 MeV, hence, the threshold voltage of the NE 4697 amplifier/analysers was set between 600 and 800 volts to include this peak. An intravenous injection of ^{125}I-fibrinogen (*circa* 0.4 MBq) produced a count rate comparable with that obtained when ^{111}In-labelled blood cells were injected.

IV.2.7 *Data Analysis*

Platelet accumulation was measured as an increase in radioactive counts/sec by the detector over the thorax. Results are expressed as a percentage change in radioactive

counts (calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts and converting this increase to a percentage of the baseline intrathoracic counts). Although abdominal counts were recorded in each case as an indication of events occurring in the peripheral circulation, for convenience this data is not presented. An example however, is shown in the results section.

IV.3 **Results**

IV.3.1 *Use of Ratios or Paired Differences*

By standardising the quantity of isotope and number of platelets injected, it has been possible to compare the use of intrathoracic accumulation due to injection of an aggregatory stimulus (maximal increase in thoracic count rate) with the use of ratios (maximal thoracic count rate/count rate in the abdomen). Inspection of the individual results shows that close correlation does not necessarily exist. For example, animals in which a ratio of 4.2:4.3 was observed in response to the injection of ADP (100 *u*g/kg) or PAF (10 *n*g/kg) had increased intrathoracic count rates that ranged between 1,690 cps and 3,575 cps. Such discrepancy probably reflects variation in the retention of platelets in abdominal regions (*eg*. liver and spleen) so that abdominal counts cannot serve as a measure of the vascular content of platelets. In the present study, thoracic counts have been used in all instances to determine platelet accumulation.

IV.3.2 *Control Responses*

The intravenous injection of saline (0.9% w/v), CFTP buffer and BSA saline, the control vehicles for ADP, collagen and PAF, respectively, produced no significant changes of the thoracic count rates in guinea-pigs that had been administered ^{111}In-labelled platelets, ^{111}In-labelled erythrocytes or ^{125}I-fibrinogen (Figure IV.2 (a)-(c)).

Figure IV.2 (a)

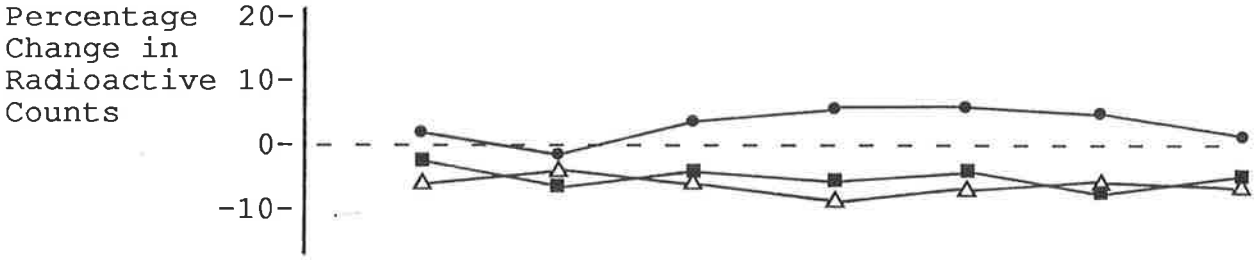


Figure IV.2 (b)

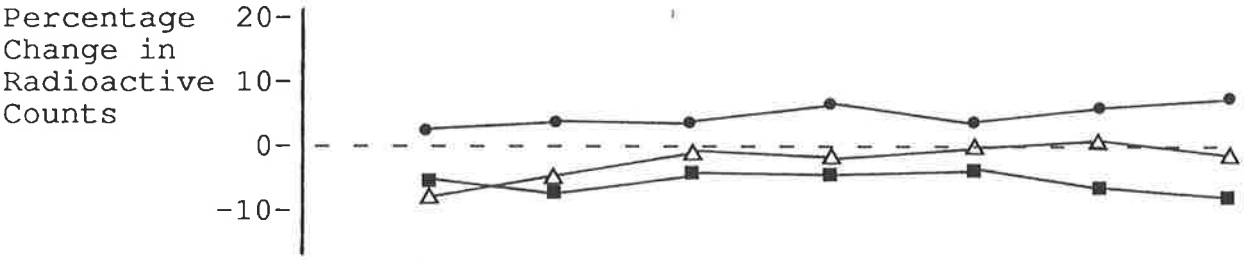


Figure IV.2 (c)

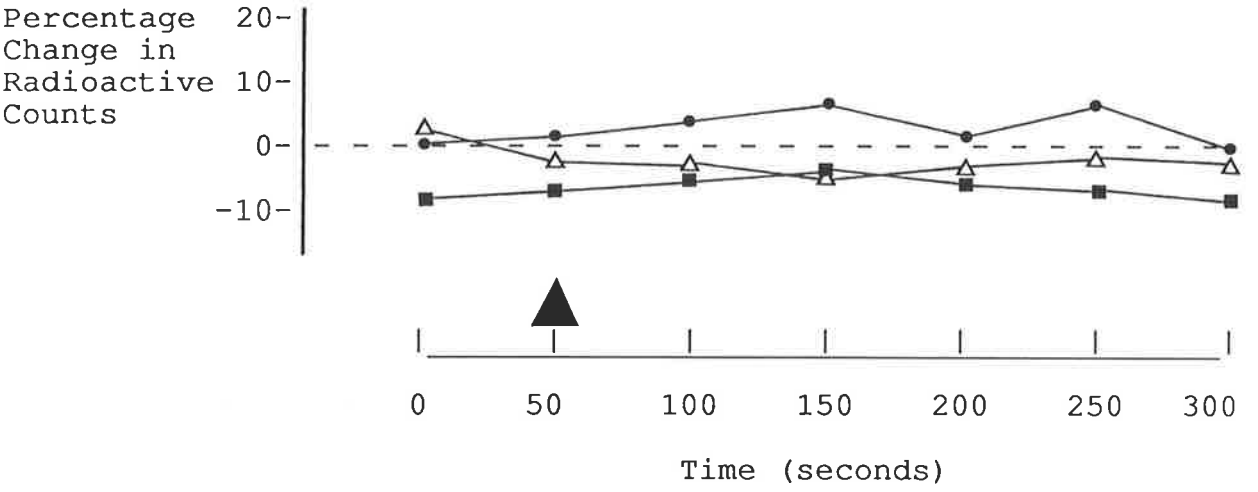


Figure IV.2 Changes in thoracic platelet-associated radioactivity over time following i.v. administration of bolus doses of (a) saline, (b) CFTP buffer and (c) BSA saline at arrow. Accumulation of platelets (•) contrasts with an absence of effect upon ^{111}In-labelled erythrocytes (■) and ^{125}I-fibrinogen (△). Changes are expressed as percentage change in radioactive counts as compared with pre-injection levels. Points depict the mean of observations from four guinea-pigs.

IV.3.3 *Response to ADP*

When ADP (100 *u*g/kg) was injected intravenously, an abrupt increase in the thoracic count rate was observed in animals that had received an intravenous injection of ^{111}In-labelled platelets, but not in animals that had received an intravenous injection of ^{111}In-labelled erythrocytes or ^{125}I-labelled fibrinogen (Figure IV.2 (d)). These observations are consistent with selective entrapment of platelets within the pulmonary vasculature. Maximal retention of platelets within the thorax was achieved within 20-30 sec after injection of ADP and returned to pre-injection levels within 5 min. When a range of ADP doses (1-180 *u*g/kg) was used, the parameters of peak height and area under the curve (AUC) increased in a dose-related fashion (Table IV.1).

IV.3.4 *Response to Collagen*

Intravenous injection of collagen fibrils (100 *u*g/kg) produced no noteworthy change of the thoracic count rate when ^{111}In-labelled erythrocytes or ^{125}I-labelled fibrinogen were used as intravascular markers (Figure IV.2 (e)), whereas thoracic count rates of ^{111}In-labelled platelets rose progressively, achieving a maximum 100 sec after injection of collagen. Intrathoracic platelet accumulation was evident following injection of collagen within the dose range 32-180 *u*g/kg. Responses were dose related, whether by reference to peak height or area under the curve (AUC) (Table IV.1).

IV.3.5 *Reponse to PAF*

When PAF (10 *n*g/kg) was injected intravenously, the thoracic count rate increased in those animals that had received an injection of ^{111}In-labelled platelets, whereas in animals that had received an intravenous injection of ^{111}In-labelled erythrocytes or ^{125}I-labelled fibrinogen, there was a reduction of thoracic count rate. An accumulation of ^{125}I-labelled fibrinogen in the abdomen was observed to parallel the fall in count rate from the

Figure IV.2 (d)

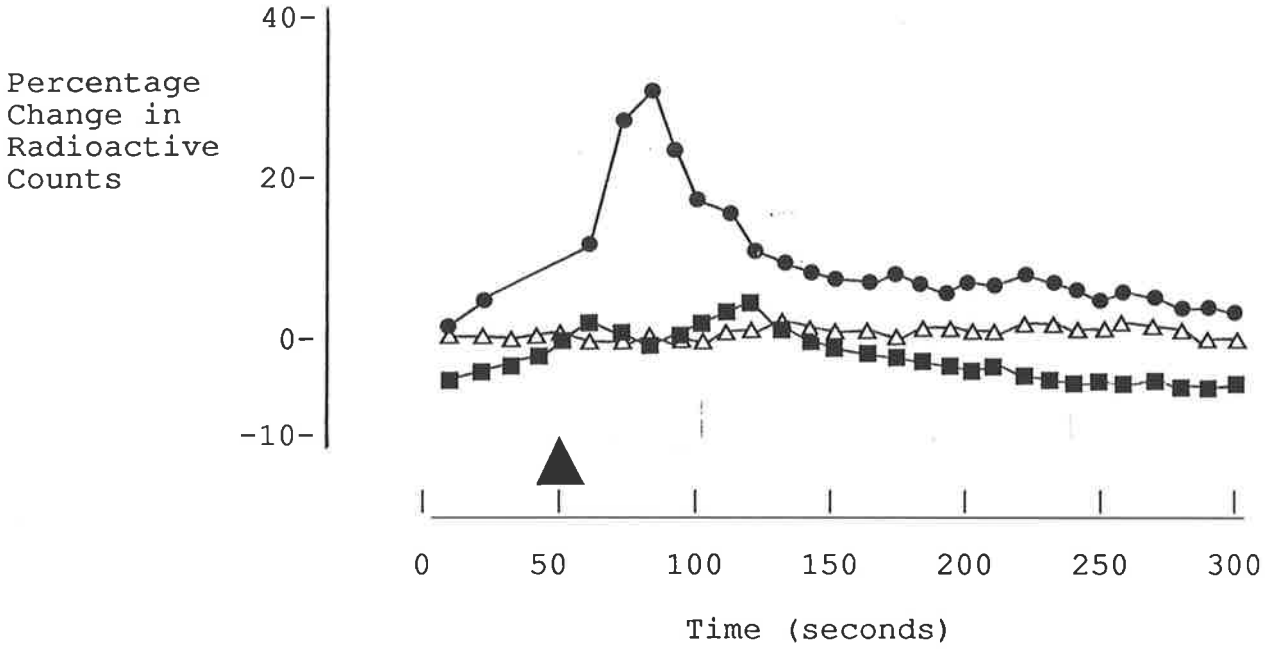


Figure IV.2 (e)

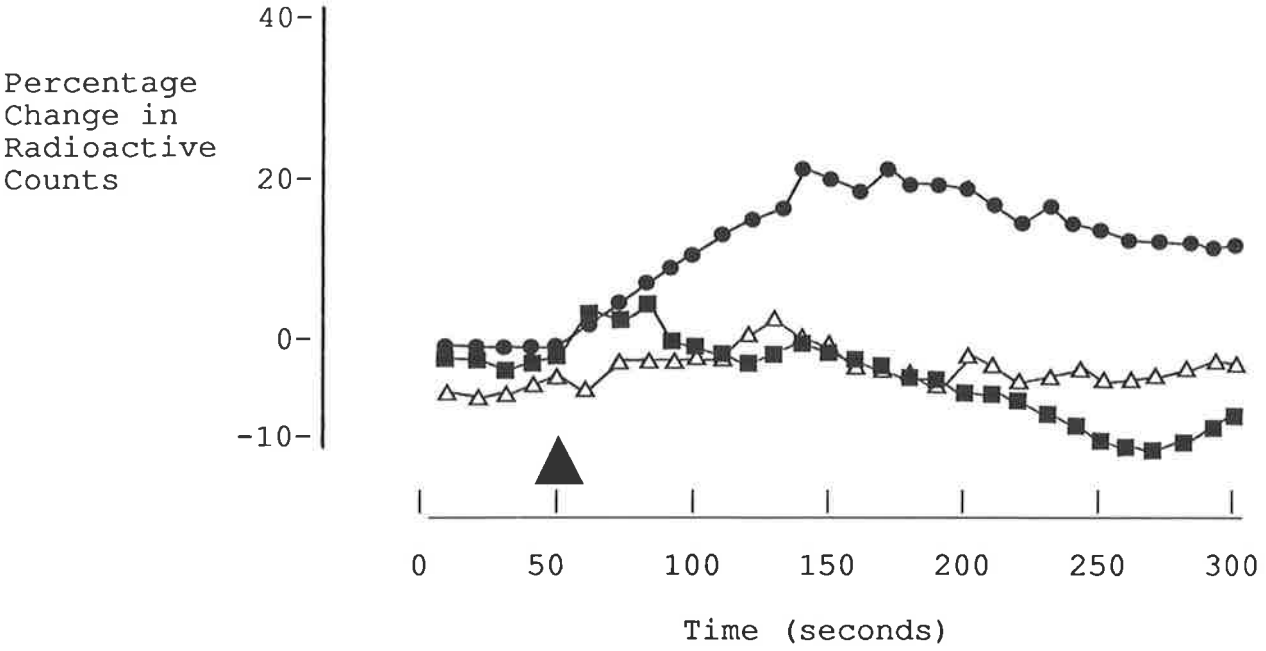


Figure IV.2 Changes in thoracic (■ ● △) platelet-associated radioactivity over time following i.v. administration of bolus doses of (d) ADP (100 *u*g/kg) and (e) collagen (100 *u*g/kg) at arrow. Accumulation of platelets (●) contrasts with an absence of effect upon ^{111}In-labelled erythrocytes (■) and ^{125}I-fibrinogen (△). Changes are expressed as percentage change in radioactive counts as compared with pre-injection levels. Points depict the mean of observations from four guinea-pigs.

AGONIST	DOSE (ug/kg)	PEAK HEIGHT (cps ± sem)	AUC (cm^2 ± sem)
ADP	1.0	1.49 ± 0.6	0.0
	3.2	11.89 ± 0.72	6.0 ± 0.7
	5.6	17.36 ± 0.42	13.9 ± 1.1
	10.0	24.07 ± 0.55	15.4 ± 0.9
	56.0	26.06 ± 0.48	15.5 ± 1.2
	100.0	27.86 ± 0.36	21.8 ± 1.3
Collagen	32.0	9.06 ± 4.0	14.2 ± 1.2
	56.0	18.05 ± 4.6	28.4 ± 1.1
	100.0	24.86 ± 4.5	42.7 ± 1.4
	180.0	27.58 ± 6.1	69.9 ± 1.4
PAF	0.001	1.08 ± 0.63	5.0 ± 0.9
	0.0032	4.74 ± 0.26	11.5 ± 1.1
	0.0056	17.33 ± 0.42	23.1 ± 1.2
	0.01	25.15 ± 0.66	25.9 ± 1.1
	0.032	29.94 ± 0.52	75.2 ± 1.3

Table IV.1 Increases in peak height and area under the curve (AUC) for each platelet agonist (mean ± sem), ADP, collagen and PAF. Peak height represents the increase in intrathoracic counts and has been calculated by subtracting the baseline thoracic counts from the maximal increase in thoracic counts observed and converted to a percentage of the baseline thoracic counts. Height and area have been estimated from mean data using at least 6 animals for each dose of agonist.

thorax; therefore, the reduction in thoracic counts reflected a loss of plasma protein into the viscera. The transient reduction of erythrocyte counts may reflect sequestration of erythrocytes at nonthoracic sites or constriction of vessels within the thorax. Consequently, when PAF is used, the incremental increase (by comparison with pre-injection levels) of intrathoracic content of ^{111}In-labelled platelets may be an underestimate of the accumulation of platelets within the thorax (Figure IV.2 (f)). Nevertheless, the present technique permitted detection of an effect of PAF upon platelets when as little as 1 *ng*/kg was injected, even though such a dose does not produce a detectable change in blood pressure or lung function in this species. Maximal intrathoracic platelet accumulation was observed between 50 and 60 sec following injection of PAF. Responses to PAF were dose related over the range 1-32 *ng*/kg, whether by reference to peak height or area under the curve (AUC) (Table IV.1).

IV.3.6 *Dose-Response Relationships*

From this data, dose-response curves in terms of peak height have been plotted for each stimulus (Figure IV.3). PAF is shown to be the most potent platelet aggregatory stimulus, being 1,000 times more potent than ADP or collagen.

IV.3.7 *Inhibition of Accumulation*

Inhibitory effects on platelet aggregation may be detected in this experimental system as either i) paired differences between successive responses in a single experimental animal to an aggregatory stimulus, or ii) as differences between contemporary experiments using treated and control animals. The response to successive doses of any of the aggregatory stimuli employed are closely comparable. Thus, successive responses (as peak height) to a fixed dose of PAF (10 *ng*/kg) were 27.50 $\pm$ 6.81% and 25.54 $\pm$ 6.27% (mean $\pm$ sem), with a co-efficient of variation for the paired differences of 1.18, which indicates that either method may be adopted. Figure IV.4 (a) shows successive

Figure IV.2 (f)

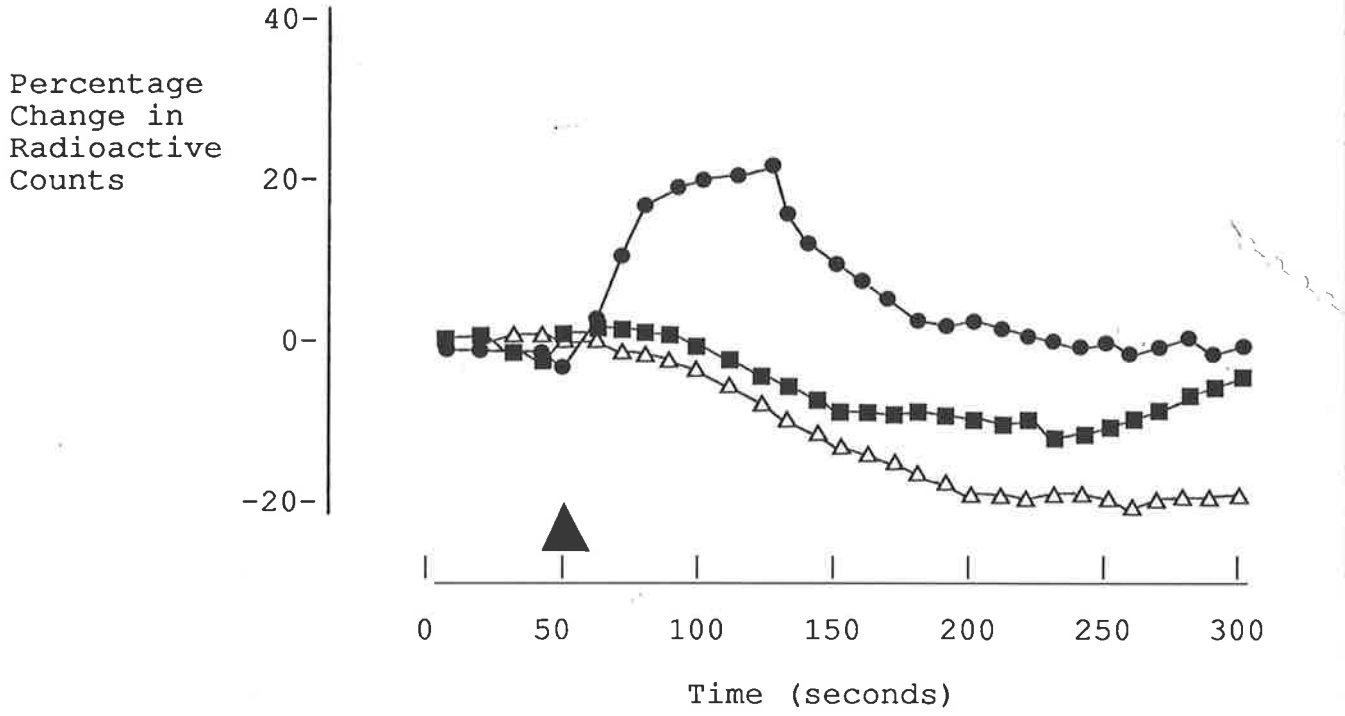


Figure IV.2 Changes in thoracic (■ ● △) platelet-associated radioactivity over time following i.v. administration of bolus doses of (f) PAF (0.1 *u*g/kg) at arrow. Accumulation of platelets (●) contrasts with an absence of effect upon ^{111}In-labelled erythrocytes (■) and ^{125}I-fibrinogen (△). Changes are expressed as percentage change in radioactive counts as compared with pre-injection levels. Points depict the mean of observations from four guinea-pigs.

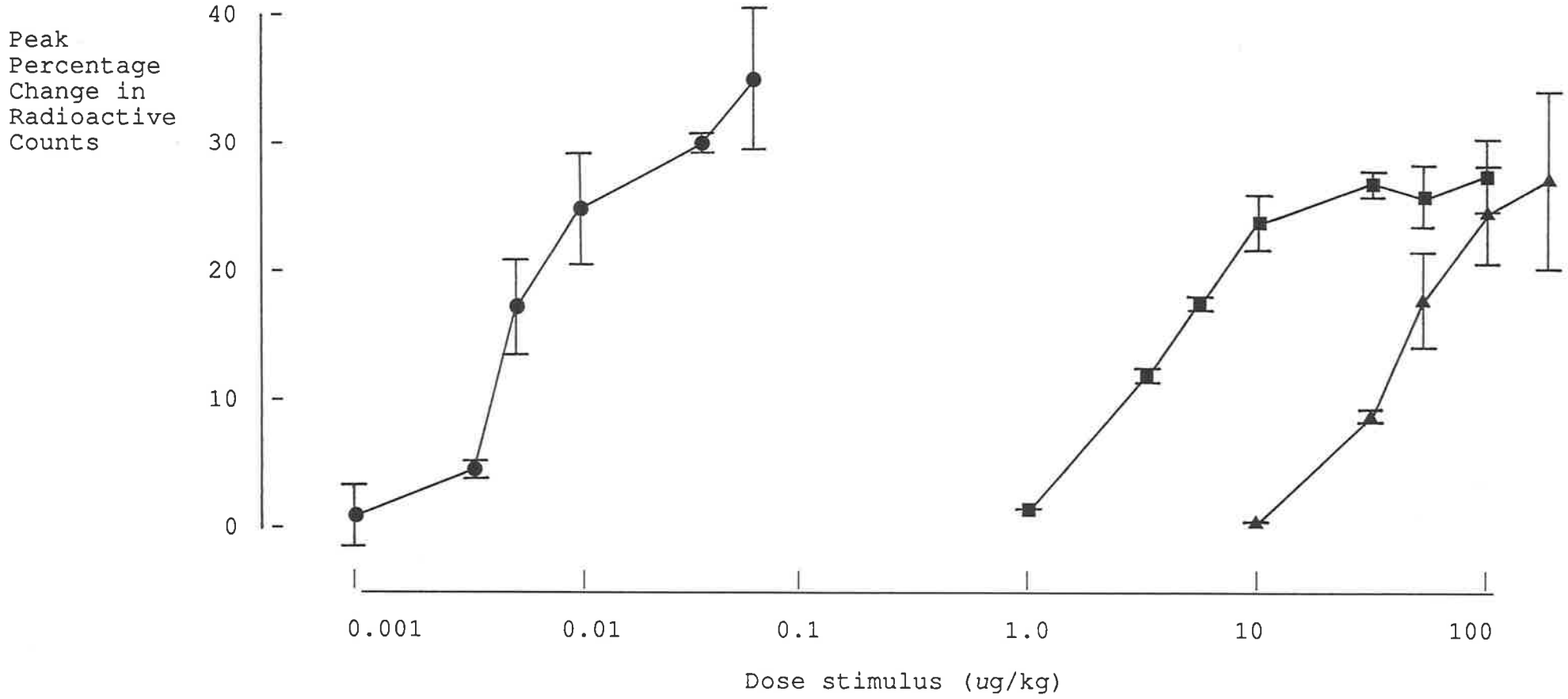


Figure IV.3 Graph showing the log dose rank order of potency of PAF (●), ADP (■) and collagen (▲) in terms of the maximal change in intrathoracic radioactive counts of the guinea-pig where ^{111}In-labelled platelets have been utilised. Each point represents the mean ± sem of at least six animals.

Figure IV.4 (a)

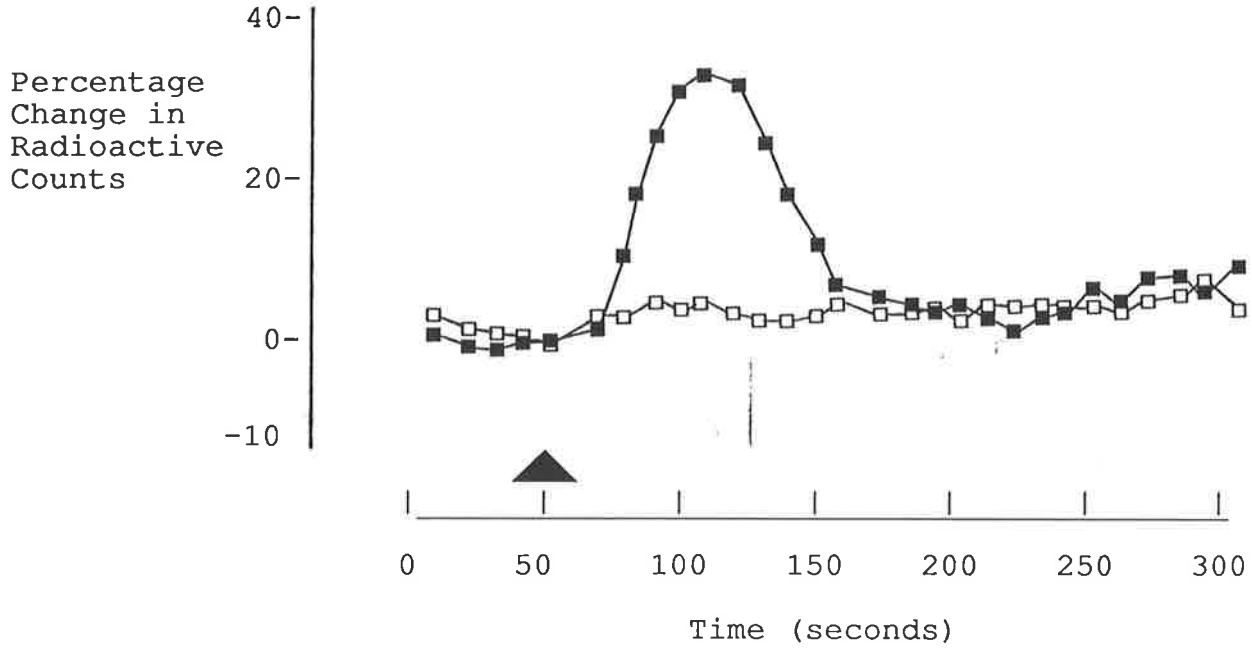


Figure IV.4 (b)

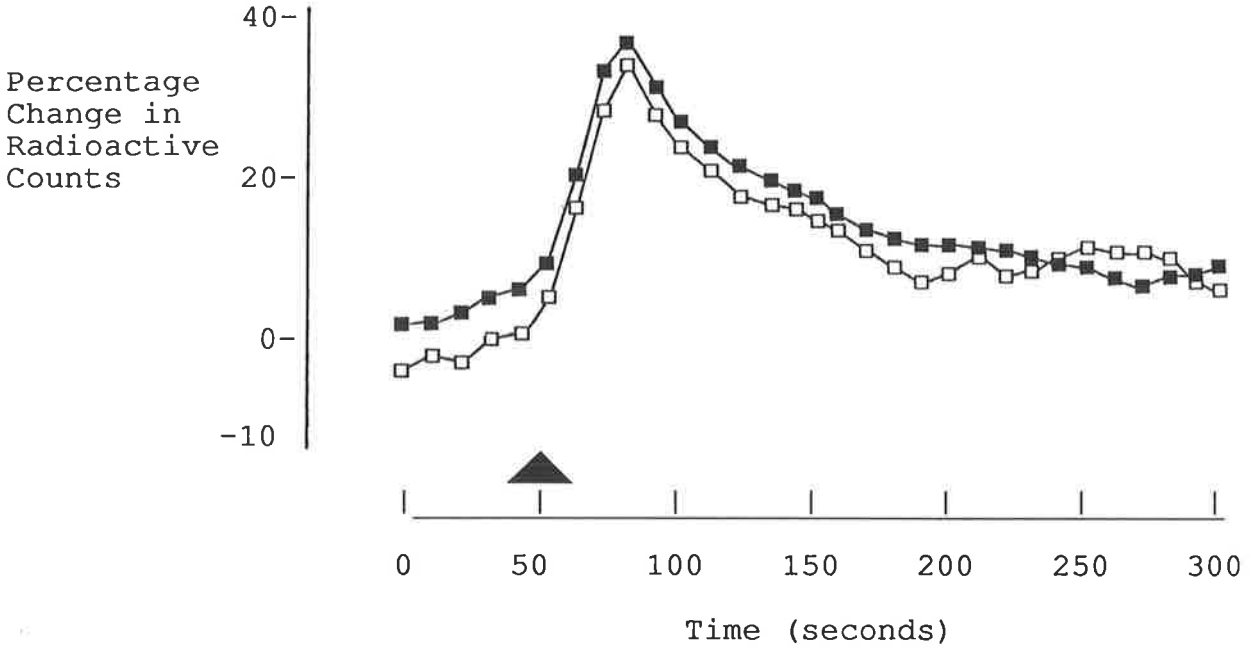


Figure IV.4 The effect of SRI 63-441 (1 mg/kg) (□) or control (■) upon intrathoracic ^{111}In-labelled platelet accumulation induced by i.v. injection of (a) PAF (10 *n*g/kg) or (b) ADP (100 *u*g/kg). Points depict the mean of observations from four guinea-pigs.

Figure IV.5 (a)

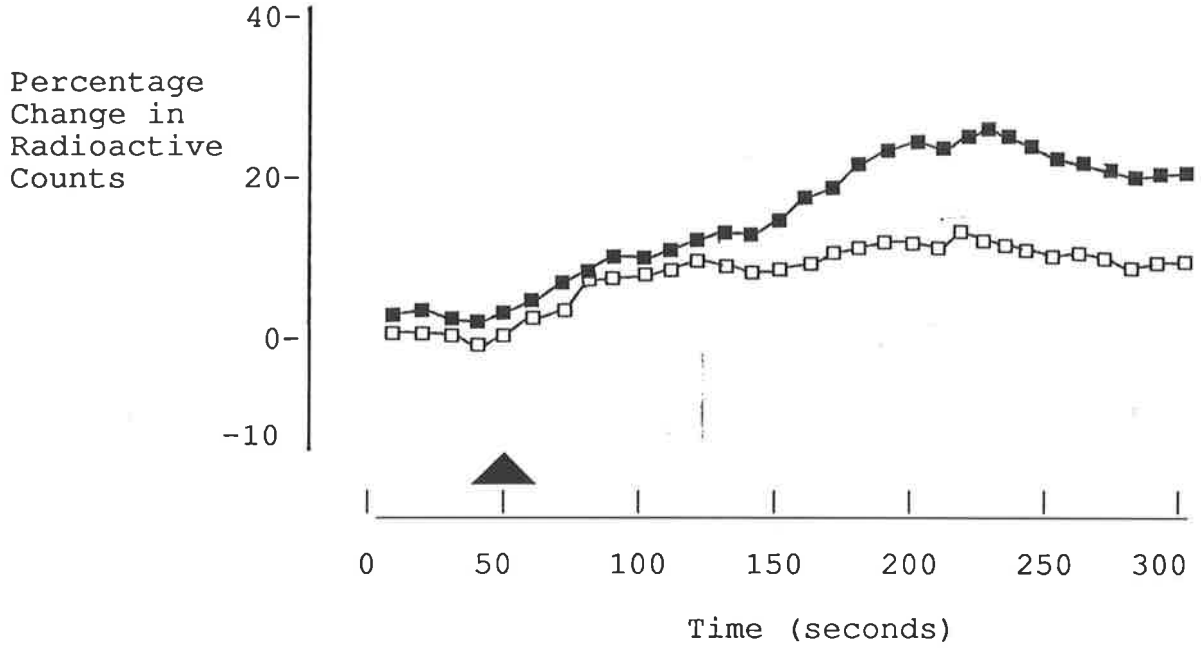


Figure IV.5 (b)

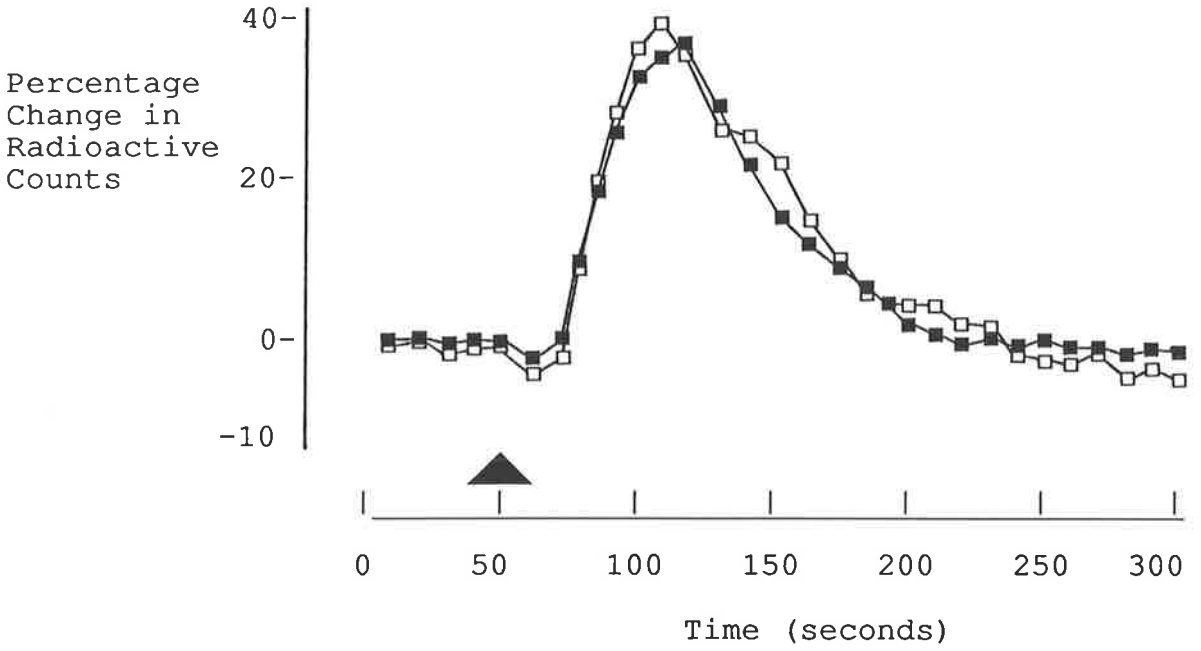


Figure IV.5 The effect of acetyl salicylic acid (1 mg/kg) (□) or control (saline) (■) upon intrathoracic ^{111}In-labelled platelet accumulation induced by i.v. injection of (a) collagen (100 *ug*/kg) or (b) PAF (10 *ng*/kg). Points depict the mean of observations from four guinea-pigs.

responses to bolus injections of PAF (10 *n*g/kg) 30 min before and 1 min after administration of a selective PAF antagonist, SRI 63-441 (1 mg/kg). Inhibition was selective, since this dose of SRI 63-441 did not diminish the response to ADP (Figure IV.4 (b)). As a corollary, acetyl salicylic acid (1 mg/kg) reduced the peak response to an intravenous injection of collagen (Figure IV.5 (a)) without influencing the amplitude of a response to PAF of comparable magnitude (Figure IV.5 (b)).

IV.4 Discussion

Continuous intrathoracic monitoring of isotopically labelled platelets was introduced as a method for the *in vivo* measurement of platelet activation (Davies *et al.*, 1981) and a preliminary evaluation of the technique was reported using a prototype computer with limited processing capacity (Page *et al.*, 1982*b*). Since that time, technological advances have enabled the recording system to evolve, so that the present system (AIMSplus) uses a co-processor to log and process data for up to 6 experiments independently of one another. Housing the computer within a standard microcomputer has allowed contemporary computing techniques to be incorporated into the experimental system in order to provide documentation of the experiment and to present data in graphical form in real time.

In an earlier study (Page *et al.*, 1982*b*), variable numbers of platelets and variable amounts of isotope were administered and results were expressed as a ratio of thoracic to abdominal counts. This procedure makes an assumption that abdominal counts reflect intravascular isotope content. Results from the present study imply that this may be unjustified, because abdominal counts may include platelets that have been damaged or have responded to activation stimuli by being retained in the spleen or liver or other abdominal structures.

Use of the present technique has permitted the detection of responses to lower doses of ADP, collagen and PAF than previously reported in the guinea-pig (Page *et al.*, 1982*b*) and has adopted optimal settings for isotope detection so that an increased sensitivity of between 3- and 10-fold was evident for these aggregatory stimuli. For PAF, responses of

platelets could be detected following injection of as little as 1 ng/kg, a dose that lacks cardiovascular and pulmonary effects in the guinea-pig. From the dose-response studies, PAF is the most potent platelet aggregatory stimulus, being 100 times more potent than ADP and 1,000 times more potent than collagen.

The time course of the responses also revealed differences, since responses to ADP were maximal earlier (approx. 30 sec) than those for collagen (100 sec) and PAF (60 sec). These differences in response characteristics are quite distinctive. The maximal rate of accumulation of ADP appears limited to a maximal value, so that it cannot be presumed that the lung acts as a passive filter but rather contributes to determining the rate of accumulation of platelet aggregates by altered blood flow (AV shunts) or endothelial activation.

The distinctive nature of the effects of ADP, collagen and PAF were clearly revealed by use of antagonists and inhibitors. For instance, the PAF receptor antagonist SRI 63-441 (Handley *et al.*, 1986) inhibited fully the response to PAF, but was without effect upon a response of comparable intensity induced by ADP. The findings involving the nonsteroidal anti-inflammatory drug, acetyl salicylic acid (ASA) complement this observation, as the response to collagen was reduced by ASA, while the responses to PAF or ADP were not affected.

AIMSplus therefore provides a simple, reproducible *in vivo* system that can complement *in vitro* aggregometry as a method to study platelet function. The use of this system in rabbits will be described in a Chapter VI of this thesis. Extension to larger mammals, including primates, or the use of solid state detectors will permit the same equipment to be employed in an analogous manner for the study of thrombi in single vessels and in models that are considered of more direct clinical relevance to thrombotic diseases (Hanson and Harker, 1987).

CHAPTER V

THE EFFECT OF VARIOUS DRUGS ON PAF-INDUCED ^{111}INDIUM-LABELLED PLATELET ACCUMULATION IN THE GUINEA-PIG

V.1 **Introduction**

The phospholipid platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), which is generated by a range of inflammatory cells following allergic or non-allergic activation, has been proposed as a mediator in asthma as it can mimic allergen-induced events including acute bronchospasm, induction of sustained non-selective airway hyperreactivity and eosinophil accumulation, in both experimental animals and man (Morley *et al.*, 1984). Were PAF to be generated during exacerbation of asthma, then a diminished response could be anticipated on testing platelets *ex vivo*. This prediction has been substantiated (Fishel and Zwemer, 1970; Solinger *et al.*, 1973; Maccia *et al.*, 1977; Thompson *et al.*, 1984), implying PAF formation in this disorder. Studies using a variety of techniques have confirmed that platelet abnormalities are a characteristic feature of asthma (Storck *et al.*, 1955; Knauer *et al.*, 1981; Gresele *et al.*, 1982; Traietti *et al.*, 1984; Wasserman and Ginsberg, 1984; Page *et al.*, 1985; Johnson *et al.*, 1986; Szczecklik *et al.*, 1986; Taytard *et al.*, 1986; Gresele *et al.*, 1987; Martin *et al.*, 1987; Taytard *et al.*, 1987). Such platelet abnormalities do not result in overt haematological sequelae, although bleeding time is increased significantly (Szczecklik *et al.*, 1986). However, such observations strongly suggest involvement of a platelet stimulus during asthma exacerbation and thereby strengthen the candidature of PAF for this role; without implicating PAF it would be difficult to account for platelet involvement in asthma.

PAF is one of the most potent *in vitro* platelet agonists yet identified, inducing calcium flux, phosphatidyl inositol turnover, protein phosphorylation, fibrinogen binding, shape change, granule content release and aggregation (Chignard *et al.*, 1986). In guinea-pigs, PAF-induced bronchoconstriction (Vargaftig *et al.*, 1980) and airway hyperreactivity

(Mazzoni *et al.*, 1985*a*) are platelet dependent since both can be abrogated by rendering animals selectively thrombocytopenic.

This chapter assesses the potential of a variety of drugs to inhibit PAF-induced intrathoracic ^{111}In-labelled platelet accumulation in the guinea-pig, with the animals acting as their own controls. The ability of PAF to induce intrathoracic platelet accumulation in the guinea-pig has been examined with the use of the Automated Isotope Monitoring System (AIMS) as described in the previous chapter (Chapter IV).

V.2 **Methods**

V.2.1 *Animals*

Male Dunkin-Hartley guinea-pigs, 400-600 g body weight were used throughout this study. Test animals were anaesthetised with urethane (1.4 g/kg i.p.) and an external jugular or dorsal foot vein was cannulated for the administration of ^{111}In-labelled blood cells and drugs or agents causing platelet activation. Intravenous cannulae with a dead space volume of at least 0.2 ml were used.

V.2.2 *Drugs/Chemicals*

Platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (Bacham, Bubensdorf, FRG) was dissolved in ethanol and kept as a stock solution (1 mg/kg), which was diluted in a solution of bovine serum albumin (BSA) (0.25% w/v) (Sigma) in saline (0.9% w/v) as required. Citrate buffer, for separation and labelling of platelets or erythrocytes, contained trisodium citrate (2.8%), sodium dihydrogen phosphate (0.015%) and glucose (0.2%) (all reagent grade) and was autoclaved at 15 lb/inch^2 for 30 min (Mollison, 1967). ^{111}Indium oxine (^{111}In, 37 MBq/ml) was obtained from Amersham International (Amersham, England).

SRI 63-441 (*cis* (+)-1-[2-[hydroxy [tetrahydro-5 [(octadecylaminocarbonyl)oxyl]

methyl]furan-2-yl]methoxy-phosphynyloxy]ethyl]-quinolinium hydroxide inner salt) (Sandoz, Basel, Switzerland), brotizolam (Sandoz), mepyramine maleate (Sandoz), cyproheptadine hydrochloride (Sigma), disodium cromoglycate (DSCG) (Fisons), ketotifen (4-(1-methyl-4-piperidylidene) 4H-benzo [4,5]-cyclohepta [1,2-b] thiophen-10(9H)-one hydrogen fumarate (Sandoz), AH 21-132 (Sandoz), prednisolone-21-sodium succinate (Sigma), aminophylline (Sandoz), isoproterenol bitartrate (isoprenaline) (Sigma), ipratropium bromide (Sigma), diltiazem hydrochloride (Sigma), verapamil hydrochloride (Sigma), iloprost (Schering) and heparin (Roche, Basel, Switzerland) were dissolved in physiological saline (0.9% w/v). Acetyl salicylic acid (ASA, aspirin) (Sandoz) and indomethacin (Sigma) were dissolved in 200 *u*l NaHCO3 and diluted with tris buffer (10% w/v). Dazmegrel (3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid) (Pfizer) was dissolved in 0.1 M NaOH. FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) (Fisons) was dissolved in 200 *u*l tris buffer (10% w/v) and diluted in saline (0.9% w/v).

V.2.3 *Preparation and Monitoring of ^{111}Indium-Labelled Platelets and ^{111}In-Labelled Erythrocytes*

^{111}In-labelled guinea-pig platelets and erythrocytes were prepared and monitored as described in Chapter IV.

V.2.4 *Experimental Protocol*

A period of 5-10 min was allowed to elapse before commencing an experiment following the intravenous administration of either ^{111}In-labelled platelets or ^{111}In-labelled erythrocytes. Recording commenced 3 min before the administration of PAF and counts were summated over 60 sec periods for 70 min. PAF (180 *n*g/kg) was infused intravenously over a 10 min period at a constant rate (0.158 ml/min) from an infusion pump (B. Braun, Melsengun). Thirty min later, a second identical PAF infusion was given.

Drugs or putative inhibitors of platelet accumulation under investigation (Table V.1) were administered as an acute intravenous bolus 1 min prior to the administration of the second PAF infusion. Drugs were injected in a volume of 0.2 ml so as to occupy the dead space in the intravenous cannula, followed by an infusion of saline (0.8 ml over 10 sec) at a constant rate (4.7 ml/min) from an infusion pump (B. Braun, Melsengun). Three experiments were undertaken concurrently and independently of one another. At the end of an experiment, animals were sacrificed by a lethal i.v. injection of sodium pentobarbitone.

V.2.5 *Data Analysis*

Platelet accumulation was measured as an increase in radioactive counts/sec by the detector over the thorax. Results are presented as a percentage change in radioactive counts (calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts and converting this increase to a percentage of the baseline intrathoracic counts). Although abdominal counts were recorded in each case as an indicator of events occurring in the peripheral circulation, for convenience this data is not presented. An example however, is shown in the results section.

Student's *t*-tests for paired observations were employed to determine the effects of drugs on ^{111}In-labelled platelet accumulation induced by PAF infusion (180 *n*g/kg over 10 min). Data are expressed as a mean percentage change $\pm$ standard error of the mean (sem) of *n* observations. A *p* value of < 0.05 was considered to be statistically significant.

V.3 **Results**

V.3.1 *Response to PAF*

The intravenous infusion of the PAF vehicle BSA saline produced no significant change in the thoracic count rates in guinea-pigs that had been administered ^{111}In-labelled platelets (Figure V.1). PAF (10 min intravenous infusions) induced a dose-dependent

DRUG	**CLASS**	**DOSE** (mg/kg)
SRI 63-441	PAF antagonists	0.01, 0.1, 1
Brotizolam		1
Mepyramine	histamine (H_1) antagonist	1
Cyproheptadine	histamine (H_1) and serotonin receptor antagonist	1
Cromoglycate	prophylactic anti-asthma drugs	10
Ketotifen		1
AH 21-132		1
Prednisolone		10
Aminophylline		10
Isoprenaline	*beta*-receptor agonist	0.01
Ipratropium bromide	anti-cholinergic	1
Diltiazem	calcium channel blockers	1
Verapamil		1
Aspirin	cyclo-oxygenase inhibitors	1
Indomethacin		1
Dazmegrel	thromboxane synthetase inhibitor	10
Iloprost	prostacyclin analogue	0.01
FPL 55712	leukotriene C_4 and D_4 antagonist	1

Table V.1 Drugs evaluated for their effect on PAF-induced ^{111}In-labelled platelet accumulation in the guinea-pig.

accumulation of ^{111}In-labelled platelets in the guinea-pig thorax (Figure V.1). ^{111}In-labelled intrathoracic platelet accumulation induced by PAF (180 *ng*/kg over 10 min) in the guinea-pig was reproducible 30 min later (Figure V.2 (a) and Table V.2).

V.3.2 *Effect of PAF Antagonists*

The two PAF antagonists investigated, brotizolam (1 mg/kg) and SRI 63-441 (0.1 mg/kg), fully inhibited the accumulation of ^{111}In-labelled platelets induced by the second PAF infusion (180 *ng*/kg over 10 min) ($p < 0.05$) (Figure V.2 (b) and Table V.2). The effect of SRI 63-441 was dose-related over the range 0.01-1.0 mg/kg i.v. (Table V.2).

V.3.3 *Effect of Histamine and Serotonin Antagonists*

Neither the selective histamine (H_1) antagonist mepyramine (1 mg/kg), nor the combinated H_1 and 5-HT antagonist cyproheptadine (1 mg/kg) had any significant effect on ^{111}In-labelled platelet accumulation induced by a PAF infusion (180 *ng*/kg over 10 min) in the guinea-pig ($p > 0.05$) (Table V.2).

V.3.4 *Effect of Prophylactic Anti-Asthma Drugs*

Disodium cromoglycate (DSCG) (10 mg/kg), ketotifen (1 mg/kg), AH 21-132 (1 mg/kg) and prednisolone (10 mg/kg) had no significant effect on PAF (180 *ng*/kg over 10 min)-induced ^{111}In-labelled platelet accumulation in the guinea-pig ($p > 0.05$) (Table V.2). The methylxanthine aminophylline (10 mg/kg) however, significantly inhibited the PAF-induced response ($p < 0.05$) (Figure V.2 (c) and Table V.2).

V.3.5 *Effect of Bronchodilators*

PAF (180 *ng*/kg over 10 min)-induced ^{111}In-labelled intrathoracic platelet

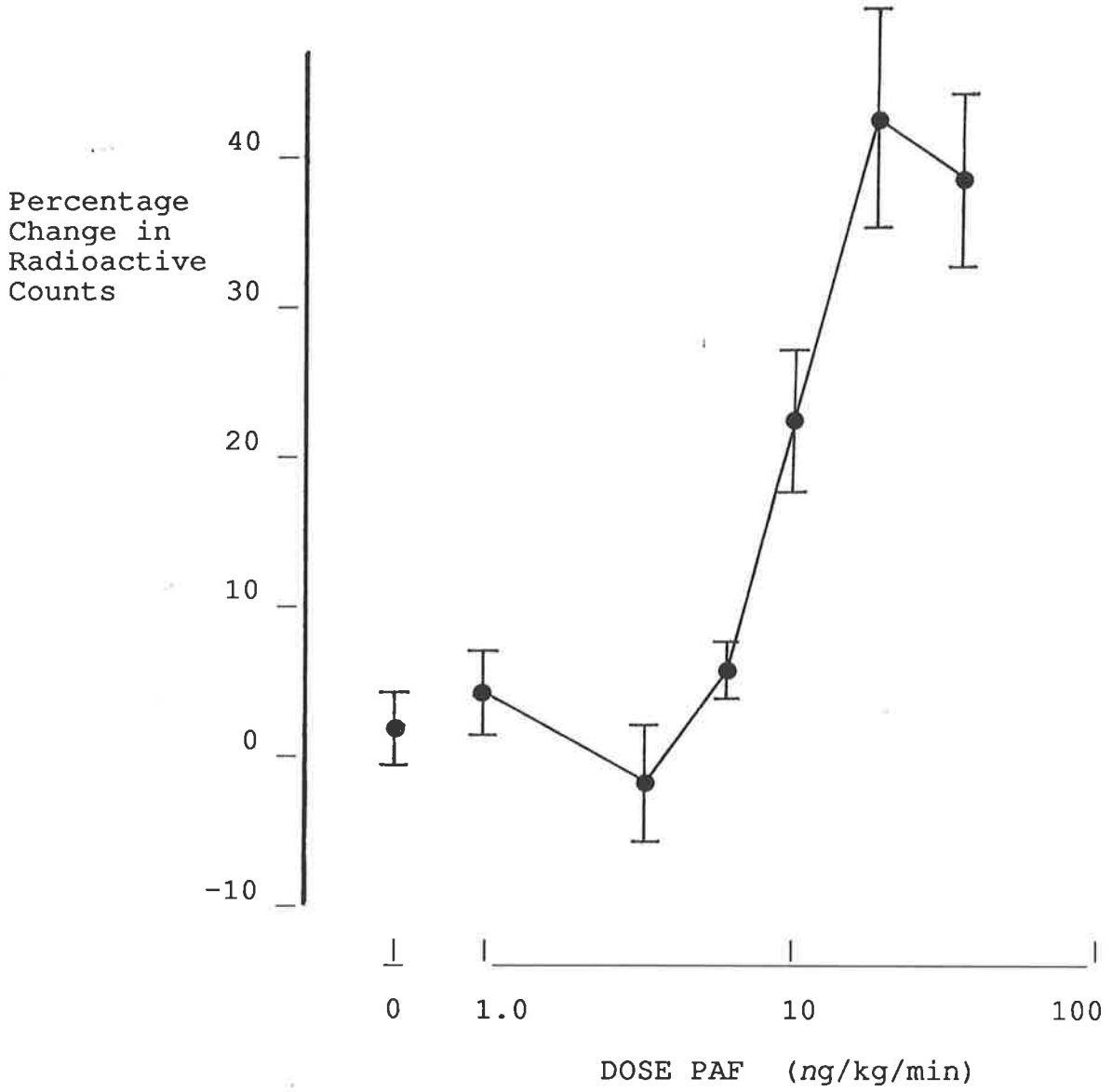


Figure V.1 The effect of PAF infusions (*ng*/kg/min for 10 min) on intrathoracic ^{111}In-labelled platelet accumulation in the guinea-pig (mean ± sem; n=6).

Figure V.2 (a)

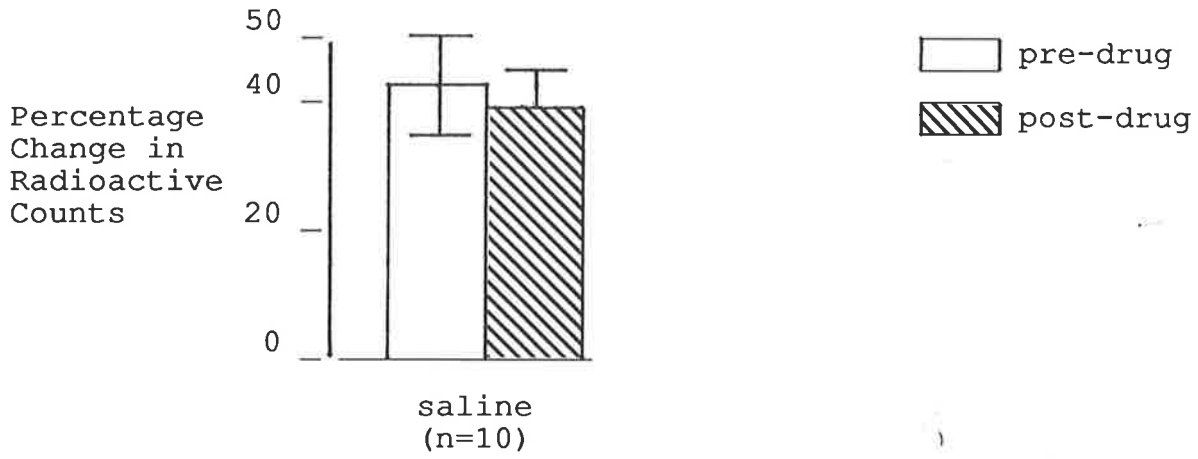


Figure V.2 (b)

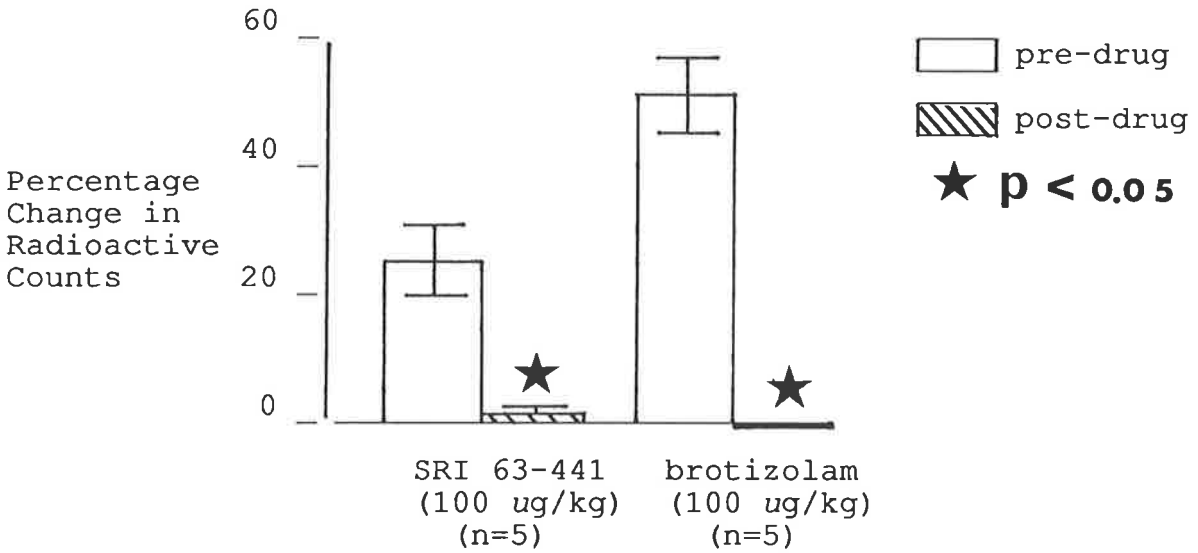


Figure V.2 (c)

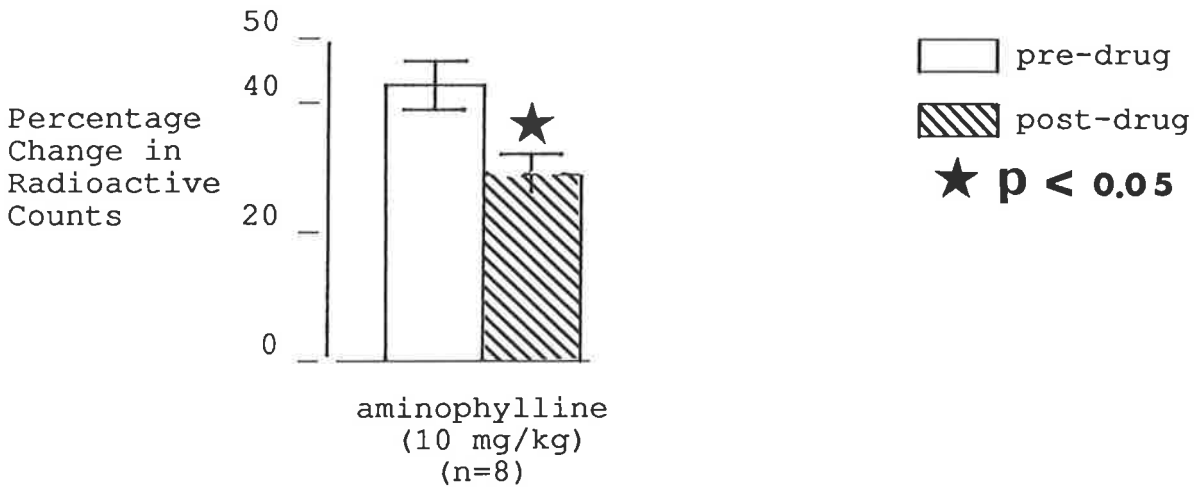


Figure V.2 (d)

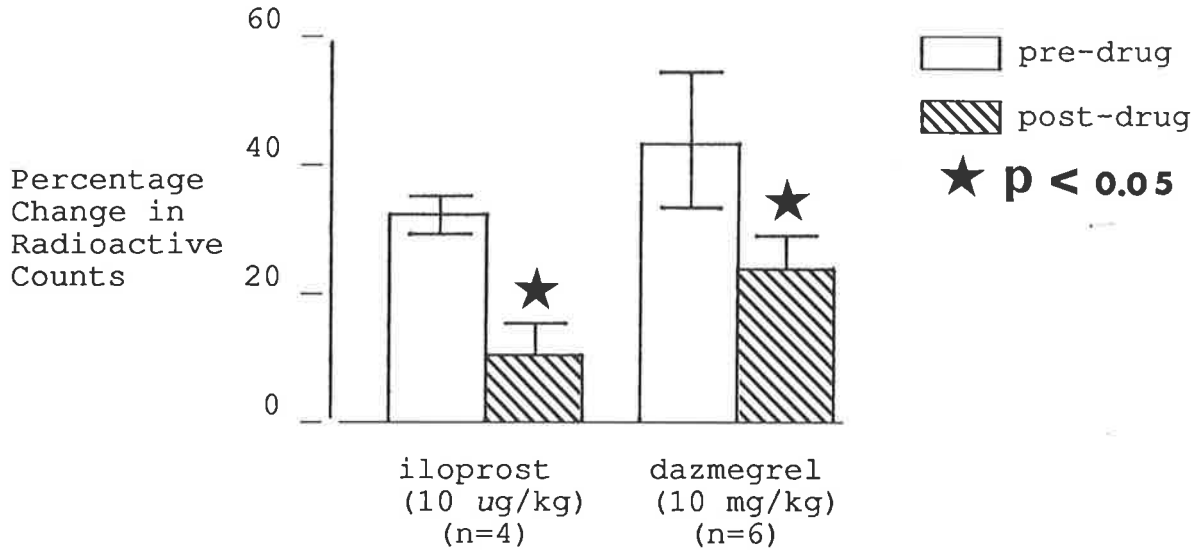


Figure V.2 (e)

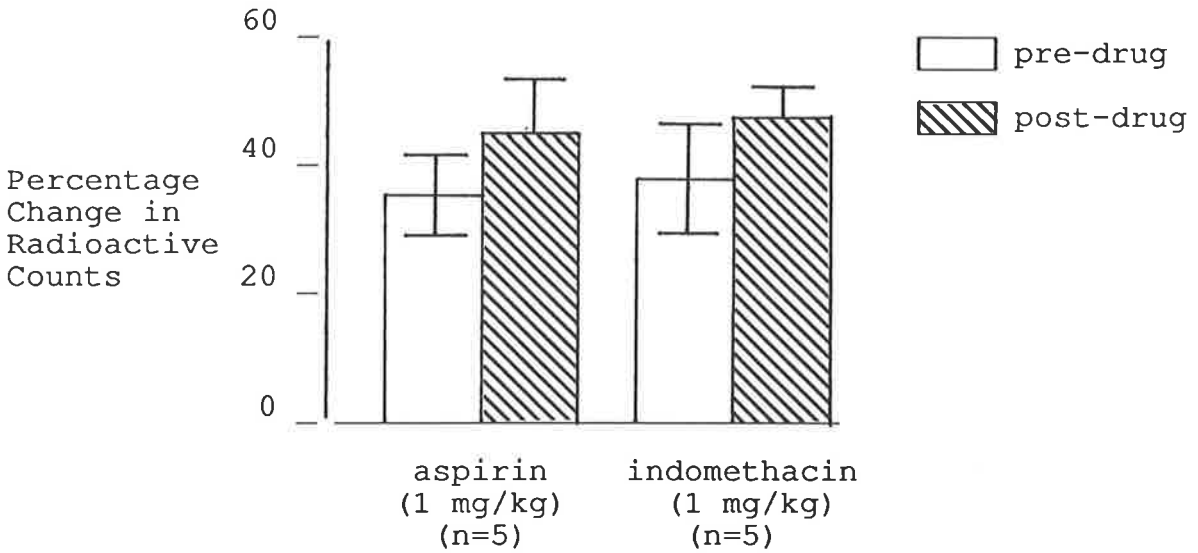


Figure V.2 The effect of (a) saline and (b)-(e) drug pretreatment upon intrathoracic ^{111}In-labelled platelet accumulation induced by i.v. infusion of PAF (180 *ng*/kg over 10 min).

DRUG	DOSE (mg/kg)	PEAK HEIGHT pre-drug (mean ± sem)	PEAK HEIGHT post-drug (mean ± sem)	n	*p*
Saline		42.69 ± 7.55	39.28 ± 5.91	10	0.6337
SRI 63-441	0.01	57.39 ± 6.26	43.25 ± 1.26	3	0.1553
	0.1	25.42 ± 4.71	0.68 ± 1.00	5	0.0097
	1	22.60 ± 5.41	-2.52 ± 1.44	6	0.0045
Brotizolam	1	50.60 ± 6.91	-0.25 ± 1.19	5	0.0025
Mepyramine	1	71.29 ± 11.91	51.11 ± 3.02	5	0.1297
Cyproheptadine	1	37.74 ± 2.70	42.72 ± 5.62	5	0.4561
Cromoglycate	10	45.18 ± 9.34	44.42 ± 3.06	6	0.9146
Ketotifen	1	37.87 ± 10.39	39.26 ± 2.73	4	0.9086
AH 21-132	1	45.41 ± 6.52	28.11 ± 2.96	5	0.1082
Prednisolone	10	25.89 ± 3.85	21.46 ± 3.91	4	0.0909
Aminophylline	10	42.82 ± 3.73	29.65 ± 2.55	8	0.0039
Isoprenaline	0.01	38.31 ± 5.64	27.92 ± 2.36	4	0.1425
Ipratropium bromide	1	29.77 + 3.35	26.89 + 4.53	4	0.5261
Diltiazem	1	40.81 ± 7.10	22.98 ± 10.11	5	0.2031
Verapamil	1	34.00 ± 7.91	25.05 ± 7.11	3	0.3196
Aspirin	1	35.62 ± 6.59	45.41 ± 8.28	5	0.0836
Indomethacin	1	38.43 ± 8.19	47.61 ± 4.93	6	0.4491
Dazmegrel	10	43.09 ± 9.13	23.50 ± 5.08	6	0.0096
Iloprost	0.01	32.59 ± 3.48	10.59 ± 5.10	4	0.0281
FPL 55712	1	42.25 ± 5.23	35.59 ± 3.25	6	0.3673

Table V.2 The effect of various drugs on PAF (18 *n*g/kg over 10 min)-induced ^{111}In-labelled platelet accumulation in the guinea-pig. Peak height has been calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts observed and represented as a percentage increase of the baseline intrathoracic counts. Drug effects were compared to saline controls in the same animal.

accumulation in the guinea-pig was not significantly altered by prior treatment with either bronchodilator, *ie.* the *beta*-adrenoceptor agonist isoprenaline (10 *u*g/kg) or the cholinergic antagonist ipratropium bromide (1 mg/kg) ($p > 0.05$) (Table V.2).

V.3.6 *Effect of Ca^{2+} Antagonists*

The two Ca^{2+}-channel blockers, verapamil (1 mg/kg) and diltiazem (1 mg/kg) had no significant effect on the PAF (180 *n*g/kg over 10 min)-induced ^{111}In-labelled platelet response in the guinea-pig ($p > 0.05$) (Table V.2).

V.3.7 *Effect of Arachidonate Metabolism Inhibition and Anti-Platelet Drugs*

Both cyclo-oxygenase inhibitors acetyl salicylic acid (1 mg/kg) and indomethacin (1 mg/kg) exerted no statistically significant effect on ^{111}In-labelled intrathoracic platelet accumulation induced by PAF (180 *n*g/kg over 10 min) ($p > 0.05$). The trend for both drugs however, was a potentiation of the response (Figure V.2 (e) and Table V.2). In contrast to this, the thromboxane synthetase inhibitor dazmegrel (10 mg/kg) and the stable prostacyclin analogue iloprost (10 *u*g/kg), significantly inhibited PAF (180 *n*g/kg over 10 min)-induced ^{111}In-labelled platelet accumulation in the guinea-pig ($p < 0.05$) (Figure V.2 (d) and Table V.2). The selective lipoxygenase inhibitor FPL 55712 (1 mg/kg), was without effect on the PAF (180 *n*g/kg over 10 min)-induced platelet response in the guinea-pig ($p > 0.05$) (Table V.2).

V.4 **Discussion**

Over the last few years PAF has gained widespread attention as a possible mediator of asthma as it can reproduce many of the characteristic features of this disease (Morley *et al.*, 1984; Cuss *et al.*, 1986) eliciting both bronchoconstriction (Vargaftig *et al.*, 1980; Cuss *et al.*, 1986) and long-lasting inflammatory changes in the lung (Camussi *et al.*, 1983*b*).

Furthermore, PAF has been detected in peripheral blood of asthmatics undergoing antigen provocation (Thompson *et al.*, 1984) and is generated in the lungs of asthma patients during exacerbations of the disorder (Page, 1988).

Although a number of mediators have inflammatory properties, the capacity of PAF to induce a sustained, non-selective increase in bronchial reactivity to spasmogens in both experimental animals (Mazzoni *et al.*, 1985*a*; Chung *et al.*, 1986) and man (Cuss *et al.*, 1986) has distinguished it from other substances that hitherto have been considered as mediators of asthma. It appears that PAF promotes hyperreactivity *via* platelet stimulation and the migration of platelets into the pulmonary parenchyma. Both the bronchospasm (Vargaftig *et al.*, 1980) and hyperreactivity (Mazzoni *et al.*, 1985*a*) that follows PAF exposure may be abolished by the selective immunological depletion of platelets. A factor released from PAF-stimulated platelets has recently been shown to induce airway hyperreactivity in thrombocytopenic guinea-pigs (Sanjar *et al.*, 1989). This mediator is PAF-specific as neither platelet disruption nor activation of platelets with ADP induced its generation. The chemical nature of this material remains unknown. Ketotifen and prednisolone have been shown to inhibit the airway hyperreactivity induced by PAF-stimulated platelet supernatants, whereas cromoglycate and aminophylline were without effect (Morley *et al.*, 1989*a*). Similarly, when ketotifen or prednisolone were incubated with PRP *in vitro* prior to the addition of PAF, the injection of supernatants into thrombocytopenic guinea-pigs resulted in reduced airway hyperreactivity (Morley *et al.*, 1989*a*).

PAF is an extremely potent platelet stimulus, since concentrations between 1-10 nM are sufficient to induce activation *in vitro* (Chignard *et al.*, 1979). *In vitro*, PAF causes shape change and Ca^{2+}-dependent release of granular contents (*eg.* serotonin and ADP) (Henson, 1976). Hence, release or generation of PAF *in vivo* will evoke a wide range of responses that are due to the biological activity of platelet activation products.

The ability of PAF to induce a progressive accumulation of ^{111}In-labelled platelets into the thoracic region of the guinea-pig has been reported in this and other studies (Chapter IV; Page *et al.*, 1982*b*; Robertson and Page, 1987; Smith *et al.*, 1989*a*). In the

present study, the systematic evaluation of a broad spectrum of drugs has been undertaken to provide information on the *in vivo* platelet response to PAF and shed some light on the role of platelets in PAF-induced pulmonary responses and hence their role in asthma.

A diverse range of chemical entities have been shown to act as PAF antagonists, including PAF analogues (Terashita *et al.*, 1983; Braquet and Godfroid, 1986; Braquet *et al.*, 1987), natural products, *eg.* kadsurenone (Shen *et al.*, 1985) and the ginkgolides (Braquet *et al.*, 1985*b*), triazolobenzodiazepines (Kornecki *et al.*, 1984; Casals-Stenzel, 1987*b*) and certain calcium channel blocking agents (Tuffin and Wade, 1985). To varying extents, all the PAF antagonists described are capable of inhibiting PAF-induced pathological effects both *in vitro* and *in vivo*, including the ability to inhibit PAF-induced hyperreactivity in guinea-pigs (Deeming *et al.*, 1985). Several PAF antagonists also inhibit allergic bronchospasm in experimental animals (Braquet *et al.*, 1985*a*; Darius *et al.*, 1986; Casals-Stenzel, 1987*a*). The ginkgolide preparation BN 52063 selectively inhibits PAF-induced wheal and flare responses in human skin and PAF-induced platelet aggregation *ex vivo* in man (Barnes *et al.*, 1987*a*).

PAF is believed to elicit platelet aggregation *via* a receptor-mediated process. Using radioligand binding assays, specific binding sites have been demonstrated on platelet membranes (Hwang *et al.*, 1983). In the present study, the PAF receptor antagonist SRI 63-441 (Handley *et al.*, 1986) inhibited ^{111}In-labelled platelet accumulation induced by an infusion of PAF in a dose-dependent manner (0.01-1 mg/kg). Brotizolam (1 mg/kg) (Casals-Stenzel, 1987*b*) also fully inhibited the PAF-induced platelet response in the guinea-pig.

The intravenous administration of selective histamine (H_1) antagonists can effect some bronchodilation in asthma; however, the suppression of allergen-induced responses by H_1 antagonists is modest in comparison with inhibition of the effects of inhaled histamine (Morley *et al.*, 1985*a*). The H_1 antagonist mepyramine is not an effective inhibitor of PAF-induced bronchospasm (Vargaftig *et al.*, 1982) and has no effect on PAF-induced hyperreactivity in the guinea-pig (Morley *et al.*, 1985*a*). Similarly, the 5-HT antagonist methysergide has no effect on PAF-induced bronchoconstriction (Vargaftig *et*

al., 1982). In the present study, neither the selective H_1 antagonist mepyramine nor the combined H_1 and 5-HT antagonist cyproheptadine, had any effect on PAF-induced ^{111}In-labelled platelet accumulation in the guinea-pig, indicating the lack of involvement of both histamine and serotonin in this response.

Clinically used prophylactic anti-asthma drugs include disodium cromoglycate (DSCG), ketotifen, glucocorticoids and theophylline. The ability of these drugs to prevent PAF-induced airway hyperreactivity in guinea-pigs (Mazzoni *et al.*, 1985*b*) by inhibition of PAF-induced pathology provides a basis for their prophylactic efficacy. This property is not shared by drugs that are ineffective as prophylactic agents in asthma (Mazzoni *et al.*, 1985*b*).

The chief effect of DSCG in animal experimental models is inhibition of mediator release in response to IgE antigen-antibody interactions and non-immunological stimuli (Orr and Cox, 1969). The paramount therapeutic effect of inhaled DSCG is its ability to suppress both immediate- and late-onset asthmatic responses after provocation by a variety of allergens (Pepys *et al.*, 1968; Booij-Noord *et al.*, 1971; Lowhagen and Rak, 1985). Cromoglycate is however, ineffective in inhibiting PAF-induced bronchoconstriction in experimental animals (Morley and Aoki, 1986). DSCG prevents the impairment of mucociliary transport induced by specific bronchial challenge (Wanner, 1979). It stabilises the mast cell membrane during the antigen-dependent and activation stages of mast cell mediator release, but the cellular actions are not understood. DSCG has also been shown to directly inhibit the activation of human neutrophils and eosinophils *in vitro* (Moqbel *et al.*, 1986). It has been postulated that DSCG inhibits the bronchoconstriction induced by a number of stimuli by modulating reflex activity in the airways (Kay, 1987).

Clinical evaluation of ketotifen, a benzocyclopheto-thiophene with potent H_1 antagonist activity (Craps, 1980), has revealed efficacy in asthma, by comparison with placebo or with other H_1 antagonists (Martin and Romer, 1978; Craps, 1980). As ketotifen lacks direct bronchodilator activity and H_1 antagonism *per se* confers no long-lasting benefit in asthma, an additional property must account for its prophylactic efficacy (Morley *et al.*, 1985*a*). Inhibition of mediator release in IgE-dependent allergic responses,

suppression of bronchoconstrictor responses to SRS-A and PAF, reversal of agonist-induced desensitisation to *beta*-adrenergic agents and impairment of calcium influx into smooth muscle have been identified as properties of ketotifen (Craps, 1980). Efficacy of ketotifen in asthma therapy could depend on these effects, but such rationale is not predictive of efficacy for other prophylactic anti-asthma drugs and does not anticipate the failure of classes that are not prophylactic in asthma (*eg.* $beta_2$-adrenergic agonists, mast cell stabilising agents and lipoxygenase enzyme inhibitors).

Corticosteroids, whether inhaled (Burge *et al.*, 1982; Kraan *et al.*, 1985) or administered orally (Booij-Noord *et al.*, 1971; Bhagat and Grunstein, 1985), inhibit the allergen-induced late asthmatic reaction and non-specific bronchial responsiveness. They are however, without effect on the bronchoconstrictor response to PAF in guinea-pigs (Morley and Aoki, 1986). Many mechanisms are involved in the anti-allergic effect of the glucocorticoids, including the formation and release of the anti-phospholipase A_2 (PLA_2) proteins *eg.* macrocortin (Blackwell *et al.*, 1982). It is thought that macrocortin is an active fragment of lipomodulin and both proteins inhibit the release of arachidonic acid by inhibiting PLA_2 on the mast cell membrane. As a consequence, in anaphylactic reactions the biosynthesis of prostaglandins and leukotrienes is inhibited. PLA_2 activity is inhibited over time by steroidal anti-inflammatory drugs (Packham and Mustard, 1986). Glucocorticoids have been shown to potentiate the *beta*-adrenergic receptor by increasing receptor density (Mano *et al.*, 1979) and to affect membrane lipids *ie.* enhance the biosynthesis of phosphatidylcholine in the lung, a surface-active agent which prevents adherence due to surface tension of the inner surface of the lung. Cholesterol and fatty acid synthesis is also affected. Therefore, these drugs exert some control over membrane fluidity and the regulation of membrane enzyme activity (Duval *et al.*, 1983). Glucocorticoids have been shown to increase neutrophil numbers and decrease numbers of lymphocytes, eosinophils, monocytes and basophils. Inhibition of aggregation of rabbit platelets *in vitro* has been reported by the steroid methylprednisolone, possibly *via* effects on platelet membrane lipids (Cazenave *et al.*, 1979).

The methylxanthines (MXs) theophylline and its ethylenediamine salt,

aminophylline, were initially thought to act only as bronchodilators, rapidly reversing bronchial smooth muscle contraction. Trials matching theophylline against DSCG however, indicate an additional long term prophylactic role (Godfrey, 1980). Other potentially significant actions of theophylline include the inhibition of PAF-induced bronchospasm in experimental animals (Morley *et al.*, 1985*a*), pulmonary vascular dilation, stimulation of mucociliary clearance, inhibition of anaphylactic mediator release and suppression of mediator-induced oedema (Persson, 1980). MXs prevent the hydrolysis of cAMP by inhibiting phosphodiesterase (PDE) activity in airway smooth muscle (Freyss-Beguin *et al.*, 1983), however, evidence suggests that this is not the mechanism responsible for the prophylactic activity of theophylline in asthma. Plasma theophylline concentrations obtained from treated patients are too low to inhibit the PDE activity isolated from human lung and bronchial smooth muscles (Holgate *et al.*, 1984*b*) and there is a weak correlation between the extent of PDE inhibition and the magnitude of relaxation of airway muscle (Fredholm *et al.*, 1979). Furthermore, dipyridamole, a potent PDE inhibitor, does not produce bronchodilatation (Ruffin and Newhouse, 1981). At concentrations 20- to 100-fold lower than those required to effectively inhibit PDE activity, MX derivatives are competitive antagonists of adenosine A_1- and A_2-receptors (Fredholm and Persson, 1982). Adenosine, a naturally occurring purine nucleoside formed from the cleavage of AMP by 5'-nucleotidase (Holgate *et al.*, 1984*b*), causes bronchoconstriction either by enhancing the IgE-dependent mediator release from mast cells, stimulating bronchoconstrictor neural reflexes or by a direct effect on airway smooth muscle (Holgate *et al.*, 1984*b*).

In the present study, aminophylline was the only prophylactic anti-asthma drug tested to significantly reduce the extent of ^{111}In-labelled platelet accumulation induced by PAF infusion in the guinea-pig thorax. DSCG, ketotifen, the new Sandoz anti-asthma drug AH 21-132 and the glucocorticoid prednisolone were all without significant effect on this response. These results are in marked contrast to those of Deeming and colleagues (1986) who reported that prophylactic anti-asthma drugs impair PAF-induced platelet accumulation in the guinea-pig.

Bronchodilators are widely used in the treatment of allergic bronchospasm. Studies

on immunologically challenged human lung fragments (Church and Young, 1983; Hughes *et al.*, 1984) and dispersed human lung cells (Peters *et al.*, 1982; Holgate *et al.*, 1984) have shown that *beta*$_2$-adrenoceptor agonists are potent inhibitors of mast cell mediator release, in clear contrast to the relatively weak inhibitory effect DSCG exerts on immunological mediator release from human lung mast cells (Peters *et al.*, 1982). *Beta*$_2$-adrenoceptor agonists inhibit the immediate asthmatic reaction to inhaled antigen (Howarth *et al.*, 1985) and PAF (Morley *et al.*, 1985*b*), although it is not known how much of this effect is due to functional antagonism of bronchoconstriction and how much is due to inhibition of mast cell mediator release. Administration of salbutamol and DSCG by inhalation to patients with allergic asthma has been found to inhibit the increase in plasma histamine in parallel with the inhibition of bronchoconstriction induced by bronchial allergen provocation (Howarth *et al.*, 1985). Thus, both *in vitro* and *in vivo* *beta*$_2$-agonists are highly potent mast cell stabilising agents. It follows that mast cells are not obligatory for either airway inflammation or non-specific bronchial responsiveness as *beta*$_2$-agonists cannot reduce these events. *Beta*$_2$-adrenoceptor agonists are unable to modify the allergen-induced late asthmatic reaction (Hegardt *et al.*, 1981) and when used for prolonged periods in high doses, unable to influence the state of non-specific bronchial responsiveness (Peel and Gibson, 1980; Burge *et al.*, 1982). It is now recognised that the mast cell is probably only involved in the pathogenesis of the immediate asthmatic reaction, whether provoked by inhaled allergen (Holgate *et al.*, 1987) or by exercise (Anderson, 1985). Therefore, as inhibitors of mast cell mediator release, *beta*$_2$-agonists are only likely to to influence this component of the response.

Both relaxation in the tracheal muscle and the inhibition of mediator release are mediated by an increase in tissue cyclic AMP. Stimulation of the *beta*$_2$-receptor activates the enzyme adenylate cyclase which converts intracellular ATP to cAMP. The increase in cAMP is thought to act by enhancing the binding of intracellular Ca^{2+} to the cell membrane and endoplasmic reticulum. The myoplasmic Ca^{2+} is reduced and relaxation occurs (Schneid *et al.*, 1979). *Beta*-adrenergic agents also reduce mucous gland secretions and increase cilial beat frequency, enhancing mucociliary clearance from the lungs.

The mechanism by which the synthetic antimuscarinic agent ipratropium bromide (a derivative of methylatropine) prevents acute asthma is thought to be inhibition of the vagal reflex elicited *via* stimulation of irritant receptors on the airways. It is also effective in suppressing the formation of TXA_2 in isolated guinea-pig lung provoked by histamine or SRS-A (Berti *et al.*, 1980). The bronchodilator effect of ipratropium bromide has been shown to be similar to that achieved with salbutamol (Leahy *et al.*, 1983). Combined use of ipratropium bromide and *beta*$_2$-agonists produces greater bronchodilatation than with either drug alone (Ward *et al.*, 1981; Pierce *et al.*, 1982; Bryant, 1985).

Neither bronchodilator evaluated in the present study, *ie.* isoprenaline or ipratropium bromide, exerted any significant effect on PAF-induced intrathoracic ^{111}In-labelled platelet accumulation in the guinea-pig.

Calcium ions entering into the cytoplasm act as a trigger for the contraction of bronchial smooth muscle cells. Ca^{2+}-channel or Ca^{2+}-entry blockers competitively antagonise the transmembrane transport of extracellular Ca^{2+} (Cohn, 1982) and have an anti-anaphylactic effect on smooth muscle tension developed in sensitised guinea-pig lung exposed to antigen (Weiss *et al.*, 1982) and inhibit histamine-induced contraction of guinea-pig (Fanta *et al.*, 1982) and human (Drazen *et al.*, 1983; Henderson *et al.*, 1983*b*) bronchial smooth muscle. They have also been shown to inhibit antigen-induced histamine release from mast cells (Ennis *et al.*, 1983) and sensitised human lung (Cerrina *et al.*, 1982). Experiments in intact animals have demonstrated inhibition of anaphylaxis (Brugman *et al.*, 1983; Russi *et al.*, 1983) and also inhibition of citric acid and methacholine-induced bronchoconstriction (Brugman *et al.*, 1983) following aerosol administration of Ca^{2+} antagonists. In human subjects, Ca^{2+} channel blockers have been shown to inhibit exercise- (Cerrina *et al.*, 1980; Barnes *et al.*, 1981; Corris *et al.*, 1983) and cold air- (Henderson *et al.*, 1983*a*) induced bronchoconstriction and to reduce antigen-induced broncoconstriction in sensitised individuals (Henderson *et al.*, 1983*b*; Miadonna *et al.*, 1983). Evidence of a direct bronchodilating effect of these agents has been reported in subjects with bronchial asthma (Patakas *et al.*, 1983).

In platelets, low concentrations of verapamil have been shown to block the

transmembrane Ca^{2+} flux in response to adrenaline (Owen *et al.*, 1980; Owen and Le Breton, 1981). When Ca^{2+} influx was blocked by verapamil, both intracellular Ca^{2+} mobilisation in response to adrenaline and *in vitro* adrenaline-induced aggregation were inhibited (Owen *et al.*, 1980; Owen and Le Breton, 1981). These findings suggest that adrenaline causes entry of Ca^{2+} into platelets to induce Ca^{2+} mobilisation from a cellular store within the platelet. In contrast, it is postulated that other aggregating agents such as ADP, collagen and the Ca^{2+} ionophore A23187 cause activation by mobilisation of Ca^{2+} from cellular stores (*eg.* superficial binding sites and the dense tubular system) without Ca^{2+} entry and much higher concentrations of verapamil are required to inhibit *in vitro* aggregation induced by these agents (Ikeda *et al.*, 1981). These higher concentrations of verapamil may be affecting cellular mobilisation of Ca^{2+} as occurs in smooth muscle (Church and Zsoter, 1980). The administration of verapamil to healthy volunteers has been demonstrated to inhibit platelet aggregation caused by adrenaline, collagen and ADP *ex vivo* (Ikeda *et al.*, 1981). In view of these findings, the failure of both verapamil and diltiazem to significantly inhibit *in vivo* platelet accumulation induced by PAF in the present study, suggests that PAF elicits aggregation *in vivo* without the requirement of Ca^{2+} influx. The secretory process that ensues following the exposure of platelets to PAF *in vitro* has been shown to be associated with a rise in intracellular Ca^{2+}. Since different platelet stimuli elevate internal Ca^{2+} by distinct processes (Sage and Rink, 1986*a*; 1986*b*), it is possible that the effect of PAF on Ca^{2+} movement includes a component unique to this stimulus which, as suggested by the present study, is resistant to calcium channel blockade. Diltiazem has been reported to display weak PAF antagonistic properties (Tuffin and Wade, 1985), however, it appears from the present study that the PAF antagonism exerted is not sufficient to prevent PAF-induced *in vivo* platelet accumulation (*cf.* SRI 63-441 and brotizolam).

Exposure of a sensitised isolated lung preparation to allergen results in the release of arachidonic acid products (Piper and Vane, 1971). PGF_{2alpha} is a potent spasmogen of airway smooth muscle and evokes bronchoconstriction when inhaled by asthmatic subjects (Mathe *et al.*, 1973). It has since been established that several cyclo-oxygenase products

may contribute to bronchoconstriction in response to allergen. PGD_2, PGE_2, PGI_2, PGF_{2alpha} and TXA_2 are generated following IgE-dependent activation of a range of cell types, including macrophages, eosinophils and platelets as well as mast cells and basophils (Morley *et al.*, 1984). There is however, no clear indication as to the extent to which these various materials are generated during the response of an asthmatic subject to allergen inhalation.

The common property of non-steroidal anti-inflammatory drugs (NSAIDs) is their capacity to inhibit metabolism of arachidonic acid by the inactivation of cyclo-oxygenase enzymes. NSAIDs show no effect on allergen-induced bronchospasm (Fairfax *et al.*, 1983), yet they are potent inhibitors of the late reaction to allergen inhalation (Salmon *et al.*, 1984). The effect of aspirin and indomethacin on the late reaction reveals that cyclo-oxygenase products (*eg.* TXA_2) may contribute to the symptoms of the sustained reaction to allergen. In the guinea-pig, aspirin and indomethacin have been shown to enhance PAF-induced increased hyperreactivity (Mazzoni *et al.*, 1985*b*; Anderson and Fennessy, 1988). It is possible that this enhancement by cyclo-oxygenase inhibitors may be due to shunting of arachidonic acid metabolism towards lipoxygenase pathways after PAF challenge.

It has been reported that guinea-pig platelets do not generate TXA_2 when stimulated with PAF *in vitro* (Vargaftig *et al.*, 1980) and that aspirin has no effect on PAF-induced aggregation or release in guinea-pig platelets *in vitro* (Vargaftig *et al.*, 1980). In accordance with these findings, neither aspirin nor indomethacin in the present study inhibited ^{111}In-labelled platelet accumulation in the guinea-pig. However, the effects of both nonsteroidal anti-inflammatory drugs evaluated in this study parallel their documented effects on PAF-induced hyperreactivity in the guinea-pig (Mazzoni *et al.*, 1985*b*; Anderson and Fennessy, 1988) *ie.* both drugs caused mild (not statistically significant) potentiation of the PAF-induced platelet response.

This augmented response may due to stimulation of the lipoxygenase pathway as a result of cyclo-oxygenase inhibition in platelet and/or endothelial cells, leading to the formation of possible platelet active lipid products, which in conjunction with PAF stimulation, enhance platelet accumulation. Alternatively, the inhibition of arachidonate

metabolism in endothelial cells may dampen the endogenous inhibitory control mechanism provided by prostacyclin (PGI_2) and result in an exaggerated response. If, on the other hand, platelet TXA_2 is an important mediator involved in PAF-induced platelet activation in the guinea-pig, the results may be explained by platelet cyclo-oxygenase being less sensitive to inhibition by aspirin and indomethacin than that of the corresponding endothelial cell enzyme. This would result in a relative abundance of the highly pro-aggregatory TXA_2 and a deficiency of the anti-aggregatory PGI_2, permitting the development of a greater extent of platelet accumulation than would be achieved by PAF alone.

In the dog, PAF-induced increased airway responsiveness is prevented by the thromboxane synthetase inhibitor OKY 046 (Chung *et al.*, 1986), suggesting the involvement of thromboxane in this species. In the present study, dazmegrel, a potent and selective thromboxane synthetase inhibitor (Vermylen *et al.*, 1981; Fitzgerald and Oates, 1984), significantly inhibited ^{111}In-labelled platelet accumulation in the guinea-pig in response to PAF. This finding may implicate the involvement of TXA_2 in PAF-induced platelet accumulation in this species, contrary to previous reports. However, as dazmegrel did not completely abolish the PAF-induced platelet response, other mechanisms of activation must be involved. Alternatively, PGD_2 and PGE_2, both produced in excess when TXA_2 production is impaired, may, respectively, potentiate and inhibit stimulation by cyclic endoperoxides (Defreyn *et al.*, 1982; Smith, 1982; Bertele *et al.*, 1984; Gresele *et al.*, 1984; Rajtar *et al.*, 1985). Thus, reorientation of cyclic endoperoxide metabolism towards other prostanoic derivatives following dazmegrel administration, may have modulated platelet function in the present study.

In guinea-pig, PGI_2 inhibits PAF-induced platelet aggregation and release *in vitro* (Vargaftig *et al.*, 1980) and ADP-induced platelet accumulation *in vivo* (Page *et al.*, 1982*b*). The finding that PGI_2 suppresses PAF-induced bronchoconstriction (Vargaftig *et al.*, 1980) yet fails to relax guinea-pig bronchial smooth muscle directly (Vargaftig and Lefort, 1979) is further evidence that PAF-induced bronchoconstriction in the guinea-pig dependent upon *in vivo* platelet stimulation. These platelet and platelet-dependent

bronchial effects of PAF are therefore under the modulating influence of cAMP-adenylate cyclase system (Best *et al.*, 1977; Gorman *et al.*, 1977; Tateson *et al.*, 1977).

Inhibition of PAF-induced hyperreactivity in the guinea-pig by the stable prostacyclin mimetic iloprost has been reported recently (Smith *et al.*, 1989*b*), even though PAF-induced intrathoracic platelet accumulation was not diminished (Smith *et al.*, 1989*b*). This finding conflicts with that of the present study where a similar dose of iloprost significantly reduced the extent of ^{111}In-labelled intrathoracic platelet accumulation in the guinea-pig. The authors suggest that hyperreactivity is mediated *via* a material generated by the interaction between PAF and platelets (Smith *et al.*, 1989*b*). This postulate was substantiated (Sanjar *et al.*, 1989) and the generation of the platelet-derived factor was inhibited by incubation of PRP with iloprost prior to activation with PAF (Sanjar *et al.*, 1989).

Leukotrienes (LTs) are synthesised by several cell types by lipoxygenase-dependent metabolism of arachidonic acid. LTB_4 and the peptido-leukotrienes LTC_4, LTD_4 and LTE_4 are present in slow-reacting substance of anaphylaxis (SRS-A), a spasmogen formed during the acute IgE-dependent reaction to allergen in various tissues, including human lung (Piper, 1980). Synthetic LTs produce sustained contraction of human airway smooth muscle *in vitro* (Leitch and Drazen, 1984). Bronchoconstriction (Holroyde *et al.*, 1981) but not increased airway hyperreactivity (Griffin *et al.*, 1983) follows the inhalation of LTs by asthmatic subjects. FPL 55712, a cromolyn derivative, is a potent and selective antagonist of SRS-A. At higher concentration it inhibits thromboxane synthetase and the antigen-induced release of histamine from rat peritoneal mast cells (Welton *et al.*, 1981). With non-atopic volunteers FPL 55712 partly inhibits LTC_4- and LTD_4-induced bronchoconstriction (Holroyde *et al.*, 1981) and inhibits antigen-induced histamine release in the rat (Buckle *et al.*, 1979). FPL 55712 has been shown to inhibit PAF-induced bronchoconstriction *in vivo*, but only when administered at a non-specific dose (10 mg/kg i.v.) (Lewis *et al.*, 1984). Prevention of PAF-induced hyperreactivity however, has been demonstrated by FPL 55712 in the guinea-pig (Anderson and Fennessy, 1988).

In the present study, FPL 55712 had no significant effect on PAF-induced

intrathoracic ^{111}In-labelled platelet accumulation. From these results it appears that the platelet accumulation induced in the guinea-pig is not dependent on the generation of the lipoxygenase products LTC_4 and LTD_4.

The ability of prophylactic anti-asthma drugs to inhibit PAF-induced airway hyperreactivity in guinea-pigs provides a basis for their efficacy, as it is a property not shared by drugs that are ineffective as prophylactic agents in asthma (Mazzoni *et al.*, 1985*b*). It appears from the present study that the anti-platelet activity of aminophylline is not related to its efficacy as a prophylactic anti-asthma agent since other prophylactic anti-asthma drugs evaluated in this system, cromoglycate, ketotifen, AH 21-132 and prednisolone were without effect on PAF-induced platelet accumulation. It follows that these drugs are not prophylactic in asthma as a result of their ability to suppress intrathoracic platelet accumulation. Similarly, the inability of inhibitors of PAF-induced bronchoconstriction to impair PAF-induced platelet accumulation implies that the benefit they confer in the immediate asthmatic response is not dependent on their ability to prevent platelet activation. In view of the fact that both PAF-induced bronchospasm (Vargaftig *et al.*, 1980) and hyperreactivity (Mazzoni *et al.*, 1985*a*) are platelet dependent, the findings of the pesent study suggest that it is not platelet accumulation *per se* that contributes to these phenomena. Release of an as yet unidentified factor from platelets induced by exposure to PAF could account for the PAF-induced pulmonary effects (Sanjar *et al.*, 1989; Smith *et al.*, 1989*b*). However, not all prophylactic anti-asthma drugs are capable of inhibiting hyperreactivity induced by this factor (Morley *et al.*, 1989*a*). It is apparent that PAF-induced bronchopulmonary responses and circulating platelets are closely linked, however, the precise relationship remains to be elucidated.

In platelets, an elevation of the intracellular level of cAMP has an inhibitory effect (Mills and Smith, 1971). Cyclic AMP is produced by the enzyme adenylate cyclase (AC) and degraded by the enzyme cAMP phosphodiesterase (PDE). Therefore, agents which either stimulate AC or block PDE will inhibit platelet activation. Aminophylline has been shown to inhibit PDE in platelets (Mills and Smith, 1971) and in the present study it inhibited intrathoracic ^{111}In-labelled platelet accumulation in the guinea-pig. Like PGI_2,

the stable prostacyclin analogue iloprost activates AC (Sturzebecher and Losert, 1987) and significantly inhibited PAF-induced platelet activity. Isoprenaline has also been shown to activate AC, yet in the present study its inhibitory action on the PAF-induced platelet response was not statistically significant.

The findings of this study strongly suggest that the PAF-induced platelet response in the guinea-pig is under the control of the cAMP-adenylate cyclase system.

CHAPTER VI

A RADIOISOTOPIC MODEL FOR INVESTIGATING PLATELET AGGREGATION IN THE RABBIT

VI.1 **Introduction**

A non-invasive technique for the automated continuous external imaging of ^{111}Indium (^{111}In)-labelled platelets in the guinea-pig has been described in Chapter IV of this thesis. Since the rabbit is a useful model for investigating a number of platelet related disorders such as atherosclerosis (Duff *et al.*, 1957; West *et al.*, 1982; Jayakody *et al.*, 1988), an attempt has been made to modify the model of *in vivo* platelet aggregation for use in this species.

There is some evidence to suggest that the subcellular localisation of ^{111}Indium in rabbit platelets is not cytosolic as in other mammalian platelets, but is associated with the platelet dense granules (Baker *et al.*, 1982) and that release of the ^{111}In may occur upon stimulation of the platelets *in vitro*. Recent studies however, reveal that ^{111}In can be used to radiolabel rabbit platelets *in vivo* and the ^{111}In is not lost from the platelet over several hours, even after repeated platelet activation (May *et al.*, 1990).

The aim of this study was therefore to characterise the *in vivo* response of ^{111}In-labelled platelets in the rabbit to the intravenous administration of various platelet agonists *ie.* adenosine diphosphate (ADP), collagen, platelet activating factor (PAF), thrombin and serotonin (5-HT). The effect of a variety of drugs on the PAF-induced response was then investigated in this model as a corollary to the findings in the guinea-pig which was described in the previous chapter (Chapter V).

VI.2 **Methods**

VI.2.1 *Animals*

New Zealand White rabbits of either sex and ranging between 1.8-3.2 kg body weight were used throughout the study. Test animals were anaesthetised with a combination of diazepam (5 mg/kg i.p.) followed 10 min later by Hypnorm (0.4 ml/kg i.m.). Two marginal ear veins were cannulated (Venisystems Butterfly-25; 19.1 mm length, 0.4 mm internal diameter) for the administration of platelet agonists and other drugs, and ^{111}In-labelled platelets or ^{111}In-labelled erythrocytes. ^{111}In-labelled blood cells were allowed to equilibrate for at least 30 min before challenge with the platelet stimuli.

VI.2.2 *Drugs/Chemicals*

Diazepam (Valium) (Hoffmann LaRoche, Basel, Switzerland); Hypnorm (0.315 mg fentanyl citrate/ml and 10 mg fluanisone/ml) (Janssen Pharmaceutica). Adenosine diphosphate (ADP) disodium salt (Sigma), serotonin (5-hydroxytryptamine, 5-HT) (Sigma), thrombin (Sigma), SRI 63-441 (*cis* (±)-1[2-[hydroxy [tetrahydro-5 [(octadecylamino-carbonyl)oxy] methyl]furan-2-yl]methoxy-phosphinyloxy]ethyl]-quinolinium hydroxide, inner salt) (Sandoz, Basel, Switzerland), iloprost (Schering) and sodium heparin (148 I.U./mg) (Hoffmann LaRoche) were dissolved in physiological saline (0.9% w/v). Platelet activating factor (PAF) (1-*o*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (Bacham, Bubensdorf, FRG) was dissolved in ethanol and kept as a stock solution (1 mg/ml), which was diluted in a solution of bovine serum albumin (BSA) (0.25% w/v) (Sigma) in saline (0.9% w/v) as required. Acetyl salicylic acid (ASA, aspirin) (Sandoz) and indomethacin (Sigma) were dissolved in 200 *u*l $NaHCO_3$ and diluted with tris buffer (10% w/v). Dazmegrel (3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid) (Pfizer) was dissolved in 0.1 M NaOH. Collagen (Hormon-Chemie, Munich, FRG) was diluted in isotonic glucose buffer (pH 2.7). PGE_1 (Sigma) was dissolved in ethanol. ^{111}Indium oxine (^{111}In, 37 MBq/ml) was obtained from Amersham International (Amersham, England).

VI.2.3 *Preparation of ^{111}Indium-Labelled Platelets*

The procedure for labelling rabbit platelets with ^{111}Indium-oxine was similar to that previously described in the guinea-pig in Chapter IV, with slight modifications. Blood from the marginal ear vein of the conscious rabbit was collected into 0.1 M trisodium citrate (10% v/v) and centifuged (200 *g*, 10 min) to yield platelet-rich plasma (PRP). PRP was then recentrifuged (1,000 *g*, 10 min) in the presence of PGE_1 (300 *n*g/ml) and the platelet pellet was resuspended in 1.5 ml calcium-free Tyrode's solution containing PGE_1 (CFTP). The platelets were incubated with ^{111}In oxine (1.5 MBq) at 37^{O}C for 1.5 min and the resultant ^{111}In-labelled platelets were recentrifuged (1,000 *g*, 10 min). The free ^{111}In oxine was removed and the ^{111}In-labelled platelet pellet was washed twice with 1 ml CFTP before being resuspended in CFTP (1 ml per recipient rabbit). One ml aliquots of the platelet suspension were administered intravenously into both the anaesthetised donor and recipient rabbits, followed by 1 ml of heparinised saline to maintain patency of the cannula.

VI.2.4 *Preparation of ^{111}Indium-Labelled Erythrocytes*

Blood was collected and PRP obtained as described for platelets. The PRP was discarded and a 1ml aliquot of packed erythrocytes was removed and washed in 10 ml CFTP. Erythrocytes were pelleted by centrifugation (1,000 *g*, 10 min) and the supernatant discarded. The pellet was washed with 1 ml CFTP and resuspended in 1 ml of the same solution by gentle shaking. ^{111}In oxine (1.5 MBq) was added to the erythrocyte suspension and incubated for 1.5 min at 37^{O}C. After centrifugation (1,000 *g*, 10 min), the supernatant containing unbound ^{111}In oxine was discarded and erythrocytes were resuspended in CFTP (1 ml per recipient animal). Aliquots of 1 ml were injected intravenously into the two anaesthetised test animal preparations as described for platelet injection.

VI.2.5 *Administration of Platelet Agonists*

Each of the platelet agonists ADP, collagen, PAF, thrombin and serotonin were

administered as a bolus intravenous dose *via* the ear vein cannula and flushed through with 0.3 ml of physiological saline (0.9% w/v).

VI.2.6 *Dose-Response Studies*

Animals received one platelet stimulus only, at a range of doses (except in the case of thrombin). In animals receiving ADP, PAF or 5-HT, a minimum time interval of 30 min was maintained between successive doses to ensure the return of platelet responsiveness. Doses were administered in a randomised fashion in different animals.

In animals receiving collagen, a period of 60 min was allowed to elapse between each dose. If thoracic count rates had not returned to pre-injection levels within this time (as often was the case at high doses), dosing was not continued in that particular animal.

An initial priming dose of ADP (100 *u*g/kg) was administered 30 min prior to the thrombin administration. Only one dose of thrombin was given *per* animal as responses did not necessarily return to pre-injection levels and hence were not reproducible if repeated in the same animal.

At the end of an experiment, animals were sacrificed by a lethal i.v. injection of sodium pentobarbitone.

VI.2.7 *Effects of Drugs*

Test drugs were administered as intravenous bolus doses 1 min prior to the administration of the platelet stimulus (ADP (100 *u*g/kg i.v.), PAF (100 *n*g/kg i.v.) or collagen (100 *u*g/kg i.v.)) to assess their effects on ^{111}In-labelled platelet responses.

VI.2.8 *Monitoring of ^{111}Indium-Labelled Blood Cells*

Spontaneously breathing rabbits were placed supine. Sodium iodide crystal scintillation detectors (1 inch crystal, DM1-1, Nuclear enterprises) were housed in lead

collimators (18 mm thick) with a circular aperture (33 mm) at the crystal face. Circulating ^{111}In-labelled platelets or ^{111}In-labelled erythrocytes were continuosly monitored in the thoracic, cranial and hindlimb regions using probes located over the thorax (to monitor the pulmonary circulation), over the hindlimb (to monitor the peripheral circulation) and against the head (to monitor the cerebral circulation) (Figure VI.1). The hindlimb was chosen in preference to the abdomen (as described for the guinea pig, Chapter IV) as it provided a large enough area to monitor platelet and red blood cell events at a distance which was removed from any possible interference from liver- or spleen-associated radioactivity. Two experiments (*ie.* 2 rabbits) were conducted simultaneously and counts were estimated as previously described in Chapter IV *ie.* with 2 dual channel gamma spectrometers and logged with the aid of a special application interface with a microcomputer (IBM AT3).

The kinetics of the intrathoracic ^{111}In-labelled platelet accumulation were not identical for every stimulus, thus different time sequences were utilised to monitor the response to each agonist, *ie.* radioactive counts were summated at 2 sec intervals over a 5 min period following administration of a bolus dose of ADP; 10 sec intervals over a 30 min period following a bolus dose of collagen; 5 sec intervals over a 15 min period following a bolus dose of PAF; 5 sec intervals over a 15 min period following a bolus dose of 5-HT; or 10 sec intervals for at least 150 min following a bolus dose of thrombin.

VI.2.9 *Data Analysis*

Platelet accumulation was measured as an increase in radioactive counts/sec by the detectors over the thoracic, hindlimb and cranial regions. Results are presented as a percentage change in radioactive counts (calculated by subtracting the baseline counts from the maximal change in counts and converting this change to a percentage of the baseline counts). Data are expressed as a mean percentage change $\pm$ standard error of the mean (sem) of n observations. Significance was assessed statistically by the Student's t-test. A p value of less than 0.05 was considered significant.

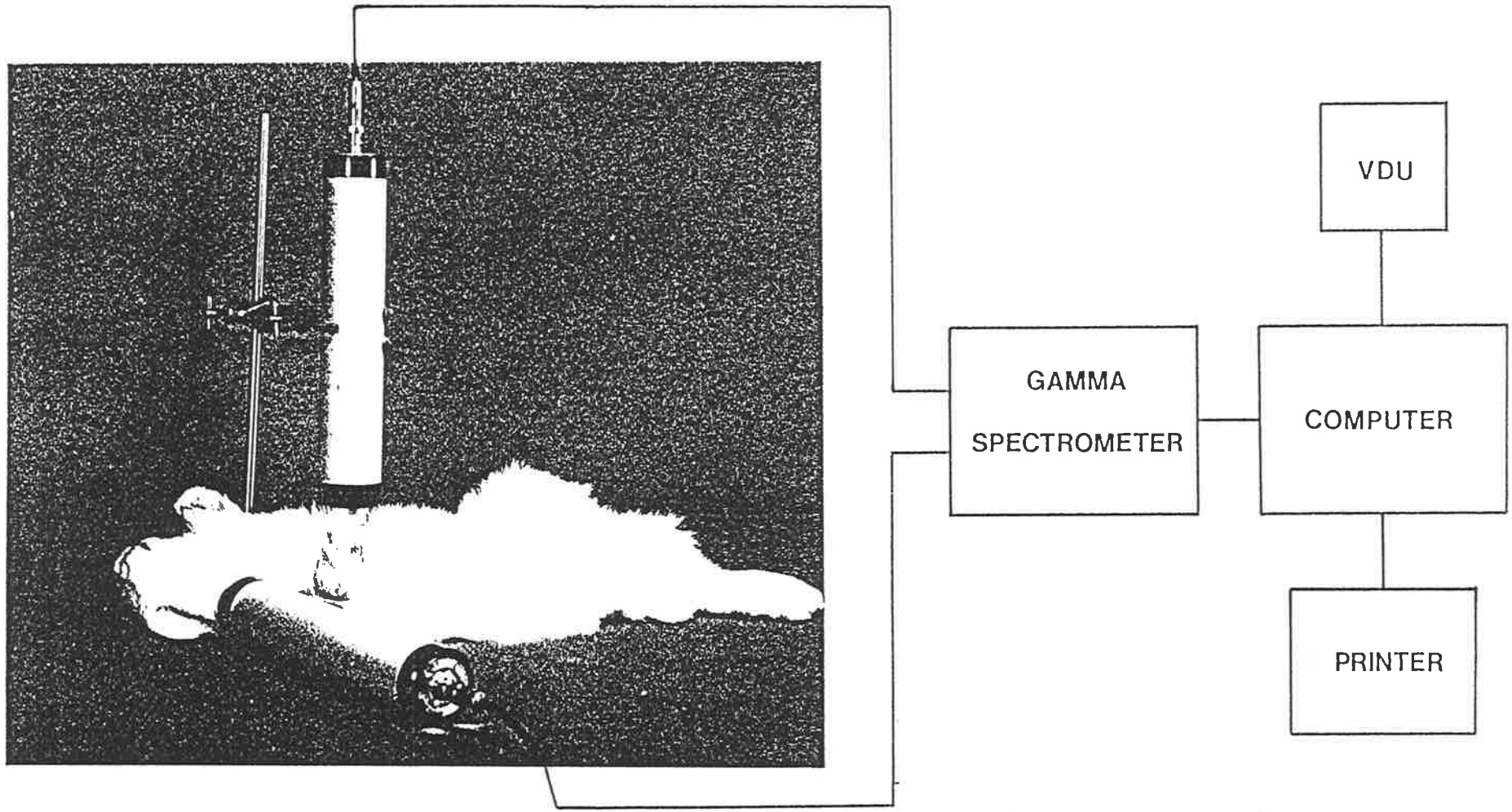


Figure VI.1 This shows the location of the crystal scintillation probes over the thoracic and cranial regions of an anaesthetised rabbit (hindlimb probe is not shown). The radioactive counts detected by these probes are estimated by a dual channel gamma spectrometer and logged with the aid of a special application interface with a microcomputer (see methods).

VI.3 **Results**

VI.3.1 *Control Responses*

The intravenous injection of saline (0.9% w/v) (the control vehicle for ADP, 5-HT and thrombin), CFTP buffer (the control for collagen) or BSA saline (the control for PAF) produced no significant changes in the thoracic, hindlimb or cranial count rates in rabbits that had been administered ^{111}In-labelled platelets (Table VI.1) or ^{111}In-labelled erythrocytes (Table VI.2).

VI.3.2 *Response to ADP*

The response to ADP administration (100 *ug*/kg) was transient. An abrupt increase of the thoracic count rate was observed in animals that had received ^{111}In-labelled platelets (Figure VI.2 (a) and Table VI.1), but not in animals that had received ^{111}In-labelled erythrocytes (Figure VI.2 (a) and Table VI.2). In both the cranial and hindlimb regions a concomitant reduction in platelet-associated radioactivity followed the i.v. ADP injection (Figure VI.2 (a) and Table VI.1), whereas there was no change in counts in these areas in animals that had received ^{111}In-labelled RBCs (Table VI.2). These observations are consistent with selective entrapment of platelets within the pulmonary vasculature. Maximal retention of platelets within the thorax (54.20 $\pm$ 6.26% ; n=11) was achieved within 20-30 sec after injection of ADP (100 *ug*/kg) and returned to pre-injection levels within 2 min. When a range of ADP doses (0.56-100 *ug*/kg) was used, the peak height of the responses in all regions were dose-related (Figure VI.3 and Table VI.1).

VI.3.3 *Response to Collagen*

Intravenous injection of collagen fibrils (100 *ug*/kg) produced a small but significant increase in the thoracic count rate when ^{111}In-labelled erythrocytes were used as an intravascular marker (30.69 $\pm$ 10.37% ; n=5) (Figure VI.2 (b) and Table VI.2). However,

AGONIST	DOSE	n	PEAK HEIGHT (mean ± sem) THORAX	HINDLIMB	HEAD
ADP	0.00	5	4.23 ± 1.00	-7.42 ± 1.37	-8.11 ± 0.68
	0.10	9	5.68 ± 1.58	-11.40 ± 1.16	-6.42 ± 0.84
(ug/kg)	0.18	4	4.20 ± 1.29	-7.54 ± 2.14	-6.24 ± 0.83
	0.32	4	8.75 ± 1.98	-10.96 ± 1.03	-6.95 ± 2.63
	0.56	5	7.97 ± 1.22	-9.25 ± 1.32	-9.08 ± 1.17
	1.00	10	17.84 ± 3.56	-15.17 ± 1.99	-13.49 ± 2.32
	1.80	4	15.13 ± 3.56	-13.32 ± 2.13	-12.81 ± 2.08
	3.20	4	23.15 ± 2.44	-13.42 ± 1.57	-14.50 ± 3.11
	5.60	5	32.40 ± 4.23	-19.13 ± 1.98	-21.71 ± 3.80
	10.00	8	42.17 ± 4.55	-26.86 ± 2.71	-28.38 ± 3.22
	100.00	11	54.20 ± 6.26	-36.09 ± 2.04	-37.01 ± 7.79
Collagen	0	6	2.83 ± 0.53	-3.89 ± 0.90	-2.11 ± 0.30
	10	5	5.12 ± 1.25	-6.43 ± 2.57	-5.01 ± 1.86
(ug/kg)	18	3	9.36 ± 3.23	-7.56 ± 1.10	-6.83 ± 2.94
	32	3	10.14 ± 2.19	-9.14 ± 1.45	-9.36 ± 3.70
	56	7	27.98 ± 11.52	-16.20 ± 3.30	-16.41 ± 3.95
	100	7	57.07 ± 12.73	-24.72 ± 3.72	-30.31 ± 5.74
	180	3	30.87 ± 4.74	-18.50 ± 1.86	-14.62 ± 0.65
PAF	0	10	4.84 ± 1.14	-7.79 ± 1.32	-6.74 ± 0.99
	1	8	7.75 ± 1.55	-7.71 ± 0.93	-12.46 ± 3.61
(ng/kg)	10	9	20.87 ± 2.83	-16.79 ± 4.42	-14.33 ± 3.27
	100	14	46.03 ± 4.62	-26.99 ± 3.00	-25.04 ± 4.80
5-HT	0	3	4.01 ± 1.11	-6.88 ± 1.67	-5.64 ± 0.82
	100	4	7.14 ± 2.66	-8.61 ± 2.36	-13.37 ± 0.86
(ug/kg)	180	4	6.10 ± 1.85	-10.22 ± 3.07	-10.26 ± 1.03
	320	3	6.52 ± 0.68	-10.15 ± 2.51	-6.50 ± 1.14
	560	3	6.77 ± 0.69	-10.68 ± 2.66	-9.52 ± 1.81
	1000	3	11.30 ± 2.19	-12.55 ± 1.15	-10.33 ± 2.01
Thrombin	0.0	5	4.87 ± 3.98	-3.01 ± 1.75	-3.24 ± 3.23
	1.0	4	6.25 ± 4.42	-5.31 ± 3.04	-4.62 ± 2.99
(I.U/kg)	3.2	4	14.62 ± 6.35	-11.77 ± 3.70	-18.57 ± 2.58
	5.6	5	18.63 ± 5.89	-20.51 ± 3.99	-23.76 ± 5.09
	10.0	5	22.27 ± 6.28	-21.89 ± 5.18	-25.87 ± 5.08
	18.0	5	27.47 ± 8.26	-28.64 ± 3.83	-25.58 ± 5.46
	32.0	4	34.74 ± 13.83	-34.20 ± 4.03	-29.44 ± 7.41

Table VI.1 Effect of each platelet agonist, ADP, collagen, PAF, serotonin and thrombin on ^{111}In-labelled platelet accumulation in the thorax, hindlimb and cranial regions of the rabbit (mean ± sem). Peak height has been calculated by subtracting the baseline counts in each region from the maximal change in counts observed in those areas and converted to a percentage of the baseline counts.

AGONIST	DOSE	n	PEAK HEIGHT (mean ± sem) THORAX	HINDLIMB	HEAD
ADP	0.0	5	4.81 ± 0.94	-11.78 ± 1.33	-5.44 ± 0.91
	1.0	5	6.87 ± 1.75	-14.56 ± 3.69	-6.18 ± 1.78
(ug/kg)	5.6	4	5.19 ± 1.13	-11.39 ± 3.65	-4.85 ± 0.42
	10.0	5	5.29 ± 0.94	-13.23 ± 3.77	-7.85 ± 2.44
	56.0	4	9.85 ± 3.84	-10.81 ± 1.10	-6.72 ± 0.97
	100.0	5	12.56 ± 3.01	-13.80 ± 2.00	-8.38 ± 1.93
Collagen	0	4	4.04 ± 1.01	-6.37 ± 2.02	-8.31 ± 1.29
	10	4	4.91 ± 1.17	-4.84 ± 2.19	-8.75 ± 3.06
(ug/kg)	56	4	22.98 ± 13.18	-10.91 ± 3.64	-12.44 ± 4.63
	100	5	30.69 ± 10.37	-15.27 ± 5.05	-15.61 ± 6.29
	180	4	36.90 ± 9.63	-16.89 ± 5.48	-20.75 ± 8.03
PAF	0	5	3.91 ± 0.28	-3.89 ± 1.44	-5.25 ± 2.78
	1	5	2.89 ± 1.06	-5.27 ± 2.28	-8.18 ± 2.99
(ng/kg)	10	5	4.59 ± 1.79	-3.91 ± 0.83	-6.94 ± 1.02
	100	5	15.88 ± 3.49	-8.46 ± 3.00	-10.67 ± 4.45
5-HT	0	4	4.70 ± 1.10	-4.28 ± 2.04	-6.55 ± 3.11
(mg/kg)	1	4	8.22 ± 3.41	-6.04 ± 3.29	-10.84 ± 4.73
Thrombin	0.0	4	3.22 ± 0.56	-8.01 ± 2.83	-2.96 ± 1.11
	10.0	5	6.49 ± 2.20	-13.45 ± 4.60	-6.06 ± 2.72
(I.U/kg)	32.0	4	10.16 ± 4.00	-15.28 ± 6.97	-8.81 ± 4.65

Table VI.2 Effect of each platelet agonist, ADP, collagen, PAF, serotonin and thrombin on ^{111}In-labelled erythrocyte accumulation in the thorax, hindlimb and cranial regions of the rabbit (mean ± sem). Peak height has been calculated by subtracting the baseline counts in each region from the maximal change in counts observed in those areas and converted to a percentage of the baseline counts.

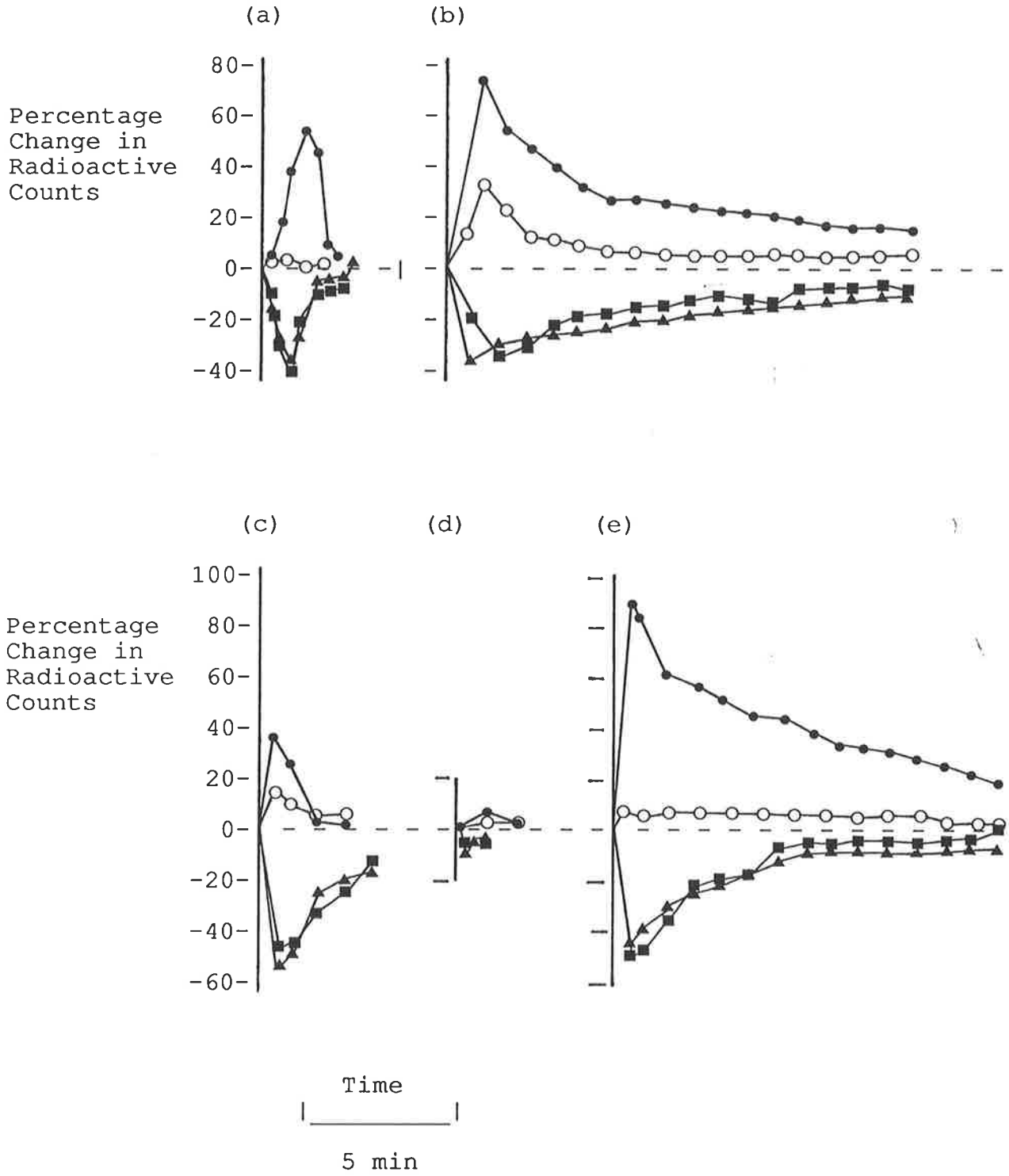


Figure VI.2 Changes in thoracic (•), cranial (▲) and hindlimb (■) platelet-associated radioactivity and thoracic erythrocyte-associated radioactivity (○) over time following i.v. administration of bolus doses of (a) ADP (10 *u*g/kg), (b) collagen (100 *u*g/kg), (c) PAF (10 *n*g/kg), (d) 5-HT (100 *u*g/kg) and (e) thrombin (32 I.U./kg) in individual rabbits. Changes are expressed as percentage change in radioactive counts as compared with pre-injection levels.

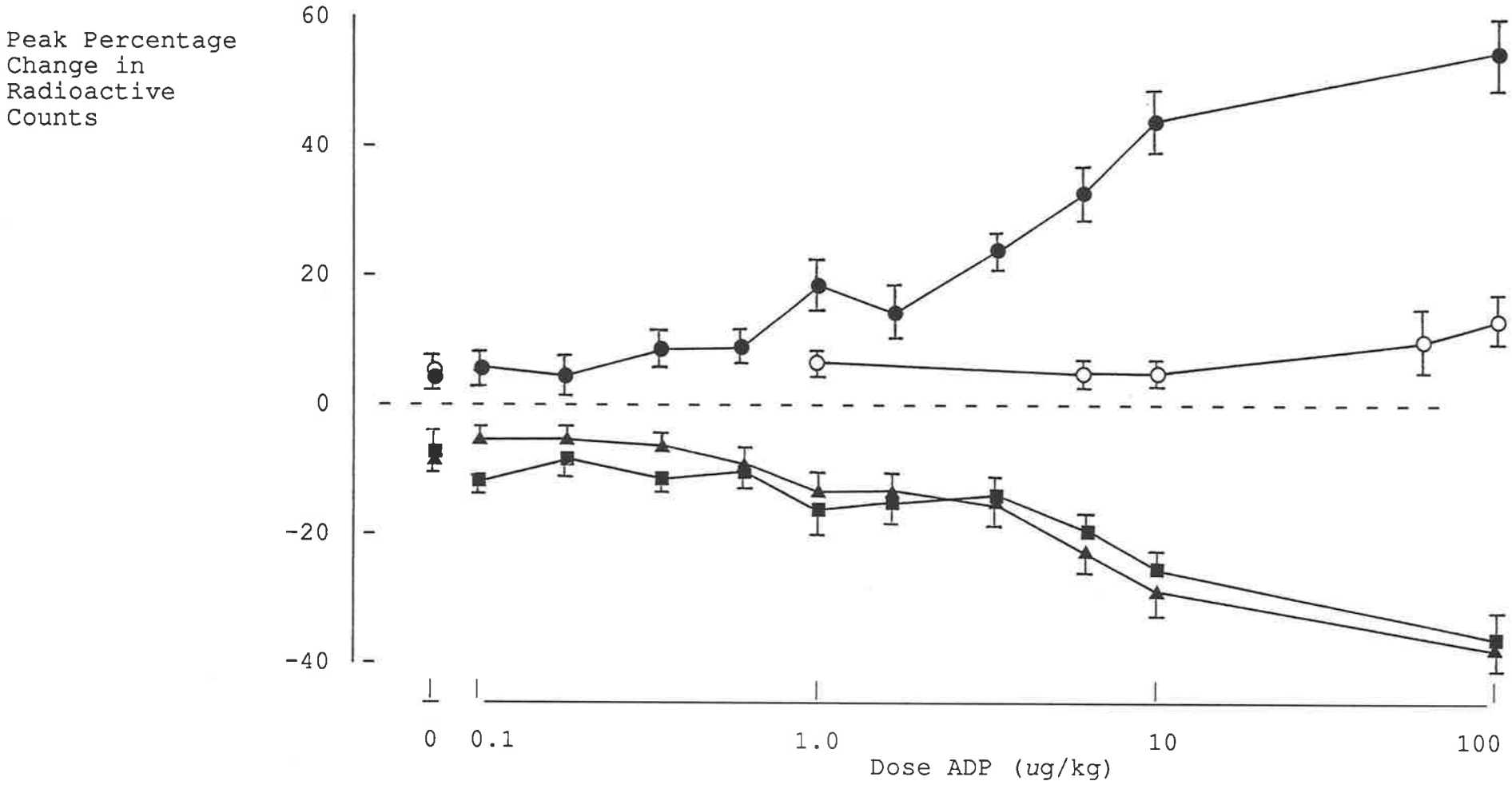


Figure VI.3 Each point represents the mean ± sem (n=4-11) of the maximal change in radioactive counts in the thoracic (●), hindlimb (■) and cranial (▲) regions of the rabbit where ^{111}In-labelled platelets have been utilised and the thoracic region only (○) of rabbits where ^{111}In-labelled erythrocytes have been utilised following i.v. administration of a bolus dose of ADP (0.1-100 *u*g/kg).

this was significantly smaller than the maximal increase achieved with ^{111}In-labelled platelets (57.07 ± 12.73% ; n=7) ($p < 0.05$) (Figure VI.2 (b) and Table VI.1). Thoracic count rates of platelet-associated radioactivity rose progressively, achieving a maximum within 3 min after injection of collagen, returning to pre-injection levels within 10-25 min. ^{111}In-labelled platelets decreased in both reference regions in response to the intrathoracic accumulation (Figure VI.2 (b) and Table VI.1). Platelet responses in the thoracic, hindlimb and cranial regions induced by collagen were dose related within the dose range 10-180 *ug*/kg (Figure VI.4 and Table VI.1).

VI.3.4 *Response to PAF*

The response to PAF (100 *ng*/kg) was fairly rapid in onset, the maximum ^{111}In-labelled platelet accumulation in the thorax (46.03 ± 4.62% ; n=14) being reached within 30-60 sec, while the return to pre-injection levels occurred within 5-15 min. Correspondingly, there was a progressive decline in the cranial and hindlimb count rates in those animals that had received an injection of ^{111}In-labelled platelets (Figure VI.2 (c) and Table VI.1). In animals that had received an intravenous injection of ^{111}In-labelled erythrocytes there was also an increase of the thoracic count rate, however this accumulation was slight in comparison with that achieved with ^{111}In-labelled platelets (15.88 ± 3.49% ; n=5) ($p < 0.05$) (Figure VI.2 (c) and Table VI.2). An effect of PAF upon platelets was detected with as little as 1 *ng*/kg, even though such a dose does not produce detectable changes in blood pressure or lung function in this species. Responses to PAF were dose related over the range 1-100 *ng*/kg (Figure VI.5 and Table VI.1).

VI.3.5 *Response to Serotonin*

A small yet significant increase of the thoracic count rate was observed following the i.v. injection of 5-HT (1.0 mg/kg) in animals that had received ^{111}In-labelled platelets ($p < 0.05$) (Figure VI.2 (d) and Table VI.1), but not in animals that had received ^{111}In-labelled

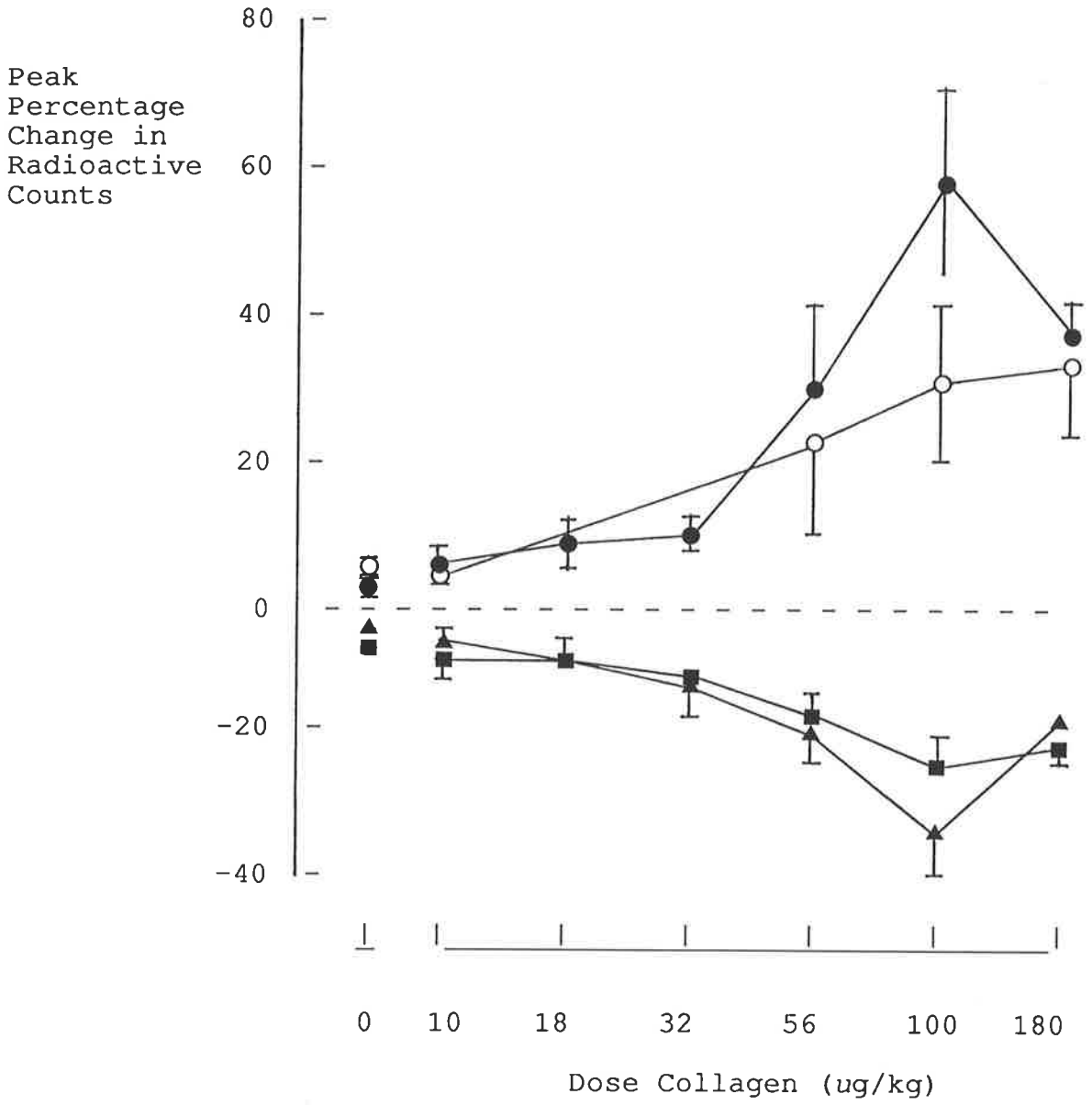


Figure VI.4 Each point represents the mean ± sem (n=4-7) of the maximal change in radioactive counts in the thoracic (●), hindlimb (■) and cranial (▲) regions of the rabbit where ^{111}In-labelled platelets have been utilised and the thoracic region only (○) of rabbits where ^{111}In-labelled erythrocytes have been utilised following i.v. administration of a bolus dose of collagen (10-180 *ug*/kg).

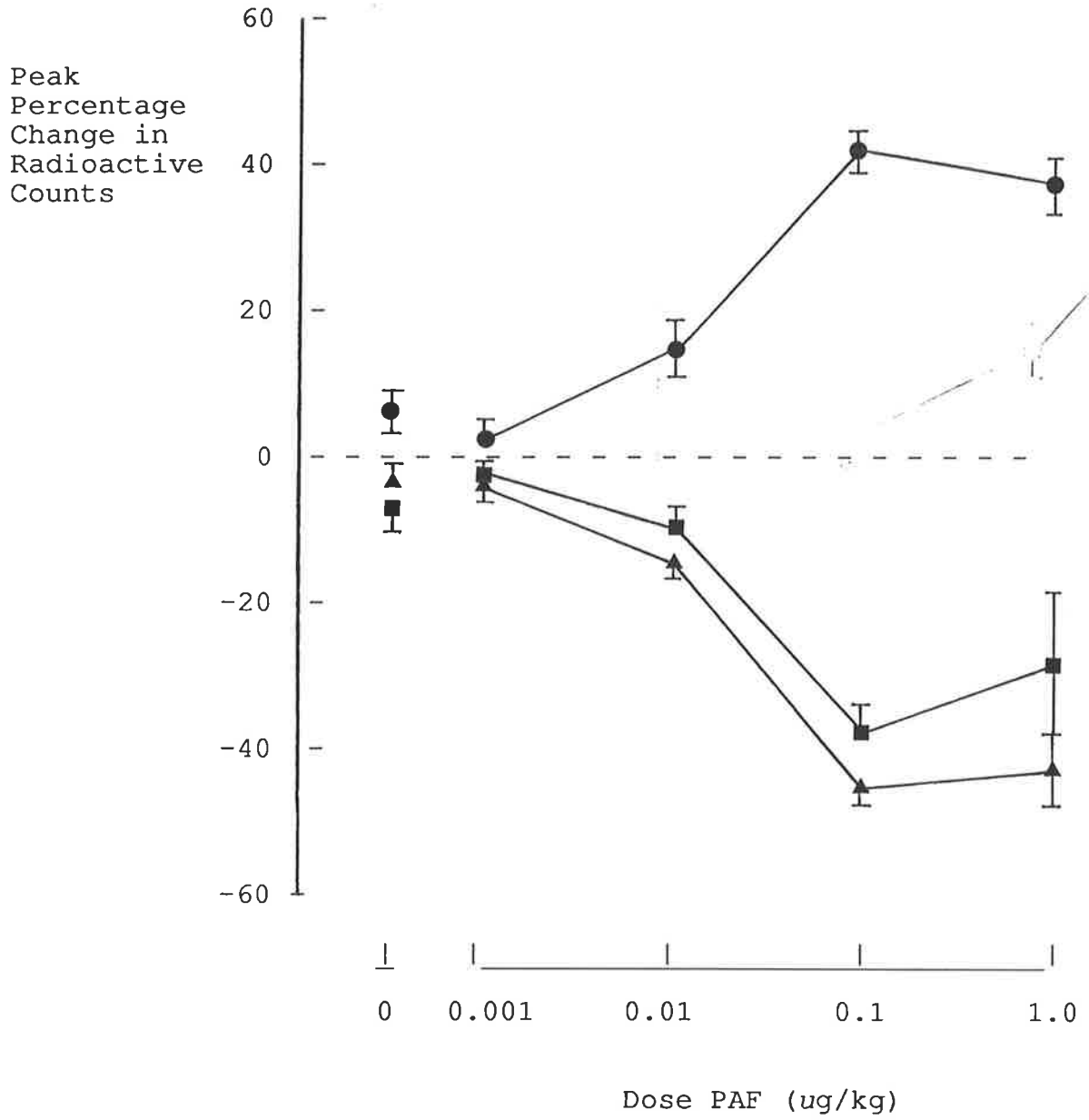


Figure VI.5 Each point represents the mean ± sem (n=4-5) of the maximal change in radioactive counts in the thoracic (●), hindlimb (■) and cranial (▲) regions of the rabbit where ^{111}In-labelled platelets have been utilised and the thoracic region only (○) of rabbits where ^{111}In-labelled erythrocytes have been utilised following i.v. administration of a bolus dose of PAF (0.001-1.0 *u*g/kg).

erythrocytes (Figure VI.2 (d) and Table VI.2). This dose of 5-HT caused a slight reduction in ^{111}In-labelled platelets in both reference areas (Figure VI.2 (d) and Table VI.1). Maximal retention of platelets within the thorax (11.30 $\pm$ 2.19% ; n=3) was achieved within 30-60 sec after injection of 5-HT and returned to preinjection levels within 5 min. Doses less than 1.0 mg/kg had no significant effect on ^{111}In-labelled platelet accumulation (Figure VI.6 and Table VI.1).

VI.3.6 *Response to Thrombin*

The response to thrombin (32 I.U./kg) was rapid in onset with maximal intrathoracic ^{111}In-labelled platelet accumulation (34.74 $\pm$ 13.83% ; n=4) (Figure VI.2 (e) and Table VI.1) reached within 1-2 min, but, in relation to other stimuli, slower to return to basal levels (10-30 min) (Figure VI.2 (e). Platelet-associated cranial and hindlimb count rates decreased progressively following thrombin injection (Figure VI.2 (e) and Table VI.1). Significant changes in erythrocyte-associated radioactivity in the thoracic region were not elicited by thrombin administration (Figure VI.2 (e) and Table VI.2). Thrombin-induced ^{111}In-labelled platelet responses in the thorax, hindlimb and head were dose-related over the range 1-32 I.U./kg with respect to peak height (Figure VI.7 and Table VI.1).

VI.3.7 *Dose-Response Relationships*

Dose-response curves in terms of peak height have been plotted for each stimulus (Figure VI.8). PAF is shown to be the most potent platelet aggregatory stimulus, being 100 times more potent than ADP and 1,000 times more potent than collagen. Serotonin proved to be an extremely weak platelet agonist when administered alone i.v. in the rabbit, with only slight accumulation induced by a high dose (1.0 mg/kg).

VI.3.8 *Effect of Drugs*

Figure VI.6

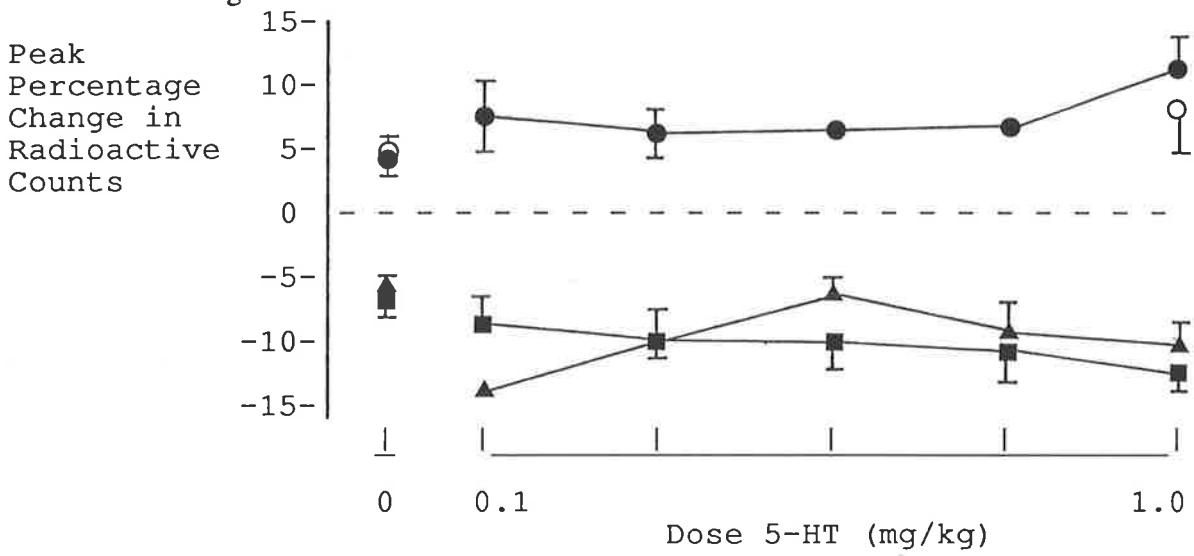
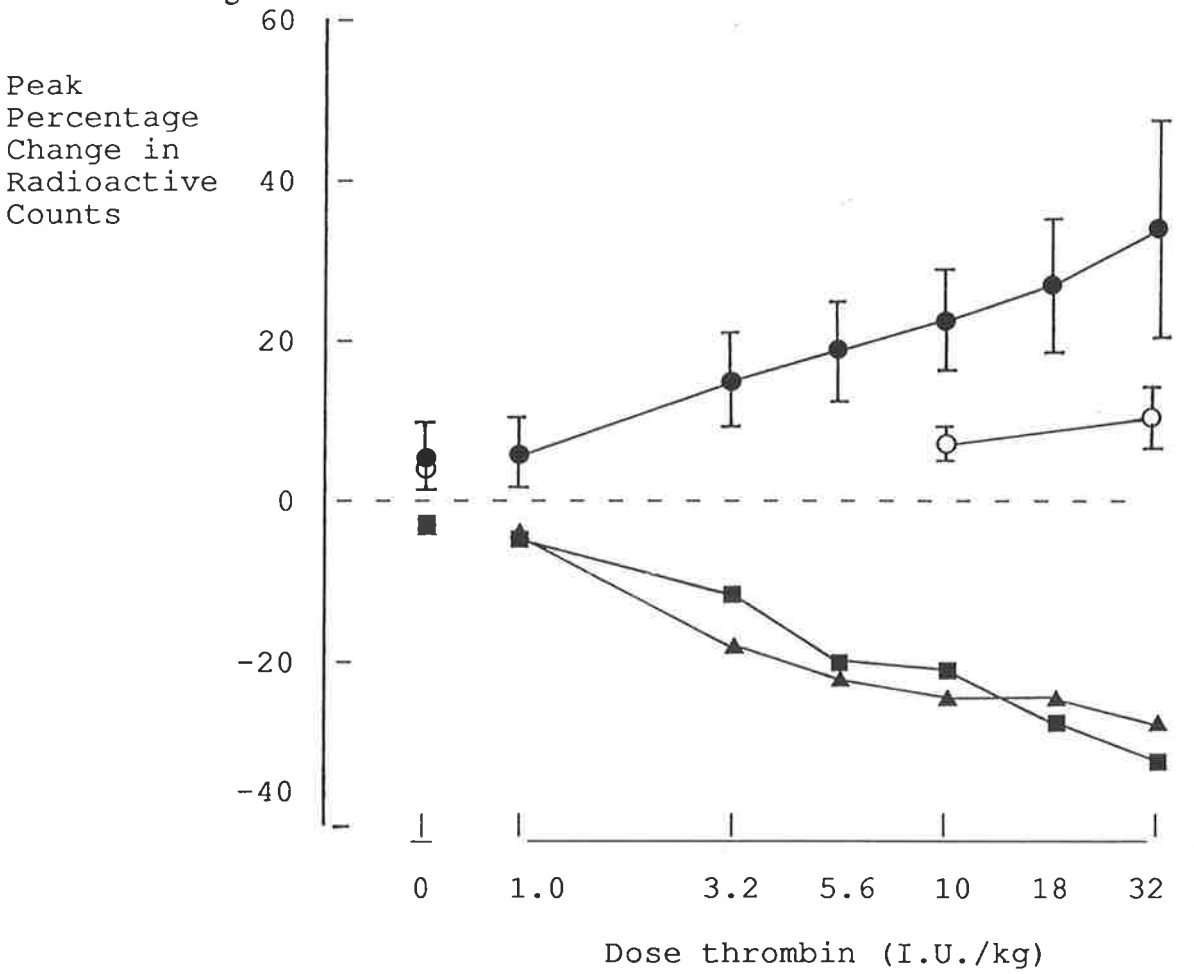


Figure VI.7



Figures VI.6 and VI.7 Each point represents the mean ± sem (n=4-5) of the maximal change in radioactive counts in the thoracic (●), hindlimb (■) and cranial (▲) regions of the rabbit where ^{111}In-labelled platelets have been utilised and the thoracic region only of rabbits where ^{111}In-labelled erythrocytes (○) have been utilised following i.v. administration of a bolus dose of 5-HT (0.1-1.0 mg/kg) and thrombin (1-32 I.U./kg).

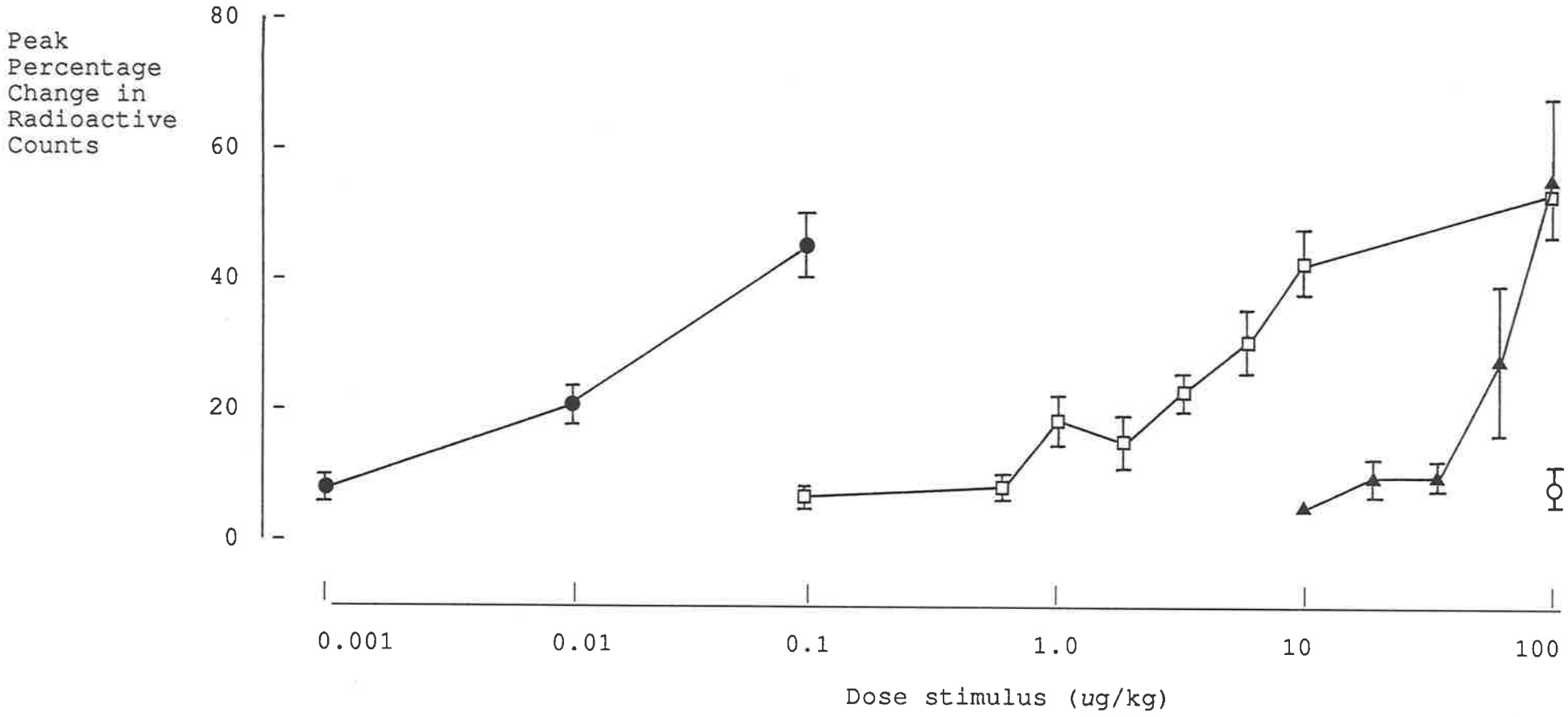


Figure VI.8 Graph showing the log dose rank order of potency of PAF (●) (n=8-14), ADP (□) (n=4-11), collagen (▲) (n=3-7) and 5-HT (○) (n=3-4). Each point represents the mean ± sem of the maximal change in intrathoracic radioactive counts of the rabbit where ^{111}In-labelled platelets have been utilised.

Figure VI.9 and Table VI.3 show the effects of various drug pretreatments on ^{111}In-labelled intrathoracic platelet accumulation induced by a bolus intravenous dose of PAF (100 *ng*/kg) in the rabbit.

VI.3.8.1 *SRI 63-441*

The PAF antagonist SRI 63-441 (1 mg/kg i.v.) significantly inhibited PAF (100 *ng*/kg)-induced ^{111}In-labelled platelet accumulation in the rabbit ($p < 0.05$) (Figure VI.9 and Table VI.3). Inhibition was selective, since this dose of SRI 63-441 did not diminish the response to ADP (100 *ug*/kg i.v.) (Figure VI.10).

VI.3.8.2 *Aspirin and Indomethacin*

Both non-steroidal anti-inflammatory drugs, acetyl salicylic acid (1 mg/kg i.v.) and indomethacin (1 mg/kg i.v.) produced significant enhancement of PAF (100 *ng*/kg)-induced ^{111}In-labelled platelet accumulation in the rabbit ($p < 0.05$) (Figure VI.9 and Table VI.3).

VI.3.8.4 *Dazmegrel*

The inhibition of ^{111}In-labelled platelet accumulation in the rabbit by the thromboxane synthetase inhibitor dazmegrel was not statistically significant ($p > 0.05$) (Figure VI.9 and Table VI.3).

VI.3.8.3 *Iloprost*

The stable prostacyclin analogue iloprost (10 *ug*/kg i.v.) exerted significant inhibition of PAF (100 *ng*/kg)-induced ^{111}In-labelled intrathoracic platelet accumulation in the rabbit ($p < 0.05$) (Figure VI.9 and Table VI.3), which was dose related over the range 0.01-100

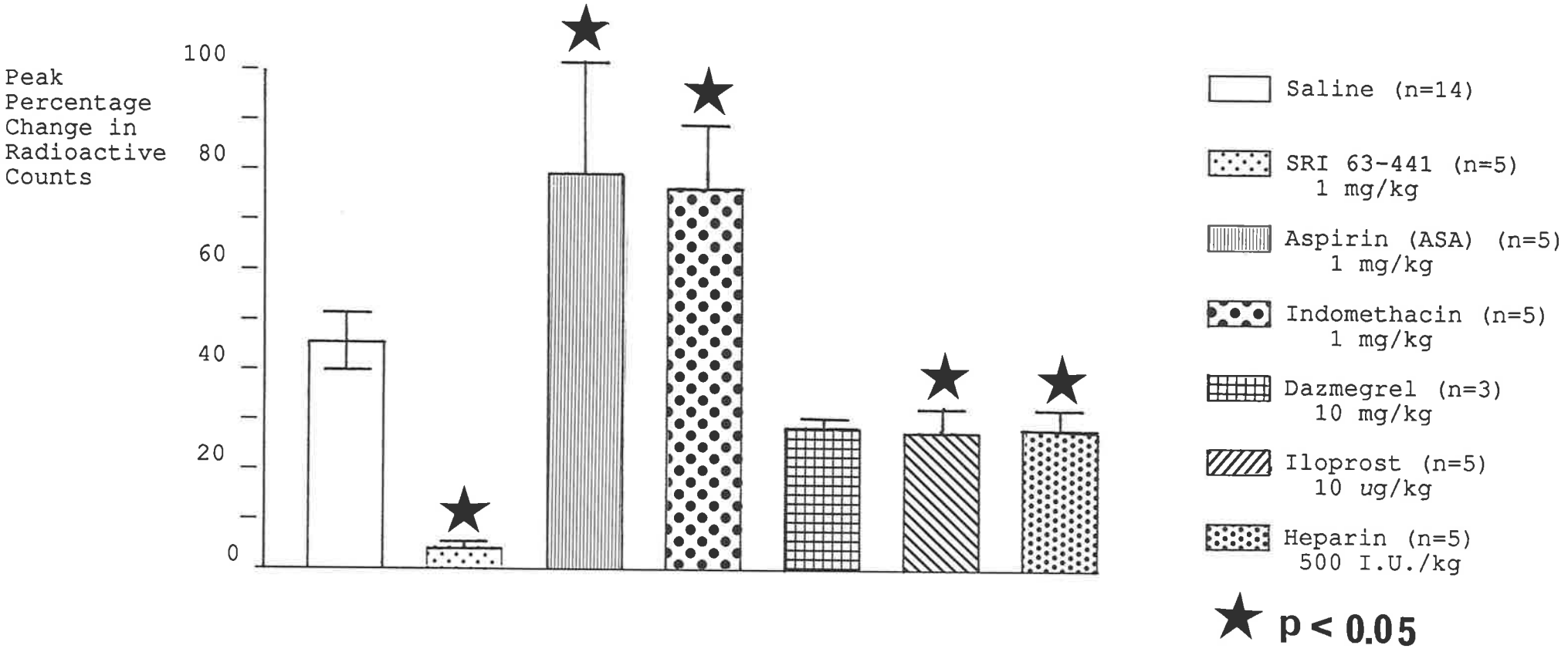


Figure VI.9 Effect of various drug pretreatments on the intrathoracic ^{111}In-labelled platelet accumulation induced by PAF (100 *n*g/kg i.v.) in the rabbit (mean ± sem).

DRUG	DOSE (mg/kg)	PEAK HEIGHT (mean ± sem)	n	p
PAF alone		46.03 ± 4.62	14	
SRI 63-441	1	3.84 ± 2.01	5	0.0001
Iloprost	0.00001	48.10 ± 3.3	5	0.8061
	0.0001	34.26 ± 5.54	5	0.1825
	0.001	37.64 ± 3.75	5	0.3175
	0.01	27.64 ± 5.06	5	0.0022
	0.1	13.37 ± 1.48	5	0.0007
Dazmegrel	10	28.81 ± 1.30	3	0.1136
Aspirin	1	79.07 ± 22.02	4	0.0309
Indomethacin	1	76.45 ± 13.44	5	0.0128
Heparin	500 I.U./kg	28.50 ± 4.35	6	0.0346

Table VI.3 The effect of various drugs on PAF (100 *ng*/kg i.v.)-induced ^{111}In-labelled platelet accumulation in the rabbit. Peak height has been calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts observed and represented as a percentage increase of the baseline intrathoracic counts. Drug effects were compared to saline controls in separate animals.

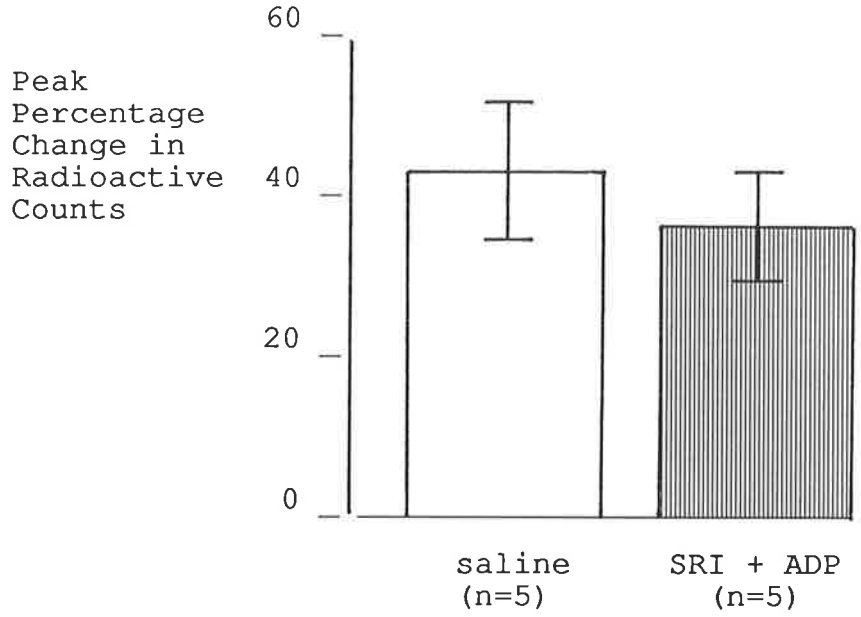


Figure VI.10 The effect of saline and SRI 63-441 (1 mg/kg i.v.) upon the maximal percentage increase in intrathoracic platelet-associated radioactivity induced by an intravenous injection of ADP (100 *ug*/kg).

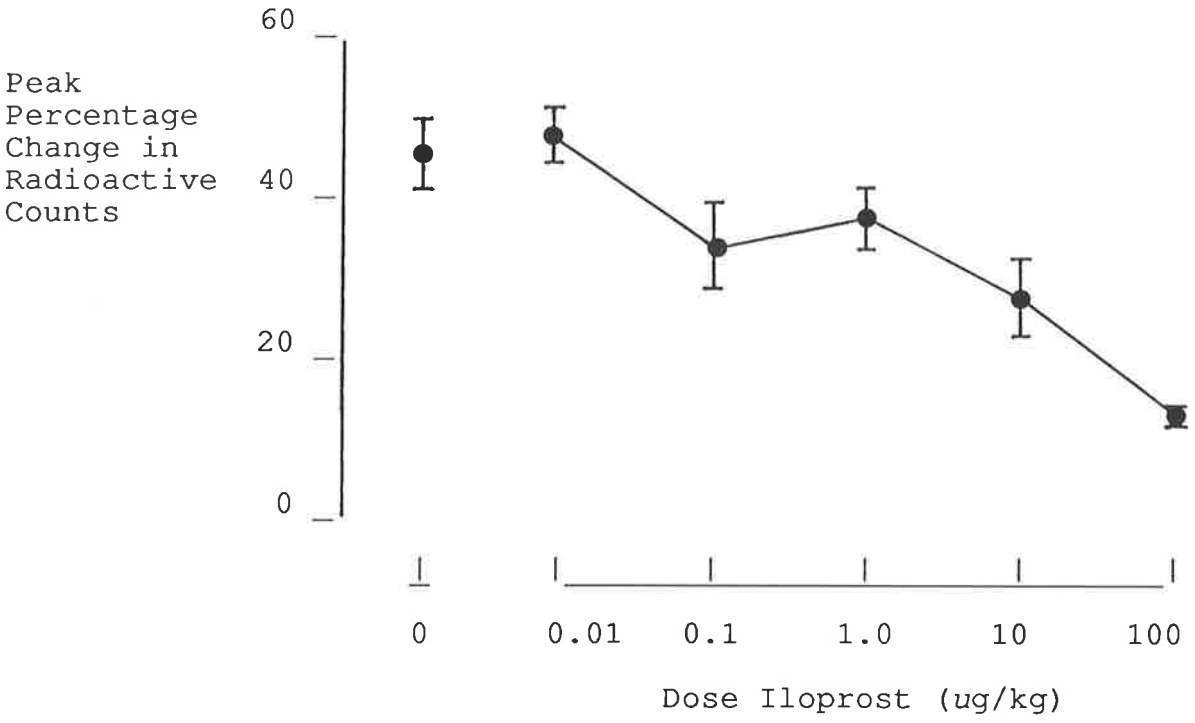


Figure VI.11 Effect of a range of doses of iloprost (*ug*/kg i.v.) on intrathoracic ^{111}In-labelled platelet accumulation induced by PAF (100 *ng*/kg i.v.) in the rabbit (n=5 for each dose).

ug/kg (Figure VI.11 and Table VI.3).

VI.3.8.5 *Heparin*

The anticoagulant heparin (500 I.U./kg i.v.), when administered alone into rabbits had no effect on ^{111}In-labelled platelet movement (data no shown). This dose of heparin significantly inhibited the PAF (100 *ng*/kg)-induced platelet response ($p < 0.05$) (Figure VI.9 and Table VI.3).

VI.4 Discussion

The intravenous administration of the platelet agonists ADP, collagen, PAF and thrombin elicited dose related accumulation of platelet-associated radioactivity in the thoracic region of the rabbit. Serotonin induced only slight platelet accumulation at a relatively high dose (1.0 mg/kg). This most likely reflects trapping of platelet aggregates within the pulmonary vasculature rather than changes in blood flow or blood volume since comparable changes were not observed when similar experiments were performed with ^{111}Indium-labelled erythrocytes. Significant accumulation of ^{111}In-labelled erythrocytes was evident however, following collagen administration. Furthermore, similar profiles have been reported with ^{111}Indium-labelled platelets in other species (Page *et al.*, 1982*b*; Oyekan and Botting, 1986; Smith *et al.*, 1989*a*) and histological sections of the lung tissue taken at the time of maximal increase in thoracic radioactivity revealed intravascular platelet aggregates throughout the pulmonary vasculature (Butler *et al.*, 1979; Dewar *et al.*, 1984). The parallel loss of platelet radioactivity from the hindlimb region is again most likely to reflect the loss of platelets from the peripheral circulation (and a resultant thrombocytopenia) rather than changes in blood flow or blood volume, since such changes were not evident following the administration of ^{111}Indium-labelled erythrocytes.

The doses of platelet agonists used in this study are similar to those reported by other investigators using different techniques to evaluate platelet function in rabbits. The doses

of PAF (Honey *et al.*, 1986; Robertson and Smith, 1986), ADP, collagen and thrombin (Honey *et al.*, 1986) correlate with those obtained using automated platelet counting. The dose of PAF is similar to that reported to induce thrombocytopenia and release of platelet factor 4 into the peripheral circulation of rabbits following the intravenous administration of PAF (McManus *et al.*, 1979*b*). In the rat, 5-HT within the dose range 10-400 *u*g/kg produced *in vivo* platelet aggregation (Oyekan and Botting, 1986). In the present study 5-HT did not induce appreciable accumulation (1 mg/kg) and thus appears not to be an *in vivo* platelet stimulus in rabbits. The doses of the platelet agonists ADP, collagen and PAF correlate with those in the guinea-pig using this automated isotope monitoring system (Chapter IV). From the dose-response studies, PAF is the most potent platelet aggregatory stimulus, being 100 times more potent than ADP, and 1,000 times more potent than collagen. Guinea-pig platelets have been reported to be more sensitive to aggregation by PAF than rabbit platelets *in vitro* (Vargaftig *et al.*, 1981*a*); however, this finding is not confirmed *in vivo* by results obtained in Chapter IV and the present study.

The time course of the responses in the rabbit are also comparable to those observed in the guinea-pig (Chapter IV) *ie.* responses to ADP were maximal earlier (20-30 sec) than those for collagen (180 sec) and PAF (60-120 sec). The maximum response to thrombin was achieved within 60-120 sec. The duration of the platelet accumulation also varied considerably. The response to ADP was transient (less than 2 min) in comparison with the response to PAF (5-15 min), whereas the response to both collagen (10-25 min) and thrombin (10-30 min) were longer lasting. These findings imply differences in the mechanism of action of the different platelet stimuli.

An interesting observation in the present study was a concomitant fall in platelet-associated radioactivity in the cranial circulation following i.v. injection of all of the platelet stimuli. Again this is unlikely to reflect changes in blood flow or blood volume, as similar changes were not observed when ^{111}Indium-labelled erythrocytes were used. This observation would suggest that platelet aggregates formed transiently in the pulmonary vasculature following i.v. administration of platelet agonists consist of "cranial circulation-derived" platelets as well as those recruited from the peripheral circulation.

A similar pattern of pulmonary platelet accumulation was observed when ADP, collagen and PAF were administered *via* the carotid artery (May *et al.*, 1990), suggesting that the platelet aggregates formed in response to these agonists must circulate as they become larger, until they finally become trapped within the pulmonary vasculature. Initially, thrombin administration *via* the carotid artery elicited a similar pattern of aggregation to that of its intravenous counterpart. However, a sustained and marked accumulation of platelet-associated radioactivity in the cranial vasculature became apparent over the following 3 hours, which was not attributable to changes in blood flow or blood volume (May *et al.*, 1990). It has been proposed that ^{111}Indium-labelled platelet accumulation induced by the intracarotid administration of thrombin in the rabbit may provide a novel model of thromboembolic stroke (May *et al.*, 1990).

It is accepted that PAF produces platelet aggregation *via* a receptor-mediated process. Specific binding sites have been demonstrated on rabbit platelets using radioligand binding studies (Hwang *et al.*, 1983). In the present study, the PAF receptor antagonist SRI 63-441 (Handley *et al.*, 1986) fully inhibited PAF-induced ^{111}In-labelled platelet accumulation in the thorax of the rabbit, but was without effect upon a response of comparable intensity induced by ADP. This closely correlates with the situation observed in the guinea-pig, as discussed in Chapter IV (Smith *et al.*, 1989*a*).

The effects of both nonsteroidal anti-inflammatory drugs were in marked contrast to those observed with the other drugs evaluated in this study. Contrary to expectation, neither aspirin nor indomethacin displayed an inhibitory action on PAF-induced platelet accumulation in the rabbit, as did SRI 63-441, iloprost and heparin. Both aspirin and indomethacin caused significant potentiation of the PAF-induced platelet response, a finding which resembles those results documented in the guinea-pig (Chapter V).

Three possibilities exist to explain this phenomenon. The first of these findings hinges on the contribution of arachidonate derived products (in platelets and endothelium) to PAF-induced platelet activation in the rabbit. The involvement of TXA_2 in PAF-induced platelet activation is not clear. In rabbit platelets *in vitro* PAF has been shown to trigger aggregation and release independently from TXA_2 formation (Vargaftig, 1978; Cazenave

et al., 1979). Accordingly, ASA and the combination cyclo-oxygenase and lipoxygenase inhibitor eicosatetraynoic acid failed to interfere with aggregation and the release reaction induced by PAF (Cazenave *et al.*, 1979; Vargaftig *et al.*, 1980). However, release of TXB_2 has been demonstrated following the intravenous administration of PAF to rabbits (McManus *et al.*, 1983; Lefer *et al.*, 1984). The same authors have documented that PAF stimulates rabbit platelets *in vitro* to produce this arachidonic acid metabolite (McManus *et al.*, 1983). Furthermore, rabbits are highly susceptible to sudden death following the i.v. administration of high doses of collagen (Mallarkey and Smith, 1985), with a profile similar to that induced by either arachidonate or high dose PAF (Lefer *et al.*, 1984). Death could be prevented by pre-treatment with Ca^{2+} entry blockers, indomethacin or trisodium citrate (Mallarky and Smith, 1985). Death is probably due to vasoconstriction caused by vasoactive agents released from platelets, with TXA_2 being the most likely mediator responsible (Robertson and Smith, 1986). In the present study, rabbits challenged with collagen at doses greater than 100 *ug*/kg incurred sudden death, yet this was not found following PAF administration.

If platelet-derived TXA_2 is not involved in PAF-induced platelet accumulation in the rabbit, the results obtained with ASA and indomethacin in the present study may be explained in terms of their effects on the vascular endothelium. Endothelial cell-derived PGI_2 may be instrumental in limiting the extent of PAF-induced platelet aggregation. Therefore, if this pathway is blocked, the inhibitory control mechanism may no longer operate, resulting in an exaggerated response. However, if platelet TXA_2 is an important mediator involved in PAF-induced platelet activation in the rabbit, the results may be explained by platelet cyclo-oxygenase being less sensitive to inhibition by aspirin and indomethacin than that of the corresponding endothelial cell enzyme. This would result in a relative abundance of the highly pro-aggregatory TXA_2 and a deficiency of the anti-aggregatory PGI_2. This situation would result in the development of a greater extent of platelet accumulation than would be achieved by PAF alone. Alternatively, inactivation of the enzyme cyclo-oxygenase may induce stimulation of the lipoxygenase pathway, leading to the formation of possible platelet active lipid products, which in conjunction with PAF

stimulation, lead to an enhanced PAF-induced ^{111}In-labelled platelet accumulation in the thoracic region of the rabbit.

In the present study, dazmegrel, a potent and selective thromboxane synthetase antagonist (Vermylen *et al.*, 1981; Fitzgerald and Oates, 1984), inhibited platelet accumulation induced by PAF in the rabbit, however the inhibition did not reach statistical significance ($p > 0.05$). This result may or may not implicate a mediatory role for TXA_2 in the PAF response, as the number of animals tested was small (n=3). Furthermore, reorientation of cyclic endoperoxide metabolism towards other prostanoic derivatives as a result of thromboxane synthetase inactivation may have modified platelet function (Defreyn *et al.*, 1982; Smith, 1982; Bertele *et al.*, 1984; Gresele *et al.*, 1984; Rajtar *et al.*, 1985).

PGI_2 has been shown to inhibit PAF-induced aggregation and the release reaction in rabbit platelets *in vitro* (Cazenave *et al.*, 1979; Vargaftig *et al.*, 1980). In the present study the PAF-induced platelet response in the rabbit was inhibited by the stable prostacyclin analogue iloprost (Sturzebecher and Losert, 1987) (10 *ug*/kg) to a similar extent as in the guinea-pig (Chapter V). The effect of iloprost was found to be dose-related. As for the guinea-pig, it appears that PAF-induced platelet activation in the rabbit *in vivo* is modulated by the adenylate cyclase-cAMP system (Best *et al.*, 1977; Gorman *et al.*, 1977; Tateson *et al.*, 1977).

Heparin is one of the most widely used anticoagulant drugs, yet there is conflicting opinion as to its effect on platelet aggregation (Mustard and Packham, 1975). Observations that heparin can induce or potentiate platelet aggregation *in vitro* have suggested that heparin exerts a pro-aggregatory effect (Eika, 1972; Mohammad *et al.*, 1981). Systemic anticoagulants are mandatory in most existing *in vivo* techniques, thus precluding analysis of the effects of heparin on platelet aggregation and disaggregation *in vivo*. The method employed in the present study does not require the use of systemic anticoagulants. In the present study, no aggregatory effects of heparin were observed on normal circulating platelets, nor was there enhancement of aggregation of platelets during activation by the intravenous injection of PAF. On the contrary, heparin induced significant inhibition of

platelet accumulation induced by PAF.

It has been demonstrated in the guinea-pig that low doses of heparin (100-250 I.U./kg) inhibit thrombin-induced platelet accumulation and activation of the coagulation cascade (as assessed by ^{125}I-labelled fibrinogen accumulation *in vivo*), yet have no effect on platelet accumulation induced by other platelet stimuli such as ADP, collagen or PAF (Barrett *et al.*, 1984). Furthermore, these other platelet agonists do not initiate coagulation suggesting that substantial platelet activation may occur in the circulation independently of the coagulation cascade (Barrett *et al.*, 1984). Larger doses of heparin (100-500 I.U./kg) have been shown to cause dose-related inhibition of platelet activation *in vivo* induced by ADP, collagen or PAF in the guinea-pig (Barrett *et al.*, 1984). The results of the present study in the rabbit parallel those findings *ie.* high dose heparin (500 I.U./kg) significantly inhibited PAF-induced platelet accumulation.

This study has characterised platelet accumulation in the rabbit to stimuli administered *via* the intravenous route using an *in vivo* automated isotope monitoring system. The results indicate that PAF-induced platelet aggregation in particular, appeared to be mediated by many physiological systems *in vivo*. These interactions could not have been considered if this phenomenon was studied *in vitro*.

CHAPTER VII

THE EFFECT OF RADIOGRAPHIC CONTRAST MEDIA (RCM) ON PLATELET FUNCTION *IN VIVO*

VII.1 **Introduction**

As outlined in Chapter III of this thesis, studies investigating the effects of contrast media on platelet function have yielded conflicting results. Both *in vitro* and *ex vivo* RCM have been shown to inhibit platelet aggregation induced by various agonists (Zir *et al.*, 1974; Shapiro *et al*, 1977; Gafter *et al.*, 1979; Belleville *et al.*, 1982; Parvez *et al.*, 1983; Paajanen *et al.*, 1984; Parvez *et al.*, 1984; Rao *et al.*, 1985; Stormorken *et al.*, 1986; Parvez and Patel, 1988). Paradoxically, RCM are reported to stimulate the release of serotonin from platelets (Fritzler *et al.*, 1978; Ring *et al.*, 1978*a*; Ring and Sovak, 1981) and whole blood (Parvez *et al.*, 1982; Fareed *et al.*, 1984*b*) *in vitro*.

As an extension to the *ex vivo* and *in vitro* studies of RCM on platelet aggregation and thromboxane A_2 production reported in Chapter III of this thesis, the effects on platelet accumulation of a high osmolality, ionic radiographic contrast medium (Urovison) and a low osmolality, non-ionic agent (Iopamiro) were studied in the *in vivo* rabbit model.

VII.2 **Methods**

VII.2.1 *Animals*

Male New Zealand White rabbits (1.8-3.2 kg body weight) were anaesthetised with a combination of diazepam (Valium, Hoffman LaRoche, Basel, Switzerland) (5 mg/kg i.p.) followed 10 min later by Hypnorm (0.315 mg fentanyl citrate/ml and 10 mg fluanisone/ml, Janssen Pharmaceutica) (0.4 ml/kg i.m.). A marginal ear vein was cannulated (Venisystems Butterfly-25; 19.1 mm length, 0.4 mm internal diameter) for the

administration of ADP and contrast agents. ^{111}Indium-labelled platelets were administered *via* a marginal vein in the opposite ear and allowed to equilibrate in the circulation for at least 30 min before challenge with the test substances.

VII.2.2 *Preparation of ^{111}Indium-Labelled Platelets*

The procedure for labelling platelets with ^{111}Indium oxine (^{111}In-oxine, 37 MBq/ml) (Amersham International, Amersham, England) was as described in Chapter VI of this thesis (May *et al.*, 1990). Briefly, blood was obtained from a marginal ear vein of a conscious rabbit into 0.1 M trisodium citrate (10% v/v). PRP obtained by centrifugation (200 *g*, 10 min) was then recentrifuged (1000 *g*, 10 min) in the presence of PGE_1 (Sigma) (300 *ng*/ml) and the platelet pellet was resuspended in 1.5 ml calcium-free Tyrode's solution containing PGE_1 (CFTP). The platelet suspension was incubated with ^{111}In-oxine (37 MBq) (5 min at room temperature), recentrifuged (1000 *g*, 10 min), the free ^{111}In-oxine removed and the ^{111}In-labelled pellet washed once with CFTP (1 ml). The ^{111}In-labelled platelet pellet was resuspended in CFTP and 1 ml of ^{111}In-labelled platelets were administered intravenously (*via* a marginal ear vein) to the previously anaesthetised donor and recipient rabbits.

III.2.3 *Administration of Contrast Agents*

Thirty min prior to the administration of the test contrast agent, a standard dose of ADP (100 *ug*/kg) known to elicit substantial platelet accumulation in the rabbit (refer Chapter VI) was injected *via* the intravenous cannula and was flushed through with 0.3 ml normal saline (0.9% w/v). This confirmed that the experimental system was functioning and provided a reference for the extent of platelet accumulation.

The contrast media under evaluation were randomly assigned to different animals. Six animals each received either saline, Urovison 58% or Iopamiro 370 at a dose of 1.5 ml/kg or 3.0 ml/kg. Saline and RCM were administered as a bolus intravenous infusion *via*

the ear vein cannula followed by 0.3 ml normal saline (0.9% w/v). Following the platelet accumulation, monitored animals were sacrificed by a lethal i.v. injection of sodium pentobarbitone.

VII.2.4 *Measurement of Platelet Accumulation In Vivo*

Circulating ^{111}In-labelled platelets were continuously monitored in the thoracic and hindlimb regions using 1 inch crystal scintillation probes located over the thorax and over the hindlimb. Counts were estimated with a dual channel gamma spectrometer (Nuclear Enterprises NE 461) and logged with the aid of a special application interface with a dedicated microcomputer (IBM AT3) (Chapter VI).

Radioactive counts were summated at 2 sec intervals over a 5 min period following administration of the bolus dose of ADP, and 5 sec intervals over 30 min following the bolus dose of the specified contrast agent.

A complete description of the radioisotopic method for investigating platelet accumulation in the rabbit model is found in Chapter VI.

VII.2.5 *Data Analysis*

Platelet accumulation in the rabbit was measured as an increase in radioactive counts/sec by the detector located over the thorax. Results are expressed as a percentage change in intrathoracic radioactive counts (calculated by subtracting the baseline counts from the maximal increase in counts and converting this increase to a percentage of the baseline counts). Although abdominal counts were recorded in each case as an indication of events occurring in the peripheral circulation, for convenience this data is not presented. Student's *t*-tests were employed to assess statistical significance.

In all cases a p value of < 0.05 was accepted as the lowest level for the expression of statistical significance.

VII.3 **Results**

ADP (100 *u*g/kg i.v.) produced a rapid, transient increase in platelet-associated radioactivity in the thoracic region of the rabbit. The maximum response (42.66 ± 6.77%) was attained within 20-30 sec and returned to pre-injection levels within 2 min. Neither Urovison nor Iopamiro at a dose of 1.5 ml/kg had any significant effect on ^{111}In-labelled platelets ($p > 0.05$) (Figure VII.1 and Table VII.1). Similarly, Iopamiro at a dose of 3.0 ml/kg had no significant effect on the platelet response ($p > 0.05$) (Figure VII.1 and Table VII.1). However, 3.0 ml/kg Urovison caused a significant accumulation of ^{111}In-labelled platelets in the thoracic region of the rabbit ($p < 0.05$) (15.69 ± 1.79%) (Figure VII.1 and Table VII.1). The extent of this response was significantly smaller than the response to ADP (100 *u*g/kg i.v.) ($p < 0.05$) (Figure VII.1). The ^{111}In-labelled platelet accumulation induced by 3.0 ml/kg Urovison was relatively slow in onset, reaching a maximum within 5 min and returning to pre-injection levels within 10 min.

VII.4 **Discussion**

In the present study, high dose Urovison caused significant intrathoracic ^{111}In-labelled platelet accumulation in the rabbit *in vivo*. No change was elicited in the thoracic region of animals that had received ^{111}In-labelled erythrocytes, indicating that the accumulation of platelets within the microvascular network of the lung was not the result of increased blood flow. The response to Urovison was slight by comparison with that achieved on administering ADP or dextran sulphate (refer Chapter VI) to the same system. The higher dose of Urovison used (*ie.* 3.0 ml/kg i.v.) is approximately twice that administered routinely to patients. Neither contrast agent administered to rabbits in a dose comparable to that used in the clinical situation had any effect on platelet accumulation.

The foregoing findings suggest that radiographic contrast media do not stimulate platelets; rather, they actually inhibit platelet function when this is studied *in vitro*. Moreover, it is apparent that non-ionic, low osmolality agents such as Iopamiro

(iopamidol) suppress platelet activation to a lesser degree than ionic, high osmolality media such as Urovison (sodium and meglumine diatrizoate).

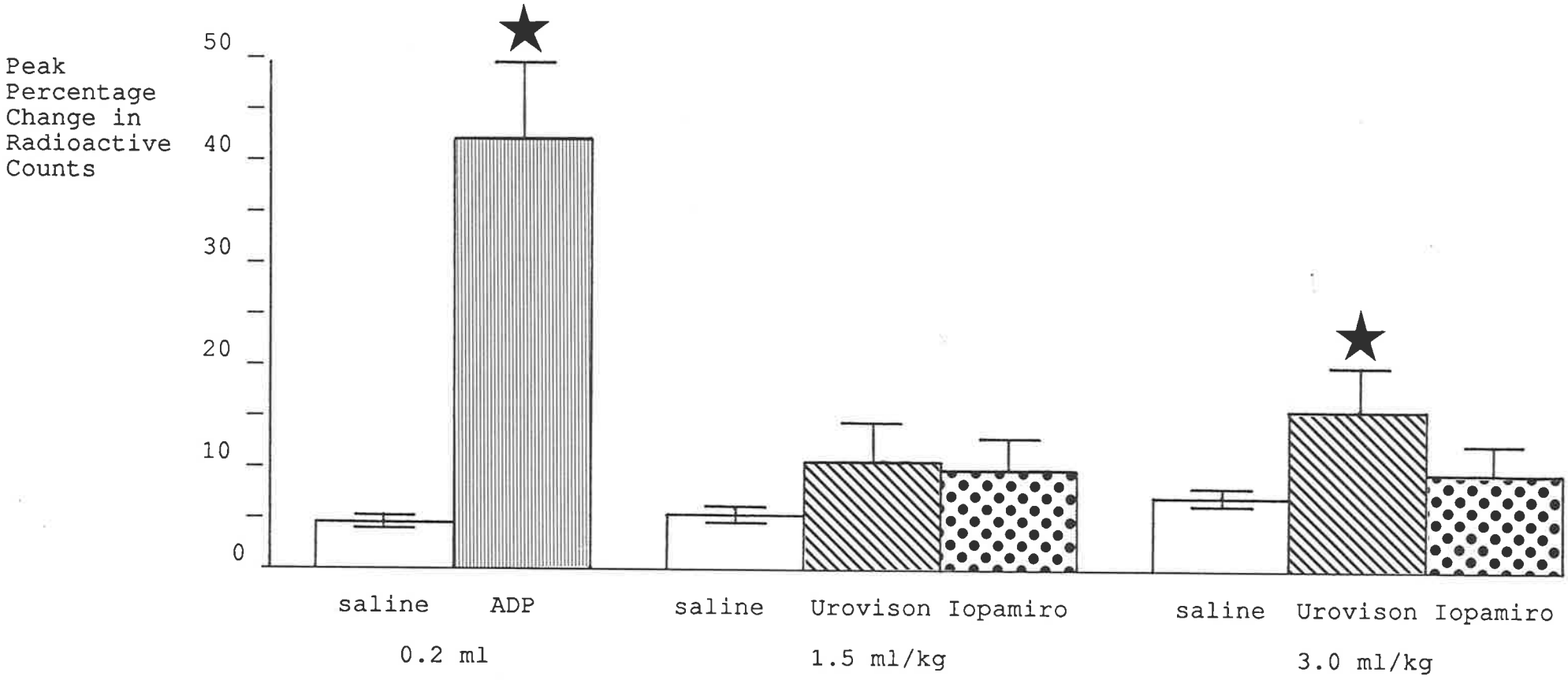


Figure VII.1 Effect of saline (0.2 ml; 1.5 and 3.0 ml/kg i.v.), ADP (100 *ul*/kg i.v.) (0.2 ml), Urovison (1.5 and 3.0 mg/kg i.v.) and Iopamiro (1.5 and 3.0 mg/kg i.v.) on intrathoracic ^{111}In-labelled platelet accumulation in the rabbit (n=6) (mean ± sem). ★ **p < 0.05**

STIMULUS	VOLUME	n	PEAK HEIGHT (mean ± sem)	p
Saline	0.2 ml	6	4.23 ± 0.81	
	1.5 ml/kg	6	5.26 ± 0.77	
	3.0 ml/kg	6	7.09 ± 0.83	
ADP (100 ug/kg)	0.2 ml	6	42.66 ± 6.77	0.0002
Urovison	1.5 ml/kg	6	11.13 ± 3.19	0.2058
	3.0 ml/kg	6	15.69 ± 1.79	0.0012
Iopamiro	1.5 ml/kg	6	9.90 ± 2.34	0.0965
	3.0 ml/kg	6	9.02 ± 2.88	0.1207

Table VII.1 The effect of saline (0.2 ml, 1.5 and 3.0 ml/kg i.v.), ADP (100 *u*g/kg i.v.), Urovison (1.5 and 3.0 mg/kg i.v.) and Iopamiro (1.5 and 3.0 mg/kg i.v.) on intrathoracic ^{111}In-labelled platelet accumulation in the rabbit. Data is presented as mean percentage increases in thoracic counts ± sem (calculated by subtracting maximal counts from baseline counts and expressed as a percentage of the baseline counts. Results obtained with ADP and RCM are compared to their respective saline controls for statistical analysis.

CHAPTER VIII

CARDIOPULMONARY AND PLATELET CHANGES INDUCED BY DEXTRAN SULPHATE IN THE RABBIT

VIII.1 Introduction

Acute hypotension can follow the intravenous injection of numerous substances. This abrupt decrease in blood pressure may be mediated by direct effects on resistance vessels or by specific antigen recognition leading to activation of mast cells and basophils (anaphylaxis). On the other hand, similar events may be initiated by non-allergic (anaphylactoid) reactions such as are seen after the intravascular administration of radiographic contrast media (RCM) (Chapter I). The precise chain of events responsible for acute hypotension induced by RCM is not fully understood (Chapter I), although histamine is thought to play an important role (Herd *et al.*, 1988; Chapter II). In the rabbit model of IgE-anaphylaxis, acute hypotension has been shown to be platelet-dependent (Benveniste *et al.*, 1972; Pinckard *et al.*, 1977) and secondary to release of platelet activating factor (PAF) (McManus *et al.*, 1979*a*).

Adverse reactions to the intravenous infusion of dextran sulphate in patients have been reported (Brisman *et al.*, 1968; Strebel and Siegler, 1968; Maddi *et al.*, 1969). The incidence of serious and fatal reactions has been reported as 0.007% for low molecular weight dextran 40 (Ring and Messmer, 1977) and 0.069% (Ring and Messmer, 1977) to 0.075% (Bauer and Ostling, 1970) for dextran 70). This anaphylactoid response includes acute hypotension, bradycardia followed by tachycardia, irregular respiration, wheezing and bronchospasm. Autopsy studies following fatal dextran-induced reactions show an occlusion of pulmonary vessels with platelet thrombi, leucocytes and fibrin.

Systemic administration of dextran in the rat produces a state of acute anaphylactoid shock and a widespread degranulation of tissue mast cells (Parratt and West, 1957). The polysaccharide also releases histamine from isolated rat peritoneal mast cells *in vitro* and,

in common with antigen-antibody stimulaion of such cells, the process is potentiated by exogenous phosphatidylserine (Goth *et al.*, 1971) and is dependent on the presence of extracelluar calcium ions (Foreman and Mongar, 1972). However, it is unclear whether the compound interacts with specific glucoreceptors on the mast cell membrane (Moodley *et al.*, 1982; Thompson *et al.*, 1987) or combines with and cross-links cell-fixed IgE antibody (Hanahoe, 1984). Histamine release by dextran has been demonstrated in human subjects but does not appear to correlate with adverse reactions (Lorenz *et al.*, 1976).

A reproducible, anaphylactoid-type response can be induced by dextran sulphate in rabbits and is prevented by platelet depletion or by a combination of vagotomy and carotid ligation (Wiggins *et al.*, 1985). This response can be mimicked by serotonin but not by histamine (Wiggins *et al.*, 1985).

The aim of the present study was to further examine the mechanisms by which intravenous dextran sulphate causes anaphylactoid reactions in rabbits. In particular, the ability of various drugs, vagotomy and platelet depletion to influence dextran sulphate-induced cardiopulmonary changes and intrathoracic ^{111}Indium-labelled platelet accumulation has been investigated.

VIII.2 **Methods**

VIII.2.1 *Animals*

Male New Zealand White rabbits (1.8-3.8 kg) were used throughout the study. Animals were anaesthetised with sodium pentobarbital (30 mg/kg i.v.) for the cardiopulmonary studies and with a combination of diazepam (Valium, Roche) (5 mg/kg i.p.) followed 10 min later by Hypnorm (Janssen Pharmaceutica) (0.4 ml/kg i.m.) (0.315 mg fentanyl citrate/ml and 10 mg fluanisone/ml) for the platelet studies. In addition, 10 rabbits were anaesthetised with sodium pentobarbital (30 mg/kg i.v.) and the platelet responses to ADP and PAF were compared with those obtained from a group of animals anaesthetised with Hypnorm. This was done in order to establish the influence of the two

procedures on platelet responses and thus to relate the results of the platelet studies with those of the cardiopulmonary studies.

At the end of an experiment, animals were sacrificed by a lethal i.v. injection of sodium pentobarbitone.

VIII.2.2 *Drugs/Chemicals*

Adenosine diphosphate (ADP) disodium salt (Sigma), dextran sulphate sodium salt (average molecular weight 500,000) (Sigma), ICS 205-930 (3*alpha*-tropanyl)-1H-indole-3-carboxylic acid ester) (Sandoz, Basel, Switzerland), SRI 63-441 (*cis* (±)-1[2-[hydroxy [tetrahydro-5 [(octadecylaminocarbonyl)oxy] methyl]furan-2-yl]methoxy-phosphinyloxy]ethyl]-quinolinium hydroxide, inner salt), (Sandoz), sodium heparin (148 I.U./mg) (Hoffmann LaRoche, Basel, Switzerland) and iloprost (Schering) were dissolved in physiological saline (0.9% w/v). Platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (Bacham, Bubensdorf, FRG) was dissolved in ethanol and kept as a stock solution (1 mg/ml), which was diluted in a solution of bovine serum albumin (BSA) (0.25% w/v) (Sigma) in saline (0.9% w/v) as required. Dazmegrel (3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid) (Pfizer) was dissolved in 0.1 M NaOH. Dexamethasone acetate (Sigma) was dissolved in ethanol (50% v/v): polyethylene glycol (50% v/v). Acetyl salicylic acid (ASA, aspirin) (Sandoz) and indomethacin (Sigma) were dissolved in 200 *u*l $NaHCO_3$ and diluted with tris buffer (10% w/v). PGE_1 (Sigma) was dissolved in ethanol. ^{111}Indium oxine (^{111}In, 37 MBq/ml) was obtained from Amersham International (Amersham, England).

VIII.2.3 *In Vivo Pulmonary and Haemodynamic Measurements*

Airways resistance (R_L) and dynamic compliance (C_{dyn}) were measured in anaesthetised, spontaneously breathing rabbits by means of a flow transducer (Fleisch, type 0000) attached to an indwelling intratracheal cannula. Coincident pressure changes within

the thorax were monitored directly *via* an intrathoracic cannula, so that the pressure difference between the trachea and the thorax could be measured and displayed (*ie.* transpulmonary pressure, TPP). From these measurements of flow and differential pressure, both R_L and and C_{dyn} were calculated breath by breath using a respiratory analyser (Buxco, model 6).

The femoral artery and external jugular vein were cannulated for measurement of arterial blood pressure and heart rate and for the administration of experimental substances, respectively.

VIII.2.4 *Platelet Depletion*

Specific rabbit lytic anti-platelet antibodies were raised in sheep. To determine the requirement of platelets in the cardiopulmonary response to dextran sulphate, 6 rabbits were rendered thrombocytopenic by the administration of the antiserum (2 ml) on 3 separate occasions (at 24 hr intervals) prior to lung function studies.

VIII.2.5 *In Vivo Platelet Accumulation Studies*

Two marginal ear veins were cannulated (Venisystems Butterfly-25; 19.1 mm length, 0.4 mm internal diameter) for the administration of ^{111}In-labelled platelets and erythrocytes, and drugs and platelet stimuli to the rabbits. Intravenous cannulae with a dead space volume of at least 0.2 ml were used. ^{111}In-labelled platelets or erythrocytes were allowed to equilibrate for at least 30 min before challenge with the platelet stimuli.

^{111}Indium-Labelled platelets were prepared and monitored in anaesthetised, spontaneously breathing rabbits using the Automated Isotope Monitoring System (AIMS) which has been fully described in Chapter VI.

An initial priming dose of ADP (100 *u*g/kg) was administered 30 min prior to the dextran administration. Only one dose of dextran was given *per* animal as responses did not necessarily return to pre-injection levels and hence were not reproducible if repeated in

the same animal.

VIII.2.6 *Effects of Drugs*

Dextran and saline controls were administered intravenously *via* an indwelling cannula in a volume of 1 mg/kg. In both experimental systems drugs were administered intravenously as a bolus dose (volume 0.2 ml) *via* the venous cannula 1 min prior to the intravenous infusion of dextran sulphate (20 mg/kg; 1 ml/kg) and flushed through with 0.3 ml of physiological saline (0.9% w/v).

VIII.2.7 *Vagotomy*

Bilateral section of the vagus nerve was performed in 10 animals 60 min prior to experimentation to determine the influence of vagal tone on dextran-induced cardiopulmonary parameters. An additional 6 animals were vagotomised to assess the influence of vagal tone on intrathoracic ^{111}In-labelled platelet accumulation.

VIII.2.8 *Data Analysis*

Cardiopulmonary data are expressed as a mean percentage change $\pm$ standard error of the mean (sem) of *n* observations.

Platelet accumulation was measured as an increase in radioactive counts/sec by the detector over the thorax. Results are expressed as a percentage change in radioactive counts (calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts and converting this increase to a percentage of the baseline intrathoracic counts). Although abdominal counts were recorded in each case as an indicator of events occurring in the peripheral circulation, for convenience this data is not presented.

Significance was assessed statistically by the Student's *t*-test. A *p* value of less than

0.05 was considered significant.

VIII.3 Results

VIII.3.1 *Pulmonary and Haemodynamic Studies*

VIII.3.1.1 *Response to Dextran Sulphate*

In the rabbit, 0.1 mg/kg dextran sulphate caused no significant changes in any of the measured parameters (n=3) ($p > 0.05$). Similarly, 1 mg/kg dextran sulphate produced no appreciable changes, with the exception of a significant decrease in mean arterial blood pressure (MAP) (n=5) ($p < 0.05$) (Table VIII.1). Dextran sulphate at a dose of 10 mg/kg i.v. (n=7) caused a significant increase in airway resistance (R_L) and respiration rate (RR) ($p < 0.05$) and a significant reduction in MAP compared to the saline volume control (n=5) ($p < 0.05$) (Table VIII.1). The increase in dynamic compliance (C_{dyn}) induced by this dose of dextran sulphate did not attain statistical significance ($p > 0.05$) (Table VIII.1). Heart rate (HR) was unaffected. Figure VIII.1 is a representative tracing obtained following the i.v. administration of dextran sulphate 20 mg/kg in an individual rabbit and demonstrates the effect on flow (V), transpulmonary pressure (TPP), tidal volume (TV), C_{dyn}, R_L, RR, MAP and HR. The effects of dextran sulphate (20 mg/kg) (n=10) on C_{dyn}, R_L, RR, MAP and HR over time are shown in Figure VIII.2. C_{dyn}, RR and MAP were significantly decreased whereas R_L and RR were significantly increased following dextran sulphate (20 mg/kg i.v.) administration compared to the saline control (n=5) ($p < 0.05$) (Table VIII.1).

VIII.3.1.2 *Effect of ICS 205-930*

The effect of a bolus i.v. dose of ICS 205-930 (1 mg/kg) on the cardiopulmonary changes (V, TPP, TV, C_{dyn}, R_L, RR, MAP and HR) induced by dextran sulphate (20

DOSE	n	MAP (mm Hg)	HR (beats /min)	RR (breaths/ min)	C_{dyn} (ml/ cm H_2O)	R_L (cm H_2O/ L/sec)
0 saline control	5	116 ± 17	245 ± 6	58 ± 9	4.5 ± 0.3	19 ± 2
0.1 mg/kg	3	125 ± 8	240 ± 6	56 ± 7	4.8 ± 0.2	20 ± 1
p		0.7248	0.5964	0.9100	0.4927	0.4387
1 mg/kg	5	58 ± 4	238 ± 8	51 ± 6	5.3 ± 0.5	22 ± 2
p		0.0086	0.5017	0.5560	0.2094	0.2461
10 mg/kg	5	55 ± 4	247 ± 18	148 ± 28	3.1 ± 0.5	28 ± 3
p		0.0032	0.9247	0.0238	0.0537	0.0240
20 mg/kg	10	58 ± 7	202 ± 10	137 ± 14	2.9 ± 0.4	35 ± 4
p		0.0018	0.0129	0.0019	0.0145	0.0214

Table VIII.1 Effect of dextran sulphate (0, 1, 10 and 20 mg/kg i.v.) on the haemodynamic and pulmonary changes induced in the rabbit. Responses are expressed as the maximal change as a percentage of the pre-injection values (mean ± sem). Treatments are compared to saline controls (1 mg/kg i.v.) for statistical analysis.

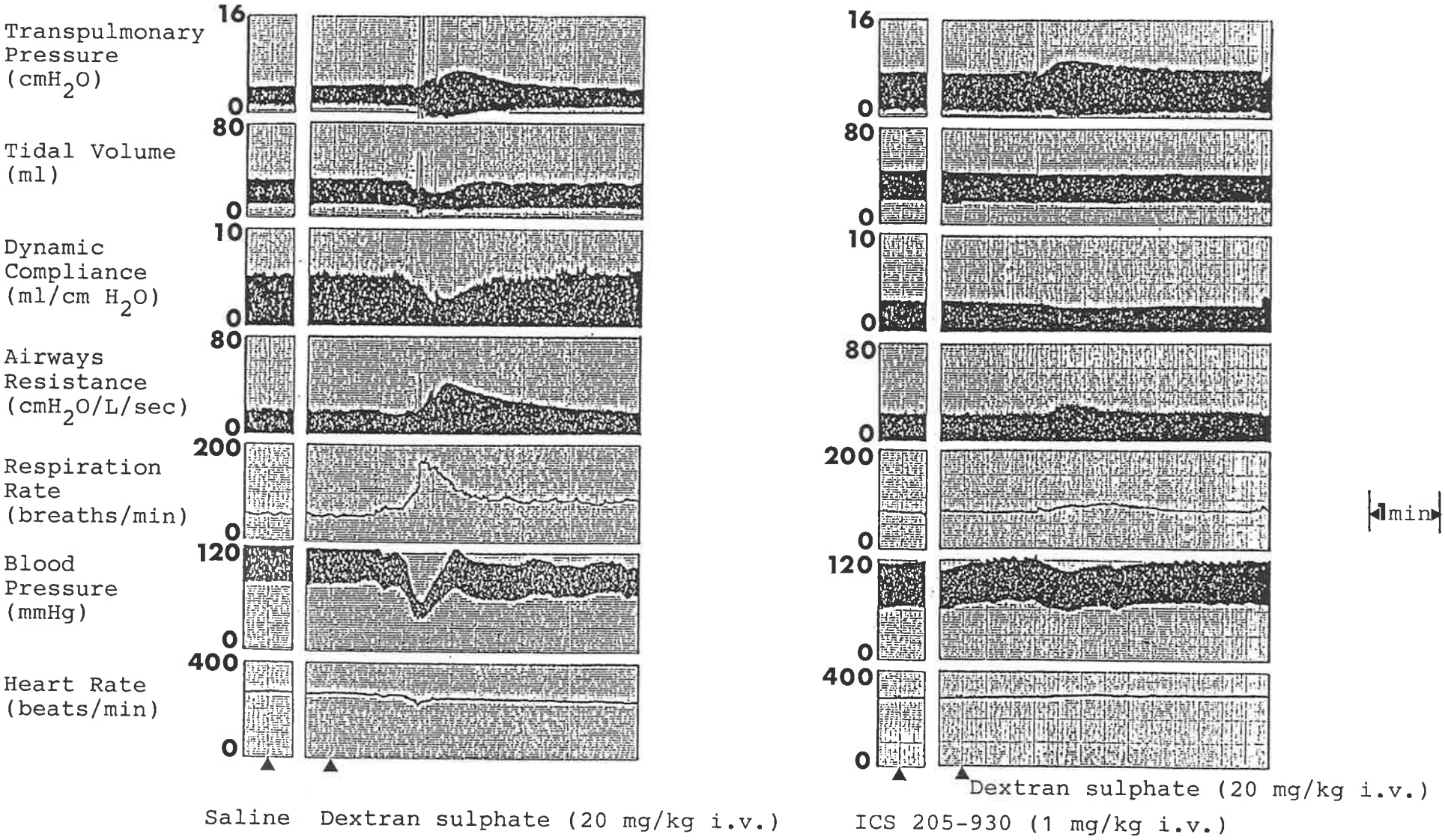


Figure VIII.1 Representative tracings of cardiopulmonary changes obtained following dextran sulphate (20 mg/kg i.v.) administration in the rabbit: saline and ICS 205-930 (1 mg/kg i.v.) pretreatment.

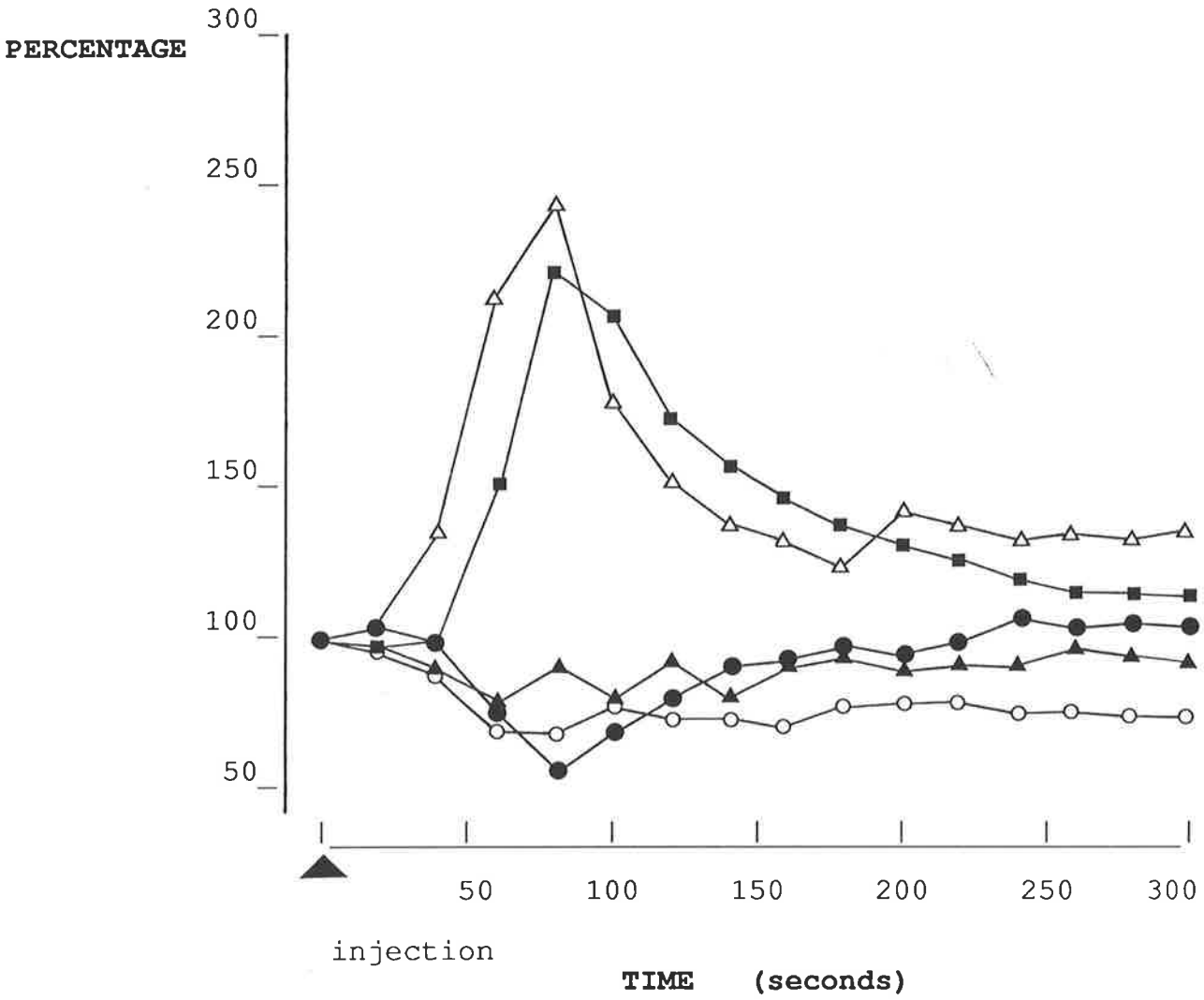


Figure VIII.2 The effect of dextran sulphate (20 mg/kg i.v.) on pulmonary dynamic compliance (C_{dyn}) (●), airway resistance (R_L) (■), respiration rate (RR) (△), mean arterial pressure (MAP) (○) and heart rate (HR) (▲)in the rabbit (n=10).

mg/kg i.v.) in an individual rabbit is shown in a representative tracing (Figure VIII.1). ICS 205-930 (1 mg/kg i.v.) injected 1 min prior to the administration of dextran sulphate (20 mg/kg i.v) resulted in the significant inhibition of all the measured dextran sulphate-induced changes (n=11) ($p < 0.05$) (Figure VIII.3 and Table VIII.2).

VIII.3.1.3 *Effect of Vagotomy*

Similar to ICS 205-930 pretreatment, vagal section significantly inhibited the pulmonary and haemodynamic changes induced by dextran sulphate (20 mg/kg i.v.) in the rabbit (n=10) ($p < 0.05$) (Figure VIII.3 and Table VIII.2).

VIII.3.1.4 *Effect of Platelet Depletion*

The percentage platelet depletion in 6 rabbits treated with anti-platelet anti-sera was 77.18 $\pm$ 2.19% (mean $\pm$ sem).

In rabbits rendered partially thrombocytopenic, all the measured dextran sulphate (20 mg/kg i.v.)-induced responses were inhibited compared to responses obtained in rabbits with normal circulating platelet counts. However, only the results obtained for HR reached statistical significance (n=6) ($p < 0.05$) (Figure VIII.3 and Table VIII.2).

VIII.3.2 *In Vivo Platelet Accumulation Studies*

VIII.3.2.1 *Responses to ADP and PAF: Pentobarbital vs Hypnorm*

The extent of intrathoracic ^{111}In-labelled platelet accumulation induced by ADP (100 *u*g/kg i.v.) and PAF (100 *n*g/kg i.v.) in rabbits anaesthetised with pentobarbital (30 mg/kg i.v.) (37.88 $\pm$ 4.11%, n=22 and 46.88 $\pm$ 3.63%, n=20, respectively) was not significantly different to the accumulation induced by the same dose of ADP and PAF in animals anaesthetised with a combination of diazepam (5 mg/kg i.p.) and Hypnorm (0.4

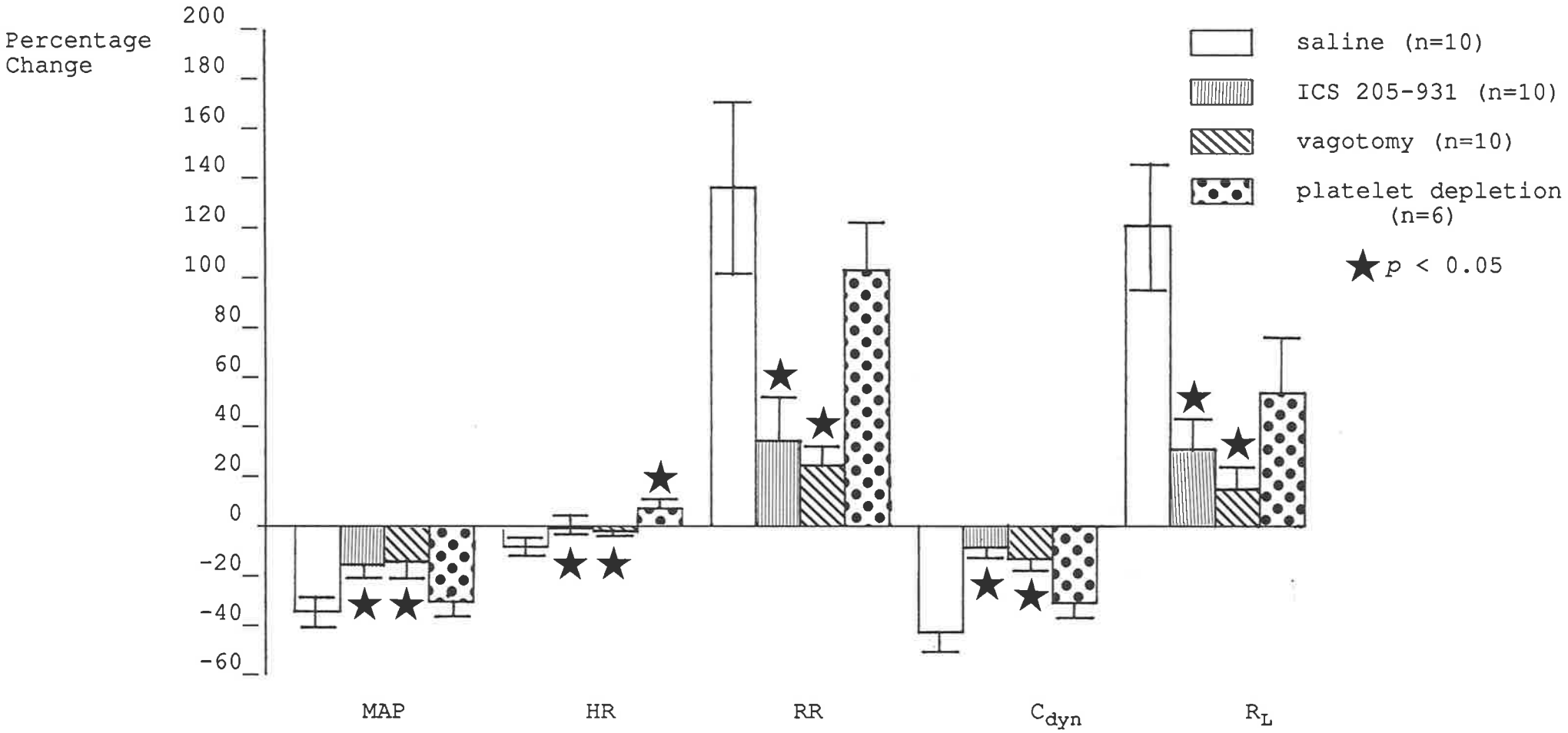


Figure VIII.3 Effect of ICS 205-930 (1 mg/kg), vagotomy and platelet depletion upon the haemodynamic (mean arterial pressure (MAP) and heart rate (HR)) and pulmonary (respiration rate (RR), dynamic pulmonary compliance (C_{dyn}) and airways resistance (R_L)) induced by dextran sulphate (20 mg/kg i.v.) in the rabbit.

TREATMENT	n	MAP	HR	RR	C_{dyn}	R_L
		(Percentage Change ± sem)				
Saline	10	-35 ± 6	-8 ± 3	136 ± 35	-44 ± 7	121 ± 25
ICS 205-930	11	-17 ± 4	-1 ± 3	33 ± 19	-8 ± 4	31 ± 12
p		0.0082	0.0384	0.0074	0.0005	0.0014
Vagotomy	10	-16 ± 5	-2 ± 2	24 ± 9	-12 ± 4	14 ± 9
p		0.0106	0.0401	0.0054	0.0005	0.0011
Platelet Depletion	6	-30 ± 6	6 ± 3	102 ± 20	-30 ± 6	54 ± 22
p		0.5284	0.0308	0.4867	0.2006	0.0783

Table VIII.2 Effect of saline (1 ml/kg i.v.), ICS 205-930 (1 mg/kg i.v.), vagotomy and platelet depletion on the haemodynamic and pulmonary changes induced by dextran sulphate (20 mg/kg i.v.) in the rabbit. Responses are expressed as the maximal change as a percentage of the pre-injection value (mean ± sem). For statistical analysis treatments are compared to the saline control responses.

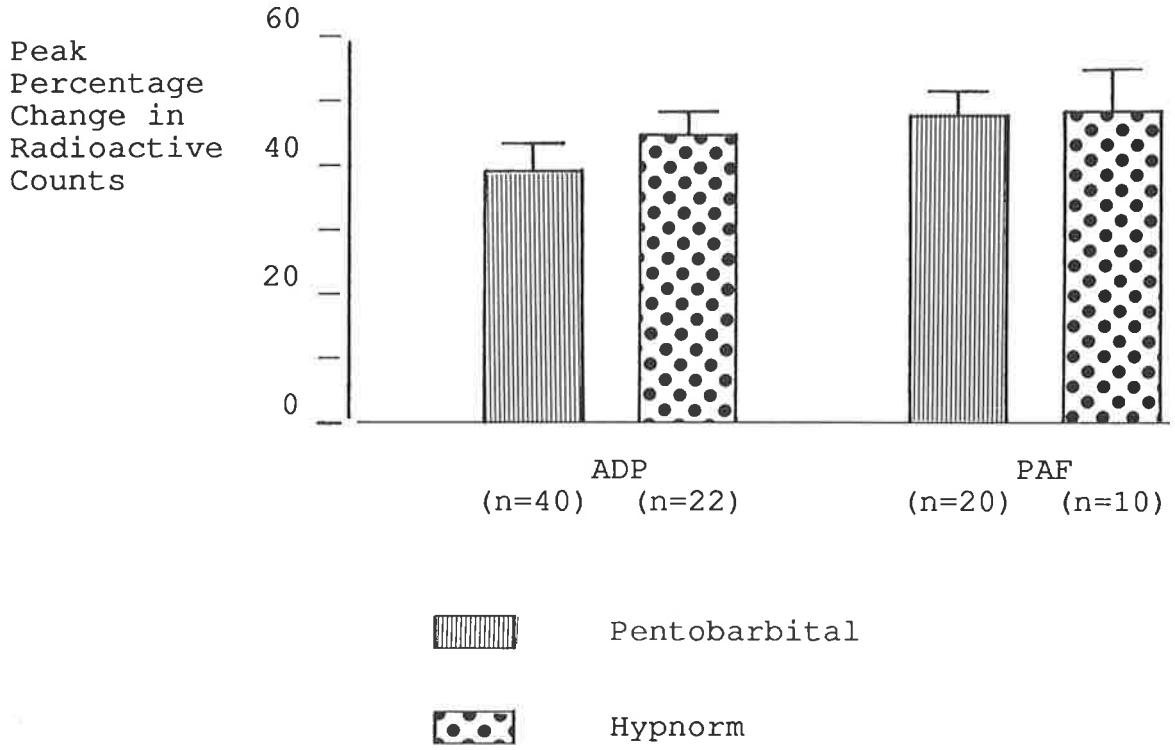


Figure VIII.4 Effect of ADP (100 *u*g/kg) and PAF (100 *n*g/kg) upon intrathoracic ^{111}In-labelled platelet accumulation in rabbits anaesthetised with pentobarbital (30 mg/kg) or Hypnorm (0.4 ml/kg).

ml/kg i.m.) (44.42 ± 3.29%, n=40 and 48.10 ± 6.38%, n=10, respectively) (Figure VIII.4).

VIII.3.2.2 *Response to Dextran Sulphate*

Dextran sulphate ^{111}In-labelled intrathoracic platelet accumulation in the rabbit was dose-related over the range 0.1-20 mg/kg i.v. (Figure VIII.5) (Table VIII.3). The intravenous administration of dextran sulphate (20 mg/kg) resulted in the progressive accumulation of ^{111}In-labelled platelets in the thoracic region of the rabbit with a concomitant reduction in the hindlimb region (results not shown). This dose of dextran sulphate elicited irreversible intrathoracic ^{111}In-labelled platelet accumulation in all animals and the maximal value (74.87 ± 12.33% ; n=10) was attained 10-15 min following administration. In animals that had received ^{111}In-labelled erythrocytes an increase in thoracic counts was also evident in response to intravenous dextran sulphate (20 mg/kg) (16.42 ± 2.53% ; n=5); however, the accumulation was small in comparison with that achieved in animals that had received ^{111}In-labelled platelets ($p < 0.05$) (Figure VIII.6 and Table VIII.3).

VIII.3.2.3 *Effect of ICS 205-930*

The prior i.v. injection of a bolus dose of ICS 205-930 (1 mg/kg) had no significant effect on the extent of ^{111}In-labelled platelet accumulation in the thoracic region of the rabbit induced by dextran sulphate (20 mg/kg i.v.) (Figure VIII.7 and Table VIII.4).

VIII.3.2.4 *Effect of Vagotomy*

Intrathoracic ^{111}In-labelled platelet accumulation induced by an intravenous bolus dose of ADP (100 *u*g/kg), PAF (100 *n*g/kg) or dextran sulphate (20 mg/kg) in rabbits that had undergone bilateral vagotomy was not significantly different to responses induced in animals with intact vagus nerves (Figure VIII.8 and Table VIII.5).

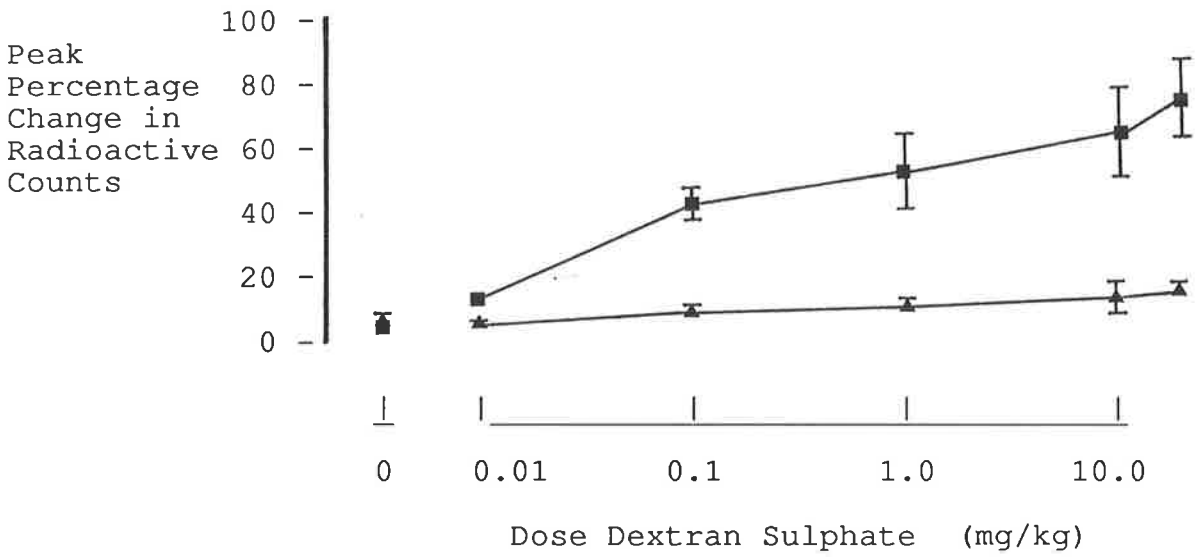


Figure VIII.5 Effect of dextran sulphate (0.01-20 mg/kg i.v.) on intrathoracic ^{111}In-labelled platelet (■) and ^{111}In-labelled erythrocyte (▲) accumulation in the rabbit (mean + sem) (n=5-10).

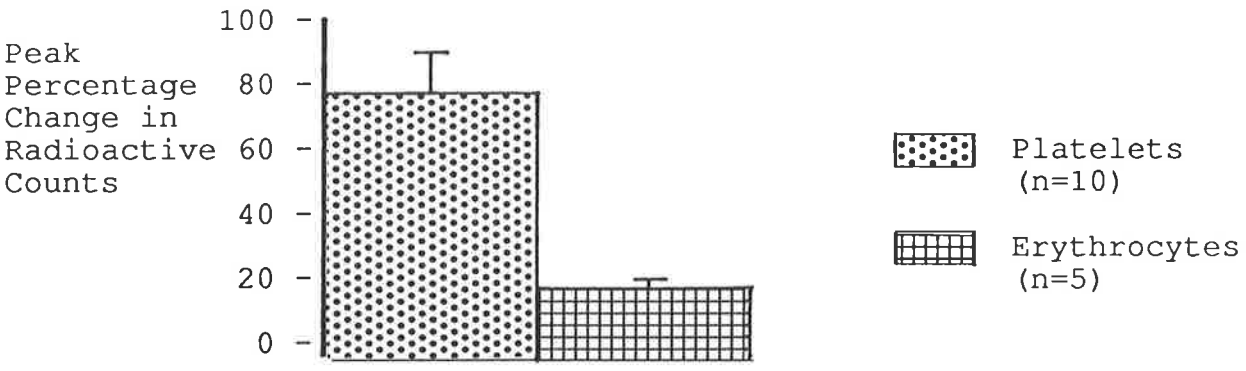


Figure VIII.6 Effect of dextran sulphate (20 mg/kg i.v.) on intrathoracic ^{111}In-labelled platelet and ^{111}In-labelled erythrocyte accumulation in the rabbit (mean + sem).

DOSE (mg/kg)	PEAK HEIGHT (mean ± sem) ^{111}In-Platelets	n	^{111}In-Erythrocytes	n
0.00	5.26 ± 0.77	5	7.24 ± 2.23	5
0.01	14.76 ± 1.35	4	5.99 ± 1.27	5
0.10	43.63 ± 4.96	5	9.74 ± 2.14	5
1.00	53.75 ± 10.95	10	10.31 ± 2.05	5
10.00	64.54 ± 12.73	9	14.91 ± 4.89	5
20.00	74.87 ± 12.33	10	16.42 ± 2.53	5

Table VIII.3 Effect of dextran sulphate (0.01-20 mg/kg i.v.) on ^{111}In-labelled platelet and ^{111}In-labelled erythrocyte accumulation in the thoracic region of the rabbit (mean ± sem). Peak height has been calculated by subtracting the baseline thoracic counts from the maximal change in thoracic counts and converted to a percentage of the baseline counts.

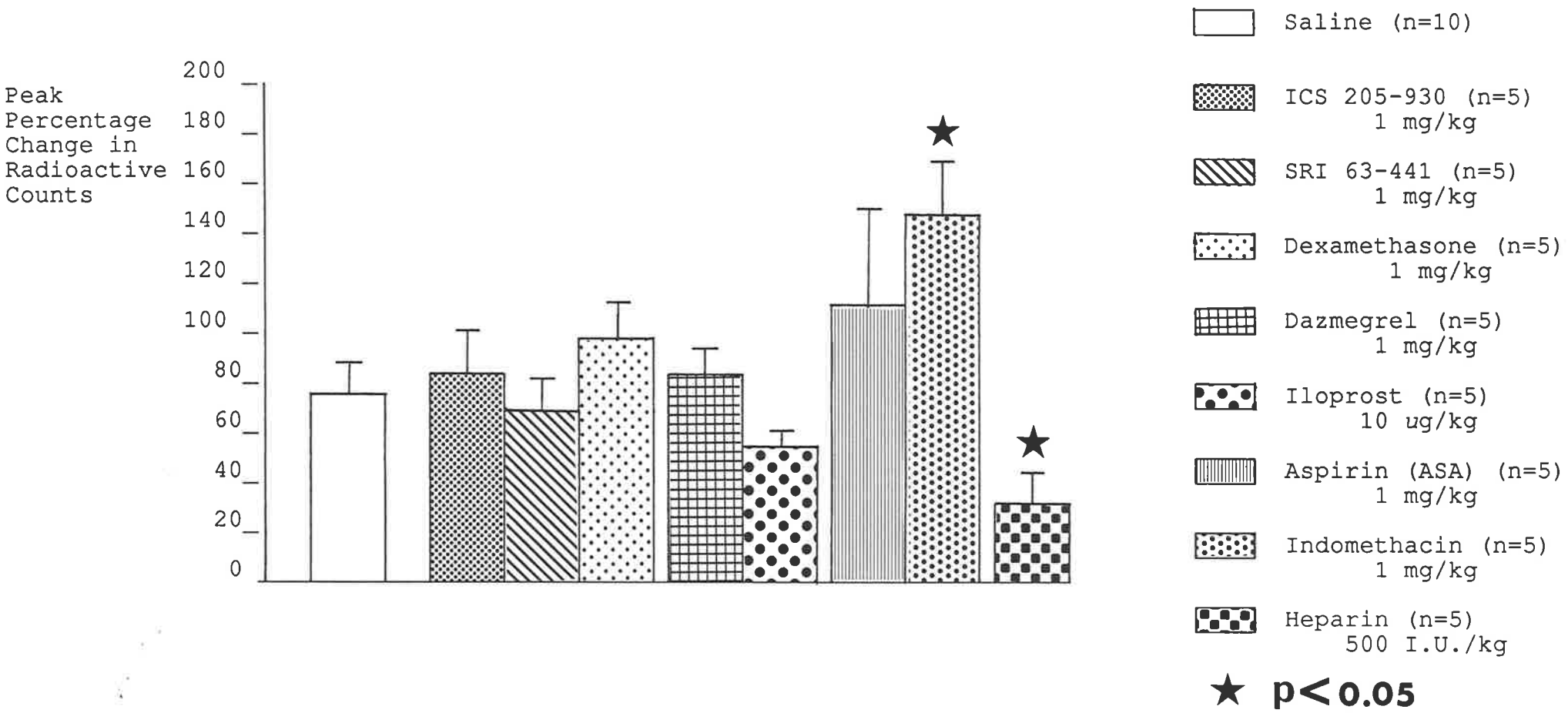


Figure VIII.7 Effect of various drug pretreatments on the intrathoracic ^{111}In-labelled platelet accumulation induced by dextran sulphate (20 mg/kg i.v.) in the rabbit (mean + sem).

DRUG	DOSE (mg/kg)	PEAK HEIGHT (mean ± sem)	n	*p*
Dextran alone		74.87 ± 12.33	10	
ICS 205-930	1	82.32 ± 15.99	5	0.7261
SRI 63-441	1	69.15 ± 11.46	5	0.7729
Dexamethasone	10	95.78 ± 13.89	4	0.3520
Dazmegrel	10	81.72 ± 10.14	4	0.7481
Iloprost	0.01	54.86 ± 6.43	6	0.2550
Aspirin	1	110.36 ± 37.28	4	0.2562
Indomethacin	1	145.32 ± 20.78	6	0.0074
Heparin	500 I.U./kg	29.91 ± 10.72	6	0.0163

Table VIII.4 The effect of various drugs on dextran sulphate (20 mg/kg i.v.)-induced ^{111}In-labelled platelet accumulation in the rabbit. Peak height has been calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts observed and represented as a percentage increase of the baseline intrathoracic counts. Drug effects were compared to the effect of dextran sulphate (20 mg/kg) alone, in a separate group of animals.

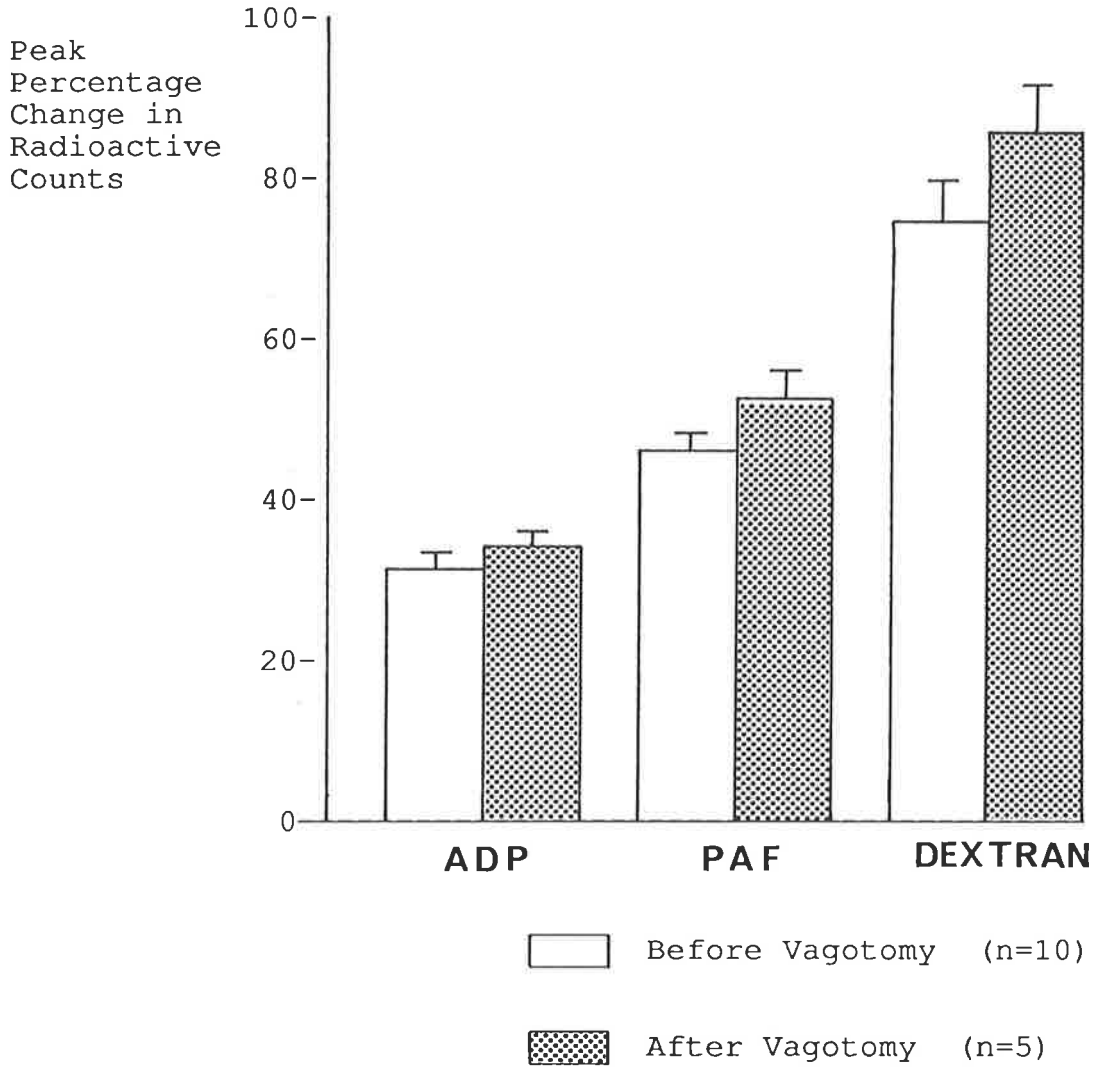


Figure VIII.8 The effect of vagotomy on ^{111}In-labelled platelet accumulation induced by ADP (100 *u*g/kg), PAF (100 *n*g/kg) and dextran sulphate (20 mg/kg) in the rabbit (mean + sem).

DRUG	PEAK HEIGHT intact vagi (mean ± sem)	PEAK HEIGHT post-vagotomy (mean ± sem)	*p*
ADP 100 *ug*/kg	31.68 ± 4.79 (n=10)	34.43 ± 4.20 (n=5)	0.3027
PAF 100 *ng*/kg	46.03 ± 4.62 (n=10)	52.78 ± 6.74 (n=5)	0.4506
Dextran 20 mg/kg	74.87 ± 12.33 (n=10)	86.12 ± 14.39 (n=5)	0.5884

Table VIII.5 The effect of bilateral vagal section on the ^{111}In-labelled platelet response induced by ADP (100 *ug*/kg i.v.), PAF (100 *ng*/kg i.v.) and dextran (20 mg/kg i.v.) in the rabbit. Peak height has been calculated by subtracting the baseline intrathoracic counts from the maximal increase in intrathoracic counts observed and represented as a percentage increase of the baseline intrathoracic counts.

VIII.3.2.5 *Effects of Other Drugs*

Pretreatment of rabbits with either the PAF antagonist SRI 63-441 (1 mg/kg i.v.), the glucocorticoid dexamethasone (1 mg/kg i.v.), the thromboxane synthetase inhibitor dazmegrel (10 mg/kg i.v.) or the prostacyclin analogue iloprost (10 *ug*/kg i.v.) had no significant effect on the extent of ^{111}In-labelled platelet accumulation induced by dextran sulphate (20 mg/kg i.v.) ($p > 0.05$) (Figure VIII.7 and Table VIII.4). The i.v. administration of either non-steroidal anti-inflammatory drug, acetyl salicylic acid (ASA) or indomethacin, caused a potentiation of the dextran (20 mg/kg i.v.)-induced platelet accumulation (Figure VIII.7 and Table VIII.4); however, the effect of ASA was not statistically significant ($p > 0.05$). Heparin (500 I.U./kg i.v.) was the only drug tested to significantly inhibit the dextran sulphate (20 mg/kg i.v.)-induced platelet response when administered 1 min prior to the stimulus ($p < 0.05$) (Figure VIII.7 and Table VIII.4).

VIII.4 Discussion

In the experiments described in the present study, an anaphylactoid reaction was induced in rabbits by the intravenous injection of the polyanion dextran sulphate. In particular, the intravenous injection of dextran sulphate (20 mg/kg) caused decreases in heart rate (HR), mean arterial blood pressure (MAP) and dynamic compliance (C_{dyn}) and increases in respiration rate (RR) and airways resistance (R_L).

Platelet depletion has been shown to prevent the hypotension caused by IgE-induced anaphylaxis in the rabbit (Benveniste *et al.*, 1972; Pinckard *et al.*, 1977; McManus *et al.*, 1979*a*). In the present study, platelet depletion inhibited dextran sulphate-induced hypotension and bradycardia and the pulmonary alterations; however, the effects were not statistically significant for all the measured parameters. This is probably due to the extent of thrombocytopenia induced by the anti-platelet anti-serum. Studies utilising anti-sera report platelet depletion of at least 95%, whereas in the present study only 77% platelet depletion was achieved. Therefore sufficient circulating platelets were present to respond

to dextran sulphate challenge. Nonetheless, the results do suggest an involvement of platelets in the dextran sulphate-induced anaphylactoid response.

Following dextran sulphate injection in the rabbit, platelets have been shown to disappear from blood and appear in the lung, where ultrastructural analysis 2 min after dextran administration showed them to be aggregated and in the process of degranulating (Wiggins *et al.*, 1985). In the present study, an irreversible accumulation of ^{111}In-labelled platelets in the thoracic region was elicited in every rabbit infused intravenously with dextran sulphate (20 mg/kg), suggestive of substantial coagulation cascade activation. Both the duration and the extent of dextran sulphate-induced accumulation was far in excess of that induced by other known platelet stimuli in the rabbit *eg.* ADP, collagen, PAF and thrombin (Chapter VI). Further evidence for the pivotal role of platelets in the response induced by dextran sulphate in the present study, is the finding that both cardiopulmonary alterations and platelet accumulation were dose-related over a similar range.

Rabbit platelets contain both histamine and serotonin in their dense granules (Da Prada *et al.*, 1981), and dextran has been shown to liberate histamine in rabbits (Haining, 1956). The finding that cimetidine infused at high doses prevented dextran-induced hypotension (Wiggins *et al.*, 1985) indicated histamine to be the important mediator. However, when histamine was injected intravenously or into the right ventricle of the rabbit, the predominant result was either no change in blood pressure or immediate hypertension with tachycardia followed by a mild hypotensive phase (Brimblecombe *et al.*, 1974; Carroll *et al.*, 1974; Wiggins *et al.*, 1985). It has been reported that high doses of cimetidine inhibit serotonin-induced cardiovascular changes in the rabbit (Wiggins and Campbell, 1983). Therefore, it is not possible to attribute dextran-induced hypotension and bradycardia solely to histamine release from platelets (or from basophils which disappeared from the blood and appeared in degranulated form in the lung following dextran injection (Wiggins et al., 1985)). In contrast, serotonin injection reproduced the immediate hypotension and bradycardia observed following dextran sulphate injection, although the decrease in blood pressure was less prolonged, even when high doses of serotonin were used (Wiggins *et al.*, 1985). The involvement of histamine in dextran

sulphate-induced haemodynamic changes was suggested by the finding that a combination of histamine and serotonin infused over 1 min appeared to reproduce more closely both the extent and duration of the events seen after dextran sulphate administration (Wiggins *et al.*, 1985). In addition to this, plasma measurements confirmed that the amount of serotonin released was sufficient to cause the hypotension and bradycardia, whereas the converse applied to histamine (Wiggins *et al.*, 1985).

In the present study, dextran sulphate-induced cardiopulmonary changes in the rabbit were significantly diminished, but not completely abolished, by bilateral vagotomy. A similar finding has been reported by Wiggins and colleagues (1985), whereby dextran sulphate-induced hypotension could be reduced by section of the vagus and depressor nerves and totally prevented by carotid ligation in addition to nerve section. Vagal nerves are not involved in these events *via* an effect on platelet function, as ^{111}In-labelled platelet responses to ADP, PAF or dextran sulphate in the present study were totally unaffected by vagotomy.

Prior treatment of the rabbits with the potent and highly selective 5-HT_3 receptor antagonist ICS 205-930 (Richardson *et al.*, 1985), significantly inhibited the dextran sulphate-induced pulmonary and haemodynamic alterations.

Taken together, the results obtained with vagotomy and 5-HT_3 receptor blockade indicate a serotonin-mediated chemoreflex as the major determinant of dextran-induced cardiopulmonary changes in the rabbit. The chemoreflexes by which serotonin induces hypotension and bradycardia were originally identified by Bezold and Hirt in 1867. Dawson and Comroe (1954) defined 3 separate chemoreflexes in the heart and lungs *ie.* a coronary reflex, a pulmonary depressor reflex and a pulmonary respiratory reflex. The afferent limb of these reflexes begins with chemoreceptors in the lungs, heart and carotid sinus (Jacobs and Comroe, 1971). Signals travel in the vagus nerve and depressor nerve in the rabbit (Winton and Bayliss, 1955), to the autonomic nerve centres in the brain. The efferent limb appears to take the form of a general increase in vagal tone and a decrease in sympathetic tone, but there are also more localised organ effects (*eg.* on lungs or cardiovascular system) depending on the site of receptor stimulation (Erspamer, 1966).

Decreases in heart rate and blood pressure have been documented not only in the rabbit, but also in the cat (Erspamer, 1966). In other studies, reflex vasodilatation due to decreased sympathetic activity has been observed in the isolated hindlimb, liver, kidney, intestine, spleen and retina (Erspamer, 1966). Increased parasympathetic activity can cause smooth muscle contraction in the bronchioles, which may explain the increase in airways resistance and decrease in dynamic compliance induced by dextran sulphate in the present study.

The mechanism by which dextran sulphate causes substantial intrathoracic platelet accumulation has not been elucidated, although dextran has previously been observed to cause platelet aggregation *in vitro* (Walton, 1953; Tiffany and Penner, 1981). Wiggins and colleagues (1985) suggested that dextran sulphate activates basophils (as evidenced by diminished blood basophil count and the appearance of degranulated basophils in the lung following dextran injection) with consequent release of platelet activating factor (PAF). This situation would thus parallel the model of IgE anaphylaxis in the rabbit (Benveniste *et al.*, 1972; Pinckard *et al.*, 1977; McManus *et al.*, 1979*a*). No evidence is provided to show that dextran interacts directly with basophils, although such an interaction may occur either by a charge or osmotic effect (Findlay *et al.*, 1981). The results of the present studies however, refute this proposal. The kinetics (*ie.* time course and extent) of dextran-induced ^{111}In-labelled platelet accumulation in the rabbit differ significantly from that of the PAF-induced response (Chapter VI). Furthermore, the specific PAF receptor antagonist SRI 63-441 (Handley *et al.*, 1986) (100 *ug*/kg i.v.) has been shown to fully inhibit PAF-induced ^{111}In-labelled intrathoracic platelet accumulation in the rabbit (Chapter VI), whereas the same dose (or higher *ie.* 1 mg/kg) of SRI 63-441 had no effect on dextran sulphate-induced platelet accumulation.

Neither NSAID, aspirin nor indomethacin, displayed an inhibitory action on dextran sulphate-induced intrathoracic ^{111}In-labelled platelet accumulation in the rabbit. Both drugs enhanced the dextran sulphate-induced platelet response, an effect similar to that observed in the rabbit (Chapter VI) and guinea-pig (Chapter V) challenged with PAF. The contribution of thromboxane A_2 (TXA_2) in the dextran sulphate-induced platelet response

is not known. If dextran sulphate induced substantial TXA_2 production *in vivo*, there would be a significant incidence of death, as rabbits are highly susceptible to sudden death following the i.v. administration arachidonate (Lefer *et al.*, 1984; refer Chapter VI). In the present study the mortality rate following dextran sulphate administration was zero. Results obtained with ASA and indomethacin in the present study may be explained in terms of their effects on the vascular endothelium. The inhibition of prostacyclin (PGI_2) synthesis due to inactivation of the enzyme cyclo-oxygenase in endothelial cells, may have caused the exaggerated responses by eliminating the endogenous inhibitory control mechanism. Alternatively, cyclo-oxygenase inactivation may have shunted arachidonic acid metabolism toward the lipoxygenase pathway, leading to the formation of possible platelet active lipid products, which led to an enhanced dextran sulphate response.

In the present study, dazmegrel, a potent and selective thromboxane synthetase antagonist (Vermylen *et al.*, 1981; Fitzgerald and Oates, 1984), was without effect on the platelet accumulation induced by dextran sulphate. This result may or may not implicate an involvement TXA_2, as thromboxane synthetase inhibition may direct metabolism towards other platelet-active prostanoic derivatives (Defreyn *et al.*, 1982; Smith, 1982; Bertele *et al.*, 1984; Gresele *et al.*, 1984; Rajtar *et al.*, 1985). Furthermore, many mechanisms may operate in the dextran sulphate-induced platelet response and TXA_2 may play only a small role.

The stable prostacyclin analogue iloprost (Sturzebecher and Losert, 1987) (10 *u*g/kg) has been shown to significantly inhibit PAF-induced intrathoracic ^{111}In-labelled platelet accumulation in the rabbit (Chapter VI). In the present study, iloprost reduced the dextran sulphate-induced platelet response; however, this effect did not reach statistical significance.

There is conflicting opinion as to the effect of heparin on platelet aggregation (Mustard and Packham, 1975). In the present study, high dose heparin (500 I.U./kg) was the only drug tested to induce significant inhibition of platelet accumulation induced by dextran sulphate in the rabbit.

The results of the present study parallel the findings in Chapter VI. The only drugs

able to inhibit either PAF- or dextran sulphate-induced ^{111}In-labelled platelet accumulation in the rabbit were iloprost and heparin. This strongly suggests multiple modes of action in the response to these two platelet agonists *in vivo*.

Rabbits may have natural antibodies against dextrans (Palosvo and Milgrom, 1981) or dextran sulphate-immunoglobulin interactions may have triggered platelet activation (Levy *et al.*, 1981). Activation of complement by dextran may also have contributed to platelet activation and thrombocytopaenia (Ulevitch and Cochrane, 1977). However, this mechanism cannot totally account for the platelet-induced effects, since C3 depletion did not prevent dextran-induced hypotension (Wiggins *et al.*, 1985).

The results of the present study, together with those of Wiggins and colleagues (1985), indicate that dextran sulphate elicits cardiopulmonary effects largely by releasing serotonin from platelets lodged in the pulmonary circulation. Once released, this serotonin stimulates 5-HT_3 receptors located on chemoafferent nerve endings resulting in a complex of reflex events. Dextran sulphate injection results in activation of many mediator systems. Evidence is provided in this study that platelet activation in the lungs causes bradycardia and hypotension by means of powerful autonomic reflexes activated by serotonin. If similar reflex mechanisms exist in humans, ICS 205-930 might be useful in preventing anaphylactoid reactions occurring in patients receiving dextran.

CHAPTER IX

GENERAL CONCLUSIONS

Adverse reactions to the intravascular administration of radiographic contrast media (RCM) have remained a significant problem since the introduction of such agents into diagnostic radiology approximately sixty years ago. The diversity of reactions and their idiosyncratic nature have led to the formulation of many different proposals regarding their aetiology. To elucidate the role of the vasoactive autacoid histamine in the production of such untoward responses, a study of four different radiographic contrast media (RCM) was undertaken in a group of 200 patients undergoing intravenous urography (IVU). The contrast agents evaluated included the commonly used ionic, high osmolality Urovison (sodium and meglumine diatrizoate), the ionic, low osmolality Hexabrix, and two newer agents, Iopamiro (iopamidol) and Omnipaque (iohexol), which are both non-ionic and have a low osmolality. The effects of these RCM were examined with respect to histamine release, cardiovascular effects and adverse drug reactions (ADRs).

Mild side-effects such as local pain at the injection site, heat, flushing and nausea were encountered by 49.5% of all the patients assessed, while 3% experienced reactions of a more severe nature. The latter included vomiting, urticaria, bronchospasm and hypotension. The conventional RCM Urovison produced the greatest number of ADRs *ie.* 80% of patients administered this agent experienced reactions of varying intensity. Iopamiro caused the least number of ADRs *ie.* 36%.

Plasma histamine concentrations were found to increase within a 20 minute period following contrast medium injection in 80% of all patients studied, with a return to pre-injection levels within 10 minutes of this rise. However, despite the fact that Urovison caused the greatest and Iopamiro the least change in plasma histamine level one minute following administration, no significant correlation between the magnitude of the change in histamine level and the production of a particular ADR could be substantiated. This may be due to both patient variables and the absence of more serious reactions in the study

group. The variability noted in this evaluation may relate to the existence of differing populations of histamine receptors or variable receptor sensitivity to histamine in the patients.

Heart rate increased significantly following the administration of Urovison, Hexabrix and Iopamiro in the absence of any appreciable change in blood pressure. Taken together, these results indicate that the use of low-osmolality contrast media are clinically advantageous, in that they minimise ADRs, histamine release and patient discomfort. However, findings from this study do not implicate histamine liberation as the sole cause for the development of adverse reactions to radiographic contrast media.

Serotonin, a potent vasoactive autacoid, is stored in the dense granules of platelets and is liberated when these blood elements are sufficiently stimulated to undergo the release reaction. In addition, upon stimulation, platelets can synthesise thromboxane and various prostaglandins and hydroxy-acids *via* the metabolism of membrane arachidonic acid, which may be involved in the development of anaphylactic and anaphylactoid reactions. The effect on platelet function of Urovison and Iopamiro was therefore examined in a further 20 patients undergoing IVU. Using *in vitro* aggregometry techniques it was noted that Urovison caused significant inhibition of platelet aggregation in response to collagen *ex vivo*, but not in response to ADP, adrenaline or arachidonate. No significant difference in aggregation induced by any of the foregoing platelet stimuli was evident following Iopamiro injection. Furthermore, neither RCM significantly altered thromboxane B_2 (TXB_2) production in response to any of the platelet agonists. In a complementary *in vitro* study, both Urovison and Iopamiro caused a dose-dependent inhibition of platelet aggregation in response to ADP and collagen. However, the ionic, high osmolality Urovison was found to have approximately four times the inhibitory potency of the non-ionic, low osmolality Iopamiro. The inhibitory action of the two RCM in this study could not be attributed to their effect on TXA_2 synthesis as both RCM could inhibit the aggregation of platelets removed from volunteers who had ingested a dose of aspirin known to maximally inhibit TXA_2 production *ex vivo*. The ability of both RCM to inhibit platelet aggregation indicates that RCM do not activate platelets directly and hence do not

release serotonin.

In vitro platelet aggregometry, a technique introduced by Born, has been extensively used since its development in the early 1960's. However, this operationally simple system may not necessarily be indicative of complex events occurring in the circulation. A substantial component of the work presented in this thesis therefore, is devoted to the modification and improvement of a method for studying platelet function *in vivo*. Homologous platelets labelled with ^{111}Indium were injected intravenously into anaesthetised, spontaneously breathing guinea-pigs and these circulating labelled platelets continuously monitored in the thoracic and abdominal regions using collimated crystal scintillation probes. The counts were collected using a dual channel gamma spectrometer incorporating a dedicated microcomputer. The increase in counts recorded over the thoracic region and the fall in counts recorded over the abdominal region (employed as a reference for events occurring in the peripheral circulation) following the intravenous administration of a platelet stimulus implies that platelets are being aggregated within the vascular system and accumulating in the pulmonary microvasculature. The finding that the same platelet stimulus produces minimal changes in counts recorded over either region when using ^{111}Indium-labelled red blood cells indicates that the effects are not due merely to alterations in blood flow or blood volume and are thus consistent with the conclusion that ^{111}Indium-labelled platelets accumulate in the lung. Dose-effect relationships were obtained for the known platelet agonists ADP, collagen and PAF *via* the intravenous route in guinea-pigs, and responses were detected at lower doses than previously reported in this species. PAF was found to be the most potent platelet stimulus, being 100 times more potent than ADP and 1,000 times more potent than collagen. Differences in kinetics were also observed, whereby the platelet response to ADP was more rapid and transient than the response to either PAF or collagen. Furthermore, the distinctive nature of these responses was highlighted by the finding that the selective PAF receptor antagonist SRI 63-441 fully inhibited the platelet accumulation induced by PAF but not by ADP. As a corollary, the platelet responses induced by collagen was diminished by aspirin, yet the PAF- and ADP-induced accumulation remained unaffected.

PAF, a phospholipid generated by a range of "activated" inflammatory cells, has been proposed as a mediator of asthma as it can elicit responses in both experimental animals and man that mimic those evident in the disorder, in particular, sustained non-selective airway hyperreactivity. PAF is an extremely potent platelet stimulus and PAF-induced airway hyperreactivity in guinea-pigs has been shown to be platelet dependent. Therefore, a wide range of drugs were assessed for their ability to modify PAF-induced intravascular ^{111}Indium-labelled platelet accumulation in the guinea-pig in an attempt to elucidate mechanisms of the PAF-induced *in vivo* platelet response.

Both PAF antagonists SRI 63-441 and brotizolam fully inhibited the PAF-induced platelet response in the guinea-pig, the former being shown to do so in a dose-related fashion. Neither disodium cromoglycate, ketotifen nor prednisolone (prophylactic anti-asthma drugs), isoprenaline (a non-selective *beta*-agonist), ipratropium bromide (a cholinergic antagonist), verapamil and diltiazem (Ca^{2+} channel blockers), aspirin and indomethacin (cyclo-oxygenase inhibitors) or FPL 55712 (a leukotriene C_4 and D_4 antagonist) had any effect on PAF-induced intrathoracic ^{111}Indium-labelled platelet accumulation in the guinea-pig. However, dazmegrel (a thromboxane synthetase inhibitor) exerted inhibition in this system, implicating a significant role of thromboxane A_2 in the PAF-induced platelet response in the guinea-pig. Furthermore, aminophylline (a methylxanthine) and iloprost (a stable prostacyclin analogue) significantly inhibited the PAF-induced response. Methylxanthines prevent the hydrolysis of cAMP by inhibiting phosphodiesterase activity, whereas iloprost activates adenylate cyclase; both of these actions in platelets inhibit activation. These findings suggest that PAF-induced platelet accumulation in the guinea-pig pulmonary vasculature is under the control of the cAMP-adenylate cyclase system. In addition, it appears that drugs employed in the treatment of asthma do not exert their beneficial action on platelet accumulation *per se*, but possibly act *via* a substance released from platelets.

The model of *in vivo* platelet aggregation was then modified for use in the rabbit. The intravenous administration of ADP, collagen, PAF and thrombin elicited dose-related accumulation of ^{111}Indium-labelled platelets in the thoracic region with concomitant

declines in radioactive counts in both the hindlimb and head *ie.* the two reference regions. Minimal changes in erythrocyte-associated radioactivity were detected in either region. Once again, these findings most likely reflect the trapping of platelet aggregates within the pulmonary vasculature. Furthermore, these platelet aggregates consist of both cranial- and peripheral-derived platelets. Unlike other stimuli evaluated in the rabbit model, serotonin was virtually without effect on intrathoracic ^{111}Indium-labelled platelet accumulation. The doses of ADP, collagen and PAF effective in the rabbit were found to correlate well with those effective in the guinea-pig using this *in vivo* method. PAF was the most potent platelet stimulus, being 100 times more potent than ADP and 1,00 times more potent than collagen. The time course of the responses in the rabbit was also comparable with those observed in the guinea-pig, with the maximal effect induced by ADP occurring earlier than those for collagen, PAF and thrombin. The response to ADP was transient by comparison with the duration of the other platelet agonist-induced responses.

The PAF-induced platelet response in the rabbit was further characterised with the use of a variety of drugs as a corollary to the study undertaken in the guinea-pig. The PAF antagonist SRI 63-441 exerted a selective inhibition of PAF-induced intrathoracic platelet accumulation, as it was without effect upon the ADP-induced response. Aspirin and indomethacin significantly potentiated the PAF-induced platelet response in the rabbit. This phenomenon may due to interference of endothelial cell prostacyclin production or by shunting of platelet arachidonate metabolism toward the lipoxygenase pathway. As was observed in the guinea-pig, iloprost inhibited intrathoracic platelet accumulation in the rabbit. Therefore, it appears that PAF induced ^{111}Indium-labelled platelet accumulation is modulated by the adenylate cyclase control system in the two species. Furthermore, a high dose of the anticoagulant heparin was found to inhibit platelet accumulation induced by PAF in the rabbit.

^{111}Indium-labelled platelet studies were also conducted in rabbits using the RCM Urovison and Iopamiro, when it was shown that neither contrast agent when injected intravenously in a dose comparable to that used in the clinical situation in man had any effect on platelet accumulation. When Urovison was administered at twice the clinical

dosage, significant ^{111}Indium-labelled platelet accumulation was achieved; however, the magnitude of this response was small by comparison with that induced by other platelet stimuli *eg*. ADP, PAF, collagen and thrombin. These findings provide evidence that radiographic contrast agents do not activate platelets directly *in vivo*, and are in accordance with the data obtained in the *ex vivo* and *in vitro* studies conducted in this thesis.

The concluding experimental chapter examined relevant aspects of the toxicity of dextran. It is well recognised that the intravenous infusion of this agent in patients is associated with a low incidence of serious and even fatal anaphylactoid reactions. A study was therefore undertaken to examine the mechanism by which dextran sulphate causes anaphylactoid reactions in rabbits. The intravenous infusion of dextran sulphate to rabbits was shown to cause a decrease in heart rate, mean arterial blood pressure and dynamic pulmonary compliance with a concomitant increase in the respiration rate and airways resistance. These effects were significantly reduced, but not completely abolished, by vagotomy or by prior treatment with the 5-HT_3 receptor antagonist, ICS 205-930. Furthermore, platelet depletion was shown to diminish these cardiopulmonary changes induced by dextran. Intravenous dextran sulphate elicited substantial intrathoracic ^{111}In-labelled platelet accumulation in the rabbit which was of greater magnitude and duration than that observed for other platelet stimuli examined in the rabbit. The only drug tested in this system to effectively reduce the extent of platelet accumulation induced by dextran was high dose heparin. The dextran-induced platelet response was resistant to inhibition by SRI 63-441, ICS 205-930, dexamethasone, dazmegrel, iloprost, aspirin and indomethacin. The foregoing results indicate that dextran sulphate elicits cardiopulmonary effects largely by releasing serotonin from platelets lodged in the pulmonary circulation. Once released, this serotonin stimulates 5-HT_3 receptors located on chemoafferent nerve endings resulting in a series of complex reflex events.

APPENDIX I

A.I HISTAMINE ASSAY

A.I.1 *Reagents and Stock Solutions*

Histamine standard: 10 *n*g/ml in distilled H_2O, freshly prepared by diluting 100 *u*l of a 1 *u*g/ml stock solution (stored at -20^{O}C) in 9.9 ml distilled H_2O.

Sodium phosphate buffer: 0.1 M, pH 7.9.

Enzyme: histamine-N-methyltransferase, prepared from rat kidneys as described by Shaff and Beaven (1979) (stored at -20^{O}C in 200 *u*l aliquots).

Enzyme-[^{3}H]-S-adenosyl-L-methionine ([^{3}H]-SAMe) mixture: prepared by mixing 100 *u*l of [^{3}H]-SAMe with each 100 *u*l of enzyme preparation. A sufficient volume of the mixture was prepared immediately prior to use.

Diamine oxidase: 1 Unit/ml, prepared as a stock solution on a weekly basis (stored at 4^{O}C). Added to plasma sample to give a final concentration of 0.5 U/ml.

NaOH: 8 N.

Chloroform/ether: 1:1 combination prepared as required.

Scintillation fluid: 4 L toluene, 33 g 1,4,-bis[2(H-methyl-5-phenyloxazolyl)] benzene (POPOP) and 1 g 2,5-diphenyloxazole (PPO) were mixed together to which was added 2 L Triton- X. Prepared as needed.

All pipetting and dilutions of histamine, tritiated SAMe, diamine oxidase and plasma samples were performed with disposable plastic pipettes and polypropylene vials, as histamine is adsorbed to glass (Shaff and Beaven, 1979).

A.I.2 *Chemicals*

Chemicals were purchased from the following companies:

Sigma Chemical Co. (St. Louis, Mo); histamine dihydrochloride, POPOP, PPO and

diamine oxidase (0.17 Units/mg solid).

BDH Chemicals (Aust.) Pty. Ltd. (Pt. Fairy, Vic.); diethyl ether, chloroform and toluene.

New England Nuclear (Boston, Mass); [^{3}H]-SAMe 10.0-20.0 Ci/mmol (low specific activity) and 78.0-85.0 Ci/mmol (high specific activity).

Laboratory Supply Pty. Ltd. (Adelaide, S.A.); Triton X-100.

Schering AG (Berlin/Bergkamen, Germany); Urovison 58% and Iopamiro 370.

May & Baker Australia Pty. Ltd. (West Footscray); Hexabrix 320.

Winthrop Laboratories Division of Sterling Pharmaceuticals Pty. Ltd.; Omnipaque 300.

A.I.3 *Assay Procedure*

The following procedure is based on methodology described by Shaff and Beaven (1979) with modifications by Head and Jonsson (1985).

A.I.3.1 *Principle*

The single enzymatic isotopic assay of histamine involves incubation of the plasma sample with a radiolabelled methyl donor, [^{3}H]-S-adenosyl-methionine and the enzyme histamine-N-methyltransferase to convert histamine to [^{3}H]-N-methyl-histamine. The labelled methyl-histamine is then separated from S-adenosyl-methionine by extraction into chloroform/ether. The source of histamine-N-methyltransferase is a partially purified preparation obtained from rat kidneys.

A.I.3.2 *Preparation of Enzyme Histamine-N-Methyltransferase*

Twelve kidneys were removed from 6 Sprague-Dawley rats and weighed. Kidneys were homogenised in 9 volumes of 0.25 M sucrose, centrifuged (40,000 *g* for 60 min at

4^{O}C) and the supernatant was collected. Ammonium sulphate was slowly added to give 45% saturation which was centrifuged (27,000 *g* for 10 min at 4^{O}C) and the supernatant collected. Ammonium sulphate was then added slowly to give 70% saturation. The preparation was again centrifuged (27,000 *g* for 10 min at 4^{O}C) and the supernatant was discarded. The precipitate was dissolved in 3-5 ml of 0.1 M sodium phoshate buffer (pH 7.4) for 15 hr then dialyzed against 4 L of 0.1 M sodium phoshate buffer (pH 7.4) for 15 hr. The preparation was dialyzed a second time in the same manner. Finally, 200 *u*l aliquots were stored in polypropylene tubes at -20^{O}C.

A.I.3.3 *Histamine Assay*

All samples were assayed in duplicate. Prepared as follows were:

(i) assay blanks - 30 *u*l distilled H_2O + 30 *u*l 0.9% saline (x4).

(ii) assay standards - 30 *u*l 0.9% saline + 30 *u*l histamine standard [10 *n*g/ml] (x2).

(iii) samples for assay - 30 *u*l distilled H_2O + 30 *u*l sample; 30 *u*l histamine standard [10 *n*g/ml] + 30 *u*l sample; 30 *u*l diamine oxidase [0.5 U/ml] + 30 *u*l sample.

(iv) ice-cold enzyme incubation mixture (EIM) for 10 tubes - 270 *u*l 0.1 M sodium phosphate buffer (pH 7.9) + 15 *u*l histamine-N-methyl-transferase + 15 *u*l ^{3}H-SAMe.

(v) 30 *u*l EIM was added to each tube, stoppered and gently vortexed.

(vi) The preparation was incubated in agitating water bath at 37^{O}C for 70 min.

(vii) The incubation was stopped with 0.7 ml 8N NaOH, the incubate vortexed then left for 20 min.

(viii) 7 ml chloroform/ether (1:1) was added.

(ix) The tubes were shaken for 10 min then centrifuge (4,000 *g* for 5 min at 4^{O}C).

(x) The aqueous layer was snap-frozen in dry ice/ethanol and the chloroform/ether was poured into a tube containing 0.5 ml 8N NaOH.

(xi) Steps (ix) and (x) were repeated.

(xii) The chloroform/ether was poured into scintillation vials, evaporated to dryness and finally assessed for radioactivity.

APPENDIX II

A.II THROMBOXANE B_2 ASSAY

A.II.1 *Reagents and Stock Solutions*

Gelatin-Tris buffer: 0.1% gelatin, 0.9% NaCl, 0.01 M Tris and 0.05% azide in distilled water, pH 7.3.

TXB_2 standard: Prepared by dissolving 1 mg TXB_2 in 100 *u*l ethanol and 900 *u*l Na_2CO_3, and further diluted in tris-gelatin buffer to give 100 *n*g/ml, which was stored at -70^{O}C.

Sodium phosphate buffer: 0.01 M, pH 7.6.

TXB_2 antiserum: Dissolved in 20 ml gelatin-Tris buffer and stored at -20^{O}C.

Dextran-coated charcoal: 0.5% dextran (molecular weight 40,000) and 0.5% activated acid washed charcoal in sodium phosphate buffer.

^{3}H-TXB_2: Stored at -20^{O}C was diluted 10 *u*l in 9.99 ml sodium phoshate buffer to give approximately 10,000 cpm, which was stored at 4^{O}C.

A.II.2 *Chemicals*

Tritiated TXB_2 was supplied by Amersham, UK and unlabelled TXB_2 by Caymen chemicals, Denver, USA. Antibody to TXB_2 was supplied by Seragen, Boston, USA. The scintillation fluid used was PCS II (Amersham).

A.II.3 *Assay Procedure*

The following procedure is based on methodology described by Fitzpatrick (1982).

A.II.3.1 *Principle*

Thromboxane A_2 (TXA_2) production was measured as its stable metabolite (hydrolysis product) thromboxane B_2 (TXB_2) by radioimmunoassay.

A.II.3.2 *Preparation of Samples After Aggregation of Platelet-Rich Plasma*

After 5 min, platelet aggregation was quenched by the addition of 540 *u*l cold ethanol to the platelet suspension. All samples were then stored at -70^{O}C until required for assay then thawed. The precipitate was removed after centrifugation and the supernatant evaporated to dryness at 37^{O}C under nitrogen. The residue was resuspended in 1.56 mM sodium bicarbonate (pH 10) and TXB_2 measured by radioimmunoassay.

A.II.3.3 *Thromboxane B_2 Assay*

Samples were diluted to within the range of the standard curve (with an automatic dilutor) and incubated overnight at 4^{O}C with 200 *u*l phosphate buffer, 100 *u*l antiserum and 100 *u*l ^{3}H-TXB_2.

Incubated identically were:

i) 100 *u*l aliquots of TXB_2 standard (5 - 500 *pg*/100 *u*l) and

ii) buffer (zero control)

^{3}H-TXB_2 (100 *u*l) was incubated also with buffer only (blank counts).

Dextran coated charcoal was added to each tube after incubation and following centrifugation (1600 *g* for 30 min at 4^{O}C) supernatants were transferred into scintillation vials to which scintillation fluid was added. Radioactivity was assessed with a Beckman scintillation counter.

All standards and samples were assayed in duplicate. To determine the percentage of ^{3}H-TXB_2 bound, duplicate counts were averaged, blank counts subtracted and the resultant counts divided by the zero control.

The TXB_2 standard *pg*/100 *u*l incubation mixture was plotted against the percentage of ^{3}H-TXB_2 bound (using a four parameter logistic curve fitting programme on a Canon

BXI computer). From the percentage bound, the amount of TXB_2 in each sample was calculated from the standard curve, corrected for sample dilution and platelet count and expressed as the amount of TXB_2 (*p*g) *per* 10^5 platelets.

REFERENCES

Aas, K.A. and Gardner, F.H. 1958. Survival of blood platelets labelled with chromium. *J. Clin. Invest.* 37:1257-1268.

Alabaster, V. and Keir, R. 1987. Comparative effect of drugs on bronchoconstriction induced by PAF, histamine and acetylcholine. *Br. J. Pharmacol.* 90:145P.

Almen, T., Aspelin, P. and Nilsson, P. 1980. Aortic and pulmonary arterial pressure after injection of contrast media into the right atrium of the rabbit. *Acta Radiol. [Suppl.]* 362:37-41.

Ameisen, J.C., Capron, A., Joseph, M., Maclouf, J., Vorng, H., Pancre, V., Fournier, E., Wallaert, B. and Tonnel, A.B. 1985. Aspirin-sensitive asthma: abnormal platelet response to drugs inducing asthmatic attacks. Diagnostic and physiopathological implications. *Int. Arch. Allergy Appl. Immunol.* 78:438-448.

Amundsen, P., Dugstad, G. and Slettebo, M. 1978. Clinical testing of Amipaque for cerebral angiography. *Neuroradiology* 15:89-93.

Anderson, S.D. 1985. Exercise-induced asthma - State of the art. *Chest* 85:191S-195S.

Anderson, G.P. and Fennessy, M.R. 1988. Lipoxygenase metabolites mediate increased airways responsiveness to histamine after acute platelet activating factor exposure in the guinea-pig. *Agents Actions* 24:8-19.

Andes, W.A. 1988. Effects of contrast media on fibrinogen and factor VIII. *Invest. Radiol.* 23:S346-S350.

Andrews, E.J. 1976. The vagus reaction as a possible cause of severe complications of radiological procedures. *Radiology* 121:1-4.

Ansell, G. 1970. Adverse reactions to contrast agents-scope of problem. *Invest. Radiol.* 5:374-384.

Ansell, G., Tweedie, M.C.K., West, C.R., Price Evans, D.A. and Couch, L. 1980. The current status of reactions to intravenous contrast media. *Invest. Radiol.* 15:S32-S39.

Archer, C.B., Cunningham, F.M. and Greaves, M.W. 1986. Comparison of the inflammatory action of C_{18} isomers and C_{16} isomers of platelet activating factor. *Br. J. Dermatol.* 113:779-780.

Archer, C.B., Frolich, W., Page, C.P., Paul., W., Morley, J. and MacDonald, D.M. 1984*a*. Synergistic interaction between prostaglandins and Paf-acether in experimental animals and man. *Prostaglandins* 27:495-501.

Archer, C.B., MacDonald, D.M., Morley, J., Page, C.P., Paul, W. and Sanjar, S.1985*a*. Effects of serum albumin, indomethacin and histamine H_1-antagonists on Paf-acether-induced inflammatory responses in the skin of experimental animals and man. *Br. J. Pharmacol.* 85:109-113.

Archer, C.B., Page, C.P., Morley, J. and MacDonald, D.M., 1985*b*. Accumulation of inflammatory cells in response to intracutaneous platelet activating factor (Paf-acether) in man. *Br. J. Dermatol.* 112:285-290.

Archer, C.B., Page, C.P., Paul, W., Morley, J. and MacDonald, D.M., 1984*b*. Inflammatory characteristics of platelet activating factor (Paf-acether) in human skin. *Br. J. Dermatol.* 110:45-50.

Ardlie, N.G. 1982. Calcium ions, drug action and platelet function. *Pharmacol. Ther.* 18:249-270.

Arnoux, B., Denjean, A., Page, C.P., Nolibe, D., Morley, J. and Benveniste, J. 1988. Accumulation of platelets and eosinophils in baboon lung following Paf-acether challenge: Inhibition with ketotifen. *Am. Rev. Respir. Dis.* 137:855-861.

Arnoux, B., Grimfeld, A., Duroux, P. and Denjean, A. 1983. Alveolar macrophages/Paf-acether. A new association in the pathogenesis of human asthma. In "Platelet Activating Factor and Structurally Related Ether-Linked Phospholipids," eds., J. Benveniste and B. Arnoux. INSERM symposium no.23, Elsevier Science Publications B.V., pp 335-341.

Arroyave, C.M. 1980. An *in vitro* assay for radiographic contrast media idiosyncrasy. *Invest. Radiol.* 15:S21-S25.

Arroyave, C.M., Bhat, K.N. and Crown, R. 1976. Activation of the alternative pathway of the complement system by radiographic contrast media. *J. Immunol.* 117:1866-1869.

Arroyave, C.M., Schatz, M. and Simon, R.A. 1979. Activation of the complement system by radiographic contrast media: studies *in vivo* and *in vitro*. *J. Allergy Clin. Immunol.* 63:276-280.

Arroyave, C.M. and Tan, E.M. 1977. Mechanism of complement activation by radiographic contrast media. *Clin. Exp. Immunol.* 29:89-94.

Aspelin, P. 1978. Effect of ionic and non-ionic contrast media on whole blood viscosity and haematocrit *in vivo*. *Acta Radiol. Diagn.* 19:977-989.

Aspelin, P. 1979. Effect of ionic and non-ionic contrast media on red cell deformability *in vitro*. *Acta Radiol. Diagn.* 20:1-12.

Aspelin, P., Birk, A., Almen, T. and Kiesewetter, H. 1980*a*. Effect of iohexol on human erythrocytes II. Red cell aggregation *in vitro*. *Acta Radiol. Diagn.* 362:123-126.

Aspelin, P. and Schmid-Schonbein, H. 1978. Effect of ionic and non-ionic contrast media on red cell aggregation *in vitro*. *Acta Radiol. Diagn.* 19:766-784.

Aspelin, P., Schmid-Schonbein, H. and Malotta, H. 1988. Do nonionic contrast media increase red cell aggregation and clot formation? *Invest. Radiol.* 23:S326-S333.

Aspelin, P., Stohr-Liessen, M. and Almen, T. 1980*b*. Effect of iohexol on human erythrocytes I. Changes of red cell morphology *in vitro*. *Acta Radiol. [Suppl.]* 362:117-122.

Aspelin, P., Teitel, P. and Almen, T. 1980*c*. Effect of iohexol on red cell deformability *in vitro*. *Acta Radiol. [Suppl]* 362:127-130.

Assem, E.S.K., Bray, K. and Dawson, P. 1983. The release of histamine from human basophils by radiological contrast agents. *Br. J. Radiol.* 56:647-652.

Assoian, R.K., Komoriya, A., Meyers, C.A., Miller, D.M. and Sporn, M.B. 1983. Transforming growth factor-*beta* in human platelets. Identification of a major storage site, purification, and characterization. *J. Biol. Chem.* 258:7155-7160.

Azuma, H., Ishikawa, M. and Sekizaki, S. 1986. Endothelium-dependent inhibition of platelet aggregation. *Br. J. Pharmacol.* 88:411-415.

Badimon, L., Fuster, V., Chesebro, J.H. and Dewanjee, M.K. 1983. New "*ex-vivo*" radioisotopic method of quantitation of platelet deposition-Studies in four animal species. *Thromb. Haemostas.* 50:639-644.

Baker, J.R.J., Butler, K.D., Eakins, M.N., Pay, G.F. and White, A.M. 1982. Subcellular localization of ^{111}In in human and rabbit platelets. *Blood* 59:351-359.

Baldini, M.G. and Ebbe, S. 1974. Platelets, production, function, transfusion and storage. Grune and Stratton, Inc., New York, pp192.

Barbaro, J.F. and Zvaifler, N.J. 1966. Antigen-induced histamine release from platelets of rabbits producing homologous PCA antibody. *Proc. Soc. Exp. Biol. Med.* 122:1245-1247.

Barnes, P.J. 1986. Asthma as an axon reflex. *Lancet* i:242-244.

Barnes, P., Chung, K.F., Dent, G., Guinot, P.H., McCusker, M. and Page, C.P. 1987*a*. Effectiveness of a specific antagonist of platelet activating factor, BN 52063, in man. *Lancet* i:248-251.

Barnes, P.J., Grandordy, B.M., Page, C.P., Rhoden, K.J. and Robertson, D.N. 1987*b*. The effect of platelet activating factor on pulmonary *beta*-adrenoceptors. *Br. J. Pharmacol.* 90:709-715.

Barnes, P.J., Wilson, N.M. and Brown, M.J. 1981. A calcium antagonist, nifedipine, modifies exercise-induced asthma. *Thorax* 36:726-730.

Barrett, P.A., Butler, K.D., Morley, J., Page, C.P., Paul, W. and White, A.M. 1984. Inhibition by heparin of platelet accumulation *in vivo*. *Thromb. Haemostas.* 51:336-370.

Basran, G.S., Page, C.P., Paul, W. and Morley, J. 1984. Platelet activating factor: a possible mediator of the dual response to allergen? *Clin. Allergy* 14:75-79.

Bauer, A. and Ostling, G. 1970. Dextran-induced anaphylactoid reactions in connection with surgery. *Acta Anaesth. Scandinav. Suppl.* 37:182-185.

Baumgartner, H.R., Muggli, R., Tschopp, T.B. and Turritto, V.T. 1976. Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. *Thromb. Haemostas.* 35:124-138.

Beasley, R., Roche, W.R., Roberts, J.A. and Holgate, S.T. 1989. Cellular events in the bronchi in mild asthma and after bronchial challenge. *Am. Rev. Respir. Dis.* 139:806-817.

Beijer, L., Botting, J., Crook, P., Oyekan, A.O., Page, C.P. and Rylander, R. 1987. The involvement of platelet activating factor in endotoxin-induced pulmonary platelet recruitment in the guinea-pig. *Br. J. Pharmacol.* 92:803-808.

Beijer, L. and Rylander, R. 1983. Macrophages and neutrophil production of PAF after exposure to endotoxin. *J. Pharmacol.* (Paris) 14:55.

Belleville, J., Baguet, J., Paul, J., Clendinnen, G. and Eloy, R. 1985. *In vitro* study of the inhibition of coagulation induced by different radiocontrast molecules. *Thromb. Res.* 38:149-162.

Belleville, J., Freyria, A.M., Pinet, A., Cornillon, B., Paul, J., Clendinnen, G. and Eloy, R. 1982. Contrast agents used for excetory urography impair platelet function in human patients. *Thromb. Res.* 28:533-544.

Benveniste, J. 1974. Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils. *Nature* 249:581-582.

Benveniste, J., Chignard, M., Le Couedic, J.P. and Vargaftig, B.B. 1982. Biosynthesis of platelet-activating factor (Paf-acether). II. Involvement of phospholipase A_2 in the formation of Paf-acether and lyso Paf-acether from rabbit platelets. *Thromb. Res.* 25:375-385.

Benveniste, J., Henson, P.M. and Cochrane, C.G. 1972. Leukocyte dependent histamine release from rabbit platelets: The role of IgE, basophils and a platelet activating factor. *J. Exp. Med.* 136:1356-1377.

Benveniste, J., Le Couedic, J.P., Polonsky, J. and Tence, M. 1977. Structural analysis of purified platelet activating factor by lipases. *Nature* 269:170-171.

Benveniste, J., Tence, M., Varenne, P., Bidault, J., Boullet, C. and Polonsky, J. 1979. Semi-synthese et structure proposee du facteur activant les plaquettes (PAF): PAF-acether, un alkyl ether analogue de la lysophosphotidylcholine. *C.R. Acad. Sci.* 289:1037-1040.

Bernstein, E.F. and Gans, H. 1966. Anticoagulant activity of angiographic contrast media. *Invest. Radiol.* 1:162-168.

Berridge, M.J. 1984. Inositol triphosphate and diacylglycerol as second messengers. *Biochem. J.* 220:345-360.

Bertele, V., Falanga, A., Tomasiak, M., Chiabrando, C., Cerletti, C. and de Gaetano, G. 1984. Pharmacologic inhibition of thromboxane synthetase and platelet aggregation: modulatory role of cyclo-oxygenase products. *Blood* 63:1460-1466.

Berti, F., Folco, G.C., Giachetti, A., Malandrino, S., Omini, C. and Vigano, T. 1980. Atropine inhibits thromboxane A_2 generation in isolated lungs of the guinea-pig. *Br. J. Pharmacol.* 68:467-472.

Best, L.C., Martin, T.J., Russell, R.G.G. and Preston, F. 1977. Prostacyclin increases cyclic AMP levels and adenylate cyclase activity in platelets. *Nature* 267:850.

Bettmann, M.A. 1982. Angiographic contrast agents: conventional and new media compared. *Am. J. Roentgenol.* 139:787-794.

Bettmann, M.A. and Higgins, C.B. 1985. Comparison of an ionic with a nonionic contrast agent for cardiac angiography. Results of a multicentre trial. *Invest. Radiol.* 20:S70-S74.

Betz, S.J., Lotner, G.Z. and Henson, P.M. 1980. Generation and release of platelet activating factor (PAF) from enriched preparations of rabbit basophils: failure of human basophils to release PAF. *J. Immunol.* 125:2749-2755.

Bezold, A. von and Hirt, L. 1867. *Unters Physiol. Lab. Wurzburg* 1:73.

Bhagat, R.G. and Grunstein, M.M. 1985. Effect of corticosteroids on bronchial responsiveness to methacholine in asthmatic children. *Am. Rev. Respir. Dis.* 131:902-906.

Bhardwaj, R. and Moore, P.K. 1987. Endothelium dependent inhibition of human platelet aggregation *in vitro*. *Br. J. Pharmacol.* 91:402P.

Bhardwaj, R. and Moore P.K. 1988. Endothelium-derived relaxing factor and the effects of acetylcholine and histamine on resistance blood vessels. *Br. J. Pharmacol.* 95:835-843.

Blackwell, G.J., Carnuccio, R., Di Rosa, M., Flower, R.J., Langham, C.S.J., Parente, L., Persico, P., Russell-Smith, N.C. and Stone, D. 1982. Glucocorticoids induce the formation and release of anti-inflammatory and anti-phospholipase proteins into the peritoneal cavity of the rat. *Br. J. Pharmacol.* 76:185-194.

Blank, M.L., Snyder, F., Byers, W.L., Brooks, B. and Muirhead, E.E. 1979. Antihypertensive activity of an alkyl ether analogue of phosphatidylcholine. *Biochem. Biophys. Res. Commun.* 90:523-534.

Bollman, G., Jager, B. and Freitag, G. 1979. *In vitro* effects of contrast media on the water content of human erythrocytes. *Acta Radiol. Diag.* 20:681-687.

Bonnet, J., Tibaudean, D. and Bessin, P. 1983. Dependency of the PAF acether induced bronchoconstriction on the lipoxygenase pathway in the guinea-pig. *Prostaglandins* 26:457-466.

Booij-Noord, H., Orie, N.G. and De Vries, K. 1971. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and predisolone. *J. Allergy Clin. Immunol.* 48:344-354.

Born, G.V.R. 1962. Quantitative investigations into the aggregation of blood platelets. *J. Physiol.* 162:67P-68P.

Born, G.V.R. 1985. Physiological and pathological involvements of platelets. In "Advances in Prostaglandin, Thromboxane and Leukotriene Research," vol. 13, eds. G.G. Neri Serneri, J. McGiff, R. Paoletti and G.V.R. Born. Raven press, New York, pp 1-11.

Born, G.V.R. and Cross, M.J. 1963. Effect of adenosine diphosphate on the concentration of platelets in circulating blood. *Nature* 197:974-976.

Born, G.V.R. and Cross, M.J. 1964. Effects of inorganic ions and of plasma proteins on the aggregation of blood platelets by adenosine diphosphate. *J. Physiol.* 170:397-414.

Botting, J.H. and Oyekan, A.O. 1986. Inhibition of intravascular platelet aggregation in rats by adrenaline. *Br J Pharmacol* 89:752P.

Bourgain, R.H. and Six, F. 1974. A continuous registration method in experimental arterial thrombosis in the rat. *Thromb. Res.* 4:599-607.

Boushey, H.A., Holzman, M.J., Sheller, J.R. and Nadel, J.A. 1980. State of the Art. Bronchial hyperreactivity. *Am. Rev. Respir. Dis.* 121:389-413.

Braquet, P. 1987. The ginkgolides: potent platelet-activating antagonists isolated from *Ginkgo biloba* L.: chemistry, pharmacology and clinical applications. *Drugs of the Future* 12:643-699.

Braquet, P., Etienne, A., Touvay, C., Bourgain, R.H., Lefort, J. and Vargaftig, B.B. 1985*a*. Involvement of platelet activating factor in respiratory anaphylaxis, demonstrated by Paf-acether inhibitor BN 52021. *Lancet* i:1501.

Braquet, P. and Godfroid, J.J. 1986. Platelet activating factor (Paf-acether) specific binding sites: 2. Design of specific antagonists. *Trends Pharmacol. Sci.* 7:397-403.

Braquet, P., Spinnewyn, B., Braquet, M., Bourgain, R.H., Taylor, J.E., Etienne, A. and Drieu, K. 1985*b*. BN 52021 and related compounds: a new series of highly specific Paf-acether receptor antagonists isolated from *Ginkgo biloba*. *Blood Vessels* 16:559-572.

Braquet, P., Touqui, L., Shen, T.Y. and Vargaftig, B.B. 1987. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* 39:97-145.

Brasch, R.C. 1980. Allergic reactions to contrast media: accumulated evidence. *Am. J. Roentgenol.* 134:797-801.

Brasch, R.C. and Caldwell, J.L. 1976. The allergic theory of radiocontrast agent toxicity: demonstration of antibody activity in sera of patients suffering major radiocontrast agent reactions. *Invest. Radiol.* 11:347-356.

Brasch, R.C., Caldwell, J.L. and Fudenberg, H.H. 1976. Antibodies to radiographic contrast agents-induction and characterization of rabbit antibody. *Invest. Radiol.* 11:1-9.

Brasch, R.C., Rockoff, S.D., Kuhn, M.D. and Chraplyvy, M. 1970. Contrast media as histamine liberators II. Histamine release into venous plasma during intravenous urography in man. *Invest. Radiol.* 5:510-513.

Brezinski, D.A., Tofler, G.H., Muller, J.E., Pohjola-Sintonen, S., Willich, S.N., Schafer, A.I., Czeisler, C.A. and Williams, G.H. 1988. Morning increase in platelet aggregability: association with assumption of the upright posture. *Circulation* 78:35-40.

Brimblecombe, R.W., Owen, D. and Parsons, M. 1974. The cardiovascular effects of histamine in laboratory animals. *Agents Actions* 4:191-192.

Brisman, R., Parks, L.C. and Haller, J.A. 1968. Anaphylactoid reactions associated with the clinical use of dextran 70. *J. Am. Med. Assoc.* 204:824-825.

Brugman, T.M., Darnell, M.L. and Hirshman, C.A. 1983. Nifedipine aerosol attenuates airway constriction in dogs with hyperreactive airways. *Am. Rev. Respir. Dis.* 127:14-17.

Bryant, D.H. 1985. Nebulized ipratropium bromide in the treatment of acute asthma. *Chest* 88:24-29.

Buchanan, M.R. and Hirsh, J. 1978. A comparison of the effects of aspirin and dipyridamole on platelet aggregation *in vivo* and *ex vivo*. *Thromb. Res.* 13:517-529.

Buckle, D.R., Outred, D.J., Ross, J.W., Smith, H., Smith, R.J., Spicer, B.A. and Gasson, B.C. 1979. Aryloxyalkyloxy- and aralkyloxy-4-hydroxy-3-nitrocoumarins which inhibit histamine release in the rat and also antagonize the effects of a slow reacting substance of anaphylaxis. *J. Med. Chem.* 22:158-168.

Bunting, S., Gryglewski, R., Moncada, S. and Vane, J.R. 1976. Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins* 12:897-928.

Burge, P.S., Efthimiou, J., Turner-Warwick, M. and Nelmer, P.T. 1982. Double-blind trials of inhaled beclomethasone diproprionate and fluocortin butyl ester in allergen-induced immediate and late asthmatic reactions. *Clin. Allergy* 12:523-531.

Busch, C. and Olson, P.S. 1973. Density distribution of 51Cr-labelled platelets within the circulating dog platelet population. *Thromb. Res.* 3:1-11.

Busse, R., Trogisch, G. and Bassenge, E. 1985. The role of endothelium in the control of vascular tone. *Basic Res. Cardiol.* 80:475-490.

Butler, K.D., Pay, G.F., Roberts, J.M. and White, A.M. 1979. The effect of sulphinpyrazone and other drugs on the platelet response during the acute phase of the active arthus reaction in guinea pigs. *Thromb. Res.* 15:319-340.

Camussi, G., Anglietta, M., Malavas, M., Tetta, C., Piacibello, W., Sanavio, F. and Bussolino, F. 1983*a*. The release of platelet-activating factor from human endothelial cells in culture. *J. Immunol.* 131:2397-2403.

Camussi, G., Pawlowski, I., Tetta, C., Roffinello, C., Alberton, M., Brentjens, J. and Andres, G. 1983*b*. Acute lung inflammation induced in the rabbit by local installation of 1-*O*-octadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine or of native platelet-activating factor. *Am. J. Pathol.* 112:78-88.

Capron, A., Ameisen, J.C., Joseph, M., Auriault, C., Tonnel, A.B. and Caen, J. 1985. New functions for platelets and their pathological implications. *Int. Arch. Allergy Appl. Immunol.* 77:107-114.

Capron, A., Dessaint, J.P., Capron, M., Joseph, M., Ameisen, J.C. and Tonnel, A.B. 1986. From parasites to allergy: a second receptor for IgE. *Immunol. Today* 7:15-18.

Cardinal, D.C. and Flower, R.J. 1980. The electronic aggregometer: A novel device for assessing platelet behaviour in blood. *J. Pharmacol. Meth.* 3:135-158.

Carr, D.H. and Walker, A.C. 1984*a*. Contrast media reactions: experimental evidence against the allergy theory. *Br. J. Radiol.* 57:469-473.

Carr, D.H. and Walker, A.C. 1984*b*. Kinin activation as a predictive assay for adverse reactions to radiographic contrast media. *Acta Radiol. Diagn.* 25:337-342.

Carroll, P., Glover, W. and Catt, N. 1974. Cardiovascular histamine receptors in the rabbit. *Aust. J. Exp. Biol. Med. Sci.* 52:577-582.

Casals-Stenzel, J. 1987*a*. Effects of WEB 2086, a novel antagonist of platelet activating factor in active and passive anaphylaxis. *Immunopharmacology* 3:7-24.

Casals-Stenzel, J. 1987*b*. The inhibitory activity of brotizolam and related compounds on platelet activating factor (PAF)-induced effects *in vitro* and *in vivo*. In "New Horizons in Platelet Activating Factor Research," vol. 29, eds C.M. Winslow and M.L. Lee. John Wiley & Sons Ltd., pp 277-285.

Cazenave, J.-P., Benveniste, J. and Mustard, J.F. 1979. Aggregation of rabbit platelets by platelet-activating factor is independent of the release reaction and the arachidonate acid pathway and inhibited by membrane-active drugs. *Lab. Invest.* 41:275-285.

Cazenave, J.-P., Packham, M.A. and Mustard, J.F. 1973. Adherence of platelets to a collagen-coated surface: development of a quantitative method. *J. Lab. Clin. Med.* 82:978-990.

Cerrina, J., Denjean, A., Alexander, G., Lockhart, A. and Duroux, P. 1980. Inhibition of exercise-induced asthma by a calcium antagonist, nifedipine. *Am. Rev. Respir. Dis.* 123:156-160.

Cerrina, J., Hadji, L., Marche, E., Duroux, P. and Benveniste, J. 1982. Effect of the Ca^{2+} antagonist nifedipine on histamine and SRS release from human lung tissue. *Am. Rev. Respir. Dis.* 125:64 (abstract).

Chahine, R.A. and Raizner, A.E. 1976. The mechanism of hypotension following angiography. *Invest. Radiol.* 11:472-478.

Chang, J.C., Lee, D. and Gross, H.M. 1989. Acute thrombocytopenia after IV administration of a radiographic contrast medium. *Am. J. Roentgenol.* 152:947-949.

Chap, H., Mauco, G., Simon, M.F., Benveniste, J. and Douste-Blazy, L. 1981. Biosynthetic labelling of platelet activating factor (Paf-acether) from radioactive acetate by stimulated platelets. *Nature* 289:312-314.

Charo, I.F., Feinman, R.D. and Detwiler, T.C. 1977. Interrelations of platelet aggregation and secretion. *J. Clin. Invest.* 60:866-873.

Chignard, M., Coeffier, E. and Benveniste, J. 1986. Role of PAF-acether and related ether-lipid metabolism in platelets. In "Advances in Medicine and Biology. Mechanisms of Stimulus Response Coupling in Platelets," eds. J. Westwick, M.F. Schully, D.E. McIntyre and V.V. Kakkav, Plenum Press, New York.

Chignard, M., Le Couedic, J.P., Tence, M., Vargaftig, B.B. and Benveniste, J. 1979. The role of platelet activating factor in platelet aggregation. *Nature* 279:799-800.

Chignard, M., Wal, F., Lefort, J. and Vargaftig, B.B. 1982. Inhibition by sulphinpyrazone of the platelet-dependent bronchoconstriction due to platelet-activating factor (Paf-acether) in the guinea-pig. *Eur. J. Pharmacol.* 78:71-79.

Chung, K.F., Aizawa, H., Leikauf, G., Ueki, I.F., Evans, T.W. and Nadel, J.A. 1986. Airway hyper-responsiveness induced by platelet activating factor: role of thromboxane generation. *J. Pharmacol. Exp. Ther.* 236:580-584.

Chung, K.F. and Barnes, P.J. 1989. Effects of platelet activating factor on airway calibre, airway responsiveness, and circulating cells in asthmatic subjects. *Thorax* 44:108-115.

Chung, K.F., Dent, G. and Barnes, P.J. 1989. Effects of salbutamol on bronchoconstriction, bronchial hyperresponsiveness, and leukocyte responses induced by platelet activating factor in man. *Thorax* 44:102-107.

Church, M.K. and Young, K.D. 1983. The characteristics of inhibition of mediator release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. *Br. J. Pharmacol.* 78:671-679.

Church, J. and Zsoter, T.T. 1980. Calcium antagonistic drugs. Mechanism of action. *Can. J. Physiol. Pharmacol.* 58:254-264.

Clawson, C.C. 1971. Platelet interaction with bacteria II. Fate of the bacteria. *Am. J. Pathol.* 65:381-398.

Clawson, C.C. 1974. Modification of neutrophil function by platelets. In "Platelets: Production, Function, Transfusion and Storage," eds. M.G. Baldini and S. Ebbe. Grune and Stratton, New York, pp 287-297.

Cockcroft, D.W., Ruffin, R.E., Dolovich, J. and Hargreave, F.E. 1977. Allergen-induced increase in non-allergic bronchial reactivity. *Clin. Allergy* 7:503-513.

Coeffier, E., Chignard, M., Delautier, D. and Benveniste, J. 1984. Cooperation between platelets and neutrophils for Paf-acether formation. *Fed. Proc.* 43:2900.

Coel, M.N. and Lasser, E.C. 1971. A pharmacological basis for peripheral vascular resistance changes with contrast media injection. *Am. J. Roentgenol.* 111:802-806.

Cogen, F.C., Norman, M.E., Dunsky, E., Hirshfeld, J. and Zweiman, B. 1979. Histamine release and complement changes following injection of contrast media in humans. *J. Allergy Clin. Immunol.* 64:299-303.

Cohan, R.H. and Dunnick, N.R. 1987. Intravascular contrast media: adverse reactions. *Am. J. Roentgenol.* 149:665-670.

Cohn, J.N. 1982. Calcium-entry blockers in coronary artery disease. Introduction. *Circulation* 65:1-2.

Corot, C., Perrin, J.M., Belleville, J., Amiel, M. and Eloy, R. 1989. Effect of iodinated contrast media on blood clotting. *Invest. Radiol.* 24:390-393.

Corris, P.A., Nariman, S. and Gibson, G.J. 1983. Nifedipine in the prevention of asthma induced by exercise and histamine. *Am. Rev. Respir. Dis.* 128:991-992.

Cote, C.J., Greenhow, D.E. and Marshall, B.E. 1979. The hypotensive response to rapid intravenous administration of hypertonic solutions in man and in the rabbit. *Anaesthesiology* 50:30-35.

Coyle, A., Sjoerdsma, K., Page, C., Brown, L. and Metzger, W. 1987. Modification of the late asthmatic response and bronchial hyperreactivity by BN 52021, a platelet activating factor antagonist. *Clin. Res.* 35:254A.

Craps, L. 1980. The prophylaxis of bronchial asthma with ketotifen. Five years of clinical investigations. *Acta Therapeutica* 6:213-225.

Cunningham, J.J. 1977. Contrast media reactions correlated with time of day. *Am. J. Roentgenol.* 129:1039-1040.

Cuss, F.M., Dixon, C.M. and Barnes, P.J. 1986. Effects of inhaled platelet activating on pulmonary function and bronchial responsiveness in man. *Lancet* ii:189-192.

Dahl, S.G., Linaker, O., Mellbye, A. and Sveen, K. 1976. Influence of the cation on the side-effects of urographic contrast media. *Acta Radiol. Diag.* 17:461-471.

Da Prada, M., Richards, J.G. and Kettler, R. 1981. Amine storage organelles in platelets. In "Platelets in Biology and Pathology," ed. J. Gordon. Amsterdam, Elsevier North Holland Biomedical Press, pp 107-144.

Darius, H., Lefer, D.J., Smith, J.B. and Lefer, A.M. 1986. Role of platelet-activating factor-acether in mediating guinea pig anaphylaxis. *Science* 232:58-60.

Davies, O.J., Morley, J., Page, C.P. and Paul, W. 1981. *In vivo* aggregation of guinea-pig platelets. *J. Physiol.* 320:3-4P.

Dawson, G.S. and Comroe, J.H. 1954. Chemoreflexes from the heart and lungs. *Physiol. Rev.* 34:167-201.

Dawson, P. and Edgerton, D. 1983. Contrast media and enzyme inhibition. I. Cholinesterase. *Br. J. Radiol.* 56:653-656.

Dawson, P., Harrison, M.J.G. and Weisblatt, E. 1983*a*. Effect of contrast media on red cell filtrability and morphology. *Br. J. Radiol.* 56:707-710.

Dawson, P., Hewitt, P., Mackie, I.J., Machin, S.J., Amin, S. and Bradshaw, A. 1986. Contrast, coagulation, and fibrinolysis. *Invest. Radiol.* 21:248-252.

Dawson, P., Turner, M.W., Bradshaw, A. and Westaby, S. 1983*b*. Complement activation and generation of C3a anaphylatoxin by radiological contrast agents. *Br. J. Radiol.* 56:447-448.

Deeming, K., Mazzoni, L., Morley, J., Page, C.P. and Sanjar, S. 1986. Prophylactic anti-asthma drugs impair platelet accumulation within the lung. *Br. J. Pharmacol.* 87:73P.

Deeming, K., Mazzoni, L., Morley, J., Page, C.P., Sanjar, S. and Smith, D.L. 1985. PAF antagonism in asthma. *Prostaglandins* 30:715.

Defreyn, G., Deckmyn, H. and Vermylen, J.A. 1982. A thromboxane synthetase inhibitor reorients endoperoxide metabolism in whole blood towards prostacyclin and prostaglandin E_2. *Thromb. Res.* 26:389-400.

de Gaetano, G., Bertele, V. and Cerletti, C. 1987. Pharmacology of antiplatelet drugs. In "Platelets in Biology and Pathology III," eds., D.E. Macintyre and J.L. Gordon. Amsterdam: Elsevier, pp. 515-573.

Demopolous, C.A., Pinckard, R.N. and Hanahan, D.J. 1979. Platelet activating factor: Evidence for 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component of platelet activating factor (a new class of lipid chemical mediators). *J. Biol. Chem.* 254:9355-9358.

De Maeyer, H., Deckmyn, H., Arnout, J., Baert, A.L. and Vermylen, J. 1985. Intravenous ionic contrast media cause local prostacyclin release in man. *Invest. Radiol.* 20:871-873.

Denjean, A., Arnoux, B., Benveniste, J., Lockhart, A. and Masse, R. 1981. Bronchoconstriction induced by intratracheal administration of platelet activating factor (PAF-acether) in baboons. *Agents Actions* 11:567-568.

Desquand, S., Touvay, C., Randon, J., Lagente, V., Vilain, B., Maridonneau-Parini, I., Etienne, A., Lefort, J. and Braquet, P. 1986. Interference of BN 52021 (ginkgolide B) with the bronchopulmonary effects of PAF-acether in the guinea-pig. *Eur. J. Pharmacol.* 127:83-95.

Dewar, A., Archer, C.B., Paul, W., Page, C.P., MacDonald, D.M. and Morley, J. 1984. Cutaneous and pulmonary histopathological responses to platelet activating factor (Paf-acether) in the guinea-pig. *J. Pathol.* 144:25-34.

Didisheim, P. and Fuster, V. 1978. Actions and clinical status of platelet-suppressive agents. *Sem. Haematol.* 15:55-72.

Drazen, J.M., Fanta, C.H. and LaCouture, P.G. 1983. Effect of nifedipine on constriction of human tracheal spirals *in vitro*. *Br. J. Pharmacol.* 78:691-697.

Duff, G.L., McMillan, G.C. and Ritchie, A.C. 1957. The morphology of early atherosclerotic lesions in the aorta demonstrated by the surface technique in rabbits fed cholesterol. *Am. J. Pathol.* 33:845-873.

Dunn, C.R., Lasser, E.C., Sell, S. and Lang, J. 1975. Failure to induce hypersensitivity reactions to opaque contrast media analogues in guinea-pigs. *Invest. Radiol.* 10:317-322.

Durham, S.R., Dawes, J. and Kay, A.B. 1985. Platelets in asthma. *Lancet* i:36.

Duval, D., Durant, S. and Homo-Delarche, F. 1983. Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. *Biochim. Biophys. Acta* 737:409-442.

Eika, C. 1972. Platelet refractory state induced by heparin. *Scand. J. Haematol.* 9:665-672.

Elias, D.J., Lazarus, S.C., Valone, F.H. and Gold, W.M. 1985. Production of platelet activating factor by dog mastocytoma cells. *Fed. Proc.* 44:984.

Ennis, M., Pearce, F.L. and Dollery, C.T. 1983. Calcium antagonists and histamine secretion from rat peritoneal mast cells. *Agents Actions* 13:144-148.

Erspamer, V. 1966. Peripheral physiological and pharmacological actions of indole alkylamines. In "Handbook of Experimental Pharmacology," vol XIX, 5-hydroxytryptamine and Related Indole Alkylamines, ed. V. Erspamer. New York, Springer-Verlag, pp 245-351.

Evans, T.W., Chung, K.F., Rogers, D.F. and Barnes, P.J. 1987. Effect of platelet activating factor on airway vascular permeability: possible mechanisms. *J. Appl. Physiol.* 63:479-489.

Fairfax, A.J., Hanson, J.M. and Morley, J. 1983. The late reaction following bronchial provocation with house dust mite allergen. Dependence on arachidonic acid metabolism. *Clin. Exp. Immunol.* 52:393-398.

Fanta, C.H., Venugopalan, C.S., LaCouture, P.G. and Drazen, J.M. 1982. Inhibition of bronchoconstriction in the guinea-pig by a calcium channel blocker, nifedipine. *Am. Rev. Respir. Dis.* 125:61-66.

Fareed, J., Baker, W.H., Walenga, J.M. and Messmore, H.L.Jr. 1984*a*. Molecular markers of hemostatic activation: future prospectives. *Sem. Thromb. Hemostas.* 10:329-332.

Fareed, J., Moncada, R., Messmore, H.L.Jr., Walenga, J.M., Hoppensteadt, D. and Wehrmacher, W.H. 1984*b*. Molecular markers of contrast media-induced adverse reactions. *Sem. Thromb. Hemostas.* 10:306-328.

Farr, R.S., Cox, C.P., Wardlow, M.L. and Jorenson, R. 1980. Preliminary studies of an acid labile factor (ALF) in human sera that inactivates platelet activating factor (PAF). *Clin. Immunol. Immunopathol.* 15:318-330.

Fennessy, M.R., Stewart, A.G. and Thompson, D.C. 1986. Aerosolized and intravenously administered leukotrienes: effects on the bronchoconstrictor potency of histamine in the guinea-pig. *Br. J. Pharmacol.* 87:741-749.

Findlay, S.R., Dvorak, A.M., Kagey-Sobotka, A. and Lichtenstein, L.M. 1981. Hyperosmolar triggering of histamine release from human basophils. *J. Clin. Invest.* 67:1604-1613.

Findlay, S.R. and Lichtenstein, L.M. 1980. Basophil "releasability" in patients with asthma. *Am. Rev. Respir. Dis.* 122:53-59.

Fishel, C.W. and Zwemer, R.J. 1970. Aggregation of platelets from B. pertussis-infected mice and atopically sensitive human individuals. *Fed. Proc.* 29:640.

Fischer, H.W. 1968. Hemodynamic reactions to angiographic media. A survey and commentary. *Radiology* 91:66-73.

Fischer, H.W., Katzberg, R.W., Morris, T.W. and Spataro, R.F. 1984. Systemic response to excretory urography. *Radiology* 151:31-33.

Fischer, H.W. and Thomson, K.R. 1978. Contrast media in coronary arteriography. A review. *Invest. Radiol.* 13:450-459.

FitzGerald, G.A. and Oates, J.A. 1984 Selective and nonselective inhibition of thromboxane formation. *Clin. Pharmacol. Ther.* 35:633-640.

Fitzpatrick, F.A. 1982. A radioimmunoassay for thromboxane B_2. In "Methods in Enzymology," eds. W.E.M. Lands and W.L. Smith. Academic Press, London, p 286.

Flavahan, N.A., Aarhus, L.L., Rimele, T.J. and Vanhoutte, P.M. 1985. Respiratory epithelium inhibits bronchial smooth muscle tone. *J. Appl. Physiol.* 58:834-838.

Foreman, J.C. and Mongar, J.L. 1972. Effect of calcium on dextran-induced histamine release from isolated mast cells. *Br. J. Pharmacol.* 46:767-769.

Fredholm, B.B., Brodin, K. and Stranberg, K. 1979. On the mechanism of relaxation of tracheal smooth muscle by theophylline and other cyclic nucleotide phosphodiesterase inhibitors. *Acta Pharmacol. Toxicol.* 45:336-344.

Fredholm B.B. and Persson, C.G.A. 1982. Xanthine derivatives as adenosine receptor antagonists. *Eur. J. Pharmacol.* 81:673-676.

Frewin, D.B., Jonsson, J.R., Head, R.J., Russell, W.J. and Beal, R.W. 1984. Histamine levels in stored human blood. *Transfusion* 24:502-504.

Frewin, D.B., Cleland, L.G., Jonsson, J.R. and Robertson, P.W. 1986. Histamine levels in human synovial fluid. *J. Rheumatol.* 13:13-14.

Freyria, A.M., Pinet, A., Belleville, J., Eloy, R. and Traeger, J. 1982. Effects of five different contrast agents on serum complement and calcium levels after excretory urography. *J. Allergy Clin. Immunol.* 69:397-403.

Freyss-Beguin, M., Griffaton, G., Lechat, P., Picken, D., Quennedey, M.C., Rouot, B. and Schwartz, J. 1983. Comparison of the chronotropic effect and the cyclic AMP accumulation induced by *beta*$_2$-agonists in rat heart cell culture. *Br. J. Pharmacol.* 78:717-723.

Friesinger, G.C., Schaffer, J., Criley, J.M., Gaertner, R.A. and Ross, R.S. 1965. Hemodynamic consequences of the injection of radiopaque material. *Circulation* 31:730-740.

Frigas, E. and Gleich, G.J. 1986. The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.* 77:527-537.

Fritzler, M.J., Ring, J., Arroyave, C.M. and Tan, E.M. 1978. Human blood cells: ultrastructural changes and mediator release after exposure to roentgenographic contrast media. *Can. Med. Assoc. J.* 119:901-906

Furchgott, R.F. and Zawadzki, J.V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 228:373-376.

Furlong, B., Henderson, A.H., Lewis, M.J. and Smith, J.A. 1987. Endothelium-derived relaxing factor inhibits *in vitro* platelet aggregation. *Br. J. Pharmacol.* 90:687-692.

Gafter, U., Creter, D., Zevin, D., Catz, R. and Djaldetti, M. 1979. Inhibition of platelet aggregation by contrast media. *Radiology* 132:341-342.

Garay, R. and Braquet, P. 1986. Involvement of K+ movements in the membrane signal induced by PAF-acether. *Biochem. Pharmacol.* 35:2811-2815.

Garcia-Estan, J., Carbonell, L.F., Garcia-Salom, M., Salazar, F.J. and Quesada, T. 1989. Hemodynamic effects of hypertonic saline in the conscious rat. *Life Sci.* 44:1343-1350.

Garcia Rafanell, J. and Form, J. 1982. Intravenous arachidonate-induced respiratory distress: Comparative study of three *in vivo* models for the measurement of antithrombotic activity. *J. Pharmacol. Meth.* 7:231-238.

Gateau, D., Arnoux, B., Deriaz, H., Viars, P. and Benveniste, J. 1984. Acute effects of intratracheal administration of Paf-acether (platelet activating factor) in humans. *Am. Rev. Respir. Dis.* 129:A3.

Gates, D.F. and Ceccarelli, F.E. 1972. Benadryl and the IVP reaction. *J. Urol.* 108:627-628.

Godfrey, S. 1980. The relative merits of cromolyn sodium and high-dose theophylline therapy in chidhood asthma. *J. Allergy Clin. Immunol.* 65:97-104.

Godfroid, J.J., Heymans, F., Michel, E., Redenilh, C., Steiner, E. and Benveniste, J. 1980. Platelet activating factor (Paf-acether): total synthesis of 1-o-octadecyl-2-o-acetyl-sn-glyceryl-3-phosphorylcholine. *FEBS Lett.* 116:161-164.

Goetzl, E.J. 1980. Mediators of immediate hypersensitivity derived from arachidonic acid. *N. Eng. J. Med.* 303:822-825.

Goldberg, M. 1984. Systemic reactions to intravascular contrast media. *Anaesthesiology* 60:46-56.

Golman, K. and Holtas, S. 1980. Proteinuria produced by urographic contrast media. *Invest. Radiol.* 15:561-566.

Gonsette, R.E. and Delmotte, P. 1980. *In vivo* activation of serum complement by contrast media: a clinical study. *Invest. Radiol.* 15:S26-S28.

Goodwin, D.A., Bushberg, J.T., Doherty, P.W., Lipton, M.J., Conley, F.K., Diamanti, C.I. and Meares, C.F. 1978. Indium-111-labeled autologous platelets for location of vascular thrombi in humans. *J. Nucl. Med.* 19:626-634.

Gorman, R.R., Bunting, S. and Miller, O.V. 1977. Modulation of human adenylate cyclase by prosyaglandin (PGX). *Prostaglandins* 13:377.

Goth, A., Adams, H.R. and Knoohuizen, M. 1971. Phosphatidyl serine: selective enhancer of histamine release. *Science* 173:1034-1035.

Greenberg, J.P., Packham, M.A., Guccione, M.A., Rand, M.L., Reimers, H.-J. and Mustard, J.F. 1979. Survival of rabbit platelets treated *in vitro* with chymotrypsin, plasmin, trypsin or neuraminidase. *Blood* 53:916-927.

Greenberger, P.A. 1984. Contrast media reactions. *J. Allergy Clin. Immunol.* 74:600-605.

Greenberger, P., Patterson, R., Kelly, J., Stevenson, D.D., Simon, R. and Lieberman, P. 1980. Administration of radiographic contrast media in high risk patients. *Invest. Radiol.* 15:S40-S43.

Greer, I.A., Winter, J.H., Gaffney, D., McLoughlin, K., Belch, J.J.F., Boyd, E. and Forbes, C.D. 1984. Platelets in asthma. *Lancet* ii:1479.

Gresele, P., Grasselli, S., Todisco, T. and Nenci, G.G. 1985. Platelets and asthma. *Lancet* i:347.

Gresele, P., Ribaldi, E., Grasselli, S., Todisco, T. and Nenci, G.G. 1987. Evidence for platelet activation in allergic asthma. In "PAF, Platelets, and Asthma," eds. M. Schmitz-Schumann, G. Menz and C.P. Page. Birkhauser Verlag, Basel, pp 119-128.

Gresele, P., Todisco, T., Merante, F. and Nenci, G.G. 1982. Platelet activation and allergic asthma. *New. Engl. J. Med.* 306:549.

Gresele, P., Van Houtte, E., Arnout, J., Deckmyn, H. and Vermylen, J. 1984. Thromboxane synthetase inhibition combined with thromboxane receptor blockade: a step forward in antithrombotic strategy? *Thromb. Haemostas.* 52:364.

Griffin, M., Weiss, J.W., Leitch, A.G., McFadden, E.R., Corey, E.J., Austen, K.F. and Drazen, J.M. 1983. Effects of leukotriene D on the airways in asthma. *N. Engl. J. Med.* 308:436-439.

Grimley, R.P., Rafiqi, E., Hawker, R.J. and Drolc, Z. 1981. Imaging of 111In-labelled platelets - a new method for the diagnosis of deep vein thrombosis. *Br. J. Surg.* 68:714-716.

Gryglewski, R.J., Korbut, R., Ocetkiewicz, A. and Stachura. 1978. *In vivo* method for quantitation of anti-platelet potency of drugs. *Naunyn-Schmeideberg's Arch. Pharmacol.* 302:25-30.

Guidollet, J., Louisot, P., Amiel, M. and Limam, K. 1988. Iodinated contrast agents: Effect on acetylcholine hydrolase: *in vitro* study. *Invest. Radiol.* 23:S171-S173.

Haining, C.G. 1956. The release of cellular histamine in rabbit blood by dextran and dextran sulphate. In "CIBA Foundation Symposium on Histamine." Churchill, London, p 160.

Halonen, M., Lohman, I.C., Dunn, A.M., McManus, L.M. and Palmer, J.D. 1985. Participation of platelets in the physiologic alterations of the AGEPC response and of IgE anaphylaxis in the rabbit. *Am. Rev. Respir. Dis.* 131:11-17.

Halonen, M., Palmer, J.D., Lohman, I.C., McManus, L.M. and Pinckard, R.N. 1980. Respiratory and circulatory alterations induced by acetyl glyceryl ether phosphorylcholine, a mediator of IgE anaphylaxis in the rabbit. *Am. Rev. Respir. Dis.* 122:915-924.

Halpern, B., Ky, N.T. and Amache, N. 1967. Diagnosis of drug allergy *in vitro* with the lymphocyte transformation test. *J. Allergy* 40:168-181.

Hamasaki, Y., Mojarad, M., Saga, T., Tai, H.H. and Said, S.I. 1984. Platelet activating factor raises airway and vascular pressures and induces edema in lungs perfused with platelet-free solution. *Am. Rev. Respir. Dis.* 129:742-746.

Hamberg, M. and Samuelsson, B. 1974. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. U.S.A.* 71:3400-3404.

Hamberg, M., Svensson, J. and Samuelsson, B. 1975. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. U.S.A.* 72:2994-2998.

Hanahoe, T.H.P. 1984. Mechanism of histamine release induced by dextran: the role of immunoglobulin E. *Agents Actions* 14:468-474.

Handley, D.A., Lee, M. and Saunders, R. 1983. Extravasation and aortic ultrastructural changes accompanying acute and sub-acute intraperitoneal administration of PAF. Platelet activating factor INSERM Symposium No. 23, pp 245-250. J. Benveniste and B. Arnoux (eds.) Elsevier Publishers, B.V. Amsterdam.

Handley, D.A., Tomesch, J.C. and Saunders, R.N. 1986. Inhibition of PAF-induced systemic responses in the rat, guinea pig, dog and primate by the receptor antagonist SRI 63-441. *Thromb. Haemostas.* 56:40-44.

Hanson, S.R. and Harker, L.A. 1987. Baboon models of acute arterial thrombosis. *Thromb. Haemostas.* 58:801-805.

Harker, L.A. 1978. Platelet survival time: its measurement and use. *Progr. Hemostas. Thromb.* 4:321-347.

Harnish, P., Mukherji, M. and Kido, D.K. 1987*a*. Physiologic effects of contrast media in the rabbit. *Invest. Radiol.* 22:901-904.

Harnish, P.P., Mukherji, M., Northington, F.K. and Kido, D.K. 1987*b*. Opiate involvement in contrast media-induced blood pressure changes. *Invest. Radiol.* 22:905-907.

Harnish, P.P., Zuniga, J., Northington, F.K., Melrose, P.A., Joseph, S.A., Kido, D.K. and Knigge, K.M. 1988. Effect of contrast media on *beta*-endorphin secretion. *Acta Radiol. Diagn.* 29:741-743.

Hartman, G.W., Hattery, R.R., Witten, D.M. and Williamson, B. Jr. 1982. Mortality during excretory urography: Mayo Clinic experience. *Am. J. Roentgenol.* 139:919-922.

Hartung, H.P., Parnham, M.J., Winkelmann, J., Engelberger, W. and Hadding, U. 1983. Platelet activating factor (PAF) induces the oxidative burst in macrophages. *Int. J. Immunopharmacol.* 5:115-121.

Haslam, R.J. and Vanderwel, M. 1982. Inhibition of platelet adenylate cyclase by 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phoshorylcholine (platelet activating factor). *J. Biol. Chem.* 157:6879-6885.

Heaton, W.A., Davis, H.H., Welch, M.J., Mathias, C.J., Joist, J.H., Sherman, L.A. and Siegal, B.A. 1979. Indium-111: a new radionuclide label for studying human platelet kinetics. *Br. J. Haematol.* 42:613-622.

Heffner, J.E., Shoemaker, S.A., Carham, E.M., Patel, M., McMurphy, I.F., Morris, H.G. and Repine, J.E. 1983. Acetyl glyceryl ether phosphorylcholine-stimulated human platelets cause pulmonary hypertension and oedema in isolated rabbit lungs. *J. Clin. Invest.* 71:351-357.

Hegardt, B., Pauwels, R. and Van Der Straten, M. 1981. Inhibitory effect of KWC 2131, terbutaline and DSCG on the immediate and late allergen-induced bronchoconstriction. *Allergy* 36:115-122.

Hellewell, P.G. and Williams, T.J. 1986. A specific antagonist of platelet activating factor suppresses oedema formation in an Arthus reaction but not oedema induced by leukocyte chemoattractants in rabbit skin. *J. Immunol.* 137:302-307.

Henderson, A.F., Heaton, R.W. and Costello, J.F. 1983*a*. Effect of nifedipine on bronchoconstriction induced by inhalation of cold air. *Thorax* 38:512-515.

Henderson, A.F., Heaton, R.W., Dunlop, L.S. and Costello, J.F. 1983*b*. Effects of nifedipine on antigen-induced bronchoconstriction. *Am. Rev. Respir. Dis.* 127:549-553.

Henocq, E. and Vargaftig, B.B. 1986. Accumulation of eosinophils in response to intracutaneous Paf-acether and allergens in man. *Lancet* ii:1378-1379.

Henson, P.M. 1976. Activation and desensitization of platelets by platelet-activating factor (PAF) derived from IgE-sensitized basophils. I. Characteristics of the secretory response. *J. Exp. Med.* 143:937-952.

Henson, P.M. 1977. Activation of rabbit platelets by platelet activating factor derived from IgE-sensitised basophils: Characteristics of the aggregation and dissociation from secretion. *J. Clin. Invest.* 60:481-490.

Herd, C.M., Robertson, A.R., Frewin, D.B. and Taylor W.B. 1988. Adverse reactions during urography: are these due to histamine release? *Br. J. Radiol.* 61:5-11.

Herd, C.M., Rodgers, S.E., Lloyd, J.V., Bochner, F., Duncan, E.M. and Tunbridge, L.J. 1987. A dose-ranging study of the anti-platelet effect of enteric coated aspirin in man. *Aust. N.Z. J. Med.* 17:195-200.

Higgins, C.B., Gerber, K.H., Mattrey, R.F. and Slutsky, R.A. 1982. Evaluation of the hemodynamic effects of intravenous administration of ionic and nonionic contrast materials. Implications for deriving physiologic measurements from computed tomography and digital cardiovascular imaging. *Radiology* 142:681-686.

Hilal, S.K. 1966. Hemodynamic changes associated with the intra-arterial injection of contrast media. *Radiology* 86:615-633.

Hilgard, P. 1982. In "Interaction of Platelets and Tumour Cells," eds. G.A. Jamieson and A.R. Scipio. Alan R. Liss Inc, pp 143-158.

Hirsch, J.G. 1960. Comparative bactericidal activities of blood serum and plasma serum. *J. Exp. Med.* 112:15-22.

Hogan, J.C., Lewis, M.J. and Henderson, A.H. 1988. *In vivo* EDRF activity influences platelet function. *Br. J. Pharmacol.* 94:1020-1022.

Holgate, S.T., Church, M.K., Cushley, M.J., Robinson, C., Mann, J.S. and Howarth, P.H. 1984*a*. Pharmacologic modulation of airway calibre and mediator release in human models of bronchial asthma. In "Asthma: Physiology, Immunopharmacology and Treatment. IIIrd International Symposium," eds. A.B. Kay, K.F. Austen and L.M. Lichtenstein. London: Academic Press, pp 391-415.

Holgate, S.T., Mann, J.S. and Cushley, M.J. 1984*b*. Adenosine as a bronchoconstrictor mediator in asthma and its antagonism by methyl xanthines. *J. Allergy Clin. Immunol.* 74:302-306.

Holgate, S.T., Robinson, C. and Church, M.K. 1988. The contribution of mast cell mediators to acute allergic reactions in human skin and airways. *Allergy (Suppl.)* 43:22-31.

Holroyde, M.C., Altounyan, R.E.C., Cole, M., Dixon, M. and Elliott, E.V. 1981. Bronchoconstriction produced in man by leukotrienes C and D. *Lancet* ii:17-18.

Honey, A.C., Lad, N. and Tuffin, D.P. 1986. Effect of indomethacin and dazoxiben on intravascular platelet aggregation in the anaesthetised rabbit. *Thromb. Haemostas.* 56:80-85.

Hook, W.A. and Siraganian, R.P. 1981. Influence of anions, cations and osmolarity on IgE-mediated histamine release from human basophils. *Immunology* 43:723-731.

Hornstra, G. 1970. Method to determine the degree of ADP-induced platelet aggregation in circulating rat blood ("Filter-Loop Technique"). *Br. J. Haematol.* 19:321-329.

Hovig, T. 1963. Release of a platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline "extract" of tendons. *Thromb. Diath. Haemorrhag.* 9:264.

Howarth, P.H., Durham, S.R., Lee, T.H., Kay, A.B., Church, M.K. and Holgate, S.T. 1985. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. *Am. Rev. Respir. Dis.* 132:986-992.

Hughes, P.J., Holgate, S.T. and Church, M.K. 1984. Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A_2-purinoceptor mediated mechanism. *Biochem. Pharmacol.* 33:3847-3852.

Humphrey, J.H. and Jaques, R. 1954. The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species. *J. Physiol.* 124:305-310.

Humphrey, J.H., McManus, L.M., Hanahan, D.J. and Pinckard, R.N. 1984. Morphological basis of increased vascular permeability induced by acetyl glyceryl ether phosphorylcholine. *Lab. Invest.* 50:16-22.

Humphrey, J.H., McManus, L.M., Satouchi, K., Hanahan, D.J. and Pinckard, R.N. 1982. Vasoactive properties of acetyl glyceryl ether phosphorylcholine and analogues. *Lab. Invest.* 46:422-427.

Hwang, S.B., Lam, M.H., Biftu, T., Beattie, T.R. and Shen, T.Y. 1985*a*. Trans-2, 5-Bis-(3,4,5-trimethotyphenyl) tetrahydrofuran: An orally active specific and competitive receptor antagonist of platelet activating factor. *J. Biol. Chem.* 260:15639-15645.

Hwang, S.B., Lam, M.H. and Shen, T.Y. 1985*b*. Specific binding sites for platelet activating factor in human lung tissues. *Biochem. Biophys. Res. Commun.* 128:972-979.

Hwang, S.B., Lee, C.-S.C., Cheah, M.J. and Shen, T.Y. 1983. Specific receptor sites for 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea-pig smooth muscle membranes. *Biochemistry* 22:4756-4763.

Ieyasu, H., Takai, Y., Kaibuchi, K., Savamura, M. and Nishizuka, Y. 1982. A role of calcium-activated, phospholipid-dependent protein kinase in platelet activating factor-induced serotonin release from rabbit platelets. *Biochem. Biophys. Res. Commun.* 108:1701-1708.

Ignarro, L.J., Lippton, H., Edwards, J.C., Baricos, W.H., Hyman, A.L., Kadowitz, P.J. and Greutter, C.A. 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for involvement of S-nitrothiols as active intermediates. *J. Pharmacol. Exp. Ther.* 218:739-749.

Ikeda, Y., Kikuchi, M., Handa, M., Yoshii, Y., Toyama, K., Yamamoto, M., Wanatabe, K. and Ando, Y. 1981. The role of calcium in platelet microtubule assembly-disassembly. *Thromb. Res.* 23:163-168.

Inarrea, P., Gomez-Cambronero, J., Nieto, M. and SanchezCrespo, M. 1984. Characteristics of the binding of platelet-activating factor to platelets of different animal species. *Eur. J. Pharmacol.* 105:309-315.

Ind, P.W., Barnes, P.J., Brown, M.J., Causon, R. and Dollery, C.T. 1983. Measurement of plasma histamine in asthma. *Clin. Allergy* 13:61-67.

Jacobs, L. and Comroe, J.H. 1971. Reflex apnoea, bradycardia and hypotension produced by serotonin and phenyldiguanide acting on the nodose ganglion of the cat. *Circ. Res.* 29:145-155.

Jayakody, L., Kappagoda, T., Senaratne, M.P.J. and Thomson, A.B.R. 1988. Impairment of endothelium-dependent relaxation: an early marker for atherosclerosis in the rabbit. *Br. J. Pharmacol.* 94:335-346.

Johnson, C.E., Belfield, P.W., Davis, S., Cooke, N.J., Spencer, A. and Davis, J.A. 1986. Platelet activation during exercise induced asthma: effect of prophylaxis with cromoglycate and salbutamol. *Thorax* 41:290-294.

Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.C., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. and Vane, J.R. 1976. The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins* 12:915-928.

Joist, J.H., Baker, R.K., Thakur, M.L. and Welch, M.J. 1978. Indium-111-labeled human platelets: uptake and loss of label and *in vitro* function of labeled platelets. *J. Lab. Clin. Med.* 92:829-836.

Joseph, M., Tonnel, A.B., Capron, A. and Dessaint, J.P. 1981. The interaction of IgE antibody and human alveolar macrophages and its participation in the inflammatory processes of lung allergy. In "Pharmacology of Inflammation and Allergy," eds. F. Russo-Marie, B.B. Vargaftig and J. Benveniste. Colloques de l'INSERM, vol 100, pp 311-318.

Jouvin-Marche, E., Cerrina, J., Coeffier, E., Duroux, P. and Benveniste, J. 1983. Effect of the calcium antagonist on the release of platelet activating factor (Paf-acether), slow reacting substance (SRS) and *beta*-glucuronidase from human neutrophils. *Eur. J. Pharmacol.* 89:19-26.

Jouvin-Marche, E., Ninio, E., Beurain, G. and Benveniste, J. 1984. Biosynthesis of Paf-acether (platelet activating factor). VII. Precursors of Paf-acether and acetyltransferase activity in human leukocytes. *J. Immunol.* 133:892-898.

Kalimo, K., Jansen, C.T. and Kormano, M. 1980. Allergological risk factors as predictors of radiographic contrast media hypersensitivity. *Ann. Allergy* 45:253-255.

Kaliner, M., Dyer, J., Merlin, S., Shelton, A., Greenhill, A., Treadwell, G., McKenna, W. and Lieberman, P. 1984. Increased urine histamine and contrast media reactions. *Invest. Radiol.* 19:116-118.

Katzen, B.T. 1980. In "Interventional diagnostic and therapeutic procedures," ed. H.G. Jacobsen. Springer Verlag.

Kay, A.B. 1987. The mode of action of anti-allergic drugs. *Clin. Allergy* 17:153-164.

Kelly, J.F., Patterson, R., Lieberman, P., Mathison, D.A. and Stevenson, D.D. 1978. Radiographic contrast media studies in high-risk patients. *J. Allergy. Clin. Immunol.* 62:181-184.

Keyzer, J.J., Udding, H. and de Vries, K. 1984. Measurement of N-methylhistamine concentrations in urine as a parameter for histamine release by radiographic contarst media. *Diagn. Imag. Clin. Med.* 53:67-72.

Khalife, J., Capron, M., Grzych, J.M., Bazin, H. and Capron, A. 1985. Extracellular release of rat eosinophil peroxidase (EPO). I. Role of anaphylactic immunoglobulins. *J. Immunol.* 134:1968-1974.

Kimball, J.P., Sansone, V.J., Diters, L.A. and Wissel, P.S. 1988. Red blood cell aggregation versus blood clot formation in ionic and nonionic contrast media. *Invest. Radiol.* 23:S334-S339.

Kinnison, M.L., Powe, N.R. and Steinberg, E.P. 1989. Results of randomized controlled trials of low- versus high-osmolality contrast media. *Radiology* 170:381-389.

Kleinknecht, D., Deloux, J. and Homburg, J.C. 1974. Acute renal failure after intravenous urography-detection of antibodies against contrast media. *Clin. Nephrol.* 2:116-119.

Kloprogge, E. and Akkerman, J.W.N. 1984. Binding kinetics of Paf-acether (1-o-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) to intact human platelets. *Biochem. J.* 223:901-909.

Knauer, K.A., Lichtenstein, L.M., Adkinson N.F.Jr. and Fish, J.E. 1981. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N. Eng. J. Med.* 304:1404-1407.

Kohler, C., Wooding, W. and Ellenbogen, L. 1976. Intravenous arachidonate in the mouse: A model for the evaluation of antithrombotic drugs. *Thromb. Res.* 9:67-80.

Kolb, W.P., Lang, J.H. and Lasser, E.C. 1978. Nonimmunologic complement activation in normal human serum induced by radiographic contrast media. *J. Immunol.* 121:1232-1238.

Kornecki, E., Ehrlich, Y.H. and Lennox, R.H. 1984. Platelet activating factor-induced aggregation of human platelets specifically inhibited by triazolobenzodiazepines. *Science* 226:1454-1456.

Kraan, J., Koeter, G.H., Mark, T.W.V.D., Sluiter, H.J. and de Vries, K. 1985. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with anti-asthma drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J. Allergy Clin. Immunol.* 76:628-636.

Lacy, J., Bober-Sorcinelli, K.E., Farber, L.R. and Glickman, M.G. 1986. Acute thrombocytopenia induced by parenteral radiographic contrast medium. *Am. J. Roentgenol.* 146:1298-1299.

Laerum, F. 1983. Acute damage to human endothelial cells by brief exposure to contrast media *in vitro*. *Radiology* 147:681-684.

Laerum, F.L. and Holm, H.A. 1981. Postphlebographic thrombosis. *Diagn. Radiol.* 140:651-654.

Lalli. A.F. 1974. Urographic contrast media reactions and anxiety. *Radiology* 112:267-271.

Lalli, A.F. 1980. Contrast media reactions: data analysis and hypothesis. *Radiology* 134:1-12.

Lalli, A.F. and Greenstreet, R. 1981. Reactions to contrast media: testing the CNS hypothesis. *Radiology* 138:47-49.

Lambrecht, G. and Parnham, M.J. 1986. Kadsurenone distinguishes between different platelet activating factor receptor subtypes on macrophages and polymorphonuclear leucocytes. *Br. J. Pharmacol.* 87:287-289.

Lampe, K.F., James, G., Erbesfeld, M., Mende, T.J. and Viamonte, M.Jr. 1970. Cerebrovascular permeability of a water-soluble contrast material, Hypaque (sodium diatrizoate). Experimental study in dogs. *Clin. Allergy* 16:73-82.

Lang, M., Hansen, D. and Hahn, H.L. 1987. Effects of the PAF antagonist CV 3988 on PAF-induced changes in mucus secretion and in respiratory and circulatory variables in ferrets. In "PAF, Platelets, and Asthma," eds. M. Schmitz-Schumann, G. Menz and C.P. Page. Birkhauser Verlag, Basel, pp245-252.

Lang, J. and Lasser, E.C. 1967. Binding of roentgenographic contrast media to serum albumin. *Invest. Radiol.* 2:396-400.

Lang, J. and Lasser, E.C. 1975. Inhibition of adenosine triphosphatase and carbonic anhydrase by contrast media. *Invest. Radiol.* 10:314-316.

Lang, J., Lasser, E.C. and Kolb, L.B. 1976. Activation of serum complement by contrast media. *Invest. Radiol.* 11:303-308.

Lang, J.H., Lasser, E.C., Talner, L.B., Lyon, S. and Coel, M. 1974. Inorganic iodide in contrast media. *Invest. Radiol.* 9:51-55.

Lapetina, E.G. 1982. Platelet activating factor stimulates the phosphatidylcholine cycle. *J. Biol. Chem.* 257:7314-7317.

Lasser, E.C. 1968. Basic mechanisms of contrast media reactions. Theoretical and experimental considerations. *Radiology* 91:63-65.

Lasser, E.C. 1971. Metabolic basis of contrast material toxicity-status 1971. *Am. J. Roentgenol.* 113:415-422.

Lasser, E.C. 1985. Etiology of anaphylactoid responses. The promise of nonionics. *Invest. Radiol.* 20:S79-S83.

Lasser, E.C. 1988. A general and personal perspective on contrast material research. *Invest. Radiol.* 23:S71-S74.

Lasser, E.C., Berry, C.C., Talner, L.B., Santini, L.C., Lang, E.K., Gerber, F.H. and Stolberg, H.O. 1987. Pretreatment with corticosteroids to alleviate reactions to intravenous contrast material. *N. Engl. J. Med.* 317:845-849.

Lasser, E.C. and Lang, J.H. 1966. Inhibition of acetylcholinesterase by some organic contrast media. *Invest. Radiol.* 1:237-242.

Lasser, E.C. and Lang, J.H. 1970. Contrast-protein interactions. *Invest. Radiol.* 5:446-451.

Lasser, E.C., Lang, J.H., Lyon, S.G. and Hamblin, A.E. 1979*a*. Complement and contrast material reactors. *J. Allergy Clin. Immunol.* 64:105-112.

Lasser, E.C., Lang, J.H., Lyon, S.G. and Hamblin, A.E. 1980. Changes in complement and coagulation factors in a patient suffering a severe anaphylactoid reaction to injected contrast material: some considerations of pathogenesis. *Invest. Radiol.* 15:S6-S12.

Lasser, E.C., Lang, J.H., Lyon, S.G., Hamblin, A.E. and Howard, M. 1981*a*. Prekallekrein-kallekrein conversion: Conversion rates as a predictor for contrast catastrophes. *Radiology* 140:11-15.

Lasser, E.C., Lang, J.H., Lyon, S.G., Hamblin, A.E. and Howard, M. 1981*b*. Glucocorticoid-induced elevations of C1-esterase inhibitor: a mechanism of protection against lethal dose range contrast challenge in rabbits. *Invest. Radiol.* 16:20-23.

Lasser, E.C., Lang, J.H. and Walters, A.J. 1970. Are contrast media histamine releasers? *Invest. Radiol.* 5:277.

Lasser, E.C., Slivka, J., Lang, J.H., Kolb, W.P., Lyon, S.G., Hamblin, A.E. and Nazareno, G. 1979*b*. Complement and coagulation: causative considerations in contrast catastrophes. *Am. J. Roentgenol.* 132:171-176.

Lasser, E.C., Sovak, M. and Lang, J.H. 1976. Development of contrast media idiosyncrasy in the dog. *Radiology* 119:91-95.

Lasser, E.C., Walters, A.J. and Lang, J.H. 1974. An experimental basis for histamine release in contrast material reactions. *Radiology* 110:49-59.

Lasser, E.C., Walters, A.J., Reuter, S.R. and Lang, J.H. 1971. Histamine release by contrast media. *Radiology* 100:683-686.

Lawton, G., Phillips, T. and Davies, R. 1982. Alterations in heart rate and rhythm at urography with sodium diatrizoate. *Acta Radiol. Diagn.* 23:107-110.

Leahy, B.C., Gomm, S.A. and Allen, S.C. 1983. Comparison of nebulized salbutamol with nebulized ipratropium bromide in acute asthma. *Br. J. Dis. Chest* 77:159-163.

Lee, T.C., Blank, M.L., Fitzgerald, V. and Snyder, F. 1981. Substrate specificity in the bio-cleavage of the 1-alkyl-2-acetyl-sn-glyceryl-3-phosphorocholine (a hypotensive and platelet activating lipid) and its metabolites. *Arch Biochem. Biophys.* 208:353-357.

Lee, T.-C., Lenihan, D.J., Malone, B., Roddy, L.L. and Wasserman, S.I. 1984. Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J. Biol. Chem.* 259:5526-5530.

Lefer, A.M., Muller, H.F. and Smith, J.B. 1984. Pathophysiological mechanisms of sudden death induced by platelet activating factor. *Br. J. Pharmacol.* 83:125-130.

Lefort, J., Rotilio, D. and Vargaftig, B.B. 1984. The platelet-independent release of thromboxane A_2 by PAF-acether from guinea-pig lungs involves mechanisms distinct from those for leukotrienes. *Br. J. Pharmacol.* 82:565-575.

Leitch, A.G. and Drazen, J.M. 1984. In "The Leukotrienes, Chemistry and Biology," eds. L.W. Chaknin and D.M. Bailey. Academic Press, London, pp 248-270.

Lellouch-Tubiana, A., Lefort, J., Pirotzky, E., Vargaftig, B.B. and Pfister, A. 1985. Ultra-structural evidence for extravascular platelet recruitment in the lung upon intravenous injection of platelet activating factor (Paf-acether) to guinea-pigs. *Br. J. Exp. Pathol.* 66:345-356.

Lellouch-Tubiana, A., Lefort, J., Simon, M.T., Pfister, A. and Vargaftig, B.B. 1988. Eosinophil recruitment into guinea-pig lungs after PAF-acether and allergen administration. *Am. Rev. Resp. Dis.* 137:948-954.

Levy, D.E., Horner, A.A. and Solomon, A. 1981. Immunoglobulin-sulfated polysaccharide interactions. *J. Exp. Med.* 153:883-896.

Lewis, A.J., Dervinis, A. and Chang, J. 1984. The effects of antiallergic and bronchodilator drugs on platelet-activating factor (PAF-acether) induced bronchospasm and platelet aggregation. *Agents Actions* 15:636-642.

Lichtenstein, L.M., Schleimer, R.P., MacGlaskan, D.W., Peters, S.P., Schulman, E.S., Proud, D., Creticos, P.S., Naclerio, R.M. and Kagey-Sobtka, A. 1984. *In vitro* and *in vivo* studies of mediator release from human mast cells. In "Asthma: Physiology, Immunopharmacology and Treatment." Academic Press Inc, pp 1-15.

Lieberman, P. and Siegle, R.L. 1979. Complement activation following intravenous contrast material administration. *J. Allergy Clin. Immunol.* 64:13-17.

Lieberman, P., Siegle, R.L. and Taylor, W.W.Jr. 1978. Anaphylactoid reactions to iodinated contrast media. *J. Allergy Clin. Immunol.* 62:174-180.

Lindgren, P. 1970. Hemodynamic responses to contrast media. *Invest. Radiol.* 5:424-435.

Littner, M.R., Rosenfield, A.T., Ulreich, S. and Putman, C.E. 1977. Evaluation of bronchospasm during excretory urography. *Radiology* 124:17-21.

Lorenz, W., Doenicke, A., Messmer, K., Reimann, H.-J., Thermann, M., Lahn, W., Berr, J., Schmal, A., Dormann, P., Regenfuss, P. and Hamelmann, H. 1976. Histamine release in human subjects by modified gelatin (Haemaccel) and dextran: an explanation for anaphylactoid reactions observed under clinical conditions? *Br. J. Anaesth.* 48:151-165.

Lorenz, W., Reimann, H.-J., Barth, H., Kusche, J., Meyer, R., Doenicke, A. and Hutzel, M. 1972. A sensitive and specific method for the determination of histamine in human whole blood and plasma. Hoppe-Seylers Z. *Physiol. Chem.* 353:911-920.

Lowhagen, O. and Rak, S. 1985. Modification of bronchial reactivity after treatment with sodium cromoglycate during pollen season. *J. Allergy Clin. Immunol.* 75:460-467.

Maccia, C.A., Gallagher, J.S., Ataman, G., Glueck, H.I., Brooks, S.M. and Bernstein, I.L. 1977. Platelet thrombopathy in asthmatic patients with elevated immunoglobulin E. *J. Allergy Clin. Immunol.* 59:101-108.

MacDonald, A.J., Moqbel, R., Wardlaw, A.J. and Kay, A.B. 1986. Platelet activating factor (PAF-acether) enhances eosinophil cytotoxicity *in vitro*. *J. Allergy Clin. Immunol.* 77:227 (abstract).

Maddi, V.I., Wyso, E.M. and Zinner, E.N. 1969. Dextran anaphylaxis. *Angiology* 20:243-248.

Magnussen, H., Litt, M., Reuss, G., Jorres, R. and Hartmann, V. 1985. Efficacy of methylxanthines in bronchial asthma: bronchodilation versus protection. *Am. Rev. Respir. Dis.* 131:A49.

Malik, A.B. 1983. Pulmonary microembolism. *Physiol. Rev.* 63:1114-1206.

Mallarkey, G. and Smith, G.M. 1985. A comparative study of the involvement of the prostaglandin H_2/thromboxane A_2 pathway in intravasculr platelet aggregation in guinea-pigs and rats. *Br. J. Pharmacol.* 84:425-430.

Mallet, A.J., Cunningham, F.M. and Daniel, F. 1985. Rapid isocratic high performance liquid chromatographic purification of platelet activating factor (PAF) and lyso PAF from human skin. *J. Chromatogr.* 309:16-164.

Mann, M.R. 1961. The pharmacology of contrast media. *Proc. Royal Soc. Med.* 54:473-476.

Mano, K., Akbarzadeh, A. and Townley, R.G. 1979. Effect of hydrocortisone on *beta*-adrenergic receptors in lung membranes. *Life Sci.* 25:1925-1930.

Marcus, A.J., Broekman, M.J., Weksler, B.B., Jaffe, E.A., Safier, L.B., Ullman, H.L. and Tack-Goldman, K. 1981. Interactions between stimulated platelets and endothelial cells *in vitro*. *Phil. Trans. R. Soc. Lond.*.B 294:343-353.

Marcus, A.J., Broekman, M.J., Safier, L.B., Ullman, H.L. and Islam, N. 1982. Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions in vitro. Biochem. Biophys. Res. Commun. 109:130-137.

Marcus, A.J., Safier, L.B., Ullman, H.L., Broekman, J., Islam, N., Oglesby, T.D. and Gorman, R.R. 1984. 12*S*,20-dihydroxyicosatetraenoic acid: a new icosanoid synthesized by neutrophils from 12*S*-hydroxyicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. *Proc. Natl. Acad. Sci U.S.A.* 81:903-907.

Margolis, G., Griffin, A.T., Kenon, P.D., Tinall, G.T., Ruggins, R. and Fort, L. 1959. Contrast medium injury to the spinal cord. *J. Neurosurg. 16:390-404.*

Mathe, A.A., Hedqvist, P., Holmgren, A. and Svanborg, N. 1973. Bronchial hyperreactivity to prostaglandin F_{2alpha} and histamine in patients with asthma. *Br. Med. J.* 1:193-196.

Martin, J.F., Slater, D.N. and Trowbridge, E.A. 1987. Platelet production in the lungs. In "PAF, Platelets, and Asthma," eds. M. Schmitz-Schumann, G. Menz and C.P. Page. Birkhauser Verlag, Basel, pp 37-57.

Martin, U. and Romer, D. 1978. The pharmacological properties of a new, orally active, antianaphylactic compound: ketotifen, a benzocycloheptathiophene. Arzneim Forsch. 5:770-782.

May, G.R., Herd, C.M., Butler, K.D. and Page, C.P. 1990. A radioisotopic model for investigating platelet aggregation in the rabbit. J. Pharmacol. Meth. (submitted).

Mazzoni, L., Morley, J., Page, C.P. and Sanjar, S. 1985*a*. Induction of airway hyper-reactivity by platelet activating factor in the guinea-pig. *J. Physiol.* 365:107P.

Mazzoni, L., Morley, J., Page, C.P. and Sanjar, S. 1985*b*. Prophylactic anti-asthma drugs impair the airway hyperreactivity that follows exposure to platelet activating factor (PAF). *Br. J. Pharmacol.* 86:571P.

McAfee, J.G. 1957. A survey of complications in aortography. *Radiology* 825-832.

McClennan, B.L., Ling, D., Rholl, K.S. and James, M. 1986. Urography with a low osmolality contrast agent. Comparison of Hexabrix with Conray 325. *Invest. Radiol.* 21:144-150.

McManus, L.M. 1987. Acute lung injury induced by intravascular platelet activating factor. In "New Horizons in Platelet Activating Factor Research," vol. 25, eds. C.M. Winslow and M.L. Lee. John Wiley & Sons Ltd., London, pp 233-25.

McManus, L.M., Fitzpatrick, F.A., Hanahan, D.J. and Pinckard, R.N. 1983. Thromboxane B_2 release following acetyl glyceryl ether phosphorylcholine (AGEPC) infusion in the rabbit. *Immunopharmacology* 5:197-207.

McManus, L.M., Hanahan, D.J., Demopolous, C.A. and Pinckard, R.N. 1979*a*. Pathobiology of the intravenous infusion of acetyl glyceryl ether phosphorylcholine (AGEPC), a synthetic platelet-activating factor (PAF), in the rabbit. *J. Immunol.* 124:2919-2924.

McManus, L.M. and Hass, D.L. 1986. Late phase pulmonary eosinophil accumulation after intravenous acetyl glyceryl ether phosphorylcholine (AGEPC) infusion in the rabbit. *Fed. Proc.* 45:995.

McManus, L.M., Morley, C.A., Levine, S.P. and Pinckard, R.N. 1979*b*. Platelet activating factor (PAF) induced release of platelet factor 4 (PF4) *in vitro* and during IgE anaphylaxis in the rabbit. *J. Immunol.* 123:2835-2841.

Mencia-Huerta, J.M. and Benveniste, J. 1981. Platelet activating factor (Paf-acether) and macrophages: phagocytosis-associated release of Paf-acether from rat peritoneal macrophages. *Cell. Immunol.* 57:281-292.

Mencia-Huerta, J.M., Lewis, R.A., Razin, E. and Austen, K.F. 1983. Antigen-initiated release of platelet activating facor (Paf-acether) from mouse bone marrow-derived mast cells sensitised with monoclonal IgE. *J. Immunol.* 131:2958-2964.

Mencia-Huerta, J.M., Ninio, E., Roubin, R. and Benveniste, J. 1981. Is platelet activating factor (Paf-acether) synthesis by murine peritoneal cells (Pc) a two-step process? *Agents Actions* 11:556-558.

Messmore, H.L., Walenga, J.M. and Fareed, J. 1984. Molecular markers of platelet activation. *Sem. Thromb. Haemostas.* 10:264-269.

Metzger, W.J., Hunninghake, G.W. and Richerson, H.B. 1985. Late asthmatic responses: inquiry into mechanisms and significance. *Clin. Rev. Allergy* 3:145-165.

Metzger, W.J, Sjoerdsma, K., Richerson, H.B., Moseley, P., Zvala, D., Monick, M. and Hunninghake, G.W. 1987. Platelets in bronchoalveolar lavage from asthmatic patients and allergic rabbits with allergen-induced late phase responses. In "PAF, Platelets, and Asthma," eds. M. Schmitz-Schumann, G. Menz and C.P. Page. Birkhauser Verlag, Basel, pp 151-159.

Miadonna, A., Tedeschi, A., Leggieri, E., Cottini, M., Restuccia, M. and Bianchini, C. 1983. Effect of verapamil on allergen-induced asthma in patients with respiratory allergy. *Ann. Allergy* 51:201-204.

Miller, W.L., Doppman, J.L. and Kaplan, A.P. 1975. Renal arteriography following systemic reaction to contrast material. *J. Allergy Clin. Immunol.* 56:291-295.

Miller, O.V., Johnson, R.A. and Gorman, P.R. 1977. Inhibition of PGE-stimulated cyclic AMP accumulation in human platelets by thromboxane A_2. *Prostaglandins* 13:600-609.

Mills, J.E, Sellick, H. and Widdicombe, J.G. 1969. Activity of lung irritant receptors in pulmonary microembolism, anaphylaxis and drug-induced bronchoconstrictions. *J. Physiol.* 203:337-357.

Mills, D.C.B. and Smith, J.B. 1971. The influence on platelet aggregation of drugs that affect the accumulation of 3',5'-cyclic monophosphate in platelets. *Biochem. J.* 121:185-196.

Mitchell, J.R.A. 1981. Prostaglandins in vascular disease: a seminal approach. *Br. Med. J.* 282:590-594.

Miyazawa, T., Nakagawa, H. and Oshino, N. 1989. Role of red blood cell deformation in toxicity of contrast media in cerebral angiography. *Invest. Radiol.* 24:383-389.

Mohammad, S.F., Anderson, W.H., Smith, J.B., Chuang, H.Y.K. and Mason, R.G. 1981. Effects of heparin on platelet aggregation and release and thromboxane A_2 production. *Am. J. Pathol.* 104:132-141.

Mollison, P.L. 1967. Labelling of red cells with radioactive chromium. In "Blood Transfusions in Clinical Medicine." Blackwell Scientific Publications, Oxford and Edinburgh, pp 721-724.

Moncada, S., Needleman, P., Bunting, S. and Vane, J. 1976. Prostaglandin endoperoxide and thromboxane generating systems and their selective inhibition. *Prostaglandins* 12:323-329.

Moncada, S. and Vane, J.R. 1978*a*. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. *Pharmacol. Rev.* 30:293-331.

Moncada, S. and Vane, J.R. 1978*b*. Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. *Br. Med. Bull.* 34:129-135.

Mondot, S., Caillard, C.G. and Sendivy, P. 1989. *In vivo* actions of 48740 RP on the cardiovascular effects of Paf-acether in dogs and cats. In "Proceedings of the IIIrd International Congress of Inflammation," eds. F. Russo-Marie, M. Chignard and J. Mencia-Huerta. Raven Press.

Moodley, I., Mongar, J.L. and Foreman, J.C. 1982. Histamine release induced by dextran: the nature of the dextran receptor. *Eur. J. Pharmacol.* 83:69-81.

Moore, S. 1976. Platelet aggregation secondary to coronary obstruction. *Circulation* 53:I-66-I-69.

Moqbel, R., Walsh, G.M., MacDonald, A.J. and Kay, A.B. 1986. Effect of disodium cromoglycate on activation of human eosinophils in neutrophils following reversed (anti-IgE) anaphylaxis. *Clin. Allergy* 16:73-82.

Moreno, R.H., Hogg, J.C. and Pare, P.D. 1986. Mechanics of airway narrowing. *Am. Rev. Respir. Dis.* 133:1171-1180.

Morley, J. and Aoki, S. 1986. A central role for platelet activating factor in asthma. In "Immunopharmacology Reviews of the 4th Symposium: PAF in Allergy and Inflammation," ed. T. Miyamoto. *Deut. Mediz. Wochenschr.* pp 17-30.

Morley, J., Chapman, I.D., Sanjar, S. and Schaeublin, E. 1989*a*. Actions of ketotifen on PAF-induced airway hyperreactivity in the anaesthetised guinea-pig. *Br. J. Pharmacol.* 96:76P.

Morley, J., Hanson, J.M. and Youlten, L.J.F. 1984*a*. In "Allergy," ed. M.H. Lessof. John Wiley & Sons Ltd., London, pp 45-72.

Morley, J., Page, C.P., Mazzoni, L. and Sanjar, S. 1985*a*. Anti-allergic drugs in asthma. *Triangle* 24:59-70.

Morley, J., Page, C.P. and Paul, W. 1983. Inflammatory actions of platelet activating factor (Paf-acether) in guinea-pig skin. *Br. J. Pharmacol.* 80:503-509.

Morley, J., Page, C.P. and Sanjar, S. 1985*b*. PAF and airway hyperreactivity. *Lancet* ii:451.

Morley, J., Sanjar, S. and Page, C.P. 1984*b*. The platelet in asthma. *Lancet* ii:1142-1144.

Morley, J., Smith, D. and Chapman, I. 1989*b*. Platelet activating factor and airway smooth muscle. *Pharmacol. Ther.* 40:373-382.

Morris, T.W., Sahler, L.G. and Fischer, H.W. 1982. Calcium binding by radiopaque media. *Invest. Radiol.* 17:501-505.

Mustard, J.F. 1981. Function of blood platelets and their role in haemostasis. *Trans Amer. Clin. Climat. Assoc.* 87:104-127.

Mustard, J.F. and Packham, M.A. 1975. Platelets, thrombosis and drugs. *Drugs* 9:19-76.

Mustard, J.F., Packham, M.A., Kinlough-Rathbone, R.L., Perry, D.W. and Regoeczi, E. 1978. Fibrinogen and ADP-induced platelet aggregation. *Blood* 52:453-466.

Nachman, R.L. and Weksler, B.B. 1980. The platelet as an inflammatory cell. In "The cell biology of inflammation," ed. G. Weissmann. Elsevier, North Holland, Amsterdam, pp. 145-162.

Najean, Y. and Ardaillou, N. 1969. The use of ^{75}Se-methionin for the *in vivo* study of platelet kinetics. *Scand. J. Haematol.* 6:395-401.

Needleman, P., Bronson, S.D., Wyche, A., Sivakoff, M. and Nicolaou, K.C. 1978. Cardiac and renal prostaglandin I_2. *J. Clin. Invest.* 61:839-849.

Neoh, S.H., Sage, M.R., Willis, R.B., Roberts-Thomson, P. and Bradley, J. 1981. The *in vitro* activation of complement by radiologic contrast materials and its inhibition with *epsilon* aminocaproic acid. *Invest. Radiol.* 16:152-158.

Ninio, E.W., Mencia-Huerta, J.M., Heymans, F. and Benveniste, J. 1982. Biosynthesis of platelet activating factor: Evidence for an acetyl transferase activity in murine macrophages. *Biophys. Biochem. Acta* 710:23-31.

Nunn, B. 1979. Collagen-induced platelet aggregation: evidence against the essential role of platelet adenosine diphosphate. *Thromb. Haemostas.* 42:1193-1206.

Oda, M., Satouchi, K., Yasunaga, K. and Saito, K. 1986. Polymorphonuclear leukocyte-platelet interactions: Acetylglyceryl ether phosphocholine-induced platelet activation under stimulation with chemotactic peptide. *J. Biochem.* 100:1117-1123.

O'Flaherty, J.T., Wykle, R.L., Miller, H., Lewis, J.C., Waite, M., Bass, D.A., McCall, C.E. and De Chatelet, L.R. 1981. 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholines: novel class of neutrophil stimulants. *Am. J. Pathol.* 103:70-79.

Olsson, O. 1951. Antihistaminic drugs for inhibiting untoward reactions to injections of contrast medium. *Acta Radiol.* 35:65-70.

Orr, T.S.C. and Cox, J.S.G. 1969. Disodium cromoglycate, an inhibitor of mast cell degranulation and histamine release induced by phopsholipase A. *Nature* 233:197-198.

Owen, R.T. 1979. Platelets. A review of selected aspects of their physiology, pharmacology and pathology, with special reference to their role in inflammation and thrombogenesis. *Drugs of Today* 15:487-516.

Owen, N.E., Feinberg, H. and Le Breton, G.C. 1980. Epinephrine induces Ca^{2+} uptake in human blood platelets. *Am. J. Physiol.* 239:H483-H488.

Owen, N.E. and Le Breton, G.C. 1981. Ca^{2+} mobilisation in blood platelets are visualised by chlortetracycline fluorescence. *Am. J. Physiol.* 241:H613-H619.

Oyekan, A.O. and Botting, J.H. 1986. A minimally invasive technique for the study of intravascular platelet aggregation in anaesthetised rats. *J. Pharmacol. Meth.* 15:271-277.

Paajanen, H., Kormano, M. and Uotila, P. 1984. Modification of platelet aggregation and thromboxane synthesis by intravascular contrast media. *Invest. Radiol.* 19:333-337.

Pace-Asciak, C. 1976. Isolation, structure and biosynthesis of 6-ketoprostaglandin F_{1alpha} in the rat stomach. *J. Am. Chem. Soc.* 98:2348-2349.

Packham, M.A. and Mustard, J.F. 1986. Interactions of platelet activating pathways: studies with inhibitors specific for individual pathways. In "Platelet Responses and Metabolism," vol. 1, ed. H. Holmsen. CRC Press, Florida, pp 267-283.

Page, C.P. 1988. The involvement of platelets in non-thrombotic processes. *Trends Pharmcol. Sci.* 9:66-71.

Page, C.P., Paul, W., Basran, G.S. and Morley, J. 1982*a*. Platelet activation in asthma. *N. Eng. J. Med.* 307:251-252.

Page, C.P., Paul, W., Basran, G.S. and Morley, J. 1985. Platelet activation in asthma. In "Bronchial Asthma. Mechanisms and Therapeutics," eds. E.B. Weiss, M.S. Siegal and M. Stein. Little, Brown and Co., Boston, pp 266-270.

Page, C.P., Paul, W. and Morley, J. 1982*b*. An *in vivo* model for studying platelet aggregation and disaggregation. *Thromb. Haemostas.* 47:210-213.

Page, C.P., Paul, W. and Morley, J. 1983. *In vivo* aggregation of guinea-pig platelets in response to synthetic platelet activating factor (PAF-acether). *Agents Actions* 13:506-507.

Page, C.P., Paul, W. and Morley, J. 1984. Platelets and bronchospasm. *Int. Arch. Allergy Appl. Immunol.* 74:347-350.

Palmer, R.M.J., Ferrige, A.G. and Moncada, S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524-526.

Palosvo, T. and Milgrom, F. 1981. Appearance of dextrans and anti-dextran antibodies in human sera. *Int. Arch. Allergy Appl. Immunol.* 65:153-161.

Parratt, J.R. and West, G.B. 1957. 5-Hydroxytryptamine and the anaphylactoid reaction in the rat. *J. Physiol.* 139:27-41.

Parvez, Z., Marsan, R.E., Moncada, R. and Patel, N. 1988. Effect of contrast media on prostaglandin synthesis *in vivo*. *Invest. Radiol.* 23:S178-S181.

Parvez, Z. and Moncada, R. 1985. Complement abnormalities during contrast media procedures. *Int. J. Immunopharmacol.* 7:123-127.

Parvez, Z., Moncada, R. and Fareed, J. 1982. Contrast media induced serotonin release in human blood. *Acta Radiol. Diagn.* 23:561-565.

Parvez, Z., Moncada, R., Fareed, J. and Messmore, H.L. 1983. Effects of nonionic contrast media (CM) on the components of coagulation and complement systems. *Invest. Radiol.* 18:279-284.

Parvez, Z., Moncada, R., Fareed, J. and Messmore, H.L. 1984. Antiplatelet action of intravascular contrast media. Implications in diagnostic procedures. *Invest. Radiol.* 19:208-211.

Parvez, Z. and Patel, N.B. 1988. Effect of a new nonionic contrast agent, ioxilan, on human erythrocytes and the hemostatic and serum complement pathways. *Invest. Radiol.* 23:S182-S185.

Patakas, D., Vlachoianni, E., Tsara, V., Louidas, G. and Argiropoulou, P. 1983. Nifedipine in bronchial asthma. *J. Allergy Clin. Immunol.* 72:269-273.

Patterson, R. and Harris, K.E. 1983. The activity of aerosolised and intracutaneous synthetic platelet activating factor (AGEPC) in rhesus monkeys with IgE-mediated airway responses and normal monkeys. *J. Lab. Clin. Med.* 102:933-938.

Paul, W., Page, C.P., Cunningham, F.,M., and Morley, J. 1984. The plasma protein extravasation response to Paf-acether is independent of platelet accumulation. *Agents Actions* 15:80-82.

Pauwels, R., van Renterghem, D., van der Straeten, M., Johannesson, N. and Persson, C.G.A. 1985. The effect of theophylline and enprophylline on allergen-induced bronchoconstriction. *J. Allergy Clin. Immunol.* 76:583-590.

Peel, E.T. and Gibson, G.J. 1980. Effects of long-term inhaled salbutamol therapy on the provocation of asthma by histamine. *Am. Rev. Respir. Dis.* 121:973-978.

Pepys, J., Hargreave, F.E., Chan, M. and McCarthy, D.S. 1968. Inhibitory effects of disodium cromoglycate on allergen inhalation tests. *Lancet* ii:134-137.

Persson, C.G.A. 1980. Some pharmacological aspects of xanthines in asthma. *Eur. J. Resp. Dis.* 61:7-16.

Peters, G.A., Hodgson, J.R. and Donovan, R.J. 1966. The effect of premedication with chlorpheniramine on reactions to methylglumine iodipamide. *J. Allergy* 38:74-82.

Peters, S.P., Schulman, E.S., Schleimer, R.P., MacGlashan, D.W., Newball, H.H. and Lichtenstein, L.M. 1982. Dispersed human lung mast cells: pharmacologic aspects and comparison with human lung fragments. *Am. Rev. Respir. Dis.* 126:1034-1038.

Pfister, R.C. and Hutter, A.M. 1980. Cardiac alterations during intravenous urography. *Invest. Radiol.* 15:239-242.

Phillips, M.J., Meyrick Thomas, R.H., Moodley, I. and Davies, R.J. 1983. A comparison of the *in vivo* effects of ketotifen, clemastine, chlorpheniramine and sodium cromoglycate on histamine and allergen induced wheals in human skin. *Br. J. Clin. Pharmacol.* 19:277-286.

Pierce, R.J., Holmes, P.W. and Campbell, A.H. 1982. Use of ipratropium bromide in patients with severe airways obstruction. *Aust. N.Z. J. Med.* 12:38-43.

Pinckard, R.N., Halonen, M., Palmer, J.D., Butler, C., Shaw, J.O. and Henson, P.M. 1977. Intravascular aggregation and pulmonary sequestration of platelets during IgE-induced systemic anaphylaxis in the rabbit: abrogation of lethal anaphylactic shock by platelet depletion. *J. Immunol.* 119:2185-2193.

Pinckard, R.N., Kniker, W.E.T., Lee, T., Hanahan, D.J. and McManus, L.M. 1980. Vasoactive effects of 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (AGEPC) in human skin. *J. Allergy Clin. Immunol.* 65:196 (abstract).

Pinon, J.F. 1984. *In vivo* study of platelet aggregation in rats. *J. Pharmacol. Meth.* 12:79-84.

Piper, P.J. (ed.) 1980. "SRS-A and Leukotrienes." John Wiley & Sons, Ltd., London.

Piper P. and Vane, J. 1971. The release of prostaglandins from lung and other tissues. *Ann. N.Y. Acad. Sci.* 180:363-385.

Pirotzky, E., Ninio, E., Bidault, J., Pfister, A. and Benveniste, J. 1984*a*. Biosynthesis of platelet activating factor. VI. Precursor of platelet activating factor and acetyl transferase activity in isolated rat kidney cells. *Lab. Invest.* 51:567-572.

Pirotzky, E., Page, C.P., Roubin, R., Pfister, A., Paul, W., Bonnet, J. and Benveniste, J. 1984*b*. Paf-acether-induced plasma exudation in rat skin is independent of platelets and neutrophils. *Microcirc. Endoth. Lymphat.* 1:107-122.

Poulsen, J., Rasmussen, F. and Georgsen, J. 1987. Hypotensive shock associated with bradycardia after intravenous injection of contrast medium. *Radiology* 164:275-276.

Powe, N.R., Kinnison, M.L. and Steinberg, E.P. 1989. Quality assessment of randomized controlled trials of contrast media. *Radiology* 170:377-380.

Radomski, M.W., Palmer, R.M.J. and Moncada, S. 1987*a*. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol.* 92:181-187.

Radomski, M.W., Palmer, R.M.J. and Moncada, S. 1987*b*. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* ii:1057-1058.

Radomski, M.W., Palmer, R.M.J. and Moncada, S. 1987*c*. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochem. Biophys. Res. Commun.* 148:1482-1489.

Raininko, R. and Ylinen, S.L. 1987. Effect of ionic and non-ionic contrast media on aggregation of red blood cells *in vitro*. *Acta Radiol. Diagn.* 28:87-92.

Rajtar, G., Cerletti, C., Castagnoli, M.N., Bertele, V. and de Gaetano, G. 1985. Prostaglandins and human platelet aggregation. Implications for the anti-aggregatory activity of thromboxane-synthetase inhibitors. *Biochem. Pharmacol.* 34:307-310.

Rao, A.K. and Walsh, P.N. 1983. Aquired qualitative platelet disorders. *Clin. Haematol.* 12:201-238.

Rao, A.K., Rao, V.M., Willis, J., Beckett, C. and Steiner, R.M. 1985. Inhibition of platelet function by contrast media: iopamidol and ioxaglate versus iothalamate. *Radiology* 156:311-313.

Rapoport, S., Bookstein, J.J., Higgins, C.B., Carey, P.H., Sovak, M. and Lasser, E.C. 1982. Experience with metrizamide in patients with previous severe anaphylactoid reactions to ionic contrast agents. *Radiology* 143:321-325.

Rapoport, R.M. and Murad, F. 1983. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.* 52:352-357.

Read, R., Vick, J. and Meyer, M. 1959. Influence of perfroate characteristics on the pulmonary vascular effects of hypertonic solutions. *Fed. Proc.* 18:124.

Renooij, W. and Snyder, F.F. 1981. Biosynthesis of 1-*o*-alkyl-2-acetyl-sn-glyceryl-phosphorylcholine (platelet activating factor and a hypotensive lipid) by choline-phosphotransferase in various rat tissues. *Biochem. Biophys. Acta* 663:545-556.

Rice, M.C., Lieberman, P., Siegle, R.L. and Mason, J. 1983. *In vitro* histamine release induced by radiocontrast media and various chemical analogs in reactor and control subjects. *J. Allergy Clin. Immunol.* 72:180-186.

Richardson, B.P., Engel., G., Donatsch, P. and Stadler, P.A. 1985. Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature* 316:126-131.

Ring, J., Arroyave, C.M., Fritzler, M.J. and Tan, E.M. 1978*a*. *In vitro* histamine and serotonin release by radiographic contrast media (RCM). Complement-dependent and -independent release reaction and changes in ultrastructure of human blood cells. *Clin. Exp. Immunol.* 32:105-118.

Ring, J., Endrich, B. and Intaglietta, M. 1978*b*. Histamine release, complement consumption, and microvascular changes after radiographic contrast media infusion in rabbits. *J. Lab. Clin. Med.* 92:584-594.

Ring, J. and Messmer, K. 1977. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* i:466-469.

Ring, J., Rothenberger, K.-H. and Clauss, W. 1985. Prevention of anaphylactoid reactions after contrast media infusion by combined histamine H_1- and H_2-receptor antagonists: results of a prospective controlled trial. *Int. Arch. Allergy Appl. Immunol.* 78:9-14.

Ring, J. and Sovak, M. 1981. Release of serotonin from human platelets *in vitro* by radiographic contrast media. *Invest. Radiol.* 16:245-248.

Robertson, H.J.F. 1987. Blood clot formation in angiographic syringes containing nonionic contrast media. *Radiology* 162:621-622.

Robertson, P.W., Frewin, D.B., Robertson, A.R., Mahar, L.J. and Jonsson, J.R. 1985. Plasma histamine levels following administration of radiographic contrast media. *Br. J. Radiol.* 58:1047-1051.

Robertson, D.N. and Page, C.P. 1987. Effect of platelet agonists on airway reactivity and intrathoracic platelet accumulation. *Br. J. Pharmacol.* 92:105-111.

Robertson, D.N. and Smith, G.M. 1986. CV 3988 inhibits *in vivo* platelet aggregation induced by PAF-acether and collagen. *Eur. J. Pharmacol.* 123:91-97.

Rockoff, S.D. and Aker, U.T. 1972. Contrast media as histamine liberators: VI. Arterial plasma histamine and hemodynamic responses following angiocardiography in man with 75% Hypaque. *Invest. Radiol.* 7:403-406.

Rockoff, S.D. and Brasch, R. 1971. Contrast media as histamine liberators III. Histamine release and some associated hemodynamic effects during pulmonary angiography in the dog. *Invest. Radiol.* 6:110-114.

Rockoff, S.D., Brasch, R., Kuhn, C. and Chraplyvy, M. 1970. Contrast media as histamine liberators I. Mast cell histamine release *in vitro* by sodium salts of contrast media. *Invest. Radiol.* 5:503-509.

Rockoff, S.D., Kuhn, C. and Chraplyvy, M. 1971. Contrast media as histamine liberators IV. *In vitro* mast cell histamine release by methylglucamine salts. *Invest. Radiol.* 6:186-190.

Rockoff, S.D., Kuhn, C. and Chraplyvy, M. 1972. Contrast media as histamine liberators: V. Comparison of *in vitro* mast cell histamine release by sodium and methylglucamine salts. *Invest. Radiol.* 7:177-181.

Rosenfield, A.T., Littner, M.R., Ulreich, S., Farmer, W.C. and Putman, C.E. 1977. Respiratory effects of excretory urography: a preliminary report. *Invest. Radiol.* 12:295-298.

Ross, R., Raines, E. and Bowen-Pope, D. 1985. Macrophages, platelets, endothelial injury, growth factors and cell proliferation. *Adv. Inflamm. Res.* 10:99-104.

Roth, G.J. and Siok, C.J. 1978. Acetylation of the NH_2-terminal serine of prostaglandin synthetase by aspirin. *J. Biol. Chem.* 253:3782-3784.

Rubin, A.-H.E., Smith, L.J. and Patterson, R. 1987. The bronchoconstrictor properties of platelet-activating factor in humans. *Am. Rev. Respir. Dis.* 136:1145-1151.

Ruffin, R.E. and Newhouse, M.T. 1981. Dipyridamole - is it a bronchodilator? *Eur. J. Resp. Dis.* 62:123-126.

Russi, E.W., Marchette, B., Yerger, L., Abraham, W.M. and Ahmed, T. 1983. Modification of allergic bronchoconstriction by a calcium antagonist: mode of action. *Am. Rev. Respir. Dis.* 127:675-679.

Sage, S.O. and Rink, T.J. 1986*a*. Kinetic differences between thrombin-induced and ADP-induced calcium influx and release from internal stores in fura-2 loaded human platelets. *Biochem. Biophys. Res. Commun.* 136:1124-1129.

Sage, S.O. and Rink, T.J. 1986*b*. Effects of ionic substitution on $[Ca^{2+}]$ rises evoked by thrombin and PAF in human platelets. *Eur. J. Pharmacol.* 128:99-107.

Salem, D.N., Findlay, S.R., Isner, J.M., Konstam, M.A. and Cohen, P.F. 1986. Comparison of histamine release effects of ionic and non-ionic radiographic contrast media. *Am. J. Med.* 80:382-384.

Salmon, J.A., Higgs, G.A., Tilling, L., Moncada, S. and Vane, J.R. 1984. Mode of action of benoxaprofen. *Lancet* i:848-849.

Sanchez-Crespo, M., Alonso, F. and Egido, J. 1980. Platelet activating factor in anaphylaxis and phagocytosis: Release from human peripheral polymorphonuclears and monocytes during the stimulation by ionophore A23187 and phagocytosis but not from degranulating basophils. *Immunology* 40:645-655.

Sanjar, S., Smith, D. and Kristersson, A. 1989. Incubation of platelets with PAF produces a factor which causes airway hyperreactivity in guinea-pigs. *Br. J. Pharmacol.* 96:75P.

Saunders, R.N., Van Valen, R.G., Klimek, J.J., Handley, D.A. and Winslow, C.M. 1983. Inhibition of the pulmonary inflammation pressure induced by platelet activating factor. *Thromb. Haemostas.* 50:1493.

Saxon, A. 1981. Immediate hypersensitivity. Appoach to diagnosis. In "Manual of Allergy and Immunology; Diagnosis and Therapy," eds., G.J. Lawlor and T.J. Fischer. Little, Brown and Co., Boston, p 15.

Schatz, M., Patterson, R., O'Rourke, J., Nickelsen, J. and Northup, C. 1975. The administration of radiographic contrast media to patients with a history of a previous reaction. *J. Allergy Clin. Immunol.* 55:358-366.

Schellenberg, R., Walker, B. and Snyder, F. 1983. Platelet-dependent contraction of human bronchi by platelet activating factor. *J. Allergy Clin. Immunol.* 71:A145.

Schinz, H.F., Baensch, W.E., Frommhold, W., Glauner, R., Uehlinger, E. and Wellauer, J. 1968. Roentgen Diagnosis. New York, Grune and Stratton, pp 206-219.

Schneid, C.R., Honeyman, T.W. and Fay, F.S. 1979. Mechanisms of *beta*-adrenergic relaxation of smooth muscle. *Nature* 277:32-35.

Schoning, B., Lorenz, W. and Doenicke, A. 1982. Prophylaxis of anaphylactoid reactions to a polypeptidal plasma substitute by H_1- plus H_2-receptor antagonists: synopsis of three randomized controlled trials. *Klin. Wochenschr.* 60:1048-1055.

Schulze, B. 1980. Serine proteases as mediators of radiographic contrast media toxicity. *Invest. Radiol.* 15:S18-S20.

Seidel, G., Groppe, G. and Meyer-Burgdorff, C. 1974. Contrast media as histamine liberators in man. *Agents Actions* 4:143-150.

Shaff, R.E. and Beaven, M.A. 1979. Increased sensitivity of the enzymatic isotopic assay of histamine: measurement of histamine in plasma and serum. *Anal. Biochem.* 94:425-430.

Shapiro, G.A., Loeb, P.M., Berk, R.N., Manton, J., Ellzey, B. and Reynolds, D. 1977. Influence of cholografin and renografin 76 on platelet function. *Radiology* 124:641-643.

Shehadi, W.H. 1975. Adverse reactions to intravascularly administered contrast media: a comprehensive study based on a prospective survey. *Am. J. Roentgenol.* 124:145-152.

Shehadi, W.H. 1982. Contrast media adverse reactions: occurrence, recurrence, and distribution patterns. *Radiology* 143:11-17.

Shehadi, W.H. and Toniolo, G. 1980. Adverse reactions to contrast media. *Radiology* 137:299-302.

Shen, T.Y., Hwang, S.B., Chang, M.N., Doebber, T.W., Lam, M.H., Wu, M.S., Wang, X., Han, G.G. and Li, R.Z. 1985. Characterisation of a platelet activating factor receptor antagonist isolated from haifenteng (*Piper futokadsura*): specific inhibition of *in vitro* and *in vivo* platelet activating factor-induced effects. *Proc. Natl. Acad. Sci. U.S.A.* 82:672-676.

Shephard, E.G., Malan, L., Macfarlane, C.M., Mouton, W. and Joubert, J.R. 1985. Lung function and plasma levels of thromboxane B_2, 6-keto prostaglandin F_{1alpha} and *beta*-thromboglobulin in antigen-induced asthma before and after indomethacin pretreatment. *Br. J. Clin. Pharmacol.* 19:459-470.

Shio, H. and Ramwell, P.W. 1972. Prostaglandin E_1 in platelet harvesting: an *in vitro* study. *Science* 175:536-538.

Shishido, M. and Katori, M. 1981. A quantitative method using continuous recording of platelet thrombus size in hamster cheek pouch. *Microvasc. Res.* 22:199-209.

Shojania, A.M. 1985. Immune-mediated thrombocytopenia due to an iodinated contrast medium (diatrizoate). *Can. Med. Assoc. J.* 133:123.

Shukla, S.D. and Hanahan, D.J. 1983. An early transient decrease in phosphatidylinositol 4,5-bisphosphate upon stimulation of rabbit platelets with acetylglycerylether phosphorylcholine (platelet activating factor). *Arch. Biochem. Biophys.* 227:626-629.

Siegle, R.L. and Lieberman, P. 1976. Measurement of histamine, complement components and immune complexes during patient reactions to iodinated contrast material. *Invest. Radiol.* 11:98-101.

Siegle, R.L., Lieberman, P., Jennings, B.R. and Rice, M.C. 1980. Iodinated contrast material: studies relating to complement activation, atopy, cellular association, and antigenicity. *Invest. Radiol.* 15:S13-S17.

Siegle, R.L., McGuire, W.L. and Peters, J.E. 1988. Development of polyclonal and monoclonal antibodies to iodinated contrast media. *Acta Radiol. Diagn.* 29:737-740.

Silver, M.J., Smith, J.B., Ingerman, C. and Kocsis, J.J. 1973. Arachidonic acid-induced human platelet aggregation and prostaglandin formation. *Prostaglandins* 4:863-875.

Simon, R.A., Schatz, M., Stevenson, D.D., Curry, N., Yamamoto, F., Plow, E., Ring, J. and Arroyave, C. 1979. Radiographic contrast media infusions. Measurement of histamine, complement, and fibrin split products and correlation with clinical parameters. *J. Allergy Clin. Immunol.* 63:281-288.

Siraganian, R. and Osler, A. 1969. Histamine release from sensitised rabbit leukocytes and associated platelet involvement. *J. Allergy* 43:167-175.

Small, P., Satin, R., Palayew, M.J. and Hyams, B. 1982. Prophylactic antihistamines in the management of radiographic contrast reactions. *Clin. Allergy* 12:289-294.

Smith. G.M. 1981. A study of intravascular platelet aggregation by continuous platelet counting. *Trends Pharmacol. Sci.* 2:105-107.

Smith, J.B. 1982. Effect of thromboxane synthetase inhibitors on platelet function: enhancement by inhibition of phosphodiesterase. *Thromb. Res.* 28:477-485.

Smith, G.M. and Freuler, F. 1973. The measurement of intravascular aggregation by continuous platelet counting. Bibl Anat 12 Karger, Basel pp. 229-234.

Smith, J.B., Ingerman, C., Kocsis, J.J. and Silver, M.J. 1973. Formation of prostaglandins during the aggregation of human blood platelets. *J. Clin. Invest.* 52:965-969.

Smith, D., Sanjar, S., Herd, C. and Morley, J. 1989*a*. *In vivo* method for the assessment of platelet accumulation. *J. Pharmacol. Meth.* 21:45-59.

Smith, D., Sanjar, S. and Morley, J. 1989*b*. Platelet activation and PAF-induced airway hyperreactivity in the anaesthetised guinea-pig. *Br. J. Pharmacol.* 96:74P.

Smith, J.B. and Willis, A.L. 1971. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature* 231:235-237.

Snyder, F. 1985. Chemical and biochemical aspects of platelet activating factor: A novel class of acetylated ether-linked choline phospholipids. *Med. Res. Rev.* 5:107-140.

Snyder, F. 1987. The significance of dual pathways for the biosynthesis of platelet activating factor: 1-alkyl-2-lyso-*sn*-glycero-3-phosphate as a branchpoint. In "New Horizons in Platelet Activating Research," eds C.M. Winslow and M.L. Lee. John Wiley & Sons, Ltd., London, pp 13-26.

Sobin, S., Frasher, W.G., Jacobsson, G. and Vanceck Loven, F.A. 1959. Nature of adverse reactions to radiopaque agents. *J. Am. Med. Assoc.* 170:1546-1547.

Solinger, A., Bernstein, I.L. and Glueck, H.I. 1973. The effect of epinephrine on platelet aggregation in normal and atopic subjects. *J. Allergy Clin. Immunol.* 51:29-34.

Spataro, R.F., Katzberg, R.W., Fischer, H.W. and McMannis, M.J. 1987. High-dose clinical urography with the low-osmolality contrast agent Hexabrix: comparison with a conventional contrast agent. *Radiology* 162:9-14.

Stanley, R.J. and Pfister, R.C. 1976. Bradycardia and hypotension following use of intravenous contrast media. *Radiology* 121:5-7.

Stewart, A.G. and Dusting, G.J. 1988. Characterization of receptors for platelet-activating factor on platelets, polymorphonuclear leukocytes and macophages. *Br. J. Pharmacol.* 94:1225-1233.

Stimler, N.P. and O'Flaherty, J.T. 1983. Spasmogenic properties of platelet-activating factor: evidence for a direct mechanism in the contractile response of pulmonary tissues. *Am. J. Pathol.* 113:75-84.

Storck, H., Hoigne, R. and Koller, F. 1955. Thrombocytes in allergic reactions. *Int. Arch. Allergy* 6:372-384.

Stormorken, H. 1988. Effects of contrast media on the hemostatic and thrombotic mechanisms. *Invest. Radiol.* 23:S318-S325.

Stormorken, H., Skalpe, I.O. and Testart, M.C. 1986. Effect of various contrast media on coagulation, fibrinolysis, and platelet function. An *in vitro* and *in vivo* study. *Invest. Radiol.* 21:348-354.

Strebel, L. and Siegler, P.E. 1968. Experience with clinical testing of dextran solutions. *Arch. Surg.* 96:471-475.

Sturzebecher C.-S. and Losert, W. 1987. Effects of iloprost on platelet activation *in vitro*. In "Prostacyclin and its Stable Analogue, Iloprost," eds. R.J. Gryglewski and G. Stock. Springer, Berlin, Heidleberg, p 39.

Svenson, R.H. 1984. Comparison of the hemodynamic effects of Hexabrix and Renografin-76 following left ventriculography and coronary arteriography. *Invest. Radiol.* 19:S333-S334.

Sweeney, M.J. and Klotz, S.D. 1983. Frequency of IgE mediated radio contrast dye reactions. *J. Allergy Clin. Immunol.* 71:147 (abstract).

Szczeklik, A., Milner, P.C., Birch, J., Watkins, J. and Martin, J.F. 1986. *Thromb. Haemostas.* 56:183-187.

Takizawa, H., Ishii, A., Suzuki, S., Shiga, J. and Miyamoto, T. 1988. Bronchoconstriction induced by platelet-activating factor in the guinea pig and its inhibition by CV-3988, a PAF antagonist: serial changes in findings of lung histology and bronchoalveolar lavage cell population. *Int. Arch. Allergy Appl. Immunol.* 86:375-382.

Tateson, J.E., Moncada, S. and Vane, J.R. 1977. Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins* 13:389-397.

Taytard, A., Guenard, H., Vuillemin, L., Bouvot, J.L., Vergeret, J., Ducassou, D., Piquet, Y. and Freour, P. 1986. Platelet kinetics in stable atopic asthma patients. *Am. Rev. Respir. Dis.* 134:983-985.

Taytard, A., Vuillemin, L., Guenard, H., Rio, P., Vergeret, J. and Ducassou, D. 1987. Kinetic of normal platelet in stable asthmatic patients. *Am. Rev. Respir. Dis.* 135:A388.

Tence, M., Polonsky, J., Le Couedic, J.P. and Benveniste, J. 1980. Release, purification and characterisation of platelet activating factor (PAF). *Biochimie* 62:251-259.

Terashita, Z., Tsushima, S., Yoshioka, Y., Nomura, H., Inada, Y. and Nishikawa, K. 1983. CV-3988, a specific antagonist of platelet activating factor (PAF). *Life Sci.* 32:1975-1982.

Thakur, M.L., Walsh, L., Malech, H.L. and Gottschalk, A. 1981. Indium-111-labeled platelets: improved method, efficacy and evaluation. *J. Nucl. Med.* 22:381-385.

Thakur, M.L., Welch, M.J., Joist, J.H. and Coleman, R.E. 1976. Indium-111 labeled platelets: studies on preparation and evaluation of *in vitro* and *in vivo* functions. *Thromb. Res.* 9:345-357.

Thiemermann, C., Lidbury, P., Thomas, R. and Vane, J.R. 1988. Endothelin exhibits *ex vivo* platelet aggregation in the rabbit. *Eur. J. Pharmacol.* 158:181-182.

Thompson, P.J., Hanson, J.M., Bilani, H., Turner-Warwick, M. and Morley, J. 1984. Platelets, platelet activating factor and asthma. *Am. Rev. Respir. Dis.* 129:A3.

Thompson, H.L., Leoutsakos, A. and Pearce, F.L. 1987. On the mechanism of dextran-induced histamine secretion from rat peritoneal mast cells. *Agents Actions* 20:169-171.

Tiffany, M.L. and Penner, J.A. 1981. Heparin and other sulfated polyanions and their interaction with the blood platelet. *Ann. N.Y. Acad. Sci.* 370:662-667.

Till, G., Rother, U. and Gemsa, D. 1978. Activation of complement by radiographic contrast media: generation of chemotactic and anaphylatoxin activities. *Int. Arch. Allergy Appl. Immunol.* 56:543-550.

Ting, S., Zweimann, B. and Lauker, R.M. 1983. Cromolyn does not modulate human allergic skin reactions *in vivo*. *J. Allergy Clin. Immunol.* 77:12-17.

Tofler, G.H., Brezinski, D., Schafer, A.I., Czeisler, C.A., Rutherford, J.D., Willich, S.N., Gleason, R.E., Williams, G.H. and Muller, J.E. 1987. Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N. Engl. J. Med.* 316:1514-1518.

Traietti, P., Marmaggi, S., Dardes, N., Moscatelli, B., Bologna, E. and Vulterini, S. 1984. Circulating platelet activation in respiratory diseases: differences between arterial and venous blood in COLD and asthmatic patients. *Respiration (Suppl.)* 46:62-63 (abstract).

Ts'ao, C.-H., Lo, R. and Raymond, J. 1976. Critical importance of citrate-blood ratio in platelet aggregation studies. *Am. J. Clin. Pathol.* 65:518-522.

Tuffin, D.P. and Wade, P.J. 1985. Calcium channel blocking drugs: a structural lead for PAF antagonists? *Prostaglandins* 30:702.

Ulevitch, R.J. and Cochrane, C.G. 1977. Complement-dependent hemodynamic and hematologic changes in the rabbit. *Inflammation* 2:199-216.

Valone, F.H. 1984. Isolation of a platelet membrane protein which binds the platelet-activating factor 1-O-hexadecyl-2-acetyl-SN-glycero-3-phosphorylcholine. *Immunology* 52:169-174.

Valone, F.H., Coles, E., Reinhold, V.R. and Goetzl, E.J. 1982. Specific binding of phospholipid platelet activating factor by human platelets. *J. Immunol.* 129:1637-1641.

Valone, F.H. and Goetzl, E.J. 1982. Specific binding by human polymorphonuclear leucocytes of the immunological mediator 1-*o*-hexadecyl/octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine. *Immunology* 48:141-149.

Vanhoutte, P.M. 1987. Airway epithelium and bronchial reactivity. *Can. J. Physiol. Pharmacol.* 65:448-450.

Vanhoutte, P. and Houston, D.S. 1985. Platelets, endothelium, and vasospasm. *Circulation* 72:728-734.

Vargaftig, B.B. 1978. The inhibition of cyclo-oxygenase of rabbit platelets by aspirin is prevented by salicylic acid and by phenanthrolines. *Eur. J. Pharmacol.* 50:231-241.

Vargaftig, B.B. and Lefort, J. 1979. Differential effects of prostacyclin and prostaglandin E1 on bronchoconstriction and thrombocytopenia during collagen and arachidonate infusions and anaphylactic shock in the guinea-pig. *Prostaglandins* 18:519-528.

Vargaftig, B.B., Chignard, M. and Benveniste, J. 1981*a*. Present concepts on the mechanisms of platelet aggregation. *Biochem. Pharmacol.* 30:263-271.

Vargaftig, B.B., Chignard, M., Benveniste, J., Lefort, J. and Wal., F. 1981*b*. Background and present status of research on platelet activating factor (Paf-acether). *Ann. N.Y. Acad. Sci.* 370:119-137.

Vargaftig, B.B., Lefort, J., Chignard, M. and Benveniste, J. 1980. Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur. J. Pharmacol.* 65:185-192.

Vargaftig, B.B., Lefort, J., Wal, F., Chignard, M. and Medeiros, M.C. 1982. Non-steroidal anti-inflammatory drugs if combined with anti-histamine and anti-serotonin agents interfere with the bronchial and platelet effects of "platelet-activating factor" (Paf-acether). *Eur. J. Pharmacol.* 82:121-130.

Verburg, K.M., Bowsher, R.R. and Henrey, D.P. 1983. A new radioenzymatic assay for histamine using purified histamine-N-methyltransferase. *Life. Sci.* 32:2855-2867.

Vermylen, J., Defreyn, G., Carreras, L.O., Machin, S.J., Van Schaeren, J. and Verstraete, M. 1981. Thromboxane synthetase inhibition as antithrombotic strategy. *Lancet* i:1073-1075.

Wahl, S.M., Hunt, D.A., Wakefield, L.M., McCartney-Francis, N., Wahl, L.M., Roberts, A.B. and Sporn, M.B. 1987. Transforming growth factor type *beta* induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. U.S.A.* 84:5788-5792.

Wakkers-Garritsen, B.G., Houwerziji, J., Nater, J.P. and Wakkers, P.J.M. 1976. IgE-mediated adverse reactivity to a radiographic contrast medium. *Ann. Allergy* 36:122-126.

Walker, A.C. and Carr, D.H. 1986. Reactions to radiographic contrast media: an attempt to detect specific anti-contrast medium antibodies in the sera of reactor patients. *Br. J. Radiol.* 59:531-536.

Walton, K.W. 1953. Observations on the effects of a series of dextran sulphates of varying molecular weight on the formed elements of the blood *in vitro*. *Br. J. Pharmacol.* 8:340-347.

Wanner, A. 1979. The role of mucociliary dysfunction in bronchial asthma. *Am. J. Med.* 67:477-485.

Ward, M.J., Fentem, P.H., Roderick Smith, W.H. and Davies, D. 1981. Ipratropium bromide in acute asthma. *Br. Med. J.* 282:598-600.

Wardlaw, A.J., Dunnette, S., Gleich, G.J., Collins, J.V. and Kay, A.B. 1988. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. *Am. Rev. Respir. Dis.* 137:62-69.

Wardlaw, A.J., Moqbel, R., Cromwell, O. and Kay, A.B. 1986. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest.* 78:1701-1706.

Wasserman, S.I. 1983. Mediators of immediate hypersensitivity. *J. Allergy Clin. Immunol.* 72:101-115.

Wasserman, S.I. and Ginsberg, M.H. 1984. Release of platelet factor 4 into the blood after cold challenge of patients with cold urticaria. *J. Allergy Clin. Immunol.* 74:275-279.

Watts, S.E., Tunbridge, L.J., Duncan, E M. and Lloyd, J.V. 1985. Storage of platelets for tests of platelet function: comparison of two methods of pH control. *Thromb. Res.* 37:73-83.

Watts, S.E., Tunbridge, L.J. and Lloyd, J.V. 1983. Storage of platelets for tests of platelet function: effects of pH on platelet aggregation and liberation of *beta*-thromboglobulin. *Thromb. Res.* 29:343-354.

Wedmore, C.V. and Williams, T.J. 1981. Platelet-activating factor (PAF), a secretory product of polymorphonuclear leucocytes, increases vascular permeability in rabbit skin. *Br. J. Pharmacol.* 74:916P-917P.

Wein, P., Handler, M. and Chadda, K.D. 1982. Severe thrombocytopenia as a result of contrast left ventricular angiography. *Cathet. Cardiovasc. Diagn.* 8:495-499.

Weiss, H.J., Aledort, L.M. and Kockwa, S. 1968. Effects of salicylates on haemostatic properties in man. *J. Clin. Invest.* 47:2169-2180.

Weiss, E.B., Markowicz, J. and Barbero, L. 1982. Effect of calcium antagonists in experimental asthma. *Allergy* 37:513-519.

Welton, A.F., Hope, W.C., Tobias, L.D. and Hamilton, J.G. 1981. Inhibition of antigen-induced histamine release and thromboxane synthase by FPL 55712, a specific SRA-A antagonist? *Biochem. Pharmacol.* 30:1378-1382.

West, C.E., Deuring, K., Schutte, J.B. and Terpstra, A.H.M. 1982. The effect of age on the development of hypercholesterolaemia in rabbits fed semi-purified diets containing casein. *J. Nutr.* 112:1287-1295.

Westaby, S., Dawson, P., Turner, M.W. and Pridie, R.B. 1985. Angiography and complement activation. Evidence for generation of C3a anaphylatoxin by intravascular contrast agents. *Cardiovasc. Res.* 19:85-88.

Wiggins, R.C. and Campbell, A.M. 1983. Inhibition of serotonin-mediated cardiovascular reflexes by cimetidine in the rabbit. *Gen. Pharmacol.* 14:469-470.

Wiggins, R.C., Glatfelter, A., Campbell, A.M., Kunkel, R.G. and Ulevitch, R.J. 1985. Acute hypotension due to platelet serotonin-induced chemoreflexes after intravenous injection of dextran sulfate in the rabbit. *Circ. Res.* 57:262-277.

Winton, F.R. and Bayliss, L.E. 1955. Human Physiology. London. J.A. Churchill Ltd. p 61.

Witten, D.M., Hirsch, F.D. and Hartman, G.W. 1973. Acute reactions to urographic contrast medium: incidence, clinical characteristics and relationships to history of hypersensitivity states. *Am. J. Roentgenol.* 119:832-840.

Woolf, N. 1981. Thrombosis and atherosclerosis. In "Haemostasis and thrombosis," eds., A.L. Bloom and D.P. Thomas. Churchill Livingstone, pp. 527-554.

Wu, K.K. and Hoak, J.C. 1974. A new method for the quantificative detection of platelet aggregates in patients with arterial insufficiency. *Lancet* ii:924-926.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. 1988. A novel, potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332:411-415.

Yasaka, T., Boxer, L.A. and Baehner, R.L. 1982. Monocyte aggregation and superoxide anion release in response to formyl-methionyl-leucyl-phenylalanine (FMLP) and platelet-activating factor (PAF). *J. Immunol.* 128:1939-1944.

Yen, S.S. and Morris, H.G. 1981. An imbalance of arachidonic acid metabolism in asthma. *Biochem. Biophys. Res. Commun.* 103:774-779.

Younger, R.E., Herrod, H.G., Lieberman, P.L., Trouy, R.L. and Crawford, L.V. 1986. Characteristics of diatrizoate-induced basophil histamine release. *J. Allergy Clin. Immunol.* 77:94-100.

Yu, S.K. and Latour, J.F. 1977. Potentiation by *alpha*- and inhibition by *beta*-adrenergic stimulations of rat platelet aggregation. A comparative study with human and rabbit platelets. *Thromb. Haemostas.* 37:413-422.

Zeman, R.K. 1977. Disseminated intravascular coagulation following intravenous pyelography. *Invest. Radiol.* 12:203-204.

Zir, L.M., Carvalho, A.C., Harthorne, J.W., Colman, R.W. and Lees, R.S. 1974. Effect of contrast agents on platelet aggregation and ^{14}C-serotonin release. *N. Engl. J. Med.* 291:134-135.

Zucker, M.B. 1980. The functioning of blood platelets. *Sci. Am.* 242:70-89.

Zucker, M.B. and Mauss, E.A. 1988. Erythrocyte aggregation in iohexol and other nonionic media. *Invest. Radiol.* 23:S340-S345.

Zweiman, B., Mishkin, M.M. and Hildreth, E.A. 1975. An approach to the performance of contrast studies in contrast material-reactive persons. *Ann. Int. Med.* 83:159-162.