PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Croonian Lecture, 1993: The Endothelium: Maestro of the Blood Circulation

J. R. Vane

Phil. Trans. R. Soc. Lond. B 1994 **343**, 225-246
doi: 10.1098/rstb.1994.0023

The Croonian Lecture, 1993. The endothelium: maestro of the blood circulation

J. R. VANE

The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.

CONTENTS

SUMMARY

The vascular endothelium plays a vital role in the control of the circulation. It metabolizes various vasoactive substances, coverts angiotensin I to angiotensin II and secretes the potent vasodilators prostacyclin and EDRF(NO) and the vasoconstrictor peptide endothelin-1. The balance between these mediators determines the responses of the cardiovascular system in diseases such as hypertension, atherosclerosis and myocardial infarction.

1. INTRODUCTION

Dr William Croone was an original Fellow of the Royal Society and the lectureship in his name was established (by his wife) in 1701 'for the advancement of natural knowledge and illustrative experiment on local motion'. The first Croonian Lecture was given by Dr Stuart on 'The motion of the heart'. It is particularly apposite, therefore, that my lecture should start with William Harvey, who discovered the circulation of the blood, and that I should go on to examine the ways in which the motions of the muscles of our arteries are locally controlled by the endothelium.

I shall describe some of the work I have been involved in over the past 40 years. In the process, I hope to show that it is not only Princes who travel the road to Serendip but also scientists. The application of simple methods, luck, happy coincidences, serendipity and noticing the unusual have all played an important role in my scientific career.

William Harvey died in 1657. Had he lived a few

Phil. Trans. R. Soc. Lond. B (1994) **343**, 225–246
Printed in Great Britain

years longer he would surely have been a Founding Fellow of the Royal Society. He was a medical student in Padua, where he would have watched candlelit dissections of the human cadaver by Fabricius in the famous lecture theatre now bearing his name.

Many other places, of course, also lay claim to William Harvey, including Cambridge where he received an M.D. in 1602, the College of Physicians where he became a Fellow in 1607, St Bartholomew's Hospital where he was a physician from 1609–1643 and Merton College, Oxford where he was Warden for a year from 1645. In founding a new Institute for Cardiovascular Research at St Bartholomew's Hospital Medical College in 1986, it was opportune to call it the William Harvey Research Institute.

In his writings, Harvey refers to work on many animal species (Harvey 1628) but one of his most famous experiments was on the heart *in situ* in a living snake. He chose a snake because as a cold blooded animal it had a slow heart beat and then proceeded to show that occluding the great vein led to draining of the heart whereas occlusion of the major artery led to engorgement. He said 'I am obliged to conclude that in animals the blood is driven round in a circuit with an unceasing circular sort of movement. . .'. Such a conclusion would have been impossible without the use of animals for experiments. It is no exaggeration to say that the whole of modern medicine, therapeutics and surgery is based on Harvey's discovery of the circulation of the blood. Now, in the 20th century, animal experiments are just as vital for making new discoveries about the heart and the circulation as they were then.

Anyone (like William Harvey) working on the living circulation would have observed that 'Blood continues fluid in the sound and living vessels, but it coagulates in dead ones. . .'. (Brücke 1857). We now know that this mysterious effect is due to the endothelial cells which, when living, keep the blood fluid.

These cells form a monolayer lining, like a carpet, in all of the blood vessels of our circulation. Initially, they were thought to act as a sort of dialysis membrane allowing nutrients from the blood stream to diffuse through to the underlying tissues without letting proteins or blood cells escape. Now, we know that these marvellously sophisticated cells are virtually in control of the blood circulation. They have a highly active metabolic function (Gryglewski *et al.* 1988) including taking up and metabolizing 5-hydroxytryptamine and inactivating PGE_1, PGE_2 and $PGF_{2\alpha}$. In addition, they have enzymes which inactivate bradykinin and which convert angiotensin I (an inactive decapeptide) into the very potent vasopressor agent, angiotensin II. Indeed it is the same enzyme which carries out both these functions. Manipulation of this enzyme led to the discovery of the angiotensin-converting enzyme (ACE) inhibitors, which are some of the most valuable drugs available today for treating hypertension and heart failure.

The story started in 1964, when Sergio Ferreira came from Brazil to my laboratories at the Royal College of Surgeons. In Brazil, he had studied under Rocha é Silva, the discoverer of bradykinin, another important peptide which Rocha é Silva had first isolated from the poisonous venom of the Brazilian snake, *Bothrops jararaca*. Working on the old principle that venoms sometimes contained not only noxious substances but also others which potentiate their effects, Ferreira isolated in 1965 from the venom of the same snake, a factor which he called bradykinin-potentiating factor (BPF) (Ferreira 1965). He came to us as a post-doctoral researcher, carrying some BPF in his pocket. At the time, we were working on the renin–angiotensin system and I suggested to Sergio that he should test his snake venom extracts on the two enzymes involved, renin and ACE. However, he had his own ideas and within a week or so he had persuaded me to work on bradykinin rather than he working on angiotensin! It was only two years later that another of my colleagues, Mick Bakhle, agreed to test the snake venom extract on a crude cell-free preparation of ACE and showed it to be a potent inhibitor (Bakhle 1971).

We followed this up on various bioassay preparations and also in the whole animal. We had already shown that the lungs contained high levels of ACE, using a simple perfused lung preparation in which the perfusate superfused a rat colon highly sensitive to the contractor activities of angiotensin (Bakhle *et al.* 1969). It was in this and similar preparations that we also showed that BPF inhibited the conversion of angiotensin I to angiotensin II (Ferreira *et al.* 1970).

At the time, I was a consultant to the drug company Squibb in New Jersey, U.S.A. and I went to them with the proposal that they should study this snake venom extract which we then knew to be a mixture of peptides. With the help of one of the purified peptides from it, I suggested that they could test the concept that angiotensin was important in high blood pressure and if it were, then they would have a starting point for a new therapy for high blood pressure. Indeed, in parallel work when he returned to Brazil (now convinced that ACE inhibition was important) Ferreira showed that BPF ablated the rise in blood pressure in cats which was caused by a massive release of renin when the blood supply to a kidney was restored after being clamped for 6 h (Krieger *et al.* 1971). It also worked in other experimental models of hypertension.

I visited Squibb three times a year and each time found that their initial enthusiasm for the project was waning, for their marketing people did not comprehend that proving a concept with an extract of a snake venom could possibly lead to a new drug. Peptides have to be injected rather than given orally and they emphatically reiterated that there was no market for an antihypertensive drug which had to be injected. They were in the business of selling drugs and not of proving concepts. Nevertheless, my two main scientific contacts at Squibb, the vice president in charge of research, Arnold Welch (with whom I spent my post-doc when he was a Professor of Pharmacology at Yale), and his deputy Chuck Smith remained enthusiastic; this eventually led to the synthesis of 1 kg of teprotide, the most active peptide from BPF. This indeed lowered blood pressure in hypertensive man,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

thus proving the concept that the production of angiotensin II contributed to high blood pressure and that ACE was an important new therapeutic target.

In 1973 I joined the Wellcome Foundation, another Pharmaceutical Company, as Director of Research and Development and so was no longer able to consult with Squibb. It was within the next two or three years that Ondetti and Cushman at Squibb solved the problem of changing a peptide inhibitor of ACE into a non-peptide inhibitor with oral activity (Ondetti *et al.* 1977). This eventually led to captopril, the first ACE inhibitor to be marketed as an antihypertensive drug. This has become a blockbuster drug of substantial importance, as have other ACE inhibitors since marketed by other companies. The total sales in 1991 around the world were more than $6B. Incidentally, but importantly, ACE inhibitors have also found other uses in man, such as in the treatment of congestive heart failure (Insel *et al.* 1989).

What a tortuous pathway this history involves! All of the positive elements of basic research – unexpected discoveries, unexpected outcomes, internationalism in science and fortuitous alliances – are there, as well as the now well-established fact that the marketing people in a drug company consistently fail to predict the usefulness of drugs (such as propranolol, cimetidine and captopril) that involve important new concepts, and because they cannot forecast commercial success, actively oppose their development.

Endothelial cells also generate various proteins like Von Willebrand's factor, tissue plasminogen activator, growth promoting factors and lipids such as platelet activating factor. An area of particular excitement to me is the generation of three potent vasoactive mediators, prostacyclin, endothelium-derived relaxing factor (EDRF) and endothelin. The first two relax blood vessels and cause vasodilatation, whereas the last one is a peptide, which potently contracts vascular smooth muscle.

I shall review each of these in turn, pointing out their functions and trying whenever possible to discuss their interactions.

2. PROSTACYCLIN

Prostacyclin is one member of the prostaglandin family of lipid mediators derived from arachidonic acid, 'the arachidonic acid cascade', of which there are now some ninety members. Some of these have been identified chemically but without as yet any known biological functions. The hydroxyeicosatetraenoic acids (HETEs) and all of the leukotrienes are made by other enzymes (the lipoxygenases) but cyclooxygenase provides the precursor (prostaglandin H_2) of the classical prostaglandins and their production is prevented by aspirin through inhibition of this enzyme (Vane 1971*a*) (figure 1).

In 1933, von Euler in Sweden, identified a smooth muscle-contracting and vasodepressor activity in seminal fluid as a lipid-soluble acid (von Euler 1934). Von Euler, who was later to share the Nobel prize for other seminal contributions in the field of the adrenergic nervous system, called the substance 'prostaglandin'

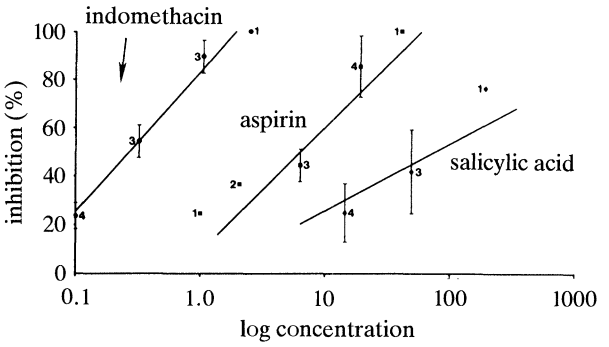

Figure 1. The inhibition of prostaglandin synthesis by aspirin-like drugs. Arachidonic acid was incubated with the supernatant from a guinea-pig lung homogenate in the absence of drug, or in the presence of various concentrations of non-steroidal anti-inflammatory drugs (concentration in $\mu g\ ml^{-1}$). Figures represent numbers of observations. (From Vane 1971*a*. Reprinted with permission from *Nature*. Copyright 1971 Macmillan Journals Limited.)

because he believed erroneously that it only came from the prostate gland.

More than 20 years were to pass before technical advances allowed the demonstration that 'prostaglandin' was in fact a family of lipid compounds of unique structure. Prostaglandin E_1 (PGE_1) and $PGF_{1\alpha}$ were isolated in crystalline form and structures were assigned in 1962 (Bergström & Samuelsson 1962). Soon, more prostaglandins were characterized and, like the others, proved to be 20-carbon unsaturated carboxylic acids with a cyclopentane ring. When the general structure of the prostaglandins became apparent, their kinship with essential fatty acids was recognized, and in 1964 Bergström and co-workers (Bergström *et al.* 1964) and van Dorp (van Dorp *et al.* 1964) and associates independently achieved the biosynthesis of PGE_2 from arachidonic acid using enzyme preparations from ram seminal vesicles. Because they are derived from 20-carbon essential fatty acids, the prostaglandins and related compounds are called eicosanoids. These essential fatty acids contain three, four or five double bonds: eicosatrienoic acid (dihomo-γ-linolenic acid), eicosatetraenoic acid (arachidonic acid) and eicosapentaenoic acid (EPA) leading to prostaglandins with one (PGE_1), two (PGE_2) or three (PGI_3) double bonds. In the 1960s there was a great interest in these potent acid lipids, especially PGE_1, PGE_2 and $PGF_{2\alpha}$ and we studied in some depth their pharmacology, especially their activity in causing blood vessels to dilate. We also helped to delineate their release from and inactivation by different organs of the body.

At the time, we were also working on the type of anaphylaxis or shock which underlies the pathology of asthma. This involved studying the chemical mediators or messengers responsible for causing the symptoms of asthma which are released during anaphylactic shock from lungs isolated from the guinea-pig. There were so many chemicals released that we had to increase our bioassay cascade to include six different tissues, each one selectively printing out in a dynamic way the release of one of these substances

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

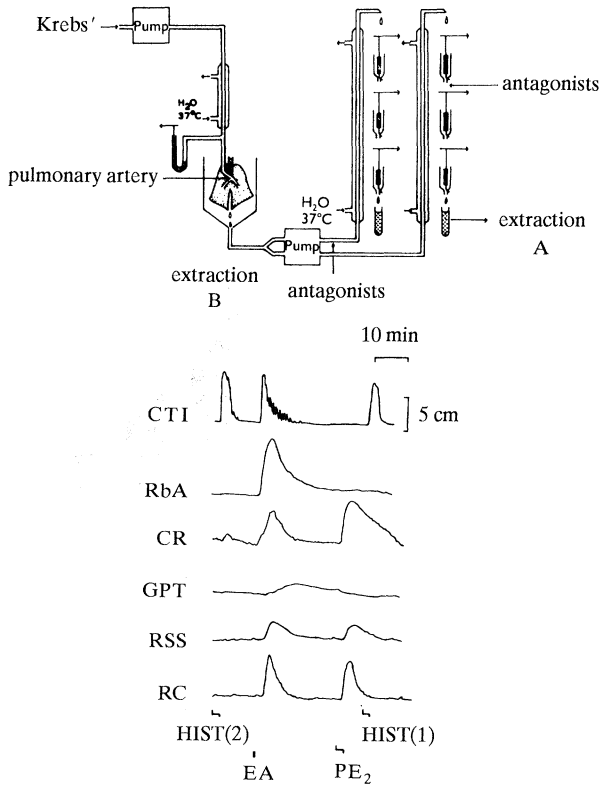

Figure 2. The detection of substances released by challenge (with egg albumen) of sensitized, perfused guinea-pig lungs. A cascade of six tissues was needed to detect the various substances released during anaphylactic shock. Histamine (HIST(2), 2 μg ml^{-1}; HIST(1), 1 μg ml^{-1}) and prostaglandin E_2 (PE_2, 50 ng ml^{-1}) were administered directly over the tissues, while egg albumen (EA, 10 mg) was given through the lungs (IA). Abbreviations used: CR, chick rectum; CTI, cat terminal ileum; GPT, segment of guinea-pig trachea; RbA, rabbit aortic strip; RC, rat colon; RSS, rat stomach strip. (From Vane 1971*b*. Reprinted from Ciba Foundation Study No. 38 with permission from the Ciba Foundation.)

from the lungs (Vane 1971*b*) (figure 2). We caused anaphylactic shock by injecting egg albumen, a protein to which the guinea-pig had previously been sensitized. We showed the release of different mediators, some of which were well known, such as histamine, and slow-reacting substances of anaphylaxis, characterized later in 1979 by Samuelsson as a mixture of lipoxygenase products of arachidonic acid (Samuelsson *et al.* 1979) which he re-named 'leukotrienes'. We also showed that prostaglandins E_2 and $F_{2\alpha}$ and a newly found substance which we called rabbit aorta contracting substance (RCS) were released during this pathological reaction. With Priscilla Piper, who was doing this work as a Ph.D. student, and had brought with her from Harry Collier's laboratory his interest in aspirin and similar drugs, we went on to show that mefenamate and other aspirin-like drugs selectively abolished the release of RCS (Piper & Vane 1969).

It was this work which led me directly to the idea that aspirin could inhibit the natural production of prostaglandins, thereby explaining its therapeutic effects. I well remember having this idea over the weekend while I was writing a review. On the Monday morning, I went into the laboratory and said to Priscilla Piper, Sergio Ferreira and others that I thought I knew how aspirin worked. This was before carrying out the actual experiment! On that Monday, I took the very tissue which had led to the concept – the guinea-pig lung – homogenized it and precipitated by centrifugation the cell debris, leaving a soup of the cell contents which I knew from the work of Änggård and Samuelsson would contain the enzyme that synthesizes prostaglandins from arachidonic acid (Änggård & Samuelsson 1965). With a little of this enzyme in different test tubes, I added the precursor arachidonic acid and then put in different doses of aspirin, salicylic acid, morphine or indomethacin. I included morphine in the protocol because it was an analgesic working by a different (central) mechanism. I measured prostaglandin formation by bioassay. By the end of that day I was convinced that aspirin (but not morphine) indeed inhibited the biosynthesis of the prostaglandins. After a further three weeks' work in which the experiment was repeated in several different ways, the evidence was compelling (figure 1).

Two new Ph.D. students then joined in this work: Rod Flower who had been with us previously as a technician before taking first class honours in Sheffield, and Salvador Moncada who came from Honduras to study with me for a Ph.D. They, together with Sergio Ferreria, over the next few years developed the concept into the now generally accepted theory that the mechanism of action of aspirin-like drugs is through inhibition of prostaglandin biosynthesis (Ferreira *et al.* 1971; Gryglewski *et al.* 1972). Clearly, this research on the biochemical events which explain the way in which aspirin works has had enormous implications. New aspirin-like drugs have been discovered and marketed and new uses for aspirin have been developed, including its use to prevent heart attacks and strokes (Meade 1992).

We now know that there are at least two forms of the cyclo-oxygenase (COX) enzyme which synthesizes prostaglandins, a constitutive enzyme (COX-1) and a structurally different enzyme (COX-2) which is induced by inflammatory stimuli (Xie *et al.* 1991; Kujubu & Herschman 1992). This important discovery offers an explanation for some of the few remaining puzzles associated with the theory of the mode of action of aspirin-like drugs and at the same time points to exciting new therapeutic targets. It should now be possible to design an aspirin-like anti-inflammatory drug (anti-COX-2) which does not have any irritant effects on the stomach (anti-COX-1). Indeed, many drug companies are re-examining their library of compounds to see whether they may already have one.

(*a*) *Discovery of prostacyclin*

We discovered prostacyclin in 1976, while investigating how the blood vessel walls made unstable prostanoids like thromboxane A_2 (Moncada *et al.* 1976*a*). Earlier, as I have mentioned, we had discovered a substance which we called rabbit aorta-contracting substance and in 1975 Bengt Samuelsson

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

characterized this chemically (Hamberg *et al.* 1975) and re-named it 'thromboxane A_2'. It was in looking for the enzyme that generates thromboxane A_2 from arachidonic acid that we came across, by serendipity, yet another previously unknown substance.

Thromboxane A_2 is generated by platelets and is a powerful vasoconstrictor substance as well as causing platelets to clump together. By this time, I had moved to Wellcome and Salvador Moncada had rejoined us as head of our prostaglandin research group. We began a search, using our classic techniques of bioassay, for tissues other than platelets that could make thromboxane A_2. We took extracts from kidney, lung, spleen and aorta and incubated them *in vitro* with the prostaglandin precursors to find out (by bioassay) whether the extracts made thromboxane A_2. Kidney and lung did not generate RCS or thromboxane A_2 but did make prostaglandins, whereas the spleen made both. However, the aorta did not seem to make either, although the precursor disappeared. This meant either that the tissue simply destroyed the precursor or, as it turned out, that our bioassay techniques were not picking up the active product. When we included other bioassay tissues such as the rabbit coeliac artery (figure 3), we found that the aortic extract was indeed manufacturing a dilator substance which did not correspond to anything then recognized. Very soon afterwards, we also found that PGX, as we called it, inhibited the clumping of platelets. This work led to the identification in 1976 of another prostaglandin. We re-named it 'prostacyclin' and, in collaboration with the Upjohn Company in Kalamazoo (who were experts in prostaglandin chemistry) we characterized its chemical structure and synthesized it.

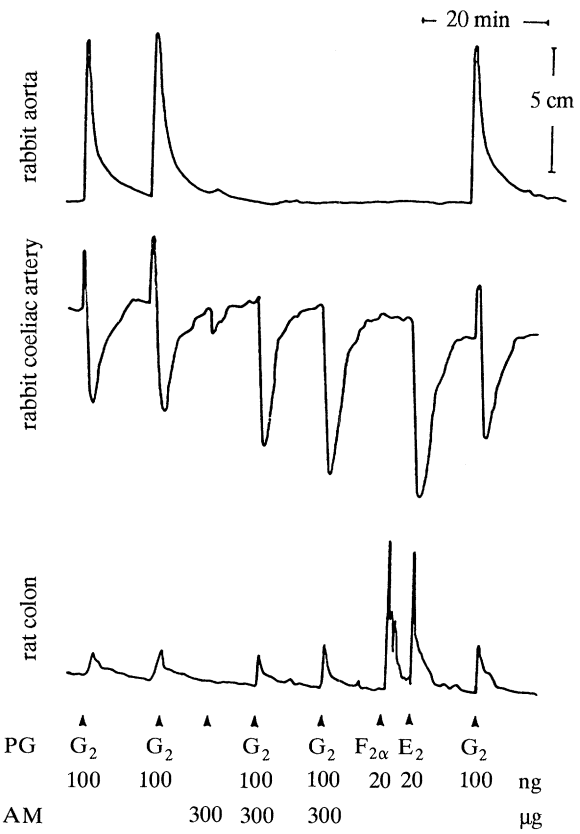

Figure 3. The synthesis of PGX (later named PGI_2 or prostacyclin) by aortic microsomes. The prostaglandin precursor PGG_2 (G_2) contracted rabbit aorta, caused a brief contraction followed by a relaxation of the coeliac artery and gave a small contraction of the rat colon. In contrast, when PGG_2 was incubated for 1 min at 22°C with pig aortic microsomes (AM) a novel product was formed (PGI_2) which did not contract the rabbit aorta or the coeliac artery but caused a profound relaxation of the rabbit coeliac artery. (From Bunting *et al.* 1976. Reprinted with permission from *Prostaglandins*. Copyright 1976, Butterworth-Heinemann.)

(*b*) *The formation and properties of prostacyclin*

Prostacyclin is the main product of arachidonic acid in all vascular tissues so far tested including those of man (Bunting *et al.* 1976) (figure 3). The ability of the large vessel wall to synthesize prostacyclin is greatest at the intimal surface and progressively decreases toward the adventitia (Moncada *et al.* 1977). Culture of cells from vessel walls also shows that endothelial cells are the most active producers of prostacyclin (Weksler *et al.* 1977; MacIntyre *et al.* 1978), but the underlying smooth muscle cells can also make it.

Prostacyclin relaxes isolated vascular strips and is a strong hypotensive agent through vasodilatation of all vascular beds studied, including the pulmonary and cerebral circulations (for a review, see Moncada & Vane 1979). Several authors have suggested that prostacyclin generation participates in, or accounts for, functional hyperaemia of the stomach mucosa (Whittle 1980*a*) and of adipose tissue (Axelrod & Levine 1981).

Prostacyclin is the most potent endogenous inhibitor of platelet aggregation yet discovered. This effect is short-lasting *in vivo*, disappearing within 30 min of cessation of intravenous administration. Prostacyclin not only prevents platelets from sticking together but also disperses existing aggregates *in vitro* (Moncada *et al.* 1976*a*; Ubatuba *et al.* 1979) and in the circulation of man (Szczeklik *et al.* 1978). Moreover, it inhibits thrombus formation in models such as the electrically damaged carotid artery of the rabbit (Ubatuba *et al.* 1979) or the constricted coronary artery of the dog (Aiken *et al.* 1979), protects against sudden death (due to platelet clumping in the lungs) induced by intravenous arachidonic acid in rabbits (Bayer *et al.* 1979), and inhibits platelet aggregation in pial venules of the mouse when applied locally (Rosenblum & El Sabban 1979).

Prostacyclin inhibits platelet aggregation by stimulating receptors on the cell surface which in turn activate the intracellular enzyme adenylate cyclase, leading to an increase in cAMP levels in the platelets (Gorman *et al.* 1977; Tateson *et al.* 1977). In this respect prostacyclin is much more potent than either PGE_1 or PGD_2 and its effect is longer-lasting. In contrast to TXA_2, prostacyclin enhances Ca^{2+} sequestration in platelet membranes (Kaser-Glanzmann *et al.* 1977). Moreover, inhibitory effects on platelet phospholipase (Lapetina *et al.* 1977; Minkes *et al.* 1977)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

and platelet cyclo-oxygenase (Malmsten *et al.* 1976) have been described. All of these effects are related to its ability to increase cAMP in platelets. Prostacyclin, by inhibiting several steps in the activation of the arachidonic acid cascade, exerts an overall control of platelet aggregability.

One of the functional characteristics of the intact vascular endothelium is its non-reactivity to platelets. As will be developed later, the generation of both prostacyclin and nitric oxide contribute to this thromboresistance. Prostacyclin inhibits platelet aggregation (platelet–platelet interaction) at much lower concentrations than those needed to inhibit adhesion (Higgs *et al.* 1978) (platelet-collagen interaction). Thus, prostacyclin may permit platelets to stick to and interact with damaged vascular tissue, so allowing platelets to participate in the repair of a vessel wall while at the same time preventing or limiting thrombus formation.

(*c*) *Prostacyclin and cytoprotection*

In addition to its well-known vasodilator and anti-aggregating actions, prostacyclin shares with some other prostaglandins a 'cytoprotective activity', as yet not clearly understood. This activity has usually been studied on ulcer formation (Whittle 1980*b*) in the rat stomach. We have suggested (Moncada & Vane 1982; Vane 1983) that this third property may be important in explaining certain therapeutic effects of prostacyclin. For instance, in models of myocardial infarction, prostacyclin reduces infarct size (Jugdutt *et al.* 1979; Ogletree *et al.* 1979; Ribeiro *et al.* 1981), arrhythmias (Starnes *et al.* 1982), oxygen demand (Ribeiro *et al.* 1981) and enzyme release from the infarcted areas (Ohlendorf *et al.* 1980). In sheep, prostacyclin protected the lungs against injury induced by endotoxin (Demling *et al.* 1981). There was also a beneficial effect in endotoxin shock in the dog (Fletcher & Ramwell 1980) and cat (Lefer *et al.* 1980) where prostacyclin improves splanchnic blood flow and reduces the formation and release of lysosomal hydrolases. The effects of hypoxic damage in the cat isolated perfused liver are also substantially reduced by prostacyclin (Araki & Lefer 1980). Canine livers can be preserved *ex vivo* for up to 48 h and then successfully transplanted using a combination of refrigeration, Sacks' solution and prostacyclin (Monden & Fortner 1982).

All these effects can be related to an observation made by Moncada and his colleagues. The addition of prostacyclin to platelets during their separation from blood and subsequent washing substantially improves their immediate functionality *in vitro*. Furthermore, whereas platelets are normally functional for about 6 h, when prepared with the addition of prostacyclin they remain functional for some 72 h (Moncada *et al.* 1982). This extended viability of platelets *in vitro* is not accompanied by a prolonged increase in levels of cAMP (Blackwell *et al.* 1982), although it may be a consequence of the initial increase.

All these results suggest that some of the therapeutic effects of prostacyclin are related to this cytoprotective effect and point to even wider indications for prostacyclin in cell or tissue preservation *in vivo* and *in vitro*.

(*d*) *Prostacyclin and atherosclerosis*

Lipid peroxides, such as 15-hydroperoxy arachidonic acid (15-HPAA), are potent and selective inhibitors of prostacyclin generation by vessel wall microsomes or by fresh vascular tissue (Bunting *et al.* 1976; Gryglewski *et al.* 1976; Moncada *et al.* 1976*b*; Salmon *et al.* 1978). There are high concentrations of lipid peroxides in atherosclerotic lesions (Glavind *et al.* 1952). Lipid peroxidation induced by free radical formation occurs in vitamin E deficiency, the ageing process and in hyperlipidaemia accompanying atherosclerosis (Slater 1972). Accumulation of lipid peroxides in atheromatous plaques could predispose to thrombus formation by inhibiting generation of prostacyclin by the vessel wall without reducing TXA_2 production by platelets. Moreover, platelet aggregation is induced by 15-HPAA and this aggregation is not inhibited by adenosine or PGE_1 (Mickel & Horbar 1974).

Human atheromatous plaques do not produce prostacyclin (D'Angelo *et al.* 1978; Sinzinger *et al.* 1979). In normal rabbits the production of prostacyclin by the luminal surface of the aorta is abolished by de-endothelialization and slowly recovers with re-endothelialization over a period of about 70 days. However, the recovery of prostacyclin formation did not occur in rabbits made moderately hypercholesterolaemic by diet (Eldor *et al.* 1982). These results suggest that it would be worth exploring whether attempts to reduce lipid peroxide formation by inhibiting peroxidation influence the development of atherosclerosis and arterial thrombosis. Vitamin E acts as an antioxidant and perhaps its empirical use in arterial disease in the past (Marks 1962; Boyd & Marks 1963; Haeger 1968) had, in fact, a biochemical rationale (Ferns *et al.* 1993).

The initiation of atherosclerosis depends upon injury to the endothelium and activation of several different cell types. Platelets adhere to the injured endothelium and monocytes, which invade the sub-endothelium, accumulate lipid and become macrophage-derived foam cells (Ross 1986). Each of these cell types releases a growth factor, probably of the same type, which acts on the underlying smooth muscle and causes proliferation of the smooth muscle cells which also take up lipid (Raines & Ross 1993).

About eight years ago, both Willis (Willis *et al.* 1987) and Hajjar (Hajjar 1985) showed that prostacyclin or one of its stable analogues prevented the release of these growth factors and inhibited the uptake of cholesterol esters into macrophages or into smooth muscle cells. This could therefore be an important function for the prostacyclin generated by the endothelial cells of the blood vessel wall. Blood pressure is not altered by administration of aspirin or indomethacin which prevent prostacyclin production, so it is unlikely that prostacyclin is contributing to the maintenance of a normal blood pressure by causing a constant vasodilatation. It must be generated by the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

endothelium for some other purpose; perhaps to stop platelets from sticking, and perhaps as a natural protection against the formation of atherosclerotic plaques.

(*e*) *Clinical applications of prostacyclin*

Prostacyclin is available as a stable freeze-dried preparation (Epoprostenol) for administration to man. It is supplied together with an alkaline buffer which allows a solution to be infused for several hours. Intravenous infusion of prostacyclin in healthy volunteers leads to a dose-related inhibition of platelet aggregation, dispersal of circulating platelet aggregates, arteriolar vasodilatation, increases in skin temperature, facial flushing (Moncada 1982; Vane 1982) and sometimes headache. Infusion of prostacyclin into patients susceptible to migraine or cluster headache induces, in most cases, a headache different from those usually experienced (Peatfield *et al.* 1981).

Extracorporeal circulation of blood brings it into contact with artificial surfaces which cannot generate prostacyclin. In the course of such procedures thrombocytopaenia and loss of platelet haemostatic function occur and make an important contribution to the bleeding problems sometimes seen, for instance, following charcoal haemoperfusion or prolonged cardiopulmonary bypass in man. Formation of microemboli during cardiopulmonary bypass may also contribute to the cerebral complications which sometimes follow this procedure. Platelet damage and thrombocytopaenia were prevented by prostacyclin both in animal models of extracorpeal circulation (Moncada 1982; Vane 1982) and in man.

Several double blind clinical trials of prostacyclin in cardiopulmonary bypass have been published (Noback *et al.* 1980; Bennett *et al.* 1981; Bunting *et al.* 1981; Chelly *et al.* 1981; Longmore *et al.* 1981; Radegran *et al.* 1981; Walker *et al.* 1981). The treatment groups showed a preservation of platelet number and function, with a reduction in the blood loss in the first 18 h after operation. In the trial by Longmore and colleagues (Longmore *et al.* 1981) the blood loss was halved. In that by Walker and coworkers (Walker *et al.* 1981), filters were used and the formation of many platelet aggregates on the filters from the placebo group contrasted strikingly with the lack of platelet adhesion to those from patients treated with prostacyclin. The heparin-sparing effect of prostacyclin was confirmed and the vasodilator effects were not troublesome; indeed, Nobak and colleagues (Noback *et al.* 1980) suggest that these effects may be utilized in controlling intra-bypass hypertension. However, perhaps because of the associated fall in perfusion pressure, prostacyclin or its analogues have not gained widespread use in extracorporeal circulations.

When I was at Wellcome, we considered the various diseases in which prostacyclin production may be reduced and where the exogenous application of prostacyclin itself or an analogue might be useful therapeutically. For example, in primary pulmonary hypertension, Higenbottam and his colleagues at Papworth Hospital (Higenbottam 1987) have shown that there is a lowering of pulmonary blood pressure by prostacyclin which is sustained for the duration of the infusion. Patients with this severe disease are normally bedridden, but when prostacyclin was infused continuously intravenously from a pump attached to their belts, the pulmonary blood pressure fell and patients were able to live a fairly active life, until such time (sometimes after two years) as a heartlung transplant was available to treat them rather more permanently (Long & Rubin 1987). Interestingly, there was no tolerance to the action of prostacyclin, and when the infusion was stopped to refill the pump, the pulmonary blood pressure shot up again, showing that this was a very evanescent effect; it depended on the presence of prostacyclin in the bloodstream.

Gryglewski and his colleagues in Cracow (Szczeklik *et al.* 1979) were the first to demonstrate the beneficial effects of infusions of prostacyclin in ischaemic disease of the legs. Szczeklik et al reported striking and prolonged benefits following intra-arterial infusion of prostacyclin in five patients with advanced atherosclerotic lower limb peripheral vascular disease (PVD). Rest pain disappeared, previously refactory ulcers healed and the muscle blood flow, as measured by $Xenon^{133}$ clearance, was significantly increased for at least 6 weeks after prostacyclin infusion. This group later reported on 55 patients with advanced peripheral arterial disease of the lower extremities (Szczeklik & Gryglewski 1981). In summary, 42% of patients treated showed a persistent, long-lasting improvement. In another 40% of patients the improvement lasted no longer than 2 months, while in the remaining 18% of patients the results were virtually negative. The authors believe that these figures do not represent the limit of efficacy of prostacyclin therapy in advanced peripheral vascular disease and that even more successful treatment will ensue when the mechanism of action of prostacyclin is better understood.

The largest double-blind placebo-controlled study of prostacyclin in patients with peripheral vascular disease was performed by Virgolini *et al.* (1990). One hundred and eight patients were randomly assigned to receive either prostacyclin ($6\ ng\ kg^{-1}\ min^{-1}$ over 8 h daily for five consecutive days) or placebo infusions (Virgolini *et al.* 1990). Prostacyclin treatment resulted in a significant increase of absolute and relative walking times which persisted for up to 6 months in some patients. Following a second infusion period in an open trial the number of positive responder patients was further increased (Virgolini *et al.* 1991).

Prostacyclin also induces significant and long-lasting improvements in Raynaud's phenomenon. Intravenous infusion of the drug for 72 h at the maximum tolerated dose (up to $10\ ng\ kg^{-1}\ min^{-1}$) produced striking reductions in the frequency, duration and severity of the disease in 21 of 24 patients. In all patients who responded, the improvement lasted for weeks (mean 9–10 weeks) and in three patients, subjective improvement was still seen 6 months after the infusion. Pain relief was a striking feature, presum-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ably associated with the increased blood flow indicated by increased temperature of the hands and fingers (Dowd *et al.* 1982). Although this was an open trial, the author's previous experience suggested that there was no placebo response to saline infusion in similar patients. Belch *et al.* (1981) have also reported successful treatment in four out of five patients and a blind clinical trial (Belch *et al.* 1983) has confirmed these results in Raynaud's phenomenon, the improvements again outlasting the infusions of prostacyclin by 6 weeks or more.

In the second half of the 1980s iloprost became available and, because it was stable and easier to use, the clinical trialists turned their attention to this analogue. The activity of iloprost is identical to that of prostacyclin and the two substances can be discussed together.

Beneficial effects of intravenous infusion of prostacyclin were obtained in nine patients with severe congestive heart failure (CHF) refractory to digitalis and diuretics (Yui *et al.* 1982). A recent pilot study in 33 patients with severe refractory CHF compared the effects of prostacyclin administered chronically by a small portable infusion pump for 12 weeks in addition to conventional therapy with the effects of conventional therapy alone. Patients who received prostacyclin in addition to their usual drugs experienced improved haemodynamics and reduced mortality. In a 6 min walk test performed 12 weeks after the start of therapy, patients who received prostacyclin walked further than patients who received conventional therapy alone (Gheorghiade *et al.* 1992). However, larger controlled trials of chronic prostacyclin therapy on survival of patients with severe, refactory CHF were stopped, for they failed to confirm the earlier promise.

The clinical use of prostacyclin has been bedevilled by two factors. First, because its mode of action in PVD is not understood, the correct dosage has been difficult to set. In the event, the maximum tolerated dose (close to that causing flushing etc) has been used and this may well be too high. Secondly, it is an unstable substance and although it has been stabilized pharmaceutically, care has to be taken by the clinician in its use.

Clearly, there are many clinical conditions which may respond to prostacyclin treatment and its place in therapeutics (and that of chemically stable analogues) is still being defined some 17 years after its discovery.

Sadly, within Wellcome, prostacyclin became a political football because of its close association with me, and the internecine strife that was generated inhibited and almost blocked its full development: even so, it earns a few million pounds a year. The overall lack of vision of the marketing advisors in a pharmaceutical company was once more demonstrated, for they could not analyse effectively the potential market. Even the medical division failed to understand the importance of the discovery and based their opinion of its possible efficacy on the vasodilator properties rather than on the anti-platelet and cytoprotective effects. 'We know vasodilators are ineffective in peripheral vascular disease,' they said. However, other drug companies, including several in Japan and Schering in Germany, picked up the baton. Schering have now developed the stable analogue, iloprost, which is about to be marketed for the treatment of obstructive diseases of the circulation. In a retrospective comparison of iloprost with other treatments in Raynaud's phenomenon, 24 of 48 patients who had not responded to any previous treatment found iloprost to be of benefit (Watson & Belcher 1991). A further double blind trial showed that a low dose of iloprost ($0.5\ ng\ kg^{-1}\ min^{-1}$) was as effective as the standard dose ($2\ ng\ kg^{-1}\ min^{-1}$) in reducing the severity of Raynaud's phenomenon and encouraging ulcer healing with fewer side effects (Torley *et al.* 1991).

As with the first prototype ACE inhibitors, orally active compounds are also on their way. Again, the timescale is inordinately protracted with two decades passing between discovery and the marketing of a compound active by mouth. Several companies have produced prostacyclin analogues which display oral activity; indeed, one (beraprost) is on the market in Japan (Sakaguchi *et al.* 1990*a*,*b*) and another (cicaprost) is being developed in Germany for its potential anti-cancer activity (Schirner & Schneider 1992). The availability of a daily tablet which would slow down or even prevent the atherosclerotic process would make a valuable contribution to medicine.

3. ENDOTHELIUM-DERIVED RELAXING FACTOR/NITRIC OXIDE

Endothelium-derived relaxing factor is another unstable vasodilator made by the endothelial cells. Its capricious effects were noted over the last century without any scientific understanding until 1980.

In 1914, Dale found that acetylcholine dilated blood vessels (Dale 1914), whereas Reid Hunt noted that it caused constriction (Hunt 1915). Then Burn and Robinson in 1951 found a biphasic action in the rabbit ear (Burn & Robinson 1951). When they injected acetylcholine repeatedly into the circulation of the perfused rabbit ear it initially caused a dilatation, but over a period of a few hours the reaction gradually changed into a constriction. Burn put forward several hypotheses to explain this biphasic activity of acetylcholine, but the actual reason came with Bob Furchgott's work in 1980 in the U.S.A. For many years, workers in his laboratory had been using strips of helically cut rabbit aorta and had consistently obtained contractions with acetylcholine. When they started to use aortic rings, they sometimes found that acetylcholine caused relaxation. This result was traced to the fact that the rings which relaxed had intact endothelial cells, whereas the helical strips, by virtue of the method of preparation, did not. Thus, the relaxation being seen with acetylcholine was due to a dilating substance released from the endothelial cells. Furchgott called this substance Endothelium Derived Relaxing Factor or EDRF. Now it was possible to explain Dale and Reid Hunt's results: presumably, Dale was a better experimentalist than Reid Hunt and preserved his endothelial cells. One could also explain Burn and Robinson's results because after three or

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

four hours the endothelial cells of the rabbit ear became so damaged that they would no longer make EDRF, the obligatory relaxing factor which transmits the effects of acetylcholine to the underlying smooth muscle.

Furchgott concluded that EDRF is released from the endothelial cells by acetylcholine and causes vasodilatation. The results of Furchgott and Zawadzki's work were published in 1980 (Furchgott & Zawadzki 1980) and many research laboratories joined in the hunt to see whether it was an amine, a peptide or a prostaglandin, all without success. Over the next ten years, many other workers joined the field and found that EDRF was released by a whole variety of mediators (including bradykinin, histamine and ADP) (Toda 1984) and by pulsatile pressure (Lamontagne *et al.* 1991). Interestingly, largely the same mediators release prostacyclin as EDRF (De Nucci *et al.* 1988).

(*a*) *Properties of EDRF*

EDRF has a half life of (depending on how it is measured) 4 to 50 s, which is even shorter than that of prostacyclin (Gryglewski *et al.* 1986). Like prostacyclin, it is a powerful vasodilator and inhibits platelet aggregation (Azuma *et al.* 1986). Unlike prostacyclin, it also inhibits platelet adhesion (Radomski *et al.* 1987*a*; Sneddon & Vane 1988). As mentioned earlier, platelet aggregation is when platelets stick to each other whereas adhesion is when they stick to something else, like collagen in the blood vessel wall, or glass.

In 1985, Furchgott found that EDRF stimulates the production of the cyclic nucleotide, cyclic GMP (Martin *et al.* 1985). It had been thought that cyclic AMP and cyclic GMP were part of a Ying Yang system having opposite effects inside cells. At this stage, there was yet another dramatic amalgamation of two hitherto separate fields. For more than 100 years substances like amyl nitrite and nitroglycerin have been used to cause a widening of the arteries in conditions such as angina. Indeed, Alfred Nobel who made his fortune out of dynamite (which stabilized glyceryl trinitrate by mixing it with kieselguhr, rather like a pharmaceutical preparation) wrote to a friend saying 'It sounds like the irony of fate that I should be ordered by my doctor to take nitroglycerin internally'. Work in the 1970s showed that this type of nitrate was converted in muscle cells to the simple molecule nitric oxide (NO) and it was this substance that brought about the relaxation of arterial and venous muscle (Katsuki *et al.* 1977). Furchgott knew that the nitrovasodilators, exert their vasodilator effects because they are metabolized to nitric oxide, which acts intracellularly on the enzyme guanylate cyclase to increase the concentrations of cyclic GMP in smooth muscle cells.

This and other similarities between EDRF and the end product of the nitrovasodilators led Furchgott (Khan & Furchgott 1987) and Ignarro (Ignarro *et al.* 1987) independently to propose at a meeting in 1986 that EDRF may be nitric oxide: a heretical proposal, because nitrates or nitric oxide were not thought to be normally generated in animals or man. My colleague of long standing, Salvador Moncada, who is now the Director of Research at Wellcome, returned from that meeting and sought a means of measuring nitric oxide. The only method which was available then was a chemiluminescence one used to measure nitric oxide in the exhaust gases of cars or as a pollutant in the atmosphere. Moncada adapted this machine and showed convincingly that nitric oxide accounted for the activity of EDRF (Palmer *et al.* 1987). Subsequently, there has been a lot of debate as to whether there is more than one EDRF or whether EDRF is a nitrosothiol (Myers *et al.* 1990), but the evidence that nitric oxide is a physiological mediator is now compelling.

(*b*) *Identification of EDRF*

Moncada's evidence for the identity of EDRF (Palmer *et al.* 1987) was obtained by growing endothelial cells on very small beads. At confluence, there were about 150 endothelial cells on each bead and about 1–3 ml of these beads covered with $2–5 \times 10^7$ endothelial cells were packed into a small water-jacketed (37°C) column. The column was perfused with Krebs' solution and the endothelial cells stimulated to release EDRF with bradykinin, ADP or another agonist. Using a cascade bioassay system they showed that the EDRF in the perfusate from the column produced the same characteristic pattern of responses as nitric oxide on the individual tissues of the cascade. With another sample of the perfusate, the nitric oxide was measured by chemiluminescence, and there was enough nitric oxide being released from the endothelial cells to account for the vasodilator activity of EDRF (Palmer *et al.* 1987).

Over the past fifty years pharmacologists have discovered many mediators; amines such as histamine, acetylcholine and catecholamines; peptides like bradykinin and angiotensin II and lipids like prostaglandins. In 1987, a different and very potent mediator of vascular relaxation and inhibition of platelet aggregation was identified as the simple chemical, nitric oxide, composed of the two main elements of the air. It is the simplest messenger of them all and as a gas, even when dissolved in body fluids, it will diffuse freely through cell membranes. When nitric oxide was first proposed as the mediator accounting for the activities of EDRF, people said that nitric oxide was only made in the atmosphere by thunder and lightning, so how can the body actually produce it?

(*c*) *Synthesis of EDRF*

It is fascinating that in the attempts to identify the synthetic pathway for nitric oxide, the clue came from yet another field of knowledge. The American scientists, Hibbs and Marletta (Hibbs *et al.* 1987; Iyengar *et al.* 1987) were investigating the effects of the amino acid, L-arginine on isolated activated macrophages. They found that these cells lost one of their important functions, their ability to kill other cells if they were starved of L-arginine. They also found that the macrophages made nitrite (NO_2^-) and nitrate (NO_3^-)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

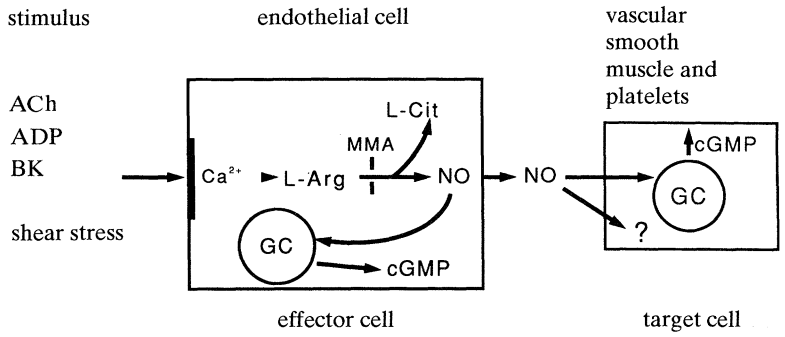

Figure 4. Release of endothelium-derived relaxing factor (nitric oxide). Receptors on the endothelial cell are stimulated by acetylcholine (Ach), adenosine diphosphate (ADP), bradykinin (BK) or shear stress which raise intracellular calcium. This activates enzymatic conversion of L-arginine (L-Arg) to nitric oxide (NO) and L-citrulline (L-Cit). Nitric oxide activates guanylate cyclase (GC) in the target cells or in the same cell and cyclic GMP (cGMP) concentrations increase. Nitric oxide synthase is inhibited by N^G-monomethyl-L-arginine (MMA).

from the guanidino group of L-arginine. The enzyme performing this function was inhibited by the simple substrate analogue, N^G-monomethyl-L-arginine. However, nitrite and nitrate did not kill cells and so the actual cytotoxicity may have been due to an unstable intermediate. For those working with the arachidonic acid cascade, it was a fairly common experience to find stable, inactive products (6-keto-$PGF_{1\alpha}$ from prostacyclin or thromboxane B_2 from thromboxane A_2) which signalled the presence of an unstable but active intermediate. The publication of Hibbs work, then, was followed by a race to find the active intermediate. It was already well established that nitric oxide breaks down to nitrite and nitrate (Sisler 1956) and the race was won by the Wellcome group under Moncada. Later, after the discovery that endothelial cells make nitric oxide, Hibbs and Marletta demonstrated that the cytotoxic effects of macrophages were indeed mediated by nitric oxide (Hibbs *et al.* 1988; Marletta *et al.* 1988).

Moncada and his group showed that endothelial cells make nitric oxide from the same amino acid L-arginine (Palmer *et al.* 1988) and that the enzyme that does this is inhibited by N^G-monomethyl-L-arginine (Rees *et al.* 1989*a*). Just as, when we found that aspirin inhibited the formation of prostaglandins, it gave us a means of understanding the functions of prostaglandins, the finding that N^G-monomethyl-L-arginine inhibits the formation of nitric oxide provided a powerful tool for investigating the actions of nitric oxide in the body.

Within my research group, we have looked at the intracellular generation of L-arginine (Mitchell *et al.* 1990) which comes from several sources, including uptake of external L-arginine. When L-arginine is metabolized by nitric oxide synthase to form nitric oxide, L-citrulline is formed (Palmer & Moncada 1989), an action which is prevented by N^G-monomethyl-L-arginine. Interestingly, unlike in other cells, L-citrulline is very quickly recycled through arginine-succinate back to L-arginine itself so the endothelial cell has a mechanism for maintaining its intracellular L-arginine concentrations (Hecker *et al.* 1990).

An enzyme which forms nitric oxide has been cloned from rat brain (Bredt *et al.* 1991) and from endothelial cells (Janssens *et al.* 1992; Sessa *et al.* 1992; Lamas *et al.* 1992). This cloned enzyme has recognition sites for various co-factors as well as phosphorylation sites, indicating that it is highly regulated. This enzyme is constitutive and requires calcium as a co-factor (Förstermann *et al.* 1991). Another type of nitric oxide synthase, which can be induced by lipopolysaccaride, has been isolated and cloned (Lowenstein *et al.* 1992; Lyons *et al.* 1992). Its induction is inhibited by glucocorticoids (Rees *et al.* 1990) and it does not need calcium as a co-factor.

(*d*) *Release of endothelium-derived relaxing factor*

Figure 4 shows a scheme of the events which take place during the release of EDRF. An effector cell (which could be an endothelial cell or one of the many cells that are now known to make nitric oxide) is stimulated with a chemical mediator such as acetylcholine, ADP or lipopolysaccaride to activate the enzyme which makes nitric oxide from L-arginine. These mediators, as well as shear stress (Lamontagne *et al.* 1992) or pulsatile pressure (Lamontagne *et al.* 1991), promote the conversion of L-arginine to nitric oxide by increasing the intracellular calcium. The nitric oxide then acts upon the guanylate cyclase of the same cell (Schröder & Schrör 1989) or diffuses to another cell such as a smooth muscle cell (Furchgott *et al.* 1984) or a platelet to increase the cyclic GMP there (Radomski *et al.* 1987*b*) and to cause muscular relaxation (Griffith *et al.* 1985) or inhibition of platelet aggregation (Radomski *et al.* 1987*b*). Nitric oxide is a gas dissolved in water, so there are no barriers (such as cell membranes) to its diffusion. Thus, one can imagine an endothelial cell releasing little puffs of nitric oxide each time it is stretched by the pulsations of the heart.

It is now possible to put the release of prostacyclin and nitric oxide into the context of damage and repair of the endothelial lining. There is co-operation between the actions of prostacyclin and nitric oxide when the blood vessel is damaged (Adelman *et al.* 1981). For example, if the inside of the carotid artery of a rabbit is damaged by clamping the outside with a metal clip, some endothelial cells are lost, and platelets collect on the subendothelium to repair the damage. Because there is no nitric oxide to inhibit their

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

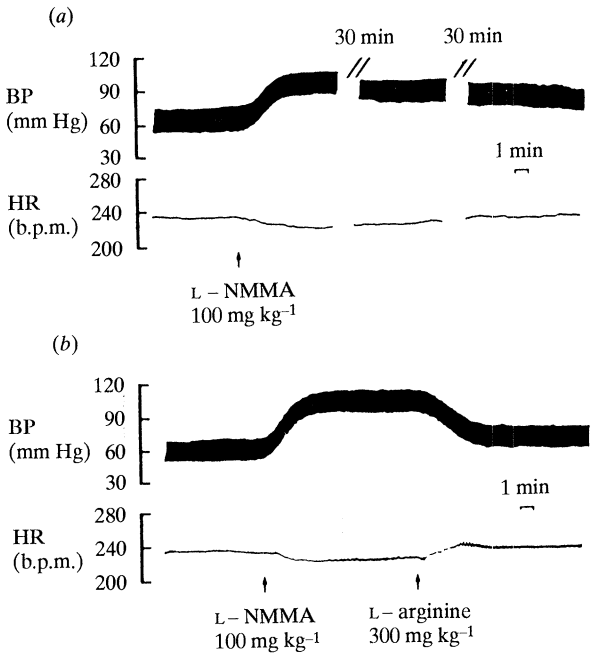

Figure 5. The effect of inhibition of nitric oxide formation by N^G-monomethyl-L-arginine (L-NMMA) on the blood pressure of a rabbit. (*a*) A single intravenous dose of L-NMMA resulted in a rise in blood pressure that lasted over 1 h. (*b*) Administration of L-arginine (but not L-arginine) reversed the rise in pressure induced by L-NMMA. BP = blood pressure; HR = heart rate. (From Rees *et al.* 1989*b*. Reprinted with permission from *Proc. natn. Acad. Sci. U.S.A.*).

adhesion the platelets form a monolayer. They are able to stick to the vessel wall and begin the repair process, but the prostacyclin which is still being made by the underlying tissue prevents this monolayer from building up into a strong aggregate.

(*e*) *Effect of EDRF on blood pressure*

Figure 5 illustrates what is probably the most important action of EDRF. It shows work from the Moncada group where an anaesthetized rabbit with a blood pressure of between 60 and 90 mm Hg, was injected with the nitric oxide synthesis inhibitor N^G-monomethyl-L-arginine (Rees *et al.* 1989*b*). There was an immediate rise in blood pressure which took a long time to return to normal. However, when the natural substrate for the nitric oxide synthase, L-arginine, was administered the elevated blood pressure very quickly returned to normal. Therefore, in the rabbit and in all other species tested as well as in man, there is a vasodilator tone due to continuous nitric oxide formation in the blood vessels which keeps the blood pressure down. When this tone is eliminated with N^G-monomethyl-L-arginine, the blood pressure goes up.

For many years, we have known that blood vessels are under the tonic control of the sympathetic nervous system, such that blocking or cutting the nerves reduces the blood pressure (Paton & Zaimis 1949). Thus, there is a tonic centrally controlled vasoconstriction. The local, tonic vasodilatation induced by nitric oxide from the endothelial cells will counteract this vasoconstriction so that the final actual blood pressure results from the interaction between these two opposing forces. Of course, this is a simplified version of events and many other factors (including angiotensin II, as described earlier) contribute to the overall picture. Nevertheless, it is probably the locally produced nitric oxide and the sympathetic nervous system which are the dominant players.

The release of EDRF or nitric oxide from endothelial cells has been shown over the last few years to be reduced in hypertensive animals (Miller *et al.* 1987; Panza *et al.* 1990) and in animal models of atherosclerosis (Förstermann *et al.* 1988; Cooke *et al.* 1991).

Thus, some forms of hypertension in man may be due to a deficiency of nitric oxide formation by the endothelium. Collier and Vallance (Vallance *et al.* 1989) infused N^G-monomethyl-L-arginine into the forearm of volunteers, which reduced blood flow by about 50%. The reduction in blood flow was due to inhibition of the continuous secretion of nitric oxide, and could be rapidly reversed with L-arginine, although the blood flow would have returned to normal after a longer time if L-arginine had not been used. Similar experiments (Calver *et al.* 1992) have shown that there is a deficiency of nitric oxide formation in the forearms of volunteers with hypertension.

(*f*) *Non-endothelial sources of nitric oxide*

As the work on nitric oxide has expanded, it has become obvious that nitric oxide acts as a second messenger or cell-to-cell messenger in many cell types.

(i) *Macrophages*

The nitric oxide generated in macrophages kills tumour cells (Stuer & Nathan 1989) and microbes (Granger *et al.* 1990). The nitric oxide synthase, which is distinct from the endothelial enzyme, has been identified in macrophages activated by cytokines (Stuer & Marletta 1985). Activation of this enzyme requires NADPH and flavins as co-factors as well as protein synthesis but is independent of calcium or calmodulin as calmodulin is tightly bound to the enzyme protein itself. The enzyme has now been cloned (Xie *et al.* 1992) and expressed in frog oocytes (Lyons *et al.* 1992).

The mechanism of the cytostatic effect of macrophages on tumour cells, fungi, mycobacteria and parasites is not clear. However, activated macrophages can inhibit a number of enzymes present in microbial and tumour cells by nitrosylating the non-haeme iron moiety (Stuer & Nathan 1989). As evidence of this, nitrosyl–iron–sulphur complexes have been detected in the culture medium after induction of nitric oxide synthesis in cytostatic macrophages (Lancaster & Hibbs 1990). Conditioned medium from these macrophages inhibits mitochondrial respiratory enzymes, DNA synthesis and aconitase activity in mouse tumour cells (Nathan 1992). The effect of nitric oxide on DNA synthesis is most likely due to inactivation of the rate limiting enzyme, ribonucleotide reductase (Lepoivre *et al.* 1991) through scavenging of its tyrosyl radical and perhaps also by the reaction of nitric oxide with non-haeme iron. The cytotoxic

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

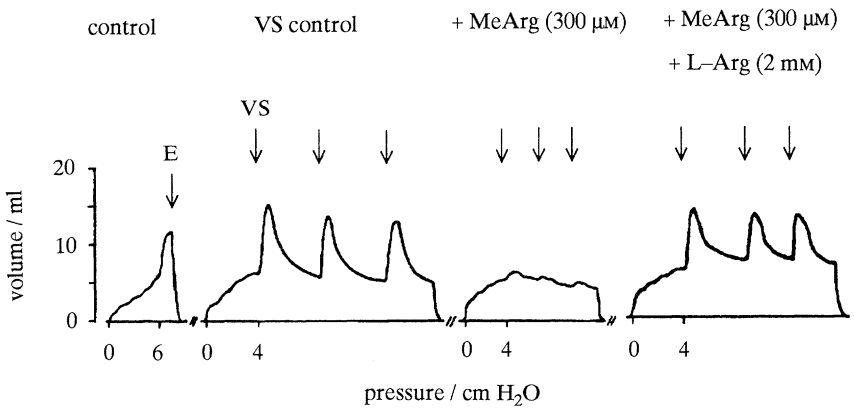

Figure 6. Changes in gastric volume at different intragastric pressures (control) and after vagal stimulation (VS). Stimulation-induced gastric relaxation was inhibited by N^G-monomethyl-L-arginine (MeArg) and partially reversed with L-arginine (L-Arg) but not D-arginine (not shown). E denotes emptying of the stomach after a response. (From Desai *et al.* 1991*b*.)

action of nitric oxide produced by macrophages and pancreatic islet cells inhibits the Krebs cycle enzyme, aconitase in mouse tumour and mouse islet cells (Welsh & Sandler 1992). An alternate theory is that nitric oxide behaves as an apoptotic stimulus, activating an endogenous endonuclease involved in the cleavage of DNA into nucleosomal fragments (Sarih *et al.* 1993).

(ii) *Mast cells*

Together with Piero Mannaioni and his colleagues in Florence, some of whom came to my department, we demonstrated the formation of nitric oxide by mast cells (Masini *et al.* 1991). When nitric oxide formation was inhibited with N^G-monomethyl-L-arginine, the spontaneous or stimulated release of histamine into the media from its storage granules in these cells was substantially increased, suggesting that the endogenous formation of nitric oxide by the mast cell was limiting the release of histamine. The application of a nitric oxide-donor like sodium nitroprusside (Salvemini *et al.* 1991) to the isolated mast cells suppresses this histamine release in a dose dependent manner, either when compound 48/80 or ionophore A23187 are used to cause the release of histamine. In this cell, then, the formation of nitric oxide helps to maintain the integrity and to prevent activation.

(iii) *Reflex relaxation of the stomach*

Some 30 years ago when I was working with Bill (now Sir William) Paton, we found that in the guinea-pig isolated stomach when we gradually increased the pressure inside, in steps of 1 cm, it gradually filled with fluid, until at a certain threshold (about 7 cm of water) the fundic end of the stomach relaxed enormously accommodating a great amount of fluid without a further rise in pressure (Paton & Vane 1963). A similar effect can also be seen after stimulation of particular vagus nerves. This has become known as adaptive or receptive relaxation. It always happens when food is taken into the stomach; without this accomodation the stomach pressure would rise and become uncomfortable. With Bill Paton we showed that this accommodation was mediated by nervous reflexes which were neither adrenergic or cholinergic in nature. This was before the term non-adrenergic, non-cholinergic (NANC) nerves was invented.

When inhibitors of nitric oxide formation became available, I asked Kash Desai, one of my Ph.D. students, to examine this accomodation in the guinea-N^G-monomethyl-L-arginine. Figure 6 shows pressure-induced adaptive relaxation (Desai *et al.* 1991*a*) and receptive relaxation induced by vagal stimulation in the presence of atropine (Desai *et al.* 1991*b*). Both are abolished when the stomach is exposed to N^G-monomethyl-L-arginine. When N^G-monomethyl-L-arginine plus L-arginine (to reverse its effects) are then placed in the isolated organ bath the reflex relaxation in the isolated organ bath the reflex relaxation returns. Thus, there are nitrergic nerves which relax the stomach and accommodate the increase in volume in response to the intake of food or fluid.

(iv) *Hypotension in endotoxaemia*

An enhanced formation of nitric oxide in response to bacterial lipopolysaccharide (LPS) is an important mediator of hypotension, peripheral vasodilatation and vascular hyporeactivity to vasoconstrictor agents in endotoxaemia (Thiemermann & Vane 1990; Szabó *et al.* 1993*a*) (figure 7). LPS induces a calcium-independent nitric oxide synthase in various cells (including macrophages and vascular smooth muscle cells *in vitro*), as well as in whole organs such as lung, liver and spleen *in vivo*, resulting in an enhanced formation of nitric oxide (see Moncada *et al.* 1991). Corticosteroids, which inhibit the induction of this calcium-independent isoform of nitric oxide synthase in response to LPS (Radomski *et al.* 1990) exert beneficial effects in circulatory shock (Thiemermann *et al.* 1993; Szabó *et al.* 1993*b*).

(*g*) *Conclusions*

The discovery of EDRF by Furchgott in 1980 has led to an explosion of work on nitric oxide. Who would have guessed then that a simple gas would turn out to have so many important functions in the body?

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

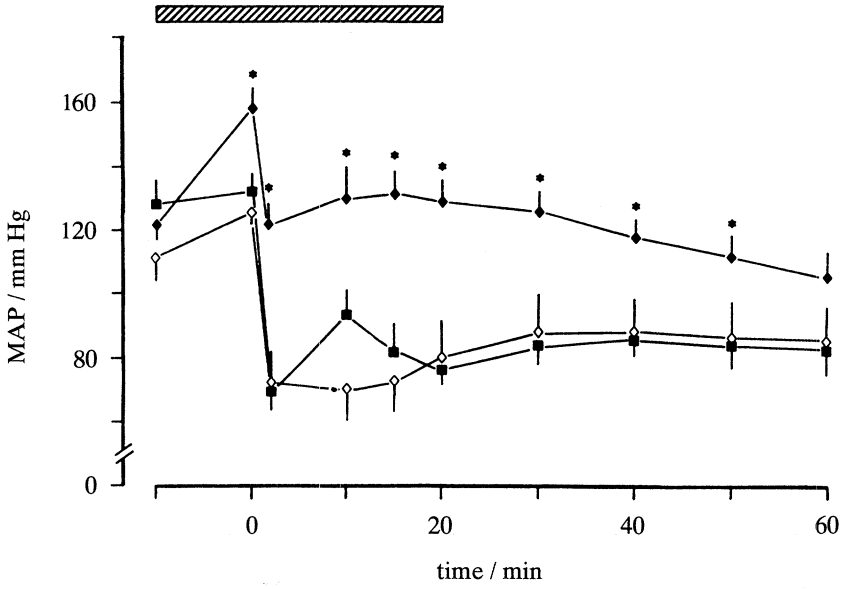

Figure 7. N^G-monomethyl-L-arginine (MeArg) attenuates the fall in mean arterial blood pressure (MAP) in response to *E. coli* lipopolysaccharide (LPS) in the anaesthetized rat. Animals received either LPS only (filled squares), MeArg and LPS (filled diamonds), or MeArg plus L-arginine and LPS (open diamonds). The hatched bar indicates the infusion period of MeArg or MeArg plus L-arginine. (From Thiemermann & Vane 1990. Reproduced with permission from Elsevier Science Publications.)

Apart from those already mentioned, nitric oxide has an important transmitter function (perhaps linked to the laying down of memory) in the brain (Garthwaite *et al.* 1988). It is also responsible for causing the changes in blood vessels locally in the penis which lead to erection (Rajfer *et al.* 1992). Clearly, with such a plethora of functions, nitric oxide is also stimulating intense interest in the drug industry.

On the one hand, there is a search for ways of increasing the effectiveness of nitric oxide to counteract a deficiency associated with the constitutive enzyme, such as in angina, hypertension and impotence. New nitric oxide donors are being sought, along with ways of making them tissue-specific. Inhibitors of the enzyme which breaks down the intracellular messenger cGMP should also be of value. On the other hand, there is increasing evidence that an excessive production of nitric oxide, probably mostly associated with the induction of a second nitric oxide synthase, can contribute to cardiovascular collapse in septic and haemorrhagic shock (Szabó *et al.* 1993*a*; Thiemermann *et al.* 1993). The development of selective inhibitors of this enzyme may be useful in the treatment of such life-threatening conditions.

4. ENDOTHELIN-1

Another major discovery in the field of the endothelial cell is that of endothelin. In the 1980s, it was shown that endothelial cells grown in culture *in vitro*, generated a vasoconstrictor substance (Hickey *et al.* 1985). A young Japanese scientist named Masashi Yanagisawa, and his Ph.D. supervisor Tomoh Masaki, concluded it was a peptide and, using techniques of molecular biology, attempted to determine its structure. On 1st April 1988, together with others, they published a paper in Nature, showing that a 21 amino acid peptide, with two disulphide bridges, accounted for the activity of this strong vasoconstrictor (Yanagisawa *et al.* 1988) (figure 8). There was also an endothelin-converting enzyme that made endothelin from a 'big endothelin' which in turn was made from a 203 amino acid preproendothelin (figure 9).

This was such a stunning paper that I rang one of my colleagues to ask him what he thought about it. He rang me back to suggest that it must be an April Fool's Joke and that the editor of Nature, John Maddox, must be having us all on as it was too perfect. But it was a true paper. We now know that there are three endothelins, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) (Inoue *et al.* 1989). ET-1 is made by the endothelial cells, ET-2 is made in the intestine (Firth & Ratcliffe 1992) and ET-3 is made in the brain particularly by the hypothalamus and the pituitary gland (Hirai *et al.* 1991).

Previously, an activity would be isolated and identified by the biologists; the chemists would then put a structure to it and thus it would be identified. Nowadays with the tools of molecular biology, mediators can be isolated and identified without initially knowing what they do or where they come from. In the case of the three endothelin mediators, it is known that only endothelin-1 is made by the endothelial cell, but the cell types making the other endothelins are not yet identified for certain. ET-2 differs by two amino acids, and ET-3 is different by five amino acids from ET-1 (Inoue *et al.* 1989). Another family of substances called the sarafotoxins also have the same generic structure. They are made by the venom glands of an Israeli snake, the burrowing asp, which kills its prey by cardiotoxicity (Kloog *et al.* 1988).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

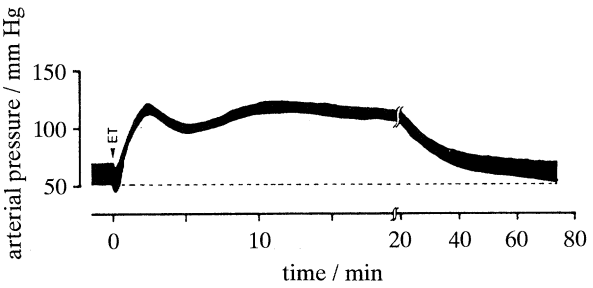

Figure 8. The effect of endothelin-1 (ET) on rat blood pressure after a single dose. The blood pressure is increased for more than 60 min. (From Yanagisawa *et al.* 1988. Reproduced with permission from *Nature*. Copyright 1988, Macmillan Journals Limited.)

(*a*) *Properties of endothelin-1*

(i) *Effects on blood pressure*

The effects of ET-1 on the blood pressure of a rat are shown in figure 8. It causes an increase in blood pressure which lasts for more than 60 min (Yanagisawa *et al.* 1988), which is an unusually long lasting effect for a peptide, especially as the circulation is cleared of ET-1 in a couple of minutes. Endothelin-1 contracts vascular smooth muscle, is more potent on veins than on arteries (D'Orléans-Juste *et al.* 1989), and is two-thirds removed by the lungs (Änggård *et al.* 1989). It is, therefore, unlikely to be a circulating hormone.

(ii) *Release of prostacyclin and EDRF*

Endothelin-1 also releases prostacyclin and EDRF, as shown in Figure 8 by the transient fall in blood pressure before the rise (Yanagisawa *et al.* 1988; Thiemermann *et al.* 1989). When the same dose of endothelin-1 is repeated after giving a cyclooxygenase inhibitor, a much bigger rise in blood pressure is achieved (figure 10) i.e. the original response was

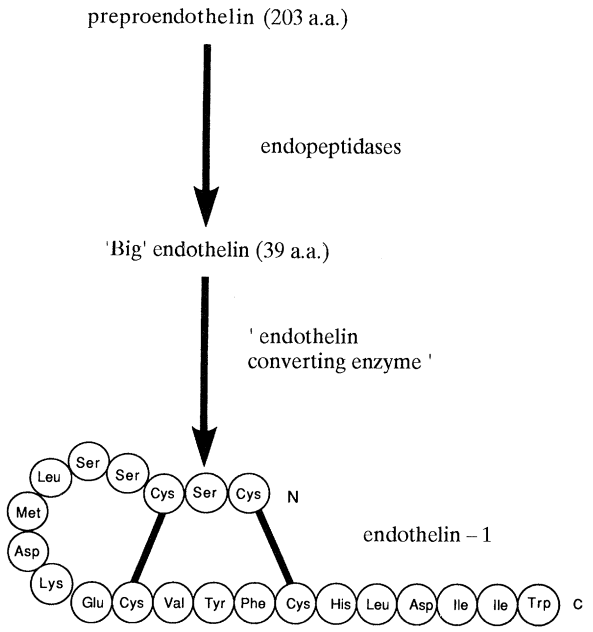

Figure 9. The proposed pathway of formation of endothelin-1 (ET-1) and its amino acid (a.a.) composition.

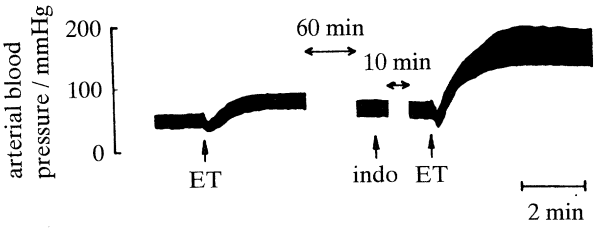

Figure 10. Potentiation of the pressor response to endothelin-1 (ET) by indomethacin (Indo) on rat blood pressure. A second dose of ET increased the blood pressure approximately twice that induced by the first challenge. (From de Nucci *et al.* 1988.)

limited by the release of prostacyclin (De Nucci *et al.* 1988). The initial vasodepressor effect is still there and this is due to the release of EDRF (or nitric oxide). It is now evident in increasingly complex systems, such as the dog kidney (Miura *et al.* 1989) or even in the human hand (Brain *et al.* 1989), that the release of these mediators by ET-1 modulates its own action.

(*b*) *Release of endothelin-1 in disease states*

We have only known about endothelin-1 for 5 years, but we are now able to measure by radioimmunoassay (Saito *et al.* 1989; Suzuki *et al.* 1989) plasma levels in man of between 0.2–5 picograms per ml. Big endothelin-1, the inactive 39 amino acid peptide, is also found in human plasma (Miyauchi *et al.* 1989). Some radioimmunoassays lack specificity and may pick up the metabolic products of endothelin-1, but raised endothelin-1 levels as measured by radioimmunoassay have been shown in various diseases such as myocardial infarction (Miyauchi *et al.* 1989) cardiogenic shock (Cernacek & Stewart 1989), heart failure (Koyama *et al.* 1989; Totsune *et al.* 1989; Cody *et al.* 1992; McMurray *et al.* 1992; Stewart *et al.* 1992), hypertension (Kohno *et al.* 1990) and hepatorenal syndrome (Moore *et al.* 1992) (see table 1).

It has been suggested that ET-1 has a role in acute renal failure (Firth *et al.* 1988) for the kidney is about ten times more sensitive to its vasoconstrictor action than other vascular regions (Pernow *et al.* 1989). In addition, plasma levels of ET-1 are elevated in renal failure (Tomita *et al.* 1989) and the medulla of the

Table 1. *Clinical significance of raised plasma levels of ET-1*

disease	reference
myocardial ischaemia	Miyauchi *et al.* (1989)
cardiogenic shock	Cernacek & Stewart (1989)
renal failure	Firth *et al.* (1988)
	Tomita *et al.* (1989)
uraemia	Koyama *et al.* (1989)
haemodialysis	Totsune *et al.* (1989)
hypertension	Kohno *et al.* (1990)
coronary vasospasm	Toyo-oka *et al.* (1991)
pre-eclapsia	Greer *et al.* (1991)
heart failure	Stewart *et al.* (1992)
	Cody *et al.* (1992)
	McMurray *et al.* (1992)
hepatorenal syndrome	Moore *et al.* (1992)
cirrhosis	Asbert *et al.* (1993)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

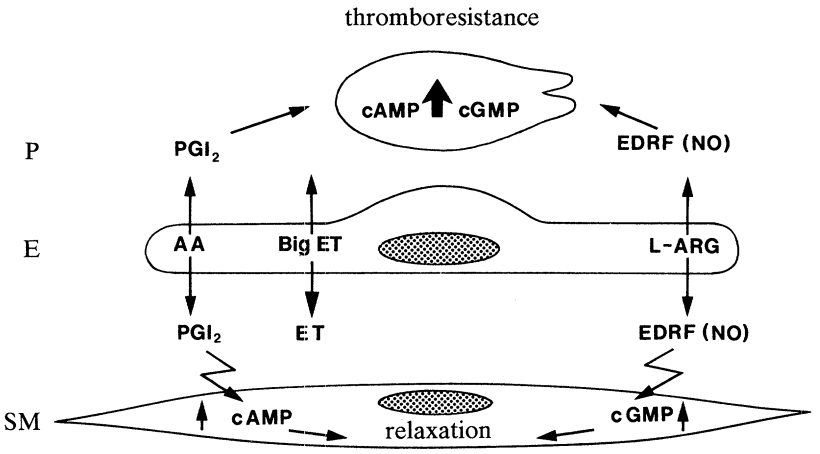

Figure 11. The vascular endothelial cell (E) and how it may affect the circulating platelet (P) and the underlying smooth muscle cells (SM). Abbreviations used: AA, arachidonic acid; EDRF, endothelium-derived relaxing factor; ET, endothelin; NO, nitric oxide; L-Arg, L-arginine; PGI_2, prostacyclin.

kidney contains a high density of endothelin receptors as well as producing substantial amounts of ET-1 (Kaw *et al.* 1993; Wellings *et al.* 1993).

An interesting finding has been the discovery of the mitogenic effects of ET-1, shown in cardiovascular smooth muscle cells (Komuro *et al.* 1988; Hirata *et al.* 1989; Nakaki *et al.* 1989), in fibroblasts (Takuwa *et al.* 1989) and in mesangial cells (Simonson *et al.* 1989). ET-1 also enhanced transcription of the c-fos and c-myc proto-oncogene, biochemical signals which are closely linked to proliferation (Komuro *et al.* 1988; Simonson *et al.* 1989). These effects suggest that ET-1 could have a trophic effect related to the development of the lesions seen in hypertension and atherosclerosis. Infusions of ET-1 increased the extent of smooth muscle hyperplasia after balloon-catheter injury of arterial vessels (Trachtenberg *et al.* 1993; Douglas & Ohlstein 1993) and atheromatous sections of saphenous vein grafts show an increase in the number of ET-1 receptors (Dashwood *et al.* 1993). Thus, ET-1 may have a role in atherosclerosis and in the re-occlusion that often follows invasive opening of occluded arteries.

(*c*) *Endothelin receptor antagonists and endothelin converting enzyme inhibitors*

Two endothelin receptors are known, the ET_A receptor (Arai *et al.* 1990) and the ET_B receptor. The ET_A receptor is selective in that ET-1 is much more active on it than is ET-3. The ET_B receptor (Sakurai *et al.* 1990) is non-selective and all the endothelins (and sarafotoxins) are equally potent.

Although it was thought that ET_A receptors mediate the contractile effects of the endothelins, whereas vasodilator effects were mediated by ET_B receptors (Sakurai *et al.* 1992), it is now clear that this division is too simple. ET_B receptors can also mediate vasoconstriction (McMurdo *et al.* 1993), for instance in the rabbit pulmonary artery and saphenous vein, the dog saphenous vein and the porcine coronary artery (Shetty *et al.* 1993; White *et al.* 1993). Interestingly, it is ET_A receptors which predominantly mediate constrictions of the renal vasculature of the rabbit (D'Orléans-Juste *et al.* 1993), but ET_B receptors are more important in that of the rat (Cristol *et al.* 1993).

The roles of the endothelins are being clarified by the development of antagonists such as BQ-123 (ET_A receptor-selective; Ihara *et al.* 1992) and PD 145065 (non-selective; Doherty *et al.* 1993). A non-peptide, orally active, non receptor-selective antagonist, Ro 46-2005 (Clozel *et al.* 1993), has also been developed which decreases the deleterious consequences of severely impaired kidney blood flow and blood clots on the brain. However, there are effects, such as the stimulation of NO release from the endothelium, that seem to be mediated by additional receptor sub-types (Warner *et al.* 1993).

The pharmaceutical industry is also actively looking for inhibitors of endothelin converting enzyme but so far the enzymes which cleave the inactive big endothelins (38–41 amino acids) into their active forms (21 amino acids) have not been isolated (or more accurately, their isolation has not been published). Clearly, as the converting enzyme which makes angiotensin II from angiotensin I became a suitable target for therapeutic antihypertensive intervention, an inhibitor of endothelin converting enzyme could be another antihypertensive substance.

The role of the endothelins in health and disease is still unfolding. The availability of inhibitors of endothelin generation or antagonists of endothelin action will help in this elucidation. They may also become important therapeutic agents.

5. CONCLUSIONS

Figure 11 shows the endothelial cells positioned between the blood stream and the smooth muscle. They can make prostacyclin from arachidonic acid and EDRF or NO from L-arginine. These evanescent mediators both inhibit platelet aggregation in what has turned out to be a synergistic way (Radomski *et al.* 1987*b*). There is no synergism between their relaxant effects on the smooth muscle cells (Lidbury *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1989). The intracellular messenger for prostacyclin is cAMP and that for NO is cGMP.

The endothelial cells also make big endothelin-1 which is converted by enzymes to the potent vasoconstrictor, endothelin-1. It is certain that if the endothelial cell is making the most potent vasoconstrictor substance yet discovered (ten times more potent than the previous record holder angiotensin II), then this substance is there for a purpose. It would not be there simply as a random peptide made innocuously, so it could well be contributing to disease states such as hypertension and atherosclerosis.

Thus, there are many areas in which the study of the endothelial cell, and its ability to release chemical mediators, have led and might lead to therapeutic interventions for various disease states. The medicines of tomorrow will arise from the research work of today, but the process between the discovery and the market is very prolonged, sometimes taking 15–20 years to reach fruition. But without basic research of the kind I have described, we will have no new medicines.

The William Harvey Research Institute is supported by Glaxo Group Research Limited, the Parke-Davis Pharmaceutical Division of Warner-Lambert, the Ono Pharmaceutical Company and Schwarz Pharma AG.

REFERENCES

Adelman, B., Stemerman, M., Merrell, D. & Handin, R. 1981 The interaction of platelets with aortic subendothelium: inhibition of adhesion and secretion by prostaglandin I_2. *Blood* **58**, 198–205.

Aiken, J.W., Gorman, R.R. & Shebuski, R.J. 1979 Prevention of blockage of partially obstructed coronary arteries with prostacyclin correlates with inhibition of platelet aggregation. *Prostaglandins* **17**, 483–494.

Änggård, E. & Samuelsson, B. 1965 Biosynthesis of prostaglandins from arachidonic acid in guinea-pig lung. *J. biol. Chem.* **240**, 3518–3521.

Änggård E., Galton, S., Rae, G., Thomas, R., McLoughlin, L., de Nucci, G. & Vane, J.R. 1989 The fate of radioiodinated endothelin-1 and endothelin-3 in the rat. *J. cardiovasc. Pharmacol.* **13** (Suppl. 5), S46–S49.

Arai, H., Hori, S., Aramori, I., Ohkubo, H. & Nakanishi, S. 1990 Cloning and expression of a cDNA encoding an endothelin receptor. *Nature, Lond.* **348**, 730–732.

Araki, H. & Lefer, A.M. 1980 Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. *Am. J. Physiol.* **238**, H176–H181.

Asbert, M., Ginès, P., Jiménez, W., Clària, J., Saló, J., Arroyo, V., Rivera, F. & Rodós, J. 1993 Circulating levels of endothelin in cirrhosis. *Gastroenterology* **104**, 1485–1491.

Axelrod, L. & Levine, L. 1981 Prostacyclin production by isolated adipocytes. *Diabetes* **30**, 163–167.

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

Bakhle, Y.S. 1971 Inhibition of angiotensin I converting enzyme by venom peptides. *Br. J. Pharmacol.* **43**, 252–254.

Bakhle, Y.S., Reynard, A.M. & Vane, J.R. 1969 Metabolism of angiotensin in isolated perfused tissues. *Nature, Lond.* **222**, 956–959.

Bayer, B.-L., Blass, K.-E. & Forster, W. 1979 Anti-aggregatory effect of prostacyclin (PGI_2) in vivo. *Br. J. Pharmacol.* **66**, 10–12.

Belch, J.J.F., Newman, P., Drury, J.K., Capell, H., Lieberman, P., James, W.B., Forbes, C.D. & Prentice, C.R.M. 1981 Successful treatment of Raynaud's syndrome with prostacyclin. *Thromb. Haemostas.* **45**, 255–256.

Belch, J.J.F., Newman, P., Drury, J.K., McKenzie, F., Capell, H., Leiberman, P., Forbes, C.D. & Prentice, C.R. 1983 Intermittent epoprostenol (prostacyclin) infusion in patients with Raynaud's syndrome. *Lancet* i, 313–315.

Bennett, J.G., Longmore, D.B. & O'Grady, J. 1981 Use of prostacyclin in cardiopulmonary bypass in man. In *Clinical pharmacology of prostacyclin* (ed. P. J. Lewis & J. O'Grady), pp. 201–208. New York: Raven Press.

Bergström, S., Danielsson, H. & Samuelsson, B. 1964 The enzymatic formation of prostaglandin E_2 from arachidonic acid. Prostaglandins and related factors 32. *Biochim. biophys. Acta* **90**, 207–210.

Bergström, S. & Samuelsson, B. 1962 Isolation of prostaglandin E_1 from human seminal plasma. *J. biol. Chem.* **237**, PC3005–3006.

Blackwell, G.J., Radomski, M., Vargas, J.R. & Moncada, S. 1982 Prostacyclin prolongs viability of washed human platelets. *Biochim. biophys. Acta* **718**, 60–65.

Boyd, A.M. & Marks, J. 1963 Treatment of intermittent claudication. A reappraisal of the value of α-tocopherol. *Angiology* **14**, 198–208.

Brain, S.D., Crossman, D.C., Buckley, T.L. & Williams, T.J. 1989 Endothelin-1: demonstration of potent effects on the microcirculation of humans and other species. *J. cardiovasc. Pharmacol.* **13** (Suppl. 5), S147–9.

Bredt, D.S., Hwang, P.M., Glatt, C.E., Lowenstein, C., Reed, R.R. & Snyder, S.H. 1991 Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature, Lond.* **351**, 714–718.

Brücke, E. 1857 An essay on the cause of the coagulation of the blood. *Br. Foreign Med. Chir. Rev.* **19**, 183–212.

Bunting, S., Gryglewski, R., Moncada, S. & 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**, 897–913.

Bunting, S., O'Grady, J., Fabiani, J.-N., Terrier, E., Moncada, S. & Vane, J.R. 1981 Cardiopulmonary bypass in man: effects of prostacyclin. In *Clinical pharmacology of prostacyclin* (ed. P. J. Lewis & J. O'Grady), pp. 181–193. New York: Raven Press.

Burn, J.H. & Robinson, J. 1951 Reversal of the vascular response to acetylcholine and adrenaline. *Br. J. Pharmacol.* **6**, 110–119.

Calver, A., Collier, J., Moncada, S. & Vallance, P. 1992 Effect of local intra-arterial N^G-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *J. Hypertension* **10**, 1025–1031.

Cernacek, P. & Stewart, D.J. 1989 Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. *Biochem. biophys. Res. Commun.* **161**, 562–567.

Chelly, J., Tricot, A., Garcia, A., Boucherie, J.-C., Fabiani, J.-N., Passelecq, J. & Dubost, Ch. 1981 Hemodynamic effects of prostacyclin infusion after coronary bypass. In *Clinical pharmacology of prostacyclin* (ed. P. J. Lewis & J. O'Grady), pp. 209. New York: Raven Press.

Clozel, I., Breu, V., Burri, K., Cassal, J.-M., Fischli, W., Gray, G.A., Hirth, G., Löffler, B.-M., Müller, M., Neldhart, W., Ramuz, H. 1993 Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. *Nature, Lond.* **365**, 759–761.

Cody, R.J., Haas, G.J., Binkley, P.F., Capers, Q. & Kelley,

R. 1992 Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. *Circulation* **85**, 504–509.

Cooke, J.P., Andon, N.A., Girerd, X.J., Hirsch, A.T. & Creager, M.A. 1991 Arginine restores cholinergic relaxation of hypercholesterolemic rabbit thoracic aorta. *Circulation* **83**, 1057–1062.

Cristol, J.-P., Warner, T.D., Thiemermann, C. & Vane, J.R. 1993 Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetised rat. *Br. J. Pharmacol.* **108**, 776–779.

D'Angelo, V., Villa, S., Mysliwiec, M., Donati, M. & de Gaetano, G. 1978 Defective fibrinolytic and prostacyclin-like activity in human atheromatous plaques. *Thromb. Diath. Haemorrh.* **39**, 535–536.

D'Orleans-Juste, P., Finet, M., de Nucci, G. & Vane, J.R. 1989 Pharmacology of endothelin-1 in isolated vessels. Effect of nicardipine, methylene blue, hemoglobin and gossypol. *J. cardiovasc. Pharmacol.* **13** (Suppl. 5), S19–S22.

D'Orléans-Juste, P., Yano, M. & Télémaque, S. 1993 ET_A-dependent pressor effects and release of prostacyclin induced by endothelins in pulmonary and renal vasculatures. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S235–S238.

Dale, H.H. 1914 The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharm. exp. Ther.* **6**, 147–190.

Dashwood, M.R., Barker, S.G.E., Muddle, J.R., Yacoub, M.H. & Martin, J.F. 1993 [^{125}I]Endothelin-1 binding to vasa vasorum of neovascularization in human and porcine blood vessels: a possible role for endothelin in intimal hyperplasia and atherosclerosis. *J. cardiovasc. Pharmacol.* **22** (Suppl. 8), S343–S347.

De Nucci, G., Gryglewski, R.J., Warner, T.D. & Vane, J.R. 1988 Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2334–2338.

De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D. & Vane, J.R. 1988 Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. natn. Acad. Sci. U.S.A.* **85**, 9797–9800.

Demling, R.H., Smith, M., Gunther, R., Gee, M., Flynn, J. 1981 The effect of prostacyclin infusions on endotoxin-induced lung injury. *Surgery* **89**, 257–263.

Desai, K.M., Sessa, W.C. & Vane, J.R. 1991*a* Involvement of nitric oxide in the reflex relaxation of the stomach to accomodate food or fluid. *Nature, Lond.* **351**, 477–479.

Desai, K.M., Zembowicz, A., Sessa, W.C. & Vane, J.R. 1991*b* Nitroxergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. *Proc. natn. Acad. Sci. U.S.A.* **88**, 11490–11494.

Doherty, A.M., Cody, W.L., He, J.X., DePue, P.L., Cheng, X.-M., Welch, K.M., Flynn, M.A., Reynolds, E.E., LaDouceur, D.M., Davis, L.S., Keiser, J.A. & Haleen, S.J. 1993 *In vitro* and *in vivo* studies with a series of hexapeptide endothelin antagonists. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S98–S102.

Dorp, D.A. van, Beerthuis, R.K., Nugteren, D.H. & Vonkeman, H. 1964 The biosynthesis of prostaglandins. *Biochem. biophys. Acta* **90**, 204–207.

Douglas, S.A. & Ohlstein, E.H. 1993 Endothelin-1 promotes neointima formation after balloon angioplasty in the rat. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S371–S373.

Dowd, P.M., Martin, M.F.R., Cooke, E.D., Bowcock, S.A., Jones, R., Dieppe, P.A. & Kirby, J.D. 1982 Treatment of Raynaud's phenomenon by intravenous infusion of prostacyclin (PGI_2). *Br. J. Dermatol.* **106**, 81–89.

Eldor, A., Falcone, D.J., Hajjar, D.P., Minick, C.R. & Weksler, B.B. 1982 Diet-induced hypercholesterolemia inhibits the recovery of prostacyclin production by injured rabbit aorta. *Am. J. Pathol.* **107**, 186–190.

Euler, U.S. von, 1934 Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrakten männlicher accessorischer Geschlectsdrüssen. *Arch. exptl Pathol. Pharmakol.* **175**, 78–84.

Ferns, G.A.A., Konneh, M., Änggård 1993 Vitamin E: the evidence for an anti-atherogenic role. *Artery* **20**, 61–94.

Ferreira, S.H. 1965 Bradykinin potentiating factor (BPF) present in venom of *Bothrops jararaca*. *Br. J. Pharmacol.* **24**, 163–169.

Ferreira, S.H., Greene, L.J., Alabaster, V.A., Bakhle, Y.S. & Vane, J.R. 1970 Activity of various fractions of bradykinin potentiating factor against angiotensin I converting enzyme. *Nature, Lond.* **225**, 379–380.

Ferreira, S.H., Moncada, S. & Vane, J.R. 1971 Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, new Biol.* **231**, 237–239.

Firth, J.D., Ratcliffe, P.J., Raine, A.E.G. & Leadingham, J.G.G. 1988 Endothelin: an important factor in acute renal failure. *Lancet* ii, 1179–1182.

Firth, J.D. & Ratcliffe, P.J. 1992 Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. *J. clin. Invest.* **90**, 1023–1031.

Fletcher, J.R. & Ramwell, P.W. 1980 The effects of prostacyclin (PGI_2) on endotoxin shock and endotoxin-induced platelet aggregation. *Circ. Shock* **7**, 299–308.

Förstermann, U., Mügge, A., Alheid, U., Haverich, U. & Frölich, J.C. 1988 Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. *Circ. Res.* **62**, 185–190.

Förstermann, U., Schmidt, H.H.H.W., Pollock, J.S., Sheng, H., Mitchell, J.A., Warner, T.D., Nakane, M. & Murad, F. 1991 Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem. Pharmacol.* **42**, 1849–1857.

Furchgott, R.F., Cherry, P.D., Zawadzki, J.V. & Jothianandan, D. 1984 Endothelial cells as mediators of vasodilatation of arteries. *J. cardiovasc. Pharmacol.* **6**, S336–S343.

Furchgott, R.F. & Zawadzki, J.V. 1980 The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, Lond.* **288**, 373–376.

Garthwaite, J., Charles, S.L. & Chess-Williams, R. 1988 Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature, Lond.* **336**, 385–388.

Gheorghiade, M., Sueta, C.A., Adams, K.F., Bourge, R.C., Murali, S., Uretsky, B.F., Pritzker, M.R., McGoon, M.D., Starling, R.C., Butman, S.M., Grossman, S. and the Epoprostenol Multicentre Research Group 1992 Multicentre, randomized trial of epoprostenol in patients with severe heart failure. *J. Am. Coll. Cardiol.* **19**, 216A (Abstract).

Glavind, J., Hartmann, S., Clemmensen, J., Jessen, K.E. & Dam, H. 1952 Studies on the role of lipoperoxides in human pathology. II. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta Pathol. Microbiol. Scand.* **30**, 1–6.

Gorman, R.R., Bunting, S., Miller, O.V. 1977 Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins* **13**, 377–388.

Granger, D.L., Hibbs, J.B. Jr, Perfect, J.R. & Durack, D.T. 1990 Metabolic fate of L-arginine in relation to micro-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

biostatic capability of murine macrophages. *J. clin. Invest.* **85**, 264–273.

Griffith, T.M., Edwards, D.H., Lewis, M.J. & Henderson, A.H. 1985 Evidence that cyclic guanosine monophosphate (cGMP) mediates endothelium-dependent relaxation. *Eur. J. Pharmacol.* **112**, 195–202.

Gryglewski, R.J., Botting, R.M. & Vane, J.R. 1988 Mediators produced by the endothelial cell. *Hypertension* **12,** 530–548.

Gryglewski, R.J., Bunting, S., Moncada, S., Flower, R.J. & Vane, J.R. 1976 Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* **12**, 685–713.

Gryglewski, R.J., Flower, R.J., Herbaczynska-Cedro, K. & Vane, J.R. 1972 Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature, new Biol.* **238**, 104–106.

Gryglewski, R.J., Palmer, R.M.J. & Moncada, S. 1986 Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature, Lond.* **320**, 454–456.

Haeger, K. 1968 The treatment of peripheral occlusive arterial disease with alpha-tocopherol as compared with vasodilator agents and anti-prothrombin (dicoumarol). *Vasc. Dis.* **5**, 199–213.

Hajjar, D.P. 1985 Prostaglandins and cyclic nucleotides: modulators of arterial cholesterol metabolism. *Biochem. Pharmacol.* **34**, 295–300.

Hamberg, M., Svensson, J. & Samuelsson, B. 1975 Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994–2998.

Harvey, W. 1628 *Exercitatio anatomica de motu cordis et sanguinis in animalibus*. Frankfurt: William Fitzeri.

Hecker, M., Sessa, W.C., Harris, H.J., Änggård, E.E. & Vane, J.R. 1990 The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. *Proc. natn. Acad. Sci. U.S.A.* **87**, 8612–8616.

Hibbs, J.B. Jr, Taintor, R., Vavrin, Z. & Rachlin, C. 1988 Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem. biophys. Res. Commun.* **157**, 87–94.

Hibbs, J.B. Jr, Taintor, R.R. & Vavrin, Z. 1987 Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science, Wash.* **235**, 473–476.

Hickey, K.A., Rubanyi, G., Paul, R.J. & Highsmith, R.F. 1985 Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am. J. Physiol.* **248**, C550–C556.

Higenbottam, T. 1987 The place of prostacyclin in the clinical management of primary pulmonary hypertension. *Am. Rev. Respir. Dis.* **136**, 782–785.

Higgs, E.A., Moncada, S., Vane, J.R., Caen, J.P., Michel, H. & Tobelem, G. 1978 Effect of prostacyclin (PGI_2) on platelet adhesion to rabbit arterial subendothelium. *Prostaglandins* **16**, 17–22.

Hirai, M., Miyabo, S., Ooya, E., Miyanaga, K., Aoyagi, N., Kimura, K., Kishida, S. & Nakai, T. 1991 Endothelin-3 stimulates the hypothalamic-pituitary-adrenal axis. *Life Sci.* **48**, 2359–2363.

Hirata, Y., Takagi, Y., Fukuda, Y. & Marumo, F. 1989 Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis* **78**, 225–228.

Hunt Reid 1915 Some physiological actions of the homocholins and of some of their derivatives. *J. Pharmacol.* **6**, 477–525.

Ignarro, L.J., Byrns, R.E., Buga, G.M. & Wood KS. 1987 Endothelium-derived relaxing factor (EDRF) released from artery and vein appears to be nitric oxide (NO) or a closely related radical species. *Fed. Proc. Fed. Am. Soc. exp. Biol.* **46**, 644p. [Abstract.]

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M. & Yano, M. 1992 Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.* **50**, 247–255.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., Masaki, T. 1989 The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three seperate genes. *Proc. natn. Acad. Sci. U.S.A.* **86**, 2863–2867.

Insel, J., Mirvis, D.M., Boland, M.J., Cinquegrani, M.P., Shanes, J., Rubin, S.A. & Whalen, J.J. 1989 A multicenter study of the safety and efficacy of benazepril hydrochloride, a long-acting angiotensin-converting enzyme inhibitor, in patients with chronic congestive heart failure. *Clin. Pharmacol. Ther.* **45**, 312–320.

Iyengar, R., Stuehr, D.J., Marletta, M.A. 1987 Macrophage synthesis of nitrite, nitrate, and N-nitrosamines: precursors and role of the respiratory burst. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6369–6373.

Janssens, S.P., Shimouchi, A., Quertermous, T., Bloch, D.B. & Bloch, K.D. 1992 Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase. *J. biol. Chem.* **267**, 14519–14522.

Jugdutt, B.F., Hutchins, G.M., Bulkley, B.H. & Becker, L.C. 1979 Infarct size reduction by prostacyclin after coronary occlusion in conscious dogs. *Clin. Res.* **27**, 177A.

Kaser-Glanzmann, R., Jakabova, M., George, J. & Luscher, E. 1977 Stimulation of calcium uptake in platelet membrane vesicles by adenosine 3, 5-cyclic monophosphate and protein kinase. *Biochim. biophys. Acta* **466**, 429–440.

Katsuki, S., Arnold, W., Mittal, C. & Murad, F. 1977 Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. cyclic Nucleot. Res.* **3**, 23–35.

Kaw, S., Warner, T.D. & Vane, J.R. 1993 The metabolism of endothelin-1 and big endothelin-1 by the isolated perfused kidney of the rabbit. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S65–S68.

Khan, M.T. & Furchgott, R.F. 1987 Similarities of behaviour of nitric oxide (NO) and endothelium-derived relaxing factor in a perfusion cascade bioassay system. *Fed. Proc. Fed. Am. Soc. exp. Biol.* **46**, 385p. [Abstract.]

Kloog, Y., Ambar, I., Sokolovsky, M., Kochva, E., Wollberg, Z. & Bdolah, A. 1988 Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science, Wash.* **242**, 268–270.

Kohno, M., Yasunari, K., Murakawa, K., Yokokawa, K., Horio, T., Fukui, T. & Takeda, T. 1990 Plasma immunoreactive endothelin in essential hypertension. *Am. J. Med.* **88**, 614–618.

Komuro, I., Kurihara, H., Sugiyama, T., Takaku, F. & Yazaki, Y. 1988 Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett.* **238**, 249–252.

Koyama, H., Nishizawa, Y., Morii, H., Tabata, T., Inoue, T. & Yamaji, T. 1989 Plasma endothelin levels in patients with uraemia. *Lancet* i, 991–992.

Krieger, M., Salgado, H., Assan, C., Greene, L.J. & Ferreira, S. 1971 Potential screening test for detection of overactivity of the renin-angiotensin system. *Lancet* i, 269–271.

Kujubu, D.A. & Herschman, H.R. 1992 Dexamethasone

inhibits mitogen induction of the TIS 10 prostaglandin synthase/cyclooxygenase gene. *J. biol. Chem.* **267**, 7991–7994.

Lamas, S., Marsden, P.A., Li, G.K., Tempst, P. & Michel, T. 1992 Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. *Proc. natn. Acad. Sci. U.S.A.* **89**, 6348–6352.

Lamontagne, D., Pohl, U. & Busse, R. 1992 Mechanical deformation of vessel wall and shear stress determine the basal release of endothelium-derived relaxing factor in the intact rabbit coronary vascular bed. *Circ. Res.* **70**, 123–130.

Lamontagne, D., Pohl, U. & Busse, R. 1991 The mechanical activity of the heart stimulates endothelium-derived relaxing factor release in the coronary vascular bed. *J. cardiovasc. Pharmacol.* **17** (Suppl. 3), S89–S94.

Lancaster, J.R. & Hibbs, J.B. Jr 1990 EPR demonstration of iron-nitrosyl complex formation by cytotoxic activated macrophages. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1223–1227.

Lapetina, E.G., Schmitges, C.J., Chandrabose, K. & Cuatrecasas, P. 1977 Cyclic adenosine 3, 5-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. *Biochem. biophys. Res. Commun.* **76**, 828–835.

Lefer, A.M., Tabas, J. & Smith, E.F. III 1980 Salutory effects of prostacyclin in endotoxin shock. *Pharmacology* **21**, 206–212.

Lepoivre, M., Fieschi, F., Coves, J., Thelander, L. & Fontecave, M. 1991 Inactivation of ribonucleotide reductase by nitric oxide. *Biochem. biophys. Res. Commun.* **179**, 442–448.

Lidbury, P.S., Antunes, E., de Nucci, G. & Vane, J.R. 1989 Interactions of iloprost and sodium nitroprusside on vascular smooth muscle and platelet aggregation. *Br. J. Pharmacol.* **98**, 1275–1280.

Long, W.A. & Rubin, L.J. 1987 Prostacyclin and PGE_1 treatment of pulmonary hypertension. *Am. Rev. Respir. Dis.* **136**, 773–776.

Longmore, D.B., Bennett, J.G., Hoyle, P.M., Smith, M.A., Gregory, A., Osivand, T. & Jones, W.A. 1981 Prostacyclin administration during cardiopulmonary bypass in man. *Lancet* i, 800–804.

Lowenstein, C.J., Glatt, C.S., Bredt, D.S. & Snyder, S.H. 1992 Cloned and expressed macrophage nitric oxide synthase contrasts with the brain enzyme. *Proc. natn. Acad. Sci. U.S.A.* **89**, 6711–6715.

Lyons, C.R., Orloff, G.J. & Cunningham, J.M. 1992 Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J. biol. Chem.* **267**, 6370–6374.

MacIntyre, D.E., Pearson, J.D. & Gordon, J.L. 1978 Localisation and stimulation of prostacyclin production in vascular cells. *Nature, Lond.* **271**, 549–551.

Malmsten, C., Granström, E. & Samuelsson, B. 1976 Cyclic AMP inhibits synthesis of prostaglandin endoperoxide (PGG_2) in human platelets. *Biochem. biophys. Res. Commun.* **68**, 569–576.

Marks, J. 1962 Critical appraisal of the therapeutic value of α-tocopherol. *Vitam. Hormones* **20**, 573–598.

Marletta, M.A., Poskyn, S.Y., Iyengar, R., Leaf, C.D. & Wishnock, J.S. 1988 Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry* **27**, 8706–8711.

Martin, W., Villani, G.M., Jothianandan, D. & Furchgott, R.F. 1985 Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. exp. Ther.* **232**, 708–716.

Masini, E., Salvemini, D., Pistelli, A., Mannaioni, P.F. & Vane, J.R. 1991 Rat mast cells synthesize a nitric oxide-like factor which modulates the release of histamine. *Agents Actions* **33**, 61–63.

McMurdo, L., Corder, R., Thiemermann, C. & Vane, J.R. 1993 Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetized rat with BQ-123 provides evidence for more than one vasoconstrictor receptor. *Br. J. Pharmacol.* **108**, 557–561.

McMurray, J.J., Ray, S.G., Abdullah, I., Dargie, H.J. & Morton, J.J. 1992 Plasma endothelin in chronic heart failure. *Circulation* **85**, 1374–1379.

Meade, T.W. 1992 Aspirin and myocardial infarction with an annotation on venous thrombosis. In *Aspirin and other salicylates* (ed. J. R. Vane & R. M. Botting), pp. 321–353. London: Chapman and Hall.

Mickel, H. & Horbar, J. 1974 The effect of peroxidized arachidonic acid upon human platelet aggregation. *Lipids* **9**, 68–71.

Miller, M.J.S., Pinto, A. & Mullane, K.M. 1987 Impaired endothelium-dependent relaxations in rabbits subjected to aortic coarctation hypertension. *Hypertension* **10**, 164–170.

Minkes, M., Stanford, M., Chi, M., Roth, G., Raz, A., Needleman, P. & Majerus, P.J. 1977 Cyclic adenosine 3, 5-monophosphate inhibits the availability of arachidonate to prostaglandin synthetase in human platelet suspensions. *J. clin. Invest.* **59**, 449–454.

Mitchell, J.A., Hecker, M. & Vane, J.R. 1990 The generation of L-arginine in endothelial cells is linked to the release of endothelium-derived relaxing factor. *Eur. J. Pharmacol.* **176**, 253–254.

Miura, K., Yukimura, T., Yamashita, Y., Shimmen, T., Okumura, M., Imanishi, M. & Yamamoto, K. 1989 Endothelin stimulates the renal production of prostaglandin E_2 and I_2 in anesthetized dogs. *Eur. J. Pharmacol.* **170**, 91–93.

Miyauchi, T., Yanagisawa, M. & Tomizawa, T. 1989 Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* ii, 53–54.

Moncada, S., Gryglewski, R., Bunting, S. & Vane, J.R. 1976*a* An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature, Lond.* **263**, 663–665.

Moncada, S., Gryglewski, R.J., Bunting, S. & Vane, J.R. 1976*b* A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. *Prostaglandins* **12**, 715–737.

Moncada, S., Herman, A.G., Higgs, E.A. & Vane, J.R. 1977 Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb. Res.* **11**, 323–344.

Moncada, S., Radomski, M. & Vargas, J.R. 1982 The use of prostacyclin for the separation and washing of human platelets from plasma. *Br. J. Pharmacol.* **75**, 165P.

Moncada, S. & Vane, J.R. 1979 Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.* **30**, 293–331.

Moncada, S. & Vane, J.R. 1982 The prostacyclin/thromboxane balance and cardiovascular disease. In *Symposium celebrating the Harvard Medical School Bicentennial* (ed. K. J. Isselbacher), pp. 83–113. New York: John Wiley and Sons.

Moncada, S. 1982 Eighth Gaddum Memorial Lecture. University of London Institute of Education. December 1980. Biological importance of prostacyclin. *Br. J. Pharmacol.* **76**, 3–31.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Moncada, S., Palmer, R.M.J. & Higgs, E.A. 1991 Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43**, 109–142.

Monden, M. & Fortner, J.G. 1982 Twenty-four and 48-hour canine liver preservation by simple hypothermia with prostacyclin. *Ann. Surg.* **196**, 38–42.

Moore, K., Wendon, J., Frazer, M., Karani, J., Williams, R. & Badr, K. 1992 Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome. *New Engl. J. Med.* **327**, 1774–1778.

Murad, F. 1986 Cyclic guanosine monophosphate as a mediator of vasodilation. *J. clin. Invest.* **78**, 1–5.

Myers, P.R., Minor, R.L. Jr, Guerra, R. Jr, Bates, J.N. & Harrison, D.G. 1990 Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature, Lond.* **345**, 161–163.

Nakaki, T., Nakayama, S. & Kato, R. 1989 Endothelin-mediated stimulation of DNA synthesis in vascular smooth muscle cells. *Biochem. biophys. Res. Commun.* **158**, 880–883.

Nathan, C. 1992 Nitric oxide as a secretory product of mammalian cells. *FASEB J.* **6**, 3051–3064.

Nizankowski, R., Krolikowski, W., Bielatowicz, J. & Szczeklik, A. 1985 Prostacyclin for ischemic ulcers in peripheral arterial disease. A random assignment, placebo controlled study. *Thromb. Res.* **37**, 21–28.

Nobak, C.R., Tinker, J.H., Kaye, M.P., Holcombe, G.R. & Pluth, J.R. 1980 Prostacyclin: a potent vasodilator during anaesthesia and cardiac surgery in man. *Circulation* **62** (Suppl. 3), 1242.

Ogletree, M.L., Lefer, A.M., Smith, J.B. & Nicolaou, K.C. 1979 Studies on the protective effect of prostacyclin in acute myocardial ischemia. *Eur. J. Pharmacol.* **56**, 95–103.

Ohlendorf, R., Perzborn, E. & Schrör, K. 1980 Prevention of infarction-induced decrease in circulating platelet count by prostacyclin. *Thromb. Res.* **19**, 447–453.

Ondetti, M.A., Rubin, B. & Cushman, D.W. 1977 Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science, Wash.* **196**, 441–444.

Palmer, R.M.J., Ferrige, A.G. & Moncada, S. 1987 Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature, Lond.* **327**, 524–526.

Palmer, R.M.J., Ashton, D.S. & Moncada, S. 1988 Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature, Lond.* **333**, 664–666.

Palmer, R.M.J. & Moncada, S. 1989 A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem. biophys. Res. Commun.* **158**, 348–352.

Panza, J.A., Quyyumi, A.A., Brush, J.E. Jr & Epstein, S.E. 1990 Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *New Engl. J. Med.* **323**, 22–27.

Paton, W.D.M. & Vane, J.R. 1963 An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. *J. Physiol.* **165**, 10–46.

Paton, W.D.M. & Zaimis, E.J. 1949 The pharmacological actions of polymethylene bistrimethylammonium salts. *Br. J. Pharmacol. Chemother.* **4**, 381–400.

Peatfield, R.C., Gawel, M.J. & Clifford Rose, F. 1981 The effect of infused prostacyclin in migraine and cluster headache. *Headache* **21**, 190–195.

Pernow, J., Boutier, J.F., Franco-Cereceda, A., Lacroix, J.S., Matran, R. & Lundberg, J.M. 1989 Potent selective vasoconstrictor effects of endothelin in the pig kidney *in vivo*. *Acta physiol. scand.* **134**, 573–574.

Piper, P.J. & Vane, J.R. 1969 Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.* **223**, 29–35.

Radegran, K., Egberg, N. & Papaconstantinou, C. 1981 Effects of prostacyclin during cardiopulmonary bypass in man. *Scand. J. Thorac. Cardiovasc. Surg.* **15**, 263–268.

Radomski, M.W., Palmer, R.M.J. & Moncada, S. 1987*a* Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* ii, 1057–1058.

Radomski, M.W., Palmer, R.M.J. & Moncada, S. 1987*b* The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br. J. Pharmacol.* **92**, 639–646.

Radomski, M.W., Palmer, R.M.J. & Moncada, S. 1990 Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc. natn. Acad. Sci. U.S.A.* **87**, 10043–10047.

Raines, E.W. & Ross, R. 1993 Smooth muscle cells and the pathogenesis of the lesions of atherosclerosis. *Br. Heart J.* **69** (Suppl. 1), S30–S37.

Rajfer, J., Aronson, W.J., Bush, P.A., Dorey, F.J. & Ignarro, L.J. 1992 Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *New Engl. J. Med.* **326**, 90–94.

Rees, D.D., Palmer, R.M.J., Hodson, H.F. & Moncada, S. 1989*a* A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.* **96**, 418–424.

Rees, D.D., Palmer, R.M.J. & Moncada, S. 1989*b* Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. natn. Acad. Sci. U.S.A.* **86**, 3375–3378.

Rees, D.D., Cellek, S., Palmer, R.M.J. & Moncada, S. 1990 Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: an insight into endotoxin shock. *Biochem. biophys. Res. Commun.* **173**, 541–547.

Ribeiro, L.G.T., Brandon, T.A., Hopkins, D.G., Reduto, L.A., Taylor, A.A. & Miller, R.R. 1981 Prostacyclin in experimental myocardial ischemia: effects on hemodynamics, regional myocardial blood flow, infarct size and mortality. *Am. J. Cardiol.* **47**, 835–840.

Rosenblum, W.I. & El Sabban, F. 1979 Topical prostacyclin (PGI_2) inhibits platelet aggregation in pial venules of the mouse. *Stroke* **10**, 399–401.

Ross, R. 1986 The pathogenesis of atherosclerosis – an update. *New Engl. J. Med.* **314**, 488–500.

Saito, Y., Nakao, K., Itoh, H., Yamada, T., Mukoyama, M., Arai, H., Hosoda, K., Shirakami, G., Suga, S., Jougasaki, M., Morichika, S. & Imura, H. 1989 Endothelin in human plasma and culture medium of aortic endothelial cells detection and characterization with radioimmunoassay using monoclonal antibody. *Lancet* i, 320–326.

Sakaguchi, S., Tanabe, T., Mishima, Y., Shionoya, Y., Katsumura, T., Kusaba, A. & Sakuma A. 1990*a* Effect of beraprost sodium (PGI_2 derivative) in Rayneaud's Disease or Syndrome. A comparative double blind study with placebo. *Clinics Res.* **67**, 234–242.

Sakaguchi, S., Tanabe, T., Mishima, Y., Shionoya, Y., Katsumura, T., Kusaba, A. & Sakuma, A. 1990*b* Effect of beraprost sodium (PGI_2 derivative) in chronic arterial occlusive disease. A comparative double blind study with Ticlopidine Hydrochloride. *Clinics Res.* **67**, 575–584.

Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K. & Masaki, T. 1990 Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature, Lond.* **348**, 732–735.

Sakurai, T., Yanagisawa, M. & Masaki, T. 1992 Mole-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

cular characterization of endothelin receptors. *Trends Pharmacol. Sci.* **13**, 103–108.

Salmon, J.A., Smith, D.R., Flower, R.J., Moncada, S. & Vane, J.R. 1978 Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. *Biochim. biophys. Acta* **523**, 250–262.

Salvemini, D., Masini, E., Pistelli, A., Mannaioni, P.F. & Vane, J. 1991 Nitric oxide: a regulatory mediator of mast cell reactivity. *J. cardiovasc. Pharmacol.* **17** (Suppl. 3), S258–S264.

Samuelsson, B., Borgeat, P., Hammarström, S. & Murphy, R.C. 1979 Introduction of a nomenclature: leukotrienes. *Prostaglandins* **17**, 785–787.

Sarih, M'h., Souvannavong, V. & Adam, A. 1993 Nitric oxide induces macrophage death by apoptosis. *Biochem. biophys. Res. Commun.* **191**, 503–508.

Schirner, M. & Schneider, M.R. 1992 Antimetastatic potential of the stable prostacyclin analogue Cicaprost. In *Prostacyclin: new perspectives in basic research and novel therapeutic indications* (ed. J. Vane & G. Rubanyi), pp. 247–275. Amsterdam: Elsevier Science Publications.

Schröder, H. & Schrör, K. 1989 Cyclic GMP stimulation by vasopressin in LLC-PK_1 kidney epithelial cells is L-arginine-dependent. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340**, 475–477.

Sessa, W.C., Harrison, J.K., Barber, C.M., Zeng, D., Durieux, M.E., D'Angelo, D.D., Lynch, K.R. & Peach, M.J. 1992 Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J. biol. Chem.* **267**, 15274–15276.

Shetty, S.S., Okada, T., Webb, R.L., DelGrande, D. & Lappe, R.W. 1993 Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. *Biochem. biophys. Res. Commun.* **191**, 459–464.

Simonson, M.S., Wann, S., Mene, P., Dubyak, G.R., Kester. M., Nakazato, Y., Sedor, J.R. & Dunn, M.J. 1989 Endothelin stimulates phospholipase C, Na^+/H^+ exchange, c-fos expression and mitogenesis in rat mesangial cells. *J. clin. Invest.* **83**, 708–712.

Sinzinger, H., Feigl, W. & Silberbauer, K. 1979 Prostacyclin generation in atherosclerotic arteries. *Lancet* ii, 469.

Sisler, H.H. 1956 Nitrogen, phosphorus, arsenic, antimony and bismuth. In *Comprehensive inorganic chemistry* (ed. M. C. Sneed & R. C. Brasted), vol. 5, pp. 60–77. Princeton and Van Nostrand.

Slater, T.F. 1972 *Free radical mechanisms in tissue injury*. London: Pion Ltd.

Sneddon, J.M. & Vane, J.R. 1988 Endothelium-derived relaxing factor reduces platelet adhesion to bovine endothelial cells. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2800–2804.

Starnes, V.A., Primm, R.K., Woosley, R.L., Oates, J.A. & Hammon, J.W. 1982 Administration of prostacyclin prevents ventricular fibrillation following coronary occlusion in conscious dogs. *J. cardiovasc. Pharmacol.* **4**, 765–769.

Stewart, D.J., Cernacek, P., Costello, K.B. & Rouleau, J.L. 1992 Elevated endothelin-1 in heart failure and loss of normal response to postural change. *Circulation* **85**, 510–517.

Stuehr, D.J. & Marletta, M.A. 1985 Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. *Proc. natn. Acad. Sci. U.S.A.* **82**, 7738–7742.

Stuehr, D.J. & Nathan, C.F. 1989 Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J. exp. Med.* **169**, 1543–1545.

Suzuki, N., Matsumoto, H., Kitada, C., Masaki, T. & Fujino, M. 1989 A sensitive sandwich-enzyme immunoassay for human endothelin. *J. immunol. Meth.* **118**, 245–250.

Szabó, C., Mitchell, J.A., Thiemermann, C. & Vane, J.R. 1993*a* Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. *Br. J. Pharmacol.* **108**, 786–792.

Szabó, C., Mitchell, J.A., Gross, S.S., Thiemermann, C. & Vane, J.R. 1993*b* Nifedipine inhibits the induction of nitric oxide synthase by bacterial lipopolysaccharide. *J. Pharmacol. exp. Ther.* **265**, 674–680.

Szczeklik, A., Gryglewski, R.J., Nizankowski, R., Musial, J., Pieton, R. & Mruk, J. 1978 Circulatory and anti-platelet effects of intravenous prostacyclin in healthy men. *Pharmac. Res. Commun.* **10**, 545–556.

Szczeklik, A., Nizankowski, R., Slawinski, S., Szczeklik, G., Gluszko, P. & Gryglewski, R.J. 1979 Successful therapy of advanced arteriosclerosis obliterans with prostacyclin. *Lancet* i, 1111–1114.

Szczeklik, A. & Gryglewski, R.J. 1981 Treatment of vascular disease with prostacyclin. In *Clinical pharmacology of prostacyclin.* (ed. P. J. Lewis & J. O'Grady) pp. 159–167. New York: Raven Press.

Takuwa, N., Takuwa, Y., Yanagisawa, M., Yamashita, K. & Masaki, T. 1989 A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. *J. biol. Chem.* **264**, 7856–7861.

Tateson, J.E., Moncada, S. & Vane, J.R. 1977 Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins* **13**, 389–397.

Thiemermann, C., Lidbury, P., Thomas, R. & Vane, J. 1988 Endothelin inhibits ex vivo platelet aggregation in the rabbit. *Eur. J. Pharmacol.* **158**, 181–182.

Thiemermann, C., Lidbury, P.S., Thomas, G.R. & Vane, J.R. 1989 Endothelin-1 releases prostacyclin and inhibits ex vivo platelet aggregation in the anesthetized rabbit. *J. cardiovasc. Pharmacol.* **13** (Suppl. 5), S138–S141.

Thiemermann, C., Szabó, C., Mitchell, J.A. & Vane, J.R. 1993 Vascular hyporeactivity to vasoconstrictor agents and hemodynamic decompensation in hemorrhagic shock is mediated by nitric oxide. *Proc. natn. Acad. Sci. U.S.A.* **90**, 267–271.

Thiemermann, C. & Vane, J. 1990 Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. *Eur. J. Pharmacol.* **182**, 591–595.

Toda, N. 1984 Endothelium-dependent relaxation induced by angiotensin II and histamine in isolated arteries of dog. *Br. J. Pharmacol.* **81**, 301–307.

Tomita, K., Ujiie, K., Nakamishi, T., Tomura, S., Matsuda, D., Ando, K., Shichiri, M., Hirata, Y. & Marumo, F. 1989 Plasma endothelin levels in patients with acute renal failure. *New Engl. J. Med.* **321**, 1127.

Torley, H.I., Madhok, R., Capell, H.A., Brouwer, R.M.L., Maddison, P.J., Black, C.M., Englert, H., Dormandy, J.A. & Watson, H.R. 1991 A double blind, randomised, multicentre comparison of two doses of intravenous iloprost in the treatment of Raynaud's phenomenon secondary to connective tissue diseases. *Ann. Rheum. Dis.* **50**, 800–804.

Totsune, K., Mouri, T., Takahashi, M., Ohneda, M., Sone, M., Saito, T. & Yoshinaga, K. 1989 Detection of immunoreactive endothelin in plasma of hemodialysis patients. *FEBS Lett.* **249,** 239–242.

Toyo-oka, T., Aizawa, T., Suzuki, N., Hirata, Y., Miyauchi, T., Shin, W.S., Yanagisawa, M., Masaki, T. & Sugimoto, T. 1991 Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation* **83**, 476–483.

Trachtenberg, J.D., Sun, S., Rapp, N.S., Choi, E.T.,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Callow, A.D. & Ryan, U.S. 1993 The effect of ET-1 infusion on the development of intimal hyperplasia following balloon catheter injury. *J. cardiovasc. Pharmacol.* **22** (Suppl. 8), S355–S359.

Ubatuba, F.B., Moncada, S. & Vane, J.R. 1979 The effect of prostacyclin (PGI_2) on platelet behaviour, thrombus formation in vivo and bleeding time. *Thromb. Haemostasis* **41**, 425–435.

Vallance, P., Collier, J. & Moncada, S. 1989 Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* ii, 997–1000.

Vane, J.R. 1971*a* Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, Lond.* **231**, 232–235.

Vane, J.R. 1971*b* Mediators of the anaphylactic reaction. In *Identification of Asthma. Ciba Foundation Study No. 38.* pp. 121–131. Edinburgh and London: Churchill Livingstone.

Vane, J.R. 1983 Clinical potential of prostacyclin. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **11**, 449–456.

Vane, J.R. 1982 Prostacyclin: a hormone with a therapeutic potential. The Sir Henry Dale Lecture for 1981. Given at the Institute of Education, London 27.11.81. *J. Endocrinol.* **95**, 3P–43P.

Virgolini, I., Fitscha, P., Linet, O.I., O'Grady, J. & Sinzinger, H. 1990 A double blind placebo controlled trial of intravenous prostacyclin (PGI_2) in 108 patients with ischaemic peripheral vascular disease. *Prostaglandins* **39**, 657–664.

Virgolini, I., Fitscha, P., Weiss, K., Linet, O.I., O'Grady, J. & Sinzinger, H. 1991 Intravenous prostacyclin (PGI_2) infusion to 108 patients with ischaemic peripheral vascular disease: Phase II-open study. *Prostaglandins* **42**, 9–14.

Walker, I.D., Davison, J.F., Faichney, A., Wheatley, D. & Davidson, K. 1981 Prostacyclin in cardiopulmonary bypass surgery. In *Clinical pharmacology of prostacyclin* (ed. P. J. Lewis & J. O'Grady) pp. 195–199. New York: Raven Press.

Warner, T.D., Allcock, G.H., Mickley, E.J., Corder, R., Vane, J.R. 1993 Comparative studies with the endothelin receptor antagonists BQ-123 and PD 142893 indicate at least three endothelin receptors. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S117–S120.

Watson, H.R. & Belcher, G. 1991 Retrospective comparison of iloprost with other treatments for secondary Raynaud's phenomenon. *Ann. Rheum. Dis.* **50**, 359–361.

Welsh, N. & Sandler, S. 1992 Interleukin-1β induces nitric oxide production and inhibits the activity of aconitase without decreasing glucose oxidation rates in isolated mouse pancreatic islets. *Biochem. biophys. Res. Commun.* **182**, 333–340.

Weksler, B.B., Marcus, A.J. & Jaffe, E.A. 1977 Synthesis of prostaglandin I_2 (prostacyclin) by cultured human and bovine endothelial cells. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3922–3926.

Wellings, R.P., Warner, T.D., Thiemermann, C., Corder, R. & Vane, J.R. 1993 Vasoconstriction in the kidney induced by endothelin-1 is blocked by PD 145065. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S103–S106.

White, D.G., Cannon, T.R., Garratt, H., Mundin, J.W., Sumner, M.J. & Watts, I.S. 1993 Endothelin ET_A and ET_B receptors mediate vascular smooth muscle contraction. *J. cardiovasc. Pharmacol.* **22**(Suppl. 8), S144–S148.

Whittle, B.J.R. 1980*a* Actions of prostaglandins on gastric mucosal blood flow. In *Gastro-intestinal mucosal blood flow* (ed. L. P. Fielding), pp. 180–191. Edinburgh and London: Churchill Livingstone.

Whittle, B.J.R. 1980*b* Role of prostaglandins in the defense of the gastric mucosa. *Brain Res. Bull.* **5** (Suppl. 1), 7–14.

Willis, A.L., Smith, D.L., Vigo, C. & Kluge, A.F. 1987 Effects of prostacyclin and orally stable mimetic agent RS-93427-007 on basic mechanisms of atherogenesis. *Lancet* ii, 682–683.

Xie, Q.-w., Cho, H.J., Calaycay, J., Mumford, R.A., Swiderek, K.M., Lee, T.D., Ding, A., Troso, T. & Nathan, C. 1992 Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science, Wash.* **256**, 225–228.

Xie, W., Chipman, J.G., Robertson, D.L., Erikson, R.L. & Simmons, D.L. 1991 Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. natn. Acad. Sci. U.S.A.* **88**, 2692–2696.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. & Masaki, T. 1988 A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature, Lond.* **332**, 411–415.

Yui, Y., Nakajima, H., Kawai, C. & Murakami, T. 1982 Prostacyclin therapy in patients with congestive heart failure. *Am. J. Cardiol.* **50**, 320–324.

Lecture delivered 11 March 1993

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES