PROSTACYCLIN IN HEALTH AND DISEASE*

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The subject of this article is the relationship between prostaglandins that are important for cardiovascular health and disease. For many years it has been known that prostaglandins are made by almost all tissues. They are released very easily from those tissues by any kind of distortion of the cell membrane, by chemicals or simply by gently touching the surface of the tissue.

Our involvement in the field of prostaglandins goes back to 1967, when Sergio Ferreira and I demonstrated that prostaglandins (PGs) E_2 and $F_{2\alpha}$ were almost completely removed during one passage through the pulmonary circulation¹⁾. It was from this observation that we proposed that prostaglandins were local hormones, which were released locally, had an activity locally, and then were destroyed before they entered the arterial circulation.

In 1969 with Priscilla Piper²⁾, we showed that during anaphylaxis of guinea pig lungs, as well as release of the usual mediators there was also a release of PGE_2 and $PGF_{2\alpha}$, together with a strange ephemeral substance which, because of its activity, we called rabbit aorta contracting substance or RCS. This substance was very unstable and disappeared from the solution within 15 minutes.

In 1971 we went on to show that aspirin and similar drugs inhibit the biosynthesis of prostaglandins and I proposed that this was the mode of action³⁾. In other words, the activity of aspirin depends upon the fact that it is removing prostaglandins from the inflammatory site. So prostaglandins contribute to inflammation, to the febrile response and to pain. The aspirin-like drugs, of which several hundred are known, all

show the same common side effects, namely a tendency to produce stomach ulcers and kidney toxicity, and prolongation of the birth process. When the same group of chemically diverse substances all show the same prime effect and the same side effects, it is fairly safe to assume that these side effects are due to the same activity as the prime effects. So we proposed that in addition to inhibiting a pathological release of the prostaglandin which was causing inflammation, fever and pain, aspirin was also removing a physiological release of a prostaglandin that was necessary to prevent ulceration of the stomach, to keep the kidney healthy, and to assist the expulsion of the uterine contents at birth. At that time, however, we could not understand the well known anti-thrombotic effects of aspirin because neither PGE_2 nor $PGF_{2\alpha}$, which were the two major prostaglandins then known, had much effect on platelet aggregation.

It was not until 1973 when Samuelsson and others isolated and biosynthesized the endoperoxide intermediates that we began to understand the actions of aspirin on platelets^{4,5)}. In 1974 it was shown that these unstable endoperoxide intermediates would cause aggregation of platelets⁶⁾.

In 1975 Samuelsson and his team elucidated the structure of a new compound made in platelets which they called thromboxane A_2 ⁷⁾ and later we showed that this was in fact the same substance that we had previously demonstrated to be released in anaphylaxis and which we had called RCS⁸⁾. It was now possible to understand the activity of aspirin in platelets.

Any stimulation of platelets to aggregate leads to the activation of platelet phospholipase which releases arachidonic acid from the phospholipid pool. This in turn leads to cyclic endoperoxide formation. There is an enzyme in the platelet

* 1980年12月, 日本動脈硬化学会 昭和55年度冬季大会において発表

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Fig. 1 Derivation and structures of prostacyclin and thromboxane A₂, with their chemical breakdown products.

Fig. 2 Inhibition of platelet aggregation by PGI₂ generated from rings of mesenteric artery from indomethacin and non-indomethacin treated rabbits. Cut rings of mesenteric artery (6 mg) from a non-indomethacin treated rabbit were incubated in 1 ml of Tris buffer (0.05 M, pH 7.5) for 3 minutes. An aliquot (20 μl) of the supernatant from the incubation mixture added to PRP was sufficient to inhibit ADP (10 μM)—induced aggregation. Similarly, arterial rings (6 mg) from a non-indomethacin treated animal, when incubated with the PRP itself for 6 minutes produced a principle which inhibited ADP-induced aggregation. Rings of mesenteric artery (6 mg) taken from an indomethacin treated rabbit and incubated in Tris buffer for 3 min did not spontaneously produce anti-aggregating activity. However, when the rings were added to PRP, inhibition of ADP-induced aggregation resulted.

“microsomal” fraction which transforms endoperoxides to thromboxane A₂ and induces platelet aggregation. Aspirin inhibits aggregation by preventing formation of the endoperoxides and

therefore of thromboxane A₂.

In 1976 the group working with me at Wellcome isolated a new prostaglandin, which had not been detected before, from the vessel walls⁹⁾.

It was potentially antiaggregatory, was also a vasodilator and, like thromboxane A_2 , was unstable. Because we did not know the structure we called it PGX, and later in the year we renamed it prostacyclin when, in collaboration with Upjohn scientists at Kalamazoo, we were able to determine the structure¹⁰.

Thus, there is the formation of two substances with opposing activities from a common precursor through the endoperoxide intermediates. In the platelet there is the formation of thromboxane A_2 which leads to platelet aggregation and is a potent vasoconstrictor substance. It is unstable with a half life of about 30 seconds and breaks down chemically to an inert substance, thromboxane B_2 . On the other hand, in the vessel wall, the same precursors are transformed by a different enzyme system into prostacyclin, which we now abbreviate to PGI_2 . This is a potent anti-aggregating substance and a potent vasodilator. It is also unstable and breaks down chemically with a half life of 2 or 3 minutes to 6-oxo- $PGF_{1\alpha}$, which is also relatively inert. (For structures see Fig. 1).

I shall now discuss the balance between these two opposing substances, one made by the blood vessel wall and one by the platelets, and how this balance can be upset in certain disease states. I will also describe how we see the therapeutic potential for prostacyclin.

First, let me tell you how some of these activities have been demonstrated. Platelet aggregation is measured using the Born aggregometer¹¹ which is a device for detecting light transmission in platelet-rich plasma. While the platelets are suspended in a fine cloud, little light goes through to the photocell. Addition of an aggregating agent causes the platelets to clump together so that more light goes through the plasma. This process of aggregation can be monitored by displaying on a pen recorder the amount of light which is transmitted.

Very low concentrations of prostacyclin will prevent aggregation induced by ADP, arachidonic acid or any other known aggregating agent. Furthermore, when the platelets have formed aggregates, the addition of a small quantity of prostacyclin disaggregates the clumps. This is an important principle because if there is a thrombus forming in the circulation, it could be disaggregated using prostacyclin.

The second important concept derives from

experiments in which we started to use small pieces of artery taken from an animal or from man as a source of prostacyclin activity. When these are incubated in a test tube on the bench, prostacyclin activity can be detected in the supernatant, showing that the arterial tissue is making prostacyclin. This can be demonstrated by transferring a small aliquot of the supernatant into the platelet cuvette and observing inhibition of platelet aggregation (Fig. 2). Pieces (or rings) of the tissue itself when added to the cuvette will also prevent platelet aggregation. Now the importance of Fig. 2 is shown in the second half, because here the artery has been treated with a prostaglandin synthetase inhibitor, indomethacin, which stops the artery from making prostacyclin from its own endogenous arachidonic acid by preventing the formation of endoperoxides from arachidonic acid. When the treated ring is incubated in a test tube, prostacyclin can no longer be detected in the supernatant. However, that same indomethacin-treated ring, when added to the platelet cuvette, can still prevent the platelets from aggregating.

The conclusion we drew from this experiment was that arterial walls can make prostacyclin from endoperoxides liberated by the platelets. This conclusion has proved controversial. There is now substantial evidence, based upon the use of labelled arachidonic acid incorporated into the platelet membranes, that when platelets are stimulated they will release labelled endoperoxides, and these can be utilized by blood vessel walls to make prostacyclin¹². Obviously this is an important biochemical co-operation which could take place only if there is close proximity between the platelets and the blood vessel walls.

I mentioned that prostacyclin is also a vasodilator and this was demonstrated in many vascular beds, including the circulation of a dog's heart¹³. An anaesthetized open chest dog, with a flow-meter on a coronary artery, was injected arterially with 1 μ g of prostacyclin giving a marked increase in coronary blood flow. Interestingly, even when prostacyclin was dripped on to the epicardial surface there was a brisk increase in blood flow, showing that it penetrates the surface very rapidly¹⁴. Penetration of surfaces could well be useful from the point of view of administering prostacyclin or one of its analogues. It will cause an increase in blood flow in every part of the circulation, including the pulmonary

circulation.

Another important property of prostacyclin is that, unlike prostaglandins E_2 and F_2 , it passes through the lungs unchanged. It is not inactivated by the pulmonary circulation¹⁵⁾. This means that it is potentially a circulating hormone. Most of the prostacyclin formed by blood vessel walls is produced by the endothelial cells. About one half of the total body endothelial cells are contained in the pulmonary circulation. It is now evident both from animal experiments and from man that prostacyclin is, or can be, liberated from the lungs into arterial blood, and is thus a circulating hormone^{16,17)}. The most convincing evidence for this comes from the Hammersmith Hospital in London where Professor Dollery and his group have shown, using cardiac catheterization, that the level of the prostacyclin breakdown product, 6-oxo-PGF_{1 α} is higher in arterial blood of unanaesthetized subjects than in venous blood¹⁸⁾. So, there must be some prostacyclin being released by the pulmonary circulation. Again, this is a controversial point. Some people do not believe that the lungs contribute to arterial concentrations of prostacyclin, but I think we shall have a definitive answer in the next two years.

Lipid peroxides are potent inhibitors of prostacyclin synthetase, the enzyme which forms prostacyclin from the endoperoxide intermediates. The first lipid peroxide we studied was 15-hydroperoxy arachidonic acid, 15-HPAA¹⁹⁾, but many different ones have very similar IC₅₀ values of about 1 μ M²⁰⁾. Indeed, every lipid peroxide that we have examined is a potent inhibitor of prostacyclin formation. Thus an antioxidant, such as vitamin E which acts as a radical scavenger, could well protect the enzyme which forms prostacyclin from this inhibition. This is another fairly important concept, especially when you remember that atheromatous plaques contain quite high concentrations of lipid peroxides. Indeed looking at atheromatous plaques from man, it has been shown that the plaque itself cannot generate prostacyclin whereas a piece of nearby vessel can²¹⁾.

The use of lipid peroxides has enabled us to examine the arterial wall to find out whether there are other mechanisms which will prevent platelet aggregation. It was shown that human arterial rings added into the platelet cuvette caused an inhibition of platelet aggregation.

When these rings are treated with a lipid peroxide (15-HPAA) they will no longer inhibit platelet aggregation, showing that this inhibition is due to the formation of prostacyclin and also showing that once you have removed the prostacyclin formation there is nothing left in that arterial wall which will prevent platelet aggregation.

When the arterial wall is dissected into the intima, internal elastic lamina, media and adventitia, and the individual components are examined, we find that although the intima accounts for only 5% of the total vessel wall weight, it accounts for some 40% of its ability to produce prostacyclin. Furthermore, the ability to produce prostacyclin progressively decreases from the intima to the adventitia. When these pieces of tissue are treated with a lipid peroxide to prevent prostacyclin formation, the pro-aggregating elements of the vessel are exposed. The adventitia will cause platelet aggregation, and the pro-aggregating activity decreases from the adventitia to the intima. Thus, across the arterial wall there are two opposing elements, the pro-aggregating factors concentrated on the outside and the anti-aggregating on the inside²²⁾.

For many years people have been saying that the arterial wall is inert to platelets or that it is smooth and slippery. However, from the point of view of flowing platelets it is anything but smooth. When there is damage to the endothelial lining the platelets come into contact with more pro-aggregating material. They adhere to the damaged area and they can begin to repair that damage.

When an arterial wall is cut, the first thing that happens is that the platelets adhere to the side of the cut and begin to form a thrombus to prevent bleeding to death. This is a very rapid, positive feedback process. As soon as platelets start clumping they begin to release thromboxane A_2 and ADP. Both of these substances enable more platelets to stick together until the whole of that gap is plugged. This is a strong, positive feedback mechanism but have you ever wondered why it doesn't continue so that the platelet clump grows until it fills the lumen of the vessel? I think that there are two reasons for this. First, the healthy endothelial cells around the edge of the cut, by making prostacyclin, will prevent further deposition of the platelets. Secondly, the blood itself might contain a very low concentration of prostacyclin.

So, thromboxane A_2 is involved physiologically in plugging breaks in vessel walls, whereas prostacyclin prevents platelets from clumping on to the lining in healthy vessels. But what happens when the lining of the vessels is not healthy? Where an atherosclerotic plaque has ruptured and platelets aggregate on to the surface of that plaque, the aggregate grows, perhaps releasing microaggregates into the microcirculation, causing blocks further downstream. Possibly it will grow to occlude the vessel fully. If this happens in a vessel supplying the heart or the brain, then it results in a heart attack or a stroke.

Something like this can be shown experimentally using anaesthetized rabbits. When the carotid artery of an anaesthetized rabbit is lifted on to a pair of electrodes and then stimulated electrically for 2 or 3 minutes, damage is caused to the inside of the artery. Over the next 30 minutes or so, the blood flow through the artery gradually decreases until suddenly it stops. When it stops and the artery is opened, a large white platelet thrombus is found inside.

When the experiment is repeated with a second carotid artery, but just before the blood flow stops an intravenous infusion of prostacyclin is started, the blood flow quickly recovers. If this artery is opened, although there is an area of damage, there is no platelet thrombus. This once more

illustrates that prostacyclin will dissolve or disaggregate a platelet thrombus²³.

This process can be examined in even more detail microscopically using a method which was developed by Baumgartner, and involves removing endothelial cells from arterial walls. After exposing the vessel walls to blood he was able to show that platelet clumps form on the de-endothelialized surface. We have repeated this and added a low concentration of prostacyclin to see how the formation of thrombi and the adhesion of platelets to this de-endothelialized surface are affected. We found that in very low concentrations prostacyclin prevents the aggregation or sticking of platelets to each other. It does not prevent the adhesion as there is still a monolayer of platelets adhering to the piece of aortic tissue. A very much higher concentration of prostacyclin is needed in order to prevent platelet adhesion²⁴.

Thus, it is our proposal that in physiological concentrations, prostacyclin might well allow platelets to adhere to vascular tissue and interact with it. Thus the platelets are able to participate in the repair process and may provide endoperoxides for prostacyclin synthesis by nearby endothelial cells, so preventing further platelets from aggregating on the vessel surface.

Another way of studying the interaction between platelets and blood vessels or collagen dynamically

EFFECT OF PROSTAGLANDINS ON cAMP CONTENT OF HUMAN PLATELET RICH PLASMA

Fig. 3 Elevation of cyclic AMP concentration in human PRP after 30 sec incubation with prostacyclin, PGD₂, PGE₁, or 6-oxo-PGF_{1α}. For each point results of 7 observations (plasma from two donors) have been combined. The cyclic AMP concentrations are expressed as multiples of the basal level.

was devised by Gryglewski in Crakow, Poland, adopting the blood bathed technique which we have used in our laboratory for many years. He takes heparinized blood from anaesthetised cats or rabbits, and passes it over tendons or blood vessels. The weight of tendon gradually increases because of deposition of platelets and this is monitored by a transducer¹⁶⁾.

When he made rabbits atherosclerotic by feeding them a diet rich in cholesterol and olive oil for 5 months and then compared aortic strips from atherosclerotic and control animals in this system, he found that the weight of atherosclerotic aortae increased considerably due to deposition of platelets, and probably other cells as well, whereas the weight of the healthy aortae did not increase. He also looked at the ability of tissues to generate prostacyclin and found that the blood vessels and heart from the atherosclerotic rabbit could not generate as much prostacyclin as tissues from a normal animal²⁵⁾.

More recently it has been proposed that this is due to the lipid peroxide content of the atheromatous tissue. It has been known for some two or three years that low density lipoproteins (LDL's) will inhibit prostacyclin production. Gryglewski and his colleagues have now shown that in people with hyperlipidemia the LDL fraction contains a high concentration of lipid peroxides, whereas the high density lipoprotein (HDL) fraction does not. Gryglewski proposes that this accounts for the deleterious effects of LDL and also accounts for the fact that it will inhibit prostacyclin biosynthesis.

How does prostacyclin prevent platelet aggregation? It has been known for some years that other prostaglandins like PGE_1 or PGD_2 prevent platelet aggregation and that they will at the same time increase the cyclic AMP concentrations inside the platelets. We have shown that just as prostacyclin is some 20 to 40 times more potent than PGE_1 or PGD_2 in preventing platelet aggregation, so it is very much more potent in stimulating formation of platelet cyclic AMP^{26,27)} (Fig. 3). As the amounts of cyclic AMP which are needed to inhibit aggregation are quite low prostacyclin is stimulating a much greater production of platelet cyclic AMP than is needed to inhibit aggregation.

What we imagine is happening in the circulation is that when a platelet tries to adhere to the

blood vessel wall, that vessel wall can make prostacyclin either from its own endogenous precursors or from the endoperoxides liberated by the platelets. In either event, that prostacyclin will increase platelet cyclic AMP and prevent other platelets from attaching to the one already there. However, if a platelet contacts an artificial surface rather than a vessel wall, that artificial surface cannot make prostacyclin. So, endoperoxides are utilized by the platelets to make thromboxane A_2 , which decreases platelet cyclic AMP and will allow other platelets to aggregate on to the first one. This is an area in which we foresee a therapeutic potential for prostacyclin. Prostacyclin is unstable, but in fact we can stabilise it pharmaceutically. By increasing the pH to 10.5 and freeze drying it, we can make a stable preparation for clinical use. However, when it gets into the circulation, it becomes unstable again. This could be an advantage if one is trying to prevent platelet deposition on to artificial surfaces only while the blood is coming into contact with an extracorporeal circulation. Prostacyclin has now been used beneficially in several systems of extracorporeal circulation, both in animals and in man. These include charcoal hemoperfusion (the artificial liver), hemodialysis (the artificial kidney), and cardiopulmonary bypass, a system used during heart operations throughout the world.

We used heparinized dogs and took the blood through a charcoal column and then passed it back into the dog. We measured the concentration of platelets in the blood going to and from the column, and measured the microaggregates which were returning to the animal. Charcoal hemoperfusion has never been used successfully on a wide scale clinically because of its effect on platelets. In our experiments heparinized blood within 30 minutes of going through the charcoal column lost some 80% of its total platelets. Those which were left became refractory to aggregation. These platelets stick to the charcoal column. However, when prostacyclin is used together with heparin there is a preservation of platelet function and platelet number²⁸⁾.

Dr. Roger Williams at King's College Hospital in London has now applied this technique to man²⁹⁾. He has been a proponent of charcoal hemoperfusion for many years but his success rate has not been very high. He has now treated

some patients with fulminant hepatic failure. He had 14 patients who started from Grade III coma and went on to grade IV, and 19 in grade IV. By hemoperfusing the 14 patients once a day for 5 or 6 days, using a charcoal column with prostacyclin to prevent platelet clumping, he achieved a 60% survival rate. With the grade IV coma patients he obtained a 30% survival rate. He is now very strongly convinced that charcoal hemoperfusion will be made possible by prostacyclin and that the success rate will be very much better than in the past.

The second system that we studied is hemodialysis. Using the same dog system which I outlined before, we were able to show a preservation of platelets during hemodialysis. What became even more exciting was when we decided to lower the dose of heparin. With the charcoal column we could not lower the dose of heparin substantially because of the very strong stimulus to coagulation caused by the charcoal. However, in the milder conditions of hemodialysis we found that we did not need heparin when we were using prostacyclin. Prostacyclin not only prevented the rise in the screen filtration pressure which is indicative of microaggregates going back into the circulation, but it also prevented coagulation during the period of hemodialysis³⁰. This can only mean that during hemodialysis the process of blood coagulation is initiated by a cell, perhaps the platelet, which is affected by prostacyclin. We do not envisage that prostacyclin will be used routinely during hemodialysis three times a week for every patient. What we do foresee is that it will be used in those patients where heparin is contraindicated, perhaps after major operations.

There are already several examples round the world of patients who have been dialysed successfully in the absence of heparin using prostacyclin. Dr. Weston in London, Dr. Dunn and Dr. Zusman³¹ in the United States, and Professor Kerr in Newcastle U. K., have all successfully used prostacyclin to dialyse patients in whom heparin was contraindicated.

The third area we have been looking at is the therapeutic potential of prostacyclin in cardiopulmonary bypass. We have sponsored clinical trials in Paris, Glasgow, London, Manchester and Hamburg. In all these clinical trials, prostacyclin or a placebo has been used on a double-blind basis.

Regarding the platelet function and platelet number, all the trials show the same general effect. That is, without prostacyclin in the placebo group there is a significant reduction in platelet count and also in platelet function. Those platelets which are left in the circulation are no longer functional. However, when prostacyclin is used during cardiopulmonary bypass, there is a significant improvement in the platelet count.

The four different trials have also given some additional information. In the Glasgow trial a filter was used in the lines going to and from the extracorporeal apparatus. Without prostacyclin some of the holes in the filter were completely blocked by platelet aggregates. Indeed many surgeons will not use filters during cardiopulmonary bypass because they believe that the filter itself will generate microaggregates which can go back into the circulation and cause cerebral or kidney damage. One filter was taken from the apparatus of a patient who received prostacyclin and on examination it was clean, apart from a few leucocytes sticking at one or two places.

In the National Heart Hospital trial³², there was also a significant reduction in postoperative blood loss. The placebo group of patients over a period of 18 hours lost almost one litre of blood, whereas this was reduced by about a half, to less than 500 ml in the group who were treated with prostacyclin. In fact, with all trials there was a reduction in blood loss in the prostacyclin group. However, this only reached statistical significance in the trials at the National Heart Hospital and in Manchester. What this tells us is that postoperative hemostasis is likely to be better in the prostacyclin group. Because the inhibitory effects of prostacyclin on platelet function wear off fairly quickly there are platelets left in the circulation which can contribute to hemostasis after the operation.

Our conclusions in cardiopulmonary bypass are that prostacyclin infusions will inhibit platelet activation and preserve platelet function and number. It will also potentiate the anticoagulant effects of heparin and this means that less heparin can be used. It is likely to improve postoperative hemostasis and to reduce platelet accumulation on filters in the lines and prevent microaggregate formation.

One of the trials I mentioned, by Dr. Pokar in Germany, is attempting to establish whether

prostacyclin can reduce the postoperative cerebral disturbances associated with cardiopulmonary bypass but this trial is not yet complete.

Thus, we believe that prostacyclin has a significant therapeutic potential in preserving platelets in those situations where blood comes into contact with an artificial surface. We also believe that there are other diseases in which prostacyclin might have a therapeutic potential. This belief is based upon experiments in which a reduced prostacyclin formation has been demonstrated in cardiovascular disease. In experimental animals and in man it has been found that in diabetes there is a reduction of prostacyclin formation^{33,34}. It has also been shown that there is a reduction in prostacyclin formation in atherosclerosis. This is seen experimentally in animals²⁵ and by looking at atherosclerotic plaques from man²¹. In thrombocytopenic purpura³⁵ and pre-eclampsia³⁶ there is also a demonstrable reduction in prostacyclin production.

I mentioned that prostacyclin might be released from the pulmonary circulation, and those of you who smoke will be interested to know that nicotine is quite a potent inhibitor of prostacyclin synthesis. There is also increasing evidence that as we get older, there is a reduction in our blood vessels' ability to form prostacyclin³⁶.

A series of clinical trials is in progress to find out whether prostacyclin is an effective treatment in peripheral vascular disease. The first of these was by Szczeklik and Gryglewski in Crakow, Poland which was on an open basis. They now have more than 70 patients who have been treated with prostacyclin. These are patients who have major occlusions of the arteries of a leg, with ischaemic ulcers, rest pain and so on. Prostacyclin was infused intra-arterially for a period of 3 days into the affected leg. In the first series of patients very striking results were obtained, and over a series of 70 patients they now find a long term benefit in about 40% of the patients. This is one of the most interesting things about this ephemeral and unstable substance, because although it is a vasodilator and although it is a potent anti-aggregating substance, both of these activities only last for a brief period. However, when the blood flow is measured in the affected limb, there is a dramatic increase during the prostacyclin infusion and when the prostacyclin is stopped the blood flow remains elevated for weeks. The rest

pain also disappears and 40% of the patients experience a long-term benefit lasting from 3 to 6 months, with healing of ulcers^{38,39}.

Dr. Negus in England has found very similar results on a series of 15 patients, and 3 double blind clinical trials of prostacyclin in peripheral vascular disease have been initiated. However, we shall not have the results of these for some months to come.

In a trial on Raynaud's phenomenon prostacyclin was also effective. Here the finger and hand temperature were measured as an indication of increase in blood flow. Temperatures were measured before infusion and at three points during the 3 day infusion. Finger and hand temperatures were measured again 2 weeks after the infusion was stopped and the temperature remained elevated. This is another striking effect which cannot be explained simply by the vasodilator and antiaggregating activities of prostacyclin. The duration of response in 80% of the patients was at least 4 weeks, in about 70% it was 8 weeks and so on, until at 24 weeks there was still a residual effect in one patient. Thus it appears that prostacyclin could well have strong potential for use in peripheral vascular disease including Raynaud's phenomenon.

Prostacyclin is also now being tested in other forms of ischaemic disease. In Poland, Gryglewski and Szczeklik are studying patients with angina and are measuring the amounts of anti-anginal drugs taken by these patients. This is done on a double blind basis with some patients receiving a placebo infusion and some an infusion of prostacyclin.

So, what are the conditions for which prostacyclin or a stable analogue might be useful? One in which we think it might be useful is decompression sickness, which is thought to be due to platelets embolizing on to the small nitrogen bubbles which form in the circulation. Prostacyclin has already been shown to be active in pulmonary hypertension associated with the new-born and we believe it is worth testing also in deep vein thrombosis and in vascular surgery.

In transplants we have some experimental evidence that is quite exciting. When dogs are sensitized to each other by cross exchange transfusions of blood, and then a kidney is transferred from one dog to another, a hyperimmune reaction can be demonstrated, so that there is an im-

mediate rejection of the kidney. The circulation closes down within one or two hours and this seems to be due to platelet accumulation in the transplanted kidney. However, when we give an infusion of prostacyclin then we can keep that kidney alive with a thriving blood flow and urine output for the duration of the experiment, which has so far been up to eight hours⁴⁰).

For some conditions prostacyclin may well be too short-lived or too unstable. Many pharmaceutical companies are trying to find stable analogues of prostacyclin which can be used either parenterally or orally. Indeed in the 1980s I believe that there will be a substantial change in the incidence and treatment of cardiovascular disease brought about by the use of prostacyclin or one of the analogues which are now being developed.

QUESTIONS AND ANSWERS

Moderator: Thank you very much, Dr. Vane. We have a few minutes, so are there any questions? How about you, Dr. Numano?

Dr. Numano: Thank you very much, Dr. Vane. It was very impressive for me to hear your good lecture. I have a few questions. First of all, I should like to know about the concept of prostacyclin as a circulating hormone. How any minutes would prostacyclin be active in the blood stream when it is released from the lung tissues? What do you think about it?

Dr. Vane: The concept of prostacyclin as a circulating hormone is an attractive one if the concentration is very low. If it were very high, then it would mean that the bleeding time will be prolonged and there will be all sorts of complications, such as reduction in blood pressure. But I believe that with a very low concentration it could be that there is enough prostacyclin in the circulation to condition slightly the platelets into an antithrombotic state.

Now, in terms of what has been measured in circulation, Dollery and his group find something like 150 pg/ml in the arterial blood. On the other hand, John Oates in the United States by measuring urinary metabolites has calculated back to what he considers the blood levels should be, and he doesn't find anything like as much. He finds only 1 or 2 pg/ml. So, it will be some time before we know what the actual concentration of prostacyclin is in the blood.

Dr. Numano: So, it is very effective when we infuse the prostacyclin intravenously. It is very effective in the arterial blood flow.

Dr. Vane: Yes, it is effective because it passes through the lungs unchanged.

Dr. Numano: May I ask one more question?

Dr. Vane: Please.

Dr. Numano: I would like to ask one question about renal disease. Could you find some decrease in the level of prostacyclin or an increase in the level of thromboxane B₂ in the blood vessels in a patient with renal disease?

Dr. Vane: We haven't looked for that, but it will be very interesting to look for increased thromboxane levels. Certainly during cardiopulmonary bypass people are finding very high thromboxane levels. There is some work we heard about yesterday from Dr. Tada in Tokyo, where he finds higher levels of thromboxane A₂ in angina, which sounds very interesting.

Dr. Numano: But you believe that in the case of a renal disease, some disturbances of prostaglandin metabolism occurs in the kidney?

Dr. Vane: I believe that's a possibility but I have no experimental levels to show that. In the USA, Dr. Needleman has shown that when kidneys of experimental animals are made hydronephrotic, they begin to generate thromboxane A₂.

Dr. Numano: Thank you.

Moderator: Is there any other question? Then let me ask one question. Is that all right?

Well, in practice, how do they make the prostacyclin as it is such an unstable substance?

Dr. Vane: In practice, it is made synthetically and formulated into a freeze dried preparation, which is then mixed with an alkaline buffer, a glycine buffer. When you do this, and keep the pH at around 10, then it remains stable. But as soon as you infuse it the pH drops down to the body pH and becomes unstable again. But you can keep the freeze dried preparation in a refrigerator for many months.

Moderator: Because he is very famous, I did not introduce Dr. Vane before his lecture. But now that I have a few minutes, let me introduce very briefly his curriculum vitae in Japanese.

Well, Dr. Vane, I should like to express our gratitude on behalf of all the members of the Society. On this occasion, I should like to express our great respect for you who over so many years have laboured so hard in the work of this field and

to express our deep appreciation for the achievements and the untiring efforts.

References

- 1) Ferreira, S. H. and Vane, J. R.: Prostaglandins: their disappearance from and release into the circulation. *Nature (London)*, **216**: 868-873 (1967).
- 2) Piper, P. J. and Vane, J. R.: Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature (London)*, **223**: 29-35 (1969).
- 3) Vane, J. R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (London)*, **231**: 232-235 (1971).
- 4) Hamberg, M. and Samuelsson, B.: Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc. Nat. Acad. Sci. U.S.A.*, **70**: 899-903 (1973).
- 5) Nugteren, D. H. and Hazelhof, E.: Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim. Biophys. Acta*, **326**: 448-461 (1973).
- 6) Hamberg, M., Svensson, J., Wakabayashi, T., and Samuelsson, B.: Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc. Nat. Acad. Sci. USA.*, **71**: 345-349 (1974).
- 7) Hamberg, M., Svensson, J. and Samuelsson, B.: Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Nat. Acad. Sci. USA.*, **72**: 2994-2998 (1975).
- 8) Bunting, S., Moncada, S. and Vane, J. R.: The effects of prostaglandin endoperoxides and thromboxane A₂ on strips of rabbit coeliac artery and other smooth muscle preparations. *Brit. J. Pharmacol.*, **57**: 462 P (1976).
- 9) Moncada, S., Gryglewski, R. J., Bunting, S. and Vane, J. R.: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature (London)*, **263**: 663-665 (1976).
- 10) Johnson, R. A., Morton, D. R., Kinner, J. H., Gorman, R. R., McGuire, J. C., Sun, F. F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. and Vane, J. R.: The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins*, **12**: 915-928 (1976).
- 11) Born, G. V. R.: Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (London)*, **194**: 927-929 (1962).
- 12) Marcus, A. J., Weksler, B. B., Jaffe, E. A. and Broekman, M. J.: Synthesis of prostacyclin (PGI₂) from platelet-derived endoperoxides by cultured human endothelial cells. *Blood*, **54**: Suppl. 1, abst. 803, p. 290a (1979).
- 13) Armstrong, J. M., Chapple, D. J., Dusting, G. J., Hughes, R., Moncada, S. and Vane, J. R.: Cardiovascular actions of prostacyclin (PGI₂) in chloralose anaesthetized dogs. *Brit. J. Pharmacol.*, **61**: 136 P (1977).
- 14) Dusting, G. J., Chapple, D. J., Hughes, R., Moncada, S. and Vane, J. R.: Prostacyclin induces coronary vasodilatation in anaesthetized dogs. *Cardiovasc. Res.*, **12**: 720-730 (1978).
- 15) Dusting, G. J., Moncada, S. and Vane, J. R.: Disappearance of prostacyclin in the circulation of the dog. *Brit. J. Pharmacol.*, **62**: 414-415 P (1977).
- 16) Gryglewski, R. J., Korbut, R. and Ocetkiewicz, A. C.: Generation of prostacyclin by lungs in vivo and its release into the arterial circulation. *Nature (London)*, **273**: 765-767 (1978).
- 17) Moncada, S., Korbut, R., Bunting, S. and Vane, J. R.: Prostacyclin is a circulating hormone. *Nature (London)*, **273**: 767-768 (1978).
- 18) Hensby, C. N., Barnes, P. J., Dollery, C. T. and Dargie, H.: Production of 6-oxo-PGF₁α by human lung in vivo. *Lancet ii*, 1162-1163 (1979).
- 19) Moncada, S., Gryglewski, R. J., Bunting, S. and Vane, J. R.: 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**: 715-733 (1976).
- 20) Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S. and Vane, J. R.: Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. *Biochim. Biophys. Acta*, **523**: 250-262 (1978).
- 21) Angelo, V. D., Villa, S., Mysliwiec, M., Donati, M. B. and de Gaetano, G.: Defective fibrinolytic and prostacyclin-like activity in human atherosclerotic plaques. *Thromb. Diath. Haemorrh.*, **39**: 535-536 (1978).
- 22) Moncada, S., Herman, A. H., Higgs, E. A. and Vane, J. R.: Differential formation of prostacyclin (PGX or PGI₂) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb. Res.*, **11**: 323-344 (1977).
- 23) Ubatuba, F. B., Moncada, S. and Vane, J. R.: The effect of prostacyclin (PGI₂) on platelet behaviour, thrombus formation in vivo and bleeding time. *Throm. Diath. Haemorrh.*, **41**: 425-434 (1979).
- 24) Higgs, E. A., Moncada, S., Vane, J. R., Caen, J. P., Michel, H. and Tobelem, G.: Effect of prostacyclin (PGI₂) on platelet adhesion to rabbit arterial subendothelium. *Prostaglandins*, **16**: 17-

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- 22 (1978).
- 25) Dembinska-Kiec, A., Gryglewska, T., Zmuda, A. and Gryglewski, R. J.: The generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rabbits. *Prostaglandins*, **14**: 1025–1034 (1977).
- 26) Gorman, R. R., Bunting, S. and Miller, O. V.: Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins*, **13**: 377–388 (1977).
- 27) Tateson, J. E., Moncada, S. and Vane, J. R.: Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins*, **13**: 389–399 (1977).
- 28) Bunting, S., Moncada, S., Vane, J. R., Woods, H. F. and Weston, M. J.: Prostacyclin improves hemocompatibility during charcoal hemoperfusion. In: *Prostacyclin*, ed. by S. Bergström and J. R. Vane, Raven Press, New York, 361–369 (1979).
- 29) Gimson, A. E. S., Hughes, R. D., Mellon, P. J., Woods, H. F., Langley, P. G., Canalese, J., Williams, R. and Weston, M. J.: Prostacyclin to prevent platelet activation during charcoal haemoperfusion in fulminant hepatic failure. *Lancet* i: 173–175 (1980).
- 30) Woods, H. F., Ash, G., Weston, M. J., Bunting, S., Moncada, S. and Vane, J. R.: Prostacyclin can replace heparin in haemodialysis in dogs. *Lancet* ii, 1075–1076 (1978).
- 31) Zusman, R. M., Rubin, R. H., Cato, A. E., Cocchetto, D. M., Crow, J. M. and Tolkoff-Rubin, N.: Haemodialysis using prostacyclin instead of heparin as the sole antithrombotic agent. *New Engl. J. Med.*, **304**: 934–939 (1981).
- 32) Longmore, D. B., Bennett, J. G., Hoyle, P. M., Smith, M. A., Gregory, A., Osivand, T. and Jones, W. A.: Prostacyclin administration during cardiopulmonary bypass in man. *Lancet* i, 800–804 (1981).
- 33) Harrison, H. E., Reece, A. H. and Johnson, M.: Decreased vascular prostacyclin in experimental diabetes. *Life Sci.*, **23**: 351–356 (1978).
- 34) Johnson, M., Harrison, H. E., Raftery, A. T. and Elder, J. B.: Vascular prostacyclin may be reduced in diabetes in man. *Lancet* i, 325–326 (1979).
- 35) Remuzzi, G., Misiani, R., Marchesi, D., Livio, M., Mecca, G., de Gaetano, G., and Donati, M. B.: Haemolytic-uraemic syndrome: deficiency of plasma factor(s) regulating prostacyclin activity. *Lancet* ii, 871–872 (1978).
- 36) Remuzzi, G., Marchesi, D., Zoja, C., Muratore, D., Mecca, G., Misiani, R., Rossi, E., Barbato, M., Capetta, P., Donati, M. B., and de Gaetano, G.: Reduced umbilical and placental vascular prostacyclin in severe preeclampsia. *Prostaglandins*, **20**: 105–110 (1980).
- 37) Jorgensen, K., Olesen, A., Dyerberg, J. and Stoffersen, E.: Aspirin and bleeding time: dependency of age. *Lancet* ii, 302 (1979).
- 38) Szczeklik, A., Nizankowski, R., Skawinski, S., Szczeklik, J., Glusko, P. and Gryglewski, R. J.: Successful therapy of advanced arteriosclerosis obliterans with prostacyclin. *Lancet* i, 1111–1114 (1979).
- 39) Szczeklik, A., Gryglewski, R. J., Nizankowski, R., Skawinski, S., Glusko, P. and Korbut, R.: Prostacyclin therapy in peripheral artery disease. *Thromb. Res.*, **19**: 191–199 (1980).
- 40) Mundy, A. R., Bewick, M., Moncada, S. and Vane, J. R.: Suppression of hyperacute renal allograft rejection in presensitized dogs with prostacyclin. *Prostaglandins*, **19**: 595–603 (1980).