few regional or supraregional units, where the necessary skill could be concentrated. The report suggested 12 such centres: nine in England, two in Scotland, and one in Northern Ireland. These recommendations are in close agreement with those of the European Dialysis and Transplant Association committee, which has defined a "specialised paediatric centre" as one which fulfils six criteria: the children are in the direct care of a paediatrician, in a children's ward, and have access to a hospital school, a paediatric social worker, a dietitian with experience in paediatrics and renal medicine, and a child psychiatrist or psychologist. Substantially fewer than half of all children dialysed or given transplants in Europe in 1979 were treated in such centres.

How important is it for an individual child to be treated in a specialist centre? Does it really matter? Analysis of the 1978 European data³ suggests that it does. Results were distinctly better in patients treated in specialised centres than those treated elsewhere: this applied to patient survival, both on hospital dialysis and after transplantation, and to cadaveric graft survival with a difference between 68% and 56% at two years. The annual death rate on hospital haemodialysis was 34 per 1000 children in specialised centres and 92 per 1000 in non-specialised centres; the excess mortality was due mainly to hyperkalaemia, cerebrovascular accidents, and cachexia.

In 1979 the British Association for Paediatric Nephrology produced a follow-up report⁴ as a supplement to its original

recommendations. Considerable progress has been made: of the original 12 suggested centres (since increased to 13), 10 had appointed at least one consultant paediatric nephrologist, though only five satisfied the European Dialysis and Transplant Association criteria for a specialist paediatric centre. The report praised the high level of co-operation between adult and paediatric services but regretted that in certain parts of Britain "services for children remain embryonic rather than formative." This is reflected in the fact that (again in 1979) 34 centres were treating at least one child but only three were treating 10 or more. Only a relatively small investment of staff and other resources is necessary for the recommendations to be carried out in full: such expenditure is justified on the grounds that no accident of geography should deprive a child in chronic renal failure of access to the best available treatment.

¹ *Proceedings of the European Dialysis and Transplant Association, 1979.* Tunbridge Wells: Pitman Medical, 1980.

² Chantler C, Houston IB, White RHR. Future care for children with chronic renal failure in the United Kingdom: a report for the British Association of Paediatric Nephrology. BAPN, 1974.

³ Chantler C, Donckerwolcke RA, Brunner FP, *et al.* Combined report on regular dialysis and transplantation of children in Europe, 1978. *Proc Eur Dial Transplant Assoc* 1979;16:74-104.

⁴ Houston IB, Barratt TM, Chantler C, White RHR. Siting of units to care for children with chronic renal failure in the United Kingdom: a progress report 1974/79. British Association for Paediatric Nephrology, 1979.

Regular Review

Prostaglandins in vascular disease: a seminal approach

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Despite the universal agreement that occlusive vascular disease is the main cause of death and disability in most developed countries, there is no such agreement about its nature, causation, prevention, and treatment. Even on the venous side of the circulation, where thrombi form in vessels whose walls are normal, we can offer only a lame theory, wrongly attributed to Virchow,¹ to explain why thrombi form at a particular site, at a particular time, and from particular blood constituents. As soon as we move to the arterial tree the difficulties increase, because disease of the wall (atheroma) and events in the lumen (thrombosis) invariably coexist,² and this has led to a century of still-unresolved controversy about the interrelation of the two processes.

The atherogenic school—The traditional view, championed by Virchow,³ was that atheroma was an intrinsic disease of the vessel wall. He believed it to be inflammatory (endarteritis deformans), but subsequent workers focused on the lipid rather than the inflammatory component of plaques, suggesting that atheroma was caused by insudation and infiltration of plasma lipids into the wall. This unsubstantiated hypothesis led to the view that atheroma must be caused by abnormalities

of the plasma lipids and that it could be prevented or made to regress by alterations in dietary lipids. Even within the atherogenic school, alternative hypotheses to the original inflammatory and the subsequent lipid-infiltration theory have been passionately espoused: that mechanical wear and tear, accentuated by hypertension, disrupted the fabric of the artery, or that the plaques were monoclonal tumours akin to uterine fibroids. All these views shared common ground in that they either ignored the existence of thrombosis or they relegated it to the role of a mere vassal obeying the will of the diseased wall. Thrombosis was thus regarded as a secondary consequence of the plaques, which instructed the hitherto normal passing blood to adhere to them.

The proponents of this "atheroma-is-all" school invariably describe clinical events such as stroke, myocardial infarction, and gangrene of a limb as "atherosclerotic diseases," and meetings may spend several days discussing the cause of these clinical conditions without the word thrombosis being mentioned. This intellectual blind spot, which is particularly prevalent in the United States, has led to attempts to prevent or modify the clinical endpoints such as stroke or myocardial

infarction by regimens aimed at a particular theoretical atherogenic mechanism—for example, by reducing plasma lipid concentrations with diet or drugs.

Such trials differ radically from the pioneering clinical trials which established the technique as an indispensable part of our armament. The cause of tuberculosis was established by Koch last century, so that when streptomycin, isoniazid, and other antituberculous agents were developed, the trials tested only the efficacy of the regimen against a disease whose cause was known. Similarly, in the Salk-versus-Sabin poliomyelitis vaccination controversy, the causal virus was already well known and all that was required was a trial of killed versus live-attenuated vaccines. In contrast to these trials of therapeutic regimens in diseases with known causation, our trials in vascular disease are taking two steps at once: they test simultaneously our causal theories and our ability to modify these possible causal mechanisms. If, as is the present case, trials of lipid reduction give negative results this may be because the lipid theories of atherogenesis are wrong or because atheroma is not, after all, the crucial process in occlusive vascular disease.

The thrombogenic school—Even while Virchow was developing his views on atherogenesis and on the ability of plaques to produce thrombi, his rival, Rokitsansky,⁴ was suggesting the converse and was claiming that thrombi caused arterial disease by being incorporated into the vessel wall and evolving into plaques. He paid dearly for this challenge to Virchow's authority: the latter retaliated fiercely by showing that plaques were often covered with endothelium. As everyone then believed that endothelium could not regenerate, this was thought to prove that the plaques must have originated in the wall and not in the lumen. Rokitsansky's hypothesis was demolished by Virchow's prestige and languished for almost a century until it was energetically revived by Duguid.⁵

We need to worry about these old and apparently sterile arguments because our whole therapeutic and preventive strategy is inextricably bound up with whether atheroma causes thrombosis or whether thrombosis causes atheroma. If the first model is true we need only to develop antiatheroma measures and the thrombi will then take care of themselves; if the second model proves correct and we could develop antithrombotic agents, these would also prevent atheroma.

Possibly, however, some 50-year-old observations on the effect of seminal fluid on the human uterus may help us to resolve the conflict. The evolution of this knowledge is a fascinating detective story in which the "villains" and the "heroes" can only be dimly discerned, the plot is not yet revealed, and the ending—whether logical or with a last-minute dénouement—is not yet known.

The search for antithrombotic agents—Irrespective of its pathogenetic mechanism, thrombosis plays a crucial part in clinical vascular disease⁶ in that it is thrombus which embolises from veins and it is thrombus which, by occluding or embolising, gives rise to myocardial infarction, completed ischaemic stroke, transient ischaemic attacks, and gangrene of the limbs. As everyone was familiar with the formation of a solid clot from shed blood by the processes of coagulation, the formation of a solid, fibrin-containing thrombus from liquid, flowing blood was initially assumed to use the same mechanisms. Trials of anticoagulant regimens appeared to vindicate this view in venous thromboembolism, but the uncertain results and the unsatisfactory design of many of the arterial disease studies led to a general disillusionment and to the belief that anticoagulants had nothing to offer on the arterial side.⁷ The pendulum had thus swung completely

away from the earlier view that arterial thrombosis was merely clotting in the wrong place. We should remember, however, that thrombi do contain fibrin, that measurements of clotting factors such as increased concentrations of fibrinogen, factor VII, and factor VIII are predictive of later myocardial infarction,⁸ and that anticoagulant treatment does appear to reduce the risk of myocardial reinfarction.⁹

Nevertheless, as the pendulum swung away from blood coagulation, the presence of platelets in thrombi produced the alternative concept that thrombosis was haemostasis in the wrong place. Clotting factors such as thrombin could still have been concerned, but the arrival on the scene of substances which were shown to be powerful platelet aggregating agents (adenosine diphosphate¹⁰; 5HT and catecholamines¹¹; collagen¹²) and which were unrelated to the clotting cascade gave further impetus to the swing. Our ignorance about the actual mechanisms of aspects of platelet behaviour such as adhesion, aggregation, and the release of stored materials led to an intense scrutiny of the biochemical properties of blood platelets. At the same time, agents such as dipyridamole,^{13,14} sulphinpyrazone,¹⁵ and aspirin¹⁶ had been found to modify platelet activity in vivo or in vitro, so the stage was being set for an unlikely convergence of the old and apparently unrelated observations on seminal fluid with the work on platelet behaviour.

Prostaglandins—In 1930 the instillation of fresh semen into the uterus was found to produce contraction,¹⁷ and studies in England¹⁸ and Sweden¹⁹ confirmed the presence of powerful stimulants of smooth muscle in human seminal plasma. Von Euler showed that the activity resided in a fraction containing lipid-soluble acids, which he named "prostaglandin." Surprisingly little general interest was shown in these observations until the 1960s, when the widespread distribution and the powerful actions of the prostaglandins in almost every organ, tissue, and cell of the body began to be appreciated.²⁰

Many of the "key words" in the wave of articles about prostaglandins which then began to appear were of direct relevance to platelet behaviour (effects on cyclic AMP, on calcium availability, and on contractile proteins), so the effects of the prostaglandins then available were studied on platelets. Prostaglandin E₁ was found to be the most powerful known inhibitor of aggregation in vitro^{21,22} and of platelet thrombus formation in animal experiments,²² but these observations could not be fitted into the normal or disordered economy of the body. At that time, however, Vane and his colleagues²³ were studying inflammation and the anti-inflammatory action of aspirin. They found that acetylsalicylic acid inhibited prostaglandin synthesis, and as acetylsalicylic acid was known to modify platelet behaviour this observation provided a vital clue to an interactive system of prostaglandins in platelets and in vessel walls.

Thromboxanes—One-half of this system emerged from the work of Samuelsson and his colleagues,²⁴ who showed that platelets generated prostaglandin endoperoxides from arachidonic acid and that these substances then gave rise to an extremely powerful aggregating agent. They named this potential "villain" thromboxane, and the figure shows how the active but unstable thromboxane A₂ is formed and breaks down to the stable thromboxane B₂. The crucial steps in this chain are mediated by the enzyme cyclo-oxygenase (which is irreversibly acetylated and inhibited by aspirin²⁵) and by thromboxane synthetase.²⁶

Thus platelets possess an amplifying system of tremendous power which enables them to turn on, when appropriately stimulated, the synthesis of the proaggregatory and vaso-

constrictive thromboxane A₂. Why then is aspirin, which cuts off the supply of thromboxane A₂ for the life span of the platelet, not a universally acclaimed antithrombotic agent? The answer lies in the vessel wall.

Prostacyclin—In 1976, a similar system, but with a diametrically opposed end product, was found in the vessel wall.²⁷ Starting with arachidonic acid, vessel-wall cells, especially endothelium,^{28, 29} can generate cyclic endoperoxides via cyclo-oxygenase in the same way as platelets. Thereafter the pathways differ (see figure), and the vessels generate an extremely powerful antiaggregatory vasodilator substance, prostacyclin (PGI₂). Two theoretical concepts stem from this discovery. Firstly, there may be a natural balance between thromboxane A₂ (the proaggregatory, vasoconstrictive “baddy” produced by platelets) and the antiaggregatory vasodilatory “goody” produced by normal vessel wall.³⁰ (This brings Virchow versus Rokitsky and atherogenesis versus thrombogenesis back into the debate in a new setting.) Secondly, the prostacyclin system may account for the failure of aspirin to modify thrombotic disease, since in conventional dosage aspirin cuts off the supply of both thromboxane and prostacyclin, thereby leaving the balance between good and evil unaltered. We do not yet know whether aspirin in minidoses can confer benefit by selectively blocking platelet cyclo-oxygenase while leaving the vessel-wall enzyme relatively unscathed—which explains why more direct ways of modifying the synthesis or receptor activity of thromboxane are also under intense scrutiny. Two notes of caution should be sounded: firstly, not all platelet behaviour is dependent on prostaglandin pathways,³¹ and, secondly, just because systems exist it does not mean that they are concerned in physiological or pathological events. We should remember how a blinkered approach led to thrombosis itself being ignored because of the entrenched belief that atheroma was the all-important process and how coagulation and then platelet aggregation were subsequently hailed as the prime cause of thrombosis. We must not concentrate on prostaglandins to the exclusion of all other possible mechanisms. We need to validate each theoretical mechanism by clinical trials, and we cannot expect research in vitro or in animals to tell us whether we should retest anticoagulants, whether we should test aspirin at different doses, or whether we should invest our clinical trial efforts in new compounds such as thromboxane synthetase inhibitors, receptor blockers, or stable prostacyclin analogues.

Prostacyclin in health and disease—If the balance between thromboxane and prostacyclin proves to be important in

thrombogenesis we may have within our grasp a biochemical key to the so-called Virchow triad of abnormalities in the blood, the blood flow, and the vessel wall. For “blood abnormality” we could read “inappropriate and excessive thromboxane A₂ generation”; for “vessel-wall abnormality” we could read “inappropriate and reduced prostacyclin generation.” Such a reduction could be produced by endothelial disruption, by plaques overlying the endothelium which prevent prostacyclin from gaining access to the blood in the lumen, or by vessel-wall disease, since we know that atheromatous plaques can contain lipid peroxides, one of which (15-hydroperoxyarachidonic acid: 15-HPAA) strongly inhibits the production of prostacyclin.³² Finally, for “flow abnormality” we could read “any disturbance in blood flow which brings ‘bad’ platelets into contact with an insufficiently ‘good’ vessel wall.”

The interactions described above are local events which cannot readily be observed in patients since activity of the vessel walls can be measured only in biopsy specimens. Large amounts of prostacyclin are, however, generated in the lungs and added to the blood which perfuses them.³⁰ This discovery led to the view that, in addition to prostacyclin being formed locally to maintain a balance between the platelet and vessel wall, prostacyclin generated elsewhere might be acting as an antithrombotic hormone throughout the circulation by maintaining platelet cyclic AMP at an enhanced level. Recent studies have, however, cast doubt on this view³³ since specific antibodies against prostacyclin can negate the effect of added prostacyclin but do not enhance aggregation when added alone to blood samples. We must seek further evidence in our quest for validation of the role of prostacyclin by looking at platelet and vessel-wall prostaglandins in circumstances known to be associated with an increased risk of thrombosis.

An association between raised plasma concentrations of cholesterol and events which have been erroneously regarded as atherosclerotic, such as myocardial infarction and stroke, is established beyond any doubt. The pathological mechanism has been assumed to depend on enhanced formation of atheroma, but if these events are really thrombotic how does hyperlipidaemia enhance the risk of thrombosis? Arachidonic acid is an unsaturated, essential fatty acid whose four double bonds are degraded to the two which characterise the platelet prostaglandins and thromboxanes shown in the figure (where the subscript 2 as in PGG₂, PGH₂, and TXA₂ reflects this configuration). Might differences in the amount and composition of dietary, plasma, and platelet-membrane lipids be associated with the formation of different amounts and types of thromboxane and prostacyclin? As we shall see, the five-double-bond fatty acid eicosapentaenoic acid, present in fish oils, generates thromboxane A₃ (which does not share TXA₂’s powerful aggregatory properties) while at the same time generating prostaglandin I₃, which is a powerful anti-aggregatory agent. Might the formation of these materials tip the balance of power in a favourable and antithrombotic direction?

A link between lipids and platelet behaviour has long been sought and seemed at one time to be within our grasp,³⁴ but could not be characterised in detail. Recent studies have shown³⁵ that incubating platelets with cholesterol-rich liposomes increases their production of thromboxane. Malondialdehyde production, which gives some indication of the generation of thromboxane, was also found to be enhanced in the infants of diabetic mothers³⁶ and was paralleled by increased platelet aggregation. In our campaign for the

accurate use of words we should note in passing that the authors chose to describe this increase as "hyperfunction." There is of course no evidence that ADP- or noradrenaline-induced aggregation in anticoagulated plasma, separated from its red cells by centrifugation and exposed to foreign surfaces such as glass or plastic, can legitimately be described as a function. The study, therefore, showed enhanced platelet activity and not hyperfunction. Studies of prostacyclin production by blood vessels from diabetics have given less clear-cut results.^{37 38} Attempts to measure circulating prostacyclin in diabetic patients have been similarly confusing, in that one study found no difference between diabetics and matched controls,³⁹ whereas another group⁴⁰ found that concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) were lower in diabetics (99 pg/l) than in controls (133 pg/l). All the observations described above are in settings with a high risk of arterial thrombosis, but raised thromboxane B_2 concentrations have been observed⁴¹ in a group of patients undergoing knee replacement under tourniquet ischaemia, where there is a very high risk of venous thrombosis.

The formidable difficulties of sampling, whether of blood or of vessel wall, and the complex techniques needed to measure substances which are biologically active at concentrations which stretch our technology to the limit, will continue to limit our ability to study the platelet/vessel-wall prostacyclin/thromboxane balance in man in health and disease. Can we short-circuit these studies by moving straight to clinical trials, in the same way that insulin was used in patients before its structure and mode of action were known and long before any measurements of plasma insulin concentrations could be made? The difference between vascular disease and diabetes is that in the latter we were able to move quickly and blindly forward because a simple test could be used as an intermediary between the disease and the potential treatment—namely, a measurement of blood glucose. Whatever diabetes was, it was associated with a high concentration of glucose in the blood and whatever insulin was, it lowered that concentration. At present we have no simple and certain test which measures the risk of thrombosis,⁴² so we cannot give ourselves the confidence we need for long-term, large-scale outcome trials by showing that the thrombotic equivalent of blood glucose is changed by our treatment.

The therapeutic potential of prostaglandins—Although prostaglandin E_1 was a powerful inhibitor of aggregation in man and of thrombosis in animals, its systemic effect on lung, bowel, and skin limited its potential value.⁴³ Prostacyclin has a more favourable ratio of effect to side effects, and is therefore being studied in a wide range of conditions.⁴⁴ Intra-arterial prostacyclin has been claimed to modify the natural history of gangrene of the legs,⁴⁵ but we cannot ignore the possibility that vasodilatation was responsible for this improvement rather than an antithrombotic effect. The use of prostacyclin in extracorporeal circulation during heart-lung bypass prevented loss of platelets in the bubble oxygenators and filters⁴⁶; prostacyclin is also capable of conserving platelets during haemodialysis.⁴⁷ It does not, however, follow that, because prostacyclin prevents platelet consumption when blood is exposed to foreign surfaces and to air bubbles during extracorporeal circulation, this validates its role in the platelet/blood-vessel interaction in the intact circulation. Other platelet-modifying agents such as sulphinpyrazone and dipyridamole prevent platelet consumption when blood is exposed to foreign surfaces such as Silastic shunts or Starr-Edwards valves.⁴² These are readily perceived to be pharmacological effects which do not immediately lead us to believe that

thrombosis is due to sulphinpyrazone or dipyridamole deficiency.

The other way of trying to make use of favourable prostaglandins in treatment is to change their endogenous production. An early attempt to do this used dietary supplements of dihomogamma-linolenic acid to increase the production of the antiaggregatory prostaglandin E_1 , instead of the aggregatory prostaglandin G_2 , prostaglandin H_2 , and thromboxane series.⁴⁸ The instability of the fatty acid used and the lack of clearcut effect on platelet behaviour show the limitations of this approach. Dietary changes rather than supplements have therefore been explored; fish oils are particularly rich in the five-double-bond eicosapentaenoic acid. Possibly this explains why fish-eating peoples, such as Eskimos, have long bleeding times and low concentrations of arachidonic acid, and why they and the Japanese have low attack rates from thrombotic disease.^{49 50} When controlled diets, rich in eicosapentaenoic acid, were fed to seven volunteers, platelet aggregation and thromboxane production in response to collagen stimulation were reduced.⁵¹ These encouraging results should lead to more extensive studies, and to early and well-designed clinical trials in vascular disease to try to validate this hypothesis.

What of the future?—The identification of the mechanisms described above represents an enormous and exciting advance in knowledge, but "the rapid progress in prostanoid biochemistry has left behind a plethora of biological questions still to be answered. . . . Will the great potential that the management of these prostanoids offers lead to an effective treatment for thrombosis? Regardless of the many questions that have been raised, a new dimension of research has been uncovered. It seems likely that continued effort in this area can do little but good."⁵²

We need to ensure that our efforts are leading in the right direction, and we should remember three things. Firstly, some very powerful platelet activities appear to be completely independent of the prostaglandin mechanisms. We must continue to study these, as well as the now-neglected thrombin-generating coagulation pathway, in case they rather than the thromboxane/prostacyclin balance contain the clues to the causation and prevention of thrombosis. Secondly, the collective record of trials in myocardial infarction, stroke, gangrene, and venous thromboembolism is very poor. All too often these trials have produced confusion rather than practical guidelines to management or the validation or rejection of the basic pathogenetic hypothesis on which the trial was based. If we have not yet resolved the role of dipyridamole, sulphinpyrazone, and aspirin,⁵³ despite the investment of hundreds of millions of pounds and of scarce manpower resources, how can we ensure that the fruits of prostaglandin research such as stable prostacyclin analogues, thromboxane synthetase inhibitors, thromboxane-receptor blockers, and prostacyclin generators are not squandered by ill-conceived and inadequately performed clinical trials?

Finally, the enduring message is the folly of concentrating exclusively on goal-orientated research. What grant-giving body, when told that an applicant wanted to study thrombosis by instilling human semen into the uterus, would have dipped into its scarce resources? Bodies which support basic research, as opposed to those which fund studies on health-care delivery and operational research, should continue to look for quality, innovation, and excitement, rather than for projects directed towards particular goals, however desirable. Might the solution to cancer come from studies on growing broad-bean shoots rather than from the full-frontal assault on the disease

advocated by goal-orientated cancer research groups? How many apparently irrelevant applications are failing to gain support even though they may stand in the same relationship to some unlooked-for goal that reproductive physiology in the 1930s now stands in relation to thrombosis in the 1980s? That is why we should remember that the prostaglandins provide us, both literally and figuratively, with a seminal approach to vascular disease.

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