The 1982 Nobel Prize in Physiology or Medicine

The Nobel Prize in Physiology or Medicine for 1982 will be awarded to Sune Bergström, Bengt Samuelsson, and John Vane for their "discoveries concerning prostaglandins and related biologically active substances." This group of compounds includes prostaglandin (PG) E_2, $PGF_{2\alpha}$, PGD_2, thromboxane A_2, prostacyclin, and the leukotrienes. All are potent chemical transmitters of intercellular and intracellular signals that mediate a diversity of physiologic and pathologic functions. They all are formed from oxygenation of arachidonic acid, a 20-carbon polyunsaturated fatty acid.

The structures of these arachidonic acid metabolites differ considerably as do their actions. Given the diversity of their effects, specific cells are highly selective in the biotransformation of arachidonic acid, usually forming an oxygenated metabolite that can be linked to a function of the cell. Thus, thromboxane A_2, formed upon activation of platelets, is a potent stimulus for platelet aggregation and vasoconstriction, whereas endothelial cells produce prostacyclin which is a powerful inhibitor of platelet aggregation and a vasodilator. The leukotrienes and prostaglandin D_2 are produced by basophils and mast cells, and participate in the allergic responses that result from immunologic activation of these cells.

Sune Bergström is recognized for his pioneering work on the isolation of the prostaglandins and the elucidation of their structures. Knowledge of the structures of the prostaglandins provided the key that opened this field of research. Sune Bergström, previously Professor and Chairman of Chemistry and later Rector of the Karolinska Institute in Stockholm, is now Chairman of the WHO Advisory Committee on Medical Research.

Bengt Samuelsson is honored for his research elucidating the mechanisms of the biosynthesis of the prostaglandins and the pathways of their metabolism. His incisive studies on the oxygenation of arachidonic acid led to the discovery of the labile intermediates in prostaglandin formation, the cyclic endoperoxides, PGG_2 and PGH_2. The isolation and identification of these intermediates provided the basis for the discovery by Bengt Samuelsson of thromboxane A_2, and for progress in many other areas. Samuelsson's work also has elucidated the biosynthesis and structures of the leukotrienes. Bengt Samuelsson is Professor and Chairman of Chemistry as well as Dean of the Karolinska Institute.

John Vane is recognized for the discovery that vascular endothelium produces the potent inhibitor of platelet aggregation, prostacyclin. Earlier, he also demonstrated that arachidonic acid is converted by guinea pig lung to labile vasoconstrictors which subsequently were found by Bengt Samuelsson to consist largely of thromboxane A_2. John Vane discovered that aspirin blocked the formation of these arachidonic acid derived vasoconstrictors and subsequently demonstrated that aspirin and related nonsteroidal antiinflammatory drugs block the enzymatic conversion of arachidonic acid to the prostaglandins. John Vane is Group Research and Development Director of the Wellcome Foundation in Beckenham, England.

Discovery and Characterization of Prostaglandins

The scientific saga of the prostaglandins had its origins in reproductive biology. In 1930, Raphael Kurzrok and Charles Leib at Columbia University discovered that human seminal plasma contracted uterine smooth muscle. Shortly thereafter, broader biological implications were realized from the research of Maurice Goldblatt in England and Ulf S. Von Euler at the Karolinska Institute who demonstrated that the compounds in semen were active on a number of smooth muscles and lowered blood pressure when injected in animals. Von Euler, who received the Nobel Prize in 1970 for his research on catecholamines, called these factors "prostaglandins" because small amounts were found in the prostate gland, and he extended the work by characterizing them as acidic lipids.

The field remained quiescent till 19 October 1945 when Sune Bergström, working in the Department of Chemistry at the Karolinska Institute, presented a lecture to the Physiological Society of the Institute regarding his research on the oxygenation of linoleic acid by soy bean lipoxygenase. Von Euler was among the audience, and after the lecture informed Sune Bergström of his research on biologically active lipids that he thought might also represent oxygenation products. The possibility of further characterization of the chemical structure of prostaglandins was engaged, but even then it was known that it would be a formidable task requiring extraction of up to 100 kilograms of ram seminal vesicles to prepare even a small amount of prostaglandins for chemical study. The challenge became compelling to Sune Bergström, however, when he further purified extracts of prostaglandins that Von Euler had prepared before the war, and found that "after purification essentially to weightlessness, they retained extraordinary activity."

Bergström's research in the prostaglandins was temporarily sidetracked when at 31 years of age he became Chairman of the Department of Biochemistry at Lund, which at the time was relatively devoid of equipment and faculty. While developing the department he concentrated on cholesterol and bile acid research, adapting to this area the reversed phase partition chromatog-

(Left to right) Bengt Samuellson, Sune Bergström, and John Vane

0036-8075/82/1119-0765$01.00/0

raphy of Howard and Martin, which proved ideally suited for the isolation of prostaglandins when Bergström undertook this in the mid-1950's. In 1958, Bergström returned to the Karolinska Institute where he, with Jan Sjövall, Bengt Samuelsson, and Ragnar Ryhage, completed the characterization of the structures of the prostaglandins including PGE_2, $PGF_{2\alpha}$, and PGD_2 (Fig. 1).

According to Bergström, the development of mass spectrometry in the Department of Chemistry at the Karolinska Institute and particularly the invention of an interface between the gas chromatograph and mass spectrometer by Ryhage were of utmost importance in these structural characterizations which were accomplished with small quantities of the potent prostaglandins. The elucidation of the prostaglandin structures with gas chromatography–mass spectrometry was the first time a completely new structure of a natural compound had been characterized with this technology, which has now found wide application in diverse fields of science.

The 20-carbon fatty acid skeleton of all prostaglandins quickly led to the deduction that their biosynthetic origin was arachidonic acid. The formation of prostaglandins from arachidonic acid was demonstrated by Bergström and Samuelsson and by David Van Dorp at the Unilever Laboratories in Holland concomitantly and in cooperation. By making the production of prostaglandins possible through this biosynthetic route and later by chemical synthesis, the elucidation of the structure of the prostaglandins made it possible for scientists throughout the world to obtain prostaglandins for their research.

Mechanisms of Biosynthesis

Bengt Samuelsson had been a student of Sune Bergström's and subsequently participated in the small group that characterized the structure of the prostaglandins. In 1961–1962, Samuelsson was a fellow in the department of chemistry at Harvard. After his return to Stockholm he began a series of studies on the biotransformation of the prostaglandins which culminated in an elucidation of their metabolic fate in humans. This information provided a basis for quantifying the production of prostaglandins in vivo by measurement of their metabolites in blood or urine.

In 1964, Samuelsson began an exploration of the mechanism of the biosynthesis of the prostaglandins. He demonstrated that the enzymatic oxygenation of arachidonic acid was initiated by withdrawal of a hydrogen at carbon 13. Then, employing $^{18}O_2$ and mass spectrometry, he demonstrated that both of the oxygen atoms on the prostaglandin ring were derived from a single molecule of oxygen. This and other experiments led him to speculate that prostaglandins D, E, and F all were formed from a common intermediate, a cyclic endoperoxide with a bisoxygen bridge between carbons 9 and 11 of the ring (see Fig. 1). Samuelsson, together with Mats Hamberg, then confirmed that biosynthesis occurred through an endoperoxide intermediate by the isolation and identification of two endoperoxides, PGG_2 and PGH_2. Not only were these endoperoxides found to be precursors of prostaglandins D, E, and F, but the characterization of these intermediates also provided the basis for the subsequent discoveries of thromboxane A_2 and prostacyclin.

Aspirin and the Discovery of Thromboxane A_2 and Prostacyclin

John Vane, working in the Department of Pharmacology of the Institute of Basic Medical Sciences, Royal College of Surgeons of England in the early 1960's, developed a dynamic bioassay system that permitted simultaneous measurement of several substances in the circulating blood or in organ effluents. He brought to this research the strong heritage of British pharmacology in which the vigorous and creative use of bioassays has implemented discovery. This scientific tradition was exemplified in the work of Sir Henry Dale and Sir John Gaddum and was imparted to Vane during his graduate studies with J. H. Burn at Oxford. Utilizing Gaddum's concepts of parallel bioassay and superfusion, Vane bathed a series of isolated organs with a cascade of blood or perfusion fluid. The organs were selected on the basis of their known contractile responses to substances of interest, and the system enabled instantaneous assay of labile compounds. With this approach, Vane had previously demonstrated the conversion of angiotensin I to the more potent angiotensin II during its traverse of the pulmonary circulation.

Vane used the selectivity of the organs in his superfusion bioassay to distinguish between the different prostaglandins, aided by the provision of these compounds to him by the Upjohn Company, which has provided prostaglandins derived from biosynthesis and chemical synthesis to investigators on a worldwide basis. Vane and Priscilla Piper, utilizing the superfusion bioassay, investigated the release of prostaglandins during anaphylaxis in the perfused guinea pig lung. They included a strip of rabbit aorta in the bioassay system for purposes of detecting 5-hydroxytryptamine, and found strong contraction of the rabbit aorta by a substance which was not 5-hydroxytryptamine but which was highly

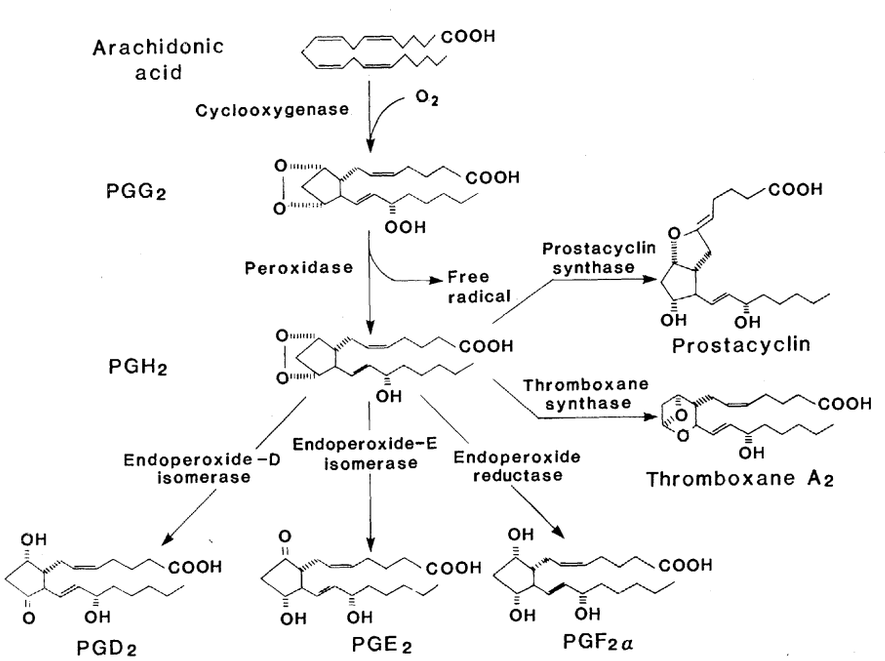

Fig. 1. The cyclooxygenase pathway of arachidonic acid metabolism. Aspirin and related antiinflammatory drugs inhibit the cyclooxygenase enzyme.

labile, losing its activity within minutes. The active material was designated "rabbit aorta contracting substance" (RCS), and they demonstrated that its release could be abolished by aspirin and other antiinflammatory drugs such as indomethacin.

This finding was extended considerably when, during a weekend in 1971, while considering the participation of prostaglandins in inflammation, Vane conceived the hypothesis that aspirin and related drugs acted by inhibiting the conversion of arachidonic acid to prostaglandins. By the following Monday evening he had completed the first experiment demonstrating that aspirin and indomethacin block the enzymatic conversion of arachidonic acid to PGE_2 and $PGF_{2\alpha}$. From subsequent investigations demonstrating that aspirin and several nonsteroidal antiinflammatory drugs all block the biotransformation of arachidonic acid to prostaglandins, Vane proposed that the known effects of aspirin in inflammation and fever could be explained by its effect on arachidonic acid oxygenation. This finding not only yielded insight into the mechanism of the pharmacologic effects of aspirin-like drugs but also provided a powerful approach to exploring the participation of prostaglandins in a variety of biologic events.

After the identification of the endoperoxide intermediates in prostaglandin synthesis, Bengt Samuelsson turned his attention to the biological activities of the endoperoxides, stimulated by the fact that they were active on the rabbit aorta, unlike the known prostaglandins. With knowledge from Vane's research that aspirin acted to block prostaglandin biosynthesis, it was interesting to Samuelsson that the inhibition of platelet aggregation by aspirin could not be explained by the known prostaglandins. Accordingly, an evaluation of the effect of endoperoxides on platelets was undertaken, which led to the observation that the endoperoxides aggregated platelets. This set in motion a series of studies by Mats Hamberg and Samuelsson in which correlation of biological activity with the observed biochemistry contributed to the discovery that the major pathway of endoperoxide metabolism in the platelet was to a novel compound, thromboxane A_2. Thromboxane A_2 was found to be a more potent stimulus for platelet aggregation than the endoperoxide. It is extremely labile and also contracts vascular smooth muscle, including that in coronary and cerebral arteries.

The demonstration that the RCS discovered by Piper and Vane was largely thromboxane A_2 pointed to the lung as an additional site of thromboxane A_2 biosynthesis. Vane with his colleagues Salvador Moncado, Richard Gryglewski, and Stuart Bunting then examined other tissues to determine which might convert the endoperoxide to thromboxane A_2, using the superfusion bioassay as their analytical tool. They examined vascular tissue because of the known contraction of severed vessels, and to their surprise found that incubation of vascular tissue with the endoperoxides did not produce effects on the superfused organs that resembled those of thromboxane A_2, PGE_2, or $PGF_{2\alpha}$. To explore the possibility that the product from vascular tissue might be PGD_2, already known as an inhibitor of platelet aggregation, they examined its effects on platelets. Indeed the product was a potent inhibitor of aggregation. Unlike PGD_2, however, this new inhibitor was labile, undergoing hydrolysis in aqueous solution with a half-life of about 3 minutes. Vane enlisted the cooperation of Roy Johnson and his colleagues at the Upjohn Company, and together they characterized the structure of the compound and, because of its bicyclic structure, called it prostacyclin (Fig. 1). Vane and his colleagues demonstrated that prostacyclin is derived primarily from the vascular endothelium where it is the principal product of arachidonic acid. From these discoveries, Vane postulated that the production of prostacyclin by endothelium cells inhibits the aggregation of platelets on their surfaces.

New Mediators of Inflammation

Just as the enigma of aspirin's action on the platelet led to the search for the platelet aggregating metabolite of arachidonic acid, new insights into the action of corticosteroids suggested that previously unrecognized metabolites of arachidonic acid might contribute to inflammatory processes. It had been demonstrated by Gryglewski and colleagues as well as by Hong and Levine that corticosteroids inhibit the liberation of free arachidonic acid. Normally, arachidonic acid exists in esterified form in membrane phospholipids. The enzymatic oxygenation of arachidonic acid to its biologically active products is initiated by the liberation of free arachidonic acid from phospholipids by phospholipases. Thus, steroids inhibit the formation of prostaglandins by preventing the libera-

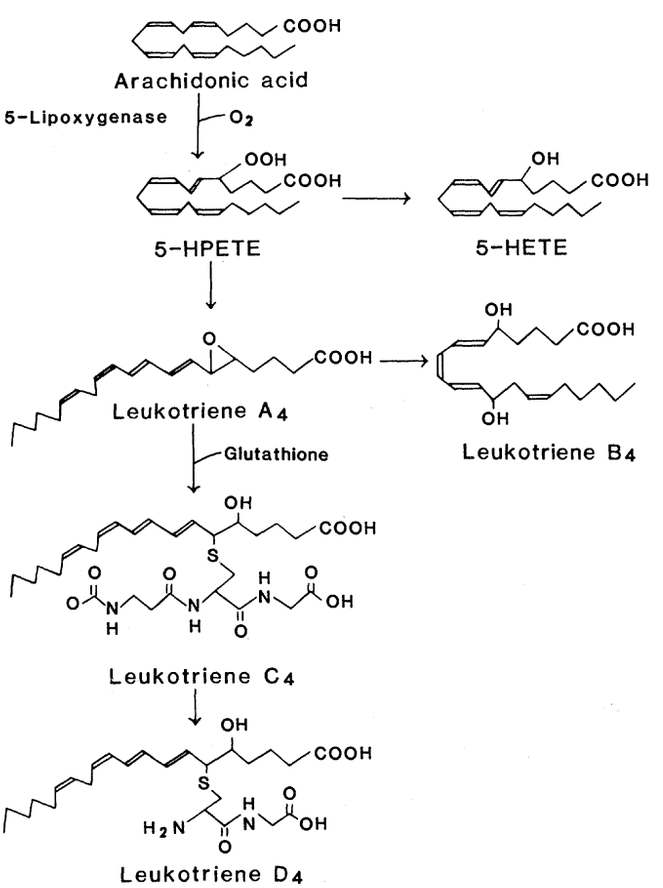

Fig. 2. The 5-lipoxygenase pathway of arachidonic metabolism, leading to the biosynthesis of leukotrienes.

tion of arachidonic acid, whereas aspirin-like drugs block their biosynthesis by inhibiting the cyclooxygenase.

The fact that corticosteroids have many antiinflammatory effects not shared by the aspirin-like drugs suggested that arachidonic acid might be metabolized to important products by pathways other than those initiated by the cyclooxygenase. Addressing this possibility, Bengt Samuelsson with Pierre Borgeat examined the metabolism of arachidonic acid in polymorphonuclear leukocytes, cells central to many inflammatory processes. They discovered that arachidonic acid was metabolized in the leukocyte largely by a 5-lipoxygenase to a series of products including dihydroxy acids with conjugated triene structures, leading to their designation as leukotrienes (LT's). One of these, a 5,12-dihydroxyeicosatetraenoic acid was designated LTB_4 (see Fig. 2) which subsequently has been found to be a potent chemotactic agent for leukocytes. It was further demonstrated that the biosynthesis of LTB_4 proceeded through a labile intermediate, the 5,6-epoxide named LTA_4. Of great interest to Bengt Samuelsson and his colleagues was the finding that the ultraviolet absorption characteristics of the leukotrienes were very similar to that reported by Howard Morris, Priscilla Piper, and colleagues for the potent bronchoconstrictor substance, slow reacting substance of anaphylaxis (SRS-A).

SRS-A, originally detected by Feldberg and Calloway in 1930, is released during anaphylaxis, and a number of experiments have implicated it as a mediator of the bronchoconstriction in asthma. Bengt Samuelsson and Robert Murphy, together with their colleagues, studied the biosynthesis of SRS-A in mast cells and found that one of the SRS-A's was derived from the conjugation of glutathione with the leukotriene epoxide LTA_4. The ultimate structural characterization of the product was made possible by the synthesis of LTA_4 by E. J. Corey at Harvard who had been in communication with Samuelsson from an early stage of the elucidation of the mechanisms of leukotriene biosynthesis. The glutathionyl conjugate was designated LTC_4 and the synthetic compound was found to be extremely potent as a bronchoconstrictor. The Stockholm group then demonstrated that LTC_4 was converted by γ-glutamyl transpeptidase to a cysteinyl glycine conjugate designated LTD_4. At essentially the same time, Priscilla Piper, Howard Morris, and their co-workers in London identified the slow-reacting substance from rat basophil leukemia cells by mass spectroscopic analysis as a cysteinyl glycine conjugate. LTD_4 also is a highly potent bronchoconstrictor. The characterization of LTC_4 and LTD_4 enables evaluation of the hypothesis that these bronchoconstrictors are important contributors to allergic asthma and that corticosteroids exert their beneficial effect by inhibiting leukotriene biosynthesis at the level of the phospholipase.

Implications of the Discoveries

The discovery of thromboxane A_2 has enhanced the understanding of platelet aggregation and revealed the potential for platelets to engender vasoconstriction through release of thromboxane A_2. The action of aspirin and related cyclooxygenase inhibitors on platelet function has been explained. The discovery of prostacyclin has uncovered a theoretical weakness in the use of aspirin as an antiplatelet drug in that aspirin doses that are maximally effective on platelets also block the biosynthesis of prostacyclin which is important in resisting platelet aggregation on vascular endothelium. Thus, more selective blockade of thromboxane biosynthesis with inhibition of thromboxane synthase (Fig. 1) is a more attractive approach, and clinical studies of thromboxane synthase inhibitors are currently under way. The potential use of such inhibitors in patients at high risk for vascular catastrophes in the coronary and cerebral circulations is attractive.

Prostacyclin has a greater and more general effectiveness in blocking platelet aggregation than does aspirin, and its inhibition of aggregation appears to be linked to an elevation in platelet adenosine 3′,5′-monophosphate. Prostacyclin has had demonstrated experimental success in preventing platelet deposition in extracorporeal circulations such as during hemodialysis, hemoperfusion, and the pump oxygenators used in cardiac surgery.

PGE_2 and its analogs block the development of experimental peptic ulcers, including those evoked by aspirin-like drugs, acting partly through a reduction in gastric acid secretion but largely by a mechanism not yet understood, which has been termed "cytoprotection."

One of the first clinical applications of the prostaglandins was the use of $PGF_{2\alpha}$ to induce labor at term, and PGE_2 has been used for second trimester abortion. A major application of analogs of these prostaglandins has been in animal husbandry; synchronization of estrus in hogs, horses, and other animals can improve the efficiency of breeding and artificial insemination. Because of its dilating effect on the ductus arteriosus, PGE_1 has been marketed for use in infants with congenital cardiac defects such as pulmonary atresia, in which ductal patency is advantageous prior to operative intervention.

The overproduction of prostaglandins is important in the pathogenesis of some human diseases. Excessive production of PGE_2 has been shown to mediate the hypercalcemia produced by certain solid tumors. PGD_2 is the principal metabolite of arachidonic acid in the mast cell, a cellular target for immunoglobulin E–mediated allergic responses. In patients with mastocytosis, a disease characterized by infiltration of tissues with excessive numbers of mast cells, an overproduction of PGD_2 frequently can be demonstrated and linked to the shocklike attacks produced by this disease.

Whereas there is evidence suggesting that leukotrienes LTC_4 and LTD_4 are mediators of bronchial asthma and that corticosteroids probably act by inhibiting their biosynthesis, the therapeutic challenge in this area remains. Knowledge of the pathways of biosynthesis and the structures of the leukotrienes enables consideration of effective pharmacologic interventions that may be more selective than corticosteroids in asthma.

Elucidation of the oxygenated metabolites of arachidonic acid and their biological importance has contributed substantially to many fields of medicine. It also has provided a basis for the development of new knowledge and therapy in the future.—JOHN A. OATES

The author is professor of medicine and pharmacology at Vanderbilt University School of Medicine, Nashville, Tennessee 37232.