

The ALLERGY ARCHIVES

Pioneers and Milestones

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The discovery and characterization of the leukotrienes

BENGT SAMUELSSON AND THE DISCOVERY OF THE LEUKOTRIENES

In November 1982, champagne corks popped in the Samuelsson laboratory after the announcement by the Nobel Foundation that Bengt Samuelsson, Sune Bergström, and Sir John Vane were awarded the Nobel Prize in Physiology or Medicine for “their discoveries concerning prostaglandins and related biologically active substances.” The author had the good fortune to be a fellow in the Samuelsson laboratory at the Karolinska Institute in Stockholm during this period and had an opportunity to attend the 1982 Nobel Fest for this astounding achievement. The Samuelsson laboratory at that time (Kemi II, Department of Chemistry, Karolinska Institute, Stockholm) was the central point for the discovery that arachidonic acid, in addition to serving as a precursor to prostaglandins, already studied in some detail by Sune Bergström, was also converted to novel bioactive substances that were proinflammatory and bronchoconstricting—namely, the 5-lipoxygenase pathway-derived leukotrienes. The laboratory that year was filled with visiting scientists, professors on sabbatical, and postdoctoral fellows from around the world, which emphasized that the Samuelsson laboratory was the focal point for investigators in this area worldwide.

Professor Samuelsson shared the distinguished Nobel Prize in Medicine in 1982 with his former mentor Sune Bergström and Sir John Vane of England for their groundbreaking research. Professor Samuelsson was born in Halmstad, Sweden, in 1934 and studied medicine at the University of Lund, located in southern Sweden and one of the oldest and largest universities in Europe. Bengt entered the Karolinska Institute in Stockholm to pursue his graduate studies in biochemistry and medical sciences when Professor Bergström’s group moved from Lund to Stockholm. He graduated in 1960 and received the medical degree a year later.

Bengt then spent a year at Harvard University visiting the Chemistry Department and returned to Stockholm to his first professorial position in Medical Chemistry at the Royal Veterinary College, where he

carried out a substantial body of research on the biosynthetic mechanisms involved in the conversion of arachidonic acid to prostaglandins. Connecting these pathways with widely used anti-inflammatories and aspirin can be traced to this earlier work from Samuelsson and colleagues that focused on the biosynthesis of unstable intermediates such as the endoperoxide prostaglandin G_2 generated by the actions of cyclooxygenase or COX, as it is widely known today. This important finding led to Samuelsson’s discovery of thromboxane, described earlier as the rabbit aortic contracting substance in elegant work from Sir John Vane’s laboratory in England. The finding also permitted Vane’s contribution of prostacyclin, a vasodilator produced by vascular endothelium. Aspirin and a battery of nonsteroidal anti-inflammatory compounds available at the time, including indomethacin, block the COX enzyme responsible for the conversion of arachidonic acid to prostaglandins and thromboxanes. This groundbreaking discovery was from Sir John Vane’s group working in London.¹

Dr Samuelsson was appointed Professor and Chairman of the Department of Physiological Chemistry at the Karolinska Institute in 1973. The photograph in Fig 1 was taken around the time of his discovery and main body of work on the leukotrienes. The first step in the elucidation of leukotrienes was the discovery by Borgeat, Hamberg, and Samuelsson² reported in 1976 that arachidonic acid was metabolized by the 5-lipoxygenase pathway. Additional studies revealed that a number of related compounds were generated that included a potent chemoattractant later termed leukotriene (LT) B_4 .^{3,4} These studies were extended, and in 1979, the group reported the discovery of the pivotal and important role of the epoxide intermediate LTA_4 , which gave a way to link the 5-lipoxygenase to the structural elucidation of the slow-reacting substances of anaphylaxis (SRS-As). These proved to be a group of cysteinyl-leukotrienes, LTC_4 , LTD_4 , and LTE_4 (Fig 1). These studies were performed with Dr Robert C. Murphy, at the time a visiting scientist from the United States, and Dr Sven Hammarström, a junior faculty member in the Samuelsson group. Because there was quite an extensive history of the elusive SRS-A, there were many hints in the literature from the group at Harvard, including the work of Orange et al,⁵ Orange and Austen,⁶ and the laboratory of Dr Charles Parker,⁷ raising the potential importance of SRS-A as a target for drug development and significance in pathophysiology. However, the structure of SRS-A remained unknown.

Discovery of the leukotrienes

Samuelsson reasoned that, because steroids and nonsteroidal anti-inflammatory drugs have significantly different anti-inflammatory effects, it seemed likely that some of these differences could be explained by the production of proinflammatory compounds produced from arachidonic acid by reactions that did not directly involve COX. This hypothesis

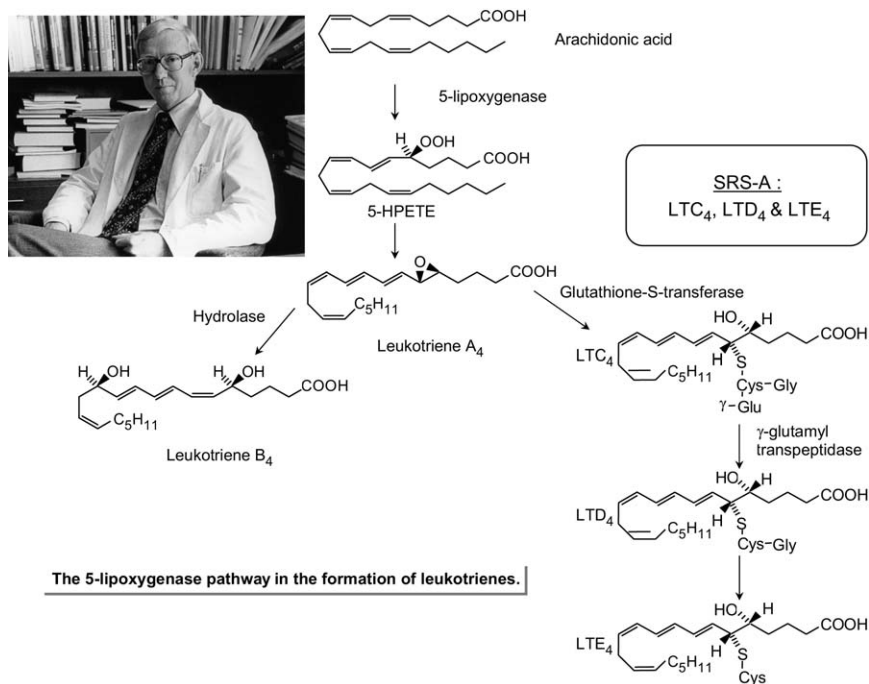


FIG 1. Bengt Samuelsson around the time of the discovery of the leukotrienes in his office at Kemi II, Karolinska Institute, Stockholm, along with the 5-lipoxygenase pathway and its role in the biosynthesis of leukotrienes as it was presented at the time of its elucidation and the 1982 Nobel Prize. Today, many components of this important system are known, including the enzymes, proteins, and genes governing their expression.

proved valid and led directly to the discovery of the leukotrienes. It is now well appreciated that the profound pro-inflammatory and bronchoconstrictor actions⁸ of leukotrienes play key roles in allergy, asthma, and inflammatory diseases.

A first important discovery in assembling the leukotriene pathway was the finding that, when rabbit peritoneal leukocytes were isolated and incubated with ¹⁴C-labeled arachidonic acid, the major product from arachidonic acid was a new lipoxygenase product: 5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-*HETE*).² Also, more polar products were formed, and structural studies demonstrated that they consisted of 5(*S*),12(*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid, including LTB₄ (Fig 1), 2 additional compounds that were epimeric at C-12, and 2 isomeric 5,6-dihydroxy-containing diols. Finding these compounds was key in unraveling the role of the epoxide intermediate termed LTA₄ in this pathway. Because these compounds all contained conjugated trienes and were isolated first from leukocytes, Samuelsson gave them the name *leukotrienes*.

Isotopic oxygen was used to demonstrate that the oxygen of the alcohol group at C-5 originated from molecular oxygen, whereas the oxygen of the alcohol group at C-12 was derived from water. Samuelsson hypothesized that leukocytes generated an unstable intermediate that would undergo nucleophilic attack by water, alcohols, and other nucleophiles. For example, they showed that when leukocytes were incubated for 30 seconds with arachidonic acid before addition of 10

volumes of methanol, they obtained trapping products; the structures were elucidated using mass spectrometry.

Trapping with methanol gave rise to 2 new, less polar compounds present in equal amounts. Their ultraviolet spectra were identical and indicated the presence of 3 conjugated double bonds. Infrared spectrometry indicated that the conjugated double bonds have *trans* geometry. Gas chromatographic-mass spectrometric analysis of several derivatives of the 2 compounds showed that they were isomeric and carried hydroxyl groups at C-5 and methoxy groups at C-12. Steric analyses demonstrated that the alcohol groups had (*S*) configuration and that they were the C-12 epimers of 5(*S*)-hydroxy-12-methoxy-trapping products. These studies led to the proposed intermediate and structure of 5,6-oxido-7,9,11,14-eicosatetraenoic acid, known today as LTA₄. E. J. Corey's group at Harvard first synthesized LTA₄ via total organic synthesis and confirmed this structure (Corey received the Nobel Prize in Chemistry in 1990). In collaboration with Samuelsson's group demonstrated that the enzymatically formed LTB₄ 5*S*,12*R*-dihydroxy acid contained 1 *cis* and 2 *trans* double bonds in the conjugated triene produced from LTA₄ (see reference⁹ for further sources). The mechanism for the biosynthesis of the epoxide LTA₄ from arachidonic acid involves initial formation of the 5-hydroperoxide via the action of the 5-lipoxygenase. Next, LTA₄ is formed from 5-hydroperoxyeicosatetraenoic acid (HPETE) by abstraction of a hydrogen at C-10 and elimination of a hydroxyl anion from the hydroperoxy group (Fig 1).



FIG 2. Samuelsson (*left*) receiving the 1982 Nobel Prize and shaking hands with the King of Sweden (*right*). Photograph used with permission from Scanpix.

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The 5-lipoxygenase link to SRS-A

In 1938, Feldberg and Kellaway introduced the name *slow-reacting substance* (SRS) for a smooth muscle contracting factor that appeared in the perfusate of guinea pig lung treated with cobra venom.⁸ Subsequent studies indicated that SRS is important as a mediator in asthma and other types of immediate hypersensitivity reactions. The immunologically generated SRS was referred to as SRS-A. SRS-A is released together with other mediators such as histamine and so forth after interactions between IgE molecules bound to membrane receptors and antigens, such as pollen. Structural work on SRS was previously limited by the lack of pure preparations of SRS. However, it has been characterized as a sulfur-containing polar lipid with UV absorption from the work of Orange et al⁵ and Orange and Austen.⁶ Also, labeled arachidonic acid might be incorporated into SRS.⁷

Earlier methods for generation of SRS gave only small amounts of the material, which precluded structural studies. To this end, Samuelsson and colleagues developed a new system employing murine mastocytoma cells for producing SRS, which was generated by challenging the cells with ionophore A23187. Using this approach, they increased their yields of spasmogenic material that was blocked with an SRS antagonist and demonstrated the incorporation of isotopically labeled precursors.^{9,10} The isolation procedure involved precipitation of protein with ethanol, alkaline hydrolysis, separation on Amberlite XAD-8 and silicic acid, and 2 steps of RP-HPLC. The isolated

material obtained showed an absorbance maximum at 280 nm and caused the typical SRS contraction of guinea pig ileum.⁹ The UV spectrum resembled the spectra Samuelsson had found earlier with the dihydroxy acids noted,^{3,11} except that the maximum wavelength was shifted higher, ~10 nm. This was consistent with a sulfur substituent in the α position, and results with labeled precursors showed that arachidonic acid and cysteine were both incorporated into SRS.

Desulfurization of SRS using Raney nickel produced 5-hydroxyarachidic acid, showing that the arachidonic acid derivative and cysteine were linked by a thioether bond. Importantly, the presence of an alcohol group at C-5 in a fatty acid reinforced their hypothesis at the time of a biogenic relation between the arachidonic acid metabolites found in leukocytes and SRS. The structure of SRS from murine mastocytoma cells was determined to be 5-hydroxy-6-*S*-glutathionyl-7,9,11,14-eicosatetraenoic acid, LTC₄, the first determination of the structure of an SRS-A. The preparation and some properties of the corresponding cysteinyl glycine derivative (LTD₄) and cysteinyl derivative (LTE₄) were reported at the same time by Professor Samuelsson at the 1979 Washington Meeting. The complete structure of LTC₄, 5(*S*)-hydroxy,6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, was confirmed by comparison with synthetic material and preparation of stereoisomers of the natural LTC₄ (Fig 1). After the 1979 meeting, several pharmaceutical companies initiated 5-lipoxygenase inhibitor programs. It is now clear from Samuelsson's seminal work with far-reaching implications that leukotrienes play an important role in

TABLE I. Key academic appointments and honors

Samuelsson's key academic appointments

Assistant Professor of Medical Chemistry, Karolinska Institutet, 1961-66
Research Fellow, Department of Chemistry, Harvard University, Cambridge, Mass, 1961-62
Professor of Medical Chemistry, Royal Veterinary College, Stockholm, Sweden, 1967-72
Professor of Medical and Physiological Chemistry, Karolinska Institutet, Stockholm, Sweden, 1973-
Chairman of the Department of Chemistry, Karolinska Institutet, Stockholm, Sweden, 1973-
Visiting Professor in Chemistry, Harvard University, Cambridge, Mass, Spring term, 1976
Dean of the Medical Faculty, Karolinska Institutet, Stockholm, Sweden, July 1, 1978-June 30, 1983
President of the Karolinska Institutet, July 1, 1983-June 30, 1995
See http://nobelprize.org/nobel_prizes/medicine/laureates/1982/samuelsson-autobio.html

Some other awards and honors

Swedish Medical Association's Jubilee Award, Stockholm, Sweden, 1968
Louisa Gross Horowitz Award, Columbia University, New York, NY, 1975
Albert Lasker Basic Medical Research Award, New York, NY, 1977
Honorary Degree of Doctor of Science, University of Chicago, Chicago, Ill, 1978
Lewis S. Rosenstiel Award in Basic Medical Research, Brandeis University, Boston, Mass, 1981
Swedish Medical Association's Jubilee Award, Stockholm, Sweden, 1981
Gairdner Foundation Award, Toronto, Ontario, Canada, 1981
Heinrich Wieland Prize, Munich, West Germany, 1981

many cell types, tissues, and organs as well as a wide range of human diseases (Fig 2). Today, the enzymes, accessory proteins, and genes that govern the expression of this pathway are known and appear to extend to cardiovascular disease.¹² Yet there remains much to learn about the intriguing roles of leukotrienes and novel lipoxygenase products in human systems.¹³

Professor Samuelsson served as the President of the Karolinska Institute, and for approximately the past 10 years, he has served as the Chairman of the Nobel Foundation (Table I). During his tenure in these positions, the Nobel Forum was created on the campus of the Karolinska Institute and the campus was transformed, with several new buildings appearing on its grounds. In 2003, Professor Samuelsson opened the 36th Annual Meeting of the Society for Leukocyte Biology (SLB) and gave an interview for the SLB newsletter. His thoughtful answers reveal his vision and commitment to scholarship.¹⁴ Just a few of Professor Samuelsson's responses from the recorded interview are given here.

Can you speak about specific influences that motivated you early in your scientific career?

Samuelsson: I started to do research very early. And, when I started to study medicine, I think biochemistry was probably the subject that I really got interested in. The Department of Biochemistry was very active, very

good. And, I was offered the possibility to do part time research while I continued to do medical studies.

It has been stated by some that the eicosanoid area is forever "green." Do you think that this is the case? Do you foresee major therapeutic breakthroughs in the near future, and do you anticipate additional breakthroughs in the area of lipid mediators?

Samuelsson: I'll restrict myself to the arachidonic cascade. I think that there will be major therapeutic breakthroughs in several areas. One area that has not been explored very much is prostaglandin receptors. A lot of knowledge has accumulated on prostaglandin receptors, and I'm sure that the antagonists and agonists will be developed. Leukotriene receptors—there are cysLT₁ receptor antagonists, but cysLT₂ is beginning to look very interesting, especially from a cardiovascular point of view. The other parts of the leukotriene pathway, like LTB₄, are very interesting. And, we have the lipoxins and the possibility of developing anti-inflammatory drugs on that basis. The most recent, exciting finding, which I actually will talk quite a lot about in the meeting in Philadelphia, will be on the membrane prostaglandin E-synthase, which is induced in a similar way to COX-2 and regulated in a similar way. And, there are now knockout studies that indicate that they have a very essential role in inflammation. I think that this will be the next target for development of anti-inflammatory drugs.

Do you have any other advice for graduate students, fellows, or junior faculty?

Samuelsson: Try to get involved in the most exciting research you can think of. And in biomedical research, go for structure and function. And try to apply the best tools. And don't try to solve problems that are not at the time approachable. Put realistic goals. I think that's very important. Of course, you can work on something that is very esoteric, but you'll never get there. Then you can work on very trivial things. You'll always get results, but they might not be very exciting. So, try to find an exciting problem that is researchable and usually try to look for structure and function. And, work long-term.

What are some of your outside interests/hobbies?

Samuelsson: I'm very interested in art—I spend a lot of time looking at art. And, I'm interested in opera and literature. I love to play golf, sail, and ski. And, dining and tasting and collecting wine is also something I appreciate.

Today, Professor Samuelsson resides in Stockholm and continues to carry out research in his laboratory at the Karolinska Institute.

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REFERENCES

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971;231:232-5.
2. Borgeat P, Hamberg M, Samuelsson B. Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes. *J Biol Chem* 1976;251:7816-20.
3. Borgeat P, Samuelsson B. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes: formation of a novel dihydroxyeicosatetraenoic acid. *J Biol Chem* 1979;254:2643-6.
4. Borgeat P, Naccache PH. Biosynthesis and biological activity of leukotriene B₄. *Clin Biochem* 1990;23:459-68.
5. Orange RP, Murphy RC, Austen KF. Inactivation of slow reacting substance of anaphylaxis (SRS-A) by arylsulfatases. *J Immunol* 1974;113:316-22.
6. Orange RP, Austen KF. Drug-induced modulation of the immunologic release of histamine and slow reacting substance of anaphylaxis. *Int Arch Allergy Appl Immunol* 1971;41:79-85.
7. Jakschik BA, Falkenhein S, Parker CW. Precursor role of arachidonic acid in release of slow reacting substance from rat basophilic leukemia cells. *Proc Natl Acad Sci U S A* 1977;74:4577-81.
8. Drazen JM, Dahlén S-E, Lee TH, editors. Five-lipoxygenase products in asthma. New York: Marcel Dekker;1998.
9. Samuelsson B. From studies of biochemical mechanisms to novel biological mediators: prostaglandin endoperoxides, thromboxanes and leukotrienes. In: *Les Prix Nobel: Nobel Prizes, presentations, biographies and lectures*. Stockholm: Almqvist & Wiksell; 1982. p. 153-74.
10. Hammarström S, Murphy RC, Samuelsson B, Clark DA, Mioskowski C, Corey EJ. Structure of leukotriene C: identification of the amino acid part. *Biochem Biophys Res Commun* 1979;91:1266-72.
11. Borgeat P, Samuelsson B. Metabolism of arachidonic acid in polymorphonuclear leukocytes: structural analysis of novel hydroxylated compounds. *J Biol Chem* 1979;254:7865-9.
12. Helgadottir A, Manolescu A, Thorleifsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, et al. The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. *Nat Genet* 2004;36:233-9.
13. Samuelsson B, Dahlén SE, Lindgren JÅ, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 1987;237:1171-6.
14. One-on-one with Professor Bengt Samuelsson: Keynote speaker, 36th annual meeting. Society for Leukocyte Biology Newsletter 2003; Spring/Summer:1.

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K. FRANK AUSTEN, MD, AND THE BIOLOGICAL CHARACTERIZATION OF SLOW-REACTING SUBSTANCE OF ANAPHYLAXIS AND ITS MOLECULAR IDENTIFICATION AS THE SULFIDOPEPTIDE LEUKOTRIENES

I had the opportunity to first meet K. Frank Austen, known as Frank, or KFA to those who have worked with him, as an intern in medicine at the old Peter Bent Brigham Hospital. I subsequently trained in his laboratory from 1972 to 1979, a period both intense and exciting in the unraveling of the mystery of slow-reacting substance of anaphylaxis (SRS-A)'s biological

properties and molecular identity. The story of this research effort was and remains instructive in the need for multidisciplinary research methods, technologic advances, and the contributions of many committed and insightful investigators over a prolonged period. It is to Frank's credit and because of his focus and determination and ability to find and nurture bright and imaginative young investigators that the prolonged saga leading to the elucidation of SRS-A as the sulfidopeptide leukotrienes (LTs) was not abandoned prematurely.

K. Frank Austen was born March 14, 1928, in Akron, Ohio. He received his MD from Harvard Medical School in 1954 and subsequently trained in Internal Medicine at the Massachusetts General Hospital, serving as Chief Medical Resident in 1958. He then undertook 3 postdoctoral training positions that served as the underpinnings of his major research interests of his career. He first spent 2 years with Elmer Becker at Walter Reed, where his interest in enzymes and the complement system was initiated. He then traveled to London to work at Mill Hill, in the Department of J. Humphrey but directly with Walter Brockelhurst, where Frank began his life-long interest in mast cell activation and mediators. His final postdoctoral experience was with Manfred Meyer at Hopkins, where Frank continued his studies of complement. Frank then returned to Harvard, first at the MGH and then as Professor and Chairman at the Robert Breck Brigham Hospital, where he was the Theodore B. Bayles Professor of Medicine from 1980-2001, and he is now the AstraZeneca Professor of Respiratory and Inflammatory Diseases at Harvard Medical School. It is important to note that while the studies described below were being conducted, the Austen laboratory also made seminal contributions to our understanding of the kinin/coagulation cascade (in work with Allen Kaplan, Jocelyn Spragg, and others) and the classical (Shaun Ruddy and Irma Gigli) and alternative complement cascades (Douglas Fearon and Mohammad Daha), including elucidating the pathway for activation and regulation of the alternative pathway of complement. Each of these efforts could easily be a subject of a separate Allergy Archives contribution.

The generation of SRS-A

An entity generated by cobra venom treatment of guinea pig lungs and with pharmacologic properties distinct from those of histamine, termed SRS, was reported in 1938.¹ Subsequent work by Brockelhurst² demonstrated that a similar principal, now called SRS-A, was generated by anaphylactic stimulation of both guinea pig and human lung tissue. On his return to Boston, Frank began his in-depth studies of this molecule with Kurt Bloch, Martin Valentine, Daniel Stechschulte, and Robert Orange.³ Their initial studies clarified the kinetics and immunologic mechanisms by which IgG and IgE antibodies mediated SRS-A generation in the rat peritoneal cavity. The latter was heat labile, mast cell dependent, and maximally effective early after passive sensitization. The former