

FROM STUDIES OF BIOCHEMICAL MECHANISMS TO NOVEL BIOLOGICAL MEDIATORS: PROSTAGLANDIN ENDOPEROXIDES, THROMBOXANES AND LEUKOTRIENES

Nobel Lecture, December 8, 1982

by

BENGT SAMUELSSON

Department of Physiological Chemistry, Karolinska Institutet,
S-104 01 Stockholm, Sweden

INTRODUCTION

Following the completion of the structural work on the prostaglandins with Sune Bergstrom and co-workers (for reviews, see refs. 1-3) I was very fortunate in being able to spend a year in the Chemistry Department at Harvard University, Cambridge, Mass. During this stay I had the opportunity to study both theoretical and synthetic organic chemistry. The year in Cambridge had a profound effect on my future research. At this time Konrad Bloch, E.J. Corey, Frank Westheimer, Robert B. Woodward and several other prominent scientists were among the faculty members of the department, and it was indeed a stimulating place for a young M.D. interested in chemistry. I was working in E.J. Corey's laboratory, and we have continued to collaborate in several areas since then.

In 1964 it was established that there was a biogenetic relationship between the polyunsaturated fatty acids and prostaglandins (4,5). This finding was of considerable biological interest and since the mechanisms of the reactions involved were unknown, I decided to study this problem in the laboratory I had established at that time.

MECHANISM OF BIOSYNTHESIS OF PROSTAGLANDINS

The conversion of 8,11,14-eicosatrienoic acid into prostaglandin E₁ (PGE₁) involves the introduction of two hydroxyl groups and one keto group. Incubation of 8,11,14-eicosatrienoic acid in an atmosphere of ¹⁸O₂ showed that the oxygen atoms of the hydroxyl groups were derived from oxygen gas whereas the keto oxygen did not contain any ¹⁸O (6,7). That this was due to exchange between the keto oxygen of the water was shown in later experiments (8). In these studies the keto group was reduced immediately with borohydride and the resulting trihydroxy acid derivative was shown to contain three atoms of ¹⁸O. These experiments were extended by carrying out the reaction in a mixture of ¹⁸O₂ (8). The reduced product was converted into the trimethoxy derivative, and the side chain carrying a hydroxyl group was cleaved off by oxidation with permanganate

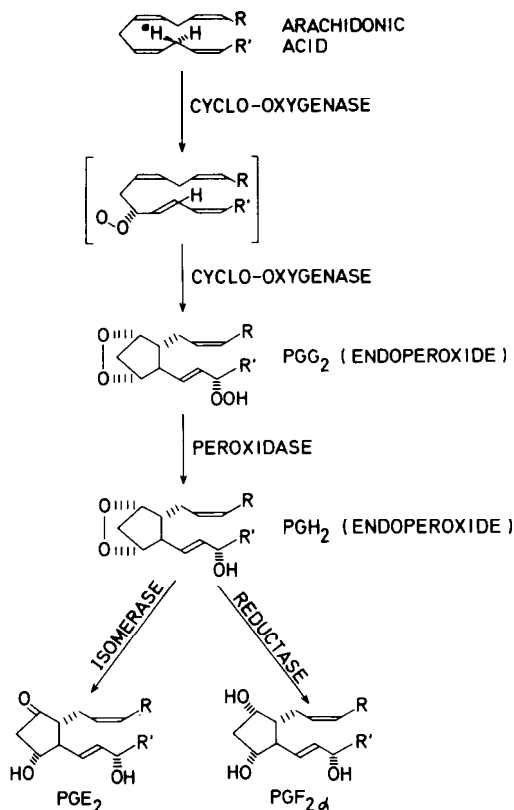


Fig. 1. Mechanism of prostaglandin biosynthesis

periodate. The resulting dicarboxylic acid ester contained the two oxygens that were introduced into the five-membered ring during the biosynthesis. Analysis of this molecule by mass spectrometry showed that it contained either two atoms of ^{18}O or two atoms of ^{16}O in the ring and that molecules with one atom of ^{18}O and one atom of ^{16}O in the ring were virtually absent. The experiment demonstrated that the oxygen atom of the hydroxyl group at C-11 and of the keto group at C-9 originated in the same molecule of oxygen (Fig. 1).

It was also shown that the hydrogens at C-8, C-11, and C-12 are retained in their original positions during the conversion of 8,11,14-eicosatrienoic acid into PGE₁, which is in agreement with the mechanism proposed (see below). Experiments with hypothetical intermediates, namely, 15-hydroperoxy-8,11,13-eicosatrienoic acid and 15-hydroxy-8,11,13-eicosatrienoic acid indicated that the initial reaction consisted of the introduction of the oxygens in the ring (9,10). However, two different mechanisms for the incorporation of the oxygen molecule seemed possible (9,10,11). In one of the pathways leading from 8,11,14-eicosatrienoic acid to the proposed intermediate, the oxygen is added across carbon atoms C-9 and C-11 with concomitant formation of the new carbon-carbon bond between C-8 and C-12. The other pathway involves a lipoxygenase-like reaction with formation of 11-peroxy-8,12,14-eicosatrienoic acid as the initial step. In

both of the pathways leading to the cyclic peroxide intermediate one hydrogen at C-13 is removed. In the latter pathway the hydrogen at C-13 is most likely removed as the initial step whereas the removal of this hydrogen occurs later in the first pathway. It was thus conceivable that the removal of the hydrogen was the rate-determining step and that substitution of tritium for hydrogen in the precursor would give a kinetic isotope effect. The resulting enrichment should appear in the precursor acid in the second pathway whereas the first pathway should produce enrichment of tritium in an oxygenated intermediate. Precursor acids, which were stereospecifically labeled with tritium at C-13 and labeled with ^{14}C at C-3, were therefore synthesized (10).

The conversion of the doubly labeled acids into PGE_2 was catalyzed by a vesicular gland preparation and the $^3\text{H}/^{14}\text{C}$ ratio of the precursor, product and the precursor remaining after the reaction was determined. It was found that $13\text{D}-^3\text{H}-^3\text{H}-^{14}\text{C}$ 8,11,14-eicosatrienoic acid retained the tritium label during the conversion to PGE_2 . The $13\text{L}-^3\text{H}-^3\text{H}-^{14}\text{C}$ -8,11,14-eicosatrienoic acid, however, was transformed into PGE_2 with essentially complete loss of tritium. The precursor isolated after 75 % conversion was significantly enriched (284 % retention) with respect to tritium. Thus, the initial step in the transformation of 8,11,14-eicosatrienoic acid into prostaglandin is the stereospecific elimination of the 13L-hydrogen. This reaction is followed by introduction of oxygen at C-11 in a lipoxigenase-like reaction to give 11-peroxy-8,12,13-eicosatrienoic acid (10,12). It is of interest in this context that soybean lipoxigenase removes the same hydrogen both specifically (08) and stereospecifically (L) (9). However, the plant lipoxigenase introduces the oxygen molecule in $\omega 6$ position whereas the lipoxigenase, which is a component of the prostaglandin synthetase, introduces the oxygen in $\omega 10$ position (9). The 11-peroxy-8,12,13-eicosatrienoic acid visualized to be formed in the initial oxygenation is subsequently transformed into an endoperoxide (8) by a concerted reaction involving addition of oxygen at C-15, isomerization of the A' double bond, formation of the new carbon-carbon bond between C-8 and C-12 and attack by the oxygen radical at C-9. This is shown in Fig. 1. Indirect evidence indicates a free radical mechanism (13,14). The endoperoxide is transformed into PGE_2 by removal of hydrogen at C-9 or into $\text{PGF}_{2\alpha}$ by a reductive cleavage of the peroxide.

When washed microsomes were used as enzyme source, eicosatrienoic acid gave rise to other products (15,16,17). These cannot act as precursors in the biosynthesis of prostaglandins; however, their structures and the fate of ^3H in labeled precursors during their formation provided additional evidence for the proposed scheme of the transformation. The monohydroxy acid fraction from an incubation with eicosatrienoic acid was shown to consist of 11-hydroxy-8,12,14-eicosatrienoic acid, 15-hydroxy-8,11,13-eicosatrienoic acid and 12-hydroxy-8(trans),10(trans)-heptadecadienoic acid. The mechanism of formation of the C acid was studied by using ^3H -eicosatrienoic acid, which also contained tritium label at C-9, C-10 or C-11 (16). These experiments showed that the conversion resulted in loss of the tritium label in these three positions. Furthermore, malonaldehyde was identified by condensation with L-arginine to give d-N-P-(pyrimidinyl)-L-ornithine or with urea to form 2-hydroxypyrimidine. The derivative

of malonaldehyde contained the ^3H label from the 9- ^3H and 11- ^3H labeled precursor whereas ^3H originally in position C-10 was lost by enolization of the malonaldehyde.

It was further found that a compound with chromatographic properties similar to those of PGE_2 , and which was transformed into $\text{PGF}_{1\alpha}$ by borohydride reduction was formed from eicosatrienoic acid (17). The structure of the new product was found to be 9 α , 15-dihydroxy-11-ketoprost-13-enoic acid (11-dehydro- $\text{PGF}_{1\alpha}$) (18). In the conversion of 9- ^3H , 3- ^{14}C - and 11- ^3H , 2- ^{14}C -8,11,14-eicosatrienoic acid to 11-dehydro- $\text{PGF}_{1\alpha}$, the latter precursor lost the ^3H label, whereas the 9- ^3H label was retained. These experiments on the structures of the various products from eicosatrienoic acid and the fate of the ^3H labels in their formation provided strong evidence for the existence of the endoperoxide intermediate.

ISOLATION AND CHARACTERIZATION OF PROSTAGLANDIN ENDOPEROXIDES

Subsequently it was possible to detect and isolate an endoperoxide from short-time incubations of arachidonic acid with the microsomal fraction of homogenates of sheep vesicular glands (19). The incubation mixtures were treated with stannous chloride in ethanol in order to reduce endoperoxide into $\text{PGF}_{2\alpha}$. This was followed by sodium borodeuteride reduction and determination of the resulting $\text{PGF}_{2\alpha}$ species by multiple-ion analysis. This method made it possible to assay PGE_2 as well as 11-dehydro- PGF_p , and $\text{PGF}_{2\alpha}$. It was of particular interest that a peak of $\text{PGF}_{2\alpha}$ appeared in the initial phase of the incubation period. No metabolic transformation of PGF compounds had been observed in preparations of sheep vesicular gland, and thus it was unlikely that the $\text{PGF}_{2\alpha}$ peak could be ascribed to enzymatic formation of $\text{PGF}_{2\alpha}$ followed by rapid metabolic degradation. Furthermore, when the SnCl_2 and sodium borodeuteride reduction was omitted, the peak of $\text{PGF}_{2\alpha}$ disappeared, indicating that $\text{PGF}_{2\alpha}$ was formed by chemical reduction of an oxygenated derivative present in the initial phase of the incubation. That an oxygenated intermediate was formed and temporally accumulated was also suggested by the finding that the rate of PGE_2 formation was slower than the rate of oxygenation of the precursor acid.

Additional support for the existence of an oxygenated intermediate that was convertible into $\text{PGF}_{2\alpha}$ by SnCl_2 reduction came from experiments in which reduced glutathione or p-mercuribenzoate was added to the microsomal suspension. The former agent increased the rate of PGE_2 formation and suppressed the peak of $\text{PGF}_{2\alpha}$, whereas the latter agent decreased the rate of PGE_2 formation with a simultaneous increase in the height and duration of the $\text{PGF}_{2\alpha}$ peak. The oxygenated intermediate detected by these experiments was also isolated. On thin-layer radiochromatographic analysis of the product (methyl esters) isolated after a 30-second incubation of labelled arachidonic acid with microsomes in the presence of p-mercuribenzoate a new radioactive derivative appeared. This derivative was identified as the methyl ester of the earlier postulated endoperoxide. In an extension of these studies the endoperoxide described above was

obtained as the free acid; in addition, an endoperoxide carrying a hydroperoxy group at C-15 was isolated (20,21). We suggested the trivial names PGG₂ for the less polar endoperoxide (15-hydroperoxy-9 α , 11 α -peroxidoprostanoic acid) and PGH₂ for the more polar endoperoxide (15-hydroxy-9 α , 11 α -peroxidoprostanoic acid). The structure of PGG₂ was established by three separate experiments. Treatment of PGG₂ with mild reducing agents such as SnCl₂ and triphenylphosphine gave PGF_{2 α} as the major product. This showed the presence of a peroxide bridge between C-9 and C-11 but did not discriminate between a hydroxy and a hydroperoxy group at C-15 since the agents used would reduce the latter group into the former. In a second experiment, PGG₂ was treated with lead tetraacetate in benzene followed by triphenylphosphine. In this case 15-keto-PGF_{2 α} was the major product. Lead tetraacetate causes dehydration in hydroperoxides into ketones, and therefore, formation of a 15-ketoprostaglandin from PGG₂ by this treatment strongly indicated the presence of a hydroperoxy group at C-15. The isomerization of PGG₂ into 15-hydroperoxy-PGE₂ in aqueous medium gave independent evidence for a peroxide group at C-15 (Fig. 1).

Two reactions are involved in the conversion of PGG₂ into PGE₂, i.e., reduction of the hydroperoxy group at C-15 into a hydroxy group (peroxidase) and isomerization of the endoperoxide structure into a β -hydroxyketone (endoperoxide isomerase) (Fig. 1). The endoperoxide isomerase was found to be almost entirely associated with the microsomal fraction. The enzymic activity was stimulated by reduced glutathione.

The endoperoxides were quite unstable ($t_{1/2} = 5$ min). However, if they were stored under anhydrous conditions in acetone they could be kept for several weeks. When their biological activity was tested on *in vitro* preparations it was found that the effects of the endoperoxides on gastrointestinal smooth muscle were comparable to those of PGE₂ and PGF_{2 α} . On the other hand, the effects on vascular (rabbit aorta) and airway (guinea pig trachea) smooth muscle were considerably greater than those of PGE₂ and PGF_{2 α} , respectively (22). Both endoperoxides were potent contractors of the isolated human umbilical artery (23). Administration of PGG₂ and PGH₂ intravenously to guinea pigs (22) produced an increase in insufflation pressure, which was more marked than that caused by corresponding doses of PGF_{2 α} . The cardiovascular effects of the endoperoxides showed a complex pattern. The blood pressure response was triphasic, i.e., a transient fall consistently followed by a shortlasting rise and then by a sustained reduction. These studies on vascular and airway smooth muscle demonstrated that the endoperoxides had effects that could not be attributed to conversion into the stable prostaglandins.

Additional studies in our laboratory showed that the two endoperoxides also had unique effects on platelets. Thus, PGG₂ and PGH₂ induced rapid and irreversible aggregation of human platelets (19,20,24).

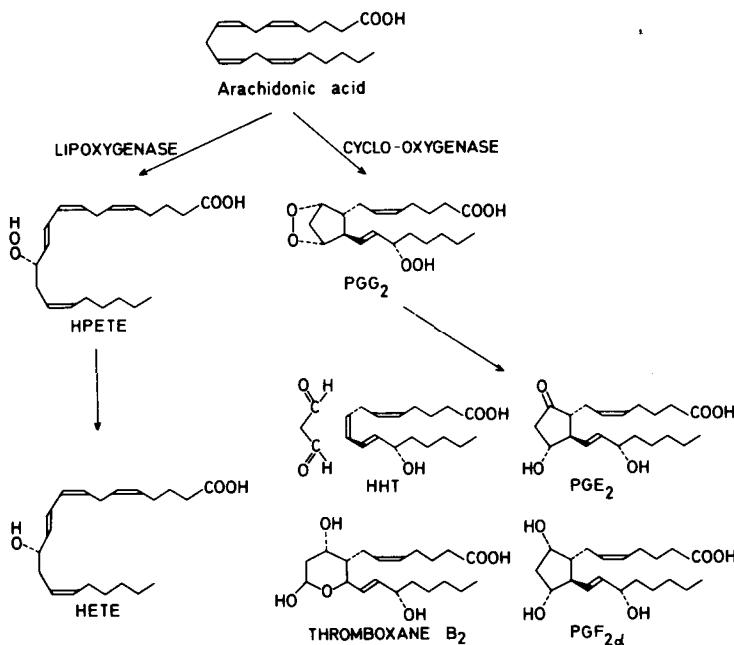


Fig. 2. Transformation of arachidonic acid in human platelets.

DISCOVERY OF AN UNSTABLE AGGREGATING FACTOR AND THE THROMBOXANE S

The biological effects of the pure endoperoxides were of particular interest in relation to other studies which demonstrated that arachidonic acid caused aggregation when added to human platelets (25,26) and that labile aggregating material (LASS) was formed from this acid when it was incubated with preparations of sheep vesicular glands (27,28,29). Furthermore, the potency of the endoperoxides in causing contractions of the isolated rabbit aorta was of particular interest in relation to the so-called rabbit aorta contracting substance (RCS) (30). RCS was reported to be formed in guinea pig lung during anaphylaxis, and was later suggested to be due to the endoperoxide intermediate in prostaglandin biosynthesis (31). We found that material with similar biological properties was formed after addition of arachidonic acid to human platelets. However, the rabbit aorta contracting substance from guinea pig lung and platelets was found to consist of one major component with a $t_{1/2}$ of about 30 seconds and a minor component of PGG₂ and/or PGF₂ with a $t_{1/2}$ of 4-5 minutes (32). The short-lived major component of RCS could be generated by addition of arachidonic acid to platelets.

We therefore incubated 1-¹⁴C-arachidonic acid with suspensions of washed human platelets in order to obtain structural information about RCS. Three major metabolites were isolated (33). One of them was found to be 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) (Fig. 2) The corresponding hydro-

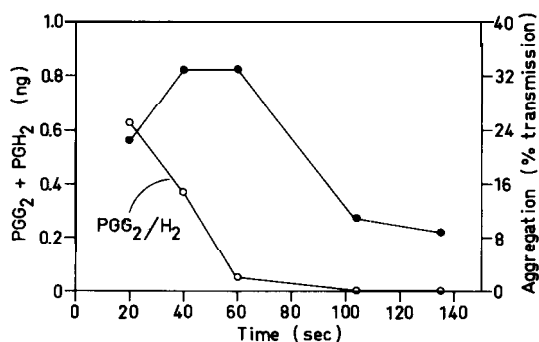


Fig. 3. Maximum aggregation induced by 0.1 ml of suspensions of washed platelets incubated for different times with 120 ng of arachidonic acid (●—●). The content of PGG₂/H₂ in these samples is also given (○—○). The platelet suspension in the aggregometer tube was preincubated for 2 min with 1.4×10^{-5} M indomethacin.

peroxide (HPETE) could be isolated after incubation of arachidonic acid with sonicated platelets. Formation of 12-HETE from arachidonic acid was also reported to occur in bovine platelets (34). A more polar metabolite was identified as 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) whereas a third component was found to be the hemiacetal derivative of 8(1-hydroxy-3-oxopropyl)-9-12L-dihydroxy-5,10-heptadecadienoic acid (thromboxane B₂, PHD). [$1-^{14}\text{C}$]-PGG₂ added to suspensions of human platelets was rapidly converted into HHT and thromboxane B₂.

All of the identified metabolites of arachidonic acid were stable compounds and could therefore not be identical to the very unstable ($t_{1/2} = 30$ seconds) RCS. Additional biological work with the platelets involving characterization of the material formed from arachidonic acid was therefore carried out. When arachidonic acid was incubated with washed platelets and an aliquot of the incubate was transferred to a suspension of platelets preincubated with indomethacin, aggregation took place. This was not due to PGG₂ or PGH₂ since the amounts found were only about one per cent of those required to explain the response. A more detailed analysis of the appearance of the aggregating factor and the endoperoxides showed that the amount of endoperoxides was highest in the very early phase of the incubation period, whereas the aggregating factor had a maximum later (35) (Fig. 3). Experiments using filtrates of incubates prepared as described above showed that the aggregating factor was very unstable. When the log dose (arbitrary units) was plotted against time of incubation at 37°C, a linear relationship was obtained. The half-life of the aggregating factor was 33-46 seconds. A factor with similar properties was also generated from the endoperoxide, PGG₂. In addition to inducing irreversible aggregation, the unstable factor also caused release of serotonin from platelets.

Further work involving $^{18}\text{O}_2$ experiments suggested that thromboxane B₂ was formed from PGG₂ by rearrangement and subsequent incorporation of one molecule of H₂O (33). It was therefore conceivable that if the rearranged inter-

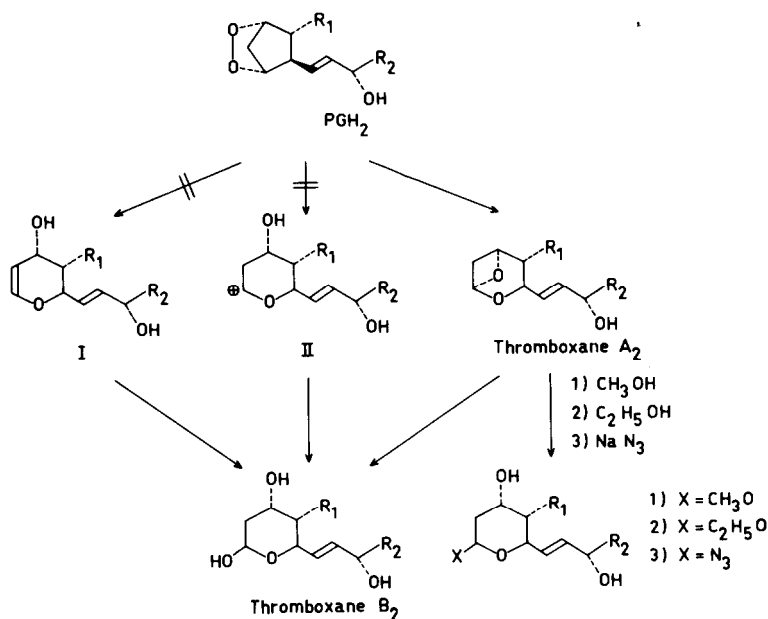


Fig. 4. Scheme of transformations of PGH_2 into thromboxane derivatives

mediate had an appreciable lifetime it should be trapped in the presence of nucleophilic reagents (36). This was found to be the case. Addition of 25 volumes of methanol to washed platelets incubated with arachidonic acid for 30 seconds gave two derivatives that were less polar than thromboxane B₂. The mass spectral data indicated that the two compounds obtained by addition of methanol were epimers of thromboxane B₂ methylated at the hemiacetal hydroxyl group (Fig. 4). The two epimers also appeared when methanol was added to platelets incubated with PGG_2 for 30 seconds. Addition of ethanol to platelets incubated with arachidonic acid for 30 seconds similarly gave rise to epimers of thromboxane B₂ ethylated at the hemiacetal hydroxyl group. Finally, addition of 5 volumes of 5M sodium azide to platelets incubated with arachidonic acid gave an azido alcohol, i.e. a derivative of thromboxane B₂ in which the hemiacetal hydroxyl group was replaced by an azido group. The trapping experiments showed the existence of a very unstable intermediate in the conversion of PGG_2 into thromboxane B₂.

In order to determine the half-life of the intermediate, the platelet suspension was incubated with [1-¹⁴C]-arachidonic acid for 45 seconds and the reaction was stopped by filtration. The clear, essentially platelet-free filtrate was kept at 37°C, and aliquots were removed after different times and immediately added to 25 ml of methanol containing tritium-labelled mono-0-methylthromboxane B₂. A linear relationship between the logarithms of the ¹⁴C/³H ratios of the purified methyl ester of mono-0-methyl-thromboxane B₂ and the times of incubation was obtained. The half-life thus obtained was 32±2 (SD) seconds.

Fig. 4 shows the proposed structure of the unstable intermediate. The acetal carbon atom binding two oxygens should be susceptible to attack by nucleophiles, e.g. H_2O (giving thromboxane B_2) as well as CH_3OH , C_2H_5OH and NH_3 (giving derivatives of thromboxane B_2 described above). Addition of C_2H_5OH to platelets incubated with arachidonic acid led to formation of mono-O-methylthromboxane B_2 lacking carbon bound 3H . This finding excluded an alternative structure of the unstable intermediate, i.e. an unsaturated oxane (I) in Fig. 4). Furthermore, the t_{1/2} of thromboxane A_2 seemed to exclude a carbonium ion structure ((II) in Fig. 4), which in aqueous medium should be considerably less stable.

The available evidence indicated that the aggregating factor and RCS were due to the same compound. Thus, they were both derived from arachidonic acid or PGG₂, their formation from arachidonic acid was blocked by indomethacin, and their half-lives were similar. It was proposed that this material is identical with the unstable intermediate detected chemically in platelets (Fig. 4) because of similar properties.

The new oxane derivatives were named thromboxanes because of their structure and origin. Thromboxane A_2 is the highly unstable bicyclic compound, and thromboxane B_2 is the stable derivative provisionally named PHD. The subscript indicates the number of double bonds, as in the prostaglandin nomenclature. The structure of thromboxane B_2 has been confirmed by synthesis (37,38,39,40).

Thromboxane A_2 has been shown to possess a variety of strong biological effects. The best known of these are induction of platelet aggregation and the release reaction (36,40) as well as strong constricting effects on vascular smooth muscle. The first vessel to be studied in this respect was the rabbit aorta (30,32); later similar contractile responses were observed in other vessels as well, such as coronary arteries (42,43,44,45,46), the mesenteric and celiac arteries (47,48), the umbilical artery (23,49), and others.

These dual effects of TXA₂, induction of vasoconstriction and platelet aggregation both come into operation after a vessel has been injured. They indicate that TXA₂ probably plays a role in normal hemostasis *in vivo* as well as in pathological conditions with an increased tendency to vasospasm and/or thrombosis. Several reviews have been written on the biological effects and possible roles of thromboxanes *in vivo* (e.g. Refs. 41,50). Thromboxane A_2 has also potent contractile effects on airways demonstrable both *in vitro* and *in vivo* (51).

Following the isolation of the endoperoxides and the discovery of thromboxane A_2 , it was found that arterial tissue converts the endoperoxide into a product with opposite effects (52). Structural work demonstrated that it was an enol ether derivative (53). This vasodilator and antiaggregating compound was named PGI₂, or prostacyclin. Thromboxane A_2 and prostacyclin probably form a hemostatic mechanism for control of the tonus of blood vessels and the aggregation of platelets *in vivo*. These platelet and vessel wall reactions, which are of considerable interest in antithrombotic therapy, are summarized in Fig. 5. The main focus is now on the development of specific thromboxane synthetase inhibitors for this purpose. For a recent review see ref. 54.

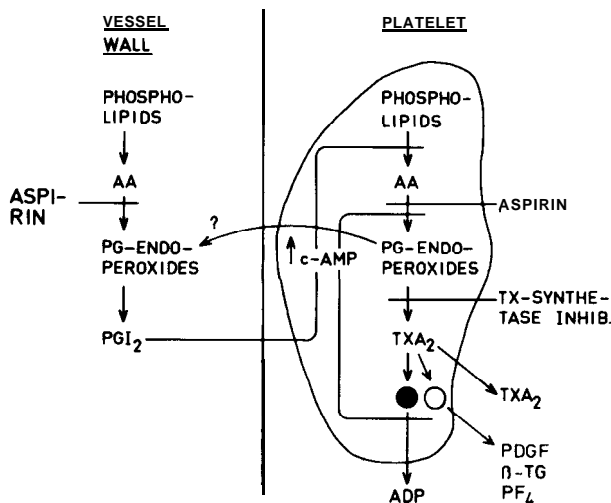


Fig. 5. Interaction between platelets and the vessel wall

DISCOVERY OF THE LEUKOTRIENE S

The role of prostaglandins in inflammation was brought into focus with the discovery by Vane and collaborators that non-steroidal anti-inflammatory drugs like aspirin inhibit the enzyme (cyclo-oxygenase) responsible for conversion of arachidonic acid into prostaglandins and thromboxanes (55). Anti-inflammatory steroids also inhibit the formation of prostaglandins; however, the mechanism of action is different. The steroids inhibit the formation of prostaglandins by blocking the release of arachidonic acid from the phospholipids. Since aspirin-like drugs and steroids have different anti-inflammatory effects it seemed conceivable to us that some of these differences might be due to formation of additional pro-inflammatory derivatives of arachidonic acid. Studies of the transformation of arachidonic acid in leukocytes, which were carried out to test this hypothesis, have recently resulted in the recognition of a novel group of compounds, the leukotrienes. These compounds seem to be of importance in both immediate hypersensitivity reactions and inflammation.

When arachidonic acid was incubated with polymorphonuclear leukocytes it was found that the major metabolite was a new lipoxygenase product, viz. 5(S)-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) (56). Additional studies also demonstrated the formation of 5(R), 12(R) dihydroxy-6,8,10,14-eicosatetraenoic acid (major product) (leukotriene B₅, c.f. below), two additional 5(S), 12-dihydroxy-6,8, 10-trans, 14-cis-eicosatetraenoic acids, epimeric at C-12, and two isomeric 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acids (Fig. 6) (57,58).

Stereochemical studies, demonstrating formation of two acids with all trans conjugated trienes and epimeric at C-12 and one major isomer (12R) with different configuration of the triene raised the question of the mechanism of

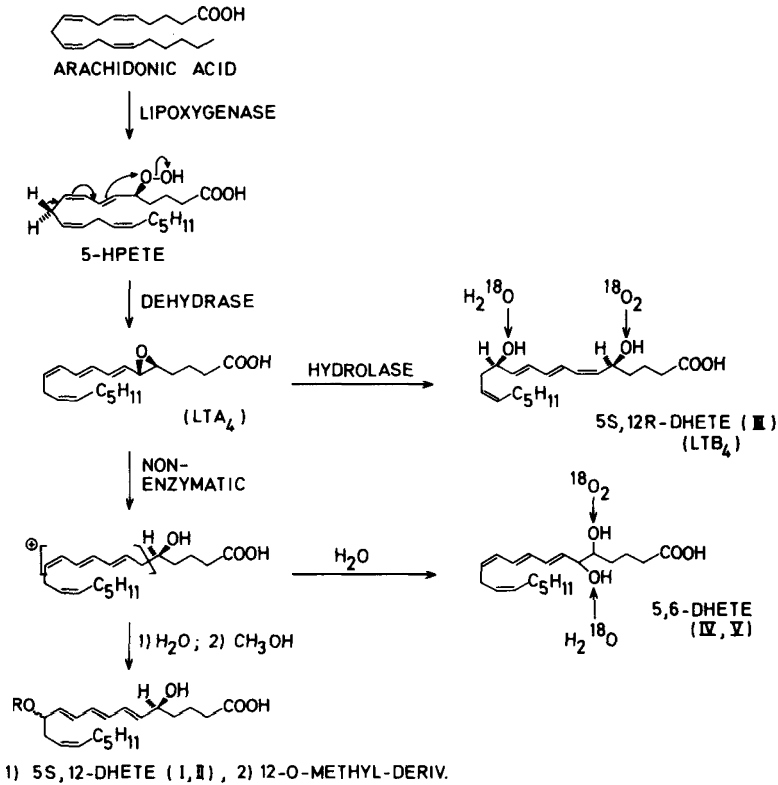


Fig. 6. Formation of dihydroxy derivatives via unstable intermediate. Origin of oxygen and trapping experiments.

formation (58). With isotopic oxygen it was demonstrated that the oxygen of the alcohol group at C-5 originated in molecular oxygen, whereas the oxygen of the alcohol group at C-12 was derived from water (Fig. 6) (59). These observations led us to develop the hypothesis that leukocytes generated an unstable intermediate which would undergo nucleophilic attack by water, alcohols, and other nucleophiles. Leukocytes were therefore incubated for 30 seconds with arachidonic acid followed by addition of 10 volumes of methanol, 10 volumes of ethanol or 0.2 volumes of N HCl. Trapping with methanol (or ethanol) yielded two new less polar compounds, which were present in equal amounts and which had ultraviolet spectra identical to those of compounds I and II (Fig. 6). Infrared spectrometry indicated that the conjugated trienes had all-trans geometry. Mass spectrometric analyses of the two compounds showed that they were isomeric and carried hydroxyl groups at C-5 and methoxy groups at C-12. The alcohol groups at C-5 had (S) configuration and it was obvious that the compounds were the C-12 epimers of 5(S)-hydroxy,12-methoxy-6,8,10,14(E.E.E.Z)-eicosatetraenoic acid (Fig.6).

These experiments demonstrated that leukocytes generated a metabolite of arachidonic acid, which can undergo a facile nucleophilic reaction with alcohols. Analysis of samples obtained from trapping experiments performed under

different conditions always showed inverse relationships between the amount of compounds I and II and their 12-O-alkyl derivatives. This result suggested that compounds I and II and the 12-O-alkyl derivatives were formed nonenzymatically from the same intermediate.

The stability of the intermediate was determined by incubating leukocytes with arachidonic acid for 45 seconds followed by addition of 1 volume of acetone in order to inactivate the enzyme. After different time intervals aliquots of the mixture were transferred to flasks containing 15 volumes of methanol. Analysis by chromatography showed that the $t_{1/2}$ of the intermediate, measured as the 12-O-methyl derivative was 3-4 min. Concomitantly with the decrease in the concentration of the intermediate the concentrations of compounds I, II, IV and V increased whereas the concentrations of compounds III and 5-hydroxy-6,8,11,14-eicosatetraenoic acid remained constant. These data suggested that the epimeric 5,6- and 1,12-dihydroxy acids (compounds I, II, IV and V) are formed by non-enzymatic hydrolysis of a common unstable intermediate, whereas compound III is generated by enzymatic hydrolysis of the same intermediate (Fig. 6). Similar experiments performed at acid and alkaline pH showed that the intermediate was acid labile and considerably more stable under alkaline conditions. It was also found that the two 5,6-dihydroxy-derivatives (IV and V) were formed non-enzymatically) from the same intermediate as the enzymatic product, 5S,12R-dihydroxy-eicosatetraenoic acid, and that ^{18}O from molecular oxygen was exclusively retained at C-5 of these derivatives whereas ^{18}O from water was introduced at C-6 or C-12. On basis of the experimental data described above, the structure 5,6-oxido-7,9,11,14-eicosatetraenoic acid (leukotriene A₄ LTA₄) (Fig. 6) was proposed for the intermediate (59).

The formation of compounds I-V from the epoxide intermediate is shown in Fig. 6. With the exception of compound III these are formed by chemical hydrolysis of the epoxide through a mechanism involving a carbonium ion. This derivative added hydroxyl anion preferentially at C-6 and C-12 to yield four isomeric products which contain the stable conjugated triene structure. The formation of compound III is enzymatic since it is not racemic at C-12 and since it is only formed by non-denatured cell preparations.

The structure, 5,6-oxido-7,9,11,14-eicosatetraenoic acid (leukotriene A₄ c.f. below) (59) proposed for the intermediate has subsequently been confirmed by chemical synthesis and the stereochemistry has been elucidated (60). The 5S,12R-dihydroxy acid formed enzymatically was earlier shown to contain one *cis* and two *trans* double bonds in the conjugated triene. The location of the *cis* double bond (A'-position) was recently established by synthetic methods (61). The allylic epoxide intermediate can exist in free form since it has been isolated from human polymorphonuclear leukocytes (62). The suggested mechanism for the biosynthesis of the epoxide from arachidonic acid (Fig. 6) involves initial formation of 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE). The epoxide is formed from 5-HPETE and subsequent abstraction of the pro-R hydrogen at C-10, and elimination of hydroxyl anion from the hydroperoxy group (63). The dehydration reaction has been found to be catalyzed by a soluble enzyme, which was recently isolated from leukocytes (64).

SLOW REACTING SUBSTANCE OF ANAPHYLAXIS (SRS-A)

The occurrence of a smooth muscle stimulating factor (SRS) appearing in the perfusate of guinea pig lung treated with cobra venom was described in 1938 (65). The factor was shown to be released also by immunological challenge (66). Biological studies of SRS suggested that it might be an important mediator of anaphylactic and other immediate hypersensitivity reactions (67,68). Characterization of SRS indicated that it was a polar lipid with UV-absorption and that it might contain sulfur (69,70,71). Studies with labelled arachidonic acid suggested that this acid might be incorporated into SRS (72,73).

Studies in our laboratory showed that treatment of human neutrophils with the ionophore A23187 resulted in stimulation of the synthesis of the 5,12-dihydroxy acid (LTB₄) described above (58). On the basis of the stimulatory effect of the ionophore on both SRS production (74) and LTB₄ formation, the UV-absorbance data and other considerations we developed the hypothesis that there was a biogenetic relationship between the unstable allylic epoxide intermediate in neutrophils and SRS generated in a variety of systems.

For production of relatively large amounts of SRS we found that murine mastocytoma cells (CXBGABMCT-1) stimulated with the calcium ionophore A23187 were more suitable than previously described systems (75). The SRS was purified by high pressure liquid chromatography. The purified material showed an absorbance maximum at 280 nm and gave a typical contraction of guinea pig ileum, which was reversed by FPL-55712 (75). The ultraviolet characteristics resembled those of the dihydroxy acids. However, the maximum was shifted to a 10 nm higher wavelength. This was in agreement with a sulfur substituent at a conjugated triene. Labelled arachidonic acid and cysteine were incorporated into the product.

Degradation of SRS by Raney nickel desulfurization gave 5-hydroxy-arachidic acid, indicating that the arachidonic acid derivative and cysteine were linked by a thioether bond (Fig. 7). This finding also supported the hypothesis that there was a biogenetic relationship between the 5-lipoxygenase pathway in leukocytes and SRS. The positions of the double bonds in SRS were determined by reductive ozonolysis. The isolation of 1-hexanol among the products indicated that the Δ^{14} -double bond of arachidonic acid had been retained. The approach used for locating the conjugated triene was based on previous studies in our laboratory that had shown that arachidonic acid and related fatty acids containing two methylene interrupted *cis* double bonds at the $\omega 6$ and $\omega 9$ positions are oxygenated to give derivatives with isomerization of the $\omega 6$ double bond to $\omega 7$. Incubation of the isolated SRS with lipoxygenase resulted in isomerization of the Δ^{14} -double bond into conjugation with the conjugated triene (forming a tetraene) since there was a bathochromic shift of 30 nm. This finding indicated that SRS contained a Δ^{11} -*cis* double bond and additional double bonds at $\Delta 7$ and $\Delta 9$. The structural work at this stage showed that SRS was a derivative of 5-hydroxy-7,9,11,14-eicosatetraenoic acid with a cysteine containing substituent in the thioether linkage at C-6. Derivatization of cysteine was suggested by the failure to isolate alanine after desulfurization.

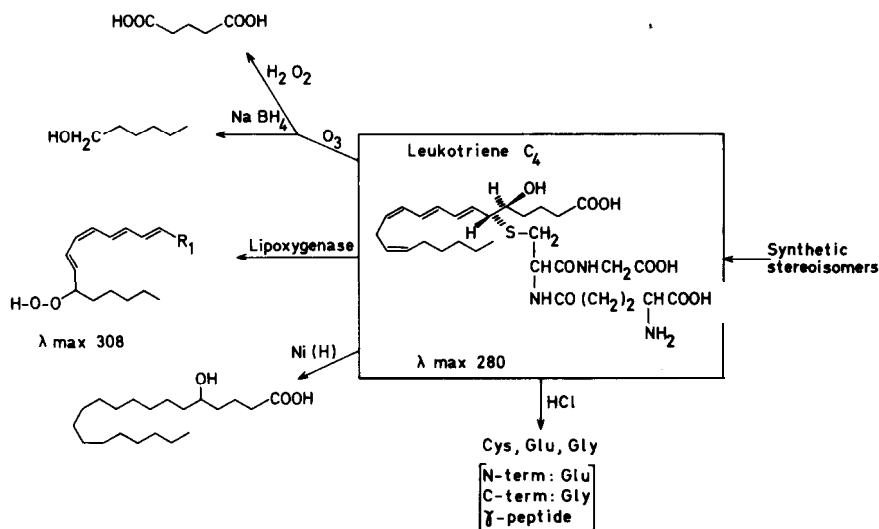


Fig. 7. Some transformations in structural studies on SRS.

The cysteine containing substituent was therefore referred to as RSH in the reports of this work (75,76,77). Further studies involving amino acid analyses of acid hydrolyzed SRS demonstrated that in addition to cysteine, one mol of glycine and one mol of glutamic acid were present per mol of SRS. End group (dansyl method and hydrozinoysis) and sequence analyses (dansyl-Edman procedure) of the peptide showed that it was γ -glutamylcysteinylglycine (glutathione). The structure of the SRS from murine mastocytoma cells was therefore 5-hydroxy-6-S-glutathionyl-7,9,11,14-eicosatetraenoic acid, leukotriene (LT)C₄ (c.f. below) (Fig. 7) (78). The structure was confirmed by comparison with synthetic material. This represented the first structure determination of an SRS-A (78). The preparation and some properties of corresponding cysteinylglycine derivative (LTD₄) and cysteinyl derivative (LTE₄) were also reported at the same time (78). These compounds have later been isolated from natural sources (see below). The proposed stereochemistry for LTC₄ was confirmed and unambiguously assigned by total synthesis including preparation of stereoisomers of LTC₄ (79). The synthetic work was carried out by E.J. Corey and co-workers. LTC₄ is thus 5(S)-hydroxy,6(R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid.

Later studies using a different cell type, the RBL-1 cells, demonstrated that the major slow reacting substance was LTD₄ (5(S)-hydroxy,6(R)-S-cysteinylglycine-7,9-trans-11,14-cis-eicosatetraenoic acid) (80).

Following the structure determination of SRS from mastocytoma cells (75,78) and synthetic preparation of LTC₄, LTD₄ and LTE₄ (78) all of these cysteine containing leukotrienes (Fig. 8) have been found in a variety of biological systems using comparison with synthetic material or partial characterization by chemical or physical methods for identification (Table 1). SRS-A is thus a

Table 1. Identification of leukotrienes from different sources

Source	LTA ₄	LTB ₄	LTC ₄	LTD ₄	LTE ₄	References
Rabbit peritoneal leukocytes	+	+				57, 59
Human peripheral leukocytes	+	+	+			86, 119, 120
Mouse mastocytoma cells	+		+			75, 77, 121
Rat basophilic leukemia cells				+	+	80, 122, 123
Rat peritoneal cells			+	+	+	124, 125, 126
Rat leukocytes		+				127, 128
Rat macrophages		+				129
Mouse macrophages			+			130
Human lung			+	+		131
Guinea pig lung				+		132
Cat paws				+	+	133

mixture of leukotrienes containing cysteine, i.e. the parent compound LTC₄ and the metabolites LTD₄ and LTE₄.

Transformation of LTA₄ into LTC₄ by enzymatic addition of glutathione has been demonstrated in both mastocytoma cells and human leukocytes pretreated with the inhibitor of arachidonic acid metabolism, BW755 (81). These studies confirm the originally proposed pathway for biosynthesis of SRS, i.e. formation of LTA₄ from arachidonic acid via 5-HPETE followed by addition of glutathione to LTA₄ with opening of the epoxide at the allylic position C-6 to give LTC₄ (75).

The biological significance of the biosynthetic pathways described and the cumbersome systematic names of the compounds involved suggested the introduction of a trivial name for these entities (76). The term "leukotriene" was chosen because the compounds were discovered in leukocytes and the common structural feature is a conjugated triene. Various members of the group have been designated alphabetically: leukotrienes A are 5,6-oxido-7,9-trans-11-cis; leukotriene B, 5(S),12(R)-dihydroxy-6-cis-8, 10-trans; leukotrienes C, 5(S)-hydroxy-6(R)-γ-glutamyl-cysteinyl-glycyl-7,9-trans-11-cis; and leukotrienes E, 5(S)-hydroxy-G(R)-S-cysteinyl-7,9-Mm-11-cis-eicosapolyenoic acids. Since precursor acids containing the Δ⁵ double bond system (i.e. 5,8,11-eicosatrienoic acid, arachidonic acid and 5,8,11,14,17-eicosapentaenoic acid) can be converted to leukotrienes containing 3-5 double bonds, a subscript denoting this number is used (82). Leukotriene A₄ is thus the epoxy derivative of arachidonic acid which can be further transformed to leukotrienes B₄, C₄ and E₄.

Leukotriene C₄ is metabolized to leukotriene D₄ by enzymatic elimination of glutamic acid by γ-glutamyl transpeptidase (80). The remaining peptide bond in leukotriene D₄ is hydrolyzed by a renal dipeptidase to give leukotriene E₄ (83). It has recently been found that LTE₄ can also function as acceptor of γ-glutamic acid forming a γ-glutamyl, cysteinyl derivative, named LTF₄ (84,85) (Fig. 8).

In addition to the 5-lipoxygenase, leukocyte preparations contain enzymes catalyzing introduction of oxygen at C-12 and C-15 (86). Recently, evidence has been obtained for leukotriene formation after initial oxygenation at either of these positions (87,88,89,90,91).

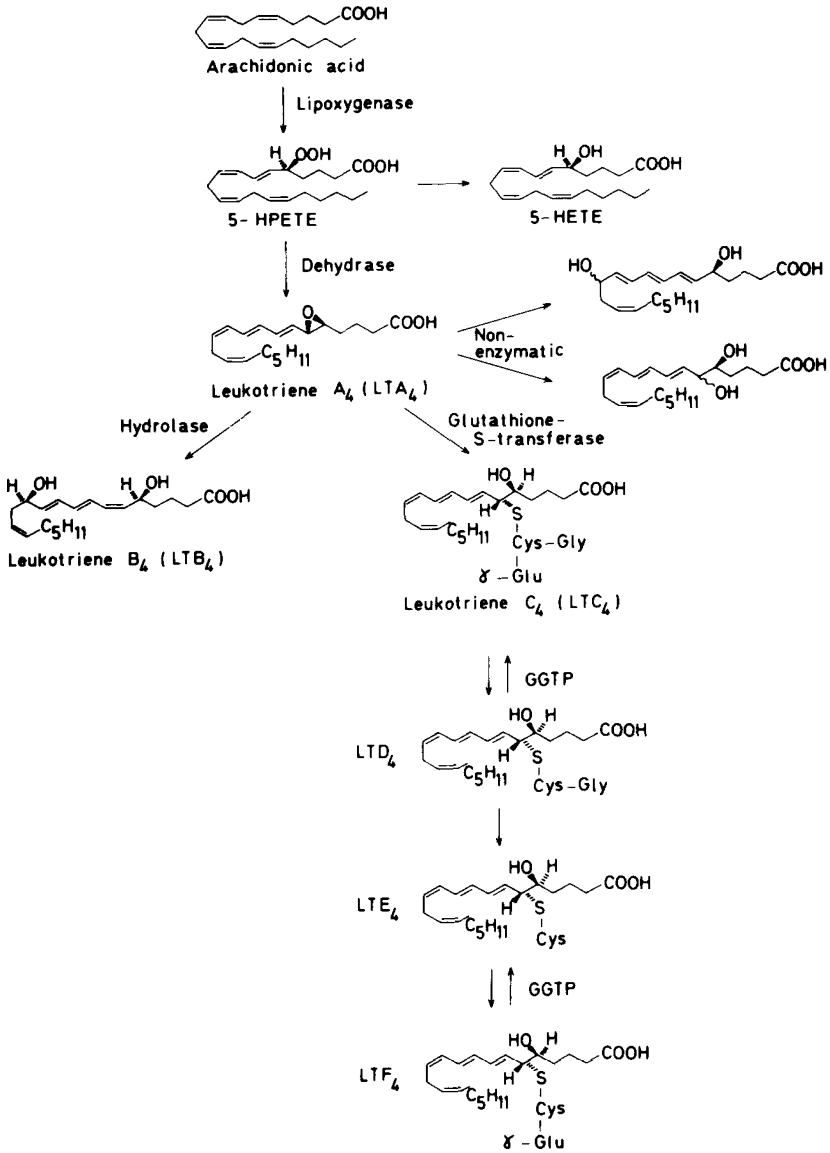


Fig. 8. Formation of leukotrienes via the 5-lipoxygenase pathway.

BIOLOGICAL EFFECTS OF THE LEUKOTRIENES

Studies with pure leukotrienes have provided detailed information about the effects of this group of compounds in different biological systems, The leukotrienes containing cystein (LTC₄, D₄ and E₄) are potent bronchoconstrictors in several species including humans, and they seem to have specific effects on the peripheral airways (92,93,94,95,96,97,98,99). They also show potent vasoconstrictor activity and have negative inotropic effects on the cardiac contractions (100).

In recent studies using bronchi from atopic patients sensitive to birch pollen (101) the relative importance of the leukotrienes as mediators of anaphylaxis has been demonstrated. Treatment of the preparation with a histamine antagonist, mepyramine, and cycle-oxygenase inhibitor, indomethacin, did not reduce the response to the specific allergen. However, benoxaprofen and a prostacyclin derivative (U-60257), both of which block leukotriene formation (102,103), inhibited the anaphylactic contraction in bronchi from asthmatics induced by birch pollen. Incubation of the atopic lung tissue with antigen resulted in a release of LTC₄, LTD₄ and LTE₄ which could be inhibited by the prostacyclin derivative U-60257 (101). These studies indicate that the leukotrienes containing cystein (LTC₄, LTD₄ and LTE₄) are major mediators of airway anaphylaxis; the finding that inhibition of leukotriene formation blocks ascaris induced asthma in monkeys also indicates that leukotriene antagonists or inhibitors of their formation could be of therapeutic value in the treatment of bronchial asthma (102).

When injected intradermally into guinea pigs LTC₄ and LTD₄ cause extravasation of Evan's blue (92,96,98). More recent studies involving intravital microscopy of the cheek pouch of the hamster (*Mesocricetus auratus*) have demonstrated specific effects of these leukotrienes on the permeability of the post-capillary venules (104). According to dose-response curves, LTC₄ and LTD₄ both induced a significant increase of vascular permeability at much lower concentrations than histamine. Leukotriene C₄ was approximately 5000 times more potent than histamine in this respect. The cysteinyl containing leukotrienes seem to increase the vascular permeability by a direct action on the vessel wall, since it occurs rapidly and does not require release of histamine or prostaglandins or the participation of polymorphonuclear leukocytes. Leukotriene B₄ also causes extravasation of plasma, although at higher concentrations. The reaction occurs with some latency and requires adhering leukocytes. Administration of a vasodilator together with leukotrienes potentiates the increase in plasma leakage caused by a submaximal dose of leukotrienes, as has been reported in the guinea pig for PGE₂ and LTD₄ (105) and in the guinea pig, rabbit and rat for PGE₂ and LTB₄ (106,107).

When LTB₄ was administered to the hamster cheek pouch in the same dose-range as LTC₄ it caused a dramatic increase in the adhesion of leukocytes to the endothelium in small venules (104). Increased adherence of human leukocytes caused by LTB₄ has also been demonstrated *in vitro* using a column of nylon fibers (108).

During superfusion with LTB₄ (6-10 min) the number of interstitial white cells increased. This finding is consistent with the chemotactic stimulant property of LTB₄. This effect of LTB₄ has been demonstrated *in vitro* using either the Boyden chamber technique or migration under agarose (109,110,111,112). *In vivo* this effect has been monitored by determining white cell accumulation in the peritoneal cavity of guinea pigs following intraperitoneal injection of LTB₄ (113). The studies described above indicate that LTB₄ might be a mediator in the migration of leukocytes from the blood to areas of inflammation. Recent work has also demonstrated that LTB₄ activates neutrophils. Addition of nano-

REFERENCES

1. Bergström, S. and Samuelsson, B.: *Ann. Rev. Biochem.* . 34, 101 (1965).
2. Samuelsson, B.: *Angew. Chemie. Int.* . 4, 410 (1965).
3. Bergström, S.: *Les Prix Nobel*, 1982.
4. Bergström, S., Danielsson H. and Samuelsson, B.: *Biochim. Biophys. Acta* , 90, 207 (1964).
5. Van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H.: *Biochim. Biophys. Acta*, 90, 104 (1964).
6. Nugteren, D.H. and Van Dorp, D.A. *Biochim. Biophys. Acta*, 98, 645 (1965).
7. Ryhage, R. and Samuelsson, B.: *Biochem. Biophys. Res. Commun.* . 19, 279 (1965).
8. Samuelsson, B.: *J. Am. Chem. Soc.* . 87, 3011, (1965).
9. Hamberg, M. and Samuelsson, B.: *J. Biol. Chem.* . 242, 5329 (1967).
10. Hamberg, M. and Samuelsson, B.: *J. Biol. Chem.* . 242, 5344 (1967).
11. Samuelsson, B.: *Progr. Biochem. Pharmacol.* 3,59-70 (1967).
12. Hamberg, M.: *Eur. J. Biochem.* . 6, 135, (1958).
13. Nugteren, D.H., Beerthuis, R.K. and van Dorp, D.A.: *In: Nobel Symposium 2, Prostaglandins*. Eds. S. Bergström and B. Samuelsson. Almqvist & Wiksell, Stockholm, p. 45-50 (1967).
14. Samuelsson, B., Granström, E. and Hamberg, M.: *In: Nobel Symposium 2, Prostaglandins*. Eds. S. Bergström and B. Samuelsson. Almqvist & Wiksell, Stockholm, P. 51-66 (1967).
15. Hamberg, M. and Samuelsson, B.: *J. Am. Chem. Soc.* . 88, 2349 (1966).
16. Hamberg, M. and Samuelsson, B.: *J. Biol. Chem.* . 242, 5344 (1967).
17. Nugteren, D.H., Beerthuis, R.K. and van Dorp, D.A.: *Res. Trav. Chim.* . 85, 405 (1966).
18. Granström, E., Lands, W.E.M. and Samuelsson, B.: *J. Biol. Chem.* . 243, 4104 (1968).
19. Hamberg, M. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA* , 70, 899 (1973).
20. Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA*, 71, 345 (1974).
21. Nugteren, D.H. and Hazelhof, E.: *Biochim. Biophys. Acta*, 326, 448 (1973).
22. Hamberg, M., Hedqvist, P., Strandberg, K., Svensson, J. and Samuelsson, B.: *Life Sci.* . 16, 451 (1975).
23. Tuvemo, T., Strandberg, K., Hamberg, M. and Samuelsson, B.: *Acta Physiol. Scand.* . 96, 145 (1976).
24. Samuelsson, B. and Hamberg, M.: *In: Prostaglandin Synthetase Inhibitors*. Eds. H.J. Robinson and J.R. Vane. Raven Press, New York, p. 107 (1974).
25. Vargaftig, B.B. and Zirinis, P.: *Nature (Lond.) New Biol.* . 244, 114 (1973).
26. Silver, M.J., Smith, J.B., Ingerman, C. and Kocsis, J.J.: *Prostaglandins* , 4, 863 (1973).
27. Willis, A.L.: *Science*, 183, 325, (1974).
28. Willis, A.L.: *Prostaglandins* , 5, 1 (1974).
29. Willis, A.L., Vane, F.M., Kuhn, D.C., Scott, C.G. and Petrin, M.: *Prostaglandins* , 8, 453 (1974).
30. Piper, P.J. and Vane, J.R.: *Nature (Lond.)*, 223, 29 (1969).
31. Gryglewski, R.J. and Vane, J.R.: *Br. J. Pharmacol.* . 45, 37 (1972).
32. Svensson, J., Hamberg, M. and Samuelsson, B.: *Acta Physiol. Scand.* . 94, 222 (1975).
33. Hamberg, M. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA* , 71, 3400 (1974).
34. Nugteren, D.H.: *Biochim. Biophys. Acta* 380, 299 (1975).
35. Samuelsson, B.: *In: Advances in Prostaglandin and Thromboxane Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 1, p. 1 (1976).
36. Hamberg, M., Svensson, J. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA* , 72, 2994 (1975).
37. Nelson, N.A. and Jackson, R.W.: *Tetr. Lett.* . 37, 3275 (1976).
38. Kelly, R.C., Schettler, I. and Stein, S.J.: *Tetr. Lett.* . 37, 3279 (1976).
39. Schneider, W.P. and Morge, R.A.: *Tetr. Lett.* . 37, 3283 (1976).
40. Corey, E.J., Schibasaki, M. and Knolle, J.: *Tetrahedron Lett.* . 19, 1625 (1977).
41. Moncada, S. and Vane, J.R.: *Pharmacol. Rev.* . 30, 293 (1979).
42. Ellis, E.F., Oelz, O., Roberts, L.J., II, Payne, N.A., Sweetman, B.J., Nies, A.S. and Oates, J.A.: *Science*, 193, 1135 (1976).

43. Needleman, P., Kulkarni, O.S. and Raz, A.: *Science*, **195**, 4090 (1977).
44. Svensson, J. and Hamberg, M.: *Prostaglandins*, **12**, 943 (1976).
45. Terashita, Z., Fukkui, H., Nishikawa, K., Hirat, M. and Kikuchi, S.: *Eur. J. Pharmacol.*, **53**, 49 (1978).
46. Wang, H.H., Kulkarni, P.S. and Eakins, K.E.: *Eur. J. Pharmacol.*, **66**, 31 (1980).
47. Bunting, S., Moncada, S. and Vane, J.R.: *Br. J. Pharmacol.*, **57**, 462 (1976).
48. Moncada, S., Ferreira, S.H. and Vane, J.R.: *In: Advances in Prostaglandin and Thromboxane Research*. Ed. J.C. Frölich. Raven Press. New York, Vol. 5, p. 211 (1978).
49. Tuvemo, T., Strandberg, K., Hamberg, M. and Samuelsson, B.: *In: Advances in Prostaglandin and Thromboxane Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 1, p. 425-428 (1976).
50. Gryglewski, R.J., Dembinska-Kiec, A. and Korbut, R.: *Acta Biol. Med. Ger.*, **37**, 715 (1978).
51. Svensson, J., Strandberg, K., Tuvemo, T. and Hamberg, M.: *Prostaglandins*, **14**, 425 (1977).
52. Moncada, S., Gryglewski, R.J., Bunting, S. and Vane, J.R.: *Nature (Lond.)*, **263**, 663 (1976).
53. 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.: *Prostaglandins*, **12**, 915 (1976).
54. Granström, E., Diczfalusy, U., Hamberg, M., Hansson, G., Malmsten, C. and Samuelsson, B.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Ed. J.A. Oates. Raven Press, New York. Vol. 10, p. 15 (1982).
55. Vane, J.R.: *In: Advances in Prostaglandins and Thromboxane Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 2, p. 791 (1976).
56. Borgeat, P., Hamberg, M. and Samuelsson, B.: *J. Biol. Chem.*, **251**, 7816 (1976).
57. Borgeat, P. and Samuelsson, B.: *J. Biol. Chem.*, **254**, 2643 (1979).
58. Borgeat, P. and Samuelsson, B.: *J. Biol. Chem.*, **254**, 7865 (1979).
59. Borgeat, P. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA*, **76**, 3213 (1979).
60. Rådmark, O., Malmsten, C., Samuelsson, B., Clark, D.A., Giichi, G., Marfat, A. and Corey, E.J.: *Biochem. Biophys. Res. Commun.*, **9**, 954 (1980).
61. Corey, E.J., Marfat, A., Goto, G. and Brion, F.: *J. Am. Chem. Soc.*, **102**, 7984 (1981).
62. Rådmark, O., Malmsten, C., Samuelsson, B., Goto, G., Marfat, A. and Corey, E.J.: *J. Biol. Chem.*, **255**, 11828 (1980).
63. Panossian, A., Hamberg, M. and Samuelsson, B.: *FEBS Lett.*, **150**, 511 (1982).
64. Rådmark, O. and Shimizu, T., in press 1982.
65. Feldberg, W. and Kellaway, C.H.: *J. Physiol. (Lond.)*, **94**, 187 (1938).
66. Kellaway, C.H. and Trethewie, E.R.: *Q. J. Exp. Physiol.*, **30**, 121 (1940).
67. Austen, K.F.: *J. Immunol.*, **121**, 793 (1978).
68. Brocklehurst, W.E.: *J. Physiol. (Lond.)*, **120**, 16P (1953).
69. Morris, H.R., Taylor, G.W., Piper, P.J., Sirois, O. and Tippins, J.R.: *FEBS Lett.*, **87**, 203 (1978).
70. Orange, R.P., Murphy, R.C., Karnovsky, M.L. and Austen, K.F.: *J. Immunol.*, **110**, 760 (1973).
71. Strandberg, K. and Uvnäs, B.: *Acta Physiol. Scand.*, **82**, 359 (1971).
72. Bach, M.K., Brashler, J.R. and Gorman, R.R.: *Prostaglandins*, **14**, 21 (1977).
73. Jakschik, B.A., Falkenheim, S. and Parker, C.W.: *Proc. Natl. Acad. Sci. USA*, **74**, 4577 (1977).
74. Conroy, M.C., Orange, R.P. and Lichtenstein, L.M.: *J. Immunol.*, **116**, 1677 (1976).
75. Murphy, R.C., Hammarström, S. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA*, **76**, 4275 (1979).
76. Samuelsson, B., Borgeat, P., Hammarström, S. and Murphy, R.C.: *Prostaglandins*, **17**, 785 (1979).
77. Samuelsson, B., Borgeat, P., Hammarström, S. and Murphy, R.C.: *In: Advances in Prostaglandin and Thromboxane Research*. Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Raven Press, New York, Vol. 6, p. 1 (1980).
78. Hammarström, S., Murphy, R.C., Samuelsson, B., Clark, D.A., Mioskowski, C. and Corey, E.J.: *Biochem. Biophys. Res. Commun.*, **91**, 1266 (1980).

79. Hammarström, S., Samuelsson, B., Clark, D.A., Goto, G., Marfat, A., Mioskowski, C. and Corey, E.J.: *Biochem. Biophys. Res. Commun.* **92**, 946 (1980).
80. Örnning, L., Hammarström, S. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA* **77**, 2014 (1980).
81. Rådmark, O., Malmsten, C. and Samuelsson, B.: *FEBS Lett.* **110**, 213 (1981).
82. Samuelsson, B. and Hammarström, S.: *Prostaglandins*, **19**, 645 (1980).
83. Bernström, K. and Hammarström, S.: *J. Biol. Chem.* **256**, 9579 (1981).
84. Anderson, M.E., Allison, R.D. and Meister, A.: *Proc. Natl. Acad. Sci. USA* **79**, 1088 (1982).
85. Uehara, N., Ormstad, K., Orrenius, S., Örnning, L. and Hammarström, S.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Raven Press, New York. Vol. 11, p. 147 (1983).
86. Borgeat, P. and Samuelsson, B.: *Proc. Natl. Acad. Sci. USA*, **76**, 2148 (1979).
87. Jubiz, W., Rådmark, O., Lindgren, J.A., Malmsten, C. and Samuelsson, B.: *Biochem. Biophys. Res. Commun.* **99**, 976 (1981).
88. Lindgren, J.A. and Samuelsson, B.: in press (1983).
89. Lundberg, U., Rådmark, O., Malmsten, C. and Samuelsson, B.: *FEBS Lett.* **126**, 127 (1981).
90. Rådmark, O., Lundberg, U., Jubiz, W., Malmsten, C. and Samuelsson, B.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 9, p. 61 (1982).
91. Maas, R.L., Brash, A.R. and Oates, J.A.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 9, p. 29 (1983).
92. Dahlén, S.-E., Hedqvist, P., Hammarström, S. and Samuelsson, B.: *Nature* **288**, 484 (1980).
93. Hedqvist, P., Dahlén, S.-E., Gustafsson, L., Hammarström, S. and Samuelsson, B.: *Acta Physiol. Scand.* **110**, 331 (1980).
94. Lewis, R.A., Austen, F.K., Drazen, J.M., Soter, M.A., Figueiredo, J.C. and Corey, E.J.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 9, p. 137 (1982).
95. Lewis, R.A., Lee, C.W., Levine, L., Morgan, R.A., Weiss, J.W., Drazen, J.M., Oh, H., Hoover, D., Corey, E.J. and Austen, K.F.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Raven Press, New York. Vol. 11, p. 15 (1983).
96. Piper, P.J. and Tippins, J.R.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Raven Press, New York. Vol. 9, p. 183 (1982).
97. Smedegård, G., Hedqvist, P., Dahlén, S.-E., Revenäs, B., Hammarström, S. and Samuelsson, B.: *Nature*, **295**, 327 (1982).
98. Drazen, J.M., Austen, F.K., Lewis, R.A., Clark, D.A., Goto, G., Marfat, A. and Corey, E.J.: *Proc. Natl. Acad. Sci. USA*, **77**, 4354 (1980).
99. Weiss, J.W., Drazen, J.M., Coles, N., McFadden, E.R., Weller, P.W., Corey, E.J., Lewis, R.A. and Austen, K.F.: *Science*, **216**, 196 (1982).
100. Levi, R., Burke, J.A. and Corey, E.J.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson and R. Paoletti. Raven Press, New York. Vol. 9, p. 215 (1982).
101. Hansson, G., Björck, T., Dahlén, S.-E., Hedqvist, P., Granström, E. and Dahlén, B.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Raven Press, New York. Vol. 12, p. 153 (1983).
102. Bach, M.K., Brashler, J.R., Fritzpatrick, F.A., Griffin, R.L., Iden, S.S., Johnson, H.G., McNee, M.L., McGuire, J.C., Smith, H.W., Smith, R.J., Sun, F.F. and Wasserman, M.A.: *In: Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Eds. B. Samuelsson, P. Ramwell and R. Paoletti. Vol. 11, p. 39 (1983).
103. Dawson, W., Boot, J.R., Harvey, J. and Walker, J.R.: *Eur. J. Rheumatol. and Infl.* **5**, 61 (1982).
104. Dahlén, S.-E., Hedqvist, P., Hammarström, S. and Samuelsson, B.: *Nature*, **288**, 484 (1980).
105. Peck, M.J., Piper, P.J. and Williams, T.J.: *Prostaglandins* **20**, 863 (1980).

106. Bray, M.A., Cunningham, F.M., Ford-Hutchinson, A.W. and Smith, M.J.H.: *Br. J. Pharmacol.* **72**, 483 (1981).
107. Wedmore, C.V. and Williams, T.J.: *Nature*, **289**, 658 (1981).
108. Palmblad, J., Malmsten, C.L., Udén, A.-M., Rådmark, O., Engstedt, L. and Samuelsson, B.: *Blood*, **58**, 658 (1981).
109. Ford-Hutchinson, A.W., Bray, M.A., Doig, M.V., Shipley, M.E. and Smith, M.J.H.: *Nature* **286**, 264 (1980).
110. Malmsten, C.L., Palmblad, J., Udén, A.-M., Rådmark, O., Engstedt, L. and Samuelsson, B.: *Acta Physiol. Scand.* **110**, 449 (1980).
111. Palmer, R.M.J., Stephney, R.J., Higgs, G.A. and Eakins, K.E.: *Prostaglandins* , **20**, 411 (1980).
112. Goetzl, E.J. and Pickett, W.C.: *J. Immunol* . **125**, 1789 (1980).
113. Smith, M.J.H., Ford-Hutchinson, A.W. and Bray, M.A.: *J. Pharm. Pharmacol* . **32**, 517 (1980).
114. Serhan, C.N., Fridovich, J., Goetzl, E.J., Dunham, P.B. and Weissmann, G.: *J. Biol. Chem.* **257**, 4746 (1982).
115. Feinmark, S.J., Lindgren, J.Å., Claesson, H.-E., Malmsten, C. and Samuelsson, B.: *FEBS Lett.* **136**, 141 (1981).
116. Serhan, C.N., Radin, A., Smolen, J.E., Korchak, H., Samuelsson, B. and Weissmann, G.: *Biochem. Biophys. Res. Commun.* **107**, 1006 (1982).
117. Blackwell, G.J., Garnuccion, R., Di Rosa, M., Flower, R.J., Parente, L. and Persico, P.: *Nature*, **287**, 147 (1980).
118. Hirata, F., Shiffmann, E., Venkatasubramanian, K., Salomon, D. and Axelrod, J.: *Proc. Natl. Acad. Sci. USA*, **77**, 2533 (1980).
119. Rådmark, O., Malmsten, C.L.: Samuelsson, B., Goto, G., Marfat, A. and Corey, E.J.: *J. Biol. Chem.* **255**, 11828 (1980).
120. Hansson, G. and Rådmark, O.: *FEBS Lett* , **122**, 87 (1980).
121. Hammarström, S. and Samuelsson, B.: *FEBS Lett.* **122**, 83 (1980).
122. Morris, H.R., Taylor, G.W., Piper, P.J., Samhoun, M.N. and Tippins, J.R.: *Prostaglandins*, **19**, 185 (1980).
123. Parker, C.W., Falkenhein, S.F. and Huber, M.M.: *Prostaglandins* , **20**, 863 (1980).
124. Bach, M.K., Brashler, J.R., Hammarström, S. and Samuelsson, B.: *J. Immunol* . **125**, 115 (1980).
125. Bach, M.K., Brashler, J.R., Hammarström, S. and Samuelsson, B.: *Biochem. Biophys. Res. Commun.* **93**, 1121 (1980).
126. Lewis, R.A., Drazen, J.M., Austen, K.F., Clark, D.A. and Corey, E.J.: *Biochem. Biophys. Res. Commun.* **96**, 271 (1980).
127. Ford-Hutchinson, A.W., Bray, M.A., Cunningham, F.M., Davidson, E.M. and Smith, M.J.H.: *Prostaglandins* , **21**, 143 (1981).
128. Siegel, M.I., McConnell, R.T., Bonser, R.W. and Cautrecasas, P.: *Prostaglandins* , **21**, 123 (1981).
129. Doig, M.V. and Ford-Hutchinson, A.W.: *Prostaglandins* , **20**, 1007 (1980).
130. Rouzer, C.A., Scott, W.H., Cohn, Z.A., Blackburn, P. and Manning, J.M.: *Proc. Natl. Acad. Sci. USA*, **77**, 4928 (1980).
131. Lewis, R.A., Austen, K.F., Drazen, J.M., Clark, D.A., Marfat, A. and Corey, E.J.: *Proc. Natl. Acad. Sci. USA*, **77**, 3710 (1980).
132. Morris, H.R., Taylor, G.W., Piper, P.J. and Tippins, J.R.: *Nature* , **285**, 104 (1980).
133. Høglum, J., Pai, J.-K., Atrache, V., Sok, K.-E. and Sih, C.J.: *Proc. Natl. Acad. Sci. USA* , **77**, 5688 (1980).