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Table of Contents

Introduction
Sune Bergström 1

1. Biosynthesis

Quantitative Aspects on Prostaglandin Synthesis in Man
Bengt Samuelsson 7

Inhibition of Prostaglandin Biosynthesis
William E. M. Lands, Paul R. LeTellier, Leonard H. Rome and *Jack Y. Vanderhoek* 15

Catecholamine Induced Increase in Prostaglandin E Biosynthesis in Homogenates of the Rat Stomach Fundus
Cecil Robert Pace-Asciak 29

Comparative Aspects of Prostaglandin Biosynthesis in Animal Tissues
E. J. Christ and *D. A. van Dorp* 35

2. Metabolism

Structures of New Metabolites of Prostaglandin E_2 in Man
Mats Hamberg and *Mary Wilson* 39

Structures of C_{14} Metabolites of Prostaglandin $F_{2\alpha}$
Elisabeth Granström 49

Purification, Properties, and Biological Significance of Prostaglandin A Isomerase
R. L. Jones and *S. Cammock* 61

3. Analytical Methods

Specific Antibodies: Reagents for Quantitative Analysis of Prostaglandins
Lawrence Levine, Rose Mary Gutierrez-Cernosek and *Helen van Vunakis* 71

Specificity of Prostaglandin Binding Sites in Rat Forestomach Tissue and Their Possible Use as a Quantitative Assay
Olga V. Miller and *Wayne E. Magee* 83

Quantitative Mass Spectrometric Analysis of Prostaglandins
Krister Greén 91

Differentiation Between Endogenous and Exogenous (Administered) Prostaglandins in Biological Fluids
Udo Axen, Lubomir Baczynskyi, David J. Duchamp, Kenneth T. Kirton and *John F. Zieserl*, Jr. 109

The Analysis of Prostaglandins Using a Computer for Plotting Ion Abundance Versus Time
C. J. Thompson and *C. N. Goode* 117

4. Round Table Discussion on Analytical Methods

Moderator: *B. Samuelsson*

B. Samuelsson, U. Axen, H. Behrman, E. Granström, K. Greén, B. M. Jaffe, K. Kirton, L. Levine, R. C. Skarness, L. Speroff and *L. S. Wolfe* 121

5. Biological Activity and Structure

The Synthesis and Biological Activity of a Series of 15-Methyl Prostaglandins
Gordon L. Bundy, Ernest W. Yankee, James R. Weeks and *William L. Miller* 125

On the Prostaglandin Inhibitory Action of Polyphloretin Phosphate
K. E. Eakins, Hans Fex, Bo Fredholm, Bertil Högeberg and *Sten Veige* 135

Structure-Activity Relationsship of Some Dibenzoxazepine Derivatives as Prostaglandin Antagonists
John H. Sanner, Richard A. Mueller and *Renata H. Schulze* 139

Relation of Prostaglandin Structure to Vascular Resistance in the Isolated Rat Pancreas
Robert N. Saunders, Richard A. Mueller, Christine A. Moser and *Daniel V. Becker* 149

6. Metabolic Effects$_1$

The Regulatory Role of the Prostaglandins on the Cyclic 3′, 5′-AMP System
F. A. Kuehl, Jr., *V. J. Cirillo, E. A. Ham* and *J. L. Humes* 155

Prostaglandins, Cyclic AMP, and Transformed Fibroblasts
George S. Johnson, Ira Pastan, David S. Oyer and *Massimo D'Armiento* 173

7. Prostaglandins and Adenylcyclase System

Prostaglandin Production by Cells in Vitro
Bernard M. Jaffe, Gordon W. Philpott, Bernd Hamprecht and *Charles W. Parker* 179

8. Metabolic Effects$_2$

Erythropoietic Effects of Prostaglandins
Peter P. Dukes 183

Prostaglandins and Sickle Cell Anemia
Malcolm Johnson, Israel Rabinowitz, Anthony L. Willis and *Paul L. Wolf* 189

Inhibition of Vasopressin Action in Toad Bladder
G. T. N. Besley, D. A. Frith and *R. S. Snart* 195

Effects of Prostaglandin E_1 and Arginine Vasotocin on Sodium Transport and Adenyl Cyclase Activity in Developing Tadpole Skin
Judith K. Jones and *I. S. Edelman* 201

Prostaglandin Modification of Membrane-Bound Enzyme Activity
Malcolm Johnson and *Peter W. Ramwell* 205

9. Respiratory System

The Effects of Inhaled Prostaglandins on Bronchial Tone in Man
A. P. Smith and *M. F. Cuthbert* 213

Opposite Effects of Prostaglandins E and F on Tracheal Smooth Muscles and Their Interaction with Calcium Ions
L. Puglisi 219

Comparative Studies on the Bronchodilating Properties of the Prostaglandin $F_{2\beta}$
Marvin E. Rosenthale, Alphonse Dervinis, Jacob Kassarich, Samuel Singer and *Melvyn I. Gluckman* 229

The Regulatory Function of Prostaglandins in the Release of Histamine and SRS-A From Passively Sensitized Human Lung Tissue
Joyce L. Walker 235

Prostaglandin $F_{2\alpha}$: Effect on Airway Conductance in Healthy Subjects and Patients with Bronchial Asthma
Aleksander A. Mathé, Per Hedqvist, Alf Holmgren and *Nils Svanborg* 241

10. Gastro-intestinal System

15-Methyl PGE_2 and 16, 16-Dimethyl PGE_2: Potent Inhibitors of Gastric Secretion
André Robert and *Barney J. Magerlein* 247

Effect of Orally and Intravenously Administered Prostaglandin 15 (R) 15-Methyl E_2 on Gastric Secretion in Man
S. M. M. Karim, D. C. Carter, D. Bhana and *P. Adaikan Ganesan* 255

PGE_2 and Gastric Acid Secretion in Man
Alan Bennett, Ian F. Stamford and *William G. Unger* 265

The Relationship Between Rat Gastric Mucosal Blood Flow and Acid Secretion During Oral or Intravenous Administration of Prostaglandins and Dibutyryl Cyclic AMP
I. H. M. Main and *B. J. R. Whittle* 271

11. Renal System

Introduction
James B. Lee 277

A Histochemical Study of the Prostaglandin Biosynthesis in the Urinary System of Rabbit, Guinea Pig, Goldhamster, and Rat
F. H. A. Janszen and *D. H. Nugteren* 287

Metabolic Mechanisms of Action of the Renal Prostaglandins
John J. Lafferty, H. Kannegiesser, James B. Lee and *Charles W. Parker* 293

Effects of PGE_2 on Renal Tubular Function
Georges Fülgraff, Gerhard Brandenbusch and *Antje Meiforth* 301

Prostaglandinlike Renal Vasodepressor Lipids and Electrolyte Exchange in the Kidney
A. A. Nekrasova, R. I. Sokolova and *L. A. Lantsberg* 307

The Effect of Prostaglandin (PGA_1) on Adrenal Function in Man
Marshal Fichman, Glenn Littenberg, Jannie Woo and *Richard Horton* 313

Occurrence of Prostaglandins in Human Urine
J. C. Frölich, B. J. Sweetman, K. Carr, J. Splawinski, J. T. Watson, E. Änggård and *J. A. Oates* 321

Effect of PGE_1 on Contrast Visualization of the Renal Vascular Bed: Comparison with Other Vasodilators
Hadi Özer, Norman K. Hollenberg and *Herbert L. Abrams* 331

Inhibition of Prostaglandin Synthesis in the Kidneys by Aspirinlike Drugs
Liliana Somova 335

Synthesis of Prostaglandins (PGs) by Renomedullary Interstitial Cells (RIC) Grown in Tissue Culture
E. Eric Muirhead, Glen S. Germain, Byron E. Leach, James A. Pitcock, Peggy Stephenson, Bennie Brooks, William L. Brosius, Jr., *Edward G. Daniels* and *Jack W. Hinman* 341

12. Circulatory System

Introduction
J. R. Weeks 349

Effects of Prostaglandin E_2 and $F_{2\alpha}$ on Cutaneous Arteries and Veins in Vivo in the Isolated Rabbit Ear
Martin Echt, Otto H. Gauer, Friedrich Kapteina and *Lothar Lange* 353

Prostaglandin Induced Vasoconstriction of Cerebral Arteries and Its Reversal by Ethanol
Y. Lucas Yamamoto, William Feindel, Leonhard S. Wolfe and *Charles P. Hodge* 359

Vasodepressor Action of Renomedullary Prostaglandins (PGE_2 and PGA_2) in the Spontaneously Hypertensive Rat (SH Rat): Evidence for a Central Action Mediated by the Vagus
E. Eric Muirhead, Byron E. Leach, Frank B. Armstrong, Jr. and *Glen S. Germain* 369

Effect of Prostaglandin E_1 on the Transmembrane Potentials of the Mammalian Heart
Valéria Kecskeméti, Károly Kelemen and *József Knoll* 373

Effects of PGE_1, PGE_2, and $PGF_{2\alpha}$ on Isolated Normal and Damaged Heart Preparations
Werner Förster and *Peter Mentz* 379

Antiarrhythmic Properties of PGE_2: Preliminary Results
Hans-Jürgen Mest, Karsten Schrör and *Werner Förster* 385

13. Inflammation

Inhibition of Prostaglandin Biosynthesis as the Mechanism of Action of Aspirinlike Drugs
John R. Vane 395

Arachidonic Acid and the Analgesic Action of Aspirinlike Drugs
H. O. J. Collier, S. A. Saeed, C. Schneider and *B. T. Warren* 413

Effect of Various Prostaglandins on Two Laboratory Models of Chronic Arthritic Inflammation
R. L. Aspinall, P. S. Cammarata, A. Nakao, J. Jiu, M. Miyano, D. E. Baker and *W. F. Pautsch* 419

Prostaglandin Release in Ocular Inflammation in Rabbits and Man
K. E. Eakins, R. A. F. Whitelocke, E. S. Perkins, A. Bennett and *G. Unger* 427

Effect of Prostaglandin E_1 on Lysosomal Enzyme Release from Human Leucocytes in the Presence of Dytochalasin B
Gerald Weissmann, Robert B. Zurier and *Sylvia Hoffstein* 435

Prostaglandins in burn injury
Carl-Evert Jonsson and *Mats Hamberg* 441

Antianaphylactic Effect of a Prostaglandin Antagonist, Polyphloretin Phosphate
Bo Fredholm and *Kjell Strandberg* 447

The Effect of Prostaglandin E_1 on the Production of Interferon
I. Mécs and *A. Maráz* 453

Prostaglandin E_2 Biosynthesis in Human Skin: Subcellular Localization and Inhibition by Unsaturated Fatty Acids and Antiinflammatory Agents
Vincent A. Ziboh, Thomas McElligott and *S. L. Hsia* 457

14. Nervous System

Prostaglandin Mediated Control of Sympathetic Neuroeffector Transmission
Per Hedqvist 461

Prostaglandins on Cyclic-AMP Formation in Cerebral Cortex of Different Mammalian Species
Ferruccio Berti, Marco Trabucchi, Virgilio Bernareggi and *Remo Fumagalli* 475

Actions of Prostaglandin E_1 on Spinal Neurons in the Frog
Flavio Coceani and *Antonio Viti* 481

The Effects of Prostaglandin E_2 on Conditioned Avoidance Response Behavior and the Electroencephalogram
W. Joseph Potts, Peter F. East, Diane Landry and *James P. Dixon* 489

Prostaglandin E_1 and Endotoxin Fever, and the Effects of Aspirin, Indomethacin, and 4-Acetamidophenol
A. S. Milton 495

Effect of Prostaglandins E_1 and $F_{2\alpha}$ on Adrenergic Responses in the Pulmonary Circulation
Philip J. Kadowitz, William J. George, Paul D. Joiner and *Albert L. Hyman* 501

15 a. Termination of Pregnancy – Extra- and Intra-amniotic Administration of Prostaglandins

Termination of Pregnancy by Extraamniotic Prostaglandins and the Synergistic Action of Oxytocin
M. P. Embrey, Keith Hillier and *P. Mahendran* 507

Induction of Abortion 1. by Intravenous and 2. by Intrauterine Administration of $PGF_{2\alpha}$ (Extra- and Intraamniotic)
A. A. Haspels and *F. Neth* 515

Intrauterine Administration of Prostaglandin $F_{2\alpha}$ for Induction of Abortion
Marc Bygdeman, Francois Beguin, Mokhtar Toppozada and *Nils Wiqvist* 525

Therapeutic Abortion by Intraamniotic Administration of Prostaglandin $F_{2\alpha}$
Rune Nyberg 533

Midtrimester Therapeutic Abortion Using Intraamniotic $PGF_{2\alpha}$
G. G. Anderson, J. C. Hobbins, V. Rajkovic, L. Goldstein, L. Speroff and *B. V. Caldwell* 539

Induction of Abortion by Intraamniotic $PGF_{2\alpha}$: a Comparison of Dose Schedules
P. G. Gillett, R. A. H. Kinch, L. S. Wolfe and *C. Pace-Asciak* 545

Intraamniotic Prostaglandin $F_{2\alpha}$ for Midtrimester Abortion
Charles A. Ballard and *Edward J. Quilligan* 551

Therapeutic Abortion by Intraamniotic Injection of Prostaglandins
G. Roberts, R. Gomersall, M. Adams and *A. C. Turnbull* 555

15 b. Termination of Pregnancy – Intravaginal Administration of Prostaglandins

Vaginal Administration of Prostaglandins to Induce Early Abortion
Ralph W. Hale, Ronald J. Pion and *Wilma B. Schiner* 561

Postconceptional Fertility Control by Prostaglandins
Mokhtar Toppozada, Francois Béguin, Marc Bygdeman, Leif Wide and *Nils Wiqvist* 567

Abortifacient Activity of Vaginally Administered Prostaglandin $F_{2\alpha}$
Robert C. Corlett, Boonlaw Sribyatta, Ian H. Thorneycroft, Daniel R. Mishell, Charles Ballard and *Robert M. Nakamura* 575

Prostaglandin $F_{2\alpha}$ Vaginal Pessaries for Midtrimester Abortion
Julian A. Elias 581

15 c. Induction of Labour – Oral and Intravenous Administration of Prostaglandins

The Results of Using Prostaglandins $F_{2\alpha}$ for Induction and Stimulation Labor
L. S. Persianinov, I. A. Manuilova and *E. A. Chernukha* 585

Oral Prostaglandin E_2 and Amniotomy for Induction of Labor
Ian Craft 593

15 d. Effects of Prostaglandins on Steroidogenesis$_1$

The Physiological Role of Prostaglandin $F_{2\alpha}$ in Corpus Luteum Regression
J. A. McCracken, B. Barcikowski, J. C. Carlson, K. Greén and *B. Samuelsson* 599

Prostaglandin F Group (PGF), Progesterone, and Estrogen Concentrations in the Uteroovarian Venous Plasma of the Conscious Ewe During the Estrous Cycle
R. I. Cox, G. D. Thorburn, W. B. Currie, B. J. Restall and *W. Schneider* 625

The Formation of Prostaglandins by the Guinea-Pig Uterus and the Effect of Indomethacin
Norman Leslie Poyser 631

Cyclic Changes in Uterine Venous and Peripheral Plasma Levels of F Prostaglandins Correlated with Peripheral Progesterone Levels in the Golden Hamster
Abubakar A. Shaikh and *Shiva K. Saksena* 635

A Comparative Study of Some Effects of Prostaglandin $F_{2\alpha}$, $F_{1\alpha}$, E_2, and E_1 on Reproductive Processes of Rats and Hamsters
A. P. Labhsetwar 641

15 e. Effects of Prostaglandins on Steroidogenesis$_2$

Prostaglandins and Steroidogenesis
Kenneth T. Kirton 645

Prostaglandins and Myometrial Contractility
Kenneth T. Kirton, L. J. Wyngarden and *K. K. Bergstrom* 651

Effect of Prostaglandin $F_{2\alpha}$ on Progestin Synthesis in the Human and Rabbit Ovary
C. P. Puri, V. Hingorani and *K. R. Laumas* 657

Induction of Abortion by Extraamniotic Administration of Prostaglandin $F_{2\alpha}$
V. Hingorani and *K. Ganesh* 665

Alterations in Biosynthesis of Progesterone in Human Corpora Lutea of Pregnancy by Prostaglandins
David F. Archer and *Edmund S. Petrilli* 669

The Effect of Prostaglandin $F_{2\alpha}$ Administration on Plasma Steroid and LH Levels in the Luteal Phase of the Menstrual Cycle
Keith Hillier, Angela Dutton and *C. S. Corker* 673

Plasma Progestin and Plasma Estrogen Levels During Infusion of $PGF_{2\alpha}$ in the Human
F. Lehmann, F. Peters, M. Breckwoldt and *G. Bettendorf* 679

Plasma Hormone Changes During Infusions of Prostaglandins $F_{2\alpha}$ and E_2 for Therapeutic Abortion
John Newton and *William Collins* 689

Effects of Prostaglandins and Gonadotrophins on Luteal Prostaglandin and Steroid Biosynthesis
Laurence M. Demers, Harold R. Behrman and *Roy O. Greep* 701

15 f. Effects of Prostaglandins on Other Ovarian Functions

Possible Role of Prostaglandins in Ovulation
David T. Armstrong, Young S. Moon and *Daniel L. Grinwich* 709

Effects of PGE_1 and 7-OXA-13-Porstynoic Acid on the Isolated Prepubertal Rat Ovary
Kurt Ahrén and *Torsten Perklev* 717

Effect of Prostaglandins E_1, E_2, and $F_{2\alpha}$ on Gonadotrophin-Induced Ovulation in Immature Female Rats
S. K. Batta, F. Piva and *L. Martini* 723

Effects of $PGF_{2\alpha}$ on Periovular Cervical Mucus
Bernard A. Eskin and *Said Azarbal* 731

15 g. Effects of Prostaglandins on the Male Reproduction

Prostaglandins (E_1 and E_2) and Reproduction in the Male Rat
Ronald J. Ericsson 737

15 h. Effects of Prostaglandins on the Human Uterine Contractility

The Effect of a β-Adrenergic Agent on Prostaglandin Stimulated Labor
J. Scher and *P. Baillie* 743

Comparative Studies of the Effect of Oxytocin and Prostaglandin $F_{2\alpha}$ in the Uterus
Karl Heinz Mosler, Roman Czekanowski and *Gisela Dronhöfer* 751

Interrelationship Between Oxytocin (Endogenous and Exogenous) and Prostaglandins
Arnold Gillespie 761

Quantitation of Uterine Activity after Vaginal Administration of Prostaglandins
M. R. Henzl, N. Arevalo-T., L. Noriega, R. Aznar, E. Ortega and *E. Segre* 767

15 i. Effects of Prostaglandins on the Animal Reproductive Tract

Tachyphylactic Response of the Rat Uterus In Vivo to Prostaglandins E_2 and $F_{2\alpha}$
James R. Weeks 773

Effects of Prostaglandins E_1 and $F_{2\alpha}$ on Uterine Hemodynamics and Motility
Kenneth E. Clark, Michael J. Ryan and *Michael J. Brody* 779

Effects of Prostaglandins and Oxytocin on the Uterine Motility of the Living Rat Under Various Hormonal Conditions
Paul Gans 783

The Role of Prostaglandin in Endotoxin-Induced Abortion and Fetal Death
M. J. K. Harper and *R. C. Skarness* 789

Immediate and Late Effect of PGE_1 on the Rabbit Oviduct (in Vivo Studies)
Moshe Salomy and *Icchak Halbrecht* 795

Effects of Prostaglandins (PGs) on Sperm and Egg Transport in the Rabbit
M. C. Chang, Dorothy M. Hunt and *C. Polge* 805

15 k. Termination of Pregnancy by Prostaglandin Analogues

Termination of Pregnancy with Prostaglandin Analogs
S. M. M. Karim, S. K. Sharma, G. M. Filshie, J. A. Salmon and *P. Adaikan Ganesan* 811

15 (S)-15-Methyl-Prostaglandin $F_{2\alpha}$: Myometrial Response and Abortifacient Efficacy
Nils Wiqvist, Francois Béguin, Marc Bygdeman and *Mokhtar Toppozada* 831

15 l. Summary of Round Table Discussion on Fertility Regulation

Moderator: *S. Bergström*

S. Bergström, Ch. Ballard, H. Behrman, M. Bygdeman, M. Embrey, A. Haspels, C. Hendricks, V. Hingorani, S. Karim, R. Kinch and *N. Wiqvist* 843

A Note on Nomenclature
M. Hamberg 847

Stability of Prostaglandins
T. J. Roseman, B. Sims and *R. G. Stehle* 851

Chairmen and Principal Lecturers 853

Speakers 873

Subject Index 881

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Introduction

Sune Bergström

Karolinska Institutet, Stockholm, Sweden

Ladies and Gentlemen, on behalf of the organizers and of the sponsor, the Schering Company, I wish you all very welcome.

I will start by telling you a little about the background of this conference.

In June 1971, Professor *Marois* invited the members of the Organizing and Program Committee to Paris, and we had a two-day session discussing how we would like to have an International Prostaglandin Conference organized. We discussed various locations from Nice and Paris to a ship sailing in the Mediterranean, and we made up a five-day program for June 1972. However, the finances for the conference had to be sought, and it was entrusted to the small Organizing Committee to try to arrange that. It was only in August that we came in contact with the Schering Company which graciously agreed to sponsor and finance the meeting. From then on, the organization of the conference has been handled most efficiently by Doctor *Silke Bernhard* and her staff in the section of Scientific Relations of the Schering Company. In October, we were able to secure a suitable place for a conference of the expected size, but by that time the original plans had to be modified. The meeting had to be delayed until September 1972 and condensed to four days. Furthermore, it has been necessary to run two parallel sessions. Unfortunately, some of the chairmen of the various sections by a misunderstanding sent out the original program. Another feature is that the attendance of this meeting had to be limited to 500, and, unfortunately, due to the local authorities, it is difficult at present to do anything for those who have turned up here now wanting to participate.

Before separating into two sections, we thought that it might be appropriate to show at least a few slides as an introduction while we are all still together.

Looking back on the history of the prostaglandins, we have indicated the first publications of the observations by *Goldblat* and by *von Euler* of the biological effects of

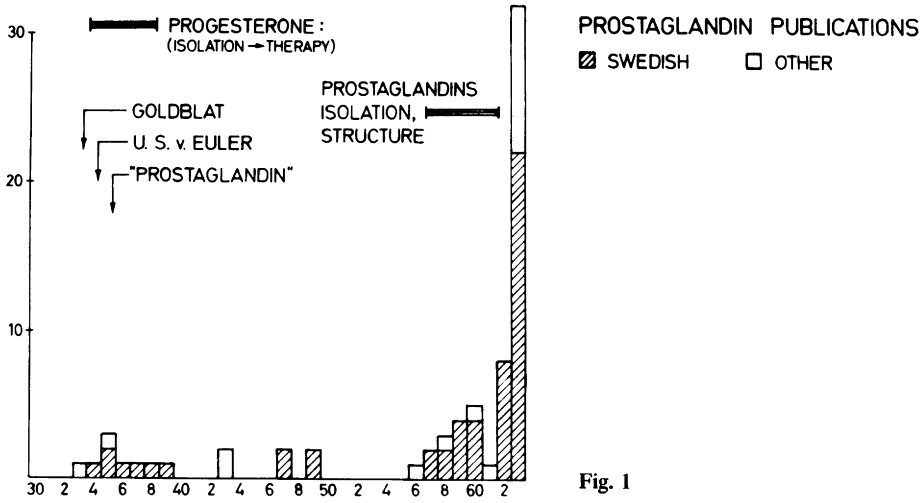

Fig. 1

the human semen. You can see how little interest, or impact, these discoveries created at that time. The reason was obviously that it was thought that these effects were due to then known compounds, and it was only when the isolation and structure work in the late fifties and early sixties proved that a new group of compounds were responsible for the effects, that the field came to life. Only then did the number of publications start to increase rapidly, even if the supply of compounds at that time was very small. However, the elucidation of the structure of these six primary prostaglandins led to the speculation that essential fatty acids might be the natural precursors for the prostaglandins. That this was indeed the case was proven simultaneously by Doctor van *Dorp*'s group in the Unilever research laboratories in Holland, by our group at Karolinska Institutet in Sweden, and by a group at The Upjohn Com-

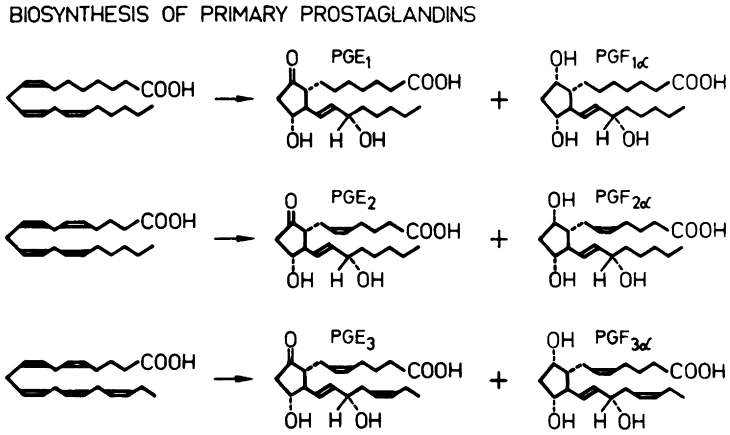

Fig. 2

pany in the USA. This discovery in its turn led to the biosynthetic production of the primary prostaglandin compounds so that they could be studied in many laboratories. This resulted in a very rapid increase in the number of publications.

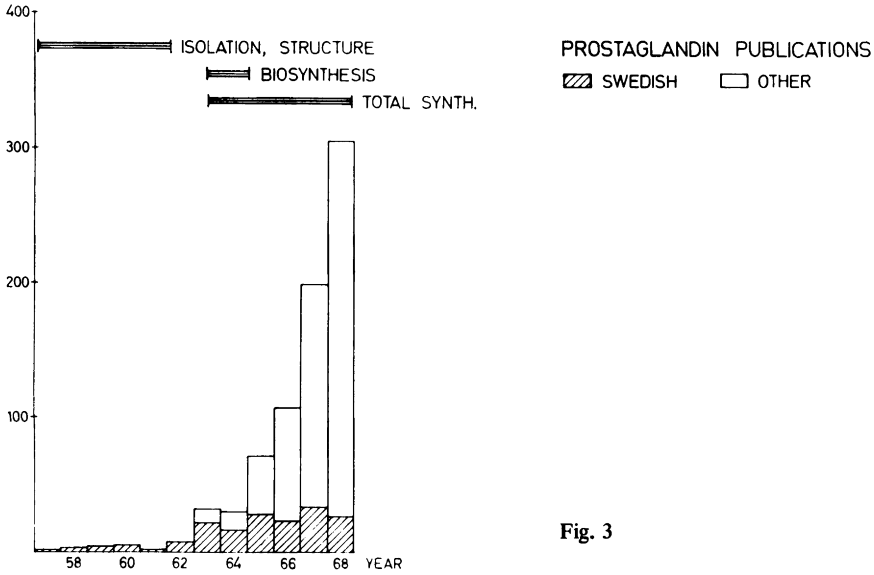

Fig. 3

The total chemical synthesis by several methods was brought to practical use in several places during the late sixties. The number of samples sent out by The Upjohn Company increased from 71 to over 2000 annually from 1965 to 1971. This material was mainly prepared by the biosynthetic method. Another surprise was, of course, the discovery of the enormously high concentrations of prostaglandins in the Gorgonia coral which has also played a certain role as raw material for production of prostaglandins during this time.

At this meeting, the first extensive discussion of new active analogs of prostaglandins will take place. It is known that the first metabolic reaction causing inactivation is the dehydrogenation of the hydroxyl at C-15, and as you have seen from the program, the first clinical evaluation of the compounds E_2 and $F_{2\alpha}$ with a methyl group at C-15 will be discussed. This, of course, is only a small beginning of a new field that will develop rapidly.

The field of the prostaglandins has really matured now, especially since we have antagonists and also inhibitors of the biosynthesis. Especially the discoveries by Professor *Vane* and his colleagues of the effect of aspirin and indomethasin and other compounds will have a tremendous impact on the study of the role of the prostaglandins in the living organism. Likewise, other compounds that can modify the rate and ratio between the different compounds formed are emerging.

The following slide is only to remind you of the great complexity of the metabolism of prostaglandins. That leads me to one of the most important parts of this conference, namely the evaluation of the various analytical methods available for the determination of the often extremely minute amounts occurring in the living organism. Much of the data published so far based on bioassay, etc., are rather unreliable. At the WHO meeting in Stockholm, January 1972, on "Prostaglandins in Fertility Control," it was agreed to do an international comparison of the radioimmunoassays that had then been published and the gas chromatography-mass spectrometry method developed in Stockholm. I think the round-table discussion on the analytical questions will be one of the most important parts of this meeting.

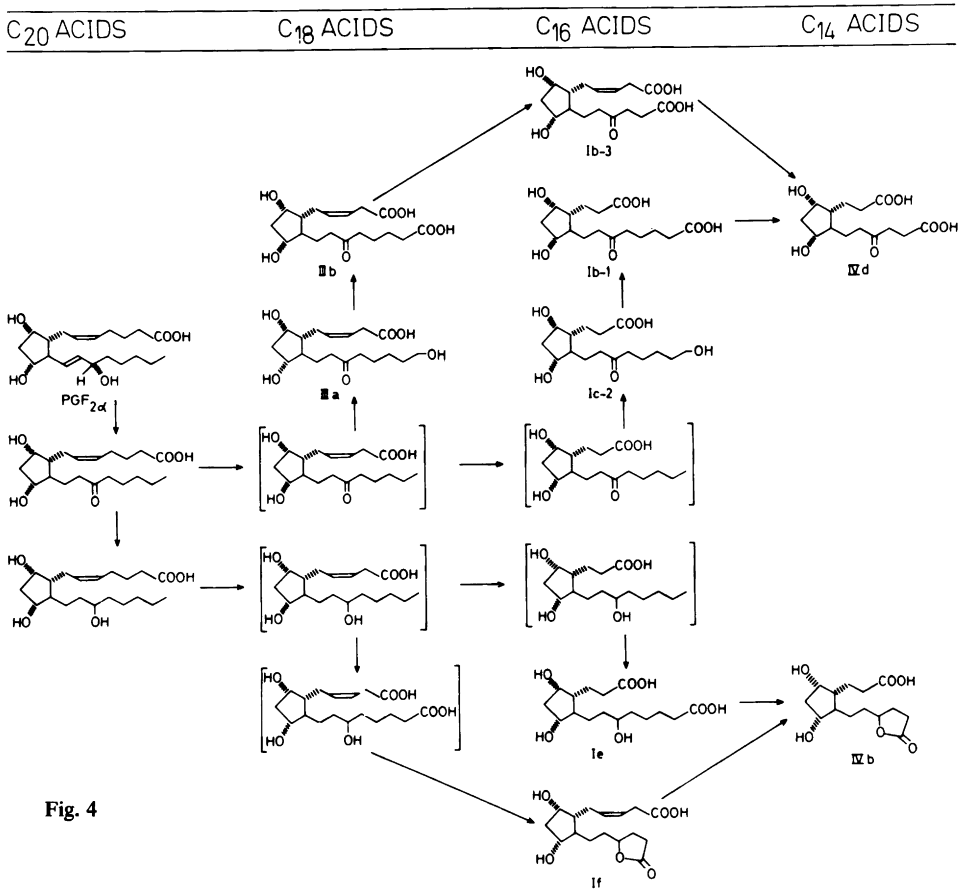

Fig. 4

You have seen how decades passed during the early history of the prostaglandins. Nowadays, it only took a few weeks after Professor *Vane*'s publication in *Nature* until the readers of the *New York Times* knew all about it. This rapid publication in the press is of course gratifying, even if it sometimes would be preferable that the data as to the clinical possibilities of the prostaglandins were more mature before being spread by the press.

The program here has, of course, to a large extent been guided by the number of papers sent in for presentation. The clinical papers in the field of control of human reproduction is by far the largest group, which is as it should be, since that is the largest problem facing mankind at present. I also think it is very gratifying that industry is participating to such a large extent at this meeting, roughly a third of the participants are from about forty pharmaceutical companies.

I believe it is only through closer cooperation in all respects between academia and the pharmaceutical industry that we can achieve what is necessary for an acceptable future of mankind.

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Quantitative Aspects on Prostaglandin Synthesis in Man

Bengt Samuelsson

Department of Chemistry, Karolinska Institutet, S–104 01 Stockholm 60, Sweden

Summary: Mass spectrometric methods have been developed and used for quantitative determination of the major prostaglandin metabolites. The synthesis (μg/24 hrs) in humans based on these measurements was for $PGE_1 + PGE_2$: 46–333 (♂, n = 10), 18–38 (♀, n = 9), and for $PGF_{1\alpha} + PGF_{2\alpha}$: 42–120 (♂, n = 9), 36–61 (♀, n = 8). During continuous infusion of $PGF_{2\alpha}$, the ratio between 15-keto-dihydro-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ ranged from 10–70. Calculated basal levels of $PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were 2 and 50 picog/ml, respectively. Reported concentrations of $PGF_{2\alpha}$ are 100–1000-fold higher than the calculated values, whereas the expected level of 15-keto-dihydro-$PGF_{2\alpha}$ is close to that found by quantitative mass spectrometry. It is concluded that the concentration of $PGF_{2\alpha}$ in peripheral plasma samples cannot be used to monitor $PGF_{2\alpha}$ synthesis in tissues and organs. In order to follow these reactions, the plasma level of the metabolite, 15-keto-dihydro-$PGF_{2\alpha}$, should be measured. Similar problems are involved in the analysis of PGE compounds.

Determination of the structure of prostaglandin metabolites, studies of their metabolic pathways, and the development of new analytical methods have recently made it possible to obtain quantitative data on the endogenous formation of prostaglandins in humans and experimental animals. The present review summarizes some of our studies in humans and deals specifically with the relationship between plasma prostaglandin levels and prostaglandin biosynthesis.

Tritium-labeled PGE_2 administered intravenously to human subjects is rapidly converted into 11α-hydroxy-9,15-diketo-prost-5-enoic acid [14]. This compound is further degraded into several metabolites, which are mainly excreted in the urine. The major urinary metabolite has been identified as 7α-hydroxy-5,11-diketo-tetranor-prostane-1,16-dioic acid [13, 14] (Fig. 1). This compound is also formed from PGE_1 and the metabolites 11α-hydroxy-9,15-diketoprostanoic acid, 11α-hydroxy-5,13-dienoic acid. Analogous transformations have been found for $PGF_{2\alpha}$ and

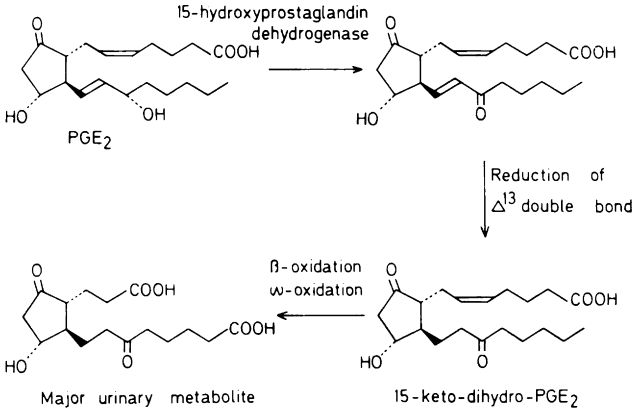

Fig. 1. Reactions involved in the formation of the major urinary metabolite (7α-hydroxy-5, 11-diketotetranor-prosta-1, 16-dioic acid) from PGE_2

$PGF_{1\alpha}$, which give rise to 3α, 7α-dihydroxy-11-ketotetranor-prostane-1,16-dioic acid as the major metabolite [7] (Fig. 2). Both the PGF and the PGE compounds are metabolized to a large number of other compounds, which have recently been identified [5, 8, 19].

Using a gas chromatographic method, the amounts of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in 24-hour samples of urine was found to range between 7–27 μg [14]. Recently improved methods for quantitative determination of both this metabolite and the major product from $PGF_{2\alpha}$ and $PGF_{1\alpha}$ have been developed [11]. These methods are based on the use of a deuterium-labeled derivative of the metabolite as internal standard and mass spectrometric determination of the ratio between unlabeled and deuterium-labeled molecules [1, 20]. A chromatogram showing both the total ion current and the intensities of ions (m/e 365 and m/e 368) representing the protium and deuterium forms is demonstrated in Fig. 3. The results of the analyses are summarized in Fig. 4. Male subjects consistently excreted larger amounts of the metabolite derived from PGE_1 and PGE_2 than female subjects. The data obtained have also been used together with information on recovery of the metabolites from their precursors to calculate the synthesis of prostaglandins of the PGE (PGE_1 and PGE_2) and the PGF ($PGF_{1\alpha}$ and $PGF_{2\alpha}$) group (Fig. 5). The PGE values, however, do not include the synthesis of PGE compounds used as precursors of PGA_1 and PGA_2. A correction has been made for the small contribution of PGE_1 and PGE_2 to the PGF metabolite.

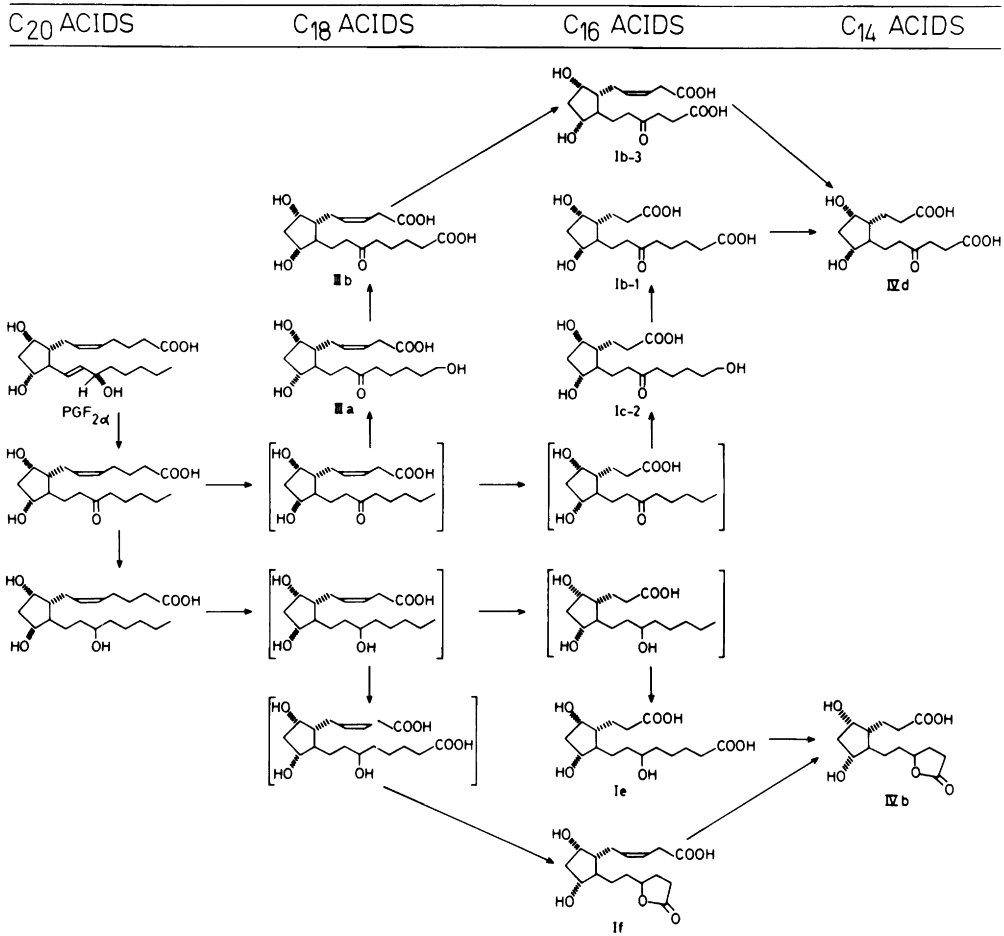

Fig. 2. Tentative pathways in the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. Compound within brackets have not been identified.

Administration of indomethacin (4x50 mg/24 hrs) to healthy human subjects resulted in a pronounced suppression of the levels of the major metabolite in urine (77–98 % inhibition, Fig. 6) [11]. Much higher doses of indomethacin (30–100 mg/kgx 24 hrs) were required to accomplish a similar inhibition in the guinea pig [15]. In man, therapeutic doses of aspirin and sodium salicylate (4x0.75 g/24 hrs) also gave a strong inhibition of the excretion of the major metabolite (Fig. 6).

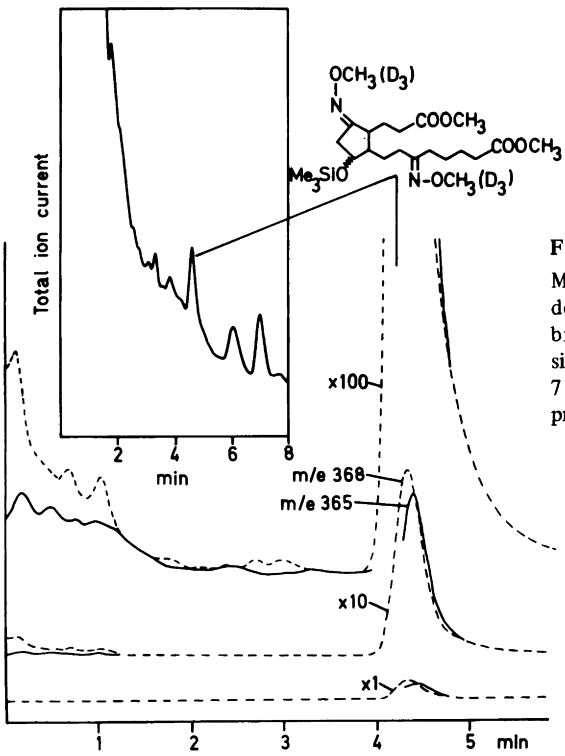

Fig. 3
Multiple-ion analysis in quantitative determination of the major isomer of bis (O-$^2H_3$-methyl-oxime)-trimethylsilyl ether derivative of dimethyl 7 α-hydroxy-5, 11-diketotetranorprostane-1, 16-dioate

Sex	Amount (μg/24 hrs)									
Males	6,5	7,3	7,4	8,1	9,6	10,9	11,9	12,3	34,0	46,7
Females	2,5	3,4	3,9	4,0	4,1	4,1	4,2	4,5	4,8	5,3

Fig. 4. Excretion of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in 20 healthy human subjects

Compounds	μg/24 hrs	
	Males	Females
$PGE_1 + PGE_2$	46–333 (N=10)	18–38 (N=10)
$PGF_{1\alpha} + PGF_{2\alpha}$	42–120 (N=9)	36–61 (N=8)

Fig. 5. Synthesis of prostaglandins in humans

Subject	Amount of metabolite (μg/24 hrs)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I[a] (♀)	4.8	4.8	1.8*	1.1*	1.5*	2.7	4.1
II[a] (♀)	3.9	4.4	0.7*	0.7*	0.7*	3.1	6.5
III[a] (♀)	3.8	3.0	0.5*	0.3*	0.3*	0.8	1.1
IV[a] (♂)	–	47.0	18.1*	3.5	–	–	–
IV[a] (♂)	24.0	23.0	6.2*	0.5*	0.5*	4.1	20.0
IV[b] (♂)	–	47.0	19.5*	6.4*	6.4*	16.6	33.3
IV[c] (♂)	–	33.5	21.2*	3.7*	0.6*	–	–
IV[c] (♂)	34.8	32.0	26.1*	9.4*	4.3*	3.9	13.3

Fig. 6. Excretion of 7α-hydroxy-5,11-diketotetranor-prostane-1,16-dioic acid in subjects receiving analgesics. Indomethacin (a, 4 x 50 mg/24 hrs), aspirin (b, 4 x 0.75 g/24 hrs), and sodium salicylate (c, 4 x 0.75 g/24 hrs) were given as indicated by asterisk.

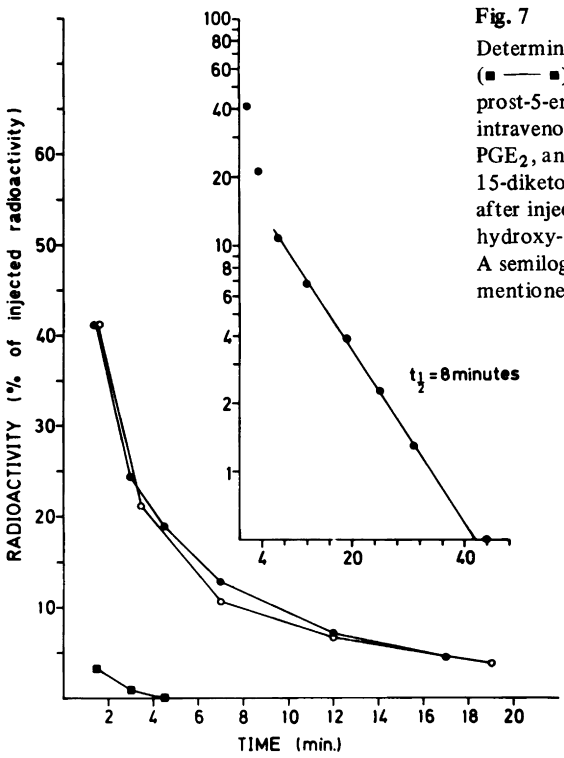

Fig. 7
Determinations of tritium-labeled PGE_2 (■ —— ■) and 11α-hydroxy-9, 15-diketo-prost-5-enoic acid (● —— ●) after intravenous injection of [17, 18-$^3H_2$] PGE_2, and of tritium-labeled 11α-hydroxy-9, 15-diketoprost-5-enoic acid (○ —— ○) after injection of [17, 18-$^3H_2$] 11α-hydroxy-9, 15-diketoprost-5-enoic acid. A semilogarithmic plot of the last-mentioned curve is also shown.

Intravenous administration of PGE_2 to human subjects results in very rapid transformation into 11α-hydroxy-9,15-diketoprost-5-enoic acid by the enzymes prostaglandin dehydrogenase and Δ^{13}-reductase [14]. Thus, 1.5 min after the injection only about 3 % was present as PGE_2 in the blood, whereas more than 40 % was recovered as the 15-keto-dihydro-metabolite (Fig. 7). Similar transformations have been observed for $PGF_{2\alpha}$ [6]. In this case, however, a dihydro-derivative was also identified.

We have recently determined the plasma levels of $PGF_{2\alpha}$, 15-keto-dihydro-$PGF_{2\alpha}$, and dihydro-$PGF_{2\alpha}$ during continuous intravenous infusion of $PGF_{2\alpha}$ to human subjects at a rate of 75 μg/min [15]. The concentration of the 15-keto-dihydro-derivative (105 ± 29 nanog/ml) was 10–70-fold higher than the parent $PGF_{2\alpha}$ (Fig. 8). The amount of $PGF_{2\alpha}$ reaching the blood was, in this experiment, about 2,000-fold that calculated to be released under normal conditions in females. Therefore, the normal basal concentration of $PGF_{2\alpha}$ should not exceed 2 picog/ml, whereas the 15-keto-dihydro-$PGF_{2\alpha}$ level might reach 50 picog/ml (Fig. 9). The latter value

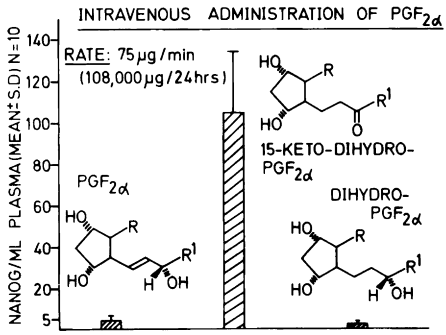

Fig. 8
Serum levels of prostaglandins during constant infusion of $PGF_{2\alpha}$

Compound	During i. v. adm. $PGF_{2\alpha}$ 75 μg/min = 108,000 μg/24 hrs	Endogenous total synthesis of $PGF_{1\alpha}$ + $PGF_{2\alpha}$ 36–61 μg/24 hrs		
	reported	calculated	reported	
			plasma	serum
$PGF_{2\alpha}$	4,450 ± 2,660	2	200–300 (RIA)	<10–990 (RIA) 500–2,500 (RIA) <200 (MS)
15-keto-dihydro-$PGF_{2\alpha}$	105,000 ± 29,300	50	20 (MS)	

Fig. 9. Concentrations of prostaglandins in females picog/ml

is of the same order of magnitude as that recently found by quantitative mass spectrometry [10]. However, reported values for $PGF_{2\alpha}$ are about 100–1000-fold higher than expected [3, 4, 16].

It has been reported that the clotting process releases prostaglandins, and that prostaglandin levels should be measured in plasma instead of serum [18]. Our data on the levels of $PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ during infusion of $PGF_{2\alpha}$ and under normal conditions, however, indicate that not even the levels of $PGF_{2\alpha}$ in samples of peripheral plasma are related to the rate of endogenous synthesis of $PGF_{2\alpha}$ in vivo in tissues and organs. The $PGF_{2\alpha}$ is probably formed by activation of the potent platelet system for prostaglandin biosynthesis even during the procedure involved in isolating blood plasma. Additional sources of $PGF_{2\alpha}$ might be leucocytes and nonenzymatic cyclization of polyunsaturated fatty acids in the blood. The latter reaction has been shown to take place in the presence of oxygen and hemin [18].

The major plasma metabolite of $PGF_{2\alpha}$, 15-keto-dihydro-$PGF_{2\alpha}$, is formed from $PGF_{2\alpha}$ in many tissues but not in blood. Both compounds can be expected to be rapidly released from the tissues. The 15-keto-dihydro-derivative, therefore, represents the compound of choice for monitoring $PGF_{2\alpha}$ biosynthesis in vivo by analysis of prostaglandins in peripheral plasma. Methods for quantitative determination of this metabolite either by mass spectrometry or by radioimmunoassay have recently been developed in our laboratory [9, 10]. Similar problems are involved in the plasma analysis of PGE compounds, and work is in progress to develop methods also for this group of prostaglandins.

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Inhibition of Prostaglandin Biosynthesis

William E. M. Lands, Paul R. LeTellier, Leonard H. Rome, and Jack Y. Vanderhoek

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

Summary: The fatty acid dioxygenase that initiates the conversion of unsaturated acids to prostaglandin may be inhibited in both reversible and irreversible ways. Reversible inhibitors such as CN^- and some antioxidants are effective only at high concentrations, and certain dithiols and Cu^{+2} chelators are effective at 10^{-3} M, suggesting a role for that ion in the activated vesicular gland dioxygenase that is not apparent with the soybean enzyme. A variety of substrate analogs also appear to compete for the active site with values ranging down to 10^{-6} M (K_I). The existence of a separate site for hydroperoxide is indicated from the reversible inhibition by added glutathione peroxidase.

All fatty acid substrates tested so far led to a self-catalyzed destruction of the dioxygenase activity in an irreversible manner regardless of their ability to form active hormone. This phenomenon would automatically tend to limit the amount of hormone produced per catalytic protein. The loss of activity is dependent upon O_2 and is blocked by reversible inhibitors of dioxygenase action. Although eicosatetraynoic acid exhibits no appreciable uptake of O_2, it destroys enzymic activity in a time-dependent and inhibitable manner analogous to that for acids that are substrates. Like the other irreversible time-dependent inhibitors of dioxygenase activity, indomethacin was effective in vitro at $1\text{-}10 \times 10^{-6}$ M. It does not, however, require the high levels of O_2 needed by the other irreversible inhibitors described above, and its mode of action appears to be different. These studies, in combination, provide more detailed insight into the overall mechanism of converting the unsaturated prostaglandin precursors into oxygenated products.

Introduction

A wide variety of agents appear to be able to modify the amount of prostaglandin that could be produced by a tissue. Since the conversion of lipid precursors to prostaglandins is a multistage enzyme-catalyzed process, we have initiated studies to

define more clearly the probable cofactors and mechanisms for the various steps, and thus understand how they may be regulated. The reactions shown in Fig. 1 illustrate the variety of steps involved. Some increase in acylhydrolase activity seems essential to the production of appreciable amounts of hormone since the non-esterified acid precursors are not normally present in significant amounts, and we found no evidence for any appreciable synthesis to occur when the acid was esterified to phospholipid [12]. The need for some additional reducing equivalents to produce the F-type prostaglandins is readily apparent, and we [13] have described a Cu^{+2}-dithiol system that produces predominantly $PGF_{2\alpha}$ and very little PGE_2, whereas the use of glutathione provides mostly PGE_2 and very little $PGF_{2\alpha}$ [11]. Reactions 4 [5] and 7 [7] appear to be side reactions that occur in the absence of the cofactors needed for reactions 5 and 6. In examining the mechanism of reaction 1, we found evidence for a more complex process than originally anticipated. The results suggest that the initial oxygenation process could be subject to a variety of physiological and biochemical control events. This report will primarily describe some results of inhibitory studies on the oxygenation reaction that give further insight into the possible regulation of prostaglandin biosynthesis.

Materials and methods

Most inhibitory agents were purchased from commercial sources. Fatty acid substrates were purchased from the Hormel Institute or Nucheck Preps, whereas the eicosatetraynoic acid was a gift from Hoffman-LaRoche; oxyphenbutazone, from Geigy Pharmaceuticals; and the indomethacin from Merck, Sharp & Dohme. The Upjohn Company gave us preparations of sheep vesicular glands used for enzymic work.

The principal method used was an automatic multisample continuous recording oxygen electrode system with 3 ml. reaction mixtures containing 0.1 M Tris chloride buffer (pH 8.5), 0.6 mM phenol, 10 to 80 μM substrate acid, and preparations of acetone powder (2 to 6 mg) of sheep vesicular glands.

Results and discussion

Figure 2 shows that although a variety of acids can serve as substrates for the vesicular gland oxygenase, the presence of the terminal (n−3) ethylenic bond seems to interfere with the normal reaction at the (n−9) bond. In the series of acids tested, only the (n−6) polyenoic acids reacted with the oxygenase. Results for other polyunsaturated acids have been described by *van Dorp*'s group [1, 20], which support the above observations. Earlier reports by *Hamberg* and *Samuelsson* have elegantly

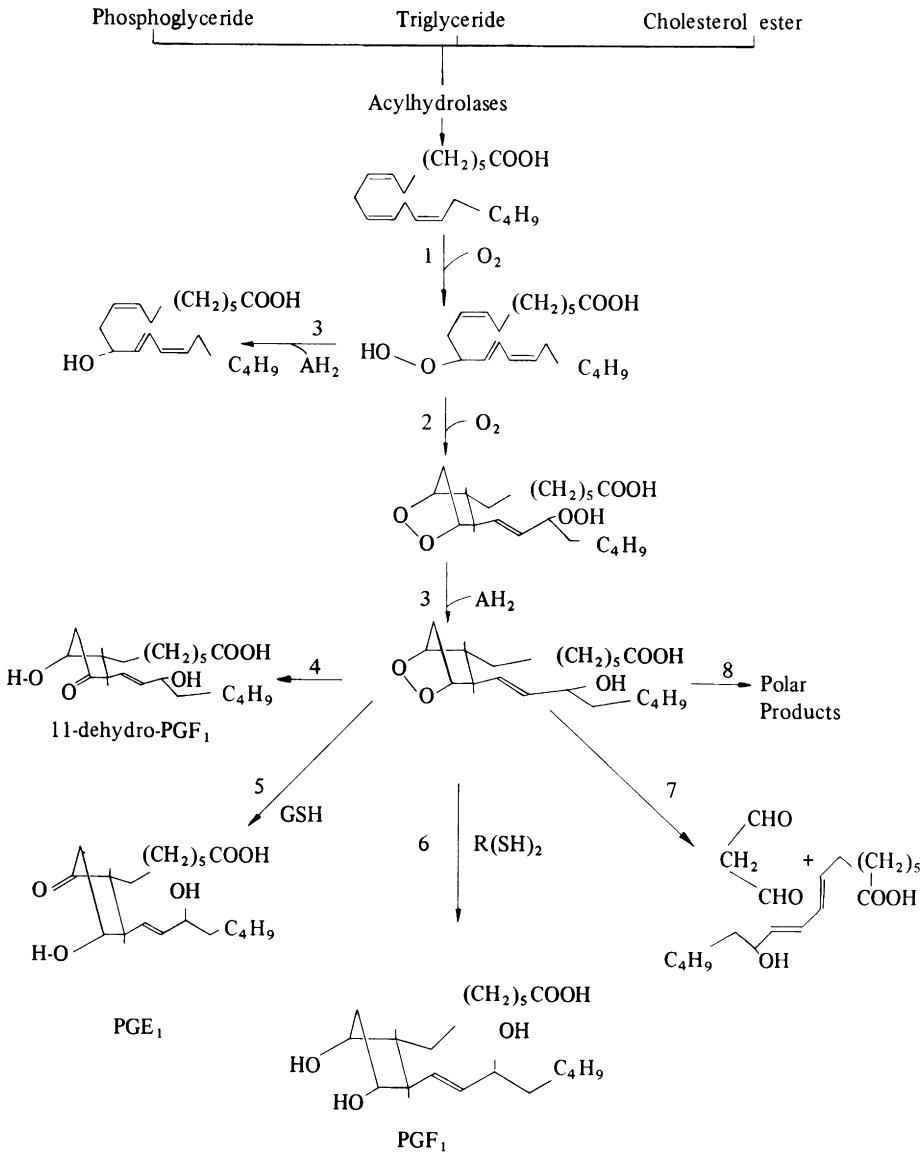

Fig. 1. The overall system for prostaglandin biosynthesis

Acid	k_3 value
18:1 (n-9)	0
18:2 (n-6)	7
18:3 (n-6)	9
18:3 (n-3)	0
20:2 (n-6)	16
20:3 (n-6)	65
20:3 (n-3)	0
20:4 (n-6)	35
20:5 (n-3)	0
22:6 (n-3)	0

$$\frac{\mu M\ O_2/min}{mg\ E/ml} = k_3$$

Fig. 2.
Average maximal velocities of oxygenation

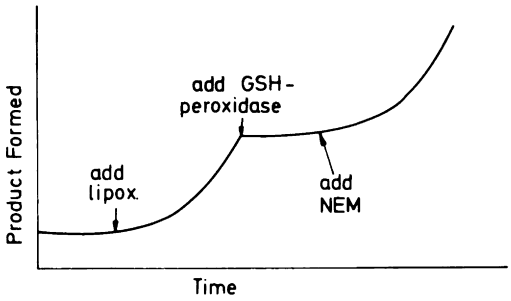

Fig. 3
Effect of glutathione peroxidase on lipoxygenase rates

described the stereospecificity of hydrogen removal [8] and oxygen addition [6] in this reaction. Interestingly, the lipoxygenase of soybean has a similar selectivity for the (n–8) proton even though the oxygen attacks the (n–6) ethylenic bond and the reaction is not inhibited by the presence of a terminal (n–3) double bond. All vesicular gland preparations tested so far converted 20:3 (the PGE_1 precursor) more rapidly than arachidonate.

Glutathione peroxidase inhibited not only prostaglandin formation [11], but also the soybean lipoxygenase (see Fig. 3), suggesting that a hydroperoxide derivative may be needed for the reaction to work. This was further supported by the extensive lags noted in attaining initial velocities (Fig. 4) and led to formulation of a possible mechanism to account for this behavior (Fig. 5) [19]. In this mechanism, we propose that the fatty acid hydroperoxide may react with oxygen to form a tetroxide which could then interact with a substrate molecule on the enzyme surface to form reactive intermediates. These intermediates would be thermodynamically favored to give the correct hydroperoxide under the stereospecific control of the enzyme. Such transient intermediates could exist and still be compatible with the isotope enrichment in substrate observed by *Hamberg* and *Samuelsson* [8].

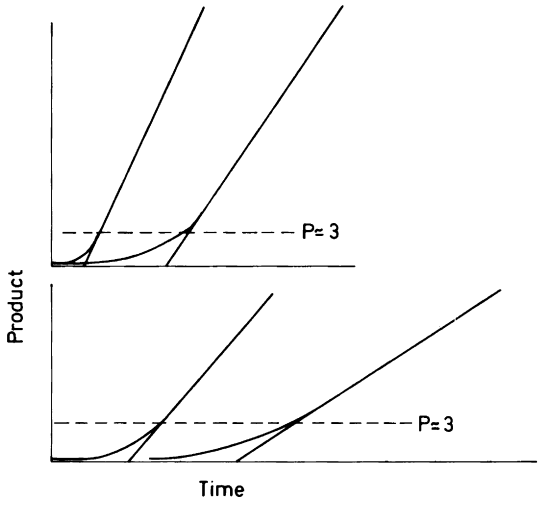

Fig. 4
Kinetic lag in lipoxygenase action on 20 : 4

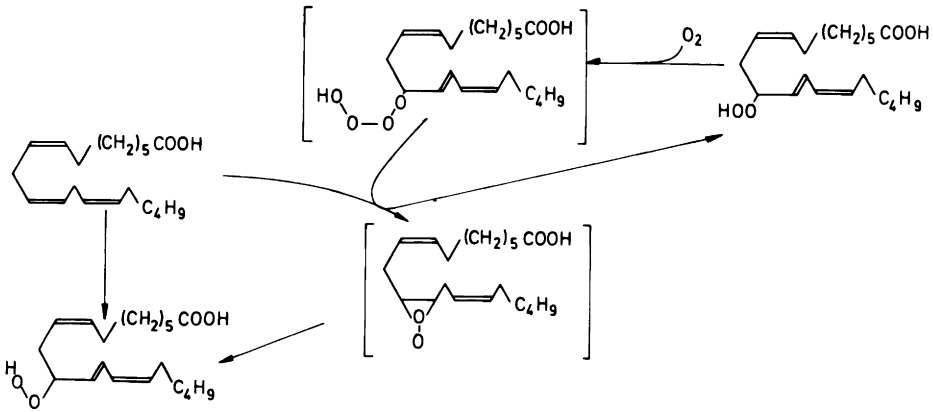

Fig. 5. A hypothetical mechanism for fatty acid oxygenase

Another unusual feature of the oxygenase reaction is its tendency to stop before all the substrate has been consumed (Fig. 6). This phenomenon appears to be due to a self-catalyzed inactivation of the enzyme as formulated in Fig. 7. Interestingly, this type of inhibition mechanism predicts that the instantaneous velocity at different times may be linearly related to the remaining substrate rather than the more familiar hyperbolic relationship [3]. We find that such a linear relationship does occur (Fig. 8) during oxygenation of fatty acids by vesicular gland preparations.

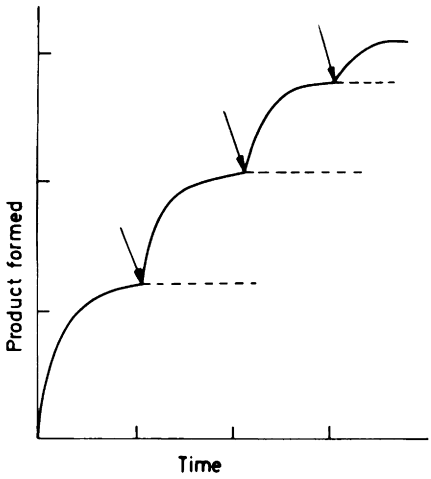

Fig. 6
Limitation of the extent of reaction by the amount of protein

$$E + S \rightleftharpoons ES \begin{cases} \xrightarrow{k_3} E + P \\ \xrightarrow{k_2} E^* + S \end{cases}$$

$$dP/dt = \frac{k_3 [E_0 - E^*]}{\frac{K_m}{S} + 1} = \frac{k_3 [E_0 - \frac{k_2 P}{k_3}]}{\frac{K_m}{S} + 1} \quad \text{and} \quad P = S_0 - S_t$$

$$v = \frac{(k_3 E_0 - k_2 S_0)}{\frac{K_m}{S} + 1} + \frac{k_2 S}{\frac{K_m}{S} + 1} \cong K' + k_2 S$$

Fig. 7. A kinetic formulation of the self-catalyzed destruction

The slope of these lines (k_2) is constant over a range of conditions and appears to be a property of the enzyme-substrate complex. The magnitude of this first-order rate constant for different acids is given in Fig. 9. Those acids which showed the largest turnover numbers (k_3) also have a tendency to destroy the catalytic activity most rapidly (k_2).

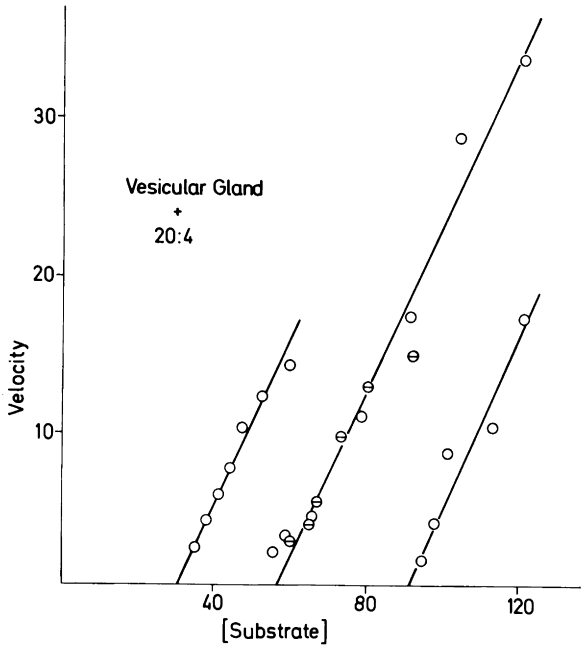

Fig. 8
The linear relationship between v_t and S_t

Acid	Estimated k_2 value (min^{-1})
18:1 (n-9)	0
18:2 (n-6)	.4
18:3 (n-6)	.3
18:3 (n-3)	0
20:2 (n-6)	.9
20:3 (n-6)	2.
20:3 (n-3)	0
20:4 (n-6)	1.
20:5 (n-3)	0
22:6 (n-3)	0

Fig. 9. Destruction of vesicular gland oxygenase

The above results support our formulation of the overall mechanism for the vesicular gland oxygenase as shown in Fig. 10. The existence of both a product and substrate binding site extends the possibilities for reversible competitive concentration-dependent inhibitions by substrate or product analogs [15]. Furthermore, the removal of all hydroperoxide from the system prevents formation of the active

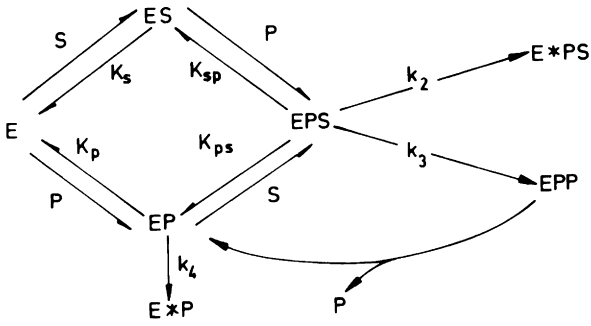

Fig. 10
A proposed formulation for oxygenase activity

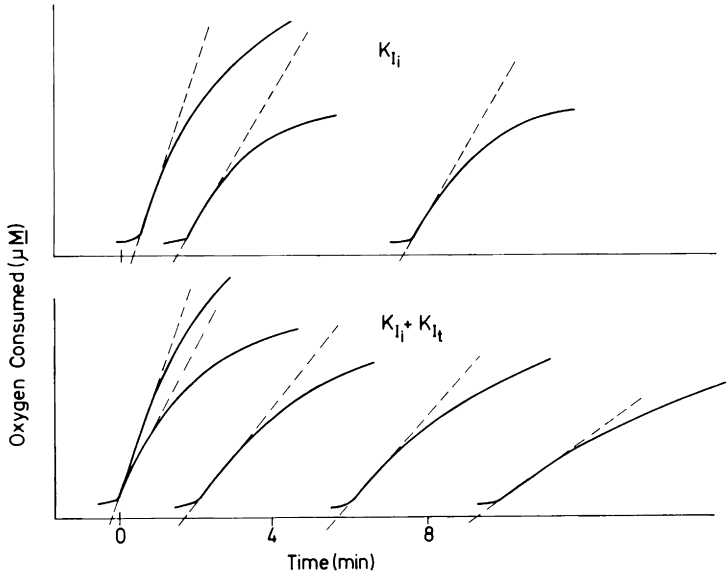

Fig. 11. Examples of instantaneous and of progressive inhibitions

intermediates that allow the substrate to react. Also, the progressive self-catalyzed destruction of enzyme (indicated by the k_2 value) places an upper limit on the maximum amount of product that can be produced by the enzyme. Unsaturated acids which are not precursors for prostaglandin formation but are present in cellular lipids may be released along with the desired precursors and inhibit the process of hormone formation. The nature and magnitude of this inhibition would then depend upon the composition of acids released. We must question whether the release of arachidonate could occur without release of those naturally occurring

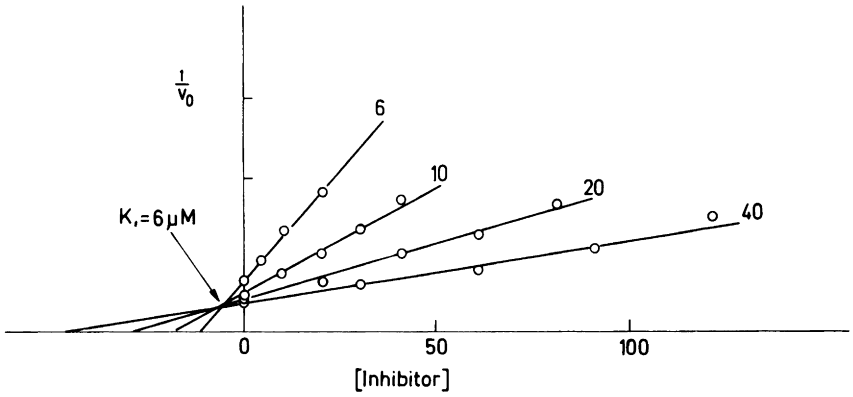

Fig. 12. 20: 3 (n–3) Inhibition of 20:4 (n–6) oxidation

Acid	μM
18:1 (n–9)	22
18:3 (n–3)	15
20:3 (n–3)	6
20:5 (n–3)	2.5
22:6 (n–3)	1.7

Fig. 13. Competitive inhibition constants (K_I)

inhibitors. Examples of the instantaneous reversible inhibition and the time-dependent nonreversible inhibition, measured in our in vitro system, are shown in Fig. 11. The observed velocities of O_2 consumption can then be plotted in the presence or absence of different amounts of inhibitor (see Fig. 12) to provide kinetic parameters for comparative purposes. In this case, we see that the (n–3) acid, 20:3, has a competitive inhibition constant of 6 μM. It can be seen in Fig. 13 that those (n–3) acids which are not substrates can still compete for binding at the substrate site. This competitive binding is stronger for the more highly unsaturated acids. On the other hand, the (n–6) acids such as linoleate (Fig. 14) cause both an instantaneous competitive inhibition and a progressive and nonreversible loss of activity. The first-order rate constant measured for this time-dependent loss is similar to that observed for the self-catalyzed destruction noted earlier. Since the loss of enzyme seen in Fig. 11 can be prevented by anaerobic conditions under which no oxygenase reaction can occur, it appears that we can protect the enzyme from itself.

We found that another method of inhibiting the oxygenation of acid and thus preventing the self-catalyzed destruction of enzyme is by adding diethyldithiocarbamate or other reagents known to complex Cu^{+2}. An early report by *Nugteren* et al. [15] stated that no inhibition was found with mM levels of several metal

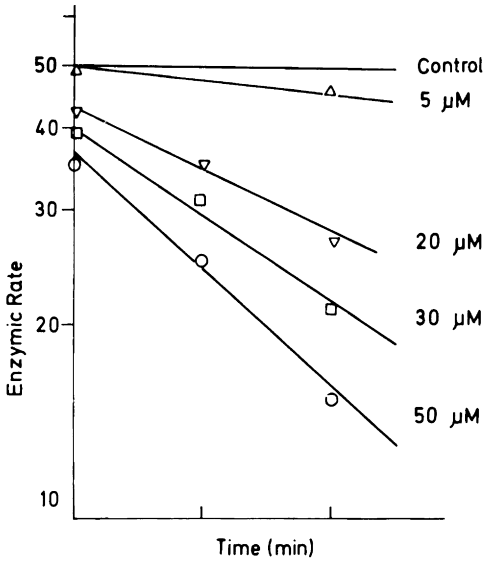

Fig. 14
Loss of vesicular gland oxygenase with 18 : 2

Complexing Agent	$[I]_{50}$ (mM)
Toluene-3,4-dithiol	0.15
Sodium cyanide	.6
Dithizone	.8
Diethyldithiocarbamate	2.
o-Phenanthroline	2.
Bathocuproine sulfonate	5.
2,2'-Bipyridine	8.
Ethyl xanthate	25.
Tiron	>50.

Fig. 15. Inhibition by complexing agents

complexing agents that we have studied. We feel that our current methods are more sensitive; Fig. 15 gives the concentrations of these agents that give 50% inhibition of the oxygenase reaction in vitro. These results, plus those described earlier with dithiols [11], lead us to believe that Cu^{+2} could play a role in the oxygenation reaction in the vesicular gland.

A variety of antioxidants were found to inhibit the vesicular gland dioxygenase. The concentrations of these antioxidants that give 50% inhibition of the oxygenase are listed in Fig. 16. We were surprised by the large differences between BHA and BHT, and also between hydroquinone and napthol-l. An early report by *Tappel* et al. [21] indicated that α-napthol, catechol, tocopherol, and hydroquinone were equally inhibitory with soybean lipoxygenase. Apparently the inhibitory ability of

Antioxidant	$[I]_{50}$ (μM)
α-Naphthol	3.5
Santoquin	6.5
BHA	6.7
NDGA	11
β-Naphthol	11
Guaicol	25
Trimethylhydroquinone	56
Quercetin	90
Propyl Gallate	120
Methylene Blue	140
Hydroquinone	180
BHT	190
Ascorbic Acid	310
α-Tocopherol	500
Sodium Citrate	>1700

Fig. 16. Inhibition by antioxidants

	1′	12′	29′
Anaerobic	16	16	15
Aerobic	12	7	2
	1′	5′	10′
Added GSH+ GSH peroxidase	18	19	19
Omitted peroxidase	10	4	2

Fig. 17
Time-dependent inhibition by eicosatetraynoic acid. The velocities are given as μM/min.

these agents is influenced by the structure of the enzyme and not solely related to their traditional antioxidant potencies [2, 22]. Our findings lead us to believe that suggestions that antioxidants may retard the aging process (e.g., [10, 16]) should be balanced against our evidence that some antioxidants may also block prostaglandin biosynthesis even when present at very low levels.

Inhibition of prostaglandin formation by eicosatetraynoic acid was first reported by *Downing* et al. [4]. Our examination of the mechanism of this inhibition indicates that it is a time-dependent process (Fig. 17). The inhibitory action is prevented by excluding either the oxygen or the lipid hydroperoxide needed to form reactive intermediates. Furthermore, diethyldithiocarbamate, which blocks the oxygenation of fatty acids by the oxygenase, also prevents the time-dependent inhibition by the tetrayne. In addition, the antioxidant α-napthol also can prevent the destructive action of eicosatetraynoic acid (Fig. 18). Thus, this acetylenic derivative appears to be functioning as a substrate analog in destroying the vesicular gland oxygenase.

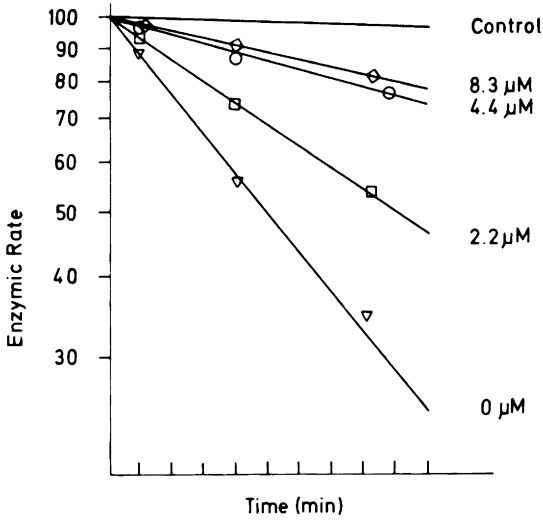

Fig. 18
α-Napthol prevents action of 6 μM eicosatetraynoic acid.

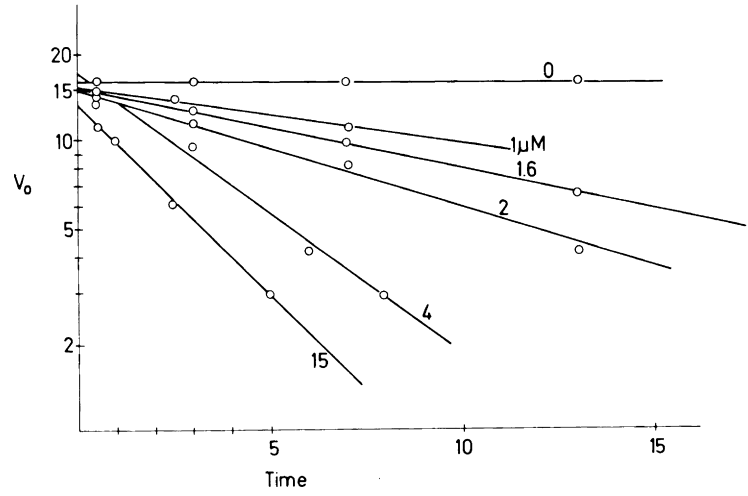

Fig. 19. Indomethacin inhibition

Another inhibitor of prostaglandin formation is indomethacin [23]. Our studies show that this drug also blocks the vesicular gland oxygenase in a concentration-dependent, time-dependent, nonreversible manner (Fig. 19). This inhibition proceeds anaerobically and is not prevented by diethyldithiocarbamate. Thus, the site of action appears to be different than that for the tetraynoic acid and other

substrate analogs. We have reported that o-phenanthroline does prevent the time-dependent destruction by indomethacin [17], and the evidence suggests that a sensitive hydrophobic site may be an added regulatory feature of the prostaglandin biosynthetic system.

Conclusion

In conclusion, the overall mechanism for active vesicular gland oxygenase that has evolved from our studies is one that provides extensive regulatory features. The phenomenon of product activation means that as the reaction begins, it enters an explosive phase of increase as the product-binding site becomes filled. In addition, the requirement of hydroperoxide allows cellular enzymes such as glutathione peroxidase to suppress the reaction in the presence of high glutathione. The balance point between these two antagonistic systems could provide a sensitive switch on hormone production. In contrast to the positive-feedback aspect of product activation, the self-catalyzed destruction constant, k_2, insures a limited yield of hormone from a given catalytic protein. When the destruction is caused by a non-precursor fatty acid, the loss of activity might be compared to an ultramicro-cauterization of the active site. This process emphasizes the need for new protein synthesis if continued production of hormone is to be maintained. Such a requirement leads in turn to an obligatory relationship between prostaglandins and those hormonal agents that stimulate transcription and production of the correct m-RNA.

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Catecholamine Induced Increase in Prostaglandin E Biosynthesis in Homogenates of the Rat Stomach Fundus

Cecil Robert Pace-Asciak

Donner Laboratory of Experimental Neurochemistry, Montreal Neurological Institute, McGill University, Montreal 112, Canada

Summary: Prostaglandin synthetase activity was demonstrated in homogenates of the rat stomach fundus by conversion of tritiated arachidonic acid into prostaglandin E_2 (PGE_2) and the isomeric 6 (9)-oxy-11,15-dihydroxyprosta-7, 13-dienoic acid [6 (9)-oxy PGF]. This activity was stimulated severalfold in a time, concentration, and temperature dependent fashion by norepinephrine. Very high concentrations of norepinephrine (10^{-2}M) abolished the stimulation in PGE synthesis observed with lower doses and resulted in the formation of labelled $PGF_{2\alpha}$. Epinephrine and dopamine were equally active in stimulating PGE synthesis, whereas related monohydroxy derivatives were less active. Desoxy derivatives were inactive. It appears from time course studies at two temperatures (37 °C, 25 °C) that norepinephrine and also other catecholamines act as protective agents, prolonging the PG synthetase in an active form.

Problem

To investigate in vitro the effects of certain neurotransmitter substances on prostaglandin synthesis and to elucidate by means of related compounds structural features required for these effects.

Materials and methods

5,6,8,9,11,12,14,15-$^3H_8$-arachidonic acid (sp. act. 19.0 mCi/μmole) was purchased from New England Nuclear and diluted to 80000 cpm/μg with unlabelled free arachidonic acid (Mann, > 99 % purity). Prostaglandin TLC standards were generously supplied by Dr. *J. E. Pike,* The Upjohn Co., Kalamazoo, Michigan. Catecholamines and other amines were purchased from Sigma as the hydrochlorides.