

# **Bronchial Asthma** **and the** **Atopic Syndrome**

## **A Biochemical Study Proposing** **a Deficit of Lipoprotein Lipase**

By Alexander Dunedin

Abstract: A thesis on bronchial asthma and the atopic conditions existing as manifestations of a breakdown in prostaglandin metabolism resultant from a chemical depletion of lipoprotein lipase (a regulating enzyme), or a deficit of the elemental components required to synthesise the enzyme.

# Bronchial Asthma and the Atopic Syndrome

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# Bronchial Asthma and the Atopic Syndrome

## Paper 1: Foreword

This document is a hypothesis of the cause of bronchial asthma and the atopic syndrome. Key terms are shown in bold and underlined. These are points of reference to published texts.

When a key term has been made in the narrative you will be able to find in the appendix the details of the author; the title of the publication; the date of and edition of publication from which it has been taken; the publisher; a surmising of the content, the page of reference; all followed by the unexpurgated reference. This has been done so the facts may be easily compared against the original texts.

The principal papers provide an index leading through an increasingly detailed definition and breakdown of atopy and its theorized cause. Two substances - **glucuronic acid [1.1]** and **glucosamine [1.2]** - are required for the construction of glycosaminoglycans, heparin and active lipoprotein lipase. Glucuronic acid is also required for the elimination of certain prostaglandins, toxins and xenobiotics from the body in a process known as **glucuronidation [1.3]**.

**A simple explanation of allergic conditions [1.4]** is inflammation. Allergic conditions are manifestations of the same type of inflammation occurring in different mucosal tissues of the body. The hypothesis here being presented is that the inflammation arises as a direct result of a deficit of **glucuronic acid [1.5]** and **glucosamine [1.6]** which one-at-the-same-time are required to control prostaglandin hormones via the enzyme lipoprotein lipase.

In **summary [1.7]**, glucuronic acid is drawn on as a resource for the elimination of toxins and xenobiotics from the body thus infringing on the production of lipoprotein lipase, hence resulting in a disorder of the prostaglandin metabolism triggered by various extrinsic toxins.

# Paper 1: Foreword

The principal papers provide an index leading through an increasingly detailed definition and breakdown of atopy and its theorized cause. Two substances - **glucuronic acid [1.1]** and glucosamine - are required for the construction of glycosaminoglycans, heparin and active lipoprotein lipase. Glucuronic acid is also required for the elimination of certain prostaglandins, toxins and xenobiotics from the body in a process known as glucuronidation.

**Paper Number: 1**  
**Reference Number: 1.1**

**Information taken from:**

Oxford Dictionary Of Biochemistry And Molecular Biology  
Revised Edition 2000  
Managing Editor Dr A D Smith University College London  
General Editors: Professor S P Datta University College London  
Dr G H Smith University College London  
Professor P N Campbell (Chairman) University College London  
Dr R Bentley University of Pittsburgh  
Dr H A McKenzie Australian Defence Force Ac.  
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Oxford University Press Inc., New York  
ISBN 0 19 850673 2  
Page 267; Glucuronic acid

## **Glucuronic Acid**

abbr. (sometimes): GA; symbol: GlcA (or (formerly) GlcU or GlcUA); the uronic acid formally derived from glucose by oxidation of the hydroxymethylene group at C-6 to a carboxyl group [1]. There are two enantiomers; D-glucuronic acid is widely distributed in plants and animals, where it usually occurs as glucuronides [2].

## **Notes:**

Glucuronic acid is a uronic acid derived from glucose by oxidation [1]. There are two enantiomers; D-glucuronic acid is widely distributed in plants and animals [2].

# Paper 1: Foreword

The principal papers provide an index leading through an increasingly detailed definition and breakdown of atopy and its theorized cause. Two substances - glucuronic acid and **glucosamine** [1.2] - are required for the construction of glycosaminoglycans, heparin and active lipoprotein lipase. Glucuronic acid is also required for the elimination of certain prostaglandins toxins and xenobiotics from the body in a process known as glucuronidation.

**Paper Number: 1**  
**Reference Number: 1.2**

**Information taken from:**

Oxford Dictionary Of Biochemistry And Molecular Biology  
 Revised Edition 2000  
 Managing Editor Dr A D Smith University College London  
 General Editors: Professor S P Datta University College London  
 Dr G H Smith University College London  
 Professor P N Campbell (Chairman) University College London  
 Dr R Bentley University of Pittsburgh  
 Dr H A McKenzie Australian Defence Force Ac.  
 © The General Editors, 1997  
 Oxford University Press Inc., New York  
 ISBN 0 19 850673 2  
 Page 265; Glucosamine

## **Glucosamine**

Symbol: GlcpN; the trivial name for the aminodeoxysugar 2-amino-2-deoxyglucopyranose [1]; there are two enantiomers. D-Glucosamine (symbol: D-GlcpN), formerly known as chitosamine, occurs in combined form in chitin, in mucoproteins, and in mucopolysaccharides [2], and is one of the most abundant natural monosaccharides [3].

## **Notes:**

Glucosamine is the name for the aminodeoxysugar 2-amino-2-deoxyglucopyranose [1]. There are two enantiomers; D-glucosamine occurs in combined form in chitin, mucoproteins, and in mucopolysaccharides [2]. It is one of the most abundant natural monosaccharides [3].

# Paper 1: Foreword

The principal papers provide an index leading through an increasingly detailed definition and breakdown of atopy and its theorized cause. Two substances - glucuronic acid and glucosamine - are required for the construction of glycosaminoglycans, heparin and active lipoprotein lipase. Glucuronic acid is also required for the elimination of certain prostaglandins, toxins and xenobiotics from the body in a process known as **glucuronidation** [1.3].

**Paper Number: 1**  
**Reference Number: 1.3**

**Information taken from:**

Toxicological chemistry and biochemistry /  
by Stanley E. Manahan.-- 3rd ed.  
Includes bibliographical references and index.  
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Lewis Publishers is an imprint of CRC Press LLC  
Page 169; Section 7.4.1 Phase II Reactions of Toxicants;  
Conjugation by Glucuronides

## **7.4 Phase II Reactions Of Toxicants**

Phase II reactions are also known as conjugation reactions because they involve the joining together of a substrate compound with another species that occurs normally in (is endogenous to) the organism. This can occur with unmodified xenobiotic compounds, xenobiotic compounds that have undergone phase I reactions, and compounds that are not xenobiotic species [1].

The substance that binds to these species is called an endogenous (present in and produced by the body) conjugating agent. Activation of the conjugating agent usually provides the energy needed for conjugation, although conjugation by glutathione or amino acids is provided by activation of the species undergoing conjugation preceding the reaction [2].

The overall process for the conjugation of a xenobiotic compound is shown in Figure 7.7. Such a compound contains functional groups, often added as the consequence of a phase I reaction, that serve as “chemical handles” for the attachment of the conjugating agent [3]. The conjugation product is usually less lipid soluble, more water soluble, less toxic, and more easily eliminated than the parent compound [4].

The conjugating agents that are attached as part of phase II reactions include glucuronide, sulfate, acetyl group, methyl group, glutathione, and some amino acids [5]. Conjugation with glutathione is also a step in mercapturic acid synthesis. Glycine, glutamic acid, and taurine are common amino acids that act as conjugating agents.

Most of the conjugates formed by these agents are more hydrophilic than the compounds conjugated, so the conjugates are more readily excreted. The exceptions are methylated and acetylated conjugates. Phase II conjugation reactions are usually rapid, and if they are performed on phase I reaction products, the rates of the latter are rate limiting for the overall process [6].

### 7.4.1 Conjugation by Glucuronides

Glucuronides are the most common endogenous conjugating agents in the body. They react with xenobiotics through the action of uridine diphosphate glucuronic acid (UDPGA). This transfer is mediated by glucuronyl transferase enzymes [7]. These enzymes occur in the endoplasmic reticulum, where hydroxylated phase I metabolites of lipophilic xenobiotic compounds are produced [8]. As a result, the lifetime of the phase I metabolites is often quite brief because the conjugating agent is present where they are produced. A generalized conjugation reaction of UDPGA with a xenobiotic compound can be represented as the following:

DIAGRAM OMITED

In this reaction  $HX-R$  represents the xenobiotic species in which  $HX$  is a functional group (such as  $-OH$ ) and  $R$  is an organic moiety, such as the phenyl group (benzene ring less a hydrogen atom). The kind of enzyme that mediates this type of reaction is UDP glucuronyltransferase. Glucuronide conjugation products may be classified according to the element to which the glucuronide is bound [9]. The atoms to which the glucuronide most readily attaches are electron rich, usually O, N, or S (nucleophilic heteroatoms in the parlance of organic chemistry) [10].

Example glucuronides involving O, N, and S atoms are shown in Figure 7.8. When the functional group through which conjugation occurs is a hydroxyl group,  $-OH$  ( $HX$  in Reaction 7.4.1), an ether glucuronide is formed [11]. A carboxylic acid group for  $HX$  gives an ester glucuronide [12]. Glucuronides may be attached directly to N as the linking atom, as is the case with aniline glucuronide [13] in Figure 7.8, or through an intermediate O atom [14].

An example of the latter is N-hydroxyacetylaminoglucuronide, for which the structure is shown in Figure 7.9. This species is of interest because it is a stronger carcinogen than its parent xenobiotic compound, N-hydroxyacetylaminofluorene, contrary to the decrease in toxicity that usually results from glucuronide conjugation [15].

The carboxylic acid ( $-CO_2H$  group) in glucuronides is normally ionized at the pH of physiological media, which is a major reason for the water solubility of the conjugates [16]. When the compound conjugated (called the aglycone) is of relatively low molecular mass, the conjugate tends to be eliminated through urine. For heavier aglycones, elimination occurs through bile [17].

Enterohepatic circulation provides a mechanism by which the metabolic effects of some glucuronide conjugates

#### **Notes:**

Phase II reactions are also known as conjugation reactions because they involve the joining of a substrate with unmodified xenobiotic compounds, xenobiotic compounds which have undergone phase I reactions, and compounds that are not xenobiotic species [1]. The substance which binds to these species is referred to as an endogenous conjugating agent [2].

The xenobiotic compound contains functional groups often added via a phase I reaction which act as 'chemical handles' for the attachment of a conjugating agent [3]. The conjugation product is usually less lipid soluble, more water soluble, less toxic, and more easily eliminated than the parent compound [4].

Conjugation agents attached as part of phase II reactions include glucuronide, sulfate, acetyl, methyl, glutathione and some amino acids [5]. Phase II conjugation reactions are usually rapid and if performed on phase I reaction products, the rates of the latter are rate limiting for the overall process [6].

Glucuronides are the most common endogenous conjugating agents in the body. They react with xenobiotics via uridine diphosphate glucuronic acid (UDPGA). This transfer is mediated by glucuronyl transferase enzymes [7]. Glucuronyl transferase enzymes occur in the endoplasmic reticulum where hydroxylated phase I metabolites of lipophilic xenobiotics are produced [8].

Glucuronide conjugation products can be classified according to the element to which the glucuronide is bound [9]. The atoms to which the glucuronide most readily attaches are electron rich, usually oxygen, nitrogen or sulfur [10]. When the functional group through which a glucuronide conjugation occurs is a hydroxyl group (-OH), an ether glucuronide is formed [11]. A carboxylic acid group gives rise to an ester glucuronide [12]. Glucuronides may be formed via attachment directly to a nitrogen as the linking atom, as is found with aniline glucuronide [13].

Glucuronides may also form attachments through an intermediate oxygen atom [14]. N-hydroxyacetylaminoglucuronide is an example of this linkage which is of note as it is more toxic than its parent compound, N-hydroxyacetylaminofluorene. This is of special interest as a decrease in toxicity usually results from glucuronide conjugation [15].

The carboxylic acid group in glucuronides is normally ionized at the pH of physiological media which is a major reason for the water solubility of the conjugates [16]. When the conjugated compound (also called the aglycone) is of relatively low molecular mass, the conjugate tends to be eliminated through the urine. With heavier conjugated compounds, elimination occurs through the bile [17].

Some glucuronide conjugates enter a recycling process in which the glucuronide conjugate released into the intestine with bile becomes deconjugated and reabsorbed. This has been known to amplify the metabolic effects of some glucuronide conjugates [18].

# Paper 1: Foreword

**A simple explanation of allergic conditions [1.4]** is inflammation. Allergic conditions are manifestations of the same type of inflammation occurring in different mucosal tissues of the body. The hypothesis here being presented is that the inflammation arises as a direct result of a deficit of glucuronic acid and glucosamine which one-at-the-same-time are required to control prostaglandin hormones via the enzyme lipoprotein lipase.

**Paper Number: 1**  
**Reference Number: 1.4**

**Information taken from:**

Delineation of hypothesis being posited  
 as the cause of allergic conditions

## **A Simplified Explanation of Allergic Conditions**

Asthma and allergic conditions are clasified in two ways according to how they are triggered. One type is triggered Intrinsically (from inside the body); the other is triggered Extrinsically (from outside the body). Both types of allergic condition trigger the same underlying reaction which results in inflammation of mast cell containing tissues.

### **Extrinsic Triggers: Toxins and Xenobiotics**

Nutrients are taken in from the environment, lifestyle and diet where upon they are broken down by the digestive system before being assembled into the bone, tissue, and chemical messengers which the body needs. A healthy body maintains and regulates all of its tissues whilst protecting them from any toxic elements.

Toxins and xenobiotics enter the body through eating, drinking and breathing, and because these toxins do not naturally have a place in the metabolism of the body, they must be chemically inactivated (detoxified) and then eliminated from the body.

To protect itself, the body utilizes certain substances (glucuronic acid) which react with the toxins and xenobiotics allowing them to be excreted. If these substances are not chemically inactivated and removed then they accumulate and interfere with the bodys natural chemical metabolism acting as irritants and poisons.

When there is a lack of available glucuronic acid the body uses the immune system to cause inflammation which in turn releases enough glucuronic acid from storage cells (mast cells) so to conjugate with and excrete the toxins.

Certain toxins trigger allergic reactions because glucuronic acid is also required for the construction of the active enzyme lipoprotein lipase. This enzyme regulates the lipid hormones known as prostaglandins which control unconscious bodily functions such as the lungs expanding. In this scenario, toxins and xenobiotics compete with prostaglandins for chemical inactivation.

### **Intrinsic Triggers: Hormones and Enzymes**

Prostaglandins are lipid hormones which stimulate unconscious functions in the body like the lungs expanding, the skin sweating, the bowel moving and the heart beating.

In the normal metabolism of the prostaglandins, these hormones require to be quickly chemically deactivated once they have served their purpose. If they are not, the functions which they stimulate do not work as they should and become overstimulated and unco-ordinated.

Examples of this breakdown in regulation are when the lungs do not deflate properly after expansion (bronchial asthma) or when the skin does not stop itching after being scratched (eczema/psoriasis).

When the body lacks the regulating enzyme (lipoprotein lipase) the body is forced to compensate; it does this by quickly requisiting resources in the body through causing inflammation with the immune system. As a result cells are damaged but the required component building blocks for the enzyme are made available and the functional deficit is balanced.

The regulating enzyme which is involved in regulating the prostaglandin hormones involved in allergic conditions is called lipoprotein lipase. It is a compound of glucuronic acid and glucosamine derived from its parent compound heparin which is stored in the mast cells.

Asthma, eczema, arthritis and Crohn's disease are all examples of the same biological compensation manifesting in different mast cell containing mucosal tissues of the body. This inflammatory compensation is specifically to acquire glucuronic acid compounds for either:

A: Production of lipoprotein lipase thus to regulate prostaglandins

B: Elimination of a toxin or xenobiotic from the body

# Paper 1: Foreword

A simple explanation of allergic conditions is inflammation. Allergic conditions are manifestations of the same type of inflammation occurring in different mucosal tissues of the body. The hypothesis here being presented is that the inflammation arises as a direct result of a deficit of **glucuronic acid [1.5]** and glucosamine which one-at-the-same-time are required to control prostaglandin hormones via the enzyme lipoprotein lipase.

**Paper Number: 1**

**Reference Number: 1.5**

**Information taken from:**

Glucuronidation of oxidized fatty acids and prostaglandins B1 and E2 by human hepatic and recombinant UDP glucuronosyltransferases  
 Joanna M. Little,\* Mika Kurkela, † Julia Sonka,\* Sirkku Jäntti, † Raimo Ketola, † Stacie Bratton,\* Moshe Finel, † and Anna Radomska-Pandya 1,  
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Copyright © 2004 by the American Society for  
 Biochemistry and Molecular Biology, Inc.  
 1694 Journal of Lipid Research Volume 45, 2004  
 Page 1696 Glucuronidation of lipid substrates

## **Glucuronidation of lipid substrates by human recombinant UGTs**

Human hepatic and intestinal microsomes from a single donor, recombinant UGT isoforms from the 1A family expressed in Sf9 cells as His tag proteins, and UGT2B7 expressed in HK293 cells were screened for their ability to glucuronidate AA, 15-HETE, 20-HETE, PGE<sub>2</sub>, and PGB<sub>1</sub>; the results are summarized in Table 1 [1].

Glucuronidation assays using UGTs from the 1A family identified UGT1A1, 1A3, 1A4, 1A9, and 1A10 as being active in the glucuronidation of both AA and 15-HETE [2]. AA, which is glucuronidated on the carboxyl function, was the best substrate for all recombinant UGTs [3]. Interestingly, 20-HETE was not accepted as a substrate by UGT1A9 or 1A10 under the experimental conditions used.

Both PGB<sub>1</sub> and PGE<sub>2</sub> were glucuronidated by all recombinant UGTs under investigation, with the exception of UGT1A4 [4]. PGB<sub>1</sub>, which contains only one hydroxyl group, as opposed to PGE<sub>2</sub>, which contains two hydroxyl groups, one on the cyclic ring and one on the side chain, was a much better substrate for all recombinant UGTs [5]. UGT1A3 was able to glucuronidate both HETE derivatives; however, 20-HETE was the better substrate by a factor of almost 2 [6].

Abbreviations: AA, arachidonic acid; GlcUA, glucuronic acid; HETE, hydroxyeicosatetraenoic acid; HI, human intestine; HL, human liver; 13-HODE, 13-hydroxyoctadecadienoic acid; LA, linoleic acid; LCMS, liquid chromatography-mass spectrometry; OFA, oxidized fatty acid; 13-OXO, 13-oxooctadecadienoic acid; PG, prostaglandin; UDPGlcUA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase; UGTs, UDP-glucuronosyltransferases

**Notes:**

Human cells were screened for their ability to glucuronidate Arachidonic Acid (AA), 15-Hydroxyeicosatetraenoic acid (15-HETE), 20-Hydroxyeicosatetraenoic acid (20-HETE), Prostaglandin 2 (PG2) and Prostaglandin B1 (PGB1) [1].

Glucuronidation assays using UDP-glucuronosyltransferases (UGTs) from the 1A family identified UDP-glucuronosyltransferase 1A1, 1A3, 1A4, 1A9 and 1A10 as being active in the glucuronication of both Arachidonic Acid (AA) and 15-Hydroxyeicosatetraenoic acid [2].

Arachidonic acid, which is glucuronidated on the carboxyl function, was the best substrate for all UDP-glucuronosyltransferases [3]. Both Prostaglandin B1 (PGB1) and Prostaglandin E2 (PGE2) were glucuronidated by all UDP-glucuronosyltransferases under investigation except UDP-glucuronosyltransferase 1A4 [4].

Prostaglandin B1 (PGB1) was a better substrate for all UDP-glucuronosyltransferases [5]. UDP-glucuronosyltransferase 1A3 was able to glucuronidate both 15- and 20-Hydroxyeicosatetraenoic acid, however 20-Hydroxyeicosatetraenoic acid was a better substrate by a factor of nearly 2 [6].

# Paper 1: Foreword

A simple explanation of allergic conditions is inflammation. Allergic conditions are manifestations of the same type of inflammation occurring in different mucosal tissues of the body. The hypothesis here being presented is that the inflammation arises as a direct result of a deficit of glucuronic acid and **glucosamine [1.6]** which one-at-the-same-time are required to control prostaglandin hormones via the enzyme lipoprotein lipase.

**Paper Number: 1**  
**Reference Number: 1.6**

**Information taken from:**

Journal of Lipid Research Volume 37,1996 p.693  
 Lipoprotein lipase and lipolysis: central roles in  
 lipoprotein metabolism and atherogenesis  
 Ira J. Goldberg  
 Department of Medicine, Columbia University  
 College of Physicians and Surgeons, 630 West 168th  
 Street, New York, NY 10032  
 Page 696; LPL hydrolysis of chylomicrons

## **LPL Hydrolysis of Chylomicrons**

The observations that heparin released LPL into the bloodstream and that LPL binding to endothelial cells was markedly decreased by HSPGdegrading enzymes [1] suggested that LPL is associated with HSPG [2]. HSPG are members of the family of proteoglycans, negatively charged polysaccharides that are components of cell membranes and the extracellular matrix, and are important in cell adhesion and growth.

The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG-carbohydrate chains) and the core proteins [4]. The major classes of sulfated GAG, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and keratan sulfate, differ in their component sugars [5]. HS is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine [6].

The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions [7]. This leads to a highly variable structure that depends on tissue of origin, molecular environment, and cell growth state [8]. Heparin differs from HS in extent of N-acetylation, N- and O-sulfation, and content of iduronate [9].

Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; HSPG, heparan sulfate proteoglycans; CS, chondroitin sulfate; DS, dermatin sulfate; GAG, glycosaminoglycans; GPI, glycosylphosphatidylinositol; PIPLC, phosphoinositol specific phospholipase C; LRP, LDL receptor-related protein; HTGL, hepatic triglyceride lipase; CAD, coronary artery disease; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein.

**Notes:**

It has been observed that heparin releases Lipoprotein Lipase (LPL) into the bloodstream and that lipoprotein lipase binding to endothelial cells is decreased by Heparin Sulfate Proteoglycan degrading enzymes [1]. This has led to the suggestion that lipoprotein lipase is associated with heparin sulfate proteoglycans [2].

Heparin sulfate proteoglycans are members of the proteoglycan family. These compounds are negatively charged polysaccharides which are components of cell membranes and the extracellular matrix. They play roles in cell adhesion and growth [3].

The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG) and the core proteins [4]. The major classes of sulfated glycosaminoglycans differ in their component sugars [5].

Heparin sulfate is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine [6]. The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions [7].

This leads to a highly variable structure that depends on the tissue of origin, molecular environment and the cell growth state [8]. Heparin differs from heparin sulfate in its extent of N-acetylation, N- and O-sulfation, and content of iduronate [9].

# Paper 1: Foreword

In **summary [1.7]**, glucuronic acid is drawn on as a resource for the elimination of toxins and xenobiotics from the body thus infringing on the production of lipoprotein lipase, hence resulting in a disorder of the prostaglandin metabolism triggered by various extrinsic toxins.

**Paper Number: 1**  
**Reference Number: 1.7**

**Information taken from:**

Aggregate notes of paper 1

## **-: Paper One - Foreword :-**

- **Glucuronic acid is a uronic acid derived from glucose by oxidation -**
- **Glucosamine is one of the most abundant natural monosaccharides -**
- **Glucuronides are the most endogenous conjugation agents found in the body -**
- **Asthma and atopy are here hypothesized as a deficit of lipoprotein lipase -**
- **Prostaglandins are lipid hormones which are metabolized via gluconidation -**
- **Heparin releases lipoprotein lipase and made of glucuronic acid and glucosamine -**

Glucuronic acid is a uronic acid derived from glucose by oxidation. There are two enantiomers; D-glucuronic acid is widely distributed in plants and animals.

Glucosamine is the name for the aminodeoxysugar 2-amino-2-deoxyglucopyranose. There are two enantiomers; D-glucosamine occurs in combined form in chitin, mucoproteins, and in mucopolysaccharides. It is one of the most abundant natural monosaccharides.

Phase II reactions are also known as conjugation reactions because they involve the joining of a substrate with unmodified xenobiotic compounds, xenobiotic compounds which have undergone phase I reactions, and compounds that are not xenobiotic species. The substance which binds to these species is referred to as an endogenous conjugating agent.

The xenobiotic compound contains functional groups often added via a phase I reaction which act as 'chemical handles' for the attachment of a conjugating agent. The conjugation product is usually less lipid soluble, more water soluble, less toxic, and more easily eliminated than the parent compound.

Conjugation agents attached as part of phase II reactions include glucuronide, sulfate, acetyl, methyl, glutathione and some amino acids. Phase II conjugation reactions are usually rapid and if performed on phase I reaction products, the rates of the latter are rate limiting for the overall process.

Glucuronides are the most common endogenous conjugating agents in the body. They react with xenobiotics via uridine diphosphate glucuronic acid (UDPGA). This transfer is mediated by glucuronyl transferase enzymes. Glucuronyl transferase enzymes occur in the endoplasmic reticulum where hydroxylated phase I metabolites of lipophilic xenobiotics are produced.

Glucuronide conjugation products can be classified according to the element to which the glucuronide is bound. The atoms to which the glucuronide most readily attaches are electron rich, usually oxygen, nitrogen or sulfur. When the functional group through which a glucuronide conjugation occurs is a hydroxyl group (-OH), an ether glucuronide is formed. A carboxylic acid group gives rise to an ester glucuronide. Glucuronides may be formed via attachment directly to a nitrogen as the linking atom, as is found with aniline glucuronide.

Glucuronides may also form attachments through an intermediate oxygen atom. N-hydroxyacetylaminoglucuronide is an example of this linkage which is of note as it is more toxic than its parent compound, N-hydroxyacetylaminofluorene. This is of special interest as a decrease in toxicity usually results from glucuronide conjugation.

The carboxylic acid group in glucuronides is normally ionized at the pH of physiological media which is a major reason for the water solubility of the conjugates. When the conjugated compound (also called the aglycone) is of relatively low molecular mass, the conjugate tends to be eliminated through the urine. With heavier conjugated compounds, elimination occurs through the bile.

Some glucuronide conjugates enter a recycling process in which the glucuronide conjugate released into the intestine with bile becomes deconjugated and reabsorbed. This has been known to amplify the metabolic effects of some glucuronide conjugates.

Asthma and allergic conditions are classified in two ways according to how they are triggered. One type is triggered Intrinsically (from inside the body); the other is triggered Extrinsically (from outside the body). Both types of allergic condition trigger the same underlying reaction which results in inflammation of mast cell containing tissues.

Asthma, eczema, arthritis and Crohn's disease are all examples of the same biological compensation manifesting in different mast cell containing mucosal tissues of the body. This inflammatory compensation is specifically to acquire glucuronic acid and glucosamine for either:

A: Production of lipoprotein lipase thus to regulate prostaglandins

B: Elimination of a toxin or xenobiotic from the body

Human cells were screened for their ability to glucuronidate Arachidonic Acid (AA), 15-Hydroxyeicosatetraenoic acid (15-HETE), 20-Hydroxyeicosatetraenoic acid (20-HETE), Prostaglandin 2 (PG2) and Prostaglandin B1 (PGB1).

Glucuronidation assays using UDP-glucuronosyltransferases (UGTs) from the 1A family identified UDP-glucuronosyltransferase 1A1, 1A3, 1A4, 1A9 and 1A10 as being active in the glucuronication of both Arachidonic Acid (AA) and 15-Hydroxyeicosatetraenoic acid.

Arachidonic acid, which is glucuronidated on the carboxyl function, was the best substrate for all UDP-glucuronosyltransferases. Both Prostaglandin B1 (PGB1) and Prostaglandin E2 (PGE2) were glucuronidated by all UDP-glucuronosyltransferases under investigation except UDP-glucuronosyltransferase 1A4.

Prostaglandin B1 (PGB1) was a better substrate for all UDP-glucuronosyltransferases. UDP-glucuronosyltransferase 1A3 was able to glucuronidate both 15- and 20-Hydroxyeicosatetraenoic acid, however 20-Hydroxyeicosatetraenoic acid was a better substrate by a factor of nearly 2.

It has been observed that heparin releases Lipoprotein Lipase (LPL) into the bloodstream and that lipoprotein lipase binding to endothelial cells is decreased by Heparin Sulfate Proteoglycan degrading enzymes. This has led to the suggestion that lipoprotein lipase is associated with heparin sulfate proteoglycans.

Heparin sulfate proteoglycans are members of the proteoglycan family. These compounds are negatively charged polysaccharides which are components of cell membranes and the extracellular matrix. They play roles in cell adhesion and growth.

The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG) and the core proteins. The major classes of sulfated glycosaminoglycans differ in their component sugars.

Heparin sulfate is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions.

This leads to a highly variable structure that depends on the tissue of origin, molecular environment and the cell growth state. Heparin differs from heparin sulfate in its extent of N-acetylation, N- and O-sulfation, and content of iduronate.

# Bronchial Asthma and the Atopic Syndrome

## Paper 2: Asthma and Atopy

Asthma [2.1] is considered an idiopathic disease which increasingly affects more and more people around the world. The asthmatic experiences varying degrees of difficulty in breathing during episodes referred to as asthma attacks. When an attack is severe it is technically referred to as Status Asthmaticus [2.2].

Asthma is a syndrome which is part of a more general term - Atopy [2.3]; this term must be defined before asthma gains its context. Atopic conditions are conditions in which the subject experiences a hypersensitive reaction known as an Allergy [2.4].

Allergies are symptoms of the response to stimulæ known as Allergens [2.5]; these are substances that cause a hypersensitive reaction. There are many different allergens which trigger an atopic response. Environmental and psychological factors can also trigger the hypersensitive reaction

Other conditions which are manifestations of Atopy are Hay Fever [2.6], Eczema [2.7], Dermatitis [2.8], Gastroenteritis [2.9], Diarrhoea [2.10], and Crohn's Disease [2.11]. All these atopic conditions have in common the biochemical events known as Anaphylaxis [2.12].

A summary definition [2.13] is given before the cells and tissues which are affected in asthma and atopy are defined.

## Paper 2: Asthma and Atopy

**Asthma** [2.1] is considered an idiopathic disease which increasingly affects more and more people around the world. The asthmatic experiences varying degrees of difficulty in breathing during episodes referred to as asthma attacks. When an attack is severe it is technically referred to as Status Asthmaticus.

**Paper Number: 2**  
**Reference Number: 2.1**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of asthma  
Page 53; asthma

### **Asthma n.**

The condition of subjects with widespread narrowing of the bronchial airways, which changes in severity over short periods of time (either spontaneously or under treatment) and leads to coughing, wheezing and difficulty in breathing [1]. Bronchial asthma may be precipitated by exposure to one or more of a wide range of stimuli, including allergens, drugs (such as aspirin and other Non Steroidal Anti-Inflammatory Drugs and beta blockers), exertion, emotion, infections, and air pollution [2].

The onset of asthma is usually early in life and in Atopic subjects (see Atopy) may be accompanied by other manifestations of hypersensitivity, such as hay fever and dermatitis; however, the onset may be delayed into adulthood or even middle or old age [3].

Treatment is with bronchodilators, with or without corticosteroids, usually administered via aerosol or dry powder inhalers, or if the condition is more severe, via nebuliser. Oral corticosteroids are reserved for patients who fail to respond adequately to these measures. Severe asthmatic attacks may need large doses of oral corticosteroids (see status asthmaticus) [4].

Avoidance of known allergens, especially the house dust mite, allergens arising from domestic pets, and food additives, will help reduce the frequency of attacks, as will the discouragement of smoking [5]. Cardiac asthma occurs in left ventricular heart failure and must be distinguished from bronchial asthma, as it is quite different. Adjective - asthmatic [6].

**Notes:**

In bronchial asthma there is a breakdown in the regular functioning of the lung manifest as narrowing of the bronchial airways [1]. An attack is triggered when contact is made with specific substances (such as aspirin and other non-steroidal anti-inflammatory drugs, and beta blockers), exertion, emotion, infections and air pollution [2].

Asthma can arise at any time of life. Asthma may be accompanied by various manifestations of hypersensitivity such as hay fever and dermatitis [3]. It is generally treated with bronchodilators, sometimes with corticosteroids [4].

Avoidance of allergens reduces the frequency of attacks. Allergens include house dust mite, allergens deriving from domestic pets and food additives [5]. It should be noted that bronchial asthma is different from cardiac asthma [6].

## Paper 2: Asthma and Atopy

Asthma is considered an idiopathic disease which increasingly affects more and more people around the world. The asthmatic experiences varying degrees of difficulty in breathing during episodes referred to as asthma attacks. When an attack is severe it is technically referred to as **Status Asthmaticus** [2.2].

**Paper Number: 2**  
**Reference Number: 2.2**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of status asthmaticus  
Page 626; status asthmaticus

### **Status Asthmaticus**

A severe attack of asthma, which often follows a period of poorly controlled asthma [1]. Patients are distressed and very breathless and may die from respiratory failure if not vigorously treated with inhaled oxygen, nebulized or intravenous bronchodilators, and corticosteroid therapy [2]; Sedatives are absolutely contraindicated. These patients need hospital care in an intensive care unit [3].

### **Notes:**

A severe asthma attack generally progresses after a time of poor control [1]. and in an acute case, complete functional breakdown of the lungs can lead to death if not acutely treated with oxygen, nebulized or intravenous bronchodilators and corticosteroid therapy [2]. Sedatives should on no account be used [3].

## Paper 2: Asthma and Atopy

Asthma is a syndrome which is part of a more general term - **Atopy** [2.3]; this term must be defined before asthma gains its context. Atopic conditions are conditions in which the subject experiences a hypersensitive reaction known as an Allergy.

**Paper Number: 2**  
**Reference Number: 2.3**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of atopy  
Page 55; atopy

### **Atopy n.**

A form of allergy in which there is a hereditary or constitutional tendency to develop hypersensitivity reactions (e.g. hay fever, allergic asthma, atopic eczema) in response to allergens (atopens). Adjective - Atopic [1].

### **Notes:**

Asthma, eczema and hayfever are all different forms of the allergic reaction which occur in hypersensitive people. Collectively these conditions are termed as atopy, or atopic conditions [1].

## Paper 2: Asthma and Atopy

Asthma is a syndrome which is part of a more general term - Atopy; this term must be defined before asthma gains its context. Atopic conditions are conditions in which the subject experiences a hypersensitive reaction known as an **Allergy** [2.4].

**Paper Number: 2**  
**Reference Number: 2.4**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of allergy  
Page 19; allergy

### **Allergy n.**

A disorder in which the body becomes hypersensitive to particular antigens (called allergens), which provoke characteristic symptoms whenever they are subsequently inhaled, ingested, injected or otherwise contacted [1].

Normally antibodies in the bloodstream destroy specific antigens without further trouble. In an allergic person, however, the reaction of allergen with tissue bound antibody (reagin) also leads, as a side effect, to cell damage, release of histamine and serotonin (5-Hydroxytryptamine), inflammation, and all the symptoms of the particular allergy [2].

Different allergies afflict different tissues and may have either local or general effects, varying from asthma and hay fever to severe dermatitis and gastroenteritis or extremely serious shock (see anaphylaxis), Adjective- allergic [3].

### **Notes:**

An allergy is a hypersensitive reaction the body takes to a particular substance [1]. Normally a healthy body does not react to these substances, however an atopic individuals body does react by causing a form of inflammation [2]. Different allergies cause inflammation in different tissues [3].

## Paper 2: Asthma and Atopy

Allergies are symptoms of the response to stimulæ known as **Allergens [2.5]**; these are substances that cause a hypersensitive reaction. There are many different allergens which trigger an atopic response. Environmental and psychological factors can also trigger the hypersensitive reaction

**Paper Number: 2**  
**Reference Number: 2.5**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of allergen  
Page 19; allergen

### **Allergen n.**

Any antigen that causes allergy in a hypersensitive person. Allergens are diverse and affect different tissues and organs [1]. Pollens, fur, feathers, mould, and dust may cause hay fever; house mites have been implicated in some forms of asthma;

drugs, dyes, cosmetics, and a host of other chemicals can cause rashes and dermatitis; some food allergies may cause diarrhoea or constipation or simulate acute bacterial food poisoning [2].

When a patient's allergen has been identified (see patch test), it may be possible to alleviate or prevent allergic attacks. Adjective - allergenic [3].

### **Notes:**

An allergen describes any substance that triggers an allergic reaction in a hypersensitive person. Allergens may affect different tissues and organs [1]. Pollens, fur, feathers, mould, dust, house mites, drugs, dyes, cosmetics, food allergies and other chemicals are amongst known allergens [2]. Allergies to substances can be identified by use of a patch test [3].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are **Hay Fever [2.6]**, Eczema, Dermatitis, Gastroenteritis, Diarrhoea, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.6**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of hay fever  
Page 295; hay fever

### **Hay Fever**

A form of allergy due to the pollen of grasses, trees, and other plants, characterised by inflammation of the membrane lining the nose and sometimes of the conjunctiva (vernal conjunctivitis) [1].

The symptoms of sneezing, running or blocked nose, and watering eyes are due to histamine release and often respond to treatment with antihistamines [2]. If the allergen is identified, it may be possible to undertake desensitisation. Medical name: allergic rhinitis.

### **Notes:**

Hay fever is the allergy which manifests as inflammation of the membrane of the nose and sometimes of the conjunctiva [1].

Hay fever often responds to treatment with antihistamines [2].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, **Eczema** [2.7], Dermatitis, Gastroenteritis, Diarrhoea, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.7**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of eczema  
Page 206; eczema

### **Eczema n.**

A common itchy skin disease characterized by reddening (erythema) and vesicle formation, which may lead to weeping and crusting [1].

It is endogenous or constitutional, i.e. outside agents do not play a primary role (compare dermatitis), but in some contexts, the terms 'dermatitis' and 'eczema' are used interchangeably [2]. There are five main types:

(1) Atopic eczema, which affects up to 20% of the population and is associated with asthma and hay fever [3]; (2) Seborrhoeic eczema (or dermatitis), involves the scalp, eyelids, nose, and lips and is associated with the presence of pityrosporum yeasts; (3) Discoid (or nummular) eczema, which is characterized by coin-shaped lesions and occurs only in adults; (4) Pompholyx, affecting the hands and feet; (5) Gravitational (or stasis) eczema, associated with poor venous circulation and incorrectly known as varicose eczema.

Treatment of eczema is with topic or systemic corticosteroids but emollients are very important, especially in treating mild cases. Adjective - eczematous [4].

### **Notes:**

Eczema is an itchy skin disease characterized by reddening and vesicle formation which may lead to weeping and crusting [1]. It is an endogenous condition [2]. Of all eczema, an estimated 20% is atopic and is associated with asthma and hay fever [3]. Treatment is topic or with corticosteroids [4].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, Eczema, **Dermatitis [2.8]**, Gastroenteritis, Diarrhoea, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.8**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of dermatitis  
Page 177; dermatitis

### **Dermatitis n.**

An inflammatory condition of the skin caused by outside agents (compare eczema, an endogenous disease in which such agents do not play a primary role) [1]. Primary irritant dermatitis may occur in anyone who has sufficient contact with such irritants as acids, alkalis, solvents, and (especially) detergents. It is the commonest cause of occupational dermatitis in hairdressers, nurses, cooks, etc. (see also napkin rash).

In allergic contact dermatitis, skin changes resembling those of eczema develop as a delayed reaction with a particular allergen, which may be present at low concentrations. The commonest example in women is nickel dermatitis from jewellery, jeans studs, etc.; in men chromium dermatitis is relatively common. Treatment of dermatitis depends upon the cause [2].

Dermatitis herpetiformis is an uncommon very itchy rash with symmetrical blistering, especially on the knees, elbows, buttocks, and shoulders. It is associated with gluten sensitivity and responds well to treatment with dapsone.

### **Notes:**

Dermatitis is extrinsically triggered inflammation of the skin. Strongly associated with eczema [1], the skin changes in dermatitis resemble those seen in eczema [2].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, Eczema, Dermatitis, **Gastroenteritis [2.9]**, Diarrhoea, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.9**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of gastroenteritis  
Page 266; gastroenteritis

### **Gastroenteritis n.**

Inflammation of the stomach and intestine. It is usually due to acute infection by viruses or bacteria or to food-poisoning toxins and causes vomiting and diarrhoea. The illness usually last 3 - 5 days. Fluid loss is sometimes severe especially in infants, and intravenous fluid replacement may be necessary [1].

### **Notes:**

Gastroenteritis is an example of the allergic reaction occurring in the stomach and intestine. The inflammation is usually triggered by infection or to toxins. The result is diarrhoea and vomiting [1].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, Eczema, Dermatitis, Gastroenteritis, **Diarrhoea [2.10]**, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.10**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of diarrhoea  
Page 182; diarrhoea

Diarrhoea n.

Frequent bowel evacuation or the passage of abnormally soft or liquid faeces. It may be caused by intestinal infections, other forms of intestinal inflammation (such as colitis or Crohn's disease), malabsorption, anxiety, and the irritable bowel syndrome. Severe or prolonged diarrhoea may lead to excess losses of fluid, salts, and nutrients in the faeces [1].

### Notes:

Diarrhoea as a result of intestinal inflammation leads to loss of fluid, salts and malabsorption of nutrients. Diarrhoea is a factor in Crohn's disease and inflammation of the colon (colitis) [1].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, Eczema, Dermatitis, Gastroenteritis, Diarrhoea, and **Crohn's Disease [2.11]**. All these atopic conditions have in common the biochemical events known as Anaphylaxis.

**Paper Number: 2**  
**Reference Number: 2.11**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of Crohn's disease  
Page 157; Crohn's disease

### **Crohn's Disease**

A condition in which segments of the alimentary tract become inflamed, thickened, and ulcerated. It usually affects the terminal part of the ileum; its acute form (acute ileitis) may mimic appendicitis. Chronic disease often causes partial obstruction of the intestine, leading to pain, diarrhoea, and malabsorption [1].

Fistulae around the anus, between adjacent loops of intestine, or from intestine to skin, bladder, etc., are characteristic complications. The cause is unknown. Treatment includes rest, corticosteroids, immunosuppressive drugs, antibiotics, dietary modification, or (in some cases) surgical removal of the affected part of the intestine. Alternative names: Regional enteritis, regional ileitis [2].

### **Notes:**

Crohn's disease is the term used to describe inflammation of various parts of the digestive tract [1]. Treatments include corticosteroids, immunosuppressive drugs, antibiotics and dietary modification [2].

## Paper 2: Asthma and Atopy

Other conditions which are manifestations of Atopy are Hay Fever, Eczema, Dermatitis, Gastroenteritis, Diarrhoea, and Crohn's Disease. All these atopic conditions have in common the biochemical events known as **Anaphylaxis [2.12]**.

**Paper Number: 2**  
**Reference Number: 2.12**

**Information taken from:**  
Oxford Reference Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of anaphylaxis  
Page 30; anaphylaxis

### **Anaphylaxis n.**

An abnormal reaction to a particular antigen, in which histamine is released from tissues and causes either local or widespread symptoms. An allergic attack (see allergy) is an example of localized anaphylaxis [1].

Rarer, but much more serious is anaphylactic shock; an extreme and generalized allergic reaction in which widespread release of histamine causes swelling (oedema), constriction of the bronchioles, heart failure, circulatory collapse, and sometimes death. Adjective - anaphylactic

### **Notes:**

Anaphylaxis is an abnormal reaction to a particular antigen. An allergic attack (allergy) is an example of localized anaphylaxis [1]. Anaphylactic shock is the term used for a generalized allergic reaction which strongly affects various tissues and systems of the body. During anaphylactic shock there is constriction of the bronchioles, heart failure, circulatory collapse, and sometimes death [2].

## Paper 2: Asthma and Atopy

A summary **definition** [2.13] is given before the cells and tissues which are affected in asthma and atopy are defined.

**Paper Number: 2**

**Reference: 2.13**

**Information taken from:**

Aggregate notes of paper 2

**-: Paper Two - Asthma and Atopy :-**

- **Bronchial asthma can be triggered by allergens -**
- **Atopic conditions are allergic conditions -**
- **Allergic conditions are manifestations of the anaphylactic reaction -**
- **Allergic responses are triggered by allergens -**
- **Allergens are physical, psychological and environmental stimuli -**
- **All allergies result in forms of inflammation -**

In bronchial asthma there is a breakdown in the regular functioning of the lung manifest as narrowing of the bronchial airways. An attack is triggered when contact is made with specific substances (such as aspirin and other non-steroidal anti-inflammatory drugs, and beta blockers), exertion, emotion, infections and air pollution.

Asthma can arise at any time of life. Asthma may be accompanied by various manifestations of hypersensitivity such as hay fever and dermatitis. It is generally treated with bronchodilators, sometimes with corticosteroids.

Avoidance of allergens reduces the frequency of attacks. Allergens include house dust mite, allergens deriving from domestic pets and food additives. It should be noted that bronchial asthma is different from cardiac asthma.

A severe asthma attack generally progresses after a time of poor control. and in an acute case, complete functional breakdown of the lungs can lead to death if not acutely treated with oxygen, nebulized or intravenous bronchodilators and corticosteroid therapy. Sedatives should on no account be used.

Asthma, eczema and hayfever are all different forms of the allergic reaction which occur in hypersensitive people. Collectively these conditions are termed as atopy, or atopic conditions.

An allergy is a hypersensitive reaction the body takes to a particular substance. Normally a healthy body does not react to these substances, however an atopic individuals body does react by causing a form of inflammation. Different allergies cause inflammation in different tissues.

An allergen describes any substance that triggers an allergic reaction in a hypersensitive person. Allergens may affect different tissues and organs. Pollens, fur, feathers, mould, dust, house mites, drugs, dyes, cosmetics, food allergies and other chemicals are amongst known allergens. Allergies to substances can be identified by use of a patch test.

Hay fever is the allergy which manifests as inflammation of the membrane of the nose and sometimes of the conjunctiva. Hay fever often responds to treatment with antihistamines.

Eczema is an itchy skin disease characterized by reddening and vesicle formation which may lead to weeping and crusting. It is an endogenous condition. Of all eczema, an estimated 20% is atopic and is associated with asthma and hay fever. Treatment is topic or with corticosteroids.

Dermatitis is extrinsically triggered inflammation of the skin. Strongly associated with eczema, the skin changes in dermatitis resemble those seen in eczema.

Gastroenteritis is an example of the allergic reaction occurring in the stomach and intestine. The inflammation is usually triggered by infection or to toxins. The result is diarrhoea and vomiting.

Diarrhoea as a result of intestinal inflammation leads to loss of fluid, salts and malabsorption of nutrients. Diarrhoea is a factor in Crohn's disease and inflammation of the colon (colitis).

Crohn's disease is the term used to describe inflammation of various parts of the digestive tract. Treatments include corticosteroids, immunosuppressive drugs, antibiotics and dietary modification.

Anaphylaxis is an abnormal reaction to a particular antigen. An allergic attack (allergy) is an example of localized anaphylaxis. Anaphylactic shock is the term used for a generalized allergic reaction which strongly affects various tissues and systems of the body. During anaphylactic shock there is constriction of the bronchioles, heart failure, circulatory collapse, and sometimes death.

# Bronchial Asthma and the Atopic Syndrome

## Paper 3: Asthma and Inflammation

The definition of Asthma [3.1] starts with the immune response. Thus the scenario begins :-  
The subject comes into contact with an allergen;

There then occurs an **immunological response to the antigenic substance [3.2]** which involves the function of the **immunoglobulins [3.3]**. Primarily it is immunoglobulin E which is involved in atopy, having the effect of sponsoring anaphylaxis in the cells and tissues.

The body synthesises immunoglobulin E and releases it into the circulation where it binds to cell surfaces, especially to **mast cells [3.4]** and circulating basophils (also called circulating mast cells).

When an allergen come into proximity with the cell cultures it combines with the cell-bound immunoglobulin E. The result of this is anaphylaxis (either localized or general) which consequently causes inflammation. The compounds and substances which are released and activated during inflammation have the clinical definition of **anaphylaxis [3.5]**.

The **summation [3.6]** of events is that the immune system causes the mast cells to release their contents into the surrounding tissues.

# Paper 3: Asthma and Inflammation

The definition of Asthma [3.1] starts with the immune response. Thus the scenario begins :-  
The subject comes into contact with an allergen;

**Paper Number: 3**  
**Reference Number: 3.1**

**Information taken from:**  
The Nutrition and Health Dictionary  
Copyright 1995  
Chapman & Hall  
Definition of asthma  
Page 33; asthma

## Asthma

A respiratory problem whose chief feature is laboured or difficult breathing. Often accompanied by characteristic wheezing or whistling sounds. The whistling or wheezing sounds of asthma are loudest when breathing air out.

The symptoms of asthma are caused by changes in the respiratory system, the system of passageways that carries air from the mouth into the lungs. In asthma, the small air passageways go into spasms, narrowing the width of the tubes and thus making the passage of air in and out of the lungs more difficult [1].

In addition to muscle spasm, there is an outpouring of mucus into the small air passages, further obstructing the flow of air [2]. Finally, the mucosa become inflamed and swollen, thus narrowing the air passageways even further, much as an accumulation of rust on the inner surface of a pipe would partially obstruct the flow of water through a pipe [3].

All these changes, the spasms, the swelling, and the out pouring of mucus, occur together, affecting to a greater or lesser degree almost all of the air passages. The resistance to the flow of air, particularly during expiration, or breathing out, is significantly increased, and the individual must work harder to move air in and out of the lungs, which results in a whistling sound. Asthma is considered to be the result of an allergy [4].

## Notes:

In asthma the muscles of the air passageways go into spasm [1], there occurs an exodus of mucus into the lungs [2], the mucosa become inflamed and swollen [3], and the resistance of air flow is significantly increased particularly whilst breathing out. Asthma is considered to be the result of an allergy [4].

# Paper 3: Asthma and Inflammation

There then occurs an **immunological response to the antigenic substance [3.2]** which involves the function of the immunoglobulins. Primarily it is immunoglobulin E which is involved in atopy, having the effect of sponsoring anaphylaxis in the cells and tissues.

**Paper Number: 3**  
**Reference Number: 3.2**

**Information taken from:**  
Basic Pharmacology  
Basic Principles in Therapeutics  
Second edition 1978  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M. D.  
The Classic Drug Reaction:  
The immunological response to an antigenic substance  
Page 957 - 958

## **The Immunological Response to an Antigenic Substance**

The physician who never uses penicillin on any patient because he has “seen” an anaphylactic reaction that appears to create unnecessary suffering is like the physician who uses it casually because he has “never had” any problems with it.

The antibodies produced by penicillin appear specific for different aspects of the chemistry of the penicillin; i.e. at least three types of immunoglobulin E antibody can be made. One is to the benzylpenicilloyle moiety (BPO), a frequent antigen and so-called “major” determinant; one is to penicilloate, and one is to the other chemicals related to penicillin that are often responsible for antigenicity, i.e., the so called “minor determinants” (Tsuji et al., 1974; Yamana et al., 1975) [1].

Although each of these antibodies can be distinctly different molecules, the antibody-combining site recognizes the whole molecule and can, in part be carrier specific. Thus it is not surprising to find cross reactivity to a variety of the chemicals that have the penicillin structure in common (Adkinson et al., 1971) [2].

Immediate hypersensitivity is clearly caused by the immunoglobulin E (IgE) molecule; there may be some types of immediate reactions that might be mediated by Immunoglobulin G. Some combination of Immunoglobulin M plus other antibodies may account for late hypersensitivity reactions and Immunoglobulin G may contribute to the accelerated response. The skin tests we will discuss usually detect tissue-fixed (skin) Immunoglobulin E and thus have proven useful in screening for those patients who are not likely to have immediate types of reactions to either the major or minor antigenic determinants of the penicillin molecule.

Degradation of the penicillin molecule to a reactive proimmunogen can proceed without enzymatic intervention. This event with penicillin is in marked contrast to most other drugs that must undergo enzymatic degradation (usually in the liver) before they become antigenic or before they conjugate with endogenous protein to form complete antigens [3].

This difference in active versus spontaneous degradation may be the reason that hypersensitivity reactions to penicillin do not express themselves in the liver. That is, INH, for instance, is degraded in the liver when the antigen or reactive metabolic intermediate may express its effects as clinically important hepatitis.

The skin-sensitizing (IgE) antibodies in penicillin allergy are most commonly developed to the penicilloyl metabolite (“major” determinant). IgE is thought to be responsible for anaphylactic reactions, and may also be produced in response to the unaltered drug or to another metabolite (e.g. penicillate, penicillamine (Levine, 1966; Turk and Baker, 1968) [4].

IgM and IgG antibodies may be produced after exposure of patients to penicillin and are usually specific for the penicilloyl metabolite (“major” determinant). The “minor” determinants (unaltered drug or other drug metabolites) may be responsible for disastrous allergic reactions, and the nomenclature (major and minor determinants) is misleading. Better terms might be “frequent” and “infrequent” determinants of antigenicity. There are many possible determinants of antigenicity after administration of penicillin.

Although IgE antibodies may cause immediate anaphylactic reactions, the significance of IgM and IgG antibodies is less clear. IgG antibodies may act as blocking antibodies to prevent or modify the course of reactions in patients who are skin-test-sensitive to antigens but who develop skin rashes rather than anaphylaxis on exposure to the allergen. In some patients, the concentration of IgG falls during the acute allergic reaction and rises during the recovery period.

Hemagglutinating antibodies (IgG) are usually specific for the penicilloyl determinant, but these antibodies may be found in 60 to 100% of populations, if sensitive methods for their detection are used. Although patients with a history of allergy have higher concentrations of IgG than normals, it is not known if these antibodies are responsible for most reactions [5].

In addition, lymphocytes have been “sensitized” to components of the penicillin’s (David, 1973, Gimenez-Camarasa, 1975; Reidenberg and Caccese, 1975). These lymphocytes, on contact with the antigen, release a substance that causes blood monocytes to migrate across endothelial linings of vessels, become converted to macrophages, and destroy tissues by release of lysosomal enzymes [6].

This hypothesis may explain those allergic reactions to penicillin that stimulate serum sicknesses. The reader is referred to Chapter 13 where a detailed discussion of the mechanisms by which humoral or cell mediated immune-generated events is presented.

### **Notes:**

During an allergic immune response the body produces antibodies known as immunoglobulins [1]. These antibodies react with molecules that have a similar chemical structure [2]. Some chemicals need to be changed before being recognized by the body as foreign matter (allergens) [3]. Immunoglobulin E is responsible for the anaphylactic reaction and can also be produced in response to the unaltered drug or to another metabolite [4].

It has been observed that Immunoglobulin G concentrations fall during the allergic reaction and rise after. Immunoglobulin G levels are higher in allergic patients [5]. Lymphocytes are immune cells that on exposure to allergens encourage the production of macrophages which digest tissues [6].

# Paper 3: Asthma and Inflammation

There then occurs an immunological response to the antigenic substance which involves the function of the **immunoglobulins** [3.3]. Primarily it is immunoglobulin E which is involved in atopy, having the effect of sponsoring anaphylaxis in the cells and tissues.

**Paper Number: 3**  
**Reference Number: 3.3**

**Information taken from:**

Joan F. Zilva  
P.R. Pannall  
Clinical Chemistry in Diagnosis and Treatment  
Third edition 1979  
Lloyd - Luke (Medical Books) Ltd  
Function of the Immunoglobulins  
Page 317 - 319 Immunoglobulin E

## **Function Of The Immunoglobulins**

The immune response mechanism of the body consists of a cellular and humoral component. Although we are concerned here only with the humoral component - the immunoglobulins - the student should remember that both are necessary: He should consult a textbook of immunology for details of the cellular mechanism. The specific functions of each class of immunoglobulin will be discussed briefly, and followed by an outline of changes in disease [1].

Immunoglobulin G (MW: 160 000);

IgG accounts for about 75 per cent of circulating immunoglobulins and contains most of the normal plasma antibodies. It is a relatively small molecule, and is present in very low concentration throughout the extracellular compartment: Its most important function is stimulated by soluble antigens such as bacterial toxins.

IgG deficiency is characterised by recurrent pyogenic infections of tissue spaces by toxin-producing organisms such as staphylococci and streptococci: pulmonary and subcutaneous infections are common. IgG can bind complement and cross the placental barrier.

In the first few months of life endogenous IgG levels are very low. Maternal IgG which has been transported across the placenta provides antibody cover during this period. Adult concentrations of this class of protein are not reached before the age of 3 to 5 years [2].

Immunoglobulin E (MW: 200 000);

IgE is synthesised by plasma cells beneath the mucosae of the gastrointestinal and respiratory tracts and by those in the lymphoid tissue of the nasopharynx [3]. It is present in nasal and bronchial secretions.

Circulating IgE is rapidly bound to cell surfaces, particularly to those of mast cells and circulating basophils, and plasma levels are therefore very low [4]. Combination of antigen with this cell-bound antibody results in the cells releasing mediators and accounts for immediate hypersensitivity reactions such as occur in hay fever [5].

Desensitisation therapy of allergic disorders aims at stimulating production of circulating IgG against the offending antigen, to prevent it reaching cell-bound IgE. - Raised concentrations are found in several diseases with an allergic component such as some cases of eczema, asthma and parasitic infestations [6].

### **Notes:**

The total immune response consists of both cellular and humoral components [1]. Immunoglobulin G is stimulated by soluble antigens such as bacterial toxins, deficiency of this antibody is characterised by pus forming (pyogenic) infections [2].

Immunoglobulin E is the antibody made by the plasma cells of the alimentary tract, the respiratory tract and by the lymphoid tissue of the nasopharynx [3]. Present in nasal and bronchial secretions, circulating immunoglobulin E rapidly binds to cell surfaces - especially of mast cells and circulating basophils [4].

Antigen combines with cell-bound Immunoglobulin E - this causes the cells to release their contents and an immediate hypersensitivity reaction (anaphylaxis) ensues [5]. Raised levels of IgE are found in allergic conditions such as asthma, eczema and parasitic infestations [6].

## Paper 3: Asthma and Inflammation

The body synthesises immunoglobulin E and releases it into the circulation where it binds to cell surfaces, especially to **mast cells** [3.4] and circulating basophils (also called circulating mast cells).

**Paper Number: 3**  
**Reference Number: 3.4**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic Principles in Therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Cellular mediators of inflammation  
Page 671 Mast cells

### **Mast Cells**

Mast cells are mainly found in loose connective tissue, frequently near vascular channels. Their most distinguishing features are their membrane-bound, spheroid, basophilic secretory granules, which may be so numerous that other cytoplasmic structures are obscured [1].

These granules contain large amounts of heparin and other mucopolysaccharides, histamine, and several proteolytic and esterolytic enzyme systems. Serotonin is present in the mast cells of some species, man being a notable exception [2].

Much is known regarding the structure and composition of mast cells, but their physiologic function remains unidentified. However it seems certain that the mast cell and the basophil (circulating mast cells) contribute to the development of an inflammatory process (Lewis et al., 1975). Changes in mast cell density and mast cell granules are thought to be characteristic of many inflammatory lesions. Mast cell preparations (either in vitro or in vivo during anaphylaxis) have been used as experimental models of inflammation [3].

The mast cell is readily degranulated (see previous section on histamine), and the granule-bound chemical substances are released into extracellular fluid [4]. Histamine and probably heparin can have important influences on an inflammatory process, but much less is known about the granule esterase's and protease's that are also released from the granules of the mast cell and basophil (Benditt, 1968; Lewis et al., 1975).

These enzymes may alter the course of inflammation by interactions with other cells or substances to release, activate, or destroy kinins, complement, and blood coagulation factors, to alter membranes of other cells such as granulocytes so that additional intracellular constituents are released, or to alter the structure and characteristics of adjacent connective tissue [5].

**Notes:**

Mast cells are mainly found in loose connective tissue often near vascular channels. They are distinguishable by copious amounts of secretory granules on the membrane [1].

The granules contain large amounts of heparin and mucopolysaccharides, histamine and several proteolytic and esterolytic enzyme systems which catalyse the splitting of esters into constituent alcohols and acids, and proteins into smaller peptides and amino acids [2].

Changes in mast cell density and mast cell granules are characteristic of many inflammatory lesions. The mast cells are intimately involved in the inflammatory process and are used as models of inflammation in anaphylaxis [3]. During the inflammatory process the mast cell degranulates (excretes its contents) into the extracellular fluid [4].

Histamine and heparin are both important factors in the inflammatory process. The digestive enzyme systems that mast cells release interact with kinins, complement, blood coagulation factors, polymorphonuclear leukocyte membranes and the structure of connective tissue [5].

## Paper 3: Asthma and Inflammation

When an allergen come into proximity with the cell cultures it combines with the cell-bound immunoglobulin E. The result of this is anaphylaxis (either localized or general) which consequently causes inflammation. The compounds and substances which are released and activated during inflammation have the clinical definition of **anaphylaxis** [3.5].

**Paper Number: 3**  
**Reference Number: 3.5**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic Principles in Therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Clinical definition of anaphylaxis  
Page 689 - 690 Inflammation

### **Anaphylaxis**

Release of mediators of inflammation with systemic effects. In man, the signs and symptoms of anaphylaxis are bronchial muscle contraction, hyperperistalsis, widespread increased capillary permeability and vasodilatation with peripheral vascular collapse due to the release of multiple chemicals during antigen - antibody interaction. Mediators that most likely participate in human disease include histamine, slow reacting substance A, and bradykinin (Kaplan et al., 1971) [1].

Although anaphylaxis shares many common pathogenetic factors with urticaria, life-threatening respiratory involvement and/or severe hypotension and subsequent cardiac abnormalities may occur at any time during the course of anaphylaxis. Major determinants of the severity of the reaction appear to be the nature, rate, and magnitude of the antigen - antibody reaction [2].

### **Notes:**

Anaphylaxis is the release of mediators of inflammation with systemic effects. Signs and symptoms are bronchial muscle contraction, hyperperistalsis, widespread increased capillary permeability and vasodilation with peripheral vascular collapse due to antibody-to-antibody interaction [1].

General anaphylaxis shares many common factors with urticaria (nettle rash) however anaphylaxis is considered life threatening due to its potent effects on respiration, blood pressure and the heart [2].

## Paper 3: Asthma and Inflammation

The **summation** [3.6] of events is that the immune system causes the mast cells to release their contents into the surrounding tissues.

**Paper Number: 3**  
**Reference Number: 3.6**

**Information taken from:**

Aggregate notes of paper 3

### **-: Paper Three - Asthma and Inflammation :-**

- Asthma is an allergic condition -
- Allergic responses are triggered by allergens -
- Antibodies and immune cells are stimulated in response to allergens -
- The body attaches antibody Immunoglobulin E to mast cells -
- When allergens react with Immunoglobulin E the mast cell degranulates -
- Anaphylaxis is the allergic reaction that causes inflammation in mast cells -

In asthma the muscles of the air passageways go into spasm, there occurs an exodus of mucus into the lungs, the mucosa become inflamed and swollen, and the resistance of air flow is significantly increased particularly whilst breathing out. Asthma is considered to be the result of an allergy.

During an allergic immune response the body produces antibodies known as immunoglobulins. These antibodies react with molecules that have a similar chemical structure. Some chemicals need to be changed before being recognized by the body as foreign matter (allergens). Immunoglobulin E is responsible for the anaphylactic reaction and can also be produced in response to an unaltered drug or to another metabolite.

It has been observed that Immunoglobulin G concentrations fall during the allergic reaction and rise after. Immunoglobulin G levels are higher in allergic patients. Lymphocytes are immune cells that on exposure to allergens encourage the production of macrophages which digest tissues.

The total immune response consists of both cellular and humoral components. Immunoglobulin G is stimulated by soluble antigens such as bacterial toxins, deficiency of this antibody is characterised by pus forming (pyogenic) infections.

Immunoglobulin E is the antibody made by the plasma cells of the alimentary tract, the respiratory tract and by the lymphoid tissue of the nasopharynx. Present in nasal and bronchial secretions, circulating immunoglobulin E rapidly binds to cell surfaces - especially of mast cells and circulating basophils.

Antigen combines with cell-bound Immunoglobulin E - this causes the cells to release their contents and an immediate hypersensitivity reaction (anaphylaxis) ensues. Raised levels of IgE are found in allergic conditions such as asthma, eczema and parasitic infestations.

Mast cells are mainly found in loose connective tissue often near vascular channels. They are distinguishable by copious amounts of secretory granules on the membrane.

The granules contain large amounts of heparin and mucopolysaccharides, histamine and several proteolytic and esterolytic enzyme systems which catalyse the splitting of esters into constituent alcohols and acids, and proteins into smaller peptides and amino acids.

Changes in mast cell density and mast cell granules are characteristic of many inflammatory lesions. The mast cells are intimately involved in the inflammatory process and are used as models of inflammation in anaphylaxis. During the inflammatory process the mast cell degranulates (excretes its contents) into the extracellular fluid.

Histamine and heparin are both important factors in the inflammatory process. The digestive enzyme systems that mast cells release interact with kinins, complement, blood coagulation factors, polymorphonuclear leukocyte membranes and the structure of connective tissue.

Anaphylaxis is the release of mediators of inflammation with systemic effects. Signs and symptoms are bronchial muscle contraction, hyperperistalsis, widespread increased capillary permeability and vasodilation with peripheral vascular collapse due to antibody-to-antibody interaction.

General anaphylaxis shares many common factors with urticaria (nettle rash) however anaphylaxis is considered life threatening due to its potent effects on respiration, blood pressure and the heart.

# Bronchial Asthma and the Atopic Syndrome

## Paper 4: Asthma and Inflammation

The clinical definition of asthma [4.1] describes the characteristic coughing and wheezing along with dyspnoea [4.2] (a word which describes the difficulty in breathing) the sufferer experiences. Along with these symptoms there is oedema [4.3] - the accumulation of fluid in the affected tissue.

On a cellular scale there is eosinophilia [4.4], which describes an increase in the number of eosinophils [4.5] - a particular kind of cell of the immune system. There is also involvement from other cells of the immune system which work in combination with each other to create a series of microscopic events in the body.

During an asthmatic episode, progressively the smooth muscle [4.6] exhibits a loss of control (dyspnoea). The smooth muscle of the body carries out unconscious actions under the regulation of the Autonomic Nervous System. The Autonomic Nervous System [4.7] is in command of many things which are not consciously managed; these include the regular beating of the heart, sweating and breathing.

Over time the immune system affects the smooth muscle, respiratory cells and connective tissue [4.8] in adverse physiological ways. Hypertrophication of the smooth muscle and a loss of vitality in the respiratory cells and connective tissue occurs. All of these tissues contain mast cells [4.9].

In synopsis [4.10]: During an asthmatic episode a breakdown in the regular control of the lungs (smooth muscle; autonomic nervous system) results in difficulty of breathing; the mast cells are targeted by the immune system (immunoglobulin E) resulting in the release of their intracellular contents into the body. The result of the immune systems action is ultimately localised inflammation of the lung (anaphylaxis) and its associated tissues.

# Paper 4: Asthma and Inflammation

**The clinical definition of asthma [4.1]** describes the characteristic coughing and wheezing along with dyspnoea (a word which describes the difficulty in breathing) the sufferer experiences. Along with these symptoms there is oedema - the accumulation of fluid in the affected tissue.

**Paper Number: 4**  
**Reference Number: 4.1**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic Principles in therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Clinical definition of asthma  
Page 483 - 484 Asthma

## **Asthma**

Bronchial asthma is characterised by episodic obstruction of airways that is clinically manifested by cough, wheezing, and dyspnoea and is frequently associated with eosinophilia in the blood and sputum [1]. Accurate identification of asthma, therefore, depends on the history, the presence of wheezing during an attack, and the findings in the blood and sputum. The diagnosis is supported by the demonstration of significant reversibility of the obstruction to airflow when aerosolised bronchodilators are used. This is measured as an improvement of 15% or more in the FEV1 [2].

The pathologic findings in the lungs of patients who died during an acute asthmatic episode are well documented. macroscopically, the lungs are over distended, and numerous tenacious mucous plugs are found in the bronchi. Histologic examination shows a dense exudate in the bronchial lumen with a mucous and serous component and many eosinophils and effete columnar respiratory cells (Dunnill, 1971). The bronchial mucous membrane and submucosa are thickened and infiltrated with eosinophils, and the bronchial smooth muscle is hypertrophied (Dunnill et al., 1969) [3].

Asthma may be related to specific allergies ("extrinsic"), if no allergy can be demonstrated, it is considered "intrinsic". Extrinsic asthma most commonly occurs in young persons with a family history of atopy and in persons who develop wheezing after exposure to house dust, pollen, or other common allergens. These patients' allergic diathesis can be verified by demonstrating a positive "type 1" reaction on prick testing of the skin with the suspected allergen (Gell and Coombs, 1968) [4].

Most atopic subjects are sensitive to several allergens. Sensitisation occurs after initial contact with antigen causes the formation of specific IgE antibodies that attack to the surface of mast cells (Ishizaka and Ishizaka, 1970) [5]. Subsequent exposure causes release of various chemical mediators by the mast cells. These include histamine, slow-reacting substance of anaphylaxis, eosinophilic chemotactic factor, bradykinin, 5-hydroxytryptamine, prostaglandin F2 alpha, and several others whose significance is not certain [6].

These mediators act directly on the bronchial wall and initiate reflexes that cause bronchoconstriction, mucosal edema, and hypersecretion [7]. As type 1 reactions occur within minutes of exposure to the allergen, the patients often give a history of almost immediate wheezing on contact with allergen.

Onset of non-atopic or intrinsic asthma commonly occurs later in life (over 35 years of age) [8]. There is no personal or family history of allergy or seasonal variation in the episodes of bronchospasm. The wheezing frequently is severe and perennial and may show marked resistance to bronchodilator therapy. It is in this group that most deaths from asthma occur (Cochrane and Clark, 1975) [9].

Table 10- 5 summarises the contrasting features of extrinsic and intrinsic asthma. Although there is much overlap in the general management of intrinsic and extrinsic asthma, many patients (particularly children) need to have their treatment tailored for their particular disease to give maximum therapeutic benefit with minimum side effects [10].

### **Notes:**

Bronchial asthma is characterised by obstructed airways manifesting coughing, wheezing and dyspnoea. The condition is associated with eosinophils in the blood, saliva and mucus [1]. Diagnosis is decided on a case history of wheezing and the ability to reverse airflow obstruction with bronchodilators [2].

A patient who has died from asthma has over inflated lungs, abnormally sticky mucus and many eosinophils in the mucus. The bronchial mucus membrane and submucosa are inflamed and the bronchial smooth muscle is abnormally enlarged [3]. If the asthma is related to specific allergies it is considered extrinsic, if no allergy can be demonstrated it is considered intrinsic.

Extrinsic asthma is the most common kind found in youths with a family history of allergy and those commonly exposed to allergens. Patients can be confirmed by a positive "type 1" reaction on a skin prick test with possible allergens [4]. Most atopic subjects are sensitive to more than two allergens. After initial contact with antigens the formation of Immunoglobulin E antibodies occurs which then attach themselves to the surface of the mast cells [5].

Subsequent exposure to the allergen causes the release of histamine, slow reacting substance of anaphylaxis, eosinophilic chemotactic factor, bradykinin, serotonin, and prostaglandin F2 alpha [6]. These mediators cause bronchoconstriction, mucosal accumulation of fluid, and hypersecretion. Immediate hypersensitivity reactions happen within minutes of contact with the allergen. Patients often give a history of this [7].

Intrinsic asthma commonly occurs in patients over 35 years of age [8], generally, there is no personal or family history of allergy nor any seasonal variation in the condition. The wheezing is often severe, lasts a long time and may show marked resistance to bronchodilators. It is in the intrinsic group that most deaths occur [9]. There is much overlap in the treatment of intrinsic and extrinsic asthma [10].

## Paper 4: Asthma and Inflammation

The clinical definition of asthma describes the characteristic coughing and wheezing along with **dyspnoea [4.2]** (a word which describes the difficulty in breathing) the sufferer experiences. Along with these symptoms there is oedema - the accumulation of fluid in the affected tissue.

**Paper Number: 4**  
**Reference Number: 4.2**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of dyspnoea  
Page 202 n. dyspnoea

### **Dyspnoea**

Laboured or difficult breathing. (The term is often used for a sign of laboured breathing apparent to the doctor, breathlessness being used for the subjective feeling of laboured breathing.) [1]. Dyspnoea can be due to obstruction to the flow of air into and out of the lungs (as in bronchitis and asthma), various disease affecting the tissue of the lung (including pneumoconiosis, emphysema, tuberculosis, and cancer), and heart disease [2].

### **Notes:**

Dyspnoea is the term used to describe apparent laboured or difficult breathing [1]. It can be due to obstruction of the flow of air into and out of the lungs as in bronchitis, asthma, or various diseases affecting the lung [2].

# Paper 4: Asthma and Inflammation

The clinical definition of asthma describes the characteristic coughing and wheezing along with dyspnoea (a word which describes the difficulty in breathing) the sufferer experiences. Along with these symptoms there is **oedema** [4.3] - the accumulation of fluid in the affected tissue.

**Paper Number: 4**  
**Reference Number: 4.3**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of oedema  
Page 456 oedema n.

## **Oedema**

Excessive accumulation of fluid in the body tissues: popularly known as dropsy. The resultant swelling may be local, as with an injury or inflammation, or more general, as in heart or kidney failure. In generalised oedema there may be collections of fluid within the chest cavity (pleural effusions), abdomen (see ascites), or within the air spaces of the lung (pulmonary oedema) [1].

It may result from heart or kidney failure, cirrhosis of the liver, acute nephritis, the nephrotic syndrome, starvation, allergy, or drugs (e.g. phenylbutazone or cortisone derivatives). In such cases the kidneys can usually be stimulated to get rid of the excess fluid by the administration of diuretic drugs.

Subcutaneous oedema commonly occurs in the legs and ankles due to the influence of gravity and (in women) before menstruation; the swelling subsides with rest and elevation of the legs.

Adjective - oedematous

## **Notes:**

Oedema is the term used to describe the excessive accumulation of fluid in the body tissues. The swelling is local in inflammation [1].

## Paper 4: Asthma and Inflammation

On a cellular scale there is **eosinophilia** [4.4], which describes an increase in the number of eosinophils - a particular kind of cell of the immune system. There is also involvement from other cells of the immune system which work in combination with each other to create a series of microscopic events in the body.

**Paper Number: 4**  
**Reference Number: 4.4**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of eosinophilia  
Page 221 n. eosinophilia

### **Eosinophilia**

An increase in the number of eosinophils in the blood. Eosinophilia occurs in response to certain drugs and in a variety of disease, including allergies, parasitic infestations and certain forms of leukaemia [1].

### **Notes:**

Eosinophilia describes an increase in the number of eosinophils in the blood. It is seen in allergies, parasitic infections, and in some forms of leukaemia [1].

# Paper 4: Asthma and Inflammation

On a cellular scale there is eosinophilia, which describes an increase in the number of **eosinophils** [4.5] - a particular kind of cell of the immune system. There is also involvement from other cells of the immune system which work in combination with each other to create a series of microscopic events in the body.

**Paper Number: 4**  
**Reference Number: 4.5**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of eosinophil  
Page 221 n. eosinophil

## **Eosinophil**

A variety of white blood cell distinguished by the presence in its cytoplasm of coarse granules that stain orange-red with Romanowsky stains [1].

The function of the eosinophil is poorly understood, but it is capable of ingesting foreign particles, is present in large numbers in lining or covering surfaces within the body, and is involved in allergic responses. There are normally  $40 - 400 \times 10^6$  to the six eosinophils per litre of blood [2].

## **Notes:**

Eosinophils are a kind of white blood cell [1] that take part in allergic responses and ingest foreign particles. Large numbers are found in the lining and covering of the surfaces within the body [2].

## Paper 4: Asthma and Inflammation

During an asthmatic episode, progressively the **smooth muscle** [4.6] exhibits a loss of control (dyspnoea). The smooth muscle of the body carries out unconscious actions under the regulation of the Autonomic Nervous System. The Autonomic Nervous System is in command of many things which are not consciously managed; these include the regular beating of the heart, sweating and breathing.

**Paper Number: 4**  
**Reference Number: 4.6**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of smooth muscle  
Page 609 smooth muscle (involuntary muscle)

### **Smooth Muscle (Involuntary Muscle)**

Muscle that produces slow long-term contractions of which the individual is unaware [1]. Smooth muscle occurs in hollow organs, such as the stomach, intestine, blood vessels, and bladder [2]. It consists of spindle-shaped cells within a network of connective tissue and is under the control of the autonomic nervous system. Compare striated muscle [3].

### **Notes:**

Smooth muscle is known as involuntary muscle and produces slow long term contractions which are not consciously controlled [1]. It is part of hollow organs such as the stomach, intestine, bladder, and blood vessels [2]. Made up of spindle shaped cells in a network of connective tissue, smooth muscle is under the control of the autonomic nervous system [3].

## Paper 4: Asthma and Inflammation

During an asthmatic episode, progressively the smooth muscle exhibits a loss of control (dyspnoea). The smooth muscle of the body carries out unconscious actions under the regulation of the Autonomic Nervous System. The Autonomic Nervous System [4.7] is in command of many things which are not consciously managed; these include the regular beating of the heart, sweating and breathing.

**Paper Number: 4**  
**Reference Number: 4.7**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of the autonomic nervous system  
Page 59 autonomic nervous system

### **Autonomic Nervous System**

The part of the nervous system responsible for the control of bodily functions that are not consciously directed, including regular beating of the heart, intestinal movements, sweating, salivation, etc [1].

The autonomic system is subdivided into sympathetic and parasympathetic nervous systems. Sympathetic nerves lead from the middle section of the spinal cord and parasympathetic nerves from the brain and lower spinal cord [2].

The heart, smooth muscles, and most glands receive fibres of both kinds and the interplay of sympathetic and parasympathetic reflex activity (the actions are often antagonistic) governs their working. Sympathetic nerve endings liberate noradrenaline as a neurotransmitter; parasympathetic nerve endings release acetylcholine [3].

### **Notes:**

The autonomic nervous system controls unconscious movements and body functions that include the regular beating of the heart, intestinal movements, sweating and salivation [1]. There are two parts to the autonomic nervous system: The sympathetic nervous system and the parasympathetic system [2].

The heart, smooth muscle and most glands are connected to both parts of the autonomic nervous system. The sympathetic portion works with the parasympathetic portion in a reflex mechanism [3].

# Paper 4: Asthma and Inflammation

Over time the immune system affects the smooth muscle, respiratory cells and **connective tissue** [4.8] in adverse physiological ways. Hypertrophication of the smooth muscle and a loss of vitality in the respiratory cells and connective tissue occurs. All of these tissues contain mast cells.

**Paper Number: 4**  
**Reference Number: 4.8**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of connective tissue  
Page 146 connective tissue

## **Connective Tissue**

The tissue that supports, binds, or separates more specialised tissues and organs, or functions as packing tissue of the body [1]. It consists of an amorphous ground substance of mucopolysaccharides in which may be embedded white (collagenous), yellow (elastic), and reticular fibres, fat cells, fibroblasts, mast cells, and macrophages [2].

Variations in chemical composition of the ground substance and in the proportions and quantities of cells and fibres give rise to tissues of widely differing characteristics, including bone, cartilage, tendons, and ligaments as well as adipose, areolar, and elastic tissues [3].

## **Notes:**

Connective tissue is the tissue that supports, binds, or provides a partition between specialised tissues and organs in the body. As well as this it functions as the packing tissue of the body [1].

Made up of a shapeless ground substance of mucopolysaccharides it may contain collagen, elastin, reticular fibres, fat cells, fibroblasts, macrophages and mast cells [2].

The chemical composition of the ground substance varies widely giving rise to a variety of tissues including bone, cartilage, tendons, ligaments as well as adipose, elastic and areolar tissues [3].

# Paper 4: Asthma and Inflammation

Over time the immune system affects the smooth muscle, respiratory cells and connective tissue in adverse physiological ways. Hypertrophication of the smooth muscle and a loss of vitality in the respiratory cells and connective tissue occurs. All of these tissues contain **mast cells** [4.9].

**Paper Number: 4**  
**Reference Number: 4.9**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of mast cell  
Page 392 mast cell

## **Mast Cell**

A large cell in connective tissue with many coarse cytoplasmic granules. These granules contain chemicals heparin, histamine and serotonin, which are released during inflammation and allergic responses [1].

## **Notes:**

The mast cell is the large cell that is found in connective tissue. The mast cell has many cytoplasmic granules which contain heparin, histamine and serotonin. During inflammation and allergic responses the granules are excreted [1].

## Paper 4: Asthma and Inflammation

In **synopsis [4.10]**: During an asthmatic episode a breakdown in the regular control of the lungs (smooth muscle; autonomic nervous system) results in difficulty of breathing; the mast cells are targeted by the immune system (immunoglobulin E) resulting in the release of their intracellular contents into the body. The result of the immune systems action is ultimately localised inflammation of the lung (anaphylaxis) and its associated tissues.

**Paper Number: 4**  
**Reference Number: 4.10**

**Information taken from:**

Aggregate notes of Paper 4

### **-: Paper Four - Asthma and Inflammation :-**

- The autonomic nervous system is stimulated -**
- The smooth muscle of the lung constricts (bronchoconstriction) -**
- Inflammation occurs in the lung -**
- Eosinophils migrate to the site of inflammation -**
- Mast cells release their contents -**
- Hypersecretion ensues -**

Bronchial asthma is characterised by obstructed airways manifesting coughing, wheezing and dyspnoea. The condition is associated with eosinophils in the blood, saliva and mucus. Diagnosis is decided on a case history of wheezing and the ability to reverse airflow obstruction with bronchodilators.

A patient who has died from asthma has over inflated lungs, abnormally sticky mucus and many eosinophils in the mucus. The bronchial mucus membrane and submucosa are inflamed and the bronchial smooth muscle is abnormally enlarged. If the asthma is related to specific allergies it is considered extrinsic, if no allergy can be demonstrated it is considered intrinsic.

Extrinsic asthma is the most common kind found in youths with a family history of allergy and those commonly exposed to allergens. Patients can be confirmed by a positive "type 1" reaction on a skin prick test with possible allergens. Most atopic subjects are sensitive to more than two allergens. After initial contact with antigens the formation of Immunoglobulin E antibodies occurs which then attach themselves to the surface of the mast cells.

Subsequent exposure to the allergen causes the release of histamine, slow reacting substance of anaphylaxis, eosinophilic chemotactic factor, bradykinin, serotonin, and prostaglandin F2 alpha. These mediators cause bronchoconstriction, mucosal accumulation of fluid, and hypersecretion. Immediate hypersensitivity reactions happen within minutes of contact with the allergen. Patients often give a history of this.

Intrinsic asthma commonly occurs in patients over 35 years of age, generally, there is no personal or family history of allergy nor any seasonal variation in the condition. The wheezing is often severe, lasts a long time and may show marked resistance to bronchodilators. It is in the intrinsic group that most deaths occur. There is much overlap in the treatment of intrinsic and extrinsic asthma.

Dyspnoea is the term used to describe apparent laboured or difficult breathing. It can be due to obstruction of the flow of air into and out of the lungs as in bronchitis, asthma, or various diseases affecting the lung.

Oedema is the term used to describe the excessive accumulation of fluid in the body tissues. The swelling is local in inflammation.

Eosinophilia describes an increase in the number of eosinophils in the blood. It is seen in allergies, parasitic infections, and in some forms of leukaemia.

Eosinophils are a kind of white blood cell that take part in allergic responses and ingest foreign particles. Large numbers are found in the lining and covering of the surfaces within the body.

Smooth muscle is known as involuntary muscle and produces slow long term contractions which are not consciously controlled. It is part of hollow organs such as the stomach, intestine, bladder, and blood vessels. Made up of spindle shaped cells in a network of connective tissue, smooth muscle is under the control of the autonomic nervous system.

The autonomic nervous system controls unconscious movements and body functions that include the regular beating of the heart, intestinal movements, sweating and salivation. There are two parts to the autonomic nervous system: The sympathetic nervous system and the parasympathetic system.

The heart, smooth muscle and most glands are connected to both parts of the autonomic nervous system. The sympathetic portion works with the parasympathetic portion in a reflex mechanism.

Connective tissue is the tissue that supports, binds, or provides a partition between specialised tissues and organs in the body. As well as this it functions as the packing tissue of the body.

Made up of a shapeless ground substance of mucopolysaccharides it may contain collagen, elastin, reticular fibres, fat cells, fibroblasts, macrophages and mast cells. The chemical composition of the ground substance varies widely giving rise to a variety of tissues including bone, cartilage, tendons, ligaments as well as adipose, elastic and areolar tissues.

The mast cell is the large cell that is found in connective tissue. The mast cell has many cytoplasmic granules which contain heparin, histamine and serotonin. During inflammation and allergic responses the granules are excreted.

# Bronchial Asthma and the Atopic Syndrome

## Paper 5: Cellular Mediators of Inflammation

At this point it becomes necessary to give definition to the series of events that constitute the inflammation initiated by the anaphylactic reaction. **The Mediators of the Inflammatory Process [5.1]** are the compounds and cells that are involved in the biochemical reactions that occur in the blood and tissues during the asthmatic episode.

One must consider the context of each chemical group as well as the **interactions between possible mediators [5.2]** to arrive at an idea of what the body is trying to achieve by its complex assertions.

The active cells which are recruited are as follows:

Mast cells  
Polymorphonuclear leukocytes  
Neutrophilic polymorphonuclear leukocytes  
Eosinophilic polymorphonuclear leukocytes  
Mononuclear cells  
Lymphocytes

The cells that are primarily targeted by the immune system are the **mast cells [5.3]** - a type of cell found in loose connective tissue (frequently near vascular channels), smooth muscle and in the blood stream as the circulating basophil.

The mast cell membranes have copious amounts of secretory granules which contain large quantities of heparin and other mucopolysaccharides, histamine and digestive enzymes. It is the mast cell that is used as a model of inflammation.

Polymorphonuclear leukocytes are the predominant immune cells found in inflamed tissues during the early phases of an inflammatory process. Two types of polymorphonuclear leukocytes are involved; neutrophilic and eosinophilic.

**Neutrophilic Polymorphonuclear Leukocytes [5.4]** are cells of the immune system which protect the body by ingesting and ultimately destroying potentially harmful organisms and foreign material. Emigrating to the sites of inflammation, they act in conjunction with the complement systems to disrupt mast cells, increase vessel permeability and release enzymes that degrade proteins, mucopolysaccharides and other substances.

**Eosinophilic Polymorphonuclear Leukocytes [5.5]** are cells of the immune system which are a prominent feature of many allergic and immediate hypersensitive reactions. They are selective to mast cells and contain many of the digestive enzymes present in neutrophils but one particular enzyme - arylsulfatase - is present in higher concentrations and is a strong inactivator of anaphylaxis. These cells degrade heparin.

**Mononuclear cells [5.6]** are cells that digest cellular debris, injured cells, some types of bacteria, fungi, protozoa and other factors that stimulate inflammation.

**Lymphocytes [5.7]** are immune cells which mediate adaptive immunity producing and secreting immunoglobulins and regulating and overall inflammatory response.

**A broad overview [5.8]** of the cellular events must be considered to develop an understanding of the chemical events which the immune system sponsors.

# Paper 5: Cellular Mediators of Inflammation

At this point it becomes necessary to give definition to the series of events that constitute the inflammation initiated by the anaphylactic reaction. **The Mediators of the Inflammatory Process [5.1]** are the compounds and cells that are involved in the biochemical reactions that occur in the blood and tissues during the asthmatic episode.

**Paper Number: 5**  
**Reference Number: 5.1**

**Information taken from:**

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Basic Principles in Therapeutics  
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Mediators of the inflammatory process  
Page 660 Table 13 - 1

## **Mediators Of The Inflammatory Process :**

Humoral :-

Amines: Histamines; Serotonin; Catecholamines  
Prostaglandins  
Peptides and Proteins: Kinins  
Activated components of complement - Anaphylatoxin, classic and alternate pathway (Properidin)  
Activated clotting and fibrinolytic system Eosinophil chemotactic factor of anaphylaxis (ECF-A)

Cellular :-

Mast Cells (Basophils): Release of granular contents  
Neutrophilic Polymorphonuclear Leukocytes: Phagocytic machinery  
Lysosomal granules  
Cytoplasmic Kallikrein  
Other protease's  
Eosinophilic Polymorphonuclear Leukocytes: Phagocytes  
Release of enzymes  
Mononuclear Cells: Mononuclear phagocytes  
Lymphocytes: T cells; B cells Helper cells  
Suppresser cells

## **Notes:**

This is an overall view and list of the chemical and cellular factors that play a role in inflammation

# Paper 5: Cellular Mediators of Inflammation

One must consider the context of each chemical group as well as the **interactions between possible mediators [5.2]** to arrive at an idea of what the body is trying to achieve by its complex assertions.

**Paper Number: 5**  
**Reference Number: 5.2**

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Interactions Between the Possible Mediators  
Page 670 Interactions Between the Possible Mediators

## **Interactions Between The Possible Mediators**

There is no single chemical substance that can initiate and sustain all, or even most, of the events observed during an inflammatory process. However, many of the mediators may interact pharmacologically; their appearance may be sequential, or all may simultaneously appear (Miller and Melmon, 1970; Webster et al., 1972) [1].

For example, the prostaglandin's can intensify the effects of other inflammatory mediators such as histamine and bradykinin; there also is evidence that inhibitors of prostaglandin synthesis inhibit the vasodilatory effects of bradykinin and potentiate the vascular effects of bradykinin and potentiate the vascular effects of angiotensin and norepinephrine (Moncada et al., 1973; Williams and Morley, 1973; Messina et al., 1975) [2].

The inhibitor of the first component of complement also inhibits plasma thromboplastin antecedent (factor XI), activated Hageman factor, Hageman factor fragments, kallikrein, and plasmin (Kaplan, 1974) [3].

In some experimental models, histamine potentiates the inflammatory response produced by serotonin and bradykinin; serotonin and bradykinin may have synergistic effects (Miller and Melmon, 1970) [4].

Activated complement components can release histamine. Hageman factor activation initiates the clotting cascade and activates prekallikrein and a plasminogen proactivator. Plasmin can initiate fibrinolysis, activate complement, act on kininogen to produce kinin peptides, and activate Hageman factor [5].

Thrombin releases serotonin from blood platelets (Zucker and Borrelli, 1955). Kallikrein, fibrinopeptides, plasmin, the prostaglandin's, and activated components of complement all possess chemotactic activity [6].

Certain cells have receptors for activated components of complement and immunoglobulins; therefore, interrelationships exist not only between the various chemical substances that may be important in the initiation and progression of inflammation, but also between these substances and the cellular components involved in an inflammatory process [7].

Undoubtedly there are additional contributors to the inflammatory process, but their roles and identification are only now emerging. There is a considerable body of knowledge related to the mechanisms of inflammation. However, we have not yet seen extensive applications of this information to therapeutics. Because use of basic pathogenetic information in other areas has been fruitful, we can logically expect major advances in anti-inflammatory therapeutics in the future.

### **Notes:**

Many chemicals are released during inflammation, these are 'humoral' mediators that are released by the cells involved in anaphylaxis. The anaphylactic reaction is a complicated series of events, which involves chemical mediators interacting and influencing each other as well as tissues to achieve a particular physiological aim. Examples of these chemical interrelationships follow [1]:

Prostaglandin's are lipid hormones that when present may increase the vasodilatory effects of histamine and bradykinin. Inhibiting the prostaglandins has the effect of inhibiting vascular dilation and the body's sensitivity to bradykinin, angiotensin and norepinephrine [2].

Inhibition of the first component of complement also inhibits components of blood coagulation (thromboplastin antecedent - factor XI; activated Hageman factor; Hageman factor fragments), kallikrein (which produces bradykinin), and plasmin (which digests the protein of blood clots - fibrin) [3].

Histamine potentiates the inflammatory response produced by serotonin and bradykinin in some models (both are known to act on smooth muscle and cause vascular dilation) [4].

The activation of complement can result in histamine release. Hageman factor, (which initiates blood clotting), also activates prekallikrein and a plasminogen proactivator. Plasmin can initiate breakdown of fibrin, activate complement, causes kinins to be produced, and activates Hageman factor in blood coagulation [5].

Thrombin (the precursor to fibrin) stimulates the release of serotonin from blood platelets. Kallikrein, fibrinopeptides, plasmin, prostaglandin's, and complement all have chemotactic activity [6].

It is plain to see that the chemical mediators of anaphylaxis interact with each other and that cells influence the chemical substances and vice versa [7]. A closer look at the cells that are the target of all the attentions; the cells which act on them, and the substances they utilise follows.

# Paper 5: Cellular Mediators of Inflammation

The cells that are primarily targeted by the immune system are the **mast cells** [5.3] - a type of cell found in loose connective tissue (frequently near vascular channels), smooth muscle and in the blood stream as the circulating basophil.

**Paper Number: 5**  
**Reference Number: 5.3**

**Information taken from:**  
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Cellular mediators of inflammation  
Page 671 Mast cells

## **Mast Cells**

Mast cells are mainly found in loose connective tissue, frequently near vascular channels. Their most distinguishing features are their membrane-bound, spheroid, basophilic secretory granules, which may be so numerous that other cytoplasmic structures are obscured. These granules contain large amounts of heparin and other mucopolysaccharides, histamine, and several proteolytic and esterolytic enzyme systems. Serotonin is present in the mast cells of some species, man being a notable exception [1].

Much is known regarding the structure and composition of mast cells, but their physiologic function remains unidentified. However it seems certain that the mast cell and the basophil (circulating mast cells) contribute to the development of an inflammatory process (Lewis et al., 1975). Changes in mast cell density and mast cell granules are thought to be characteristic of many inflammatory lesions. Mast cell preparations (either in vitro or in vivo during anaphylaxis) have been used as experimental models of inflammation [2].

The mast cell is readily degranulated (see previous section on histamine), and the granule-bound chemical substances are released into extracellular fluid [3]. Histamine and probably heparin can have important influences on an inflammatory process, but much less is known about the granule esterase's and protease's that are also released from the granules of the mast cell and basophil (Benditt, 1968; Lewis et al., 1975).

These enzymes may alter the course of inflammation by interactions with other cells or substances to release, activate, or destroy kinins, complement, and blood coagulation factors, to alter membranes of other cells such as granulocytes so that additional intracellular constituents are released, or to alter the structure and characteristics of adjacent connective tissue [4].

## **Notes:**

Mast cells are cells mainly found in loose connective tissue (see smooth muscle) and are often near vascular channels. They are distinguished by copious amounts of secretory granules on the membrane.

The secretory granules contain large amounts of heparin and mucopolysaccharides (both of which are compounds of glucuronic acid and glucosamine), histamine and several enzyme systems which catalyse the splitting of esters into constituent alcohol's and acids, and proteins into smaller peptides and amino acids [1].

The mast cells are the target cells intimately involved in the inflammatory process and are used as models of inflammation caused by anaphylaxis [2]. During inflammation the mast cell is stimulated to excrete its granules into the extracellular fluid [3].

Histamine and heparin are both important influences in the inflammatory process. The enzyme systems of mast cells may act on kinins, complement, blood coagulation factors, leukocyte membranes and on the structure and characteristics of connective tissue [4].

# Paper 5: Cellular Mediators of Inflammation

**Neutrophilic Polymorphonuclear Leukocytes [5.4]** are cells of the immune system which protect the body by ingesting and ultimately destroying potentially harmful organisms and foreign material. Emigrating to the sites of inflammation, they act in conjunction with the complement systems to disrupt mast cells, increase vessel permeability and release enzymes that degrade proteins, mucopolysaccharides and other substances.

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**Reference Number: 5.4**

**Information taken from:**  
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Cellular mediators of inflammation  
Page 671 Neutrophilic Polymorphonuclear Leukocytes

## **Neutrophilic Polymorphonuclear Leukocytes**

Neutrophilic leukocytes protect the body by ingesting and ultimately destroying potentially harmful organisms and foreign material. Phagocytosis occurs after the leukocytes have margined along the endothelium at the site of the inflammatory process [1].

They then emigrate through the vessel wall, attracted by many substances released or activated by the inflammatory stimulus, and ingest and digest or kill the "noxious" agent. Unfortunately, little is known of the mechanisms responsible for each of these steps, but they are probably directly influenced by the presence of serum factors such as complement [2].

The leukocytes can also influence other aspects of the inflammatory response in addition to the destruction of a harmful stimulus. Leukocytes are essential in the Schwartzman phenomenon, Arthus' reaction, the acute inflammatory process of gout, and probably in other conditions. Although the extent of the participation of leukocytes is not fully defined, release of their chemical contents appears to be one mechanism of action [3].

The lysosomes of leukocytes contain a protease that can cleave C5, the fifth component of complement, to yield a product that, in turn, selectively releases other lysosomal enzymes from intact leukocytes. Therefore, activation of either the classic or alternate complement pathway results in the production of factors capable of stimulating lysosomal release (Goldstein et al., 1973; Goldstein and Weissmann, 1974a) [4].

The lysosomal substances influence inflammatory processes by disrupting mast cells, increasing the permeability of vessels, furthering chemotaxis, or injuring normal tissue. Lysosomal constituents can degrade collagen, elastin, cartilage, hyaluronate, chondroitin sulphate, nucleic acids, complement components, fibrin, plasminogen, coagulation factors, and kininogen [5].

A series of nonenzymatic factors have also been isolated from lysosomes that induce capillary permeability and provoke fever (Goldstein and Weissmann, 1974a). Some proteins in the lysosome are fungicidal and bactericidal (Weissmann, 1967) [6].

Lysosomal enzymes are released by two mechanisms. One mechanism is called "regurgitation during feeding"; the lysosomal contents are released into the surrounding medium by cells engaging in endocytosis (Weissmann et al., 1971).

There is no leakage of cytoplasmic constituents and no evidence of cellular injury. Another mechanism of discharge of lysosomal enzymes from intact leukocytes involves selective release (exocytosis) of lysosomal constituents when leukocytes encounter immune complexes on solid surfaces; The process has been termed "reverse endocytosis" (Weissmann et al., 1972) [7].

The release of lysosomal contents can be modified by pharmacologic agents that alter intracellular concentrations of cyclic nucleotides (cAMP, cGMP) and agents that promote or reduce assemble of microtubules (Goldstein and Weissmann, 1974b; Ignarro, 1975) [8].

Plasmin-generating activity of a kallikrein activator is present on the cell surface and a kininase is found in the granules of neutrophils and eosinophils (Melmon and Cline, 1968). The kinin-generating capability of these cells is likely due to plasmin production and probably can account for the delayed appearance of kinin-like substances in experimental inflammatory processes (Kellermeyer and Graham, 1968; Miller et al., 1975) [9].

Although the optimal pH of kinin generation from the cytoplasm is 7 to 8, cathepsins are active in the potentially acid environment of inflammatory foci, and granulocyte kinin production continues at pH 5.5 (Greenbaum et al., 1966) [10].

### **Notes:**

Neutrophilic Polymorphonuclear Leukocytes (neutrophils) are a kind of immune cell that act to digest bacteria and foreign materials at the endothelium of the inflamed tissue [1]. These cells migrate through vessel walls attracted by the noxious substances released during inflammation [2]. Neutrophils are involved in inflammatory reactions, are essential in the Schwartzman phenomenon, Arthus' reaction and the inflammation of gout. They act by releasing their chemical contents [3].

The digestive enzymes neutrophils release affect complement, which in turn prompts other leukocytes to release their lysosomal enzymes [4]. The enzymes they release disrupt mast cells, make blood vessels more permeable for cell migration and digest substances such as collagen, elastin, cartilage, hyaluronate, chondroitin sulphate, nucleic acids, complement, fibrin, plasminogen, coagulation factors and kininogen [5].

Neutrophils also release fungicidal and bactericidal proteins as well as non-enzymic substances that act to increase capillary permeability and body temperature [6]. The enzymes are released in two ways. One is termed 'Regurgitation During Feeding', where lysosomal enzymes are discharged into the surrounding tissue whist neutrophils digest substances internally. There is no leakage of cytoplasmic constituents and no evidence of cellular injury.

The other mechanism of discharge is termed 'Reverse Endocytosis'. Intact leukocytes selectively release lysosomal constituents (exocytosis) when they encounter immune complexes on solid surfaces [7]. Enzyme release is influenced by chemicals that alter intracellular cyclic nucleotide levels and substances that influence the formation of microtubules ("intracellular veins") [8].

The surface of the neutrophil cell can stimulate the formation of plasmin and kinins, as well as this, an enzyme which digests kinins is found in the granules of both neutrophilic and eosinophilic leukocytes [9].

The pH value of the tissue environment affects the production of kinins but cathepsins (enzymes which digest proteins) and kinins produced by granulocytes (white blood cells which possess granules in their cytoplasm - neutrophils, eosinophils, basophils) continue to be synthesised where there is pH variation [10].

# Paper 5: Cellular Mediators of Inflammation

**Eosinophilic Polymorphonuclear Leukocytes [5.5]** are cells of the immune system which are a prominent feature of many allergic and immediate hypersensitive reactions. They are selective to mast cells and contain many of the digestive enzymes present in neutrophils but one particular enzyme - arylsulfatase - is present in higher concentrations and is a strong inactivator of anaphylaxis. These cells degrade heparin.

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 Cellular mediators of inflammation  
 Page 672 Eosinophilic Polymorphonuclear Leukocytes

## **Eosinophilic Polymorphonuclear Leukocytes**

Eosinophilic leukocytes are a prominent feature of many allergic and immediate hypersensitive reactions. In addition to activated complement factors and kallikrein, an eosinophil chemotactic factor of anaphylaxis (ECF-A) released from sensitised mast cells selectively attracts eosinophils (Kay et al., 1971) [1].

Eosinophils phagocytize and have membrane bound granules that contain many of the hydrolytic enzymes present in neutrophils. One enzyme present in higher concentrations than in polymorphonuclear cells is arylsulfatase, which is a potent inactivator of slow reacting substance of anaphylaxis (SRS-A) (Wasserman et al., 1975) [2]. Other factors in eosinophils inhibit histamine release, neutralise heparin, and activate plasminogen; thus, these cells may play a role in limiting allergic reactions (Goetzl et al., 1975) [3].

## **Notes:**

Eosinophilic Polymorphonuclear Leukocytes (eosinophils) are immune cells that play a major part in allergic and immediate hypersensitivity reactions. They are attracted by complement, kallikrein and a substance released by sensitised mast cells [1].

These leukocytes engulf and digest bacteria and foreign materials in a similar way to neutrophils. Eosinophils have a higher level of the enzyme - arylsulfatase - which strongly inactivates anaphylactic events by acting on aromatic and sulphur containing compounds [2].

Eosinophils inhibit histamine release, neutralise heparin (sulphur compound) and activate plasminogen. These cells play an important role in limiting allergic reactions [3].

# Paper 5: Cellular Mediators of Inflammation

**Mononuclear cells** [5.6] are cells that digest cellular debris, injured cells, some types of bacteria, fungi, protozoa and other factors that stimulate inflammation.

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Cellular mediators of inflammation  
Page 672 Mononuclear cells

## **Mononuclear Cells**

Mononuclear phagocytes. Polymorphonuclear leukocytes are the predominant cells in inflamed tissues during the early phases of an inflammatory process. Later, monocytes and macrophages are more numerous [1].

The factors that attract and stimulate the mononuclear cells seem to differ from those that influence the polymorphonuclear leukocytes. The mononuclear phagocytes (monocytes and macrophages) ingest cellular debris, injured cells, some types of bacteria, fungi, protozoa, and other factors that stimulate inflammation [2].

The mononuclear phagocytes contain many potent hydrolytic enzyme systems that are capable of degrading most of the known macromolecular constituents of tissues and bacteria (Cohn, 1965). Mononuclear phagocytes probably contribute to various immune phenomena by processing antigens into immunogenic complexes (Cohn, 1965; Sell and Asofsky, 1968; Weiser et al., 1969) [3].

The monocytic phagocytes are important factors in the bodies resistance against certain types of infection as well as during tissue repair and healing. Although the role of the monocyte in inflammation has been extensively described, we have little information on ways to influence its function by pharmacologic means [4].

## **Notes:**

Mononuclear cells are involved more in the later stages of inflammation [1]. There are two types of mononuclear cell; monocytes and macrophages. Both ingest undesirable substances (phagocytosis) [2]. Mononuclear phagocytes have enzymes capable of breaking down most tissues and bacteria [3] and are involved in immune resistance to infection, tissue repair and healing [4].

# Paper 5: Cellular Mediators of Inflammation

**Lymphocytes** [5.7] are immune cells which mediate adaptive immunity producing and secreting immunoglobulins and regulating and overall inflammatory response.

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Cellular mediators of inflammation  
Page 672 - 674 Lymphocytes

## **Lymphocytes**

The lymphocytes mediate both types of responses necessary for adaptive immunity: humoral and cell mediated immunity. Humoral immunity involves the production and secretion of antibody (immunoglobulins can combine with and frequently neutralise specific antigens) [1].

The reaction of the antibody and antigen is called an immediate reaction or immediate hypersensitivity. The clinical manifestations of this reaction (anaphylaxis, serum sickness, Arthus reaction) will depend upon the type of antibody produced and the tissues in which the reaction occurs [2].

Antibodies are produced by plasma cells derived from specialised lymphocytes called B cells. Other lymphocytes, called T cells, are involved in cellular immunity, which instead is mediated by cells specifically altered as a result of antigenic stimulation. The cell mediated type of immunity is responsible for the rejection of tissue grafts and for delayed hypersensitivity reactions, such as the tuberculin reaction [3].

Both types of lymphocytes (T and B) are derived from hematopoietic stem cells that migrate to the primary lymphoid organs, the thymus and "bursa" or its phylogenetic counterpart. The stem cells differentiate along lymphoid cell lines in these tissues [4].

Thymus derived cells, T cells, populate the thymus dependent areas of lymphoid tissues and recirculate from blood to lymph. Non thymus derived cells (B cells) colonise the thymus independent areas of the secondary lymphoid organs such as the lymph follicle [5].

After interaction with antigen, the T cells differentiate into cells involved in cellular immunity and B cells to antibody producing cells. For optimal function of either the T or B lymphocytes, both types of cells are necessary. For example, if a deficiency of T helper cells exist, the B cells cannot effectively produce antibodies after antigenic stimulation. Neither type of cell has a pure function nor can one type function independently of the other cell type [6].

The mature, antigen sensitive T lymphocyte is capable of specific antigen recognition and can initiate immune responses (Basten et al., 1971). The thymus derived lymphocytes appear to have multiple functions in immunity: (1) Helper cells and suppresser cells can modulate B cell production of antibody; (2) They modulate the extent of effector T cell expression of delayed hypersensitivity, such as mixed lymphocyte reactions, and graft versus host reactions [7].

T cells (Paul and Bencerraf, 1977) may play an important role on B cell responses, as evidenced by procedures that deplete T cells (such as thymectomy, treatment of cells with anti-lymphocyte serum) or separation of a sub-population of T cells by virtue of their adherence to insolubilised conjugate of histamines with albumin (Mozes et al., 1974; Shearer et al., 1974).

These manoeuvre result in increased antibody production against T independent antigens (Kerbel and Eiding, 1971; Shearer et al., 1974). In tolerant animals, T cells exert an active suppresser effect on IgG antibody production (Gershon and Kondo, 1971; Basten et al., 1974; Segal et al., 1974).

The mechanism of suppression of the immune response by T cells is unknown, but in addition to their effects on antibody production, it appears that T suppresser cells are also involved in regulation of T cell mediated delayed hypersensitivity (Zembala and Asherson, 1973). In antibody production, however, it is not known whether the suppressive effect is exerted directly on B cells, on macrophages that process antigens, or on other T cells that would ordinarily facilitate the B cell response.

Knowledge of the mechanism of T cell suppression may be required to understand surveillance against antigens. Such knowledge also can help with proper design of experiments to effectively modulate T and B cell function pharmacologically (e.g. with the drugs that increase or decrease their cyclic AMP concentrations) and with the effective design of unconventional new drugs that might selectively alter the functions of the cells that serve as targets for the T suppresser population.

Transfer factor is a low molecular weight RNA complex from leukocyte extracts that converts "naive" lymphocytes to antigen responsive cells. This results in a cascade effect so that a large population of antigen responsive cells is recruited to deal more effectively with the noxious agent (Lawrence, Harvey Lectures, in press) [8].

Transfer factors have specificity for subgroups of antigen sensitive T cells as opposed to specificity for various antigens. Transfer factors are now being purified to the extent that they can be chemically characterised and perhaps synthesised [9].

Their clinical efficacy has not been established, but preliminary studies suggest their anti-tumour and anti-infective actions (e.g. against coccidioidomycosis and lepromatous leprosy) and their use in therapy of immunodeficiency states (Bullock et al., 1972; Spitler et al., 1972; Ammann et al., 1974; Catanzaro et al., 1974; Levin et al., 1975). Further testing of these agents is required before recommendation of their use is possible.

The T cells are believed to be regulators of inflammatory responsiveness, because they either promote or suppress functions of other lymphocytes in both cellular and humoral immunity; They activate macrophages and enhance microbicidal activity; They produce factors (such as lymphokines) that can enhance migration and function of other mononuclear and polymorphonuclear leukocytes (Bloom and Bennett, 1970; McDevitt et al., 1974; Gershon, 1974; Mitchell, in press) [10].

Almost any process that causes tissue injury can trigger the series of events included in the inflammatory and possibly the immune process. Such stimuli may not produce inflammation or an immune response by identical mechanisms. The release or activation of histamine, serotonin, bradykinin, complement components, and coagulation factors may contribute to inflammation [11];

Many other factors (e.g. slow reacting substances, prostaglandin's changes in hydrogen ion concentration and oxygen tension), undoubtedly play some part in an inflammatory process. The emigration of granulocytes and mononuclear cells and their interaction have early direct and indirect effects by causing release of cellular contents. In a later phase of inflammation, these cells may be primarily involved in the immune process [12].

**Notes:**

Lymphocytes are immune cells involved in adaptive immunity and the production of immunoglobulins (antibodies) [1]. When an antibody combines with an antigen an immediate hypersensitivity reaction results. How the reaction manifests itself depends on the antibody and tissue involved [2].

There are two kinds of lymphocyte:- The B cell lymphocyte which produces the antibodies involved in 'humoral immunity' - and the - T cell lymphocyte which is involved in 'cellular immunity' by adapting to the antigens they encounter. The T cells are responsible for tissue rejection and delayed hypersensitivity [3].

The lymphocytes are produced by the process of 'haemopoiesis' in the bone marrow and then migrate to lymphoid tissues and bursa where they mature [4]. T cells are named thus because they are found in the thymus dependent tissues whereas the B cells are found in tissues that are independent of the thymus organ [5]. After contact with antigens T cells adapt to the antigen and B cells produce antibodies. Both T and B lymphocytes act in a co-ordinated manner to provide effective immunity [6].

Matured antigen sensitive T cells act to specific substances and initiate appropriate immune responses. T cells influence the antibody production of B cells and the extent of the hypersensitive reaction [7]. They are made antigen sensitive by way of ribonucleic acid complex termed 'transfer factor' [8]. Transfer factors are specific to groups of T cells that have antigen sensitive qualities [9].

T cells act to either promote or suppress the lymphocyte population involved in cellular and humoral immunity, as well as activate macrophage activity [10]. Many processes that produce tissue injury involve inflammation and the immune system. Many humoral substances are involved [11]. Granulocytes and mononuclear cells have early actions in releasing cellular contents, and in the later phase of the inflammatory response, are a primary aspect [12].

# Paper 5: Cellular Mediators of Inflammation

A broad overview [5.8] of the cellular events must be considered to develop an understanding of the chemical events which the immune system sponsors.

**Paper Number: 5**  
**Reference Number: 5.8**

**Information taken from:**

Aggregate notes of Paper 5

## **-: Paper Five - Cellular Mediators of Inflammation :-**

- **The allergic reaction is a complex series of chemical reactions initiated and controlled by the immune cells to cause inflammation -**
- **Mast cell cultures are the tissue targeted by the immune system -**
- **Mast cell cultures are abundant in smooth muscle and vascular channels -**
- **Eosinophils, neutrophils, and monocytes digest heparin -**
- **Lymphocytes co-ordinate and potentiate the allergic reaction by producing immunoglobulin E -**
- **Eosinophils are enzymically particularly equipped to metabolise heparin with arylsulfatase -**

This is an overall view and list of the chemical and cellular factors that play a role in inflammation

Many chemicals are released during inflammation, these are 'humoral' mediators that are released by the cells involved in anaphylaxis. The anaphylactic reaction is a complicated series of events, which involves chemical mediators interacting and influencing each other as well as tissues to achieve a particular physiological aim. Examples of these chemical inter-relationships follow:

Prostaglandin's are lipid hormones that when present may increase the vasodilatory effects of histamine and bradykinin . Inhibiting the prostaglandins has the effect of inhibiting vascular dilation and the body's sensitivity to bradykinin, angiotensin and norepinephrine.

Inhibition of the first component of complement also inhibits components of blood coagulation (thromboplastin antecedent - factor XI; activated Hageman factor; Hageman factor fragments), kallikrein (which produces bradykinin), and plasmin (which digests the protein of blood clots - fibrin).

Histamine potentiates the inflammatory response produced by serotonin and bradykinin in some models (both are known to act on smooth muscle and cause vascular dilation).

The activation of complement can result in histamine release. Hageman factor, (which initiates blood clotting), also activates prekallikrein and a plasminogen proactivator. Plasmin can initiate breakdown of fibrin, activate complement, causes kinins to be produced, and activates Hageman factor in blood coagulation.

Thrombin (the precursor to fibrin) stimulates the release of serotonin from blood platelets. Kallikrein, fibrinopeptides, plasmin, prostaglandin's, and complement all have chemotactic activity.

It is plain to see that the chemical mediators of anaphylaxis interact with each other and that cells influence the chemical substances and vice versa. A closer look at the cells that are the target of all the attentions; the cells which act on them, and the substances they utilise follows.

Mast cells are cells mainly found in loose connective tissue (see smooth muscle) and are often near vascular channels. They are distinguished by copious amounts of secretory granules on the membrane.

The secretory granules contain large amounts of heparin and mucopolysaccharides (both of which are compounds of glucuronic acid and glucosamine), histamine and several enzyme systems which catalyse the splitting of esters into constituent alcohol's and acids, and proteins into smaller peptides and amino acids.

The mast cells are the target cells intimately involved in the inflammatory process and are used as models of inflammation caused by anaphylaxis. During inflammation the mast cell is stimulated to excrete its granules into the extracellular fluid.

Histamine and heparin are both important influences in the inflammatory process. The enzyme systems of mast cells may act on kinins, complement, blood coagulation factors, leukocyte membranes and on the structure and characteristics of connective tissue.

Neutrophilic Polymorphonuclear Leukocytes (neutrophils) are a kind of immune cell that act to digest bacteria and foreign materials at the endothelium of the inflamed tissue. These cells migrate through vessel walls attracted by the noxious substances released during inflammation. Neutrophils are involved in inflammatory reactions, are essential in the Schwartzman phenomenon, Arthus' reaction and the inflammation of gout. They act by releasing their chemical contents.

The digestive enzymes neutrophils release affect complement, which in turn prompts other leukocytes to release their lysosomal enzymes. The enzymes they release disrupt mast cells, make blood vessels more permeable for cell migration and digest substances such as collagen, elastin, cartilage, hyaluronate, chondroitin sulphate, nucleic acids, complement, fibrin, plasminogen, coagulation factors and kininogen.

Neutrophils also release fungicidal and bactericidal proteins as well as non-enzymic substances that act to increase capillary permeability and body temperature. The enzymes are released in two ways. One is termed 'Regurgitation During Feeding', where lysosomal enzymes are discharged into the surrounding tissue whilst neutrophils digest substances internally. There is no leakage of cytoplasmic constituents and no evidence of cellular injury.

The other mechanism of discharge is termed 'Reverse Endocytosis'. Intact leukocytes selectively release lysosomal constituents (exocytosis) when they encounter immune complexes on solid surfaces. Enzyme release is influenced by chemicals that alter intracellular cyclic nucleotide levels and substances that influence the formation of microtubules ("intracellular veins").

The surface of the neutrophil cell can stimulate the formation of plasmin and kinins, as well as this, an enzyme which digests kinins is found in the granules of both neutrophilic and eosinophilic leukocytes.

The pH value of the tissue environment affects the production of kinins but cathepsins (enzymes which digest proteins) and kinins produced by granulocytes (white blood cells which possess granules in their cytoplasm - neutrophils, eosinophils, basophils) continue to be synthesised where there is pH variation.

Eosinophilic Polymorphonuclear Leukocytes (eosinophils) are immune cells that play a major part in allergic and immediate hypersensitivity reactions. They are attracted by complement, kallikrein and a substance released by sensitised mast cells.

These leukocytes engulf and digest bacteria and foreign materials in a similar way to neutrophils. Eosinophils have a higher level of the enzyme - arylsulfatase - which strongly inactivates anaphylactic events by acting on aromatic and sulphur containing compounds.

Eosinophils inhibit histamine release, neutralise heparin (sulphur compound) and activate plasminogen. These cells play an important role in limiting allergic reactions.

Mononuclear cells are involved more in the later stages of inflammation. There are two types of mononuclear cell; monocytes and macrophages. Both ingest undesirable substances (phagocytosis). Mononuclear phagocytes have enzymes capable of breaking down most tissues and bacteria and are involved in immune resistance to infection, tissue repair and healing.

Lymphocytes are immune cells involved in adaptive immunity and the production of immunoglobulins (antibodies). When an antibody combines with an antigen an immediate hypersensitivity reaction results. How the reaction manifests itself depends on the antibody and tissue involved.

There are two kinds of lymphocyte:- The B cell lymphocyte which produces the antibodies involved in 'humoral immunity' - and the - T cell lymphocyte which is involved in 'cellular immunity' by adapting to the antigens they encounter. The T cells are responsible for tissue rejection and delayed hypersensitivity.

The lymphocytes are produced by the process of 'haemopoiesis' in the bone marrow and then migrate to lymphoid tissues and bursa where they mature. T cells are named thus because they are found in the thymus dependent tissues whereas the B cells are found in tissues that are independent of the thymus organ. After contact with antigens T cells adapt to the antigen and B cells produce antibodies. Both T and B lymphocytes act in a co-ordinated manner to provide effective immunity.

Matured antigen sensitive T cells act to specific substances and initiate appropriate immune responses. T cells influence the antibody production of B cells and the extent of the hypersensitive reaction. They are made antigen sensitive by way of ribonucleic acid complex termed 'transfer factor'. Transfer factors are specific to groups of T cells that have antigen sensitive qualities.

T cells act to either promote or suppress the lymphocyte population involved in cellular and humoral immunity, as well as activate macrophage activity. Many processes that produce tissue injury involve inflammation and the immune system. Many humoral substances are involved. Granulocytes and mononuclear cells have early actions in releasing cellular contents, and in the later phase of the inflammatory response, are a primary aspect.

# Bronchial Asthma and the Atopic Syndrome

## Paper 6: Humoral Mediators of Inflammation

Many substances are released during inflammation and each compound serves a specific role in the body. The following are mediators of anaphylaxis:

**Histamine [6.1]** as a substance is concerned with vascular dilation and it increases the permeability of blood vessels and tissues. It facilitates oedema and the migration of immune cells and compounds across tissue barriers. Histamine produces pruritus but not pain.

**Serotonin[6.2]** as a substance causes vasodilation and increases blood flow. It is involved in the functioning of smooth muscle and non-immediate communication of pain.

The **catecholamines [6.3]**: **Adrenaline [6.4]** and **noradrenaline [6.5]** are suggested to contribute to the development of hemorrhagic lesions as well as to act as local anti-inflammatory hormones. These substances affect the sympathetic portion of the autonomic nervous system and relax the smooth muscle.

**Peptides and Proteins [6.6]**: This category covers the intrinsic coagulation system, fibrinolytic system, components of complement and components of the kinin generation system. These groups are inter-utilized by the body sharing key components. The groups work in chain reaction existing under suppression by inhibitors. They play roles in oedema.

**Kinins [6.7]** are polypeptides which function to produce arterial and venular dilation by direct action on smooth muscle. They are involved in pain production and are suggested to induce leukocyte adherence and migration during inflammation.

**Activated complement components [6.8]** is a group of compounds which act to cause late-acting, extensive cellular damage by fixing to cells and causing their mediators to be released and activated.

Both leukocytes and lung macrophages are receptive to components of complement. Complement causes the degranulation of mast cells which occurs during smooth muscle contraction. It enhances phagocytosis and changes in vascular permeability.

**Components of the blood clotting system [6.9]** interact with other plasma proteins such as the components of complement, fibrin, and the kinin system. Their function in the body is to clot - a suppressing factor being heparin.

**Prostaglandins [6.10]** are lipid compounds which produce an array of metabolic effects. Widely distributed throughout the body, each prostaglandin has its own spectrum of activities which is tissue specific. Prostaglandins have chemical properties which are not dose-dependent and are physiologically potent substances important to inflammation.

**Heparin [6.11]** is a naturally occurring anticoagulant in the blood and other tissues. It is comprised of glucosamine, glucuronic acid and varying proportions of sulphate and acetyl groups. The heparin content of tissues correlates directly with the number of mast cells present.

Preventing the coagulation of fibrinogen, heparin also functions as the structural precursor of lipoprotein lipase; an enzyme involved in the metabolism of lipids in the body.

**Mucopolysaccharides [6.12]** are a class of compound that consist in general of a sugar (example: glucosamine), a uronic acid (example: glucuronic acid) and a protein which is covalently linked. They provide the tissues with resistance to compression and act as lubricants. They are part of a broader group known as the glycosaminoglycans.

In **summary [6.13]** the immune system during inflammation is causing the deliberate degranulation and damaging of specific cells - The mast cells. The body employs cells of the immune system which have the ability to attack and digest not only allergens and bacteria but the bodies own tissues.

# Paper 6: Humoral Mediators of Inflammation

**Histamine [6.1]** as a substance is concerned with vascular dilation and it increases the permeability of blood vessels and tissues. It facilitates oedema and the migration of immune cells and compounds across tissue barriers. Histamine produces pruritus but not pain.

**Paper Number: 6**  
**Reference Number: 6.1**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
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Bailliere, Tindall london  
Functional action of histamine  
Page 660 Histamine

## **Histamine**

The notable actions of histamine are its effects on the vascular system, smooth muscle, and exocrine glands. Histamine dilates arterioles and venules in most species. Histamine induced vasodilation is independent of innervation and is only partly suppressed by antihistamines, but many can be completely overcome by sympathomimetic amines (Goodman and Gilman, 1975) [1].

Increases in capillary permeability have been attributed to histamine, but there is no evidence that it affects the smallest blood vessels (those containing a single layer of endothelium with a basement membrane). Histamine increases the permeability of small veins (up to 50 micrometers in diameter) by causing separation of the endothelial cells (Majno and Palade, 1961; Majno et al., 1961; Marchesi, 1962) [2].

When histamine is injected into human skin, a characteristic triple response occurs (Lewis, 1927). This series of events involves the local venules, arterioles, capillaries (vessels up to 50 micrometers in diameter that may lack thick muscle coats), and sensory nerves.

The triad consists of : - (1) A localised red spot, or "flush", representing the immediate and direct vasodilatory effect of histamine; - (2) A bright red flush or "flare" of irregular outline extending for 1cm or more beyond the original red spot (believed to be produced by the reflex vasodilation of the adjacent small vessels) - (3) A localised collection of edema fluid (a wheal) secondary to the extravasation of plasma fluid through the abnormally permeable walls of the small vessels [3].

Other important effects of histamine include pruritis, but not pain, when the amine is applied to a blister base or infected intradermally; unusually high doses of the amine may produce diapedesis of a small number of leukocytes (Spector and Willoughby, 1964a) [4].

In many species, repeated doses of histamine result in tachyphylaxis (Naranjo, 1966). Histamine markedly enhances the endothelial deposition of immune complexes; antihistamines will retard their deposition (Cochrane, 1963) [5].

The properties of histamine make it a candidate for influencing the acute phase of inflammation. Antihistamines, if useful at all, would have their major effects in this early phase. Whether late phases of inflammation are cause-and-effect related to early phases is unknown, but his consideration may become fundamental for the proper use of anti-inflammatory agents.

Histamine is formed by decarboxylation of histidine and is widely distributed in the body. Free histamine is found only in trace amounts in most tissues but may be quite active in terms of the role it can play during an inflammatory process (Ivy and Bachrach, 1966; Erjavec et al., 1967) [6]. Mast cells provide the major storage site for histamine, but the amine is also present in granulocytes. Histamine is stored in the mast cell (Uvnas, 1964, 1967) [7].

The extrusion of granules is associated with events that alter the mast cell membrane, such as decreases in pH and changes in the ionic milieu and temperature. Factors involved in the release from the granulocyte include similar changes, plus phagocytosis and damage of its cell membrane produced by antigen-antibody reaction [8]. Release of histamines of substance contained in the granular fraction of the cell (lysozyme, myeloperoxidase, alkaline phosphatase, cathepsin, etc.) is capable of contributing to inflammation and is inhibited by drugs that activate the adenylyl cyclase system or interfere with degranulation [9].

These observations, and the fact that inhibitors of proteolytic enzymes decrease granule extrusion, indicate an enzymatic basis for release of granules and their contents, presumably including histamine (Lichenstein and Margolis, 1968; Miller and Melmon, 1970; Bourne and Melmon, 1971) [10].

Anoxia and lack of glucose slow the extrusion of granules; therefore, the process requires energy. Histamine release may involve activation of an enzyme, located on the mast cell membrane, that alters the permeability characteristics of the membrane and results in entry of the mast cell granules into the extracellular fluid. Almost any agent that causes tissue injury also liberates histamine (Spector and Willoughby, 1963a, 1964a, 1964b; Beraldo et al., 1966) [11].

Proof of the relevance of release of a mediator in the inflammatory process requires serial measurements and correlation of changes in concentration of the substance with morphologic abnormalities (Reichgott and Melmon, 1971). The role of histamine during an inflammatory process may be defined in part by the effects of pharmacologic antagonists on the peripheral effects of the amine. By heating the skin of a guinea pig, an early and delayed phase of increased small vessel permeability was demonstrated (Sevitt, 1958).

Two phases of inflammation may be observed in many other types of injury. The initial phase can be suppressed by pre-treatment of the experimental animals with antihistamines (Spector and Willoughby, 1965) [12]. In some types of inflammation, such as severe tissue destruction produced by high temperatures or prolonged heating, the early response is less noticeable, and the overall response may appear to be an accelerated late phase that is not altered by antihistamines [13].

X-ray damage to rat intestine causes abnormal vascular permeability within 24 hours. If antihistamines are given at the time or irradiation, the increased vascular permeability is delayed for an additional 24 hours; however, by 72 hours, the severity of the reaction is equal to that of controls (Spector and Willoughby, 1964a).

The role histamine plays in the development of an inflammatory process is early, transient, incomplete, and not essential for the development of the most characteristic changes that produce lasting tissue alteration [14]. Therefore, antihistaminic's or inhibitors of histidine decarboxylase have limited but specific usefulness as anti-inflammatory agents. Conversely, if an agent that alters mast cell or granulocyte function also affects the development or maintenance of an inflammatory process, the drug may affect substances in addition to or other than histamine [15].

Finding that T lymphocytes with histamine receptors may act as suppresser cells inhibiting immune responsiveness suggests that histamines role in inflammatory disorders is more complex than was previously thought (Shearer et al., 1972; Mozes et al., 1974; Roszkowski et al., 1977) [16]. Histamine, along with prostaglandin's and adrenergic catecholamines, may play a major role in modulating the inflammatory response in both delayed and immediate hypersensitivity (Figure 13-1; Table 13-2) [17].

The demonstration of two subclasses of histamine receptors based on studies of agonists (2-methyl-histamine or 4-methyl-histamine) and antagonists (mempyramine, i.e., "classic" antihistamine or burimamide) as either H1 or H2 with selective tissue distribution and second messenger mediators has simultaneously clarified and complicated the pharmacology of histamine (Black et al., 1972; Chand and Eyre, 1975; Beaven, 1976) [18].

Histamine receptors on leukocytes that are involved in blocking further histamine release or in inhibiting lymphocyte-mediated cytotoxicity appear to be of the H2 type (Plaut et al., 1971; Lichtenstein and Gillespie, 1973; Plaut et al., 1975; Roszkowski et al., 1977) [19].

### **Notes:**

Histamine is a substance that acts on the vascular system, smooth muscle and secretory glands. It dilates both minor arteries and small veins acting independently of the motor nervous system. It can be partially suppressed by antihistamine drugs but is fully suppressed by amines such as noradrenaline [1].

Histamine increases the permeability of veins of up to 50 micrometers in diameter by separating the endothelial cells [2]. When it is injected a characteristic triple response reaction occurs. First a localised red spot appears with immediate vasodilation, then follows a bright red flush extending out from the primary site for about a centimetre or more. Thirdly oedemic fluid gathers in a localised area, termed a 'wheal', as the leakage of plasma through hyperpermeable vessel walls occurs [3].

Histamine produces itching but no pain and in high doses allows the migration of leukocytes through vessel walls [4]. Repeated doses cause a reduction in its chemical potential. Histamine acts to enhance the placement of immunoglobulins on endothelial tissues and antihistamines hinder this process [5].

Histamine is made by the decarboxylation of the essential amino acid histidine which is found widely distributed throughout the body. Free histamine is present only in trace amounts [6] but rather is stored in mast cells (also granulocytes) [7].

Mast cell degranulation is associated with changes in pH levels, ionic environment and temperature. Granulocytes degranulate to these factors, phagocytosis and antibody-antigen reactions [8].

The release of histamine contributes to the inflammatory process [9]. Inhibiting protein digesting enzymes results in lessened degranulation [10] and inadequate glucose or oxygen slows down cell degranulation. Almost any substance that causes tissue injury results in histamine release as well [11].

Two stages of inflammation can be observed in many types of injury, antihistamines act to suppress the first [12]. In inflammation caused by high or prolonged temperatures causative of severe tissue destruction, the early stage is less prominent than the late stage [13].

Histamine's role in inflammation is preparatory and is not essential for the development of lasting tissue changes [14]. Antihistamine drugs and inhibitors of histidine decarboxylase have limited usefulness as anti-inflammatory treatments [15].

T cell lymphocytes possess histamine receptors [16]. Histamine, prostaglandin's and adrenergic catecholamines are three major factors that act in delayed and immediate hypersensitivity reactions [17]. Of the two types of histamine receptor (h1 or h2) [18], the leukocytes have the h2 type. Leukocytes limit histamine release by suppressing lymphocyte immune actions (lymphocyte mediated cell damage) [19].

# Paper 6: Humoral Mediators of Inflammation

**Serotonin**[6.2] as a substance causes vasodilation and increases blood flow. It is involved in the functioning of smooth muscle and non-immediate communication of pain.

**Paper Number: 6**

**Reference Number: 6.2**

**Information taken from:**

Kenneth L. Melmon, M.D.

Howard F. Morrelli, M.D.

Clinical Pharmacology

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Functional action of serotonin

Page 661 Serotonin (5-hydroxytryptamine)

## **Serotonin (5-Hydroxytryptamine)**

The cardiovascular effects of exogenously administered serotonin are complex and variable because the direct and reflex actions of the amine may occur sequentially or sometimes almost simultaneously. In addition, the response varies depending on the route, speed and frequency of administration.

When serotonin is administered, the resultant vasodilation and increased blood flow resemble the changes observed during inflammation. Infusion of serotonin into the brachial artery of normal subjects causes the fingers to redden and then become a dusky blue colour. The colour changes are thought to be related to dilation and later constriction of the minute vessels of the skin [1].

In rodents, the combination of arteriolar dilation, venular constriction, and separation of endothelial cells occurring after subcutaneous administration of serotonin produces leakage of plasma from venules. In man serotonin has no prominent effects on vascular permeability. When applied to the base of a blister, serotonin causes severe pain, which may be delayed in onset but persists for long periods (Spector and Willoughby, 1965) [2]. The amine has only a modest and most likely unimportant effect on the emigration of leukocytes from blood vessels (Spector and Willoughby, 1964b) [3].

The results of studies on inflammation in different species show variability between species and often little relation to the changes seen in man. Implied is the need for studies of inflammation in man, the need for scepticism about facts extrapolated from animals to man but not tested in man, and the need to recognise the inadequacies of in vitro preparations used to screen new anti-inflammatory drugs.

Most serotonin in man (90-95%) is synthesised and localised in the enterochromaffin cells of the gastrointestinal mucosa and the serotonergic cells of the brain; some is present in blood platelets and spleen [4].

Platelets acquire the free amine by active transport from the blood. Once inside the platelet, serotonin is protected from metabolism until the platelet disintegrates. The mechanism for the release of stored serotonin is not well understood but appears to be related to platelet aggregation (possibly dependent on ATP and ADP), platelet breakdown (related to death, physical damage, or antigen-antibody alteration of its membrane), or direct damage to enterochromaffin cells [5].

Serotonin is present in inflammatory exudates for as long as one hour after injury. Whenever serotonin is present in inflammatory exudates, histamine is also present. Inhibitors of serotonin fail to influence the vascular changes of an inflammatory process in man (Spector and Willoughby, 1963) [6].

The limited permeability enhancing properties of serotonin are species dependent. During inflammatory processes in man, this property seems negligible. However, because of the limited information available regarding possible interaction of serotonin with other vasoactive substances, the precise role of the amine in inflammation must be kept open for review. The active contribution of a mediator may not be dramatic, but it may be critical for the direct effects of other mediators.

There is ample evidence that the mediators are physically, chemically, and pharmacologically interrelated (Miller and Melmon, 1970; Kaplan et al., 1971). Under such circumstances it would not be surprising to find that anti-serotonin activities may be manifested inconsistently as anti-histaminic or anti-adrenergic effects.

### **Notes:**

Serotonin has the action of causing vascular dilation and increasing blood flow [1]. When serotonin is applied to the base of a blister severe pain occurs which is delayed in onset but lasts for long periods [2]. Serotonin mildly effects the migration of leukocytes [3].

Over 90% of serotonin is made and localised in the gastrointestinal mucosa and the brain, but some is present in the spleen and blood platelets [4]. Platelets take free serotonin from the blood by way of active transport where it is stored until the platelet expires.

The release of stored serotonin involves platelet aggregation, breakdown of platelets or damage to cells in the intestine that contain catecholamines [5]. Serotonin is found in inflammatory secretions up to an hour after initiation, and when serotonin is present so is histamine. Inhibiting serotonin has no effect on the vascular system during inflammation [6].

# Paper 6: Humoral Mediators of Inflammation

The **catecholamines** [6.3]: Adrenaline and noradrenaline are suggested to contribute to the development of hemorrhagic lesions as well as to act as local anti-inflammatory hormones. These substances affect the sympathetic portion of the autonomic nervous system and relax the smooth muscle.

**Paper Number: 6**  
**Reference Number: 6.3**

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Howard F. Morrelli, M.D.  
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Functional action of catecholamines  
Page 663 Catecholamines

## **Catecholamines**

The catecholamines are not generally considered as mediators of inflammation. In certain situations, however, they, like serotonin, may alter the manifestations of inflammation. Epinephrine may contribute to the development of hemorrhagic lesions observed in some types of inflammatory processes, such as the Schwartzman phenomenon (McKay et al., 1969).

The catecholamines may act locally during inflammatory processes as endogenous anti-inflammatory hormones (see chapter 6) (Miller and Melmon, 1970) [1]. Experimental studies suggest that tissue injury results in augmented synthesis and destruction of catecholamines (Spector and Willoughby, 1965) [2].

The presence of receptors for catecholamines on leukocytes that are coupled to adenylyl cyclase suggests that catecholamines are capable of modulating the inflammatory response in vivo (Bourne et al., 1974). More information is required, however, before the direct and indirect roles of the catecholamines during inflammatory processes can be defined [3].

## **Notes:**

The catecholamines act locally as anti-inflammatory hormones [1]. Studies show that tissue injury results in increased catecholamine metabolism [2]. Leukocytes are known to possess receptors for catecholamines which are coupled to adenylyl cyclase [3].

# Paper 6: Humoral Mediators of Inflammation

The catecholamines: **Adrenaline** [6.4] and noradrenaline are suggested to contribute to the development of hemorrhagic lesions as well as to act as local anti-inflammatory hormones. These substances affect the sympathetic portion of the autonomic nervous system and relax the smooth muscle.

**Paper Number: 6**  
**Reference Number: 6.4**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of adrenaline  
Page 12 n. adrenaline

## **Adrenaline (Epinephrine)**

An important hormone secreted by the medulla of the adrenal gland. It has the function of preparing the body for 'fright, flight, or fight' and has widespread effects on circulation, the muscles, and sugar metabolism [1]. The action of the heart is increased, the rate and depth of breathing are increased, and the metabolic rate is raised; the force of muscular contraction improves and the onset of muscular fatigue is delayed [2].

At the same time the blood supply to the bladder and intestines is reduced, their muscle walls relax and the sphincters contract [3]. Sympathetic nerves were originally thought to act by releasing adrenaline at their endings, and were therefore called adrenergic nerves. In fact the main substance released is the related substance noradrenaline, which also forms a portion of the adrenal secretion [4].

Adrenaline given by injection is valuable for the relief of bronchial asthma, because it relaxes constricted airways. It is also used during surgery or by injection through an endoscope to reduce blood loss by constricting vessels in the skin or mucous membranes. It is included in some local anaesthetic solutions, particularly those used in dentistry, to prolong anaesthesia [5].

## **Notes:**

Adrenaline is a catecholamine hormone secreted by the adrenal medulla which acts on the circulation, muscles and sugar metabolism [1]. It increases the heart action, rate and depth of breathing, and metabolic rate whilst reinforcing muscular contraction and delaying muscle fatigue [2]. It reduces the blood supply to the bladder and intestines, relaxes their smooth muscle (involuntary) and makes the sphincter muscle (voluntary) contract [3].

It is the sympathetic nerves of the autonomic nervous system that release adrenaline in conjunction with noradrenaline [4]. Adrenaline is used to relieve bronchial asthma by relaxing the constricted smooth muscle airways. It is also used during surgical procedures to limit blood loss by constricting blood vessels in the skin and mucous membranes [5].

# Paper 6: Humoral Mediators of Inflammation

The catecholamines: Adrenaline and **noradrenaline** [6.5] are suggested to contribute to the development of hemorrhagic lesions as well as to act as local anti-inflammatory hormones. These substances affect the sympathetic portion of the autonomic nervous system and relax the smooth muscle.

**Paper Number: 6**  
**Reference Number: 6.5**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth edition 1994  
Oxford University Press  
Concise definition of adrenaline  
Page 449 n. noradrenaline

## **Noradrenaline (Norepinephrine)**

A hormone, closely related to adrenaline and with similar actions, secreted by the medulla of the adrenal gland and also released as a neurotransmitter by sympathetic nerve endings [1].

Among its many actions are constriction of small blood vessels leading to an increase in blood pressure, increased blood flow through the coronary arteries and a slowing of the heart rate, increase in the rate and depth of breathing, and relaxation of the smooth muscle in intestinal walls [2].

## **Notes:**

Noradrenaline is a catecholamine hormone closely related to adrenaline. Secreted by the adrenal medulla it has similar actions to adrenaline and acts as a neurotransmitter [1]. Among its actions are causing the constriction of small blood vessels, increasing blood pressure and circulation through the coronary arteries. It slows heart rate, increases respiration and relaxes smooth muscle [2].

# Paper 6: Humoral Mediators of Inflammation

**Peptides and Proteins [6.6]:** This category covers the intrinsic coagulation system, fibrinolytic system, components of complement and components of the kinin generation system. These groups are inter-utilized by the body sharing key components. The groups work in chain reaction existing under suppression by inhibitors. They play roles in oedema.

**Paper Number: 6**  
**Reference Number: 6.6**

**Information taken from:**  
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Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic principles in Therapeutics  
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Bailliere, Tindall london  
Functioning action of peptides and proteins  
Page 663 Peptides and Proteins

## **Peptides And Proteins**

Understanding the roles of peptides and proteins during the inflammatory process is a fascinating but difficult task. They are intimately involved in the pathogenesis of inflammation. The intrinsic coagulation system, fibrinolytic system, components of complement, and components of the kinin generating system are complex serum proteins [1].

Each system is usually suppressed by inhibitors, and activation requires a number of intermediary steps. The systems and processes of activation are interrelated and share key components of the other systems. For example, kallikrein and c'1 esterase probably have a common inhibitor [2].

Activation of Hageman factor can result in three effects: (1) Activation of prekallikrein to kallikrein and production of the kinin peptides; (2) Interaction with a plasminogen proactivator eventually leading to plasmin production; and (3) Inhibition of the coagulation cascade as the first step in the intrinsic clotting system. Each of these processes can make important contributions to the production of an inflammatory condition.

## **Notes:**

The blood coagulation system, fibrinolytic system, parts of the complement system and parts of the kinin generation system are all complexes of protein [1]. Each system has inhibitors and the systems and activators of them share key components [2].

# Paper 6: Humoral Mediators of Inflammation

**Kinins** [6.7] are polypeptides which function to produce arterial and venular dilation by direct action on smooth muscle. They are involved in pain production and are suggested to induce leukocyte adherence and migration during inflammation.

**Paper Number: 6**  
**Reference Number: 6.7**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
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Functioning action of peptides and proteins  
Page 663 Kinins

## **Kinins**

The term "kinin" refers to several polypeptides similar to bradykinin in structure and pharmacologic effect. The three that occur naturally in man include bradykinin, lysyl-brady-kinin (kallidin), and methionyllysyl-bradykinin [1].

Bradykinin may be considered a prototype for the kinins. It is a linear nonapeptide with a molecular weight of 1060 that has been isolated from plasma and synthesised (Webster and Pierce, 1963). In man, bradykinin is one of the most potent endogenous vasodilators known. The peptide produces arterial and venular dilation by direct action on smooth muscle (Kellermeyer and Graham, 1968) [2].

Bradykinin increases venular permeability, causing formation of wheal at intradermal concentrations as low as  $10^{-9}$  M. On a molar basis, the peptide is said to be 15 times more active than histamine in producing this effect [3].

Bradykinin is a very powerful pain producing agent when applied to a blister base or injected intra-arterially or intradermally in humans (Kellermeyer and Graham, 1968). The peptide may also cause leukocyte adherence and migration during inflammation, but the evidence for these effects is inconclusive and may be dependent on the model chosen for experimental study [4].

Potentially, 4 to 11 mg of bradykinin can be derived from 1 litre of human plasma. Under normal conditions almost all of the peptide exists in an inactive precursor form and is liberated from a plasma alpha-2-globulin (kininogen) by the peptidase or esterolytic action of enzymes called kallikrein's [5].

Presumably, kininogen is produced by the liver, but the factors controlling its production have not been defined. The highest concentrations of kallikrein's are in glandular tissue (parotids, pancreas, sweat glands, etc.), plasma, and urine in man [6].

Many reactions involved in kinin generations are well characterised (Erdos, 1966b; Schachter, 1969; Pisano and Austen, 1976). Plasma kallikrein ordinarily exists in an inactive form called prekallikrein and requires activation by other enzymes. Activation of kallikrein is usually associated with initiation of coagulation, temperature changes, immune mechanism, and local tissue factors [7].

Hageman factor (factor XII) is usually activated first and is responsible for the conversion of prekallikrein to kallikrein. In order for Hageman factor to be completely activated, prekallikrein and high molecular weight kininogen are required [8]. Deficiency of prekallikrein (Fletcher factor deficiency) is characterised by low or diminished activity of Hageman factor, abnormalities of coagulation, fibrinolysis, chemotactic activity, and kinin production (Weiss et al., 1974) [9].

Deficiency of high molecular weight kininogen (Williams trait) will also result in abnormalities of Hageman factor-dependant pathways (Colman et al., 1975, 1976) [10]. As with histamine release, almost any process causing tissue injury can trigger the series of events resulting in the production of bradykinin. All such stimuli may not effect kinin generation by similar sequences, but activation of surface active clotting factors, plasmin, or thrombin or disturbances of granulocyte membranes, can contribute to kinin generation (Kaplan et al., 1971; Miller 1975) [11].

Once formed, bradykinin has a very short half life in the circulation (measured in seconds). Blood plasma, erythrocytes, granulocytes and most tissues contain enzymes called kininase's, which are capable of rapidly inactivating bradykinin (Erdos, 1966a) [12]. A bradykinin like substance that produces pain has been isolated from human blister fluid and from inflammatory exudates and synovial fluid during acute arthritides of varying etiologies (Nies and Melmon, 1968) [13].

Bradykinin may play a role in the development of thermic edema (Rocha et Silva, 1964) and may be important in the pathogenesis of endotoxic shock (Nies et al., 1968a; 1968b; Nies and Melmon, 1971; Nies et al., 1971) [14].

Pulmonary and renal angiotensin converting enzymes probably are also important kininase's. Although the significance of this association is not clear at the represent time, it does point out the potential for interactions between the two systems in inflammation where the vascular state and subsequent reactions determine the intensity and course of the inflammatory process [15].

Furthermore, urinary kallikrein seems to have an important homeostatic role related to salt metabolism and blood pressure (Geller et al., 1972; Margolius et al., 1972a, 1972b, 1974; Webster et al., 1976) [16]. Plasma kallikrein might, independent of kinin formation, contribute to altered vascular permeability in inflammatory conditions.

Finally, although it is speculative, it is conceivable that patients with inflammation may develop abnormalities in homeostasis for blood pressure and those with abnormalities of blood pressure could have anomalous responses to an inflammatory stimulus [17].

## **Notes:**

Three kinins occur in man, each is similar in structure and action to bradykinin [1]. Bradykinin is considered to be the prototype for the kinins and is one of the most powerful natural dilators of the vascular system. It produces arterial and venular dilation by directly acting on the smooth muscle [2].

Bradykinin increases the permeability of venules, producing wheal and flare like that of histamine, however 15 times less is needed for the same reaction [3]. Bradykinin is a strong pain producing agent and may cause the adherence and migration of leukocytes during inflammation [4]. Existing as the inactive precursor kininogen it becomes activated by the peptidase or esterolytic enzymes known as kallikreins [5].

The highest concentrations of kallikreins in the glandular tissues, plasma and urine [6]. Kallikreins exist as the inactive precursor prekallikrein and is activated by blood coagulation, temperature changes, immunoactivity and local tissue factors [7].

Hageman factor (involved in blood coagulation) is usually activated first and brings about, prekallikrein's conversion to kallikrein. Both kininogen and prekallikreins are required to fulfil the activation of Hageman factor [8].

Deficiency of prekallikrein results in the low activity of Hageman factor, abnormal coagulation, fibrinolysis, chemotactic activity and kinin production [9]. Deficiency of kininogen also results in abnormalities of Hageman factor reliant systems [10].

Almost any tissue injury results in the production of bradykinin. Surface active clotting factors, plasmin, thrombin, and granulocyte activity influences kinin production [11]. Bradykinin has a short half life in circulation. Blood plasma, erythrocytes, granulocytes and most tissue contain enzymes that deactivate bradykinin's [12].

Blister fluid, inflammatory exudates and synovial fluid all exhibit a bradykinin like substance capable of producing pain [13]. Bradykinin has an involvement in heat induced oedema and anaphylaxis [14].

Lung and kidney enzymes involved in angiotensin metabolism (a hormone which affects blood pressure) are likely to be involved in interactions with kinin production during inflammation [15].

Urinary kallikrein has an important role in maintaining a balance between salt metabolism and blood pressure [16]. Plasma kallikrein that is independent of kinin formation may contribute to alterations in vascular permeability during inflammatory conditions [17].

# Paper 6: Humoral Mediators of Inflammation

**Activated complement components [6.8]** is a group of compounds which act to cause late-acting, extensive cellular damage by fixing to cells and causing their mediators to be released and activated. Both leukocytes and lung macrophages are receptive to components of complement. Complement causes the degranulation of mast cells which occurs during smooth muscle contraction. It enhances phagocytosis and changes in vascular permeability.

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**Reference Number: 6.8**

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Kenneth L. Melmon, M.D.  
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Functioning action of peptides and proteins  
Page 664 Activated Complement Components

## **Activated Complement Components**

The human complement system consists of at least 11 distinct serum proteins and three inhibitors (those of C1, C3 and C6). The 11 complement proteins in the classic pathway have been divided into three functional units that react in fixed sequences and are directed against cell membranes [1]: The Recognition Unit (C1q, C1r, C1s);- The Activation unit (C2, C3, C4);- The Membrane Attack Unit (C5, C6, C7, C8, C9) (Alper and Rosen, 1975; Muller-Eberhard, 1975; Volvyrn, 1976) [2].

An alternate (or properdin-dependent) pathway for complement activation also exists. The alternative pathway involves at least five proteins with activation of C3 that bypasses C1, C4 and C2 [3]. The alternate pathway is initiated by complex polysaccharides, aggregates of IgA, and some bacterial endotoxins; the activation may be independent of immunoglobulin (Ruddy et al., 1972; Vogt, 1974; Fearon and Austen, 1975). The mechanism of activation of the alternate pathway is not well understood [4].

C3, once activated by either the classic or alternate pathway, interacts with other serum components to activate the late acting complement factors that cause extensive cellular damage. Some cells such as leukocytes, lung macrophages, and those in the glomerulus appear to possess receptors for components of complement (Arnaiz-Vellena and Hay, 1975; Gelfand et al., 1975; Rabellino and Mecalfé, 1975; Reynolds et al., 1975) [5].

When cells such as platelets or mast cells that contain biologically active mediators are the target of a complement reaction, their mediators may be released and activated. Activation of complement is not restricted to cell membranes of individual circulating cells [6].

Blood vessel walls are also sites (Arthus reaction and glomerulonephritis) in which deposition of immune complexes and fixation of complement have been demonstrated. Activation of the complement system need not inevitably progress to its full expression of cell membrane lesions and cytotoxicity, but intermediate reaction products and complexes are formed that have pharmacologic properties important to the inflammatory process [7].

Anaphylatoxin, a compound generated from either the C3 or C5 components of complement, is a substance of low molecular weight that can release histamine from mast cells, cause smooth muscle contraction, and change in vascular permeability. It has been implicated in the development of anaphylaxis and other allergic reactions, but its precise biologic significance has not been fully determined [8].

The activated C1 component of complement promotes activation of other complement components and causes degranulation of mast cells, resulting in histamine release and in smooth muscle contraction. Activated components of complement can also participate in the inflammatory process by virtue of their chemotactic properties and their ability to enhance phagocytosis [9]. In addition to forming anaphylatoxin, C3 causes immune adherence, conglutination, chemotaxis, and enhanced phagocytosis by granulocytes. C5, C6 and C7 can form complexes that are chemotactic and can release histamine independently of anaphylatoxin formation (Muller-Eberhard, 1968) [10].

Principle: The complex interrelationships among proteins, peptides and amine demonstrate that there is no simple approach to the inflammatory process or its treatment. Deficiency of C1 esterase inhibitor can be demonstrated in a unique clinical syndrome: hereditary angioneurotic edema (Donaldson and Rosen, 1966; Schur and Austin, 1968; Donaldson, 1972). Patients with this genetically determined serum protein abnormality may have life threatening symptoms and signs similar to those of severe allergy and are unresponsive to the therapy used for the more common varieties of angioneurotic edema (Thorvaldsson et al., 1969).

Although the biochemical hallmark of this disease is deficiency of C1 esterase, the relationship of the protein deficiency to the expressions of the angioneurotic edema remains unclear (Frank et al., 1976). Thus not only may the active components of a system be involved in an inflammatory response, but malfunction of the modulators of the system must also be considered as key disturbances in an inflammatory reaction.

### **Notes:**

The complement system is made up of upwards of 11 serum proteins and 3 inhibitors. These act in sequence directly against cell membranes [1]. The proteins are divided into a recognition unit, an activation unit and a membrane attack unit [2]. An alternative activation unit also exists [3], which is initiated by complex polysaccharides, IgA aggregates and various bacterial toxins [4].

Leukocytes, lung macrophages, and kidney macrophages all possess receptors for complement [5]. Complement can release and activate cell contents from platelets and mast cells [6]. Blood vessel walls are also targets of immune complexes and complement [7].

Anaphylatoxin is a substance generated from complement which acts to release mediators from mast cells, cause smooth muscle contraction and influence vascular permeability. Anaphylatoxin is involved in the complement reaction of anaphylaxis and allergic reactions [8]. Complement of the recognition unit promotes the activation of the other complement units that act to degranulate mast cells, release histamine and contract smooth muscle.

Activated complement is involved in the inflammatory process due to its ability to attract immune cells (chemotaxis) and enhance phagocytosis [9]. The complement component involved in forming anaphylatoxin also causes immune adherence, conglutination, chemotaxis and enhances phagocytosis by granulocytes [10].

# Paper 6: Humoral Mediators of Inflammation

**Components of the blood clotting system [6.9]** interact with other plasma proteins such as the components of complement, fibrin, and the kinin system. Their function in the body is to clot - a suppressing factor being heparin.

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**Reference Number: 6.9**

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Kenneth L. Melmon, M.D.  
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Functioning action of peptides and proteins  
Page 667 Components of the Blood Clotting System

## **Components Of The Blood Clotting System**

The coagulation system is extremely complex. Activation of the intrinsic clotting system is initiated by activation of Hageman factor (factor XII); this agent then interacts with the complement and kinin-kallikrein systems [1]. In addition to promoting the generation of vasoactive substances, activated Hageman factor by itself is capable of producing increased vascular permeability [2].

Other components of the clotting system may have important functions in the development of an inflammatory process, e.g. fibrin, which can be leukotactic and is an essential component for the development of the classic Schwartzman reaction (Vassalli and McCluskey, 1964; McKay et al., 1969). Plasmin interacts with other plasma proteins such as components of complement, fibrin and the kinin system. Such interactions might be significant in terms of an inflammatory process, but proof of the relevance of these interactions to the inflammatory process has not yet been obtained (Eisen, 1969; Hamberg, 1969) [3].

In disseminated intravascular coagulation (e.g. associated with endotoxemia), the inter-relationships of the inflammatory process with abnormalities of coagulation may be critical to the outcome of the disease. The best "anti inflammatory agent" in such a clinical setting is the drug that can stop the coagulation process - heparin [4].

## **Notes:**

Blood coagulation is complicated. Hageman factor initiates the intrinsic clotting system and goes on to interact with complement and kinin systems [1]. Activated Hageman factor promotes vasoactive substances and vascular permeability [2]. Plasmin, the enzyme which digests fibrin also interacts with complement and the kinin system [3]. Heparin is used in clinical settings as an anti-inflammatory agent.[4].

# Paper 6: Humoral Mediators of Inflammation

**Prostaglandins [6.10]** are lipid compounds which produce an array of metabolic effects. Widely distributed throughout the body, each prostaglandin has its own spectrum of activities which is tissue specific. Prostaglandins have chemical properties which are not dose-dependent and are physiologically potent substances important to inflammation.

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Functioning action of prostaglandins  
Page 667 Prostaglandins

## **Prostaglandin's**

The term "prostaglandin's" was first introduced by Von Euler in 1935 to describe a smooth muscle stimulating lipid that he believed to be derived from the prostate. The term is now the generic name for a family of naturally occurring lipids characterised by a unique oxygenated fatty acid structure incorporating a five-membered ring (Bergstrom et al., 1963, 1965, 1968; Caton, 1973) [1].

More than a dozen compounds are recognised members of the principal classes of prostaglandin's, their precursors, and metabolites. Prostaglandin's are named by letters according to specific functional groups attached to the five membered ring of "prostanic acid" and the first numerical subscript denoting the number of double bonds contained in the side chains. The alpha and beta subscripts for PFG designate whether the hydroxyl group at C9 is on the same side of the molecule as the carboxylic acid group, (alpha), or is on the opposite side, (beta). The naturally occurring F prostaglandin's all have the alpha configurations (Pike, 1972; Caton, 1973) [2].

The prostaglandin's are considered pathogenically important in a number of settings, but they have not been definitely proven essential to any of them. As a group, they can produce an array of metabolic effects. Individually, each prostaglandin has its own spectrum of activities that is both tissue and species specific. Even in the same organ system prostaglandin's can oppose the action of others [3].

For example, different prostaglandin's F1 alpha and F2 alpha contract all segments of human fallopian tubes; PFG3 relaxes the same segments; PGE1 and PGE2 contract the uterine end but relax the ovarian end (Sandberg et al., 1965). PGE1 stimulates the uterus of the human, cat, guinea pig and rat, but relaxes the rabbit uterus (Berti and Naimzada, 1965; Horton et al., 1965).

The diverse activity of the prostaglandin's in a single organ system is confusing enough, but, in addition, they can produce both direct and indirect effects in virtually every mammalian tissue studied [4]. The prostaglandin's have been suggested as mediators of inflammation. They are released during inflammatory reactions (Greaves et al., 1971). Small amounts of PGE1 and PGE2 produce a wheal and flare at the site of injection partially by release of vasoactive amines (Crunkhorn and Willis, 1971; Sondergaard and Greaves, 1971) [5].

There are intricate relationships between intracellular calcium ions, adenylyl cyclase activity, biologic effects of cyclic AMP, and intracellular prostaglandin concentrations (Silver and Smith, 1975) [6]. Perturbation of tissue membranes may change the biosynthesis of prostaglandin's and their precursors that also have potent biologic effects. All can contribute to the intensity and duration of responses to an inciting inflammatory stimulus [7].

Since low concentrations of cyclic AMP can stimulate the activity of at least one of the enzymes involved in the metabolism of the prostaglandin's, a negative feedback control mechanism may be operative (Pike, 1972) [8]. In some tissues certain prostaglandin's inhibit cyclic AMP production in response to hormonal stimulation (Hittelman and Butcher, 1973; Kahn and Lands, 1973) [9].

There is no direct evidence for an essential role for prostaglandin's in the cellular responses we are considering, but their potency, presence, and the effects of drugs that reverse their actions all suggest that they are important mediators in the inflammatory response.

Detailed information concerning the biosynthesis of prostaglandin's is available elsewhere (Goodman and Gilman, 1975). Prostaglandin's are formed from membrane bound stores of polyunsaturated essential fatty acids by the series of complex reactions. The requirements for the enzymatic synthesis are 20-carbon fatty acids that have multiple unsaturated sites. Molecular oxygen, co-factors acting as reducing agents, and the multi-enzyme complex prostaglandin synthetase are also required. The enzymes that synthesize prostaglandin's are in all mammalian tissues except red blood cells (Ramwell and Shaw, 1970) [10].

At each step the reaction can be inhibited or stimulated. The mechanisms controlling biosynthesis of the prostaglandin's are not known. Recent detailed studies of the biosynthesis of the prostaglandin's have resulted in the isolation and identification of new intermediates in this pathway as well as some untested antagonists of specific intermediates [11].

These highly reactive, but short lived, intermediates, called endoperoxides and thromboxanes, have potent biological effects intracellularly. In some systems, the effects previously ascribed to prostaglandin's result from the endoperoxides. These substances are primarily metabolised to nonprostanoate structures with only a small amount converted to prostaglandin (Hamberg et al., 1974a, 1974b). The mechanism by which the prostaglandin's and prostaglandin intermediates reach the extracellular environment is unknown [12].

The edema produced by the prostaglandin's is not dose dependent and is substantially less than can be produced with equimolar quantities of histamine or bradykinin (Williams and Morley, 1973) [13]. Leukocytes produce prostaglandin's; their production is enhanced during phagocytosis. PGE1 in minute amounts is chemotactic for leukocytes; by virtue of their ability to synthesise prostaglandin's, leukocytes can significantly contribute to the production of prostaglandin's found in some inflammatory lesions (Higgs et al., 1975) [14].

In addition, increased prostaglandin's are produced during antigenic stimulation of the spleen (Webb and Osheroff, 1976) and by dispersed synovial cells derived from patients with rheumatoid arthritis (Dayer et al., 1976) [15].

Different prostaglandin's synthesised locally can contribute to both the continuation and termination of an inflammatory process depending on the specific prostaglandin synthesised. For example, PGE1 decreases inflammation by inhibiting aggregation of leukocytes and lysosomal release from them (Emmons et al., 1967; Robison et al., 1971; Weissmann et al., 1975) [16].

However PGE1 can also increase the intensity of an inflammatory process by virtue of its chemotactic properties (Higgs et al., 1975). PGE2 stimulates platelet aggregation and may contribute to the development of an inflammatory process.

These mixed effects of prostaglandin's, including their ability to increase the concentration of cellular cyclic AMP in a variety of formed elements of the blood, may help to explain the anti inflammatory properties of some inflammatory exudates (Atkinson et al., 1971) [17]. Prostaglandin's are released during fever and can produce fever themselves when administered into the cerebral ventricles in experimental animals or when given systematically to human subjects (Feld Fledberg and Gupta, 1973). Pyrogen induced fever can be diminished by administration of inhibitors of prostaglandin synthesis such as aspirin or indomethacin [18].

The prostaglandin's can influence the course of inflammation through their interaction with other mediator substances, e.g. additive effects with histamine or bradykinin and alteration in vascular and metabolic responses to norepinephrine, bradykinin, and angiotensin (Steinberg et al., 1964; Moncada et al., 1973; Williams and Morley, 1973; Messina et al., 1975).

In concentrations likely to be found at sites of inflammation, the prostaglandin's do not cause pain themselves, but can sensitise the pain receptors to mechanical and chemical stimulation. The analgesic action of drugs such as aspirin may be partly due to the reversal of prostaglandin induced sensitisation of nerves during an inflammatory process (Ferreira et al., 1973; Moncada et al., 1973) [19].

As mentioned previously, the prostaglandin's can stimulate cyclic AMP production, but, in addition, the prostaglandin's also modify the second messenger system for other hormones (the adenylyl cyclase - cyclic AMP - effector system) (Pike, 1972) [20].

The prostaglandin's are rapidly metabolised in the liver, lungs and other tissues (Samuelsson et al., 1971). Their biologic half life in the circulation is only a few minutes (Raz, 1972). More than 80% of prostaglandin's E1, E2 and F2 alpha is metabolised during a single passage through the lungs or liver, but even small amounts of these potent substances may be pharmacologically or therapeutically important (Vane, 1969) [21].

Generally, human tissues do not store prostaglandin's; rather it appears that their pathophysiologic effects occur as a result of immediate synthesis and release from cells and tissues whose membranes are stimulated (Ramwell and Shaw, 1970; Silver et al., 1972, 1973). Prostaglandin's are local hormones; they apparently influence the metabolic events in the same cells or the neighbours of cells that synthesise and metabolise them [22].

The presence of the asymmetric centres of the prostaglandin molecules enables chemists to synthesise many isomers and congeners. In some cases, the analogues have entirely different pharmacologic effects and pharmacokinetic behaviour.

These findings are being used to seek analogues for therapeutic uses either in mimicking selected effects of prostaglandin's, in competitively blocking some of their effects, or in designing agonists or antagonists that are tissue specific. Generally, metabolism of the prostaglandin's results in diminished biologic activity; 15 keto-PGF2 alpha has potent bronchoactivity and is a notable exception [23].

### **Notes:**

Prostaglandins are a family of smooth muscle stimulating lipids made from oxygenated fatty acids [1]. More than a dozen compounds are considered prostaglandins [2] and collectively they produce various metabolic effects. Individually each prostaglandin has wide activity which is specific to tissues, even in the same system of the body the actions of prostaglandin's can oppose each other [3]. They can produce direct and indirect effects in virtually all mammalian tissues [4].

They are released during inflammatory reactions and are known to produce wheal and flare reactions by the release of vasoactive amines [5]. There are interrelations between prostaglandin's, calcium, adenylyl cyclase and cyclic AMP [6]. Disturbance of the tissue membrane influences prostaglandin synthesis and as substances they contribute to the intensity and duration of the inflammatory reaction [7].

Low cyclic AMP levels can stimulate the activity of at least one of the enzymes involved in prostaglandin metabolism [8]. In some tissues prostaglandins inhibit cyclic AMP production in response to hormonal stimulation [9].

Formed from polyunsaturated essential fatty acids that are bound to cell membranes, they require 20-carbon fatty acids which have multiple unsaturated sites, molecular oxygen, cofactor reducing agents and the multi-enzyme complex prostaglandin synthetase. The enzymes required to make prostaglandins are present in all mammalian tissues except in red blood cells [10], and synthesis can be inhibited or stimulated at each metabolic stage [11].

Intermediates of the prostaglandin family have potent, highly reactive, short-lived actions and are mainly metabolised to non-prostaglandin related substances; only a small proportion are converted to formal prostaglandins [12]. The oedema caused by prostaglandin's is not dose-dependent. Molecule for molecule substantially less prostaglandin's are required to produce oedema in comparison to histamine or bradykinin [13].

Prostaglandin production in leukocytes is enhanced during phagocytosis having the effect of attracting leukocytes to the place of formation. Leukocytes can contribute to prostaglandin levels during inflammation [14]. Levels increase when tissues are stimulated by allergens. Prostaglandin's are released by the cells of joints in rheumatoid arthritis [15].

Local prostaglandin levels influence the continuation or cessation of inflammation [16]. Understanding the action of the prostaglandins may explain the anti-inflammatory properties of inflammatory exudates [17].

Prostaglandins affect the body's temperature and inhibitors of prostaglandins (such as aspirin) reduce body temperature [18]. Prostaglandin's also sensitise pain receptors to stimulus, and inhibitors (such as aspirin) possess an analgesic effect [19].

Prostaglandin's stimulate cyclic AMP levels and change the 'second messenger' system which acts to carry a chemical message from a hormone into the cell [20].

Rapidly metabolised in the liver, lungs and other tissues, a large proportion of these lipid hormones are metabolised during a single passage through the lungs or liver [21]. Generally they are formed after a cell or a tissue membrane is stimulated as opposed to the body having stores of them.

Prostaglandins are local hormones which have influence in the same or neighbouring cells that produced them [22]. In general the metabolism of the prostaglandin's leads to a decline in activity but the prostaglandin 15 keto PGF<sub>2</sub> alpha (which has a potent action on the lungs) is an exception [23].

# Paper 6: Humoral Mediators of Inflammation

**Heparin [6.11]** is a naturally occurring anticoagulant in the blood and other tissues. It is comprised of glucosamine, glucuronic acid and varying proportions of sulphate and acetyl groups. The heparin content of tissues correlates directly with the number of mast cells present. Preventing the coagulation of fibrinogen, heparin also functions as the structural precursor of lipoprotein lipase; an enzyme involved in the metabolism of lipids in the body.

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**Reference Number: 6.11**

**Information taken from:**  
 Percy J. Russell  
 Anita Williams  
 The Nutrition and Health Dictionary  
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 Chapman & Hall  
 Definition of heparin  
 Page 213 Heparin

## **Heparin**

The naturally occurring anticoagulant in blood and other tissues containing glucosamine, glucuronic acid, and varying proportions of sulphate and acetyl groups. Its structure is not entirely clear [1]. It is produced by the mast cells of the connective tissue and is stored as granules within the cells. The heparin content of tissues correlates with the number of mast cells present [2].

Heparin is secreted into the intercellular substance and functions there to prevent the fibrinogen that escapes from capillaries from forming fibrin clots [3]. It also functions in the formation or activation of lipoprotein lipase, which clears chylomicrons from the blood plasma [4].

## **Notes:**

Heparin is a natural anti-coagulant in the body consisting of glucosamine, glucuronic acid, sulphate and acetyl groups [1]. It is produced and stored by the mast cells in connective tissue, the heparin content of tissues serves as a relative index to the number of mast cells present [2]. Heparin is secreted into the intercellular substance to prevent fibrinogen clotting [3]. A function of heparin is to make lipoprotein lipase which is an enzyme which metabolises lipids in the body [4].

# Paper 6: Humoral Mediators of Inflammation

**Mucopolysaccharides [6.12]** are a class of compound that consist in general of a sugar (example: glucosamine), a uronic acid (example: glucuronic acid) and a protein which is covalently linked. They provide the tissues with resistance to compression and act as lubricants. They are part of a broader group known as the glycosaminoglycans.

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**Reference Number: 6.12**

**Information taken from:**

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 A. R. Leech  
 Biochemistry For The Medical Sciences  
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 Definition and function of Glycosaminoglycans  
 Page 596 Glycosaminoglycans

## **Glycosaminoglycans**

A large number of the polysaccharides that occur in mammalian connective tissue are glycosaminoglycans. They form a fairly homogenous group of carbohydrates and are broadly defined by possession of the following characteristics [1]:

- (1) They contain a repeating disaccharide unit, consisting of a sugar (such as glucosamine, usually N-acetylated) and a uronic acid (except in keratan sulphate).
- (2) The uronic acid is usually esterified with sulphate (except in hyaluronate).
- (3) The polysaccharide is usually covalently linked to a protein to form a proteoglycan (also known as a mucopolysaccharide) [2].

Most glycosaminoglycan chains contain fewer than 100 monosaccharide units but there may be as many as 50 chains attached to a single core protein in the proteoglycan. Hyaluronate chains are longer and may contain 5000 monosaccharide residues .

## **Notes:**

Glycosaminoglycans refers to a large number of polysaccharides that are found in connective tissues. They are all fairly uniform having similar characteristics which are [1]: They contain a repeating sugar unit (such as glucosamine) and a uronic acid (such as glucuronic acid) which is often esterified with sulphate. If the polysaccharide is covalently linked to a protein it makes a mucopolysaccharide [2].

# Paper 6: Humoral Mediators of Inflammation

In **summary [6.13]** the immune system during inflammation is causing the deliberate degranulation and damaging of specific cells - The mast cells. The body employs cells of the immune system which have the ability to attack and digest not only allergens and bacteria but the bodies own tissues.

**Paper Number: 6**  
**Reference Number: 6.13**

**Information taken from:**

Aggregate notes for Paper 6

## **-: Paper Six - Humoral Mediators of Inflammation :-**

- **Histamine, serotonin and kinins are released which dilate the vascular system by acting on the smooth muscle -**
  - **Adrenaline and noradrenaline are released which both act to relax smooth muscle -**
  - **Components of complement act to degranulate mast cells and contract smooth muscle -**
  - **The blood clotting system shares components with the complement system and the kinin generating system -**
- **The prostaglandins are lipid hormones that act on smooth muscle and contribute to the intensity and duration of the inflammatory reaction -**
  - **Heparin and mucopolysaccharides are released from mast cells during the ensuing inflammatory reaction -**
  - **Control of smooth muscle is the major theme in anaphylactic reactions -**

Histamine is a substance that acts on the vascular system, smooth muscle and secretory glands. It dilates both minor arteries and small veins acting independently of the motor nervous system. It can be partially suppressed by antihistamine drugs but is fully suppressed by amines such as noradrenaline.

Histamine increases the permeability of veins of up to 50 micrometers in diameter by separating the endothelial cells. When it is injected a characteristic triple response reaction occurs. First a localised red spot appears with immediate vasodilation, then follows a bright red flush extending out from the primary site for about a centimetre or more. Thirdly oedemic fluid gathers in a localised area, termed a 'wheal', as the leakage of plasma through hyperpermeable vessel walls occurs.

Histamine produces itching but no pain and in high doses allows the migration of leukocytes through vessel walls. Repeated doses cause a reduction in its chemical potential. Histamine acts to enhance the placement of immunoglobulins on endothelial tissues and antihistamines hinder this process.

Histamine is made by the decarboxylation of the essential amino acid histidine which is found widely distributed throughout the body. Free histamine is present only in trace amounts but rather is stored in mast cells (also granulocytes).

Mast cell degranulation is associated with changes in pH levels, ionic environment and temperature. Granulocytes degranulate to these factors, phagocytosis and antibody-antigen reactions.

The release of histamine contributes to the inflammatory process. Inhibiting protein digesting enzymes results in lessened degranulation and inadequate glucose or oxygen slows down cell degranulation. Almost any substance that causes tissue injury results in histamine release as well.

Two stages of inflammation can be observed in many types of injury, antihistamines act to suppress the first. In inflammation caused by high or prolonged temperatures causative of severe tissue destruction, the early stage is less prominent than the late stage.

Histamine's role in inflammation is preparatory and is not essential for the development of lasting tissue changes. Antihistamine drugs and inhibitors of histidine decarboxylase have limited usefulness as anti-inflammatory treatments.

T cell lymphocytes possess histamine receptors. Histamine, prostaglandin's and adrenergic catecholamines are three major factors that act in delayed and immediate hypersensitivity reactions. Of the two types of histamine receptor (h1 or h2), the leukocytes have the h2 type. Leukocytes limit histamine release by suppressing lymphocyte immune actions (lymphocyte mediated cell damage).

Serotonin has the action of causing vascular dilation and increasing blood flow. When serotonin is applied to the base of a blister severe pain occurs which is delayed in onset but lasts for long periods. Serotonin mildly effects the migration of leukocytes.

Over 90% of serotonin is made and localised in the gastrointestinal mucosa and the brain, but some is present in the spleen and blood platelets. Platelets take free serotonin from the blood by way of active transport where it is stored until the platelet expires.

The release of stored serotonin involves platelet aggregation, breakdown of platelets or damage to cells in the intestine that contain catecholamines. Serotonin is found in inflammatory secretions up to an hour after initiation, and when serotonin is present so is histamine. Inhibiting serotonin has no effect on the vascular system during inflammation.

The catecholamines act locally as anti-inflammatory hormones. Studies show that tissue injury results in increased catecholamine metabolism. Leukocytes are known to possess receptors for catecholamines which are coupled to adenylyl cyclase.

Adrenaline is a catecholamine hormone secreted by the adrenal medulla which acts on the circulation, muscles and sugar metabolism. It increases the heart action, rate and depth of breathing, and metabolic rate whilst reinforcing muscular contraction and delaying muscle fatigue. It reduces the blood supply to the bladder and intestines, relaxes their smooth muscle (involuntary) and makes the sphincter muscle (voluntary) contract.

It is the sympathetic nerves of the autonomic nervous system that release adrenaline in conjunction with noradrenaline. Adrenaline is used to relieve bronchial asthma by relaxing the constricted smooth muscle airways. It is also used during surgical procedures to limit blood loss by constricting blood vessels in the skin and mucous membranes.

Noradrenaline is a catecholamine hormone closely related to adrenaline. Secreted by the adrenal medulla it has similar actions to adrenaline and acts as a neurotransmitter. Among its actions are causing the constriction of small blood vessels, increasing blood pressure and circulation through the coronary arteries. It slows heart rate, increases respiration and relaxes smooth muscle.

The blood coagulation system, fibrinolytic system, parts of the complement system and parts of the kinin generation system are all complexes of protein. Each system has inhibitors and the systems and activators of them share key components.

Three kinins occur in man, each is similar in structure and action to bradykinin. Bradykinin is considered to be the prototype for the kinins and is one of the most powerful natural dilators of the vascular system. It produces arterial and venular dilation by directly acting on the smooth muscle.

Bradykinin increases the permeability of venules, producing wheal and flare like that of histamine, however 15 times less is needed for the same reaction. Bradykinin is a strong pain producing agent and may cause the adherence and migration of leukocytes during inflammation. Existing as the inactive precursor kininogen it becomes activated by the peptidase or esterolytic enzymes known as kallikrein's.

The highest concentrations of kallikreins in the glandular tissues, plasma and urine. Kallikreins exist as the inactive precursor prekallikrein and is activated by blood coagulation, temperature changes, immunoactivity and local tissue factors.

Hageman factor (involved in blood coagulation) is usually activated first and brings about, prekallikrein's conversion to kallikrein. Both kininogen and prekallikreins are required to fulfil the activation of Hageman factor.

Deficiency of prekallikrein results in the low activity of Hageman factor, abnormal coagulation, fibrinolysis, chemotactic activity and kinin production. Deficiency of kininogen also results in abnormalities of Hageman factor reliant systems.

Almost any tissue injury results in the production of bradykinin. Surface active clotting factors, plasmin, thrombin, and granulocyte activity influences kinin production. Bradykinin has a short half life in circulation. Blood plasma, erythrocytes, granulocytes and most tissue contain enzymes that deactivate bradykinin's.

Blister fluid, inflammatory exudates and synovial fluid all exhibit a bradykinin like substance capable of producing pain. Bradykinin has an involvement in heat induced oedema and anaphylaxis.

Lung and kidney enzymes involved in angiotensin metabolism (a hormone which affects blood pressure) are likely to be involved in interactions with kinin production during inflammation.

Urinary kallikrein has an important role in maintaining a balance between salt metabolism and blood pressure. Plasma kallikrein that is independent of kinin formation may contribute to alterations in vascular permeability during inflammatory conditions.

The complement system is made up of upwards of 11 serum proteins and 3 inhibitors. These act in sequence directly against cell membranes. The proteins are divided into a recognition unit, an activation unit and a membrane attack unit. An alternative activation unit also exists, which is initiated by complex polysaccharides, IgA aggregates and various bacterial toxins.

Leukocytes, lung macrophages, and kidney macrophages all possess receptors for complement. Complement can release and activate cell contents from platelets and mast cells. Blood vessel walls are also targets of immune complexes and complement.

Anaphylatoxin is a substance generated from complement which acts to release mediators from mast cells, cause smooth muscle contraction and influence vascular permeability. Anaphylatoxin is involved in the complement reaction of anaphylaxis and allergic reactions. Complement of the recognition unit promotes the activation of the other complement units that act to degranulate mast cells, release histamine and contract smooth muscle.

Activated complement is involved in the inflammatory process due to its ability to attract immune cells (chemotaxis) and enhance phagocytosis. The complement component involved in forming anaphylatoxin also causes immune adherence, conglutination, chemotaxis and enhances phagocytosis by granulocytes.

Blood coagulation is complicated. Hageman factor initiates the intrinsic clotting system and goes on to interact with complement and kinin systems. Activated Hageman factor promotes vasoactive substances and vascular permeability. Plasmin, the enzyme which digests fibrin also interacts with complement and the kinin system. Heparin is a known anti-inflammatory agent..

Prostaglandins are a family of smooth muscle stimulating lipids made from oxygenated fatty acids. More than a dozen compounds are considered prostaglandins and collectively they produce various metabolic effects. Individually each prostaglandin has wide activity which is specific to tissues, even in the same system of the body the actions of prostaglandin's can oppose each other. They can produce direct and indirect effects in virtually all mammalian tissues.

They are released during inflammatory reactions and are known to produce wheal and flare reactions by the release of vasoactive amines. There are interrelations between prostaglandin's, calcium, adenylyl cyclase and cyclic AMP. Disturbance of the tissue membrane influences prostaglandin synthesis and as substances they contribute to the intensity and duration of the inflammatory reaction.

Low cyclic AMP levels can stimulate the activity of at least one of the enzymes involved in prostaglandin metabolism. In some tissues prostaglandins inhibit cyclic AMP production in response to hormonal stimulation.

Formed from polyunsaturated essential fatty acids that are bound to cell membranes, they require 20-carbon fatty acids which have multiple unsaturated sites, molecular oxygen, cofactor reducing agents and the multi-enzyme complex prostaglandin synthetase. The enzymes required to make prostaglandins are present in all mammalian tissues except in red blood cells, and synthesis can be inhibited or stimulated at each metabolic stage.

Intermediates of the prostaglandin family have potent, highly reactive, short-lived actions and are mainly metabolised to non-prostaglandin related substances; only a small proportion are converted to formal prostaglandins. The oedema caused by prostaglandin's is not dose-dependent. Molecule for molecule substantially less prostaglandin's are required to produce oedema in comparison to histamine or bradykinin.

Prostaglandin production in leukocytes is enhanced during phagocytosis having the effect of attracting leukocytes to the place of formation. Leukocytes can contribute to prostaglandin levels during inflammation. Levels increase when tissues are stimulated by allergens. Prostaglandin's are released by the cells of joints in rheumatoid arthritis.

Local prostaglandin levels influence the continuation or cessation of inflammation. Understanding the action of the prostaglandins may explain the anti-inflammatory properties of inflammatory exudates.

Prostaglandins affect the body's temperature and inhibitors of prostaglandins (such as aspirin) reduce body temperature. Prostaglandin's also sensitise pain receptors to stimulus, and inhibitors (such as aspirin) possess an analgesic effect.

Prostaglandin's stimulate cyclic AMP levels and change the 'second messenger' system which acts to carry a chemical message from a hormone into the cell.

Rapidly metabolised in the liver, lungs and other tissues, a large proportion of these lipid hormones are metabolised during a single passage through the lungs or liver. Generally they are formed after a cell or a tissue membrane is stimulated as opposed to the body having stores of them.

Prostaglandins are local hormones which have influence in the same or neighbouring cells that produced them. In general the metabolism of the prostaglandin's leads to a decline in activity but the prostaglandin 15 keto PGF<sub>2</sub> alpha (which has a potent action on the lungs) is an exception.

Heparin is a natural anti-coagulant in the body consisting of glucosamine, glucuronic acid, sulphate and acetyl groups. It is produced and stored by the mast cells in connective tissue, the heparin content of tissues serves as a relative index to the number of mast cells present.

Heparin is secreted into the intercellular substance to prevent fibrinogen clotting. A function of heparin is to make lipoprotein lipase which is an enzyme which metabolises lipids in the body.

Glycosaminoglycans refers to a large number of polysaccharides that are found in connective tissues. They are all fairly uniform having similar characteristics which are: They contain a repeating sugar unit (such as glucosamine) and a uronic acid (such as glucuronic acid) which is often esterified with sulphate. If the polysaccharide is covalently linked to a protein it makes a mucopolysaccharide.

# Bronchial Asthma and the Atopic Syndrome

## Paper 7: Hypothesis of Biological Adaptation

In a state of deficiency the body draws progressively upon more important stores and resources of substances it requires to balance an immediate metabolic deficit; The body draws upon biologically less important substances to manufacture from them biologically more important and immediately required substances.

There is a hierarchical system in the biology of the organism. In the 'Selfish Brain Theory [7.1]' a model newly describes a 'principle of balance' in biological systems. The various organs and tissues must compete for allocation of a limited number of resources.

The principle is that the body will look after a more important tissue before it looks after a less important tissue.

Importance in these terms refers to a relative scale of absolute requirement and immediacy of need. The economics of alternative/opportunity costs and the logistics biology must be taken as factors in the interpretation of biological function.

The body draws upon the least important and most plentiful resources first. The body prioritizes the functions, organs, tissues and chemical stores according to how essential they are in the functioning of the body. In short there is a hierarchy amongst the tissues of the body.

We are literally an assembly of what we eat, drink and breath. Ultimately the body relies upon the diet for all its building blocks and fuel.

A medical condition manifests itself when the first metabolic action is impaired - that is to say - a metabolically active substance is negatively affected and the function which it supports is impeded forcing a biological adaptation of normal metabolism to occur.

As deficiency progresses the body is forced to requisite resources so to supply more vital functions with the components which are required to support the biological action. An increasing amount of symptoms occur with an increasing deficit of resources. When a biological function breaks down it is related as a symptom.

The more active the substance that is requisited for reassignment of its constituents - the more important the function that is affected in the running of the body - the more dramatic is the symptom of deficit.

The greater the stage of deficiency  
the more important the function or  
tissue which is affected.

Deficit may be brought about by lack of the substance in the diet, by increased utilization of the substance so that demand outstrips supply, or by the destruction of the substance.

# Paper 7: Hypothesis of Biological Adaptation

There is a hierarchical system in the biology of the organism. In the ‘[Selfish Brain Theory](#) [7.1]’ a model newly describes a ‘principle of balance’ in biological systems. The various organs and tissues must compete for allocation of a limited number of resources.

**Paper Number: 7**  
**Reference Number: 7.1**

**Information taken from:**

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The selfish brain: competition for energy resources.

Peters A, Schweiger U, Pellerin L, Hubold C, Oltmanns KM,  
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The brain occupies a special hierarchical position in the organism [1]. It is separated from the general circulation by the blood-brain barrier has high energy consumption and a low energy storage capacity, uses only specific substrates, and it can record information from the peripheral organs and control them. Here we present a new paradigm for the regulation of energy supply within the organism. The brain gives priority to regulating its own adenosine triphosphate (ATP) concentration [2]. In that postulate, the peripheral energy supply is only of secondary importance [3]. The brain has two possibilities to ensure its energy supply: allocation or intake of nutrients. The term ‘allocation’ refers to the allocation of energy resources between the brain and the periphery [4].

Neocortex and the limbic-hypothalamus-pituitary–adrenal (LHPA) System control the allocation and intake. In order to keep the energy concentrations constant, the following mechanisms are available to the brain: (1) high and low-affinity ATP-sensitive potassium channels measure the ATP concentration in neurons of the neocortex and generate a ‘glutamate command’ signal.

This signal affects the brain ATP concentration by locally (via astrocytes) stimulating glucose uptake across the blood-brain barrier and by systemically (via the LHPA system) inhibiting glucose uptake into the muscular and adipose tissue.(2) High-affinity mineralocorticoid and low-affinity glucocorticoid receptors determine the state of balance, i.e. the set point, of the LHPA system.

This set point can permanently and pathologically be displaced by extreme stress situations (chronic metabolic and psychological stress, traumatization,etc.), by starvation, exercise, infectious diseases, hormones, drugs, substances of abuse, or chemicals disrupting the endocrine system [5]. Disorders in the ‘energy on demand’ processor the LHPA-system can influence the allocation of energy and in so doing alter the body mass of the organism.

In summary, the presented model includes a newly discovered ‘principle of balance’ of how pairs of high and low-affinity receptors can originate set points in biological systems. In this ‘Selfish Brain Theory’, the neocortex and limbic system play a central role in the pathogenesis of diseases such as anorexia nervosa and obesity [6].

How does the human organism control its energy supply? The answer to this question is the key to treating many diseases: obesity and the so-called metabolic syndrome with diabetes mellitus, hyperlipoproteinemia, hypertension and cardiovascular diseases belonging to these disorders.

Gynecological diseases including polycystic ovaries or Psychiatric disorders such as depression or eating disorders are also associated with disrupted regulation of energy supplies. Two different processes can be distinguished that regulate energy metabolism: energy supply (appetite, intake of foods) and allocation (assignment). The various organs of the body must compete for the allocation of a limited number of energy resources [7].

### **Notes:**

There is a hierarchical system in the biology of the organism. The brain occupies a special position in this scheme [1]. To illustrate this, brain gives priority to regulating its own ATP concentration [2].

It has been postulated that the peripheral energy supply is only of secondary importance [3]. The brain has two possibilities to ensure energy supply: allocation or intake. ‘Allocation’ refers to the allocation of energy resources between the brain and the periphery [4].

System control can be permanently displaced by extreme stress, starvation, exercise, infectious diseases, hormones, drugs, substances of abuse, or chemical disruption of the endocrine system [5].

In the ‘Selfish Brain Theory’ a model newly describes a ‘principle of balance’ in biological systems [6]. The various organs and tissues must compete for allocation of a limited number of resources [7].

# Bronchial Asthma and the Atopic Syndrome

## Paper 8: The Hormonal Pathology of Asthma

After death has been caused by asthma, **pathological examination [8.1]** of the asthmatic lungs shows them to be over-distended and numerous tenacious mucous plugs are found in the bronchi. There is a dense exudate in the bronchial lumen with many eosinophils and effect columnar respiratory cells. The bronchial mucous membrane and submucosa are thickened and infiltrated with eosinophils and the bronchial smooth muscle is hypertrophied.

The lungs are hollow organs which are under smooth muscle control. **Smooth muscle [8.2]** is under the control of the autonomic nervous system which utilizes prostaglandin hormones.

**Prostaglandins [8.3]** are a group of locally acting lipid hormones which regulate multiple functions in the body. **Edema [8.4]** produced by prostaglandins is not **dose-dependent [8.5]** and they hold immediate and powerful physiological effects in the tissues and body.

**Eicosanoids [8.6]** collectively refers to the compounds prostaglandins, thromboxane, leukotrienes and epoxyeicosatrienoic eicosanoids. Eicosanoids are so named due to the twenty carbon atoms derived from the fatty acid arachidonic acid (eicosa: Greek; the number twenty).

The cysteinyl-leukotrienes and prostaglandins are collectively known as **eicosanoids [8.7]**. They represent a family of lipid mediators which have an important role in the pathophysiology of asthma.

**Prostaglandins [8.8]** require to be deactivated very quickly after they have been made active. If these hormones are not deactivated quickly and metabolised, the function the prostaglandin performs continues to be stimulated. In example; if the lungs expand and subsequently only recieve the stimulus to expand, they will remain expanded until the instigating factor is metabolised.

The fact that in asthma the lungs are fully expanded at the time of **death [8.9]** is an indication that the chemical stimulus which caused the expansion was not effectively metabolised. This could be viewed as a breakdown in the metabolic regulation of smooth muscle stimulation.

As a chemical family, prostaglandins have diverse roles in the body activated by, and reacting to, a great deal of stimuli ranging across emotion, temperature, immune response, exertion, blood pressure and many more factors. All these **stimuli [8.10]** have been indicated as triggers of the anaphylactic reaction found at the heart of all atopic conditions.

The tenacious exudate in the lungs is an indication of a lack of the agent that maintains the mucus from becoming too sticky. It is feasible that heparin may play some role in the structure or in maintaining the glycosaminoglycan structures in mucus so that it may hold water molecules.

To **iterate [8.11]**, the hypothetical scheme presented in this thesis focuses on the interactions between glucuronic acid and its compounds and the metabolism of prostaglandins. Glycosaminoglycans, heparin and lipoprotein lipase are all compounds of glucuronic acid. Heparin is the structural precursor to lipoprotein lipase which is involved in regulating prostaglandin hormone metabolism and hence smooth muscle function.

Glucuronic acid is responsible for the conjugation and elimination from the body of various toxins. As well as this role, it also serves as a structural component of heparin and lipoprotein lipase.

Anaphylactic reactions all exhibit inflammation of mast cell cultures producing degranulation of their cellular contents. Heparin is stored in mast cell granules. Mast cell population is directly proportional to heparin content of tissue.

**The asthmatic condition appears to be manifesting a deficit of:**

**The enzyme that degrades specific prostaglandins (lipoprotein lipase)**

**The agent that keeps mucous from becoming too sticky (heparin)**

**The substance required to eliminate toxins and prostaglandins from the body (glucuronic acid)**

# Paper 8: The Hormonal Pathology of Asthma

After death has been caused by asthma, **pathological examination [8.1]** of the asthmatic lungs shows them to be over-distended and numerous tenacious mucous plugs are found in the bronchi. There is a dense exudate in the bronchial lumen with many eosinophils and effete columnar respiratory cells. The bronchial mucous membrane and submucosa are thickened and infiltrated with eosinophils and the bronchial smooth muscle is hypertrophied.

**Paper Number: 8**  
**Reference Number: 8.1**

**Information taken from:**  
 Kenneth L. Melmon, M.D  
 Howard F. Morrelli, M.D  
 Clinical Pharmacology  
 Basic Principles in therapeutics  
 Second edition 1978  
 Bailliere, Tindall london  
 Clinical definition of asthma  
 Page 483 - 484 Asthma

The pathologic findings in the lungs of patients who died during an acute asthmatic episode are well documented. macroscopically, the lungs are over distended [1], and numerous tenacious mucous plugs are found in the bronchi [2]. Histologic examination shows a dense exudate in the bronchial lumen [3] with a mucous and serous component and many eosinophils and effete columnar respiratory cells (Dunnill, 1971). The bronchial mucous membrane and submucosa are thickened and infiltrated with eosinophils, and the bronchial smooth muscle is hypertrophied (Dunnill et al., 1969) [4].

## Notes:

Post mortem examination of the lungs of a patient who died during an acute asthma episode show that the lungs are over-distended [1] and numerous tenacious mucous plugs are found in the bronchi [2].

A dense exudate is found in the bronchial lumen which has a mucous and serous component [3]. Eosinophils are found to have infiltrated the bronchial lumen as well as the bronchial mucous membrane and submucosa. The bronchial smooth muscle is hypertrophied [4].

# Paper 8: The Hormonal Pathology of Asthma

The lungs are hollow organs which are under smooth muscle control. **Smooth muscle [8.2]** is under the control of the autonomic nervous system which utilizes prostaglandin hormones.

**Paper Number: 8**  
**Reference Number: 8.2**

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 Airway smooth muscle prostaglandin-EP1 receptors directly modulate  
 $\beta$ 2-adrenergic receptors within a unique heterodimeric complex

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PGE2 is a cyclooxygenase-derived product of arachidonic acid metabolism that plays a major role in regulating such diverse physiological processes as smooth muscle tone, inflammation, and pain (reviewed in ref. 17) [1]. The biological effects of PGE2 are often multifaceted and can appear contradictory to traditional signaling paradigms. As introduced earlier, some of these properties of PGE2 are due to the fact that it is an endogenous agonist for 4 receptor subtypes coupled to several signal transduction cascades.

The EP1 receptor is linked to IP3 and [Ca<sup>2+</sup>]<sub>i</sub> turnover through Gq-mediated signal transduction (18), whereas the EP2 and EP4 receptors stimulate adenylyl cyclase and cAMP production through Gs (19–21). Splice variants of the EP3 receptor give rise to multiple isoforms that apparently can couple to Gi, Gs, or Gq (22, 23).

Since one or more of these receptors may be expressed by a cell or tissue, the integrated response to PGE2 often represents the net effect of additive or opposing signal transduction events. Moreover, temporal and spatial changes in patterns of EP receptor expression in response to various stimuli (e.g., injury and inflammation) provide an additional level of dynamic regulation and complexity to PGE2-regulated processes in the airways.

For example, recent studies of the PGE2-mediated fibroproliferative response due to intratracheal bleomycin have shown an approximately 50% decrease in fibroblast EP2 receptor mRNA without significant change in the other EP subtypes (24), whereas FITC-mediated fibrosis was associated with decreases in both EP2 and EP4 transcript expression (24).

Because PGE<sub>2</sub> is produced endogenously within the lung and activates pathways that may both enhance and inhibit ASM contraction, it may have particular relevance in asthma. PGE<sub>2</sub> is produced by ASM cells, bronchial and alveolar epithelial cells, fibroblasts, and inflammatory cells in the lung [2], and increased levels have been reported in some studies with human asthma (25, 26) [3].

Furthermore, an initial enzyme for prostanoid receptor synthesis (COX-2), as well as PGE<sub>2</sub> precursors, are increased in asthmatic airways (27, 28) [3]. Following its discovery, PGE<sub>2</sub> was found to relax ASM and thus has generally been considered to be a bronchoprotective prostanoid. The bronchodilatory effect of PGE<sub>2</sub> appears to be due to activation of the EP<sub>2</sub> receptor subtype, since PGE<sub>2</sub>-mediated bronchodilation is present in EP<sub>1</sub>-, EP<sub>3</sub>-, and EP<sub>4</sub>-null mice but absent in EP<sub>2</sub>-null mice (29).

Interestingly, in nonasthmatic humans, PGE<sub>2</sub> exerts a net bronchodilation; in asthmatics, though, the response is highly variable, sometimes resulting in significant bronchoconstriction (30) [4]. The complex nature of PGE<sub>2</sub> action in the asthmatic milieu is further illustrated in that it may serve as an intermediary through which inflammatory cytokines can modulate other receptor systems that govern airway tone [5]. In particular, PGE<sub>2</sub> appears to mediate the inhibitory effects of the inflammatory cytokines TGF $\beta$  and interleukin-1 on  $\beta$ 2AR signal transduction in cultured ASM (31–34) [6].

These inflammatory cytokines stimulate COX-2 and production of PGE<sub>2</sub> by ASM, and it has been proposed that the resulting increase in cAMP production following EP<sub>2</sub> receptor activation leads to desensitization of the  $\beta$ 2AR (3) [7]. However, the mechanism(s) by which this inhibition occurs has not been completely elucidated, nor has the role of other EP receptor subtypes in mediating this effect been explored.

### **Notes:**

Prostaglandin E<sub>2</sub> is a cyclooxygenase-derived product of arachidonic acid which plays a major role in smooth muscle tone, inflammation and pain [1]. Prostaglandin E<sub>2</sub> is produced endogenously by airway smooth muscle cells, bronchial and alveolar epithelial cells, fibroblasts, and inflammatory cells in the lung [2].

Increased levels have been reported in human asthma [3]. Cox-2, an initial enzyme for prostanoid receptor synthesis, and prostaglandin E<sub>2</sub> precursors are also found increased in asthmatic airways [3].

In nonasthmatic humans, prostaglandin E<sub>2</sub> exerts a net bronchodilation, however, in asthmatics the response is highly variable, sometimes resulting in significant bronchoconstriction [4]. Prostaglandin E<sub>2</sub> may act as an intermediary through which inflammatory cytokines can modulate other receptor systems which govern airway tone [5].

Specifically, prostaglandin E<sub>2</sub> appears to mediate the inhibitory effects of the inflammatory cytokines TGF beta and interleukin-1 on Beta-2 adrenergic receptor signal transduction in cultured airway smooth muscle [6]. These inflammatory cytokines stimulate COX-2 and production of prostaglandin E<sub>2</sub> by airway smooth muscle [7].

# Paper 8: The Hormonal Pathology of Asthma

**Prostaglandins [8.3]** are a group of locally acting lipid hormones which regulate multiple functions in the body. Edema produced by prostaglandins is not dose-dependent and they hold immediate and powerful physiological effects in the tissues and body.

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**Reference Number: 8.3**

**Information taken from:**

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Leukotrienes and prostaglandins in asthma.

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Leukotrienes and prostaglandins possess properties which are central in the asthmatic reaction. They are bronchoconstrictors, they inhibit the mucociliary clearance, increase blood flow and permeability and thereby induce edema formation, and they attract and activate leukocytes [1]. They are formed partly by allergic reactions and partly by a large number of other more non-specific reactions [2].

Finally, the concentration of prostanoids has been found increased in the asthmatic reaction in vivo. The leukotrienes have not been traced in vivo in asthmatic attacks so far, but have been found in vivo in man in a specific type I allergic conjunctival reaction. Much evidence suggests that these mediators are relevant in asthmatic diseases, even though prostaglandin inhibitors have no effect in asthma.

There still remains the need to investigate the influence on asthmatic diseases by as yet unavailable leukotriene blocking agents. Even though leukotrienes are judged today to be important mediators in asthma, it does not seem reasonable to expect that a single mediator is responsible for asthmatic diseases.

Rather, it seems quite likely that asthma is caused by a complex interplay of a large number of mediators, circulating hormones, nervous mechanisms, receptor abnormalities, intracellular metabolic defects, etc.

Despite this complexity, investigations in recent years have increased the knowledge of the biochemistry and human physiological effects of leukotrienes and prostaglandins which has created an improved understanding of the asthmatic reaction's pathophysiology, contributed a pharmacological rationale for previously used therapy, and stimulated new perspectives for specific pharmacological research.

**Notes:**

Leukotrienes and prostaglandins are central to the asthmatic reaction. They act as bronchoconstrictors, inhibit mucociliary clearance, increase blood flow and permeability, induce edema as well as attract and activate leukocytes [1].

Prostaglandins and leukotrienes are formed partly by allergic reactions and part by many non-specific reactions [2]. The concentration of prostanoids has been found increased in the asthmatic reaction in vivo, however prostaglandin inhibitors have not been found to have any effect in asthma [3].

# Paper 8: The Hormonal Pathology of Asthma

Prostaglandins are a group of locally acting lipid hormones which regulate multiple functions in the body. **Edema [8.4]** produced by prostaglandins is not dose-dependent and they hold immediate and powerful physiological effects in the tissues and body.

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**Reference Number: 8.4**

**Information taken from:**

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 Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology  
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## **Inflammation**

Scientists have been grappling for years over the specific mechanisms of how prostaglandins mediate their effects on the cardinal signs of acute inflammation [1]: pain, vasodilation (swelling and redness), and fever. COX-1 is expressed in nearly all tissues, whereas COX-2 is absent in most (some exceptions are the glomerulus and certain brain regions) until induced by various inflammatory insults in monocytes or mast cells or by shear stress in endothelium [2].

In most instances, COX-1 expression is marginally affected by inflammatory stimuli. However, exceptions to the “constitutive” mode of COX-1 prostanoid synthesis are known (e.g., both COX-1 and COX-2 are expressed in the inflamed synovia of joints) [3].

Most of the traditional NSAIDs do not distinguish between the two COX isoforms [4]. Coxibs, however, were developed specifically with the promise that they would selectively block synthesis of “proinflammatory” prostaglandins derived from the induced COX-2 enzyme while leaving intact the COX-1-derived “homeostatic” prostaglandins involved in renal water and electrolyte balance, gastric cytoprotection, and platelet aggregation (40) [5].

Two years of clinical use in pain management indicate that coxibs are as effective as traditional nonselective NSAIDs and also reveal a 50 % reduction in adverse gastrointestinal events (40). Although indications of potentially deleterious actions of COX-2 inhibitors (e.g., causing acute tubulointerstitial nephritis or decreased cardioprotection) have been reported (49, 50), the case to support an increased incidence of adverse events compared with traditional NSAIDs has not been developed.

Vasodilation and increased permeability of postcapillary venules, early events in the inflammatory response, reflect the effects of COX-2–derived prostaglandins and leukotrienes at sites of inflammation [7]. Prostaglandins synergize with other mediators (e.g., bradykinin, histamine) to elicit enhanced vascular permeability and edema.

These molecules can be viewed within the context of a complex milieu of parenchymal and inflammatory cells, an array of cytokine and other noneicosanoid mediators, and extracellular matrix interactions combined with the overall physiological status of the host. To complicate matters, prostaglandins may act as both proinflammatory and anti-inflammatory mediators depending on the context, which is due in part to the array of EP-type prostaglandin receptors with opposing signal transduction pathways [8].

How tissues and cells sort out the mixed signals has been reviewed recently (48). The temporal sequence of events in acute inflammation may be governed by eicosanoid profile switching such that eicosanoids made during the initial phase are gradually replaced by other lipid mediators in the resolution phase (51).

In vitro evidence indicates that monocytes and/or macrophages can undergo a shift in eicosanoid products (52, 53), a process perhaps mediated by altered gene expression of the synthases downstream of COX-1 and COX-2 or by specific compartmentalization of the enzymes to various stimuli.

Combined data from several murine inflammation models support a complex regulatory network in eicosanoid signaling (48, 51, 54). The 5-LO pathway leading to leukotriene formation has long been recognized as a proinflammatory cascade. LTB<sub>4</sub> promotes neutrophil chemotaxis and adhesion to vascular endothelium through specific integrins.

The cysteinyl leukotrienes cause plasma leakage from postcapillary venules and enhance mucus secretion [9]. LTD<sub>4</sub> and another 5-LO– derived eicosanoid, 5-oxo-ETE, are eosinophil chemoattractants [10]. The use of 5-lipoxygenase, FLAP-, LTA<sub>4</sub>H-, and LTC<sub>4</sub>S-deficient mice has enabled a detailed examination of the leukotrienes in murine models, firmly establishing their roles in allergic inflammation .

### **Notes:**

For years scientists have been trying to understand the specific mechanisms of how prostaglandins mediate acute inflammation [1]. Cyclooxygenase 1 (COX-1) expression is expressed in nearly every tissue, whilst cyclooxygenase 2 (COX-2) is absent in most until it is induced by various inflammatory insults in monocytes or mast cells, or by stress in endothelium [2].

Generally, cyclooxygenase 1 expression is marginally affected by inflammatory stimuli, however exceptions are known such as in the inflamed synovia of joints where both cyclooxygenase 1 and 2 are found expressed [3]. Most traditional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) do not distinguish between the two COX isoforms [4].

Selective inhibitors of cyclooxygenase-2 (coxibs) were developed to specifically block synthesis of ‘proinflammatory’ prostaglandins derived from the cyclooxygenase 2 enzyme whilst not affecting the cyclooxygenase 1 derived ‘homeostatic’ prostaglandins involved in renal water and electrolyte balance, gastric cytoprotection and platelet aggregation [5].

Selective inhibitors of cyclooxygenase 2 were found to be as effective as traditional NSAIDs as well as being able to produce a 50% reduction in adverse gastrointestinal events [6].

Vasodilation and increased permeability of postcapillary venules are early events in the inflammatory response and reflect the effect of cyclooxygenase 2 derived prostaglandins and leukotrienes at sites of inflammation [7]. Prostaglandins can act as both proinflammatory and anti-inflammatory mediators depending on the context [8].

The 5-lipoxygenase pathway (5-LO) leading to leukotriene formation has been noted as a proinflammatory cascade. The cysteinyl leukotrienes are known to cause plasma leakage from postcapillary venules and enhance mucus secretion. Leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-E<sub>4</sub>E) are eosinophil chemoattractants [10].

# Paper 8: The Hormonal Pathology of Asthma

Prostaglandins are a group of locally acting lipid hormones which regulate multiple functions in the body. Edema produced by prostaglandins is not **dose-dependent** [8.5] and they hold immediate and powerful physiological effects in the tissues and body.

**Paper Number: 8**  
**Reference Number: 8.5**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic principles in Therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Functioning action of prostaglandin's  
Page 667 Prostaglandin's

At each step the reaction can be inhibited or stimulated. The mechanisms controlling biosynthesis of the prostaglandin's are not known. Recent detailed studies of the biosynthesis of the prostaglandin's have resulted in the isolation and identification of new intermediates in this pathway as well as some untested antagonists of specific intermediates.

These highly reactive, but short lived, intermediates, called endoperoxides and thromboxanes, have potent biological effects intracellularly. In some systems, the effects previously ascribed to prostaglandin's result from the endoperoxides. These substances are primarily metabolised to nonprostanoate structures with only a small amount converted to prostaglandin (Hamberg et al., 1974a, 1974b). The mechanism by which the prostaglandin's and prostaglandin intermediates reach the extracellular environment is unknown.

The edema produced by the prostaglandin's is not dose dependent [1] and is substantially less than can be produced with equimolar quantities of histamine or bradykinin (Williams and Morley, 1973). Leukocytes produce prostaglandin's; their production is enhanced during phagocytosis. PGE1 in minute amounts is chemotactic for leukocytes; by virtue of their ability to synthesise prostaglandin's, leukocytes can significantly contribute to the production of prostaglandin's found in some inflammatory lesions (Higgs et al., 1975). In addition, increased prostaglandin's are produced during antigenic stimulation of the spleen (Webb and Osheroff, 1976) and by dispersed synovial cells derived from patients with rheumatoid arthritis (Dayer et al., 1976) [3].

## Notes:

Edema produced by prostaglandins is not dose dependent [1]. Leukocytes produce prostaglandins and can significantly contribute to the production of prostaglandins in some inflammatory lesions [2]. Increased prostaglandins are produced during antigenic stimulation of the spleen as well as by dispersed synovial cells derived from patients with rheumatoid arthritis [3].

# Paper 8: The Hormonal Pathology of Asthma

**Eicosanoids [8.6]** collectively refers to the compounds prostaglandins, thromboxane, leukotrienes and epoxyeicosatrienoic eicosanoids. Eicosanoids are so named due to the twenty carbon atoms derived from the fatty acid arachidonic acid (eicosa: Greek; the number twenty).

**Paper Number: 8**  
**Reference Number: 8.6**

**Information taken from:**

Color Atlas of Physiology  
5th edition, completely revised and expanded  
Professor Agamemnon Despopoulos, M.D.  
Formerly: Ciba Geigy, Basel  
Professor Stefan Silbernagl, M.D.  
Institute of Physiology, University of Wuerzburg  
Wuerzburg, Germany  
186 color plates by Ruediger Gay and Astrid Rothenburger  
Thieme: Stuttgart · New York  
Despopoulos, Color Atlas of Physiology © 2003 Thieme  
Page 269 Eicosanoids

Eicosanoids. Prostaglandins (PG), thromboxane (TX), leukotrienes and Epoxyeicosatrienoic eicosanoids (Greek eicosa- = twenty [C atoms]) derived in humans from the fatty acid arachidonic acid (AA). (Prostaglandins derived from AA have the index number 2) [1].

AA occurs as an ester in the phospholipid layer of the cell membranes and is obtained from dietary sources (meat), synthesized from linoleic acid, an essential fatty acid, and released by phospholipase A2 (p. 252) [2].

## Notes:

Eicosanoids include the compounds prostaglandins, thromboxane, leukotrienes and epoxyeicosatrienoic eicosanoids. Eicosa is twenty in Greek. Eicosanoids are so named due to the twenty carbon atoms derived from the fatty acid arachidonic acid [1].

Arachidonic acid is found as an ester in the phospholipid layer of cell membranes and is obtained from dietary sources, synthesized from the essential fatty acid linoleic acid, and released by the action of phospholipase A2 [2].

# Paper 8: The Hormonal Pathology of Asthma

The cysteinyl-leukotrienes and prostaglandins are collectively known as **eicosanoids** [8.7]. They represent a family of lipid mediators which have an important role in the pathophysiology of asthma.

**Paper Number: 8**  
**Reference Number: 8.7**

**Information taken from:**

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Internet address: [www.atsjournals.org](http://www.atsjournals.org)

Induced Sputum Eicosanoid Concentrations In Asthma

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We have shown that induced sputum cysteinyl-leukotriene concentration was significantly greater in subjects with asthma than in normal control subjects, but there were no significant differences in concentrations of other eicosanoids. Sputum cysteinyl-leukotriene concentrations were greater in subjects with more persistent and severe asthma, suggesting that they might be more functionally important in these groups. Most of these subjects were treated with inhaled corticosteroids, supporting the theory that corticosteroids do not directly reduce cysteinyl-leukotriene production (14) [1].

We found considerable within-subject variability of most sputum eicosanoid concentrations, which could reflect variable airway production of these mediators or problems with the methodology. The fact that sputum PGE2 could be measured repeatably is more in keeping with the former. We have addressed a number of potential problems with using induced sputum to assess airway eicosanoid production by performing appropriate control experiments.

Ex vivo production, or breakdown of, seems unlikely since immediate incubation of sputum with leukotriene and prostaglandin biosynthesis and breakdown inhibitors did not affect sputum eicosanoid concentrations. Similarly, important interference in the cysteinyl-leukotriene assay by DTT is unlikely since concentrations were not significantly different in sputum treated with and that treated without DTT.

We cannot exclude the possibility that the cysteinyl-leukotriene concentration in sputum from subjects with asthma might be increased by the effect of hypertonic saline on mast cells and other mediator-producing cells in the airway. However, sputum concentrations of the mast cell product PGD2 were not elevated and subjects were pretreated with albuterol before sputum induction, which would be expected to reduce mediator release.

Furthermore, concentrations of cysteinyl-leukotrienes were greatest in spontaneously produced sputum from subjects with acute severe asthma [2]. It is possible that our inclusion of spontaneous sputum has biased our comparisons and correlations, but we consider this unlikely since inflammatory cell counts and the concentration of most inflammatory mediators have been shown to be similar in induced and spontaneous sputum samples (15).

A further concern was variable dilution of sputum eicosanoids by contaminating saliva. It is difficult to design control spiking experiments because of the different physical properties of sputum and contaminating fluid, so we attempted to minimize this by selecting sputum from surrounding fluid. Our median squamous cell contamination was less than 5%, suggesting that any effect of salivary contamination was small.

Earlier investigators measured slow-reacting substance-type activity in sputum from asthmatics using a bioassay or by high pressure liquid chromatography with mixed results (16, 17). More recently cysteinyl-leukotrienes have been shown to be present in increased concentrations in bronchial wash and bronchoalveolar lavage (BAL) samples from subjects with stable asthma (3), after allergen challenge in atopic asthma (14, 18) and after aspirin challenge in aspirin-sensitive asthma (12) [3].

Our findings using newer techniques to obtain and process sputum support these earlier findings. The relatively noninvasive nature of sputum induction and the fact that cysteinyl-leukotriene concentrations are present in considerably greater concentration in induced sputum than in BAL suggest that this technique has a number of advantages over bronchoscopic methods [4].

LTE<sub>4</sub>, the end product of LTC<sub>4</sub> and D<sub>4</sub> metabolism, can be measured in the urine, and there is increasing interest in the use of measures of urinary excretion of LTE<sub>4</sub> to assess airway cysteinyl-leukotriene production (5, 6, 19) [5]. Intervention studies show that urinary LTE<sub>4</sub> concentrations increase after allergen challenge in atopic asthma (5) and after aspirin challenge in subjects with aspirin-induced asthma (6) [6].

Urinary LTE<sub>4</sub> concentrations may also be increased in subjects with acute severe asthma (5, 20). Only 4 to 9% of an intravenous or inhaled dose of LTC<sub>4</sub> appears in the urine (19), suggesting that this technique might be relatively insensitive to minor differences or changes in airway cysteinyl-leukotriene concentrations. In support of this, urinary LTE<sub>4</sub> excretion is similar in subjects with stable asthma and in normal control subjects (5).

Furthermore, exercise-induced asthma, which is effectively inhibited by treatment with leukotriene receptor antagonists, suggesting it is at least partly due to airway cysteinyl-leukotriene production (1, 21), is not always associated with significant changes in urinary LTE<sub>4</sub> excretion (21, 22). We have shown that induced sputum cysteinyl-leukotriene concentration are significantly greater in subjects with stable asthma, suggesting that measurement in sputum is more sensitive than measurement in urine.

Although the magnitude of the differences in sputum concentrations of PGD<sub>2</sub>, PGF<sub>2a</sub>, and TXB<sub>2</sub> was similar to the differences in cysteinyl-leukotrienes, there was more within-category variability and the differences were not statistically significant. This is in keeping with the variable effect of nonsteroidal anti-inflammatory drugs on airway function and responsiveness in stable asthma (2).

Our findings contrast with those of a number of bronchoscopy studies showing increased concentrations or proportions of bronchoconstrictor prostaglandins in bronchial wash and BAL in stable asthma and after allergen challenge (4, 14, 23) [8].

Possible explanations for the difference include greater precision of bronchoscopic measurements, difference in subjects studied, or spurious elevation of airway prostaglandin production caused by trauma of the inflamed airway wall in asthma during bronchoscopy. Assessment of airway prostaglandin production using both techniques in the same subjects might help resolve this issue.

Our technique allows us to obtain simultaneous information on cellular markers of airway inflammation. We did not find a positive correlation between the sputum eosinophil count and the sputum cysteinyl-leukotriene or bronchoconstrictor prostaglandin concentration in our heterogeneous population of subjects with asthma, suggesting that additional cell types might be an important source or that the cell producing these eicosanoids is not proportionately represented in sputum.

We did find a negative correlation between the sputum eosinophil count and the supernatant PGE2 concentration [9]. One interpretation of this relationship is that PGE2 exerts an anti-inflammatory effect in the airway. The fact that PGE2 and its analogues have a number of bronchoprotective and anti-inflammatory effects in vitro (24), and inhaled PGE2 inhibits the late bronchoconstrictor (25) and inflammatory (26) response to allergen in atopic asthma, would support such a role.

Another possible explanation for the relationship between the sputum eosinophil count and PGE2 concentration is that active eosinophilic airway inflammation damages cells producing PGE2 such as airway epithelial cells. However, sputum concentrations of PGF2a (which is produced by similar cell types to PGE2) (27) correlated positively with the sputum eosinophil count, arguing against this interpretation [10].

Finally we have considered whether the reduced PGE2 concentration in the sputum supernatant of samples containing a high proportion of eosinophils may be due to reduced numbers of macrophages since the macrophage is a potential source of PGE2 (27). The lack of correlation between the sputum differential macrophage count and sputum supernatant PGE2 concentration suggests that this is not the case.

In conclusion, we have shown that eicosanoids are present in high concentrations in induced sputum and that concentrations of cysteinyl-leukotrienes are present in significantly (Pavord, Ward, Woltmann, et al.: *Induced Sputum Eicosanoid Concentrations in Asthma* 1909) higher concentrations in subjects with asthma than in normal control subjects [11].

We suggest that this technique is a potentially useful method to assess airway production of eicosanoids and to relate this to the underlying cellular inflammation. The noninvasive nature of sputum induction suggests that this technique might be particularly useful for serial assessment after intervention with drugs and/or bronchial challenge. Our estimates of within-subject repeatability of sputum eicosanoid concentrations will help in the planning of these studies.

### **Notes:**

Cysteinyl-leukotriene concentration was found increased in asthmatics. Sputum cysteinyl-leukotriene concentrations were greater in subjects with persistent and more severe asthma [1]. Concentrations of cysteinyl-leukotrienes were highest in spontaneously produced sputum from subjects with acute severe asthma [2].

Cysteinyl-leukotrienes are found increased in bronchial wash and bronchoalveolar lavage samples from asthmatics [3]. Cysteinyl-leukotriene concentrations are significantly greater in induced sputum than in bronchoalveolar lavage samples [4].

leukotriene E4 (LTE4) is the end product of leukotriene C4 (LTC4) and leukotriene D4 (LTD4). Leukotriene E4 can be measured in the urine [5]. Urinary leukotriene E4 concentrations are found increased after allergen challenge in atopic asthma and after aspirin challenge in subjects with aspirin induced asthma [6].

Exercise induced asthma is effectively inhibited by treatment with leukotriene receptor antagonists [7]. Studies showed increased concentrations or proportions of bronchoconstrictor prostaglandins in bronchial wash and bronchoalveolar lavage in stable asthma and after allergen challenge [8].

A negative correlation was found between sputum eosinophil count and supernatant prostaglandin E2 (PGE2) concentration [9]. Sputum concentrations of prostaglandin F2 alpha (PGF2a) correlated positively with the sputum eosinophil count [10].

Eicosanoids are present in high concentrations in induced sputum, and concentrations of cysteinyl-leukotrienes are present in significantly higher concentrations in those with asthma than in normal control subjects [11].

# Paper 8: The Hormonal Pathology of Asthma

**Prostaglandins [8.8]** require to be deactivated very quickly after they have been made active. If these hormones are not deactivated quickly and metabolised, the function the prostaglandin performs continues to be stimulated. In example; if the lungs expand and subsequently only receive the stimulus to expand, they will remain expanded until the instigating factor is metabolised.

**Paper Number: 8**  
**Reference Number: 8.8**

**Information taken from:**

Biochemistry: the Molecular Basis of Life  
by McKee, Trudy; McKee, James R.  
Publisher: Oxford University Press, USA  
ISBN-13: 9780195305753  
ISBN: 0195305752  
Page 338 The Eicosanoids

## The Eicosanoids

The eicosanoids are a diverse group of extremely powerful hormone-like molecules produced in most mammalian tissues. They mediate a wide variety of physiological processes. Examples include smooth muscle contraction, inflammation, pain perception, and blood flow regulation [1]. Eicosanoids are also implicated in several diseases such as myocardial infarct and rheumatoid arthritis [2].

Because they are generally active within the cell in which they are produced, the eicosanoids are called autocrine regulators instead of hormones [3]. Most eicosanoids are derived from arachidonic acid (20:4A5-8-u-14), which is also called 5,8,11,14-eicosatetraenoic acid. (Arachidonic acid is synthesized from linoleic acid by adding a three-carbon unit followed by decarboxylation and desaturation.) [4]

Production of eicosanoids begins after arachidonic acid is released from membrane phospholipid molecules by the enzyme phospholipase A2 [5]. The eicosanoids, which include the prostaglandins, thromboxanes, and leukotrienes (Figure 11 A), are extremely difficult to study because they are active for short periods (often measured in seconds or minutes) [6]. In addition, they are produced only in small amounts [7].

## Notes:

The eicosanoids are a diverse group of extremely powerful hormone-like molecules produced in most tissues. They mediate many physiological processes including smooth muscle contraction, inflammation, pain perception, and blood flow regulation [1]. Eicosanoids have been implicated in diseases including myocardial infarct and rheumatoid arthritis [2].

As they generally act in the cell in which they are synthesized, they are autocrine regulators rather than hormones [3]. Arachidonic acid is synthesized from linoleic acid via addition of a three carbon unit, decarboxylation and desaturation. Arachidonic acid is also known as 5,8,11,14-eicosatetraenoic acid [4].

Eicosanoids are produced after arachidonic acid is released from membrane phospholipid molecules by the action of phospholipase A2 [5]. The eicosanoids (prostaglandins, thromboxanes, and leukotrienes) have been hard to study due to the fact that they are active for very short periods (seconds or minutes) [6]. They are also produced in small quantities [7].

# Paper 8: The Hormonal Pathology of Asthma

The fact that in asthma the lungs are fully expanded at the time of **death [8.9]** is an indication that the chemical stimulus which caused the expansion was not effectively metabolised. This could be viewed as a breakdown in the metabolic regulation of smooth muscle stimulation.

**Paper Number: 8**  
**Reference Number: 8.9**

**Information taken from:**  
 Kenneth L. Melmon, M.D.  
 Howard F. Morrelli, M.D.  
 Clinical Pharmacology  
 Basic Principles in therapeutics  
 Second edition 1978  
 Bailliere, Tindall london  
 Clinical definition of asthma  
 Page 483 Asthma

The pathologic findings in the lungs of patients who died during an acute asthmatic episode are well documented. Macroscopically, the lungs are over distended [1], and numerous tenacious mucous plugs are found in the bronchi [2]. Histologic examination shows a dense exudate in the bronchial lumen with a mucous and serous component and many eosinophils and effete columnar respiratory cells (Dunnill, 1971). The bronchial mucous membrane and submucosa are thickened and infiltrated with eosinophils [3], and the bronchial smooth muscle is hypertrophied (Dunnill et al., 1969) [4].

## Notes:

The post mortem examination of asthmatic shows that the lungs are abnormally expanded or increased in size [1]. Numerous sticky mucous plugs are found in the bronchi, the exudate is dense in the bronchial lumen with many eosinophils and effete columnar respiratory cells [2].

The bronchial mucous membrane and submucosa are swollen and infiltrated with eosinophils [3]. The bronchial smooth muscle is found hypertrophied [4].

# Paper 8: The Hormonal Pathology of Asthma

As a chemical family, prostaglandins have diverse roles in the body activated by, and reacting to, a great deal of stimuli ranging across emotion, temperature, immune response, exertion, blood pressure and many more factors. All these **stimuli [8.10]** have been indicated as triggers of the anaphylactic reaction found at the heart of all atopic conditions.

**Paper Number: 8**  
**Reference Number: 8.10**

**Information taken from:**  
Oxford Reference  
Concise Medical Dictionary  
Fourth Edition 1994  
Oxford University Press  
Concise definition of asthma  
Page 53 asthma n.

ASTHMA N.

The condition of subjects with widespread narrowing of the bronchial airways, which changes in severity over short periods of time (either spontaneously or under treatment) and leads to coughing, wheezing and difficulty in breathing. Bronchial asthma may be precipitated by exposure to one or more of a wide range of stimuli, including allergens, drugs (such as aspirin and other Non Steroidal Anti Inflammatory Drugs and beta blockers), exertion, emotion, infections, and air pollution [1].

## **Notes:**

Amongst the stimuli cited to invoke an asthmatic episode are allergens, drugs, exertion, emotion, infections and air pollution [1].

# Paper 8: The Hormonal Pathology of Asthma

To **iterate** [8.11], the hypothetical scheme presented in this thesis focuses on the interactions between glucuronic acid and its compounds and the metabolism of prostaglandins. Glycosaminoglycans, heparin and lipoprotein lipase are all compounds of glucuronic acid. Heparin is the structural precursor to lipoprotein lipase which is involved in regulating prostaglandin hormone metabolism and hence smooth muscle function.

**Paper Number: 8**  
**Reference Number: 8.11**

**Information taken from:**

Aggregate notes for Paper 8

## **-: Paper Eight - The Hormonal Pathology of Asthma :-**

- **Post mortem exam shows lungs over distended with numerous tenacious mucous plugs -**
- **A dense exudate is found in the bronchial lumen -**
- **Eosinophils are found infiltrated in the bronchial lumen -**
- **Increased prostaglandins and leukotrienes are central to the asthmatic reaction -**
- **Cysteinyl leukotrienes are found in increased levels in asthmatics -**
- **Edema produced by prostaglandins is not dose dependent -**

Post mortem examination of the lungs of a patient who died during an acute asthma episode show that the lungs are over-distended and numerous tenacious mucous plugs are found in the bronchi.

A dense exudate is found in the bronchial lumen which has a mucous and serous component. Eosinophils are found to have infiltrated the bronchial lumen as well as the bronchial mucous membrane and submucosa. The bronchial smooth muscle is hypertrophied.

Prostaglandin E2 is a cyclooxygenase-derived product of arachidonic acid which plays a major role in smooth muscle tone, inflammation and pain. Prostaglandin E2 is produced endogenously by airway smooth muscle cells, bronchial and alveolar epithelial cells, fibroblasts, and inflammatory cells in the lung. Increased levels have been reported in human asthma. Cox-2, an initial enzyme for prostanoid receptor synthesis, and prostaglandin E2 precursors are also found increased in asthmatic airways.

In nonasthmatic humans, prostaglandin E2 exerts a net bronchodilation, however, in asthmatics the response is highly variable, sometimes resulting in significant bronchoconstriction. Prostaglandin E2 may act as an intermediary through which inflammatory cytokines can modulate other receptor systems which govern airway tone.

Specifically, prostaglandin E2 appears to mediate the inhibitory effects of the inflammatory cytokines TGF beta and interleukin-1 on Beta-2 adrenergic receptor signal transduction in cultured airway smooth muscle. These inflammatory cytokines stimulate COX-2 and production of prostaglandin E2 by airway smooth muscle.

Leukotrienes and prostaglandins are central to the asthmatic reaction. They act as bronchoconstrictors, inhibit mucociliary clearance, increase blood flow and permeability, induce edema as well as attract and activate leukocytes.

Prostaglandins and leukotrienes are formed partly by allergic reactions and part by many non-specific reactions. The concentration of prostanoids has been found increased in the asthmatic reaction in vivo, however prostaglandin inhibitors have not been found to have any effect in asthma.

Edema produced by prostaglandins is not dose dependent. Leukocytes produce prostaglandins and can significantly contribute to the production of prostaglandins in some inflammatory lesions. Increased prostaglandins are produced during antigenic stimulation of the spleen as well as by dispersed synovial cells derived from patients with rheumatoid arthritis.

For years scientists have been trying to understand the specific mechanisms of how prostaglandins mediate acute inflammation. Cyclooxygenase 1 (COX-1) expression is expressed in nearly every tissue, whilst cyclooxygenase 2 (COX-2) is absent in most until it is induced by various inflammatory insults in monocytes or mast cells, or by stress in endothelium.

Generally, cyclooxygenase 1 expression is marginally affected by inflammatory stimuli, however exceptions are known such as in the inflamed synovia of joints where both cyclooxygenase 1 and 2 are found expressed. Most traditional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) do not distinguish between the two COX isoforms.

Selective inhibitors of cyclooxygenase-2 (coxibs) were developed to specifically block synthesis of 'proinflammatory' prostaglandins derived from the cyclooxygenase 2 enzyme whilst not affecting the cyclooxygenase 1 derived 'homeostatic' prostaglandins involved in renal water and electrolyte balance, gastric cytoprotection and platelet aggregation.

Selective inhibitors of cyclooxygenase 2 were found to be as effective as traditional NSAIDs as well as being able to produce a 50% reduction in adverse gastrointestinal events. Vasodilation and increased permeability of postcapillary venules are early events in the inflammatory response and reflect the effect of cyclooxygenase 2 derived prostaglandins and leukotrienes at sites of inflammation. Prostaglandins can act as both proinflammatory and anti-inflammatory mediators depending on the context.

The 5-lipoxygenase pathway (5-LO) leading to leukotriene formation has been noted as a proinflammatory cascade. The cysteinyl leukotrienes are known to cause plasma leakage from postcapillary venules and enhance mucus secretion. Leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and 5-oxo-6,8, 11,14-eicosatetraenoic acid (5-oxo-E<sub>4</sub>) are eosinophil chemoattractants.

Eicosanoids include the compounds prostaglandins, thromboxane, leukotrienes and epoxyeicosatrienoic eicosanoids. Eicosa is twenty in Greek. Eicosanoids are so named due to the twenty carbon atoms derived from the fatty acid arachidonic acid.

Arachidonic acid is found as an ester in the phospholipid layer of cell membranes and is obtained from dietary sources, synthesized from the essential fatty acid linoleic acid, and released by the action of phospholipase A<sub>2</sub>.

Cysteinyl-leukotriene concentration was found increased in asthmatics. Sputum cysteinyl-leukotriene concentrations were greater in subjects with persistent and more severe asthma. Concentrations of cysteinyl-leukotrienes were highest in spontaneously produced sputum from subjects with acute severe asthma.

Cysteinyl-leukotrienes are found increased in bronchial wash and bronchoalveolar lavage samples from asthmatics. Cysteinyl-leukotriene concentrations are significantly greater in induced sputum than in bronchoalveolar lavage samples. Leukotriene E<sub>4</sub> (LTE<sub>4</sub>) is the end product of leukotriene C<sub>4</sub> (LTC<sub>4</sub>) and leukotriene D<sub>4</sub> (LTD<sub>4</sub>). Leukotriene E<sub>4</sub> can be measured in the urine. Urinary leukotriene E<sub>4</sub> concentrations are found increased after allergen challenge in atopic asthma and after aspirin challenge in subjects with aspirin induced asthma.

Exercise induced asthma is effectively inhibited by treatment with leukotriene receptor antagonists. Studies showed increased concentrations or proportions of bronchoconstrictor prostaglandins in bronchial wash and bronchoalveolar lavage in stable asthma and after allergen challenge.

A negative correlation was found between sputum eosinophil count and supernatant prostaglandin E2 (PGE2) concentration. Sputum concentrations of prostaglandin F2 alpha (PGF2a) correlated positively with the sputum eosinophil count. Eicosanoids are present in high concentrations in induced sputum, and concentrations of cysteinyl-leukotrienes are present in significantly higher concentrations in those with asthma than in normal control subjects.

The eicosanoids are a diverse group of extremely powerful hormone-like molecules produced in most tissues. They mediate many physiological processes including smooth muscle contraction, inflammation, pain perception, and blood flow regulation. Eicosanoids have been implicated in diseases including myocardial infarct and rheumatoid arthritis.

As they generally act in the cell in which they are synthesized, they are autocrine regulators rather than hormones. Arachidonic acid is synthesized from linoleic acid via addition of a three carbon unit, decarboxylation and desaturation. Arachidonic acid is also known as 5,8,11,14-eicosatetraenoic acid.

Eicosanoids are produced after arachidonic acid is released from membrane phospholipid molecules by the action of phospholipase A2. The eicosanoids (prostaglandins, thromboxanes, and leukotrienes) have been hard to study due to the fact that they are active for very short periods (seconds or minutes). They are also produced in small quantities.

The post mortem examination of asthmatic shows that the lungs are abnormally expanded or increased in size. Numerous sticky mucous plugs are found in the bronchi, the exudate is dense in the bronchial lumen with many eosinophils and effete columnar respiratory cells.

The bronchial mucous membrane and submucosa are swollen and infiltrated with eosinophils. The bronchial smooth muscle is found hypertrophied. Amongst the stimuli cited to invoke an asthmatic episode are allergens, drugs, exertion, emotion, infections and air pollution.

# Bronchial Asthma and the Atopic Syndrome

## Paper 9: The Metabolism of Prostaglandins

There are more than a dozen recognized members of the prostaglandin family. Prostaglandin's are named by letters according to the chemical groups attached to the five membered ring of "prostanic acid" - the first numerical subscript gives the number of double bonds contained within the side chains.

The alpha and beta subscripts for prostaglandin's tell if the hydroxyl group is on the same side of the molecule as the carboxylic acid group (alpha), or is on the opposite side (beta). The naturally occurring F prostaglandins all have alpha configuration.

Prostaglandins are formed from membrane-bound stores of polyunsaturated essential fatty acids by a series of complex reactions. The requirements for synthesis are 20-carbon fatty acids that have multiple unsaturated sites; molecular oxygen; co-factor reducing agents, and the multi-enzyme complex prostaglandin synthetase. The enzyme that synthesizes prostglandin's is found in all mammalian tissues except red blood cells.

Individually, each prostglandin has its own spectrum of activities that is tissue specific. Even in the same organ prostglandin's can oppose the action of other prostaglandins. They can produce both direct and indirect effects in virtually every mammalian tissue studied.

Generally, human tissues do not store prostaglandins; their effects occur as a result of immediate synthesis and release from the cells whose membranes are stimulated. Prostaglandins are local hormones; they influence events in the same or neighbouring cells that synthesize and metabolize them.

Prostaglandins are rapidly metabolized in the liver, lungs and other tissues. Their biologic half-life in the circulation is only a few minutes. More than 80% of prostaglandins E1, E2 and F2 alpha are metabolized during a single passage through the lungs or liver, but even in small amounts these substances have potent effect.

Generally, metabolism of the prostaglandins results in diminished biological activity, but 15 keto-PGF<sub>2</sub> alpha, which possesses a potent action on the lungs, is a notable exception.

Various endogenously produced **prostaglandins [9.1]** require glucuronic acid to be metabolized prior to being excreted from the body. **Glucuronic acid [9.2]** is also required to eliminate chemical and bacterial toxins. Endogenous compounds have to compete with exogenous **compounds [9.3]** for glucuronidation.

**Glucuronidation[9.4]** is the process whereby glucuronic acid is used to conjugate with chemical and bacterial substances so they may be metabolized and excreted from the body.

Compounds of glucuronic acid found in the body include glycosaminoglycans, heparin and lipoprotein lipase. Heparin is stored in mast cell granules and **lipoprotein lipase [9.5]** is derived from heparin.

**Glucuronic acid [9.6]** is the chemical moiety of the enzyme lipoprotein lipase which conjugates with the hydroxyl group and the carboxylic acid group found in prostaglandins. Once conjugated the lipid autocrine regulator (prostaglandin) is inactivated and can then be metabolized and excreted as a waste product.

Conjugation of prostaglandins with glucuronic acid is a significant part of the regular functioning of the lungs and tissues. A breakdown in this mechanism results in an asthmatic episode constituting smooth muscle dysfunction resulting in dyspnoea and immune system mediated inflammation technically referred to as anaphylaxis.

It is proposed that forms of the anaphylactic reaction manifest a biological adaptation to require more glucuronic acid from mast cells via heparin release and subsequent lipoprotein lipase synthesis so that prostaglandin hormones may be metabolized as normal and excreted from the body, thus enabling smooth muscle to function as normal.

In **summary [9.7]** prostaglandins are an intrinsic example of chemical sensitivity. Glucuronic acid is required to detoxify many intrinsic and extrinsic chemicals and toxins we come into contact with, thus competitively interfering with the formation of lipoprotein lipase and glucuronidation via depletion of available resources.

## Paper 9: The Metabolism of Prostaglandins

Various endogenously produced **prostaglandins** [9.1] require glucuronic acid to be metabolized prior to being excreted from the body. Glucuronic acid is also required to eliminate chemical and bacterial toxins. Endogenous compounds have to compete with exogenous compounds for glucuronidation.

**Paper Number: 9**  
**Reference Number: 9.1**

**Information taken from:**

Glucuronidation of oxidized fatty acids and prostaglandins B1 and E2 by human hepatic and recombinant UDP glucuronosyltransferases

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Although it has previously been reported that 20-HETE glucuronide is present in urine from normal human subjects at concentrations much higher than PGE<sub>2</sub> (23), this is the first report that 20-HETE glucuronide is formed in vitro by human UGT isoforms [1]. 20-HETE is formed as a result of the cytochrome P450-mediated metabolism of AA [2]. 20-HETE is a potent vasoconstrictor (41–43) and may also play an important role in renal function and the pathogenesis of hypertension (44)[3]. hydroxylated derivatives of AA such as 15- and 20-HETE are usually produced in the body after the consumption of foods rich in fats or under certain pathological conditions (25) [4].

The glucuronidation of HETEs can be considered a biotransformation process that results in the excretion of these lipids from the body after conversion to more soluble compounds [5]. However, because HETEs are also important signaling molecules, their biotransformation via glucuronidation could be, as in the case of PGs, considered a termination of biological activity [6].

Our studies have identified human recombinant UGT2B7 as being capable of forming a PGE<sub>2</sub> glucuronide. It has been demonstrated previously that metabolism of PGE<sub>2</sub> in isolated hepatocytes leads to a series of products including glucuronide conjugates (27) [7]. However, the in vitro formation of this compound is a new discovery. PGE<sub>2</sub> is a major product of AA metabolism via cyclooxygenase-2 and is also the major prostanoid product of AA metabolism in colorectal tissue (45) [8].

Because PGE<sub>2</sub> is actively glucuronidated by human intestinal mucosa, one can speculate about the significance of this reaction. Because UGT2B7 is the only isoform that has been identified to date that is able to glucuronidate PGE<sub>2</sub>, and because it is a major isoform expressed in human colon, the increased expression of UGT2B7 in the colon may protect against the accumulation of this PG [9].

On the other hand, lower expression may promote the accumulation of this PG and, thereby, the development of cancer. As presented in Fig. 4, the glucuronidation of PGE2 in small intestinal segment S-2 and colon is relatively high in some donors but demonstrates very significant individual variation. High expression of UGT2B7 in small intestine and colon, and the ability of this isoform to glucuronidate PGE2, could be a very important colon cancer protective pathway.

Additionally, we examined the glucuronidation of another PG, PGB1, for which the physiological role is not yet clearly defined. The fact that PGB1 is glucuronidated with relatively high affinity by UGT1A1, and with slightly lower affinity by UGT2B7, is also a new discovery [10]. To our knowledge, this is the first demonstration of glucuronidation of PGB1 by human UGTs and demonstrates a new biotransformation pathway for this product of AA metabolism.

This may represent a detoxification step or, if PGB1 is a ligand for nuclear receptors, as are other OFAs, may provide additional evidence that UGTs, like other drug-metabolizing enzymes, are involved in controlling steady-state concentrations of signaling molecules and/or ligands for nuclear receptors, as has been discussed by Nebert (46, 47) [11]. In summary, our present studies have identified novel substrates for in vitro glucuronidation: AA, PGB1, PGE2 and 20-HETE [12]. We have demonstrated previously that LA and its derivatives, LA-9,10- and LA-12,13-diols, as well as 13-HODE and 13-OXO, are excellent substrates for UGT2B7 (7, 8, 28) [13].

This work establishes the role of human recombinant UGT2B7 and several isoforms from the UGT1A family in the glucuronidation of physiologically and pharmacologically important lipid compounds, such as PGs, HETEs, and AA [14]. Because glucuronidation is the most effective detoxification process, it can be postulated that UGT2B7 is involved in the glucuronidation of these lipids when they accumulate above normal physiological concentrations [15]. On the other hand, the UGTs from the 1A family, especially 1A1 and 1A9, may be responsible for the glucuronidation of oxidized lipid substrates at physiological concentrations [16].

### **Notes:**

20-hydroxyeicosatetraenoic acid (20-HETE) glucuronide is present in normal human urine and is formed in vitro by human UDP-glucuronosyltransferase (UGT) isoforms [1]. 20-HETE is produced by the action of cytochrome P450 metabolism of arachidonic acid [2]. 20-HETE is a potent vasoconstrictor and has been postulated to play a role in renal function and the pathogenesis of hypertension [3].

Hydroxylated derivatives of arachidonic acid such as 15- and 20-hydroxyeicosatetraenoic acid are produced in the body after consumption of fat-rich foods or under certain pathological conditions [4]. Glucuronidation of hydroxyeicosatetraenoic acids is a biotransformation process which results in the excretion of these lipids from the body after conversion into more soluble compounds [5].

Hydroxyeicosatetraenoic acids (HETEs) are important signaling molecules and their biotransformation via glucuronidation (as in the case of prostaglandins) is considered a termination of their biological activity [6]. Human recombinant UDP-glucuronosyltransferase-2B7 (UGT2B7) can form a prostaglandin E2 glucuronide and metabolism of PGE2 leads to a series of products including glucuronide conjugates in isolated hepatocytes [7].

Prostaglandin E2 is a major product of arachidonic acid metabolism via cyclooxygenase-2 and is the major prostanoid product of arachidonic acid metabolism in colorectal tissue [8]. Prostaglandin E2 is actively glucuronidated by human intestinal mucosa.

UDP-glucuronosyltransferase-2B7 (UGT2B7) is the isoform which has been identified to glucuronidate prostaglandin E2 and is the major isoform expressed in human colon. The increased expression of this enzyme in the colon has been suggested to protect against the accumulation of this prostaglandin [9].

Prostaglandin B1 is glucuronidated with relatively high affinity for UDP-glucuronosyltransferase-1A1 (UGT1A1), and a lower affinity for UDP-glucuronosyltransferase-2B7 (UGT2B7) [10]. This has been interpreted to possibly represent a detoxification step and contribute to evidence that UDP-glucuronosyltransferases (like other drug-metabolizing enzymes) are involved in controlling steady state concentrations of signaling molecules and/or ligands for nuclear receptors [11].

Substrates for glucuronidation which have been identified include arachidonic acid, prostaglandin B1, prostaglandin E2, 20-hydroxyeicosatetraenoic acid [12]. Linoleic acid and its derivatives, linoleic acid-9,10- and linoleic acid-12,13-diols, as well as 13-oxooctadecadienoic acid (13-HODE) and 13-hydroxyoctadecadienoic acid (13-OXO) have also been identified as substrates for glucuronidation via UDP-glucuronosyltransferase-2B7 (UGT2B7) [13].

Human recombinant UDP-glucuronosyltransferase-2B7 (UGT2B7) and several isoforms from the UDP-glucuronosyltransferase-1A family are established in their role of glucuronidating physiologically and pharmacologically important lipid compounds such as prostaglandins, hydroxyeicosatetraenoic acids, and arachidonic acid [14].

As glucuronidation is the most effective detoxification process, it has been postulated that UDP-glucuronosyltransferase-2B7 (UGT2B7) is involved in the glucuronidation of these lipids when they accumulate above normal levels [15]. It is also recognized that other UDP-glucuronosyltransferases from the 1A family (esp. 1A1 and 1A9) may also be responsible for the glucuronidation of oxidized lipid substrates [16].

# Paper 9: The Metabolism of Prostaglandins

Various endogenously produced prostaglandins require glucuronic acid to be metabolized prior to being excreted from the body. **Glucuronic acid [9.2]** is also required to eliminate chemical and bacterial toxins. Endogenous compounds have to compete with exogenous compounds for glucuronidation.

**Paper Number: 9**  
**Reference Number: 9.2**

**Information taken from:**  
Linda Lazarides  
The Nutritional Health Bible  
First edition copyright 1997  
Harper Collins Publishers  
Page 93 Glucuronic Acid

## Glucuronic Acid

A substance derived from glucose, which can combine with chemical and bacterial toxins and convert them to a form ready for excretion [1].

## Notes:

Glucuronic acid is a substance derived from glucose that is required for the excretion of chemical and bacterial toxins [1].

## Paper 9: The Metabolism of Prostaglandins

Various endogenously produced prostaglandins require glucuronic acid to be metabolized prior to being excreted from the body. Glucuronic acid is also required to eliminate chemical and bacterial toxins. Endogenous compounds have to compete with exogenous **compounds** [9.3] for glucuronidation.

**Paper Number: 9**  
**Reference Number: 9.3**

**Information taken from:**  
 Biochemical Pharmacology

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Endotoxin inhibits glucuronidation in the liver :  
 An effect mediated by intercellular communication

Endotoxin [lipopolysaccharide (LPS) 50 µg/mL] added to the perfusion medium increased glucose production and inhibited the glucuronidation of p-nitrophenol in perfused mouse liver both in recirculating and non-recirculating systems, while sulfation of p-nitrophenol was unchanged [1].

The effects of endotoxin could be prevented by the addition of cyclooxygenase inhibitors, while PGD2 and PGE2 also caused a decrease in p-nitrophenol glucuronidation in perfused liver. In isolated hepatocytes endotoxin failed to affect p-nitrophenol conjugation, while PGD2 and PGE2 decreased the rate of it [2]. Our results suggest that endotoxin inhibits glucuronidation through an intercellular communication presumably mediated by eicosanoids [3].

### Notes:

Endotoxin was shown to inhibit the glucuronidation of p-nitrophenol in perfused mouse liver [1]. Prostaglandin D2 and E2 also caused a decrease in p-nitrophenol glucuronidation in perfused liver [2]. It has been suggested that endotoxin inhibits glucuronidation through intracellular communication mediated by eicosanoids [3].

# Paper 9: The Metabolism of Prostaglandins

**Glucuronidation [9.4]** is the process whereby glucuronic acid is used to conjugate with chemical and bacterial substances so they may be metabolized and excreted from the body.

**Paper Number: 9**  
**Reference Number: 9.4**

**Information taken from:**  
Linda Lazarides  
The Nutritional Health Bible  
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Harper Collins Publishers  
Page 60 Glucuronidation

## Glucuronidation

Glucuronic acid is a metabolite of glucose. It can conjugate with chemical and bacterial toxins such as alcohols, phenols, enols, carboxylic acid, amines, hydroxyamines, carbamides, sulphonamides and thiols, as well as some normal metabolites in a process known as glucuronidation [1]. For most individuals glucuronidation is a supplementary detoxification pathway. It is a secondary, slower process than sulphation or glycination, but is important if the latter pathways are diminished or saturated [2].

Obese people seem to have an enhanced capacity to detoxify molecules that can use the glucuronidation pathway. However, damage to the capacity for oxidative phosphorylation, which takes place in the mitochondria, is likely to diminish the capacity for glucuronide conjugation. If the livers detoxification pathways are excessively stimulated and overly utilised, they eventually become depleted or begin to respond poorly - being suppressed by toxic chemicals [3].

## Notes:

Glucuronic acid is a metabolite that conjugates with chemical and bacterial toxins in a process known as glucuronidation. It is known to conjugate alcohols, phenols, enols, carboxylic acid, amines, hydroxyamines, carbamides, sulphonamides and thiols [1].

As a detoxification pathway it is secondary to sulphation or glycination and is a slower process. When these other pathways become saturated it becomes primarily important [2]. If detoxification pathways are excessively stimulated and over utilized they become depleted and respond poorly. Toxic chemicals suppress these pathways [3].

# Paper 9: The Metabolism of Prostaglandins

Compounds of glucuronic acid found in the body include glycosaminoglycans, heparin and lipoprotein lipase. Heparin is stored in mast cell granules and **lipoprotein lipase [9.5]** is derived from heparin.

**Paper Number: 9**  
**Reference Number: 9.5**

**Information taken from:**

E. A. Newsholme  
A. R. Leech  
Biochemistry For The Medical Sciences  
Reprinted January 1992  
John Wiley & Sons  
Definition and function of Glycosaminoglycans  
Page 596 Glycosaminoglycans

A large number of the polysaccharides that occur in mammalian connective tissue are glycosaminoglycans. They form a fairly homogenous group of carbohydrates and are broadly defined by possession of the following characteristics [1]:

They contain a repeating disaccharide unit, consisting of a sugar (such as glucosamine, usually N-acetylated) and a uronic acid (except in keratan sulphate). The uronic acid is usually esterified with sulphate (except in hyaluronate) [2]. The polysaccharide is usually covalently linked to a protein to form a proteoglycan (also known as a mucopolysaccharide) [3].

Most glycosaminoglycan chains contain fewer than 100 monosaccharide units but there may be as many as 50 chains attached to a single core protein in the proteoglycan. Hyaluronate chains are longer and may contain 5000 monosaccharide residues.

#### Functions of Glycosaminoglycans

Glycosaminoglycans possess an exceptionally large negative charge density and therefore assume a random, extended, conformation. This accounts for the highly viscous solutions and gels (in which water molecules are 'trapped' in a loose framework of polysaccharide molecules) which these substances form and also for the lubricant action of, for example, hyaluronate, in synovial fluid [4].

In combination with protein, the sulphates of chondroitin, dermatan and keratan (each with a distinctive monosaccharide composition) form the ground substance of connective tissue. They provide the resistance of such tissues to compression, while the protein fibres of collagen and elastin embedded within them provide, respectively, the resistance to stretch and the elasticity characteristic of particular connective tissues.

Heparin, although structurally similar to other glycosaminoglycans, does not occur in connective tissue but is stored in mast cells beneath the epithelium of blood vessels. Here it acts as an anticoagulant and as a structural component of lipoprotein lipase [5].

Like all other cell constituents, glycosaminoglycans undergo continual turnover in the tissues. Their degradation is brought about by lysosomal enzymes and a number of diseases are caused by the failure of this system to work smoothly.

In rheumatoid arthritis, excessive release of lysosomal enzymes degrades the articular cartilage covering the surfaces of bone in apposition in the joints. A number of rare inborn errors of metabolism are known in which particular lysosomal enzymes are absent so that partially degraded glycosaminoglycans accumulate and cause problems (often by damaging the lysosomes so that their contents leak into the cytosol).

### **Notes:**

A large number of polysaccharides which occur in mammalian connective tissue are glycosaminoglycans [1]. They form a fairly homogenous group of carbohydrates which generally contain a repeating disaccharide unit consisting of a sugar (such as n-acetylglucosamine) and a uronic acid (often esterified with sulphate) [2]. When the polysaccharide is covalently linked to a protein they form a proteoglycan (mucopolysaccharide) [3].

Glycosaminoglycans have an exceptionally large negative charge density and assume a random, extended conformation of gels in which water molecules are trapped. It is this chemical property which contributes to the lubricant action [4]. Heparin is a glycosaminoglycan which is stored in mast cells. Heparin acts as an anticoagulant and as a structural component of lipoprotein lipase [5].

Glycosaminoglycans undergo continual turnover in the tissues via lysosomal enzyme action. Various diseases are caused by failures of this system [6]. In rheumatoid arthritis there is excessive release of lysosomal enzymes which degrade the articular cartilage covering the surfaces of bone in the joints [7].

# Paper 9: The Metabolism of Prostaglandins

**Glucuronic acid** [9.6] is the chemical moiety of the enzyme lipoprotein lipase which conjugates with the hydroxyl group and the carboxylic acid group found in prostaglandins. Once conjugated the lipid autocrine regulator (prostaglandin) is inactivated and can then be metabolized and excreted as a waste product.

**Paper Number: 9**  
**Reference Number: 9.6**

**Information taken from:**  
John Daintith  
Oxford Dictionary of Chemistry  
Third edition 1996  
Oxford University Press  
Page 225 Glucuronic Acid

## Glucuronic Acid

A compound -  $C_6H_9O_6$  - derived from the oxidation of glucose. It is an important constituent of gums and mucilages. Glucuronic acid can combine with hydroxyl (-OH), carboxyl (-COOH), or amino (-NH<sub>2</sub>) groups to form a glucuronide [1].

The addition of a glucuronide group to a molecule (glucuronidation) generally increases the solubility of a compound; hence glucuronidation plays an important role in the excretion of foreign substances [2].

## Notes:

Glucuronic acid is an important part of gums and mucilages. As a substance it combines with hydroxyl groups (-OH), carboxyl groups (-COOH), and amino groups (-NH<sub>2</sub>) to form a glucuronide [1]. Glucuronidation increases the solubility of chemicals which utilize the pathway for excretion [2].

# Paper 9: The Metabolism of Prostaglandins

In **summary** [9.7] prostaglandins are an intrinsic example of chemical sensitivity. Glucuronic acid is required to detoxify many intrinsic and extrinsic chemicals and toxins we come into contact with, thus competitively interfere with the formation of lipoprotein lipase and glucuronidation via depletion of available resources.

**Paper Number: 9**  
**Reference Number: 9.7**

**Information taken from:**

Aggregate notes from paper 9

## **-: Paper Nine - The Metabolism of Prostaglandins :-**

- **Glucuronidation of fatty acids is a biotransformation process which increases their solubility -**
- **Prostaglandin glucuronidation is considered a termination of their biological activity -**
- **Glucuronidation is the most effective detoxification process -**
- **Detoxification pathways may become depleted and respond poorly -**
- **Glucuronosyltransferase enzymes are suggested to protect against prostaglandin accumulation -**
- **Heparin, a glycosaminoglycan, contains glucuronic acid and is a structural precursor to lipoprotein lipase -**

20-hydroxyeicosatetraenoic acid (20-HETE) glucuronide is present in normal human urine and is formed in vitro by human UDP-glucuronosyltransferase (UGT) isoforms. 20-HETE is produced by the action of cytochrome P450 metabolism of arachidonic acid. 20-HETE is a potent vasoconstrictor and has been postulated to play a role in renal function and the pathogenesis of hypertension.

Hydroxylated derivatives of arachidonic acid such as 15- and 20-hydroxyeicosatetraenoic acid are produced in the body after consumption of fat-rich foods or under certain pathological conditions. Glucuronidation of hydroxyeicosatetraenoic acids is a biotransformation process which results in the excretion of these lipids from the body after conversion into more soluble compounds.

Hydroxyeicosatetraenoic acids (HETEs) are important signaling molecules and their biotransformation via glucuronidation (as in the case of prostaglandins) is considered a termination of their biological activity. Human recombinant UDP-glucuronosyltransferase-2B7 (UGT2B7) can form a prostaglandin E2 glucuronide and metabolism of PGE2 leads to a series of products including glucuronide conjugates in isolated hepatocytes.

Prostaglandin E2 is a major product of arachidonic acid metabolism via cyclooxygenase-2 and is the major prostanoid product of arachidonic acid metabolism in colorectal tissue. Prostaglandin E2 is actively glucuronidated by human intestinal mucosa.

UDP-glucuronosyltransferase-2B7 (UGT2B7) is the isoform which has been identified to glucuronidate prostaglandin E2 and is the major isoform expressed in human colon. The increased expression of this enzyme in the colon has been suggested to protect against the accumulation of this prostaglandin.

Prostaglandin B1 is glucuronidated with relatively high affinity for UDP-glucuronosyltransferase-1A1 (UGT1A1), and a lower affinity for UDP-glucuronosyltransferase-2B7 (UGT2B7). This has been interpreted to possibly represent a detoxification step and contribute to evidence that UDP-glucuronosyltransferases (like other drug-metabolizing enzymes) are involved in controlling steady state concentrations of signaling molecules and/or ligands for nuclear receptors.

Substrates for glucuronidation which have been identified include arachidonic acid, prostaglandin B1, prostaglandin E2, 20-hydroxyeicosatetraenoic acid. Linoleic acid and its derivatives, linoleic acid-9,10- and linoleic acid-12,13-diols, as well as 13-oxooctadecadienoic acid (13-HODE) and 13-hydroxyoctadecadienoic acid (13-OXO) have also been identified as substrates for glucuronidation via UDP-glucuronosyltransferase-2B7 (UGT2B7).

Human recombinant UDP-glucuronosyltransferase-2B7 (UGT2B7) and several isoforms from the UDP-glucuronosyltransferase-1A family are established in their role of glucuronidating physiologically and pharmacologically important lipid compounds such as prostaglandins, hydroxyeicosatetraenoic acids, and arachidonic acid.

As glucuronidation is the most effective detoxification process, it has been postulated that UDP-glucuronosyltransferase-2B7 (UGT2B7) is involved in the glucuronidation of these lipids when they accumulate above normal levels. It is also recognized that other UDP-glucuronosyltransferases from the 1A family (esp. 1A1 and 1A9) may also be responsible for the glucuronidation of oxidized lipid substrates.

Glucuronic acid is a substance derived from glucose that is required for the excretion of chemical and bacterial toxins.

Endotoxin was shown to inhibit the glucuronidation of p-nitrophenol in perfused mouse liver. Prostaglandin D2 and E2 also caused a decrease in p-nitrophenol glucuronidation in perfused liver. It has been suggested that endotoxin inhibits glucuronidation through intracellular communication mediated by eicosanoids.

Glucuronic acid is a metabolite that conjugates with chemical and bacterial toxins in a process known as glucuronidation. It is known to conjugate alcohols, phenols, enols, carboxylic acid, amines, hydroxyamines, carbamides, sulphonamides and thiols.

As a detoxification pathway it is secondary to sulphation or glycination and is a slower process. When these other pathways become saturated it becomes primarily important. If detoxification pathways are excessively stimulated and over utilized they become depleted and respond poorly. Toxic chemicals suppress these pathways.

A large number of polysaccharides which occur in mammalian connective tissue are glycosaminoglycans. They form a fairly homogenous group of carbohydrates which generally contain a repeating disaccharide unit consisting of a sugar (such as n-acetylglucosamine) and a uronic acid (often esterified with sulphate). When the polysaccharide is covalently linked to a protein they form a proteoglycan (mucopolysaccharide).

Glycosaminoglycans have an exceptionally large negative charge density and assume a random, extended conformation of gels in which water molecules are trapped. It is this chemical property which contributes to the lubricant action. Heparin is a glycosaminoglycan which is stored in mast cells. Heparin acts as an anticoagulant and as a structural component of lipoprotein lipase.

Glycosaminoglycans undergo continual turnover in the tissues via lysosomal enzyme action. Various diseases are caused by failures of this system. In rheumatoid arthritis there is excessive release of lysosomal enzymes which degrade the articular cartilage covering the surfaces of bone in the joints.

Glucuronic acid is an important part of gums and mucilages. As a substance it combines with hydroxyl groups (-OH), carboxyl groups (-COOH), and amino groups (-NH<sub>2</sub>) to form a glucuronide. Glucuronidation increases the solubility of chemicals which utilize the pathway for excretion.

# Bronchial Asthma and the Atopic Syndrome

## Paper 10: Heparin and it's Actions

Heparin is the best known **anti-inflammatory agent** [10.1].

Heparin is a member of the glycosaminoglycan family which is commonly known for its natural anticoagulant properties which is found especially in the **lungs** [10.2] and blood vessels.

**Heparin sulfate** [10.3] is a polymer of repeating disaccharide units of a hexuronic acid (glucuronic acid or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulfated, and both hexuronate and glucosamine residues can be O-sulfated in varying positions.

Heparin sulfate is a polymer of repeating disaccharide units of a hexuronic acid (glucuronic acid or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulfated, and both hexuronate and glucosamine residues can be O-sulfated in varying positions.

Heparin sulfate, chondroitin sulfate, dermatan sulfate and keratan sulfate are all part of the sulfated glycosaminoglycan family. Proteoglycan molecules consist of glycosaminoglycans and core proteins. Heparin sulfate proteoglycans are members of the proteoglycans that are components of the cell membranes and extracellular matrix.

Glycosaminoglycans determine the affinity of low density lipoprotein (LDL) for heparin sulfate proteoglycan. Low density lipoprotein (LDL) is associated with Heparin sulfate proteoglycans. **Lipoproteins** [10.4] are compounds of lipid and protein found in the blood and lymph.

Specificity of protein binding to heparin sulfate proteoglycan often depends on the structural features of the glycosaminoglycan chains. Low density lipoprotein binds more tightly to highly sulfated heparin glycosaminoglycan. Low density lipoprotein binds more tightly to **highly sulfated heparin glycosaminoglycan** [10.5].

Low density lipoprotein binding to endothelial cells is decreased by heparin sulfate degrading enzymes. Heparin causes release of lipoprotein lipase into the bloodstream. Heparin differs from heparin sulfate in the extent of N-acetylation, N- and O-sulfation and content of iduronate.

Heparin is stored in mast cells where it acts as an anticoagulant and a structural **component [10.6]** of lipoprotein **lipase [10.7]**.

During an asthmatic episode, inflammation and degranulation of the mast cells is produced by the action of the immune system. The body increases the numbers of eosinophils especially, which contain exceptionally high levels of the enzyme **arylsulfatase [10.8]**.

Arylsulfatase acts to split the sulfate groups from heparin sulfate and similar compounds. This acts to increase heparin levels and switch the specificity of lipoprotein away from acting on low density lipoprotein to some other mechanism of action which is involved in the termination of the anaphylactic inflammation and ultimately the symptoms of the atopic condition.

The **hypothesis [10.9]** here presented suggests the mechanism of action of this termination of the anaphylactic series of events as the making available resources of glucuronic acid for phase II conjugation and biological inactivation of both eicosanoids (prostaglandins, prostacyclins, leukotrienes, etc) and xenobiotic allergens which have competitively inhibited the normal metabolism and excretion of autocrine regulators.

# Paper 10: Heparin and it's Actions

Heparin is the best known **anti-inflammatory agent** [10.1].

Heparin is a member of the glycosaminoglycan family which is commonly known for its natural anticoagulant properties which is found especially in the lungs and blood vessels.

**Paper Number: 10**  
**Reference Number: 10.1**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic principles in Therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Functioning action of peptides and proteins  
Page 667 Components of the Blood Clotting System

## COMPONENTS OF THE BLOOD CLOTTING SYSTEM

The coagulation system is extremely complex. Activation of the intrinsic clotting system is initiated by activation of Hageman factor (factor XII); this agent then interacts with the complement and kinin-kallikrein systems. In addition to promoting the generation of vasoactive substances, activated Hageman factor by itself is capable of producing increased vascular permeability.

Other components of the clotting system may have important functions in the development of an inflammatory process, e.g. fibrin, which can be leukotactic and is an essential component for the development of the classic Schwartzman reaction (Vassalli and McCluskey, 1964; McKay et al., 1969).

Plasmin interacts with other plasma proteins such as components of complement, fibrin and the kinin system. Such interactions might be significant in terms of an inflammatory process, but proof of the relevance of these interactions to the inflammatory process has not yet been obtained (Eisen, 1969; Hamberg, 1969).

In disseminated intravascular coagulation (e.g. associated with endotoxemia), the interrelationships of the inflammatory process with abnormalities of coagulation may be critical to the outcome of the disease. The best "anti inflammatory agent" in such a clinical setting is the drug that can stop the coagulation process - heparin [1].

### Notes:

Interrelationships between the inflammatory process with abnormalities of coagulation have been noted. The best known anti-inflammatory agent in such clinical settings is heparin which can stop the coagulation process [1].

# Paper 10: Heparin and it's Actions

Heparin is a known anti-inflammatory agent. Heparin is a member of the glycosaminoglycan family which is commonly known for its natural anticoagulant properties which is found especially in the **lungs** [10.2] and blood vessels.

**Paper Number: 10**  
**Reference Number: 10.2**

**Information taken from:**  
Oxford Reference  
Concise Science Dictionary  
New edition 1991  
Oxford University Press  
Definition of heparin  
Page 320 Heparin

Heparin

A mucopolysaccharide with anticoagulant properties, occurring in vertebrate tissues, especially the lungs and blood vessels [1].

## **Notes:**

Heparin is the mucopolysaccharide which has anticoagulant properties. It is found especially in the lungs and blood vessels [1].

# Paper 10: Heparin and it's Actions

**Heparin sulfate [10.3]** is a polymer of repeating disaccharide units of a hexuronic acid (glucuronic acid or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulfated, and both hexuronate and glucosamine residues can be O-sulfated in varying positions.

**Paper Number: 10**  
**Reference Number: 10.3**

**Information taken from:**

Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis  
 Journal of Lipid Research Volume 37,1996 Page 696  
 Ira J. Goldberg  
 Department of Medicine, Columbia University  
 College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032

The observations that heparin released LPL into the bloodstream [1] (4) and that LPL binding to endothelial cells was markedly decreased by HSPGdegrading enzymes [2] (51) suggested that LPL is associated with HSPG. HSPG are members of the family of proteoglycans, negatively charged polysaccharides that are components of cell membranes and the extracellular matrix, and are important in cell adhesion and growth [3].

The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG-carbohydrate chains) and the core proteins [4]. The major classes of sulfated GAG, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and keratin sulfate, differ in their component sugars.

HS is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine [5]. The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions (52) [6].

This leads to a highly variable structure that depends on tissue of origin, molecular environment, and cell growth state. Heparin differs from HS in extent of N-acetylation, N- and O-sulfation, and content of iduronate [7].

## Notes:

Heparin causes release of lipoprotein lipase in the bloodstream [1]. Lipoprotein lipase binding to endothelial cells was markedly decreased by Heparin Sulfate Proteoglycan (HSPG) degrading enzymes [2]. Heparin sulfate proteoglycans are members of the proteoglycan family which are negatively charged polysaccharides known to be components of cell membranes and the extracellular matrix[3].

Proteoglycan molecules are composed of glycosaminoglycan chains and core proteins [4]. Heparin sulfate is a polymer composed of repeating disaccharide units of hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine [5].

The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions [6]. Heparin differs from Heparin Sulfate in the extent of N-acetylation, N- and O-sulfation and content of iduronate [7].

# Paper 10: Heparin and it's Actions

Glycosaminoglycans determine the affinity of low density lipoprotein (LPL) for heparin sulfate proteoglycan. Low density lipoprotein (LPL) is associated with Hparin sulfate proteoglycans. **Lipoproteins [10.4]** are compounds of lipid and protein found in the blood and lymph.

**Paper Number: 10**  
**Reference Number: 10.4**

**Information taken from:**  
 Percy J. Russell  
 Anita Williams  
 The Nutrition and Health Dictionary  
 Copyright 1995  
 Chapman & Hall  
 Definition of lipoproteins  
 Page 249 Lipoproteins

## Lipoproteins

Conjugated proteins that incorporate lipids as a part of their structure. Lipoproteins are associated with a wide variety of complexes that are significant in both structure and function of cells [1]. Lipoproteins appear to be of two types. Those that exist as relatively discrete and identifiable macromolecules are called "soluble types". Those that are aggregates of the complex membrane structure called "membrane types" of structural lipoproteins [2].

Most soluble lipoproteins circulate in the blood plasma. Some are involved in blood clotting [3]. The ratio of lipid to protein in the serum lipoprotein varies widely, and these variations affect their density. Lipoproteins differ in their densities and flotation rates (Sf values), depending upon the kind and quantity of lipid associated with the protein [4].

Lipoproteins fall into four general classifications which depend on their density [5]. The HDL (high density lipoprotein) and LDL (low density lipoprotein) levels in the serum are more sensitive indexes of coronary heart disease than total cholesterol [6].

The cholesterol in LDL has a direct relationship to coronary heart disease whereas the cholesterol in HDL has an inverse relationship. In others, it is the distribution of the total cholesterol between the LDL and HDL rather than the total blood cholesterol that better defines the risk of coronary heart disease[7].

## Notes:

Lipoproteins are compounds that contain both protein and lipids [1]. They are divided into two types; soluble types and membrane types [2]. Soluble lipoproteins are found in the blood plasma and some are involved in blood clotting [3].

Lipoproteins have different densities and floatation rates according to the proportions of lipid and proteins incorporated in them [4], lipoproteins are classed according to their densities [5].

High and low density lipoproteins present in blood serum are good indexes of coronary heart disease [6]. The cholesterol in low density lipoproteins has a direct relationship to coronary heart disease whereas the cholesterol in high density lipoproteins has an inverse relationship [7].

# Paper 10: Heparin and it's Actions

Specificity of protein binding to heparin sulfate proteoglycan often depends on the structural features of the glycosaminoglycan chains. Low density lipoprotein binds more tightly to highly sulfated heparin glycosaminoglycan. Low density lipoprotein binds more tightly to **highly sulfated heparin glycosaminoglycan [10.5]**.

**Paper Number: 10**  
**Reference Number: 10.5**

**Information taken from:**

Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis  
Journal of Lipid Research Volume 37,1996 Page 697  
Ira J. Goldberg  
Department of Medicine, Columbia University  
College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032

Specificity of protein binding to HSPG often depends on the structural features of the GAG chains [1]. Specific oligosaccharides with high affinity for antithrombin and several growth factors have been sequenced. LPL is no exception to this pattern; GAG also determine the affinity of LPL for HSPG [2]. LPL binds more tightly to highly sulfated heparin GAG [3] (59) and treatment of cells with chlorate, which reduces sulfate of GAG, decreases LPL binding to adipocytes [4] (60).

## **Notes:**

The specificity of protein binding to Heparin Sulfate Proteoglycan (HSPG) often depends on the structural features of the glycosaminoglycan chains [1]. The glycosaminoglycan chain determines the affinity of lipoprotein lipase (LPL) for heparin sulfate proteoglycan [2].

Lipoprotein lipase binds more tightly to highly sulfated heparin glycosaminoglycan [3]. Treatment of cells with chlorate reduces the sulfate of glycosaminoglycans and decreases lipoprotein lipase binding to adipocytes [4].

# Paper 10: Heparin and it's Actions

Heparin is stored in mast cells where it acts as an anticoagulant and a structural **component [10.6]** of lipoprotein lipase.

**Paper Number: 10**  
**Reference Number: 10.6**

**Information taken from:**  
E. A. Newsholme  
A. R. Leech  
Biochemistry For The Medical Sciences  
Reprinted January 1992  
John Wiley & Sons  
Definition and function of  
Glycosaminoglycans  
Page 596 Glycosaminoglycans

Heparin, although structurally similar to other glycosaminoglycans, does not occur in connective tissue but is stored in mast cells beneath the epithelium of blood vessels. Here it acts as an anticoagulant and as a structural component of lipoprotein lipase [1].

## **Notes:**

Heparin is structurally similar to other glycosaminoglycans and stored in mast cells where it acts as an anticoagulant and as a structural component of lipoprotein lipase [1].

# Paper 10: Heparin and it's Actions

Heparin is stored in mast cells where it acts as an anticoagulant and a structural component of lipoprotein **lipase** [10.7].

**Paper Number: 10**  
**Reference Number: 10.7**

**Information taken from:**

Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis

Ira J. Goldberg

Department of Medicine, Columbia University

College of Physicians and Surgeons, 630

West 168th Street, New York, NY 10032

Journal of Lipid Research Volume 37,1996 Page 696

The observations that heparin released LPL into the bloodstream (4) [1] and that LPL binding to endothelial cells was markedly decreased by HSPGdegrading enzymes (51) suggested that LPL is associated with HSPG [2]. HSPG are members of the family of proteoglycans, negatively charged polysaccharides that are components of cell membranes and the extracellular matrix, and are important in cell adhesion and growth [3].

The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG-carbohydrate chains) and the core proteins [4]. The major classes of sulfated GAG, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and keratin sulfate, differ in their component sugars [5].

HS is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine [6]. The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions (52). This leads to a highly variable structure that depends on tissue of origin, molecular environment, and cell growth state [7].

Heparin differs from HS in extent of N-acetylation, N- and O-sulfation, and content of iduronate [8]. Vascular endothelial cells synthesize a variety of HSPG whose core proteins are integral membrane proteins or are membrane-associated through a glycosylphosphatidylinositol (GPI) linkage. In other cell types, some proteoglycans associate with cell surfaces by binding to receptors for either the GAG or core protein [9].

## Notes:

Heparin releases Lipoprotein Lipase (LPL) into the bloodstream [1] and lipoprotein lipase binding to endothelial cells was markedly decreased by Heparin Sulphate Proteoglycan (HSPG) degrading enzymes suggests lipoprotein lipase is associate with heparin sulphate proteoglycans [2].

Heparin sulphate proteoglycans are members of the family of proteoglycans composed of negatively charged polysaccharides which are constituents of cell membranes and the extracellular matrix as well as being involved in cell adhesion and growth [3].

The two main parts of the proteoglycan molecule are the glycosaminoglycans and the core proteins [4]. The classes of sulphated glycosaminoglycan differ in their component sugars; chondroitin sulphate, dermatan sulphate, heparin sulphate, and keratin sulphate are all members of this class [5].

Heparin sulphate is a polymer of repeating disaccharide units of a hexuronic acid (glucuronic or iduronic acid) and glucosamine [6]. The glucosamine residues are either N-acetylated or N-sulphated and both hexuronate and glucosamine residues may be O-sulphated in varying positions [7].

Heparin differs from heparin sulphate in extent of N-acetylation, N- and O-sulphation and content of iduronate [8]. Vascular endothelial cells synthesize a variety of heparin sulphate proteoglycans whose core proteins are membrane proteins or are associated to the membrane through a glycosylphosphatidylinositol linkage. In other cell types, some proteoglycans associate with cell surfaces by binding to receptors for either the glycosaminoglycan or the core protein [9].

# Paper 10: Heparin and it's Actions

During an asthmatic episode, inflammation and degranulation of the mast cells is produced by the action of the immune system. The body increases the numbers of eosinophils especially, which contain exceptionally high levels of the enzyme **arylsulfatase [10.8]**.

**Paper Number: 10**  
**Reference Number: 10.8**

**Information taken from:**

The Heparin/Heparan Sulfate 2-O-Sulfatase from *Flavobacterium heparinum*  
 A Structural And Biochemical Study Of The Enzyme  
 Active Site And Saccharide Substrate Specificity  
 Received for publication, November 8, 2002, and in revised form, December 16, 2002  
 Published, JBC Papers in Press, January 7, 2003, DOI 10.1074/jbc.M211425200  
 Rahul Raman<sup>‡§</sup>, James R. Myette<sup>‡¶</sup>, Zachary Shriver<sup>‡</sup>, Kevin Pojasek<sup>‡</sup>,  
 Ganesh Venkataraman<sup>\*\*</sup>, and Ram Sasisekharan<sup>‡ ‡‡</sup>  
 From the <sup>‡</sup>Division of Biological Engineering and <sup>\*\*</sup>  
 Division of Health Sciences and Technology,  
 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Heparin and heparin sulfate glycosaminoglycans (HSGAGs) are structurally complex linear polysaccharides (1, 2) composed of repeating disaccharides of uronic acid (L-iduronic or D-glucuronic) linked to -D-glucosamine [1]. This structural complexity derives principally from the variable chemical modifications made to the polysaccharide chain. Such modifications include acetylation or sulfation at the N-position of the glucosamine, epimerization of glucuronic acid to iduronic acid, and additional O-sulfation at the 2-O-position of the uronic acid in addition to the 3-O, 6-O-position of the adjoining glucosamine [2].

It is a highly variable sulfation pattern, in particular, which ascribes to each GAG chain a unique structural signature. In turn, this signature dictates specific GAG-protein interactions underlying critical biological processes related to cell and tissue function [3]. Given this critical structure-function relationship of GAG sulfation, enzymes that can hydrolyze these sulfates in a structurally specific manner are important tools for the determination of GAG fine structure to better ascertain these structure-function relationships.

In the previous paper (21), we described the cloning, recombinant expression, and biochemical characterization of one such sulfatase, the 2-O-sulfatase from *Flavobacterium heparinum*. As members of a large enzyme family, the sulfatases hydrolyze a wide array of sulfate esters (for a review, see Refs. 3 and 4) [4].

Their respective substrates include sulfated complex carbohydrates such as the glycosaminoglycans (GAGs), steroids, sphingolipids, xenobiotic compounds, and amino acids such as tyrosine [5]. Additionally, many of these enzymes are able to hydrolyze in vitro smaller synthetic substrates (e.g. 4-nitrophenyl sulfate and catechol sulfate) [6].

It is for this reason that these enzymes are often generically described as “arylsulfatases” (even when their preferred in vivo substrate is ill defined). Despite their disparate substrate specificities, the members of this enzyme family share both considerable structural homology and a common catalytic mechanism with one another (5) [7].

**Notes:**

Heparin and heparin sulfate glycosaminoglycans (HSGAGs) are structurally complex linear polysaccharides composed of repeating disaccharides of uronic acid (either L-iduronic acid or D-glucuronic acid) linked to a D-glucosamine [1].

Structural complexity derives from the various chemical modifications made to the polysaccharide chain such as acetylation or sulfation of the N-position of the glucosamine, epimerization of glucuronic acid to iduronic acid, and additional O-sulfation at the 2-O-position of the uronic acid as well as the 3-O, 6-O position of the adjacent glucosamine [2].

The sulfation pattern is highly variable which gives each glycosaminoglycan chain a unique structure. The structure dictates the interactions which occur between the glycosaminoglycans and proteins [3].

The sulfatases are members of a large enzyme family and hydrolyze a wide variety of sulfate esters [4]. Their substrates include sulfated complex carbohydrates such as the glycosaminoglycans, steroids, sphingolipids, xenobiotic compounds, and amino acids such as tyrosine [5]. Many can hydrolyze small synthetic substrates in vitro such as 4-nitrophenyl sulfate and catechol sulfate [6].

For this reason these enzymes are generally described as arylsulfatases. Despite having disparate substrate specificities the arylsulfatases share considerable structural homology and a common catalytic mechanism [7].

# Paper 10: Heparin and it's Actions

The **hypothesis [10.9]** here presented suggests the mechanism of action of this termination of the anaphylactic series of events as teh making available resources of glucuronic acid for phase II conjugation and biological inactivation of both eicosanoids (prostaglandins, prostacyclins, leukotrienes, etc) and xenobiotic allergens which have competitively inhibited the normal metabolism and excretion of autocrine regulators.

**Paper Number: 10**  
**Reference Number: 10.9**

**Information taken from:**

Aggregate notes of paper 10

## **-: Paper Ten - Heparin and it's Actions :-**

- **Heparin is both an anti inflammatory agent and an anticoagulant -**
- **Heparin causes the release of lipoprotein lipase -**
- **Heparin is a structural component of lipoprotein lipase -**
- **The sulfation pattern gives each glycosaminoglycan chain a unique structure -**
- **Heparin sulfate and related glycosmainoglycans are composed of glucuronic acid -**
- **Sulfatases hydrolyze a wide variety of sulfate esters -**

Interrelationships between the inflammatory process with abnormalities of coagulation have been noted. The best known anti-inflammatory agent in such clinical settings is heparin which can stop the coagulation process.

Heparin is the mucopolysaccharide which has anticoagulant properties. It is found especially in the lungs and blood vessels.

Heparin causes release of lipoprotein lipase in the bloodstream. Lipoprotein lipase binding to endothelial cells was markedly decreased by Heparin Sulfate Proteoglycan (HSPG) degrading enzymes. Heparin sulfate proteoglycans are members of the proteoglycan family which are negatively charged polysaccharides known to be components of cell membranes and the extracellular matrix.

Proteoglycan molecules are composed of glycosaminoglycan chains and core proteins. Heparin sulfate is a polymer composed of repeating disaccharide units of hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine.

The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions. Heparin differs from Heparin Sulfate in the extent of N-acetylation, N- and O-sulfation and content of iduronate.

Lipoproteins are compounds that contain both protein and lipids. They are divided into two types; soluble types and membrane types. Soluble lipoproteins are found in the blood plasma and some are involved in blood clotting.

Lipoproteins have different densities and floatation rates according to the proportions of lipid and proteins incorporated in them, lipoproteins are classed according to their densities.

High and low density lipoproteins present in blood serum are good indexes of coronary heart disease. The cholesterol in low density lipoproteins has a direct relationship to coronary heart disease whereas the cholesterol in high density lipoproteins has an inverse relationship.

The specificity of protein binding to Heparin Sulfate Proteoglycan (HSPG) often depends on the structural features of the glycosaminoglycan chains. The glycosaminoglycan chain determines the affinity of lipoprotein lipase (LPL) for heparin sulfate proteoglycan.

Lipoprotein lipase binds more tightly to highly sulfated heparin glycosaminoglycan. Treatment of cells with chlorate reduces the sulfate of glycosaminoglycans and decreases lipoprotein lipase binding to adipocytes.

Heparin is structurally similar to other glycosaminoglycans and stored in mast cells where it acts as an anticoagulant and as a structural component of lipoprotein lipase.

Heparin releases Lipoprotein Lipase (LPL) into the bloodstream and lipoprotein lipase binding to endothelial cells was markedly decreased by Heparin Sulphate Proteoglycan (HSPG) degrading enzymes suggests lipoprotein lipase is associate with heparin sulphate proteoglycans.

Heparin sulphate proteoglycans are members of the family of proteoglycans composed of negatively charged polysaccharides which are constituents of cell membranes and the extracellular matrix as well as being involved in cell adhesion and growth.

The two main parts of the proteoglycan molecule are the glycosaminoglycans and the core proteins. The classes of sulphated glycosaminoglycan differ in their component sugars; chondroitin sulphate, dermatan sulphate, heparin sulphate, and keratin sulphate are all members of this class.

Heparin sulphate is a polymer of repeating disaccharide units of a hexuronic acid (glucuronic or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulphated and both hexuronate and glucosamine residues may be O-sulphated in varying positions.

Heparin differs from heparin sulphate in extent of N-acetylation, N- and O-sulphation and content of iduronate. Vascular endothelial cells synthesize a variety of heparin sulphate proteoglycans whose core proteins are membrane proteins or are associated to the membrane through a glycosylphosphatidylinositol linkage. In other cell types, some proteoglycans associate with cell surfaces by binding to receptors for either the glycosaminoglycan or the core protein.

Heparin and heparin sulfate glycosaminoglycans (HSGAGs) are structurally complex linear polysaccharides composed of repeating disaccharides of uronic acid (either L-iduronic acid or D-glucuronic acid) linked to a D-glucosamine.

Structural complexity derives from the various chemical modifications made to the polysaccharide chain such as acetylation or sulfation of the N-position of the glucosamine, epimerization of glucuronic acid to iduronic acid, and additional O-sulfation at the 2-O-position of the uronic acid as well as the 3-O, 6-O position of the adjacent glucosamine.

The sulfation pattern is highly variable which gives each glycosaminoglycan chain a unique structure. The structure dictates the interactions which occur between the glycosaminoglycans and proteins.

The sulfatases are members of a large enzyme family and hydrolyze a wide variety of sulfate esters. Their substrates include sulfated complex carbohydrates such as the glycosaminoglycans, steroids, sphingolipids, xenobiotic compounds, and amino acids such as tyrosine. Many can hydrolyze small synthetic substrates in vitro such as 4-nitrophenyl sulfate and catechol sulfate.

For this reason these enzymes are generally described as arylsulfatases. Despite having disparate substrate specificities the arylsulfatases share considerable structural homology and a common catalytic mechanism.

# Bronchial Asthma and the Atopic Syndrome

## Paper 11: Glucuronidation

In hypothesis, the antagonistic qualities of allergenic chemicals can be attributed to the compounds possessing the same chemical groups that the prostaglandin hormones possess. A deficit of lipoprotein lipase arises through a competitive requirement of glucuronic acid which is needed both to conjugate with and excrete specific chemical toxins from the body, and bioregulate the eicosanoid metabolism through glucuronidation.

As the prostaglandin hormones require the same method of detoxification as extrinsic allergens, when a deficit of glucuronic acid arises prostaglandin hormones remain active longer than they should because they have to compete with toxins for glucuronidation and biological inactivation.

The body which exists in significant deficit will possess sparse stores of glucuronic acid which are drawn upon in two instances:

[A] Stimulus causes prostaglandin hormone levels to increase which in turn increases the requirement for lipoprotein lipase, heparin, glycosaminoglycans and glucuronic acid.

[B] Certain Xenobiotics, toxins and waste substances raise the requirement for glucuronic acid because the substances require to be conjugated with glucuronic acid and excreted from the body via the glucuronidation pathway.

A deficiency of glucuronic acid results in the overstimulation of smooth muscle systems controlled by prostaglandins. This overstimulation exists until the body requisites enough glucuronic acid from mast cells (by the action of the immune system causing inflammation via anaphylaxis) to detoxify the molar sum total of the active eicosanoids and xenobiotic toxins which require glucuronidation.

Glucuronic acid compounds in the body are classed as **Acid Polysaccharides [11.1]**. As well as being an important constituent of glycosaminoglycans and mucopolysaccharides, glucuronic acid is used to conjugate foreign matter so it may be excreted as **Organic Acids in the Urine [11.2]**.

Glucuronidation is an important pathway for the metabolism of **Foreign Organic Compounds [11.3]**; these conjugates are termed glucuronides.

The specific chemical groups that require glucuronic acid for excretion possess allergenic qualities. These include alcohols, phenols, enols, carbamides, sulphonamides, thiols, some antibiotics, female sex hormones, hydroxyl containing compounds (prostaglandins), carboxylic acid containing compounds (prostaglandins), amine containing compounds and benzoic acid. These substances and chemical groups are found naturally in the body, in the diet and in our environment.

All of the above mentioned substances command relative resources of glucuronic acid for detoxification purposes. Compounds have been shown to have **Direct Chemical Interactions with Heparin [11.4]** and demonstrate that chemicals have a direct negative effect on lipoprotein lipase precursors and other compounds of glucuronic acid.

This negative effect results in an immune reaction to access relevant resources with the inflammatory process thus to compensate for a rise in glucuronic acid demand.

**Summary [11.5]**: Bronchial asthma and atopic conditions are hypothesized as manifestations of a deficiency of glucuronic acid and its compounds heparin and lipoprotein lipase.

# Paper 11: Glucuronidation

Glucuronic acid compounds in the body are classed as **Acid Polysaccharides [11.1]**. As well as being an important constituent of glycosaminoglycans and mucopolysaccharides, glucuronic acid is used to conjugate foreign matter so it may be excreted as organic acids in the urine.

**Paper Number: 11**  
**Reference Number: 11.1**

**Information taken from:**

S. P. Datta  
J. H. Ottaway  
Biochemistry  
Copyright 1965  
Bailliere, Tindall and Cassell  
Chemistry of the Carbohydrates  
Page 25 Acid Polysaccharides

## Acid Polysaccharides

There is a wide range of polysaccharides containing acid groups, either as sulphate esters or as uronic acids. They appear to play a structural role, as distinct from the storage of carbohydrate for energy as with starch and glycogen. They form gels which can be very rigid, particularly if metal ions are present [1].

The most important of them in the body are based on a repeating unit of acetyl galactosamine sulphate and glucuronic acid. Chondroitin Sulphate: This, together with a protein, forms the chondromucoid and osteomucoid which are the ground substances of cartilage and bone respectively. Mucoitin Sulphate: This is a chain of acetyl-glucosamine-sulphate-glucuronic acid units. It is found in the lubricating mucoproteins, such as the mucin of saliva. Heparin (the anticoagulant): This is very similar to mucoitin sulphate [2].

Hyaluronic acid. This is equivalent to mucoitin sulphate without the sulphate, i.e. its basic structure is acetyl-glucosamine-glucuronic acid. It forms the cement of interstitial tissue generally, and of hyalin. It is depolymerized by the enzyme hyaluronidase (the 'spreading factor') present in sperm and in some bacteria and some snake venoms [3].

Blood group substances, and capsular polysaccharides. These are rather more complex polysaccharides along the same lines, which are found in red cells and in the capsules of some bacteria (e.g. pneumococci). They are highly antigenic [4].

## **Notes:**

There are many polysaccharides which contain acid groups that are sulphate esters or uronic acids. They have a function distinct from other carbohydrates that are used for energy. They form gels that have a structural role and have an involvement with metal ions [1].

The most important of the acid polysaccharides are based on acetyl galactosamine sulphate and glucuronic acid. Chondroitin sulphate is important for bone and cartilage, Mucoitin sulphate is important to the lubricating mucoproteins and heparin is an important anticoagulant [2].

Hyaluronic acid is the acid which forms the 'cement' of interstitial tissue and of hyalin [3]. There are also complex acid polysaccharides found in red cells [4].

# Paper 11: Glucuronidation

Glucuronic acid compounds in the body are classed as Acid Polysaccharides. As well as being an important constituent of glycosaminoglycans and mucopolysaccharides, glucuronic acid is used to conjugate foreign matter so it may be excreted as **Organic Acids in the Urine [11.2]**.

**Paper Number: 11**  
**Reference Number: 11.2**

**Information taken from:**  
William Veale Thorpe  
Biochemistry for Medical Students  
Sixth edition 1955  
J. & A. Churchill Ltd.  
Organic acids in urine  
Page 497 Glucuronic acid.

## Organic Acids In Urine

Glucuronic acid in the form of conjugated compounds (glucuronides), is present in normal urine. The conjugated acids are formed from phenols produced by bacterial decomposition in the large intestine [1].

The amount is normally less than 0.15g per day. The amount is increased either by excessive intestinal putrefaction or by drugs, or metabolites of drugs, which are conjugated with glucuronic acid [2], for example: Acetanilide, antipyrine, borneol, camphor, chloral, chloroform, menthol, morphine, naphthol, phenol, thymol and some sulphonamides, e.g. sulphapyridine [3].

## Notes:

Glucuronic acid conjugates with chemicals, for example phenols, so they can be excreted in the urine. These conjugates are called glucuronides [1]. On average, in 1955, less than 150 milligrams of glucuronides were found in the urine per day. The amount is increased by intestinal putrefaction and by drugs which require glucuronic acid for excretion [2]. Some sulphonamide antibiotics require glucuronic acid for excretion [3].

# Paper 11: Glucuronidation

Glucuronidation is an important pathway for the metabolism of **Foreign Organic Compounds [11.3]**; these conjugates are termed glucuronides.

**Paper Number: 11**  
**Reference Number: 11.3**

**Information taken from:**  
William Veale Thorpe  
Biochemistry for Medical Students  
Sixth edition 1955  
J. & A. Churchill Ltd.  
Metabolism of foreign organic compounds  
Page 334 Glucuronic acid

## Glucuronic Acid

Conjugation with glucuronic acid occurs in all species. This acid can combine with many substances which contain (or form by oxidation or reduction) hydroxyl groups (alcoholic or phenolic) as well as with acids like benzoic or phenylacetic [1]. Examples of these two types of compound are phenylglucuronic acid and benzoylglucuronic acid. Conjugates with glucuronic acid are usually called glucuronides [2].

Female sex hormones which are excreted in urine usually found as glucuronides. The origin the glucuronic acid is probably from three carbon compounds e.g. trioses, but the mechanism of its formation is not understood [3].

## Notes:

Glucuronic acid conjugates with compounds that have alcoholic and phenolic hydroxyl groups as well as with acids such as benzoic acid and phenylacetic acid [1]. These compounds are excreted as the glucuronides phenylglucuronic acid and benzoylglucuronic acid [2]. Glucuronic acid also conjugates with the lipid female sex hormones before they are excreted [3].

# Paper 11: Glucuronidation

All of the above mentioned substances command relative resources of glucuronic acid for detoxification purposes. Compounds have been shown to have **Direct Chemical Interactions with Heparin [11.4]** and demonstrate that chemicals have a direct negative effect on lipoprotein lipase precursors and other compounds of glucuronic acid.

**Paper Number: 11**  
**Reference Number: 11.4**

**Information taken from:**  
Kenneth L. Melmon, M.D.  
Howard F. Morrelli, M.D.  
Clinical Pharmacology  
Basic principles in Therapeutics  
Second edition 1978  
Bailliere, Tindall london  
Direct chemical interactions with heparin  
Page 985 Direct Chemical or Physical Interactions

## Direct Chemical Or Physical Interactions

Our appreciation of drugs as therapeutic agents may diminish our awareness of their important chemical or physical properties. These properties account for interactions that may be therapeutically useful or harmful. For example, the anticoagulant effect of heparin, an acid, may be reversed by protamine, a base [1].

Other basic drugs, such as antihistamines, phenothiazine, and certain antibiotics, if in stoichiometrically appropriate quantities, may also counter heparin's effects, as shown in vitro, and in vivo in dogs (Nelson et al., 1959) [2].

## Notes:

The properties of some drugs have a negative effect on the glucuronic acid compound Heparin; for example protamine [1]. Relative amounts of antihistamines, phenothiazines and certain antibiotics also have a negative effect on Heparin [2].

# Paper 11: Glucuronidation

**Summary [11.5]:** Bronchial asthma and atopic conditions are hypothesized as manifestations of a deficiency of glucuronic acid and its compounds heparin and lipoprotein lipase.

**Paper Number: 11**  
**Reference Number: 11.5**

**Information taken from:**

Aggregate notes of paper 11

## **-: Paper Eleven - Glucuronidation :-**

- **The most important acid polysaccharides are those based on glucuronic acid and acetyl galactosamine sulfate -**
- **Glucuronic acid conjugates with various chemicals to allow their excretion as glucuronides -**
- **The amount of glucuronides in the urine is increased by intestinal putrefaction and by drugs -**
- **Glucuronic acid is known to conjugate alcoholic and phenolic hydroxyl groups -**
- **Glucuronic acid conjugates with the lipid female sex hormones for excretion -**
- **Various drugs such as antihistamines, phenothiazines and certain antibiotics have a negative effect on heparin -**

There are many polysaccharides which contain acid groups that are sulphate esters or uronic acids. They have a function distinct from other carbohydrates that are used for energy. They form gels that have a structural role and have an involvement with metal ions.

The most important of the acid polysaccharides are based on acetyl galactosamine sulphate and glucuronic acid. Chondroitin sulphate is important for bone and cartilage, Mucoitin sulphate is important to the lubricating mucoproteins and heparin is an important anticoagulant. Hyaluronic acid is the acid which forms the 'cement' of interstitial tissue and of hyalin. There are also complex acid polysaccharides found in red cells.

Glucuronic acid conjugates with chemicals, for example phenols, so they can be excreted in the urine. These conjugates are called glucuronides. On average, in 1955, less than 150 milligrams of glucuronides were found in the urine per day.

The amount is increased by intestinal putrefaction and by drugs which require glucuronic acid for excretion. Some sulphonamide antibiotics require glucuronic acid for excretion.

Glucuronic acid conjugates with compounds that have alcoholic and phenolic hydroxyl groups as well as with acids such as benzoic acid and phenylacetic acid.

These compounds are excreted as the glucuronides phenylglucuronic acid and benzoylglucuronic acid. Glucuronic acid also conjugates with the lipid female sex hormones before they are excreted.

The properties of some drugs have a negative effect on the glucuronic acid compound Heparin; for example protamine. Relative amounts of antihistamines, phenothiazines and certain antibiotics also have a negative effect on Heparin.

# Bronchial Asthma and the Atopic Syndrome

## Paper 12: Hypothesis on Atopy's Treatment

Heparin, an essential component of lipoprotein lipase, is a substance that successfully brings to an end the series of reactions which have come to be known as anaphylactic inflammation.

When heparin is injected, lipoprotein lipase is released into the bloodstream where it acts on chylomicrons and various fatty substances.

Heparin is composed of glucosamine, glucuronic acid, iduronic acid (the isomer of glucuronic acid), and varying acetyl and sulphate groups. Heparin is stored in mast cell granules.

The eosinophil splits the sulphate from heparin and sulphated glycosaminoglycans with the release of the enzyme arylsulfatase. The mast cell also releases arylsulfatase along with the secreted heparin as a part of the anaphylactic reaction.

Glucuronic acid and glucosamine in combination are potential avenues of therapeutic approach in bronchial asthma and atopic conditions by virtue of inductive reasoning of the following.

Atopic conditions are all different manifestations of the anaphylactic reaction causing localized regions of inflammation in different mast cell containing tissues. The mast cell has been used as the model of anaphylaxis.

Heparin, an essential precursor of lipoprotein lipase, is a known **Anti-inflammatory Agent [12.1]** used in a clinical setting.

When the **Effects of Heparin on Lipoprotein Lipase [12.2]** are observed it proposes that when the precursor metabolite, heparin, into the body, lipoprotein lipase is produced as a result.

**Heparin [12.3]** is a compound of glucuronic acid, iduronic acid, sulphate groups and acetyl groups. The acetyl group are attached to the glucosamine units and the sulphate groups are attached to the **Glucuronic Acid Residues [12.4]**.

The **Eosinophilic Leukocytes [12.5]** and mast cells possess an enzyme (arylsulfatase) which acts to cleave the sulphate from the glucuronic acid thus creating the enzyme lipoprotein lipase.

In extension of the hypothesis, by introducing the basic biochemical components of lipoprotein lipase into the diet the body can and will only assemble the components according to how the body normally deals with the biochemical components.

Therefore, by introducing glucuronic acid and glucosamine into the diet, the body would utilize these resources to create lipoprotein lipase and its relative precursor compounds in the relative tissues.

It is likely by comparing knowledge of the metabolism of each component to each other that glucuronic acid plays the pivotal role in the termination of the anaphylactic reaction by heparin and heparin like compounds.

This line of thought is justified by noting the extensive work done by independent scientific groups on the glucuronidation detoxification pathway.

It could be inferred that as glucuronidation is a required metabolic process for the chemical compounds that are known to trigger anaphylactic reactions and instigate allergic episodes in the atopic conditions.

Glucuronidation is used to make compounds more water soluble allowing them to be excreted in the urine. It has been firmly established that both endogenous compounds (eicosanoids, prostaglandins and leukotrienes) and exogenous compounds (**oxazepam, morphine and 3-hydroxyantipyrine [12.6]**). A competitive relationship for phase II conjugation reactions seems likely.

The molecules glucuronic acid and n-acetylglucosamine together make up a major part of the compositional basis of lipoprotein lipase, heparin, glycosaminoglycans and mucopolysaccharides.

If systematically fed to atopic subjects, it is proposed that tissues underpinning the supply of glucuronic acid for phase II conjugation reactions would become replenished and thus lipoprotein lipase activity would not be compromised when a xenobiotic load was encountered.

Glucuronic acid compounds in abundance would negate the effect that competitive inhibition has on eicosanoid regulated systems and the atopic condition would resolve.

**Hyaluronic Acid [12.7]** is a polymer store of glucuronic acid and glucosamine. It is a part of the ground substance in connective tissue. Hyaluronic acid in the context of atopy, is a more homogenous resource for the production of lipoprotein lipase and heparin.

In **Summary [12.8]** glucuronic acid and glucosamine are potential avenues of therapeutic approach in bronchial asthma and atopic conditions.

# Paper 12: Hypothesis on Atopy's Treatment

Heparin, an essential precursor of lipoprotein lipase, is a known **Anti-inflammatory Agent [12.1]** used in a clinical setting.

**Paper Number: 12**  
**Reference Number: 12.1**

**Information taken from:**

Bench-to-bedside review: The role of glycosaminoglycans in respiratory disease

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## Heparin and inflammation

There is increasing evidence that heparin has a wide range of biological properties that can be considered beneficial in the context of the regulation of the inflammatory response [1]. Heparin can inhibit the influx of neutrophils into certain tissues and inhibit T-cell trafficking [2], partly by an inhibitory effect on the heparinase enzyme secreted by T cells .

Furthermore, heparin has been shown to be released from human lung mast cells in response to allergen exposure [3], and increased levels of a heparin-like substance have been reported in the plasma of asthmatic individuals [4]. Heparin can also inhibit allergen-induced eosinophil infiltration into the airways of experimental animals [5].

After the inflammatory cells have passed through the lung tissue, it is recognized that there is a number of stages involved, including adhesion to the vascular endothelium, diapedesis across the endothelial cells and chemotaxis within tissues.

It is clear that heparin can inhibit all stages of cell migration, including the carbohydrate-selectin interactions between endothelial cells and leucocytes, the presentation of specific chemoattractants to activated leucocytes, and leucocyte trafficking [6]. Although the mechanisms that underlie the effect of heparin on neutrophil migration are well understood, the ability of heparin to interfere with eosinophil adherence is less well understood.

Nonetheless, heparin is able to inhibit the actions of several important eosinophil chemoattractants, such as platelet factor-4 [7]. Although the precise mechanism of the anti-inflammatory effects of heparin is not established, it has been suggested that inhibition of the interaction between proinflammatory cytokines and membrane-associated glycosaminoglycans may provide a mechanism for inducing clinically useful immunosuppression [8].

**Notes:**

Heparin has a wide range of biological properties which are beneficial in regulating the inflammatory response [1]. Heparin inhibits the movement of neutrophils into certain tissues. Heparin inhibits T-cell trafficking [2].

Heparin is released from mast cells in response to exposure to allergens [3]. Heparin as well as heparin-like substances have been identified in the plasma of asthmatic individuals [4]. Heparin is also known to inhibit allergen induced eosinophil infiltration into the airways of experimental animals [5].

Heparin inhibits all stages of cell migration, including carbohydrate-selectin interactions between endothelial cells and leucocytes, presentation of chemoattractants to activated leucocytes and leucocyte trafficking [6].

Heparin inhibits several eosinophil chemoattractants such as platelet factor -4 [7]. The mechanism of the anti-inflammatory actions of heparin have been suggested as involving the interaction between proinflammatory cytokines and glycosaminoglycans [8].

# Paper 12: Hypothesis on Atopy's Treatment

When the Effects of Heparin on Lipoprotein Lipase [12.2] are observed it proposes that when the precursor metabolite, heparin, into the body, lipoprotein lipase is produced as a result.

**Paper Number: 12**  
**Reference Number: 12.2**

**Information taken from:**

E. A. Newsholme  
A. R. Leech  
Biochemistry for the Medical Sciences  
Reprinted January 1992  
John Wiley & Sons  
Effect of heparin on lipoprotein lipase production  
Page 256 hydrolysis of triacylglycerol

## The Effects Of Heparin On Lipoprotein Lipase

Triacylglycerol, whether in the form of chylomicrons or other lipoproteins, is not taken up directly by any tissue. It must be hydrolysed outside the cell to fatty acids and glycerol, which can enter the cell. This hydrolysis is carried out by lipoprotein lipase (which is also known as clearing factor lipase). In extrahepatic tissues including adipose tissue, skeletal muscle, heart lung and mammary gland, the enzyme is attached to the outer surface of the endothelial cells lining the capillaries.

In the liver, the lipoprotein lipase is attached to the outer surface of the hepatocytes. At this site, the triacylglycerol is hydrolysed to glycerol and fatty acids and the latter taken up by the cells of the tissue in which the hydrolysis occurs.

The glycerol is transported in the blood to the liver and kidney for further metabolism. Evidence for this extracellular location of lipoprotein lipase is provided by the ability of heparin, injected intravenously, to release the enzyme into the bloodstream. This effect may not be of physiological significance but is useful in diagnosis; the activity of the lipase can be measured in a sample of blood taken from a patient after injection of heparin [1].

Post heparin lipolytic activities (abbreviated PHLA) are important in diagnosis of type 1 hyperlipoproteinaemia. Chylomicrons obtained directly from the lymphatic duct are a poor substrate for extrahepatic lipoprotein lipase. In order to become an effective substrate for extrahepatic lipoprotein lipase, chylomicrons must acquire apolipoprotein C from the high density lipoproteins in the blood.

## Notes:

If heparin is injected into a subject lipoprotein lipase is released into the bloodstream [1].

# Paper 12: Hypothesis on Atopy's Treatment

**Heparin [12.3]** is a compound of glucuronic acid, iduronic acid, sulphate groups and acetyl groups. The acetyl group are attached to the glucosamine units and the sulphate groups are attached to the Glucuronic Acid Residues.

**Paper Number: 12**  
**Reference Number: 12.3**

**Information taken from:**  
Percy J. Russell  
Anita Williams  
The Nutrition and Health Dictionary  
Copyright 1995  
Chapman & Hall  
Definition of heparin  
Page 213 Heparin

Heparin

The naturally occurring anticoagulant in blood and other tissues containing glucosamine, glucuronic acid, and varying proportions of sulphate and acetyl groups. Its structure is not entirely clear. It is produced by the mast cells of the connective tissue and is stored as granules within the cells. The heparin content of tissues correlates with the number of mast cells present [1].

Heparin is secreted into the intercellular substance and functions there to prevent the fibrinogen that escapes from capillaries from forming fibrin clots. It also functions in the formation or activation of lipoprotein lipase, which clears chylomicrons from the blood plasma [2].

## **Notes:**

Heparin is a natural anticoagulant in the body made up of glucosamine, glucuronic acid, sulphate and acetyl groups [1]. Heparin functions to make lipoprotein lipase which acts to metabolise lipids in the body [2].

# Paper 12: Hypothesis on Atopy's Treatment

Heparin is a compound of glucuronic acid, iduronic acid, sulphate groups and acetyl groups. The acetyl group are attached to the glucosamine units and the sulphate groups are attached to the Glucuronic Acid Residues [12.4].

**Paper Number: 12**  
**Reference Number: 12.4**

**Information taken from:**

E. A. Newsholme  
A. R. Leech  
Biochemistry For The Medical Sciences  
Reprinted January 1992  
John Wiley & Sons  
Definition and function of Glycosaminoglycans  
Page 596 Glycosaminoglycans

## Glycosaminoglycans

A large number of the polysaccharides that occur in mammalian connective tissue are glycosaminoglycans. They form a fairly homogenous group of carbohydrates and are broadly defined by possession of the following characteristics [1]:

- (1) They contain a repeating disaccharide unit, consisting of a sugar (such as glucosamine, usually N-acetylated) and a uronic acid (except in keratan sulphate).
- (2) The uronic acid is usually esterified with sulphate (except in hyaluronate).
- (3) The polysaccharide is usually covalently linked to a protein to form a proteoglycan (also known as a mucopolysaccharide) [2].

Most glycosaminoglycan chains contain fewer than 100 monosaccharide units but there may be as many as 50 chains attached to a single core protein in the proteoglycan. Hyaluronate chains are longer and may contain 5000 monosaccharide residues .

## Notes:

Glycosaminoglycans are a large number of polysaccharides found in connective tissues. They all have similar having characteristics which are [1]:

They contain a repeating sugar unit such as glucosamine (usually n-acetylated) and a uronic acid such as glucuronic acid (often esterified with sulphate). If the polysaccharide is covalently linked to a protein it forms a mucopolysaccharide [2].

# Paper 12: Hypothesis on Atopy's Treatment

The **Eosinophilic Leukocytes** [12.5] and mast cells possess an enzyme (arylsulfatase) which acts to cleave the sulphate from the glucuronic acid thus creating the enzyme lipoprotein lipase.

**Paper Number: 12**  
**Reference Number: 12.5**

**Information taken from:**

Kenneth L. Melmon, M.D.  
 Howard F. Morrelli, M.D.  
 Clinical Pharmacology  
 Basic Principles in Therapeutics  
 Second edition 1978  
 Bailliere, Tindall london  
 Cellular mediators of inflammation  
 Page 672 Eosinophilic Polymorphonuclear Leukocytes

## Eosinophilic Polymorphonuclear Leukocytes

Eosinophilic leukocytes are a prominent feature of many allergic and immediate hypersensitive reactions. In addition to activated complement factors and kallikrein, an eosinophil chemotactic factor of anaphylaxis (ECF-A) released from sensitised mast cells selectively attracts eosinophils (Kay et al., 1971) [1].

Eosinophils phagocytize and have membrane bound granules that contain many of the hydrolytic enzymes present in neutrophils. One enzyme present in higher concentrations than in polymorphonuclear cells is arylsulfatase, which is a potent inactivator of slow reacting substance of anaphylaxis (SRS-A) (Wasserman et al., 1975) [2]. Other factors in eosinophils inhibit histamine release, neutralise heparin, and activate plasminogen; thus, these cells may play a role in limiting allergic reactions (Goetzl et al., 1975) [3].

## Notes:

Eosinophilic Polymorphonuclear Leukocytes (eosinophils) are a major part of allergic and immediate hypersensitivity reactions. They are attracted by complement, kallikrein and a substance which is released by sensitised mast cells [1].

They engulf and digest bacteria and foreign materials by similar means to neutrophils but have levels of the digestive enzyme arylsulfatase which is a strong inactivator of anaphylactic events. Arylsulfatase acts on aromatic and sulphur containing compounds [2]. Eosinophils inhibit histamine release, neutralise heparin and work to promote plasmin. These cells play an important role in stopping allergic reactions [3].

## Paper 12: Hypothesis on Atopy's Treatment

Glucuronidation is used to make compounds more water soluble allowing them to be excreted in the urine. It has been firmly established that both endogenous compounds (eicosanoids, prostaglandins and leukotrienes) and exogenous compounds (**oxazepam, morphine and 3-hydroxyantipyrine [12.6]**). A competitive relationship for phase II conjugation reactions seems likely.

**Paper Number: 12**  
**Reference Number: 12.6**

**Information taken from:**

Glucuronidation of Drugs by Hepatic Microsomes Derived from  
 Healthy and Cirrhotic Human Livers<sup>1</sup>

Valerie Furlan, Sylvie Demirdjian, Olivier Bourdon, Jacques Magdalou, and Anne-Marie Taburet  
 Department of Clinical Pharmacy, Bicêtre Hospital, Assistance Publique,  
 Hôpitaux de Paris, Paris, France (V.F., S.D., O.B., A.-M.T.);  
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 Scientifique-University Henri Poincaré, Nancy I, Nancy, France (J.M.)  
 Accepted for publication January 12, 1999

It is commonly thought that phase II pathways of drug metabolism (conjugation reactions) are unaltered in liver dysfunction. The elimination as glucuronides of drugs [1] slowly extracted by the liver (such as oxazepam [2]) or highly extracted (such as morphine [3]) has never been reported to be extensively impaired in patients with liver cirrhosis (Shull et al., 1976; Patwardhan et al., 1981). More recent investigations have shown that, indeed, this was not a general rule.

Conjugation of 3-hydroxyantipyrine [4] was reduced in patients with hepatic failure (Teunissen et al., 1984), and the oral clearance of zomepirac [5], a drug that undergoes extensive glucuronidation, was decreased by 50% in cirrhosis (Witassek et al., 1983). Zidovudine [6], which is excreted mainly as 59-O-glucuronide in humans, presented a 70% decrease of oral clearance in patients with grade B or C cirrhosis (Taburet et al., 1990).

Those values are in the same range as those mentioned for drugs eliminated through phase I pathways of biotransformation, with extensive extraction by liver, such as midazolam, nifedipine, or verapamil, whose clearance is decreased by 48, 60, and 65%, respectively (Howden et al., 1988).

The decrease of hepatic clearance of drugs has been explained by a reduction of the activity and expression of enzyme isoforms responsible for their metabolisms and by the presence of portosystemic shunts (Howden et al., 1988; Morgan and Mc Lean, 1995). Because glucuronidation is a main phase II metabolic pathway of drugs in humans [7], it is important to determine to what extent this reaction is sensitive to liver failure.

Glucuronidation is supported by UDP-glucuronosyltransferases (UGT, EC 2.4.1.17) [8]. This multigenic family of enzymes catalyzes the binding of glucuronic acid, from the high-energy donor UDP-glucuronic acid (UDPGA), on the hydroxyl, carboxyl, amine, or thiol group of chemically unrelated substances [9].

**Notes:**

Certain drugs are eliminated from the body as glucuronides [1]. Drugs which utilize phase II conjugation reactions of glucuronic acid include oxazepam [2], morphine [3], 3-hydroxyantipyrine [4], zomepirac [5], zidovudine [6].

Glucuronidation is a main phase II metabolic pathway of drugs in humans[7]. Glucuronidation is supported by UDP-glucuronosyltransferases (UGT, EC 2.4.1.17) [8].

These enzymes catalyze the binding of glucuronic acid from the high energy donor UDP glucuronic acid (UDPGA), to the hydroxyl, carboxyl, amine or thiol group of chemically unrelated substances [9].

# Paper 12: Hypothesis on Atopy's Treatment

**Hyaluronic Acid [12.7]** is a polymer store of glucuronic acid and glucosamine. It is a part of the ground substance in connective tissue. Hyaluronic acid in the context of atopy, is a more homogenous resource for the production of lipoprotein lipase and heparin.

**Paper Number: 12**  
**Reference Number: 12.7**

**Information taken from:**  
Percy J. Russell  
Anita Williams  
The Nutrition and Health Dictionary  
Copyright 1995  
Chapman & Hall  
Definition of Hyaluronic Acid  
Page 219 Hyaluronic Acid

## Hyaluronic Acid

A mucopolysaccharide that is a component of the ground substance of intercellular material. The human placenta, cattle synovial fluid (synovium), and vitreous fluids (eye) are the most common sources of hyaluronic acid, but it is widely distributed and is found in most connective tissues [1].

Its name is derived from hyaloid (vitreous) and uronic acid. Hyaluronic acid is composed of equimolar proportions of D-glucuronic acid and acetyl glucosamine occupying alternating positions in the molecule [2].

## Notes:

Hyaluronic acid is a mucopolysaccharide. It is present in connective tissues and is a part of the ground substance that is widely distributed throughout the body [1]. Hyaluronic acid is made up of alternating molecules of glucuronic acid and glucosamine [2].

# Paper 12: Hypothesis on Atopy's Treatment

In **Summary [12.8]** glucuronic acid and glucosamine are potential avenues of therapeutic approach in bronchial asthma and atopic conditions.

**Paper Number: 12**  
**Reference Number: 12.8**

**Information taken from:**

Aggregate notes of paper 12

## **-: Paper Twelve - Hypothesis on Atopy's Treatment :-**

- Heparin is released from mast cells in response to exposure to allergens -**
- Heparin inhibits all stages of cell migration -**
- If heparin is injected into a subject, lipoprotein lipase is released into the bloodstream -**
- Arylsulfatase is released by neutrophils, acts on aromatic and sulfur containing compounds and is a strong inactivator of anaphylactic events -**
- Glucuronidation is main phase II metabolic pathway of drugs in humans -**
- UDP-glucuronosyltransferases are enzymes which catalyze the binding of glucuronic acid to hydroxyl, carboxyl, amino or thiol groups of chemically unrelated substances -**

Heparin has a wide range of biological properties which are beneficial in regulating the inflammatory response. Heparin inhibits the movement of neutrophils into certain tissues. Heparin inhibits T-cell trafficking. Heparin is released from mast cells in response to exposure to allergens. Heparin as well as heparin-like substances have been identified in the plasma of asthmatic individuals. Heparin is also known to inhibit allergen induced eosinophil infiltration into the airways of experimental animals.

Heparin inhibits all stages of cell migration, including carbohydrate-selectin interactions between endothelial cells and leucocytes, presentation of chemoattractants to activated leucocytes and leucocyte trafficking.

Heparin inhibits several eosinophil chemoattractants such as platelet factor 4. The mechanism of the anti-inflammatory actions of heparin have been suggested as involving the interaction between proinflammatory cytokines and glycosaminoglycans.

If heparin is injected into a subject lipoprotein lipase is released into the bloodstream. Heparin is a natural anticoagulant in the body made up of glucosamine, glucuronic acid, sulphate and acetyl groups. Heparin functions to make lipoprotein lipase which acts to metabolise lipids in the body.

Glycosaminoglycans are a large number of polysaccharides found in connective tissues. They all have similar having characteristics which are:

They contain a repeating sugar unit such as glucosamine (usually n-acetylated) and a uronic acid such as glucuronic acid (often esterified with sulphate). If the polysaccharide is covalently linked to a protein it forms a mucopolysaccharide.

Eosinophilic Polymorphonuclear Leukocytes (eosinophils) are a major part of allergic and immediate hypersensitivity reactions. They are attracted by complement, kallikrein and a substance which is released by sensitised mast cells.

They engulf and digest bacteria and foreign materials by similar means to neutrophils but have levels of the digestive enzyme arylsulfatase which is a strong inactivator of anaphylactic events. Arylsulfatase acts on aromatic and sulphur containing compounds.

Eosinophils inhibit histamine release, neutralise heparin and work to promote plasmin. These cells play an important role in stopping allergic reactions.

Certain drugs are eliminated from the body as glucuronides. Drugs which utilize phase II conjugation reactions of glucuronic acid include oxazepam, morphine, 3-hydroxyantipyrine, zomepirac, zidovudine.

Glucuronidation is a main phase II metabolic pathway of drugs in humans. Glucuronidation is supported by UDP-glucuronosyltransferases (UGT, EC 2.4.1.17).

These enzymes catalyze the binding of glucuronic acid from the high energy donor UDP glucuronic acid (UDPGA), to the hydroxyl, carboxyl, amine or thiol group of chemically unrelated substances.

Hyaluronic acid is a mucopolysaccharide. It is present in connective tissues and is a part of the ground substance that is widely distributed throughout the body. Hyaluronic acid is made up of alternating molecules of glucuronic acid and glucosamine.

# **Bronchial Asthma and the Atopic Syndrome**

## **Paper 13: Recapitulation of Hypothesis**

The vital statistics of asthma propose the importance of understanding and researching the possible remedying of atopic conditions. Asthma is a serious condition which must firstly be managed and controlled through established and proven means as judged by a qualified medical practitioner.

Any hypothesis proposing a therapy should be subject to the rigors of objective scientific method, it should be falsifiable, and it should be able to demonstrate a firm foundation in rational thought based on peer reviewed work which has been carried out by independent teams.

A holistic perspective should also take a look at the environment and lifestyles of the modern person. This seems a pertinent aspect of atopic conditions as their incidence seems to generally be on the increase in modern industrial societies. The thought to improve standards of health and the welfare of future generations seems to underpin the general ethos of medicine.

In the years 1986 through to 1993, the Office of Population Censuses and Surveys at the General Register Office of Scotland reported a total of 1190 deaths. The mortality statistics of asthma amount to a considerable motive for thorough and impartial research based on known technique.

This literature review is an attempt to make a contribution to the discussion of the causes of atopic conditions and their potential treatments. As well as this, the layout of this document has been developed in an attempt to explore the presentation and clarification of scientific literature reviews.

What follows is a recapitulation of the work through the bullet points established in the summary section of each chapter. This way, each statement may be easily traced back to its original text and compared. In conclusion to the study, the deductive assertion will be used to make a hypothesis on the basis of an inductive line of reasoning.

**-: Paper One - Foreword :-**

- **Glucuronic acid is a uronic acid derived from glucose by oxidation -**
- **Glucosamine is one of the most abundant natural monosaccharides -**
- **Glucuronides are endogenous conjugation agents found in the body -**
- **Asthma and atopy are here hypothesized as a deficit of lipoprotein lipase -**
- **Prostaglandins are lipid hormones which are metabolized via glucuronidation -**
- **Heparin releases lipoprotein lipase and is made of glucuronic acid and glucosamine -**

**1: Prostaglandins are lipid hormones which are metabolized via glucuronidation. Heparin, a compound of glucuronic acid, releases lipoprotein lipase.**

**-: Paper Two - Asthma and Atopy :-**

- **Bronchial asthma can be triggered by allergens -**
- **Atopic conditions are allergic conditions -**
- **Allergic conditions are manifestations of the anaphylactic reaction -**
- **Allergic responses are triggered by allergens -**
- **Allergens are physical, psychological and environmental stimuli -**
- **All allergies result in forms of inflammation -**

**2: Atopic conditions are allergic conditions which are manifestations of the anaphylactic reaction which results in inflammation**

**-: Paper Three - Asthma and Inflammation :-**

- Asthma is an allergic condition -
- Allergic responses are triggered by allergens -
- Antibodies and immune cells are stimulated in response to allergens -
  - The body attaches antibody Immunoglobulin E to mast cells -
- When allergens react with Immunoglobulin E the mast cell degranulates -
- Anaphylaxis is the allergic reaction that causes inflammation in mast cells -

**3: Allergic responses are triggered by allergens which stimulate antibodies and immune cells to react. The antibody immunoglobulin E gets attached to mast cells to degranulate them during the allergic reaction.**

**-: Paper Four - Asthma and Inflammation :-**

- The autonomic nervous system is stimulated -
- The smooth muscle of the lung constricts (bronchoconstriction) -
  - Inflammation occurs in the lung -
- Eosinophils migrate to the site of inflammation -
  - Mast cells release their contents -
  - Hypersecretion ensues -

**4: In asthma, the smooth muscle constricts, inflammation occurs, eosinophils migrate to the inflammation, mast cells release their contents and hypersecretion ensues.**

**-: Paper Five - Cellular Mediators of Inflammation :-**

- The allergic reaction is a complex series of chemical reactions initiated and controlled by the immune cells to cause inflammation -
- Mast cell cultures are the tissue targeted by the immune system -
- Mast cell cultures are abundant in smooth muscle and vascular channels -
  - Eosinophils, neutrophils, and monocytes digest heparin -
- Lymphocytes co-ordinate and potentiate the allergic reaction by producing immunoglobulin E -
- Eosinophils are enzymically particularly equipped to metabolise heparin with arylsulfatase -

**5: Mast cells are targeted by the immune system. Lymphocytes produce immunoglobulin E to co-ordinate and potentiate the allergic reaction. Eosinophils, neutrophils and monocytes digest heparin.**

**-: Paper Six - Humoral Mediators of Inflammation :-**

- Histamine, serotonin and kinins are released which dilate the vascular system by acting on the smooth muscle -
- Adrenaline and noradrenaline are released which both act to relax smooth muscle -
- Components of complement act to degranulate mast cells and contract smooth muscle -
  - The blood clotting system shares components with the complement system and the kinin generating system -
- The prostaglandins are lipid hormones that act on smooth muscle and contribute to the intensity and duration of the inflammatory reaction -
  - Heparin and mucopolysaccharides are released from mast cells during the ensuing inflammatory reaction -
- Control of smooth muscle is the major theme in anaphylactic reactions -

**6: Prostaglandins act on smooth muscle contributing to the intensity and duration of the inflammatory reaction. Heparin and mucopolysaccharides are released from mast cells during the ensuing reaction.**

**-: Paper 7 - Hypothesis of Biological Adaptation :-**

- There is a hierarchical system in the biology of the organism. The brain occupies a special position in this scheme. To illustrate this, brain gives priority to regulating its own ATP concentration. It has been postulated that the peripheral energy supply is only of secondary importance -
- The brain has two possibilities to ensure energy supply are allocation or intake. 'Allocation' refers to the allocation of energy resources between the brain and the periphery -
- System control can be permanently displaced by extreme stress, starvation, exercise, infectious diseases, hormones, drugs, substances of abuse, or chemical disruption of the endocrine system -
- In the 'Selfish Brain Theory' a model newly describes a 'principle of balance' in biological systems. The various organs and tissues must compete for allocation of a limited number of resources -

**7: There is a hierarchical system in the biology of the organism. In the 'Selfish Brain Theory' a model newly describes a 'principle of balance' in biological systems. The various organs and tissues must compete for allocation of a limited number of resources**

**-: Paper Eight - The Hormonal Pathology of Asthma :-**

- Post mortem exam shows lungs over distended with numerous tenacious mucous plugs -
- A dense exudate is found in the bronchial lumen -
- Eosinophils are found infiltrated in the bronchial lumen -
  - Increased prostaglandins and leukotrienes are central to the asthmatic reaction -
- Cysteinyl leukotrienes are found in increased levels in asthmatics -
- Edema produced by prostaglandins is not dose dependent -

**8: Post mortem, asthmatic lungs are overdistended, eosinophil infiltrated and have a dense exudate with mucous plugs. Increased prostaglandins and leukotrienes are central to the asthmatic reaction**

**-: Paper Nine - The Metabolism of Prostaglandins :-**

- **Glucuronidation of fatty acids is a biotransformation process which increases their solubility -**
- **Prostaglandin glucuronidation is considered a termination of their biological activity -**
- **Glucuronidation is the most effective detoxification process -**
- **Detoxification pathways may become depleted and respond poorly -**
- **Glucuronosyltransferase enzymes are suggested to protect against prostaglandin accumulation -**
- **Heparin, a glycosaminoglycan, contains glucuronic acid and is a structural precursor to lipoprotein lipase -**

**9: Glucuronidation of prostaglandins represents a termination of their biological activity. Glucuronidation is the most effective detoxification process and may become depleted: Glucuronosyltransferase enzymes may act to prevent prostaglandin accumulation**

**-: Paper Ten - Heparin and it's Actions :-**

- **Heparin is both an anti inflammatory agent and an anticoagulant -**
- **Heparin causes the release of lipoprotein lipase -**
- **Heparin is a structural component of lipoprotein lipase -**
- **The sulfation pattern gives each glycosaminoglycan chain a unique structure -**
- **Heparin sulfate and related glycosaminoglycans are composed of glucuronic acid -**
- **Sulfatases hydrolyze a wide variety of sulfate esters -**

**10: Heparin is both an antiinflammatory agent and anticoagulant which causes the release of lipoprotein lipase. Heparin is a structural component of lipoprotein lipase and is a compound of glucuronic acid**

**-: Paper Eleven - Glucuronidation :-**

- The most important acid polysaccharides are those based on glucuronic acid and acetyl galactosamine sulfate -
- Glucuronic acid conjugates with various chemicals to allow their excretion as glucuronides -
- The amount of glucuronides in the urine is increased by intestinal putrefaction and by drugs -
- Glucuronic acid is known to conjugate alcoholic and phenolic hydroxyl groups -
- Glucuronic acid conjugates with the lipid female sex hormones for excretion -
- Various drugs such as antihistamines, phenothiazines and certain antibiotics have a negative effect on heparin -

**11: Glucuronic acid conjugates various chemicals to allow their excretion as glucuronides. The amount of glucuronides in the urine is increased by intestinal putrefaction and by drugs**

**-: Paper Twelve - Hypothesis on Atopy's Treatment :-**

- Heparin is released from mast cells in response to exposure to allergens -
- Heparin inhibits all stages of cell migration -
- If heparin is injected into a subject, lipoprotein lipase is released into the bloodstream -
- Arylsulfatase is released by neutrophils, acts on aromatic and sulfur containing compounds and is a strong inactivator of anaphylactic events -
- Glucuronidation is main phase II metabolic pathway of drugs in humans -
- UDP-glucuronosyltransferases are enzymes which catalyze the binding of glucuronic acid to hydroxyl, carboxyl, amino or thiol groups of chemically unrelated substances -

**12: Heparin inhibits all stages of cell migration. Arylsulfatase is released by neutrophils, acts on aromatic and sulfur containing compounds and is a strong inactivator of anaphylactic events. UDP-glucuronosyltransferases are enzymes which catalyze the binding of glucuronic acid to hydroxyl, carboxyl, amine or thiol groups of chemically unrelated substances**

## Synthesis

Prostaglandins are lipid hormones which are metabolized glucuronidation. Heparin, a compound of glucuronic acid, releases lipoprotein lipase [1]. Atopic conditions are allergic conditions which are manifestations of the anaphylactic reaction which results in inflammation [2].

Allergic responses are triggered by allergens which stimulate antibodies and immune cells to react. The antibody immunoglobulin E gets attached to mast cells to degranulate them during the allergic reaction [3].

In asthma, the smooth muscle constricts, inflammation occurs, eosinophils migrate to the inflammation, mast cells release their contents and hypersecretion ensues [4]. Mast cells are targeted by the immune system. Lymphocytes produce immunoglobulin E to co-ordinate and potentiate the allergic reaction. Eosinophils, neutrophils and monocytes digest heparin [5].

Prostaglandins act on smooth muscle contributing to the intensity and duration of the inflammatory reaction. Heparin and mucopolysaccharides are released from mast cells during the ensuing reaction [6].

There is a hierarchical system in the biology of the organism. In the 'Selfish Brain Theory' a model newly describes a 'principle of balance' in biological systems. The various organs and tissues must compete for allocation of a limited number of resources [7].

Post mortem, asthmatic lungs are overdistended, eosinophil infiltrated and have a dense exudate with mucous plugs. Increased prostaglandins and leukotrienes are central to the asthmatic reaction [8].

Glucuronidation of prostaglandins represents a termination of their biological activity. Glucuronidation is the most effective detoxification process and may become depleted: Glucuronosyltransferase enzymes may act to prevent prostaglandin accumulation [9].

Heparin is both an antiinflammatory agent and anticoagulant which causes the release of lipoprotein lipase. Heparin is a structural component of lipoprotein lipase and is a compound of glucuronic acid [10].

Glucuronic acid conjugates various chemicals to allow their excretion as glucuronides. The amount of glucuronides in the urine is increased by intestinal putrefaction and by drugs [11].

Heparin inhibits all stages of cell migration. Arylsulfatase is released by neutrophils, acts on aromatic and sulfur containing compounds and is a strong inactivator of anaphylactic events. UDP-glucuronosyltransferases are enzymes which catalyze the binding of glucuronic acid to hydroxyl, carboxyl, amine or thiol groups of chemically unrelated substances [12].

Here we have a reduction of the deducible facts derived from the concorded literature review which forms the basis of this study. From this set of data, a line of inductive rationale behind the autoimmune conditions which manifest anaphylactic inflammation, or type 1 hypersensitivity reactions.

It is proposed that through depletion of glucuronic acid and the poor response of systems which require glucuronic acid, eicosanoid metabolism becomes unregulated affecting, amongst other systems, smooth muscle in asthma for example.

In an effort to resolve the local deficits brought about through increases in demand, it is proposed that the immune system acts in a logistical capacity to speedily requisit glucuronic acid resources from tissue through the aciton of anaphylactic inflammation.

A chief resource of glucuronic acid is that of the maast cell. The mast cell is an abundant source of sulfated glycosaminoglycans including heparin which is a structural precursor to lipprotein lipase. Glycosaminoglycans, heparin and lipoprotein lipase all represent resources of glucuronic acid.

Any increase in a demand for glucuronidation in a system deficient of glucuronic acid, caused either by xenobiotics or endogenous lipid hormones, will require a trade-off in opportunity costs between chemically inactivating foreign compounds or chemically regulating endogenous hormones.

The body thus is forced to keep itself alive by the relatively violent action of immune cells migrating to the site of inflammation to render glucuronic acid from glycosaminoglycan molecules in the tissues. Mast cells are even specifically tagged by immunoglobulin E produced by lymphocytes to facilitate anaphylaxis as a course of action.

It is suggested that the body does this act of forced reallocation via use of large amounts of arylsulfatase found in th emast cell, eosinophil and neutrophil. Arylsulfatase acts to cleave aromatic and sulfur containing compounds, helping to liberate the glucuronic acid necessary to fill the metabolic demand.

Once enought glucuronic acid has been released in its active form it is suggested that the anaphylactic inflammation subsides having the resources to both regulate any lipid hormones released or eliminate from the system xenobiotics.

According to this scheme of thought, an anaphylactic event can be brought about (a) through endogenous production of eicosanoid hormones sufficient to outstrip supply of glucuronic acid (exercise, emotion, cold) or (b) through toxic challenge of a xenobiotic load sufficient to outstrip supply of glucuronic acid and impinge on the balanced functioning of the eicosanoid hormones.

This scheme leaves adequate room to suggest that both the histamine reaction and anaphylactic reaction in general are essential mechanisms of human biology. Histamine seems to be integral to facilitating the migration of immune cells to the tissue, and the immune reaction as a whole is proposed as an important self preservation mechanism designed to keep the organism alive in states of deficit.

In a state of deficiency the body draws progressively upon more important stores and resources of substances it requires to balance an immediate metabolic deficit; The body draws upon biologically less important substances to manufacture from them biologically more important and immediately required substances.

There is a hierarchical system in the biology of the organism. In the 'Selfish Brain Theory [7.1]' a model newly describes a 'principle of balance' in biological systems. The various organs and tissues must compete for allocation of a limited number of resources. The principle is that the body will look after a more important tissue before it looks after a less important tissue.

Importance in these terms refers to a relative scale of absolute requirement and immediacy of need. The economics of alternative/opportunity costs and the logistics biology thus must be taken as factors in the interpretation of biological function.

The body draws upon the least important and most plentiful resources first. The body prioritizes the functions, organs, tissues and chemical stores according to how essential they are in the functioning of the body. In short there is a hierarchy amongst the tissues of the body.

We are literally an assembly of what we eat, drink and breath. Ultimately the body relies upon the diet for all its building blocks and fuel. A medical condition manifests itself when the first metabolic action is impaired - that is to say - a metabolically active substance is negatively affected and the function which it supports is impeded forcing a biological adaptation of normal metabolism to occur.

As deficiency progresses the body is forced to requisite resources so to supply more vital functions with the components which are required to support the biological action. An increasing amount of symptoms occur with an increasing deficit of resources. When a biological function breaks down it is related as a symptom.

The more active the substance that is requisited for reassignment of its constituents - the more important the function that is affected in the running of the body - the more dramatic is the symptom of deficit.

The greater the stage of deficiency the more important the function or tissue which is affected. Deficit may be brought about by lack of the substance in the diet, by increased utilization of the substance so that demand outstrips supply, or by the destruction of the substance.

# Bronchial Asthma and the Atopic Syndrome

## Paper 14: Addendum - Extended Analysis

Now a deductive study of our data set has allowed the formulation of a hypothesis through a process of inductive reasoning, it remains for some form of falsification to be made of said hypothesis so to begin to test its rationale as a reliable model. Various forms of falsification are available but for the purposes of this project, a type of falsification method which is literature based is most cogent with the overall framework 'a study of the anatomy of a study'.

This paper represents the taking of a focused data set and applying the treatment of language found in the concorded literature review which prefaces this paper. As the treatment of language has been illustrated, this style of arranging and reporting omits the concorded text but includes its sources for reference via bibliography.

The style of annotation indicates the absolutely finite lineage of language back to a specific piece of text. Where there is not found a reference number, it is an authored statement. It is in the study of the common instrument of science, that of the mode of communication and codification of information in such a way that it not just holds information but also makes that information available thus making communication and communicative endeavour possible; the basis of all peer review and literature based learning.

It is in the collection of a further data set a form of falsification can be made not only to get rid of incorrect assertions but to gain guidance as to whether the interpretation of the available data is moving closer to a truthful representation or further away. It is as much to rule out wasting time studying the wrong thing as to answer the question in mind as it is to make a scrupulous study of the only relevant factors.

The natural coalescence of information is enough to make a prospective study in something. Corroboration between data sets advances the study further down the road towards reliable knowledge and performs the task of providing a second landscape which must be conjunctive not only with the first data set in a mutual verification and should also provide an articulated obstacle for any hypothesis made before its collection.

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## **Bronchial Asthma**

Asthma is a common chronic inflammatory condition of the lung airways whose cause is incompletely understood. Symptoms are cough, wheeze, chest tightness and shortness of breath, often worse at night. It has three characteristics: airflow limitation which is usually reversible spontaneously or with treatment; airway hyperresponsiveness to a wide range of stimuli; inflammation of the bronchi with eosinophils, T lymphocytes and mast cells with associated plasma exudation, oedema, marked smooth muscle hypertrophy, mucus plugging and epithelial damage [32].

In chronic asthma, inflammation may be accompanied by irreversible airflow limitation. The underlying pathology in pre-school children may be different, in that they may not exhibit appreciable bronchial hyperreactivity. There is no evidence that chronic inflammation is the basis for the episodic wheezing associated with viral infections [32].

In many countries the prevalence of asthma is increasing, particularly in the second decade of life where this disease affects 10-15% of the population. There is also a geographical variation, with asthma being common in more developed countries, some of the highest rates being in New Zealand, Australia and the UK, but being much rarer in Far Eastern countries such as China and Malaysia, and in Africa and Central and Eastern Europe [32].

Long-term follow-up in developing countries suggests that the disease may become more frequent as individuals become more 'westernized'. Studies of occupational asthma suggest that a high percentage of the workforce, perhaps up to 20%, may become asthmatic if exposed to potent sensitizers [32].

Asthma can be divided into Extrinsic (implying a definite external cause) and Intrinsic or Cryptogenic (when no causative agent can be identified) forms. Extrinsic asthma occurs most frequently in atopic individuals who show positive skin-prick reactions to common inhalant allergens. Positive skin-prick tests to inhalant allergens are shown in 90% of children and 50% of adults with persistent asthma [32].

Childhood asthma is often accompanied by eczema. An overlooked cause of late-onset asthma in adults is sensitization to chemicals or biological products in the workplace. Intrinsic asthma often starts in middle age ('late onset'). Nevertheless, many patients with adult-onset asthma show positive skin tests and on close questioning give a history of respiratory symptoms compatible with childhood asthma [32].

Non-atopic individuals may develop asthma in middle age from extrinsic causes such as sensitization to occupational agents or aspirin intolerance, or because they were given adrenoceptor-blocking agents for concurrent hypertension or angina. Extrinsic causes must be considered in all cases of asthma [32].

There are two major factors involved in the development of asthma and many other stimuli that can precipitate attacks. The term 'atopy' was used by clinicians at the beginning of the century to describe a group of disorders, including asthma and hayfever, that appeared to run in families; to have characteristic wealing skin reactions to common allergens in the environment; to have circulating antibody in their serum that could be transferred to the skin of non-sensitized individuals [32].

The term is best used to describe those individuals who readily develop antibodies of IgE class against common materials present in the environment. Such antibodies are present in 30-40% of the UK population, and there is a link between serum Immunoglobulin E (IgE) levels and both the prevalence of asthma and airway hyperresponsiveness [32].

Genetic and environmental factors affect serum IgE levels. The use of DNA microsatellite markers to scan the entire genome has uncovered 22 chromosomal regions of interest containing candidate genes. Some of these, in combination with environmental factors, may turn out to play a key role in the development of asthma [32].

The genes controlling the production of the cytokines Interleukin 3 (IL-3), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 9 (IL-9), Interleukin 13 (IL-13) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) - which in turn affect mast and eosinophil cell development and longevity as well as immunoglobulin E production - are present in a cluster on chromosome 5q31-33 (the IL-4 gene cluster) [32].

A gene on chromosome 2, PHF11, which controls IgE synthesis is strongly associated with atopy whereas ADAM33 on chromosome 20 is more strongly associated with airway hyperresponsiveness and the tissue changes of remodelling. Early childhood exposure to allergens and maternal smoking have a major influence on IgE production [32].

Much current interest focuses on the role of intestinal bacteria and childhood in shaping the immune system in early life. It has been suggested that growing up in a relatively 'clean' environment may predispose towards an immunoglobulin E response to allergens (the hygiene hypothesis) [32].

Conversely, growing up in a 'dirtier' environment may allow the immune system to avoid developing allergic responses. Components of bacteria (e.g. lipopolysaccharide endotoxin; immunostimulatory CpG DNA sequences), viruses (e.g. DS RNA) and fungi (e.g. chitin, a cell wall component) are able to stimulate receptors expressed on immune cells to direct the immune and inflammatory response away from the allergic pathways [32].

Thus early life exposure to inhaled and ingested products of microorganisms may be critical in helping shape the subsequent risk of a child becoming allergic and/or developing asthma. The allergens involved in asthma are similar to those in rhinitis. Allergens from the faecal particles of the house-dust mite are the most significant extrinsic cause of asthma world-wide [32].

Cockroach allergy has been implicated in asthma in United States inner-city children, while allergens from furry pets are also becoming increasingly common causes of asthma, rhinitis and urticaria. The fungal spores from *Aspergillus fumigatus* give rise to a complex series of lung disorders, including asthma. Many allergens including those from *Aspergillus* have intrinsic biological properties, e.g. proteolytic enzymes that facilitate their passage through the airway epithelium to increase their sensitizing capacity [32].

Bronchial hyperresponsiveness can be demonstrated by asking the patient to inhale gradually increasing concentrations of either histamine or methacholine (bronchial provocation tests). This induces transient airflow limitation in susceptible individuals (approximately 20% of the population); the dose of the agonist (provocation dose) necessary to produce a 20% fall in FEV<sub>j</sub> is known as the PD<sub>20</sub> FEV<sub>j</sub> (or PC<sub>20</sub> FEV<sub>1</sub>) [32].

Patients with clinical symptoms of asthma respond to very low doses of methacholine; i.e. they have a low PC<sub>20</sub> FEV<sub>1</sub> (< 11 μmol). In general, the greater the degree of hyperreactivity, the more persistent the symptoms and the greater the need for treatment. Some patients also react to methacholine but at higher doses and include those with attacks of asthma only on extreme exertion; wheezing or prolonged periods of coughing following a viral infection; cough variant asthma; seasonal wheeze in pollen season; allergic rhinitis, but not complaining of any lower respiratory symptoms until specifically questioned; some subjects with no respiratory symptoms [32].

Although the degree of hyperresponsiveness can itself be influenced by allergic mechanisms, its pathogenesis involves a combination of airway inflammation and tissue remodelling. Over 200 materials encountered at the workplace give rise to occupational asthma. The causes are recognized occupational diseases in the United Kingdom, and patients in insurable employment are therefore eligible for statutory compensation provided they apply within 10 years of leaving the occupation in which the asthma developed [32].

Asthma due to flour, organic dusts and other large protein molecules involves specific immunoglobulin E antibodies. In contrast, reactive chemicals such as isocyanates and acid anhydrides bond chemically to epithelial cells to activate them as well as provide haptens recognized by T cells [32].

The risk of developing some forms of occupational asthma increases in smokers. The proportion of employees developing occupational asthma depends primarily upon the level of exposure. Proper enclosure of industrial processes or appropriate ventilation greatly reduces the risk. Atopic individuals develop occupational asthma more rapidly when exposed to agents causing the development of specific. Non-atopic individuals can also develop asthma when exposed to such agents, but after a longer period of exposure [32].

The characteristic feature of bronchial hyperresponsiveness in asthma means that, as well as reacting to specific antigens, the airways will also respond to a wide variety of non-specific direct and indirect stimuli such as cold air and exercise. Most asthmatics wheeze after prolonged exercise. Typically, the attack does not occur while exercising but afterwards. The inhalation of cold, dry air will also precipitate an attack [32].

Exercise-induced wheeze is driven by histamine and leukotrienes which are released from mast cells when the epithelial lining fluid of the bronchi becomes hyperosmolar owing to drying and cooling during exercise. The phenomenon can be shown by exercise, cold air and hypertonic (e.g. saline or mannitol) provocation tests [32].

Many patients with asthma experience worsening of symptoms on contact with cigarette smoke, car exhaust fumes, strong perfumes or high concentrations of dust in the atmosphere. Major epidemics have been recorded when large amounts of allergens are released into the air, e.g. soy bean epidemic in Barcelona [32].

Asthma exacerbations increase in both summer and winter air pollution episodes associated with climatic temperature inversions. Epidemics of the disease have occurred in the presence of high concentrations of ozone, particulates and NO<sub>2</sub> in the summer and particulates, Nitrogen Dioxide (NO<sub>2</sub>) and Sulphur Dioxide (SO<sub>2</sub>) in the winter [32].

Increased intakes of fresh fruit and vegetables, or unprocessed diets have been shown to be protective. Genetic variation in antioxidant enzymes is associated with more severe asthma. It is well known that emotional factors may influence asthma, but there is no evidence that patients with the disease are any more psychologically disturbed than their non-asthmatic peers [32].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), particularly aspirin and propionic acid derivatives, e.g. indometacin, have a major role in the development and precipitation of attacks in approximately 5% of patients with asthma. This effect is especially prevalent in those individuals who have both nasal polyps and asthma [32].

It is thought that treatment with these drugs leads to an imbalance in the metabolism of arachidonic acid. non-steroidal anti-inflammatory drugs inhibit arachidonic acid metabolism via the cyclo-oxygenase (COX) pathway, preventing the synthesis of prostaglandins. It has been suggested that under these circumstances there is a reduced production of prostaglandin E<sub>2</sub> and an overproduction of cysteinyl leukotrienes by eosinophils, mast cells and macrophages [32].

In such patients there is evidence for polymorphisms involving the promoter region of the Leukotriene C4 synthase gene that controls the level of activity of this terminal enzyme of the leukotriene-generating pathway. Interestingly, asthma in intolerant patients is not precipitated by COX-2 inhibitors, indicating that it is blockade of COX-1 that is linked to impaired prostaglandin E2 production [32].

The airways have a direct parasympathetic innervation that tends to produce bronchoconstriction. There is no direct sympathetic innervation of the smooth muscle of the bronchi, and antagonism of parasympathetically induced bronchoconstriction is critically dependent upon circulating epinephrine (adrenaline) acting through M2-receptors on the surface of smooth muscle cells [32].

Inhibition of this effect by  $\beta$ -adrenoceptor blocking drugs such as propranolol leads to bronchoconstriction and airflow limitation, but only in asthmatic subjects. The so-called selective  $\beta$ -adrenergic blocking drugs such as atenolol may still induce attacks of asthma; their use to treat hypertension or angina in asthmatic patients is best avoided [32].

The experimental inhalation of allergen by atopic asthmatic individuals leads to the development of different types of reaction. Immediate asthma (early reaction): Airflow limitation begins within minutes of contact with the allergen, reaches its maximum in 15-20 minutes and subsides by 1 hour [32].

Following an immediate reaction many asthmatics develop a more prolonged and sustained attack of airflow limitation (Dual and late-phase reactions) that responds less well to inhalation of bronchodilator drugs such as salbutamol [32].

Isolated late-phase reactions with no preceding immediate response can occur after the inhalation of some occupational sensitizers such as isocyanates. The development of the late-phase reaction is associated with an increase in the underlying level of airway hyperresponsiveness such that individuals may show continuing episodes of asthma on subsequent days [32].

The pathogenesis of asthma is complex and not fully understood. It involves a number of cells, mediators, nerves and vascular leakage that can be activated by several different mechanisms, of which exposure to allergens is among the most significant [32].

The varying clinical severity and chronicity of asthma is dependent on an interplay between airway inflammation and airway wall remodelling. The inflammatory component is driven by Th2-type T lymphocytes which facilitate immunoglobulin E synthesis through production of Interleukin-4 and eosinophilic inflammation through Interleukin-5 [32].

Asthma is one of the most common chronic diseases in industrialized countries, affecting 10% or more of young children in some countries. An atopic disease with high IgE levels, asthma is induced by small particles of antigen, which are able to penetrate deep into the lungs. About 80% of asthmatic children are allergic to house mites. Animal danders are another major cause [21].

The incidence of asthma appears to be increasing in many modern societies, but the reasons are unclear, and the increase doesn't appear to be linked to air pollution. A chronic atopic condition usually precedes an acute attack of asthma [21]

In addition to IgE and eosinophils there are excessive numbers of neutrophils with high-affinity IgE receptors in the airway tissues. Prostaglandin D<sub>2</sub> released from mast cells may play a role in triggering an attack. Cytokines, nitric oxide, and nerve growth factor may also participate in the response [21].

The presence of a high concentration of glutathione and of glutathione peroxidase, whose concentration increases in asthmatic lungs, may reflect the action of the antioxidant system in combating inflammation. Concentrations of surfactant proteins SP-A and SP-D are Ca<sup>2+</sup>-dependent lectins which serve as regulators of the innate immune response increase in asthma [21].

As defined by the management guidelines issued in 1999 by the National Heart, Lung and Blood Institute standard asthma therapeutics include oral and inhaled corticosteroids, leukotriene antagonists, short acting and long acting  $\beta$ -antagonists, cromolyn and nedocromil [12].

Several studies in the 1960s first reported subjective improvement in asthmatic symptoms with use of IV heparin. Bardana et al performed the first trial of inhaled heparin reporting subjective but not objective improvements [12].

Elevated levels of heparin-like anticoagulants have been demonstrated in atopic asthmatic patients, and have been induced in some patients after antigen challenge [12].

Bronchial asthma is recognized as an inflammatory disease which is characterized by bronchial hyperresponsiveness, excessive mucus production, mucosal oedema and recruitment of inflammatory cells into the airways [10].

Aerosolized heparin may be helpful in alleviating symptoms of asthma although no definitive bronchodilating activity has been observed [11a]. It is suggested that heparin may be a natural "anti-asthmatic" molecule [7][11b].

The anti-asthmatic activity of heparin is thought not to be due to its anticoagulant properties as the agent did not prolong the partial thromboplastin time measured one hour or three hours after inhalation. The administration of heparin to sheep failed to change the partial thromboplastin time for up to 12 hours after inhalation [11a].

Heparin's ability to inhibit airway responsiveness is unlikely to be related solely to its highly anionic nature as similar effects were not observed with the linear anionic molecule polyglutamic acid [10].

### **Exercise induced Asthma**

Inhaled heparin prevents bronchoconstriction response in exercise induced asthmatic subjects [5][7]. Heparin prevents Exercise-Induced Asthma (EIA) but not histamine induced bronchoconstriction. Heparin attenuates post exercise decrease of the specific airway resistance [7].

Inhaled heparin attenuates acute bronchoconstrictor response to exercise. Heparin completely, or partially inhibited exercise induced bronchoconstriction in 75% of subjects. Inhaled low molecular weight heparin, enoxaparin attenuates exercise-induced bronchoconstriction in a dose dependent manner [5].

Inhaled heparin inhibits early response to exercise induced bronchoconstriction in subject with asthma [6].

Part of the immune response consists of the release from stimulated neutrophils, macrophages, and other cells of Platelet Activating Factor (PAF: 1-O-alkyl-2-acetyl-sn-glycerophosphocholine), a material that "activates" blood platelets. The principal interest in the platelet-activating factor has arisen from its powerful effect in inducing inflammation. It is a remarkably potent compound, its effects on platelets are observed at concentrations of  $10^{-11}$  to  $10^{-10}$  M [22].

One effect of platelet activating factor on platelets is to induce a rapid (5–10s) cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C to give diacylglycerol and inositol 1,4,5-trisphosphate. The subsequent effects of these two substances cause a rapid influx of  $Ca^{2+}$  and induce a series of secondary responses [22].

Among these responses are the release of the materials stored in the platelet's granules. Platelet activating factor also appears to inhibit adenylate cyclase and causes vasodilation, a property not expected for a compound that stimulates clotting. Stimulated platelets release arachidonic acid rapidly from their phospholipids, apparently as a result of activation of phospholipase A2. The released arachidonate can in turn be metabolized to endoperoxides and thromboxane A2 [22].

These compounds are also potent activators of platelets and cause a self-activating or autocrine effect. While platelet activating factor has a beneficial function, it can under some conditions contribute in a dangerous way to inflammation and to allergic responses including anaphylaxis, asthma and cold-induced urticaria [22].

Heparin inhibits exercise induced bronchoconstriction possibly by altering inositol triphosphate (IP3) levels. Inhibition of Platelet Activating Factor (PAF)-induced airway hyperresponsiveness and cell infiltration by heparin cannot be explained by heparin acting merely as a platelet activating factor antagonist [10].

Heparin prevents exercise-induced asthma without influencing histamine induced bronchoconstriction. Various forms of exercise can induce post exercise bronchoconstriction in asthmatics attributed to large volumes of inadequately conditioned air which leads to a loss of heat and water from respiratory mucosa. The mechanism whereby airway cooling leads to bronchoconstriction is not clear [11a].

Mast cell stabilizing agents (i.e. cromolyn sodium) are known to attenuate exercise induced asthma. Inhaled heparin prevents post-exercise bronchoconstriction in subjects with exercise induced asthma [11a]. Pharmacologic agents including beta agonists, cromolyn sodium, atropine, furosemide and calcium-channel blockers offer varying degrees of protection against exercise-induced asthma or asthma due to hyperventilation with cold dry air. Heparin was found to be more effective than cromolyn in preventing post exercise bronchoconstriction [11a][11c].

Inhaled heparin preserves specific airway conductance (sGaw) in exercise induced asthma better than did 20 mg inhaled cromolyn. Inhaled heparin was superior in preserving specific airway conductance in exercise induced asthma when administered up to 3 hours prior to exercise [12].

Mast cell mediators, including histamine and leukotrienes have been implicated in the pathogenesis of exercise-induced asthma and H-histamine receptor antagonists as well as leukotriene D-receptor or 5-lipoxygenase inhibitors partly attenuate bronchoconstrictor responses induced by exercise or cold dry air [11a]. Heparin prevents the bronchoconstrictor response in subjects with exercise induced asthma [11c].

Exercise induced asthma is characterized by transient airway obstruction typically occurring after hyperventilation with cold dry air. Airway Surface Fluid (ASF) osmolality increases during hyperventilation and correlates with the magnitude of obstruction [13].

Changes in airway surface fluid osmolality in canine peripheral airways are associated with bronchial mucosal injury, mast cell degranulation and the elaboration of bronchoactive mediators. Inhaled heparin inhibits Hyperventilation Induced Bronchoconstriction (HIB) in humans. Numerous animal and human studies suggest that a variety of eicosanoids contribute to the development of hyperventilation induced bronchoconstriction. Inhalation of heparin attenuates hyperventilation induced bronchoconstriction [13].

Mast cell degranulation of lung by conditions other than anaphylaxis, such as hypoxia or exercise in certain asthmatics may release Prostaglandin-generating factor of anaphylaxis (PGF-A) and lead to prostaglandin formation [20].

A novel mediator of human lung anaphylaxis termed prostaglandin-generating factor of anaphylaxis (PGF-A) has been described which may be partly responsible for prostaglandin generation accompanying allergic reactions. PGF-A is an oligopeptide of 1,450 daltons that induces the production of  $\text{PGF}\alpha$ , PGE, TXB2 and 5-, 12-, and 15-hydroxyeicosatetraenoic acid (HETE) from human lung parenchyma and airways [23].

Experimental evidence suggests that this factor is not preformed (like histamine), but newly synthesized or rapidly generated by the anaphylactic event (like SRS-A). Mediators other than histamine and PGF-A such as SRS-A and bradykinin have also been shown to be capable of causing prostaglandin formation [23].

Reduction in hyperventilation induced airway obstruction caused by heparin is accompanied by a concomitant decrease in eicosanoid mediator concentrations. Heparin inhibits hyperventilation induced bronchoconstriction via the inhibition of either hyperventilation induced eicosanoid production or release [13].

Use of a 5-lipoxygenase activating protein antagonist confirmed that leukotrienes significantly contributed to the development of hyperventilation induced bronchoconstriction. Heparin attenuates hyperventilation induced bronchoconstriction in asthmatics and acute antigen-induced bronchoconstriction in sheep without modifying the effects of histamine [13].

Hyperventilation induced increases in Leukotriene C4 (LTC4), Leukotriene E4 (LTE4) and Prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) are inhibited and Prostaglandin D2 (PGD2) and Thromboxane B2 (TxB2) remain at baseline levels in heparin treated/dry air challenged airways [13].

Heparin tends to reduce the number of epithelial cells recovered in Broncho-Alveolar Lavage Fluid (BALF) after dry air challenge. Inhaled heparin inhibits eicosanoid mediator production and release caused by hyperventilation with dry air and significantly attenuates hyperventilation induced bronchoconstriction [13].

## Allergic Challenge Asthma

Heparin decreased the response to vagally induced bronchoconstriction in sensitized antigen challenged animals [4]. Inhaled heparin attenuates antigen induced bronchoconstriction in allergic sheep as well as prevents bronchoconstriction response to antigen in asthmatic human subjects [5].

Inhaled heparin prevents antigen induced bronchoconstriction and airway responsiveness by modulation of mast cell mediator release [5]. Inhaled heparin inhibits early response to allergen induced bronchoconstriction in subjects with asthma [6].

Heparin attenuates bronchoconstrictor response and immediate cutaneous reaction to antigen in allergic sheep. Heparin inhibits the acute cutaneous reaction and bronchoconstriction due to allergens in allergic subjects [7].

Compound 48/80 is a polymer produced by the condensation of N-methyl-p-methoxyphenethylamine with formaldehyde. It promotes histamine release. In biochemical research, compound 48/80 is used to promote mast cell degranulation. Heparin attenuates the effect of compound 48/80 and antigen-induced acute bronchoconstrictor responses in sheep without modifying the effects of histamine [11a].

Heparin attenuated antigen induced bronchoconstriction in a dose dependent manner, and inhibited bronchoconstriction responses to compound 48/80. Heparin also inhibits bronchoconstriction by inhaled dust mite extract in allergic asthmatics [7]. In asthma, heparin in a single dose reduced early asthmatic response to inhaled allergen (house dust mite extract) [11b].

Ether linked phospholipid platelet activating factor (PAF) plays an important role in the pathogenesis of asthma as it can reproduce many of the features of the disease. Unfractionated heparin and the low molecular weight heparinoid Org 10172 can inhibit airway hyperresponsiveness induced by aerosolized platelet activated factor in neonatally immunized adult rabbits [10].

Both heparin and low molecular weight heparinoid, Org 10172, possess anti-inflammatory activity in the lung and the ability to inhibit Platelet Activated Factor (PAF)-induced airway hyperresponsiveness in the rabbit. Heparin also inhibits platelet activated factor induced airway hyperresponsiveness and eosinophil infiltration in guinea pigs [10].

The ability of heparin and Org 10172 heparinoid to inhibit airway responsiveness to histamine following Bovine Serum Albumen (BSA) challenge suggests an action not attributable merely to the ability to bind histamine [10].

Heparin had no effect on histamine induced bronchoconstriction [11a]. Heparin does not alter airway reactivity to histamine in human asthmatics or sheep. Heparin attenuates hyperventilation induced bronchoconstriction in asthmatics and acute antigen induced bronchoconstriction in sheep without modifying the effects of histamine [13].

Inhaled heparin prevents allergic bronchoconstriction in sheep and inhibits anti-IgE-mediated release of histamine from mast cells in vivo [11a].

Heparin attenuates allergic bronchoconstriction in sheep, inhibits anti-IgE mediated histamine release in isolated mast cells and prevents the bronchoconstrictor response in subjects with exercise induced asthma [11c].

Analysis of the time course of the appearance of prostaglandins during human lung anaphylaxis reveals a close and parallel relationship to histamine release. Histamine acts through an H1 receptor causing bronchial smooth muscle constriction which produces prostaglandin E [20].

A close direct relationship exists between the intensity of anaphylaxis induced as reflected by the concentration of histamine released from human lung during anaphylaxis and the quantity of prostaglandin generating factor of anaphylaxis released [20].

A portion of the prostaglandin generation of anaphylaxis of human lung is due to histamine. Addition of exogenous histamine generates about 50% of the prostaglandins produced during anaphylaxis and H1 histamine receptor antagonists prevent about 50% [20].

Histamine and other mediators released from human mast cells undergoing anaphylaxis are able to induce prostaglandin generation and are found in the supernatant from lung anaphylaxis [20].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, slow reacting substance of anaphylaxis, eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandins F2a, E2, and thromboxane A2 and kallikrein of anaphylaxis [20].

Anti-allergic actions of heparin may be related to the inhibition of mediator release from mast cells. Heparin attenuates antigen induced bronchoconstriction in humans with asthma and can inhibit histamine release from isolated human mast cells. Heparin also inhibits histamine release from blood cells induced by antigen, trypsin and other proteases [11a].

Platelets factor 4 (PF4) and related member of the IL-8 supergene family, RANTES are chemoattractants for eosinophils. Heparin inhibits the actions of platelets factor 4, a cationic protein shown to be released following antigen challenge in sensitized rabbits and human asthmatics [10].

Pre-treatment with 1000 U/kg heparin attenuated the increase in the lung resistance of previously sensitized sheep by 91% on allergen exposure [12]. Inhaled heparin attenuates antigen-induced bronchoconstriction and airway hyperresponsiveness in sheep [13]. An N-desulfated heparin failed to prevent antigen induced bronchoconstriction [11a].

Antigen induced hyperresponsiveness is associated with increased release of acetylcholine from vagus nerves [4]. Inhibitory M2 muscarinic receptors control release of acetylcholine from human pulmonary parasympathetic nerves. In asthmatics the M2 acetylcholine muscarinic receptors do not function [4][6].

Heparin restores M2 receptor function by binding to and neutralizing major basic protein and polycationic substances [4]. Heparin reverses allergen induced inhibitory M2 receptor dysfunction [6]. Heparins inhibition of bronchoconstriction is co-incident with return to normal of M2 receptor function [4].

Heparin can modulate allergen airway hyperresponsiveness in guineapigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein Major Basic Protein (MBP) on M2 receptor function on airway ganglia [10]. Heparin failed to inhibit acetylcholine induced tracheal smooth muscle contraction in tissue culture [7]. Inhaled heparin inhibits methacholine-induced bronchoconstriction and is suggested to act directly on airway smooth muscle [7][13].

## **Mast cells**

Mast cells occur particularly often as single cells, but more frequently in small groups, in the vicinity of small vessels. Mast cells (diameter 6–12µm) contain a rounded nucleus. Their cytoplasm is loaded with basophilic, metachromatic granules. Paul Ehrlich (1877) interpreted these as alimentary storage granules (Ehrlich's mast cells) [24].

Mast cells synthesize, store and extrude the acid and sulfatized glycosaminoglycan heparin, the biogenic amine histamine, and also additional factors which play a role in anaphylactic reactions. Histamine is released in large amounts during allergic reactions. It causes a widening of the capillaries [24]. Heparin inhibits anti-IgE mediated histamine release in isolated mast cells [11c] and can inhibit histamine release from isolated human mast cells [11a].

Stimulation of mast cells starts a series of cellular events and culminates in mediator release. Mast cell stabilizing agents (i.e. cromolyn sodium) attenuate exercise induced asthma. Both non immunologic and immunologic stimuli can degranulate mast cells [11a].

Breakdown of inositol phospholipids leads to the generation of 1,4,5-inositol triphosphate which binds to receptors on the endoplasmic reticulum and causes internal release of calcium in mast cells and many other cells. Inositol triphosphate can degranulate mast cells and cause histamine release [11a].

Changes in Airway Surface Fluid (ASF) osmolality in canine peripheral airways are associated with bronchial mucosal injury, mast cell degranulation and the elaboration of bronchoactive mediators [13].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D2 and the 5-lipoxygenase products leukotrienes C4 and D4 previously identified as slow acting substance of anaphylaxis [18].

Histamine and other mediators released from human mast cells undergoing anaphylaxis are able to induce prostaglandin generation and are found in the supernatant from lung anaphylaxis [20].

Mast cell degranulation of lung by conditions other than anaphylaxis, such as hypoxia or exercise in certain asthmatics may release Prostaglandin Generating Factor of Anaphylaxis (PGF-A) and lead to prostaglandin formation. Prostaglandin generation secondary to prostaglandin generating factor of anaphylaxis and other mast cell derived mediators might contribute to alterations in lung function associated with variety of conditions which involve mast cell degranulation [20].

Mast cells produce Prostaglandin D2 (PGD2) and Prostaglandin Prostacyclin (PGI2) but not the Prostaglandin F2α (PGF2α), Prostaglandin E (PGE), and Thromboxane A2 (TxA2) produced by lung anaphylaxis [20].

Eosinophilic granulocytes occur not only in blood (1–4% of the leukocytes) but also in connective tissue. Eosinophilic granulocytes measure about 12 $\mu$ m in diameter. That makes them slightly larger than neutrophil cells. They have a bilobed nucleus (spectacle form). In the figure, the connection between the two lobes is not visible [25].

The characteristic marker for eosinophils are eosin-stained acidophilic or eosinophilic granules. In electron microscopy, these show a remarkably regular and characteristic structure. The oval, discus-shaped granules contain a central, oblong crystalloid body, termed internum. At higher magnification, the internum reveals a lamellar structure. A layer of lesser electron density was named externum or matrix. It surrounds the crystalloid inner body [25].

These acidophilic granules are modified lysosomes, which contain various acid hydrolases, such as acid phosphatase, cathepsin, peroxidase, arylsulfatase and ribonuclease, among others. There are few cytoplasmic organelles. Eosinophilic granulocytes recognize and phagocytose antigen-antibody complexes, and they show ameba-like mobility [25].

Eosinophils have been mentioned as source of growth factors in diseases characterized by chronic inflammation, such as asthma [2]. The safety of heparin and its capacity to inhibit eosinophils may represent a therapeutic target in asthma [3].

Heparin inhibits Platelet Activating Factor (PAF) induced airway hyperresponsiveness and eosinophil infiltration in guinea pigs. Eosinophils may not be a prerequisite for the induction of airway hyperresponsiveness. Heparin can modulate allergen airway hyperresponsiveness in guineapigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein Major Basic Protein (MBP) on M2 muscarinic receptor function on airway ganglia [10].

Platelets factor 4 (PF4) and related members of the IL-8 supergene family, RANTES are chemoattractants for eosinophils. Heparin inhibits the actions of platelets factor 4, a cationic protein shown to be released following antigen challenge in sensitized rabbits and human asthmatics [10].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, Slow Reacting Substance of Anaphylaxis (SRS-A), eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandins F<sub>2a</sub>, E<sub>2</sub>, and TxA<sub>2</sub> and kallikrein of anaphylaxis [20].

Eicosanoids include prostaglandins, thromboxanes, leukotrienes and epoxyeicosatrienoates. Eicosanoids (Greek eicosa- = twenty carbon atoms) are derived in humans from the fatty acid arachidonic acid [28]. Numerous animal and human studies suggest that a variety of eicosanoids contribute to the development of Hyperventilation Induced Bronchoconstriction (HIB) [13].

Use of a 5-lipoxygenase activating protein antagonist confirmed that leukotrienes significantly contributes to the development of hyperventilation induced bronchoconstriction. Hyperventilation induced increases in Leukotriene C4 (LTC4) Leukotriene E4 (LTE4) and Prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α) are inhibited and Prostaglandin D2 (PGD2) and Thromboxane B2 (TxB2) remain at baseline levels in heparin treated/dry air challenged airways [13].

Inflammatory cells and airway tissues may release prostaglandins and other mediators which affect response to nerve stimulation [4]. Interruption of leukotriene cascade results in inhibition of exercise induced bronchoconstriction some but not other patients [5].

Mast cell mediators, including histamine and leukotrienes have been implicated in the pathogenesis of exercise-induced asthma. H-histamine receptor antagonists as well as leukotriene D-receptor or 5-lipoxygenase inhibitors partly attenuate bronchoconstrictor responses induced by exercise or cold dry air [11a].

Prostaglandin D2 and Prostacyclin (PGI<sub>2</sub>) were quantitatively predominant in the anaphylactic reactions of human lung parenchyma and were 3 to 7 fold greater than other arachidonic acid cyclooxygenase metabolites [19].

Reduction in hyperventilation induced airway obstruction caused by heparin is accompanied by a concomitant decrease in eicosanoid mediator concentrations. Heparin inhibits hyperventilation induced bronchoconstriction via the inhibition of hyperventilation induced eicosanoid production or release [13].

Inhaled heparin inhibits eicosanoid mediator production and release caused by hyperventilation with dry air and significantly attenuates hyperventilation induced bronchoconstriction [13].

A portion of the prostaglandin generation of anaphylaxis of human lung is due to histamine. Addition of exogenous histamine generates about 50% of the prostaglandins produced during anaphylaxis and H1 histamine receptor antagonists prevent about 50%. Prostaglandins are generated during anaphylaxis in human and guinea pig lung. Prostaglandin F<sub>2</sub>α, prostaglandin E, and thromboxane A2 are the predominant derivatives of arachidonic acid found [20].

Analysis of the time course of the appearance of prostaglandins during human lung anaphylaxis reveals a close and parallel relationship to histamine release. Histamine acts through an H1 receptor causing bronchial smooth muscle constriction which produces prostaglandin E [20].

Histamine and other mediators released from human mast cells undergoing anaphylaxis are able to induce prostaglandin generation and are found in the supernatant from lung anaphylaxis. Mast cells produce prostaglandin D2 and prostacyclin but not the prostaglandin F2 $\alpha$ , prostaglandin E, and thromboxane A2 produced by lung anaphylaxis [20].

Anaphylaxis of human lung generates a novel mediator Prostaglandin Generating Factor of Anaphylaxis (PGF-A), which is capable of causing prostaglandin synthesis. Prostaglandin generating factor of anaphylaxis is a novel mediator distinct from other molecules so far recognized as products of human lung anaphylaxis [20].

A close direct relationship exists between the intensity of anaphylaxis induced as reflected by the concentration of histamine released from human lung during anaphylaxis and the quantity of PGF-A released [20].

Mast cell degranulation of lung by conditions other than anaphylaxis, such as hypoxia or exercise in certain asthmatics may release PGF-A and lead to prostaglandin formation [20].

Prostaglandin generation secondary to PGF-A and other mast cell derived mediators might contribute to alterations in lung function associated with variety of conditions which involve mast cell degranulation [20].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, Slow Reacting Substance of Anaphylaxis (SRS-A) eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandins F2a, E2, and thromboxane A2 and kallikrein of anaphylaxis [20].

Anaphylaxis of guinea pig lung induces the release of Rabbit Aorta Contracting Substance-Releasing Factor (RCS-RF), whereas rat peritoneal anaphylaxis induces the appearance of arachidonic acid releasing factor. Prostaglandin formation can clearly be demonstrated during anaphylaxis of lung in vivo or in vitro. Prostaglandins may be demonstrated to influence many lung functions including smooth muscle tone, vascular permeability and tone, mucous secretion, mast cell mediator release and others [20].

## Allergic Response

One in 10 persons, ~ 22 million people, in the United States have allergies. Ten million of these suffer from the nasal discomfort of “hay fever” and six million from the more serious asthma. Substantial numbers of people in the United States die of allergic reactions to insect stings (more than 30 per year) or to injections of penicillin (300 per year in 1970) [30].

Foods, drugs, pollens, mold spores, mites in house dust, and even heat or cold can evoke serious allergic reactions. Among these eczema (atopic dermatitis) is very common. A major cause of allergic reactions has been traced to molecules of immunoglobulins IgE, which bind to the basophils in the blood and to the related mast cells of tissues [30].

Binding of an antigen to these IgE molecules activates them. These activated antibodies bind to the  $\alpha$ - $\beta$  subunits of the Fc $\alpha$ RI, a transmembrane receptor on basophil or mast cell surfaces. If two or more IgE molecules bind to a mast cell, they may aggregate and activate the mast cell to release its histamine-containing granules [30].

The granules also release cytokines and arachidonate, which is converted primarily into prostaglandin D<sub>2</sub> and into products of the 5-lipoxygenase pathway. The products include the chemotactic leukotriene B<sub>4</sub> and leukotrienes C<sub>4</sub> and D<sub>4</sub>. The latter two constitute the slow-reacting substance of anaphylaxis. The result is a rapid inflammatory response with dilation of blood vessels, increased vascular permeability, infiltration of leukocytes, and destruction of tissues [30].

IgE is involved in killing of schistosomes, and elevated IgE levels are seen in patients infected with various parasites. The killing of schistosomes seems to be mediated by blood platelets as well as by neutrophils and eosinophils with the help of mast cells [30].

Allergic persons often have an IgE level over ten times normal levels. This increase makes the individual especially sensitive to IgE-mediated reactions, a condition called atopy (meaning “strange disease”). Allergies may also be accompanied by increased B cell levels. This can sometimes be responsible for the sudden and sometimes fatal systemic reaction of anaphylaxis. T-cell responses may also cause anaphylaxis [30].

In addition to IgE and eosinophils there are excessive numbers of neutrophils with high-affinity IgE receptors in the airway tissues. Prostaglandin D<sub>2</sub> released from mast cells may play a role in triggering an attack. Cytokines, nitric oxide, and nerve growth factor may also participate in the response [30].

Most allergy-inducing antigens are proteins, but proteins vary widely in their antigenicity. Only a few natural proteins are major allergens. Many of these are relatively small, with molecular masses of 5 – 50 kDa. Most are soluble, and some are glycoproteins. Mites carry allergens that are among the most important causes of asthma and allergic dermatitis. The allergens are 125- to 129-kDa proteins crosslinked by three disulfide bridges. Cockroaches and other insects also form many allergens. Among them are the hemoglobins of small flies of the chironomid family [30].

Proteins of cat saliva dry and flake off as dander, which contains major indoor allergens linked to asthma. Dogs, horses, cattle, and other animals also provide several allergens, some of which are lipocalins. Other allergens are provided by fungi that live on skin or nails [30].

Plants provide a host of allergens. Major allergens are found in pollen of rye-grass, of many other grasses, of ragweed, and of olive trees. Natural rubber latex would appear to be a harmless high polymer, but it contains antigenic proteins, which have been blamed for 1100 anaphylactic attacks with at least 15 deaths between 1988 and 1992 [30].

Diet is a major source of allergens and is often overlooked. Food allergies may be hard to diagnose and symptoms such as headache, diarrhea, itching, and asthma may be attributed to other causes. However, the occasional rapid death from anaphylactic shock, for example, from exposure to peanuts, is a reminder that unrecognized food allergies exist [30].

About 100 – 200 persons die annually of food allergies. About 90% of recognized food allergies involve milk, eggs, fish, crustacea, peanuts, tree nuts, soybeans, and wheat. No structural generalization can be applied to all food allergens. The muscle protein tropomyosin is a well-known allergen whose allergenicity varies among different sources. Tropomyosin from beef, pork, and chicken is usually not highly allergenic, but that from shrimp often is [30].

Asthma is one of the most common chronic diseases in industrialized countries, affecting 10% or more of young children in some countries. An atopic disease with high IgE levels, asthma is induced by small particles of antigen, which are able to penetrate deep into the lungs. About 80% of asthmatic children are allergic to house mites. Animal danders are another major cause [30].

The incidence of asthma appears to be increasing in many modern societies, but the reasons are unclear, and the increase doesn't appear to be linked to air pollution. A chronic atopic condition usually precedes an acute attack of asthma [30].

Heparin modulates allergic responses both in skin and in the respiratory system. Studies date back to the 1910s reporting that heparin inhibits anaphylaxis [7]. Van de Carr and Williams demonstrated in 1928 that heparin could modulate allergic responses in the skin and respiratory system [10]. Observations of the effect of heparin in antigenic challenge are equivocal [7].

Many biological actions of heparin including the anti-allergic activity are molecular weight dependent. Anti-allergic activity of fractionated heparins is molecular weight dependent and an inverse relationship between molecular weight and the anti-allergic activity was observed [5].

It has been suggested that the greater potency of enoxaparin is related to the presence of a higher percentage of oligosaccharide chains possessing anti-allergic activity. The antiallergic activity of inhaled heparin is independent of its anticoagulant properties [5].

An increase in levels of endogenous "heparin-like material" have been found in the plasma of allergic patients plasma [7][10]. Perfusates from inflamed skin of allergic contact eczema contain nanogramme concentrations of prostaglandins. In contrast prostaglandins could not be detected by direct examination of perfusates from normal skin though ethyl acetate extraction revealed the presence of trace amounts [14].

Prostaglandins E1, E2, F1a and F2 $\alpha$  have been isolated from inflamed skin of patients with allergic contact eczema [14]. Prostaglandin generating factors are an important phylogenetic concomitant of anaphylaxis although the specific molecular form of the factors differs between species [20].

Anaphylaxis of guinea pig lung induces the release of Rabbit Aorta Contracting Substance-Releasing Factor (RCS-RF), whereas rat peritoneal anaphylaxis has been found to induce the appearance of arachidonic acid releasing factor. Rat peritoneal anaphylaxis induces the release of an arachidonic acid releasing factor that is separable from Slow Reacting Substance of Anaphylaxis (SRS-A) [20].

Antigen induced hyperresponsiveness is associated with increased release of acetylcholine from vagus nerves [4]. Heparins anti-allergic actions may be related to inhibition of mediator release from mast cells [13]. Heparin inhibits types 1 hypersensitivity reactions in allergic sheep, suggested to be via an inhibitory effect on mast cell degranulation [10].

Pollenosis is the best clinical example of the type 1 or the immediate hypersensitivity reaction. In patients with allergic rhinitis provocation by allergens evokes both early and late reactions characterized by the release of inflammatory mediators detectable in nasal lavage [18].

In atopic patients, exposure to high concentrations of aero-allergens not only leads to the active sensation of mast cells but also increases their numbers in the mucosa of the respiratory tract [18].

Experimental nasal provocation with allergens induces gross degranulation of mast cells both on the mucosal surface and in the submucosa. The activation of mast cells by IgE leads to the release of preformed mediators including histamine, exoglycosides, the neutral protease tryptase, as well as eosinophil and neutrophil chemotactic factors [18].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D<sub>2</sub> and the 5-lipoxygenase products leucotrienes C<sub>4</sub> and D<sub>4</sub> which have been previously identified as Slow Acting Substance of Anaphylaxis [18].

Histamine causes direct and reflex dilation of post capillary venules leading to nasal obstruction, it also stimulates irritant receptors causing sneezing and stimulates secretion from goblet cells and submucosal glands [18].

## **Inflammation**

Although asthma often improves in children as they reach their teens, it is now realized that the disease frequently returns in the second, third and fourth decades. In the past the data indicating a natural decrease in asthma through teenage years have led to childhood asthma being treated as an episodic disorder [31].

However, airway inflammation is present continuously from an early age and usually persists even if the symptoms resolve. Moreover, airways remodelling accelerates the process of decline in lung function over time. This has led to reappraisal of the treatment strategy for asthma, mandating the early use of controller drugs and environmental measures from the time asthma is first diagnosed [31].

Allergy or hypersensitivity was initially defined by Von Pirquet in 1906 as 'Specifically changed reactivity of an host to an agent on a second or subsequent occasion'. This definition would apply to all specific immune responses, and allergy is now taken to mean a damaging reaction [31].

Hypersensitivity reactions underlie a number of autoimmune and allergic conditions. The scheme devised by Gell and Coombs is useful to group conditions with a similar underlying pathogenesis. Allergic Disease is thus categorised Type I Reaction or Immediate Hypersensitivity Reactions [31].

The type I reaction is an allergic response produced within 5-10 minutes of exposure to a specific allergen. Type I reactivity is mediated by IgE, although later in the reaction other mechanisms of inflammation including infiltration with eosinophils and lymphocytes may contribute [31].

Allergens (antigens that evoke allergic responses), e.g. house-dust mite, pollens, animal danders or moulds, elicit IgE reactions in individuals who are said to be atopic. Atopic diseases include, extrinsic asthma, some forms of eczema, allergic rhinitis/conjunctivitis, food allergies, anaphylaxis and angio-oedema [31].

The diagnosis is made by a typical clinical history and examination in conjunction with either skin-prick testing (when a type I wheal and flare reaction is elicited by pricking the skin through a solution of the test antigens) or by measuring specific IgE in the serum [31].

High-affinity Fc $\alpha$ RI on mast cells tightly bind locally produced allergen-specific IgE. On subsequent allergen exposure, cross-linkage of the surface IgE molecules causes degranulation of the mast cell and release of preformed (granule-derived) and newly formed (membrane derived) mediators [31].

These initiate the allergic response through increasing vascular permeability (causing swelling of the tissue), inducing chemotaxis of neutrophils and eosinophils and later lymphocytes (inflammation) and increasing airways hyperactivity (bronchoconstriction) [31].

Heparin possesses multiple noncoagulant properties including anti-inflammatory and anti-complement activity, modulation of proteases and regulation of mast cell tryptase. Heparin can inhibit lymphocyte activation, trafficking and delayed hypersensitivity responses which are lymphocyte driven [7].

Heparin and Org 10172 inhibits the infiltration of inflammatory cells into the airways following platelet activating factor challenge however this action is not dependent on the anti-inflammatory effect as polyglutamic acid also substantially inhibits cell infiltration without the associated airway hyperresponsiveness [10].

The recognition of the presence of high concentrations of heparin in preformed cytoplasmic mast cell granules in endobronchial tissue first led to the speculation about its involvement in airway inflammation. Heparin binds and inhibits a variety of cytotoxic and inflammatory mediators including eosinophilic cation protein and peroxidase [12].

In patients with allergic rhinitis provocation by allergens evokes both early and late reactions characterized by the release of inflammatory mediators detectable in nasal lavage. Chemotactic recruitment of secondary effector cells such as eosinophils, neutrophils, and basophils to the sites of mast cell degranulation, together with their activation, releases further mediators to compound the inflammatory response which leads to a state of chronic nasal hyperreactivity [18].

Eosinophils are posited as a source of growth factors in diseases characterized by chronic inflammation such as asthma [2]. A range of glycosaminoglycans inhibit the trafficking of inflammatory cells into the airway in vivo induced by both platelet activating factor (PAF) and antigen/antibody interactions [8].

Evidence indicates that prostaglandins participate in the pathogenesis of inflammation. Perfusates from inflamed skin of allergic contact eczema contain nanogramme concentrations of prostaglandins. In contrast prostaglandins could not be detected by direct examination of perfusates from normal skin though ethyl acetate extraction revealed the presence of trace amounts [14].

It has been suggested that the increased concentrations of inflamed skin could be due to increased biosynthesis or alternatively prostaglandins could be released from cell membrane lipids after activation of tissue phospholipases [14].

The presence of a pharmacologically active fatty acid with prostaglandin like properties have been identified in delayed cutaneous inflammation due to exposure to ultraviolet radiation. It has been suggested that prostaglandins are mediators of human cutaneous inflammation [14].

Prostaglandin synthesis in inflammatory bowel disease has been seen in terms of genesis of mucosal inflammation and a global increase in the synthesis of prostanoids [15]. Rheumatoid arthritis is characterized by systemic and local inflammation resulting in cartilage and bone destruction. Prostaglandin E2 is associated with some of the proinflammatory affects of rheumatoid arthritis specifically with the edema and erosion of cartilage and juxta-articular bone [16].

Glycosaminoglycans can be anti-inflammatory. A range of glycosaminoglycans inhibit the trafficking of inflammatory cells into the airway in vivo induced by both Platelet Activating Factor (PAF) and antigen/antibody interactions. The anti-inflammatory effects of glycosaminoglycans are as yet unexplained. Derivatives of heparin lacking anticoagulant activity inhibit inflammatory processes such as delayed hypersensitivity reactions [8].

Heparin has been studied as an anti-inflammatory agent [3]. Inhaled heparin possesses anti-inflammatory and immunoregulatory properties [5][6][13]. As well as heparin having anti-inflammatory actions, it also demonstrates anticomplement activity. The anti-inflammatory activity of heparin is not related to its anticoagulant properties [7].

Heparin possesses multiple non-anticoagulant properties which include modulation of various proteases, anticomplement activity and anti-inflammatory action as well as inhibition of cell growth [11a].

Both heparin and low molecular weight heparinoid Org 10172 possess anti-inflammatory activity in the lung and the ability to inhibit platelet activating factor induced airway hyperresponsiveness in the rabbit [10].

Heparin possesses anti-inflammatory properties which are dependent on the dose, timing and route of administration in animal studies [11b].

## **Inositol**

There are transmembrane receptors for which the reception of the signal and activation take place on the inner side of the membrane. An example is the receptors for inositol triphosphate which are localized in the membrane of Ca<sup>2+</sup> storage organelles and also have the character of ligand-controlled ion channels [38].

Inositol triphosphate is an intracellular messenger substance that binds to the cytosolic side of the corresponding receptor located in the membrane of cell organelles. Ligand binding leads to opening of the ion channel via a conformational change and thus to influx of Ca<sup>2+</sup> ions from the storage organelle into the cytosol [38].

The process of platelet activation involves interaction of the stimulus (e.g. thrombin) with a receptor, activation of G proteins, stimulation of phospholipase C, and liberation from phosphatidylinositol bisphosphate of inositol triphosphate and diacylglycerol [39].

These two second messengers result in an elevation of intracellular Ca<sup>2+</sup> and activation of protein kinase C. In addition, activation of phospholipase A2 produces arachidonic acid that can be converted to a variety of biologically active eicosanoids. The process of activation of neutrophils is essentially similar. They are activated, via specific receptors, by interaction with bacteria, binding of chemotactic factors, or antibody-antigen complexes [39].

The resultant rise in intracellular Ca<sup>2+</sup> affects many processes in neutrophils, such as assembly of microtubules and the actin-myosin system. These processes are respectively involved in secretion of contents of granules and in motility, which enables neutrophils to seek out the invaders. The activated neutrophils are now ready to destroy the invaders by mechanisms that include production of active derivatives of oxygen [39].

Inositol triphosphate (IP3) can degranulate mast cells and cause histamine release. Breakdown of inositol phospholipids leads to the generation of 1,4,5-inositol triphosphate which binds to receptors on the endoplasmic reticulum and causes internal release of calcium in mast cells and many other cells [11a].

Heparin is a specific blocker of inositol triphosphate mediated calcium release in various cell types [13]. Heparin blocks inositol 1,4,5-triphosphate receptors and inositol triphosphate mediated calcium release in vascular and airway smooth muscle [7].

Heparin (in vitro) binds to inositol triphosphate receptors and inhibits the inositol triphosphate induced release of calcium in various tissues, including vascular and airway smooth muscle, the cerebellum and the liver [11a].

Heparin binds to inositol triphosphate receptors and inhibits inositol triphosphate induced release of calcium in various tissues including airway smooth muscle [13]. Heparin inhibits both exercise and allergen-induced bronchoconstriction possibly by altering inositol triphosphate levels [10].

## **Histamine**

Histamine, an important mediator (local signaling substance) and neurotransmitter, is mainly stored in tissue mast cells and basophilic granulocytes in the blood. It is involved in inflammatory and allergic reactions. “Histamine liberators” such as tissue hormones, type E immunoglobulins, and drugs can release it [33].

Histamine acts via various types of receptor. Binding to H1 receptors promotes contraction of smooth muscle in the bronchia, and dilates the capillary vessels and increases their permeability. Via H2 receptors, histamine slows down the heart rate and promotes the formation of HCl in the gastric mucosa. In the brain, histamine acts as a neurotransmitter [33].

In pollenosis, histamine causes direct and reflex dilation of post capillary venules leading to nasal obstruction, it stimulates irritant receptors causing sneezing and stimulates secretion from goblet cells and submucosal glands. Histamine, leukotriene C4, and THAME esterase are also generated during the late reaction [18].

Analysis of the time course of the appearance of prostaglandins during human lung anaphylaxis reveals a close and parallel relationship to histamine release [20]. Arachidonic acid cyclooxygenase metabolites are released in an antigen dose dependent manner and reach maximal release at antigen concentrations lower than those required for maximal histamine release [19].

Prostaglandin generation/release has been considered a secondary event in anaphylaxis, and mast cell derived histamine is visualized as stimulating surrounding tissues to induce prostaglandin generation. Other mediators such as Slow Reacting Substance of Anaphylaxis (SRS-A) and bradykinin are also capable of causing prostaglandin formation [20].

A close direct relationship exists between the intensity of anaphylaxis induced as reflected by the concentration of histamine released from human lung during anaphylaxis and the quantity of Prostaglandin Generating Factor of Anaphylaxis (PGF-A) released [20].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, slow reacting substance of anaphylaxis, eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandins F2a, E2, and thromboxane A2 and kallikrein of anaphylaxis [20].

Histamine acts through an H1 receptor causing bronchial smooth muscle constriction which produces Prostaglandin E. Peripheral lung is relatively free of airways and responds to histamine with prostaglandin generation due to H1 stimulation. Addition of exogenous histamine generates about 50% of the prostaglandins produced during anaphylaxis and H1 histamine receptor antagonists prevent about 50% [20].

Heparin can bind histamine. The ability of heparin and Org 10172 heparinoid to inhibit airway responsiveness to histamine following Bovine Serum Albumin (BSA) challenge suggests an action not attributable merely to the ability to bind histamine [10].

Heparin attenuates the effect of compound 48/80 and antigen-induced acute bronchoconstrictor responses in sheep without modifying the effects of histamine. Heparin protects against the lethal effects of compound 48/80 which releases histamine, and also prevents mast cell degranulation induced by this compound in the subcutaneous tissue of mice [11a].

Heparin inhibits anti-IgE mediated histamine release in isolated mast cells [11c]. Heparin selectively inhibited the anti-IgE-induced release of histamine from human uterine mast cells without altering the effects of calcium ionophore A2318720. By increasing the preincubation period of heparin in vitro from 20 to 60 minutes, the protective effect of heparin on anti-IgE-mediated histamine release and mast cell membranes was increased by 50% [11a].

The activation of mast cells by IgE leads to the release of preformed mediators including histamine, exoglycosides, the neutral protease tryptase, and eosinophil and neutrophil chemotactic factors [18]. Heparin inhibits histamine release from blood cells induced by antigen, trypsin and other proteases [11a].

Heparin can inhibit histamine release from isolated human mast cells [11a][13]. Heparin inhibits anti-immunoglobulin E induced histamine release from isolated human uterine mast cells [13].

Heparin attenuates acute antigen-induced bronchoconstriction in sheep without modifying the effects of histamine [13]. Heparin prevents exercise-induced asthma also without influencing histamine induced bronchoconstriction [11a][7][13]. Inhaled heparin in sheep is known to inhibit anti-IgE-mediated release of histamine from mast cells in vivo [11a].

Mast cell mediators, including histamine and leukotrienes have been implicated in the pathogenesis of exercise-induced asthma. H1-histamine receptor antagonists and leukotriene D-receptor or 5-lipoxygenase inhibitors partly attenuate bronchoconstrictor responses induced by exercise or cold dry air [11a].

A portion of the prostaglandin generation of anaphylaxis of human lung is due to histamine. Histamine and other mediators released from human mast cells undergoing anaphylaxis are able to induce prostaglandin generation and are found in the supernatant from lung anaphylaxis [20].

## **Eosinophil**

The human body contains five types of white blood cells: monocytes, neutrophils, basophils, eosinophils, and lymphocytes. Each type of white blood cell plays a specific role in the body's immune defense system. Under a microscope, three kinds of white blood cells appear to contain granules within their cytoplasm. These three types are the neutrophils, basophils, and eosinophils [34].

Together, these three types of white blood cells are called the granular leukocytes. The granules are specific chemicals released by these white blood cells during the immune response. The other two types of white blood cells, the monocytes and lymphocytes, do not contain granules [34].

Eosinophils, which comprise 2-4% of the total composition of white blood cells, are believed to counteract the effects of histamine and other inflammatory chemicals. They also phagocytize bacteria tagged by antibodies. All white blood cells arise in the red bone marrow [34].

Experimental nasal provocation with allergens induces gross degranulation of mast cells both on the mucosal surface and in the submucosa. The activation of mast cells by IgE leads to the release of preformed mediators including histamine, exoglycosides, the neutral protease tryptase, and eosinophil and neutrophil chemotactic factors [18].

Cytological examination of nasal and conjunctival secretions of patients with active pollenosis shows many basophils and eosinophils [18].

Chemotactic recruitment of secondary effector cells such as eosinophils, neutrophils, and basophils to the sites of mast cell degranulation, together with their activation, releases further mediators to compound the inflammatory response which leads to a state of chronic nasal hyperreactivity [18].

All cells expressing Heparin-binding EGF-like Growth Factor (HB-EGF; a member of the epidermal growth factor family) mRNA are eosinophils and correlate in levels and number. Eosinophils have been posited as source of growth factors in diseases characterized by chronic inflammation such as in the condition of asthma [2]. Eosinophil numbers and the state of their activation are related to symptoms. The safety of heparin and its capacity to inhibit eosinophils has been suggested to represent a therapeutic target in asthma [3].

Heparin affects eosinophils and neutrophils [7]. Heparin dose dependently inhibits eosinophil accumulation [3]. Eosinophils, Major Basic Protein (MBP) and airway hyperresponsiveness are all positively correlated [4].

Cationic peroxidases such as major basic protein and eosinophil peroxidase are neutralized by the highly anionic nature of heparin [7]. Heparin may inactivate eosinophil cationic protein [3], a toxin secreted by activated human eosinophils that has anti-parasitic, antibacterial, neurotoxic activities and also has ribonuclease activity as well as structural homology to other mammalian ribonucleases [35].

The unfractionated heparin preparation multiparin, the low molecular weight heparin preparation Fragmin and the non-anticoagulant O-desulphated heparin inhibited the adhesion of polymorphonuclear leukocytes (PMNs) stimulated with formyl methionine leucyl phenylalanine (fMLP) to unstimulated Human Umbilical Vein Endothelial Cells (HUVECs) [8].

Unfractionated heparin preparation inhibits adhesion of unstimulated Poly-Morpho-Nuclear leukocytes (PMN) to endothelial cells stimulated with cytokines (IL-1 $\beta$  or TNF $\alpha$ ) or the bacterial product lipopolysaccharide (LPS). Heparin in a polymorphonuclear leucocytes suspension reduced adhesion of these cells to activated HUVECs stimulated with cytokines and lipopolysaccharide [8].

Heparin and two related compounds can inhibit the adhesion of polymorphonuclear leucocytes to endothelial cells induced by a variety of stimuli via a mechanism which doesn't involve inhibition of Intercellular Adhesion Molecule 1 (ICAM-1) or E-selectin expression on endothelial cells, involvement of polyanionic nature, involvement of anticoagulant actions; and which involves interference with the adhesive processes of both neutrophils and endothelial cells [8].

Heparin inhibits activation of the complement system, neutrophil chemotaxis and eosinophil infiltration. Heparin protects tissues against damage induced by cationic leukocyte-derived cationic mediators such as the specific eosinophil-derived mediators major basic protein, eosinophilic cationic protein and eosinophil peroxidase [10].

Heparin inhibits Platelet Activating Factor (PAF) induced airway hyperresponsiveness and eosinophil infiltration in guinea pigs. Eosinophils may not be a prerequisite for the induction of airway hyperresponsiveness. Heparin can modulate allergen airway hyperresponsiveness in guinea pigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein major basic protein on M2 muscarinic receptor function on airway ganglia [10].

Platelets factor 4 (PF4) and related member of the IL-8 supergene family, RANTES are chemoattractants for eosinophils. Heparin inhibits the actions of platelets factor 4, a cationic protein shown to be released following antigen challenge in sensitized rabbits and human asthmatics [10].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, Slow Reacting Substance of Anaphylaxis (SRS-A), eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandins F<sub>2a</sub>, E<sub>2</sub>, and thromboxane A<sub>2</sub> and kallikrein of anaphylaxis [20].

In vivo and in vitro studies show heparin acts as a chemoattractant for monocytes and neutrophils [13]. Aerosolized unfractionated heparin GM1060 significantly inhibits allergen induced eosinophil infiltration into the airways of guinea pigs [11d].

Heparin and some related glycosaminoglycans can inhibit allergen induced eosinophil infiltration when directly administered to airways [11d]. Glycosaminoglycans affect endothelial cell function and polymorphonuclear leucocyte function [8].

Heparin binds the leucocyte adhesion molecule Mac-1. Heparin binds directly to neural cell adhesion molecule (N-CAM) via a heparin binding domain located on the second immunoglobulin domain [8].

Intravenous administration of various glycosaminoglycans, including heparin, causes an inhibition of leucocyte 'rolling' (a step essential for subsequent adhesion and extravasation) in the mesenteric vasculature of the rabbit; sulfation was deemed essential for this action. Negative charge is not a requisite for the inhibitory effect of glycosaminoglycans on leucocyte 'rolling'. Protamine sulphate, which is positively charged is also able to inhibit leucocyte 'rolling' [8].

Heparin binds and inhibits a variety of cytotoxic and inflammatory mediators including eosinophilic cation protein and peroxidase [12].

## Arylsulfatase

Mammalian arylsulfatases include a microsomal enzyme, arylsulfatase C, and two lysosomal enzymes, designated arylsulfatases A and B, which are widely distributed in mammalian tissues. The arylsulfatase B enzymes have reported apparent molecular weights in the range of 50,000 to 60,000 and are noncompetitively inhibited by sulfate ions, whereas arylsulfatase A enzymes are of ~100,000 mol wt and are competitively inhibited by sulfate ions [36].

The eosinophil expresses the greatest arylsulfatase activity of the various leukocytes and the enzyme has been characterized as being type B. Histochemical studies have localized arylsulfatase B to both the large crystalloid-containing granule and to a smaller granule of the eosinophil. Human eosinophil arylsulfatase B has been demonstrated to consist of four subunits, and shown to function enzymatically as an associating-dissociating oligomeric enzyme [36].

Multiple sulfatase deficiency results in accumulation of sulfogalactosylceramide, steroid sulfates, and proteoglycans owing to a combined deficiency of arylsulfatases A, B, and C and steroid sulfatase [37]. Arylsulfatase A and B are associated with mast cell rich portions of human lung and inactivate Slow Reacting Substance of Anaphylaxis (SRS-A) in a time and dose dependent fashion. Mast cells contain more arylsulfatase than eosinophils [1].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D<sub>2</sub> and the 5-lipoxygenase products leukotrienes C<sub>4</sub> and D<sub>4</sub> previously identified as slow acting substance of anaphylaxis [18].

Heparin binds directly to Neural Cell Adhesion Molecule (N-CAM) via a heparin binding domain located on the second immunoglobulin domain. Intravenous administration of various glycosaminoglycans including heparin causes an inhibition of leucocyte 'rolling' (a step essential for subsequent adhesion and extravasation) in the mesenteric vasculature of the rabbit. Sulfation was deemed essential for this action [8].

A selectively O-desulphated derivative of heparin lacks anticoagulant activity. O-desulphated heparin inhibits allergen induced eosinophil infiltration to the lungs of guinea pigs [8], however, an N-desulfated heparin failed to prevent antigen induced bronchoconstriction [11a].

An O-desulphated derivative of heparin which lacks anticoagulant activity inhibits polymorphonuclear leucocyte adhesion suggesting the inhibitory effect of glycosaminoglycans on cell adhesion is not related to the well known effect of heparin to inhibit the coagulation cascade [8].

Secretory Leukocyte Protease Inhibitor (SLPI) functions to inhibit elastase, cathepsin G and other proteases thereby protecting tissue from self-degradation by these enzymes. Protease inhibition by secretory leukocyte protease inhibitor is potentiated by the sulfated polysaccharides [9].

The heparin fraction with highest affinity for secretory leukocyte protease inhibitor contained an increased amount of undersulfated disaccharides arising from unsulfated iduronic or glucuronic residues [9].

Exoglycosidases such as  $\beta$  glucuronidase,  $\beta$  hexoaminidase,  $\beta$  galactosidase, arylsulfatase and neutral proteases disrupt the integrity of the mucous membrane through their specific actions on tissue ground substance [18].

## Prostaglandins

The eicosanoids are a diverse group of extremely powerful hormone-like molecules produced in most mammalian tissues. They mediate a wide variety of physiological processes. Examples include smooth muscle contraction, inflammation, pain perception, and blood flow regulation. Eicosanoids are also implicated in several diseases such as myocardial infarct and rheumatoid arthritis [29].

As they are generally active within the cell in which they are produced, the eicosanoids are called autocrine regulators instead of hormones. Most eicosanoids are derived from arachidonic acid, which is also called 5,8,11,14-eicosatetraenoic acid [29].

Production of eicosanoids begins after arachidonic acid is released from membrane phospholipid molecules by the enzyme phospholipase A2. The eicosanoids, which include the prostaglandins, thromboxanes, and leukotrienes, are extremely difficult to study because they are active for very short periods (often measured in seconds or minutes). In addition, they are produced in very small amounts [29].

Prostaglandins are arachidonic acid derivatives that contain a cyclopentane ring with hydroxy groups at C-11 and C-15. Molecules belonging to the E series of prostaglandins have a carbonyl group at C-9, whereas the F series molecules have an hydroxyl group at the same position. The subscript number in a prostaglandin name indicates the number of double bonds in the molecule [29].

Prostaglandins are involved in a wide range of regulatory functions. Prostaglandins promote inflammation, an infection-fighting process that produces pain and fever; they are also involved in reproductive processes (e.g., ovulation and uterine contractions during conception and labor); and they are also involved in digestion (e.g., inhibition of gastric secretion).

Prostaglandin metabolism is complex because there are many types of prostaglandins, the types and amounts of prostaglandins are different in each tissue or organ, and certain prostaglandins have opposite effects in different organs, that is, their receptors are tissue-specific. For example, several E-series prostaglandins cause smooth muscle relaxation in organs such as the intestine and uterus. The same molecules promote contraction of the smooth muscle in the cardiovascular system [29].

The thromboxanes are also derivatives of arachidonic acid. They differ from other eicosanoids in that their structures have a cyclic ether. Thromboxane A2 (TxA2), the most prominent member of this group, is primarily produced by platelets. Once released, thromboxane A2 promotes platelet aggregation and vasoconstriction.

The leukotrienes are linear (non-cyclic) derivatives of arachidonic acid whose synthesis is initiated by a peroxidation reaction. The leukotrienes differ in the position of this peroxidation step and the nature of the thioether group attached near the site of peroxidation [29].

The name leukotrienes stems from their early discovery in white blood cells (leukocytes) and the presence of a triene (three conjugated double bonds) in their structures. The term conjugated indicates that carbon-carbon double bonds are separated by one carbon-carbon single bond. The subscript in a leukotriene name indicates the total number of double bonds in the molecule [29].

Leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> have been identified as components of slow-reacting substance of anaphylaxis (SRS-A). Anaphylaxis is an unusually severe allergic reaction that results in respiratory distress, low blood pressure, and shock. During inflammation (a normal response to tissue damage) these molecules increase fluid leakage from blood vessels into affected areas [29].

LTB<sub>4</sub>, a potent chemotactic agent, attracts infection-fighting white blood cells to damaged tissue. Chemotactic agents are also referred to as chemoattractants. Other effects of leukotrienes include vasoconstriction and bronchoconstriction (both caused by the contraction of smooth muscle in blood vessels and the air passages in the lungs) and edema (increased capillary permeability that causes fluid to leak out of blood vessels) [29].

Standard asthma therapy, as defined by the management guidelines issued in 1999 by the National Heart, Lung and Blood Institute includes oral and inhaled corticosteroids, leukotriene antagonists, short acting and long acting  $\beta$ -antagonists, cromolyn and nedocromil [12]. Evidence indicates that prostaglandins participate in the pathogenesis of inflammation [14].

Interruption of the leukotriene cascade results in inhibition of exercise induced bronchoconstriction in some but not other patients [5]. Use of a 5-lipoxygenase activating protein antagonist confirmed that leukotrienes significantly contributed to the development of hyperventilation induced bronchoconstriction [13].

Heparin either inhibits or abolishes the release of eicosanoid mediators in vivo [13]. Heparin does not act as a leukotriene receptor antagonist [13].

Heparin inhibits the increased vascular permeability induced by a variety of inflammatory mediators including histamine, bradykinin, prostaglandin E<sub>1</sub>, and cationic proteins [10]. Unfractionated heparin inhibits adhesion of radiolabelled polymorphonuclear leucocytes to human umbilical vein endothelial cells pre-stimulated with interleukin 1 $\beta$  [8].

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) act by inhibiting cyclooxygenase (COX) activity and blocking downstream production of prostanoids including PGE<sub>2</sub> [16].

Rat peritoneal anaphylaxis induces the release of an arachidonic acid releasing factor that is separable from Slow Reacting Substance of Anaphylaxis (SRS-A). Prostaglandin generating factors are an important phylogenetic concomitant of anaphylaxis although the specific molecular form of the factors differs between species [20].

Prostaglandin generation/release has been considered a secondary event in anaphylaxis, thus mast cell derived histamine is visualized as stimulating surrounding tissues to induce prostaglandin generation. Other mediators such as slow reacting substance of anaphylaxis and bradykinin are also capable of causing prostaglandin formation [20].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D<sub>2</sub> and the 5-lipoxygenase products leukotrienes C<sub>4</sub> and D<sub>4</sub> previously identified as slow acting substance of anaphylaxis [18].

Analysis of the time course of the appearance of prostaglandins during human lung anaphylaxis reveals a close and parallel relationship to histamine release. Histamine acts through an H<sub>1</sub> receptor causing bronchial smooth muscle constriction which produces prostaglandin E [20].

Histamine and other mediators released from human mast cells undergoing anaphylaxis are able to induce prostaglandin generation and are found in the supernatant from lung anaphylaxis. Addition of exogenous histamine generates about 50% of the prostaglandins produced during anaphylaxis and H<sub>1</sub> histamine receptor antagonists prevent about 50% [20].

Anaphylaxis of human lung generates a novel mediator Prostaglandin Generating Factor of Anaphylaxis (PGF-A), which is capable of causing prostaglandin synthesis. A close direct relationship exists between the intensity of anaphylaxis induced as reflected by the concentration of histamine released from human lung during anaphylaxis and the quantity of prostaglandin generating factor of anaphylaxis released [20].

Prostaglandin generating factor of anaphylaxis has been described which may be partly responsible for prostaglandin generation accompanying allergic reactions. It is an oligopeptide of 1,450 daltons that induces the production of prostaglandin F<sub>α</sub> (PGF<sub>α</sub>), prostaglandin E (PGE), thromboxane B<sub>2</sub> (TxB<sub>2</sub>) and 5-, 12-, and 15-hydroxyeicosatetraenoic acid (HETE) from human lung parenchyma and airways [23].

Experimental evidence suggests that this factor is not preformed like histamine, but newly synthesized or rapidly generated by the anaphylactic event like slow reacting substance of anaphylaxis. Mediators other than histamine and PGF-A, such as SRS-A and bradykinin, have also been shown to be capable of causing prostaglandin formation [23].

Prostaglandin generating factor of anaphylaxis is a novel mediator distinct from other molecules hitherto recognized as products of human lung anaphylaxis. Bradykinin is susceptible to carboxypeptidase B degradation whereas prostaglandin generating factor of anaphylaxis is resistant [20].

Prostaglandin generation secondary to prostaglandin generating factor of anaphylaxis and other mast cell derived mediators might contribute to alterations in lung function associated with variety of conditions which involve mast cell degranulation [20].

Prostaglandin D2 release is dependent on mast cell activation. Thromboxane A2, prostaglandin D2 and Prostacyclin (PGI2) have potent effects on smooth muscle [19]. Prostaglandin D2 is a potent vasodilator causing vascular engorgement of the mucosa while leukotriene C4 increases capillary permeability causing exudation [18].

Inflammatory cells and airway tissues may release prostaglandins and other mediators which affect response to nerve stimulation [4]. Mast cell mediators, including histamine and leukotrienes have been implicated in the pathogenesis of exercise-induced asthma. H-histamine receptor antagonists as well as leukotriene D-receptor or 5-lipoxygenase inhibitors partly attenuate bronchoconstrictor responses induced by exercise or cold dry air [11a].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D2 and the 5-lipoxygenase products leukotrienes C4 and D4 previously identified as slow acting substance of anaphylaxis [18].

Prostaglandin D2 is a relatively mast cell specific product [13]. Mast cells produce prostaglandin D2 and prostacyclin but not the Prostaglandin F2 $\alpha$ , prostaglandin E, and thromboxane A2 produced by lung anaphylaxis [20].

Local prostaglandin synthesis has potent effects upon the functional characteristics of intramucosal lymphocytes. There is an intimate relationship between macrophage prostaglandin synthesis and lymphocyte function [15].

Unfractionated heparin (multiparin), low molecular weight heparin (fragmin) and the non-anticoagulant O-desulphated derivative of heparin all inhibited polymorphonuclear leucocyte adhesion to endothelial cells stimulated with interleukin 1 $\beta$ , the bacterial product lipopolysaccharide (LPS) or Tumor necrosis factor  $\alpha$  [8].

Numerous animal and human studies suggest that a variety of eicosanoids contribute to the development of Hyperventilation Induced Bronchoconstriction (HIB) [13]. Leukotriene inhibitors attenuate exercise induced bronchoconstriction [5].

Inhaled heparin inhibits eicosanoid mediator production and release caused by hyperventilation with dry air and it significantly attenuates hyperventilation induced bronchoconstriction [13].

Reduction in hyper ventilation induced airway obstruction caused by heparin is accompanied by a concomitant decrease in eicosanoid mediator concentrations. Hyperventilation induced increases in leukotriene C4 (LTC4), leukotriene E4 (LTE4) and prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) are inhibited and prostaglandin D2 (PGD2) and Thromboxane B2 (TxB2) remain at baseline levels in heparin treated/dry air challenged airways [13].

Prostaglandin formation can clearly be demonstrated during anaphylaxis of lung and vivo or in vitro. Prostaglandins may be demonstrated to influence many lung functions including smooth muscle tone, vascular permeability and tone, mucous secretion, mast cell mediator release and others [20].

Mast cell degranulation of lung by conditions other than anaphylaxis, such as hypoxia or exercise in certain asthmatics may release prostaglandin generating factor of anaphylaxis and lead to prostaglandin formation [20].

Antigen challenge of passively sensitized chopped human lung resulted in the generation of several arachidonic acid cyclo-oxygenase metabolites thromboxane A<sub>2</sub> as measured by its stable metabolite thromboxane B<sub>2</sub>, prostaglandin D<sub>2</sub>, prostacyclin as measured by its stable metabolite 6-keto-PGF<sub>1</sub>α, prostaglandin F<sub>2</sub>α and prostaglandin E [19].

All arachidonic acid cyclooxygenase metabolites were released in an antigen dose-dependent manner and reached maximal release at antigen concentrations lower than those required for maximal histamine release [19].

Prostaglandin D<sub>2</sub> and prostacyclin were predominant quantitatively in the anaphylactic reactions of human lung parenchyma and were 3 to 7 fold greater than other arachidonic acid cyclooxygenase metabolites. Thromboxane B<sub>2</sub> was generated in quantities comparable to prostaglandin E and prostaglandin F<sub>2</sub>α [19].

Rabbit antisera directed at human IgE molecules induce mediator release which in the lung result in the release or formation of histamine, slow reacting substance of anaphylaxis, eosinophil chemotactic factors of anaphylaxis, superoxide radicals, prostaglandin F<sub>2</sub>α, prostaglandin E<sub>2</sub>, and thromboxane A<sub>2</sub> and kallikrein of anaphylaxis [20].

Anaphylaxis of guinea pig lung induces the release of Rabbit Aorta Contracting Substance-Releasing Factor (RCS-RF), whereas rat peritoneal anaphylaxis induces the appearance of arachidonic acid releasing factor [20].

The prostaglandins which are generated during anaphylaxis in human and guinea pig lung are prostaglandin F<sub>2</sub>α, prostaglandin E, and thromboxane A<sub>2</sub>, and are the predominant derivatives of arachidonic acid found. A portion of the prostaglandin generation of anaphylaxis of human lung is due to histamine. Peripheral lung is relatively free of airways and responds to histamine with prostaglandin generation due to H<sub>1</sub> stimulation [20].

Evidence indicates that prostaglandins participate in the pathogenesis of inflammation. Perfusates from inflamed skin of allergic contact eczema contain nanogramme concentrations of prostaglandins. In contrast prostaglandins could not be detected by direct examination of perfusates from normal skin though ethyl acetate extraction revealed the presence of trace amounts [14].

Prostaglandins E<sub>1</sub>, E<sub>2</sub>, F<sub>1a</sub> and F<sub>2a</sub> have been isolated from inflamed skin of patients with allergic contact eczema. Prostaglandins are highly vasoactive in human skin, prostaglandin E being more potent than prostaglandin F. Intradermal injections of prostaglandin E<sub>1</sub> in concentrations as low as 10ng/ml caused pronounced erythema of a sustained quality [14].

It has been suggested that the increased concentrations of inflamed skin could be due to increased biosynthesis or alternatively prostaglandins could be released from cell membrane lipids after activation of tissue phospholipases. The presence of a pharmacologically active fatty acid with prostaglandin like properties has also been isolated from delayed cutaneous inflammation due to exposure to ultraviolet radiation [14].

Evidence suggests that prostaglandin E2 contributes to the pathogenesis of rheumatoid arthritis. Synovial fluid from patients with rheumatoid arthritis shows increases in virtually all the prostanoids. Pharmacological evidence from rheumatoid arthritis trials and preclinical models of arthritis points to the importance of cox metabolites in the pathogenesis of the disease [16].

Non Steroidal Anti-Inflammatory Drugs (NSAIDs), inhibitors of prostaglandin E2 and other prostanoids, are used to treat this disease. NSAIDs act by inhibiting cyclooxygenase (COX) activity and blocking downstream production of prostanoids including prostaglandin E2 [16].

Prostaglandin E2 is associated with some of the proinflammatory effects of rheumatoid arthritis specifically with the edema and erosion of cartilage and juxta-articular bone. There is also an association between prostanoid EP4 receptors, interleukin 6, and arthritis. Interleukin 6 deficient mice develop significantly less arthritis than do wild type controls [16].

There is enhanced thromboxane synthesis by macrophages in Crohn's disease. Prostaglandin synthesis in inflammatory bowel disease has been seen in terms of genesis of mucosal inflammation and a global increase in the synthesis of prostanoids [15].

Several studies have shown increased synthesis of prostanoids such as prostaglandin E2, prostaglandin F2a, thromboxane B2 and 6-keto PGF1 $\alpha$  (a stable hydrolysis product of prostacyclin) during relapse of ulcerative colitis. Fundamental differences from normal have not been shown in remission. Peripheral blood mononuclear cells synthesize increased amounts of prostaglandin E2 and thromboxane B2 during relapse of Crohn's disease [15].

Prostaglandin E was extracted from nasal secretions of individuals with hay fever and from nasal washings of normal subjects [17]. Prostaglandin D2 and leukotrienes C4 and B4 are released during the early reaction in parallel with histamine and a proteolytic THAME esterase activity capable of generating bradykinin [18].

Histamine, leukotriene C4, and THAME esterase are also generated during the late reaction, but the absence of prostaglandin D2 (whose release is specific for mast cells) suggests that basophils rather than mast cells are responsible for the secretion of mediators [18].

## **M2 Acetylcholine Muscarinic Receptors**

In the central nervous system muscarinic acetylcholine receptors are more abundant than nicotinic receptors. They consist of single-chain proteins of mass  $\sim 70$  kDa. They are not ion channels but are 7-helix receptors homologous in sequence with  $\beta$ -adrenergic receptors and with rhodopsin. Five different subtypes (M1–M5) have been characterized [27].

The M1, M3, and M5 receptors are coupled to the Gq/G11 family of G proteins, and M2 and M4 are coupled to Gi/Go proteins. Their effects are slower and of longer duration than those of the nicotinic receptors. It has been difficult to assign functions to the individual types. Most regions of the brain contain more than one type, but they are thought to be involved in locomotion, learning, memory, thermoregulation, and cardiac and pulmonary functions [27].

Many drugs, some of which are used in treatment of Parkinson and Alzheimer diseases, epilepsy, and asthma, affect muscarinic receptors. The M2 receptors predominate in the heart where they help to regulate the beating frequency and atrial contractility. Sudden infant death may sometimes result from a defect in muscarinic receptors [27].

Knockout mice lacking M2 receptors also have problems with movement control, body temperature, and pain responses. Mice lacking M3 receptors are lean with very low levels of serum leptin and insulin. Many of the muscarinic receptors activate adenylate cyclase, while others are coupled to the phosphoinositide cascade. Some indirectly activate K<sup>+</sup> channels [27].

In the lungs, release of acetylcholine from the vagus nerves is under the local control of inhibitory muscarinic autoreceptors on the postganglionic nerves. Acetylcholine released from the vagus nerve stimulates both M3 muscarinic receptors on airway smooth muscle, causing contraction and bronchoconstriction, and M2 muscarinic receptors on the nerves, decreasing further release of acetylcholine [26].

These neuronal M2 receptors are present in most species studied, including humans. In the guinea pig, the function of these inhibitory receptors is markedly impaired after acute viral infection, acute ozone exposure, and antigen challenge of sensitized animals [26].

Loss of function of inhibitory M2 receptors is characterized by airway hyperresponsiveness to electrical stimulation of the vagus nerves. Furthermore, airway hyperresponsiveness to histamine in antigen challenged guinea pigs is due to increased vagally mediated reflex bronchoconstriction as a result of M2 receptor dysfunction. Airway M2 receptors are dysfunctional in some, but not all, patients with asthma [26].

Antigen induced hyperresponsiveness is associated with increased release of acetylcholine from vagus nerves [4]. Inhibitory M2 muscarinic receptors control release of acetylcholine from human pulmonary parasympathetic nerves. In asthmatics the M2 acetylcholine muscarinic receptors do not function [4][6].

Heparin restores M2 receptor function by binding to and neutralizing major basic protein and polycationic substances [4]. Heparin reverses allergen induced inhibitory M2 receptor dysfunction [6]. Heparin's inhibition of bronchoconstriction is co-incident with return to normal of M2 receptor function [4].

Heparin can modulate allergen airway hyperresponsiveness in guineapigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein Major Basic Protein (MBP) on M2 receptor function on airway ganglia [10]. Heparin failed to inhibit acetylcholine induced tracheal smooth muscle contraction in tissue culture [7]. Inhaled heparin inhibits methacholine induced bronchoconstriction and is suggested to act directly on airway smooth muscle [7][13].

Positive correlations have been made between post-hyperventilation Broncho-Alveolar Lavage Fluid (BALF) prostanoid and epithelial cell concentrations. Hyperventilation induced bronchoconstriction is prevented in the presence of eicosanoid and muscarinic receptor blockade [40].

Muscarinic cholinergic signaling plays an essential role in the control of the normal airway functions and in the development of pulmonary pathologies including asthma. The airways of mice deficient in a cAMP-specific phosphodiesterase (PDE4D) were markedly resistant to the development of methacholine induced Airway Hyper-Responsiveness (AHR) because of reduced muscarinic receptor function. Airway hyperreactivity that follows exposure to antigen is also abolished in PDE4D2/2 mice, despite an apparently normal lung inflammatory infiltration [41].

The loss of cholinergic responsiveness was specific to the airway, not observed in the heart, and was associated with a loss of signaling through muscarinic receptors with an inability to decrease cAMP accumulation. Phosphodiesterase thus plays an essential role in cAMP homeostasis and cholinergic stimulation of the airway. It also plays an important role in the development of hyperreactivity [41].

Many of the events and mechanisms involved in asthma pathogenesis are inhibited by the activation of the cyclic nucleotide-signaling pathway. An increase in intracellular cAMP interferes with lymphocyte, eosinophil, and mast cell activation, and blocks cytokine production, cell replication, and cell chemotaxis to sites of inflammation [41].

Activation of the cAMP signaling pathway in airway smooth muscle cells promotes relaxation and blocks smooth muscle cell replication, thus preventing the airway remodeling observed in chronic asthmatic patients. Genetic studies show that defective  $\beta_2$ -adrenergic receptor signaling, which results in reduced cAMP levels, is associated with increased airway hyperresponsiveness and nocturnal asthma [41].

Similarly, aberrant enhanced expression of phosphodiesterases (PDEs), which degrade and inactivate cAMP, may also be associated with atopy, although this concept has been recently challenged. In line with the inhibitory function of cAMP, inhibitors of phosphodiesterases block many of the symptoms of asthma by increasing cAMP levels in the airway and in inflammatory cells [41].

## Heparin

An increase in levels of endogenous "heparin-like material" has been found in allergic patients plasma [7][10]. Heparin is an endogenous glycosaminoglycan widely known for its anticoagulant properties [12]. Heparin has multiple non-anticoagulant properties which include interaction with various growth factors; regulation of cellular proliferation and angiogenesis, modulation of proteases and enzymes [5][7] including regulation of mast cell tryptase [7]. Inhaled heparin possesses anti-inflammatory and immunoregulatory properties [5].

Heparin has been studied as an anti-inflammatory agent [3][6][7][13]. Both heparin and low molecular weight heparinoid Org 10172 possess anti-inflammatory activity in the lung and the ability to inhibit Platelet Activating Factor (PAF)-induced airway hyperresponsiveness in the rabbit [10].

Derivatives of heparin lacking anticoagulant activity inhibit inflammatory processes such as delayed hypersensitivity reactions [8]. The antiallergic activity of inhaled heparin is independent of its anticoagulant properties [5].

Heparin possesses anti-inflammatory properties which are dependent on the dose, timing and route of administration in animal studies [11b]. Studies date back to the 1910s reporting that heparin inhibits anaphylaxis [7]. Van de Carr and Williams demonstrated in 1928 heparin could modulate allergic responses in the skin and respiratory system [10][7].

Heparin inhibits the increased vascular permeability induced by a wide range of agonists acting via specific receptors located on the vascular endothelial cells [7]. Heparin inhibits f-methionine-leucyl-phenylalanine (fMLP) activated neutrophil adhesion to resting endothelial cells [8].

Heparin inhibits activation of the complement system, neutrophil chemotaxis, modulate T cell function [13] and eosinophil infiltration [10][12][7][13].

Heparin can modulate allergen airway hyperresponsiveness in guineapigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein MBP on M2 receptor function on airway ganglia [10].

It has been suggested that heparin is a natural "anti-asthmatic" molecule [7] and as a potential anti-asthma therapy [11b]. Aerosolized heparin may be helpful in alleviating symptoms of asthma although no definitive bronchodilating activity has been observed [11a]. Heparin has no bronchodilating effect suggesting it does not act directly on baseline airway smooth muscle tone [13].

The effect of heparin on antigen challenge is reported to be equivocal [7]. Heparin does not inhibit oedema formation [3]. Heparin may modulate smooth muscle growth [13] and may also act directly on smooth muscle [7].

Heparin is biosynthesized in mast cells as a proteoglycan consisting of a central core protein from which multiple glycosaminoglycan chains extend. On isolation and purification from lung tissue, heparin is released from its protein core and isolated as a glycosaminoglycan [9].

Glycosaminoglycan heparin is a polydisperse, highly sulphated, linear polysaccharide comprised of repeating 1-4 linked uronic acid and glucosamine residues with a molecular weight range of 5,000 - 40,000 and an average molecular weight of 14,000. Low molecular weight heparin has an average weight of 5,000 and is prepared from heparin by chemical or enzymatic treatment as a clinical antithrombotic agent [9].

Heparin is found in the granules of mast cells which are present in substantial quantities in the human lung [9]. Elevated levels of heparin-like anticoagulants have been demonstrated in atopic asthmatic patients, and have been induced in some patients after antigen challenge [12]. Heparin can bind many substances which can either inactivate or enhance their effects [3].

Many biological actions of heparin, are molecular weight dependent. Anti-allergic activity of fractionated heparins is molecular weight dependent and an inverse relationship between molecular weight and the anti-allergic activity was observed. Both the degree of sulfation and molecular chain length influence the anticoagulant, antiproliferative and elastase inhibitory activity of heparin [5].

A selectively O-desulphated derivative of heparin lacks anticoagulant activity. Partially desulphated derivatives of heparin were able to inhibit non-activated neutrophil adhesion to endothelial cells stimulated by Platelet Activating Factor (PAF) or thrombin. Inhibition was observed only when glycosaminoglycans were present in the neutrophil suspension and not when added to the endothelial cells [8].

The anti-inflammatory activity of heparin is not related to its anticoagulant properties. Inhaled heparin has been shown not to prolong plasma partial thromboplastin time [7].

The anti-asthmatic activity of heparin is thought not to be due to its anticoagulant properties, as it did not prolong the partial thromboplastin time measured one hour or three hours after inhalation. The administration of heparin to sheep failed to change the partial thromboplastin time for up to 12 hours after inhalation [11a].

Heparin did not prolong the partial thrombin time, bleeding time or coagulation time during experiments with Dry Air Challenge (DAC) [13]. It has been suggested the greater potency of Enoxaparin (a low molecular weight heparin) is related to the presence of a higher percentage of oligosaccharide chains possessing anti-allergic activity [5].

Heparin attenuated antigen induced bronchoconstriction in a dose dependent manner [7]. Heparin's anti-inflammatory activity is a time-dependent phenomenon [13].

Heparin binds directly to Neural Cell Adhesion Molecule (N-CAM) via a heparin binding domain located on the second immunoglobulin domain. Heparin also binds the leucocyte adhesion molecule Mac-1 [8].

Intravenous administration of various glycosaminoglycans including heparin causes an inhibition of leucocyte 'rolling' (a step essential for subsequent adhesion and extravasation) in the mesenteric vasculature of the rabbit (sulfation was deemed essential) [8].

Heparin and two related compounds can inhibit the adhesion of Poly-Morpho-Nuclear leucocytes (PMNs) to endothelial cells induced by a variety of stimuli via a mechanism which doesn't involve inhibition of intercellular Adhesion Molecule 1 (ICAM-1) or E-selectin expression on endothelial cells, involvement of polyanionic nature, involvement of anticoagulant actions and involves the interference with the adhesive processes of both neutrophils and endothelial cells [8].

Heparin's ability to inhibit airway responsiveness is unlikely to be related solely to its highly anionic nature as similar effects were not observed with the linear anionic molecule polyglutamic acid. Heparin and Org 10172 inhibits the infiltration of inflammatory cells into the airways following Platelet Activating Factor-challenge but this action is not dependent on this anti-inflammatory effect as polyglutamic acid substantially inhibits cell infiltration without the associated airway hyperresponsiveness [10].

Heparin's flexible structure and high anionic charge allow heparin to interact with a variety of molecules *in vivo* [12]. Heparin increases inhibition of chymotrypsin by Secretory Leukocyte Protease Inhibitor (SLPI). Secretory leukocyte protease inhibitor functions to inhibit elastase, cathepsin G and other proteases thereby protecting tissue from self degradation by these enzymes [9].

The heparin fraction with highest affinity of Secretory leukocyte protease inhibitor contained an increased amount of undersulfated disaccharides arising from unsulfated iduronic or glucuronic acid residues. Heparin fractions binding most tightly to secretory leukocyte protease inhibitor are enriched in unsulfated iduronic and glucuronic acid residues [9].

The effect of heparin on the K-alpha of the protease interaction, particularly human lung elastase suggests its therapeutic application to elastase, based diseases of the lung such as asthma. 10mg SLPI and 16.7mg heparin provided protection against the development of hyperresponsiveness [9].

Heparin binds and inhibits a variety of cytotoxic and inflammatory mediators including eosinophilic cation protein and peroxidase. Heparin increases the association rate of secretory leukocyte protease inhibitor with human neutrophil elastase and cathepsin G reducing their activity [12].

Heparin protects tissues against damage induced by cationic leukocyte-derived cationic mediators such as the specific eosinophil-derived mediators Major Basic Protein (MBP), eosinophilic cationic protein and eosinophil peroxidase [10].

Cationic peroxidases such as major basic protein and eosinophil peroxidase are neutralized by the highly anionic heparin [7]. Heparin inhibits the increased vascular permeability induced by a variety of inflammatory mediators including histamine, bradykinin, prostaglandin E1, and cationic proteins [10].

Heparin selectively inhibits the anti-IgE-induced release of histamine from human uterine mast cells without altering the effects of calcium ionophore A2318720. Heparin possesses multiple non-anticoagulant properties which include modulation of various proteases, anticomplement activity and anti-inflammatory action as well as inhibition of cell growth [11a].

Heparin regulates the activity of mast cell tryptase [7]. Heparin attenuates antigen-induced bronchoconstriction in sheep and humans with asthma and can inhibit histamine release from isolated human mast cells [11a].

Heparin can bind histamine. The ability of heparin and Org 10172 heparinoid to inhibit airway responsiveness to histamine following Bovine Serum Albumin (BSA) challenge suggests an action not attributable merely to the ability to bind histamine [10].

Heparin inhibits both exercise and allergen-induced bronchoconstriction possibly by altering inositol triphosphate (IP3) levels [10]. Heparin is a specific blocker of inositol triphosphate (IP3) mediated calcium release in various cell types. Heparin binds to inositol triphosphate receptors and inhibits inositol triphosphate induced release of calcium in various tissues including airway smooth muscle [13].

Heparin blocks Inositol 1,4,5-triphosphate (IP3) receptors and inositol triphosphate mediated calcium release in vascular and airway smooth muscle [7]. Heparin (in vitro) binds to inositol triphosphate receptors and inhibits the inositol triphosphate induced release of calcium in various tissues, including vascular and airway smooth muscle, the cerebellum and the liver [11a].

Inhibition of platelet activating factor-induced airway hyperresponsiveness and cell infiltration by heparin cannot be explained by heparin acting merely as a platelet activating factor antagonist [10]. Heparin was more effective than cromolyn in preventing post-exercise bronchoconstriction. Inhaled heparin does not act as an antagonist of mediator receptors [11a].

Heparin protects against the lethal effects of compound 48/80 which releases histamine, and also prevents mast cell degranulation induced by this compound in the subcutaneous tissue of mice [11a]. Heparin inhibits bronchoconstriction responses to compound 48/80 [7]. Heparin interacts and inhibits compound 48/80, antigen and bradykinin [3].

Heparin attenuates the effect of compound 48/80 and antigen induced acute bronchoconstrictor responses in sheep without modifying the effects of histamine. An N-desulfated heparin however failed to prevent antigen induced bronchoconstriction [11a].

Heparins inhibition of bronchoconstriction is co-incident with return to normal of M2 receptor function [4][6]. Heparin restores M2 receptor function by binding to and neutralizing major basic protein and polycationic substances [4].

Inhaled heparin inhibits methacholine-induced bronchoconstriction and is suggested to act directly on airway smooth muscle [7][13]. Heparin failed to inhibit acetylcholine induced tracheal smooth muscle contraction in tissue culture [7].

Heparin can modulate allergen airway hyperresponsiveness in guineapigs via a mechanism related to reversing the effects of the eosinophil derived cationic protein Major Basic Protein on M2 acetylcholine muscarinic receptor function on airway ganglia [10].

Inhaled heparin inhibits eicosanoid mediator production and release caused by hyperventilation with dry air and significantly attenuates Hyperventilation Induced Bronchoconstriction (HIB) [13].

Unfractionated heparin preparation inhibits adhesion of unstimulated polymorphonuclear leukocytes to endothelial cells stimulated with cytokines (IL-1 $\beta$  or TNF $\alpha$ ) or the bacterial product lipopolysaccharide (LPS). Heparin in a polymorphonuclear leucocytes suspension reduced adhesion of these cells to activated Human Umbilical Vein Endothelial Cells (HU-VECs) stimulated with cytokines and lipopolysaccharide [8].

Inhalation of heparin attenuates hyperventilation induced bronchoconstriction. Reduction in hyperventilation induced airway obstruction caused by heparin is accompanied by a concomitant decrease in eicosanoid mediator concentrations. Heparin inhibits hyperventilation induced bronchoconstriction via the inhibition of either hyperventilation induced eicosanoid production or release [13].

Heparin does not act as a leukotriene receptor antagonist. Heparin either inhibits or abolishes the release of eicosanoid mediators in vivo. Hyperventilation induced increases in Leukotriene C4 (LTC<sub>4</sub>), Leukotriene E<sub>4</sub> (LTE<sub>4</sub>) and Prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\alpha$ ) are inhibited and Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) and Thromboxane B<sub>2</sub> (TxB<sub>2</sub>) remain at baseline levels in heparin treated/dry air challenged airways [13].

Heparin is actively taken up by endothelial cells. Heparin displaces the binding of chemokines to proteoglycans on endothelial surfaces [3]. Low molecular weight heparins have a lower binding affinity to endothelial cells and reduced non-renal cellular mechanism of clearance, resulting in increased bioavailability and prolonged in vivo biological activity [5].

The actions of heparin involve the interference with the adhesive processes of both neutrophils and endothelial cells [8]. The recognition of the presence of high concentrations of heparin in preformed cytoplasmic mast cell granules in endobronchial tissue first led to the speculation about its involvement in airway inflammation [12].

Inhaled heparin prevents antigen-induced bronchoconstriction and airway responsiveness by modulation of mast cell mediator release [5][6]. Heparin attenuates anti-IgE induced mast cell degranulation in vitro [5].

Heparin's inhibition of type 1 hypersensitivity reactions in allergic sheep is suggested to be via an inhibitory effect on mast cell degranulation [10]. Heparin attenuates antigen-induced bronchoconstriction in sheep and humans with asthma and also inhibits histamine release from isolated human mast cells. Anti-allergic actions of heparin may be related to the inhibition of mediator release from mast cells [11a].

Heparin selectively inhibited the anti-IgE-induced release of histamine from human uterine mast cells without altering the effects of calcium ionophore A2318720. By increasing the preincubation period of heparin in vitro from 20 to 60 minutes, the protective effect of heparin on anti-IgE-mediated histamine release and mast cell membranes was increased by 50% [11a].

During incubation, heparin may bind to mast cell membranes and may then become internalized - an active uptake of intratracheally administered heparin by mast cells has been observed [11a]. Heparin attenuates allergic bronchoconstriction in sheep, inhibits anti-IgE mediated histamine release in isolated mast cells and prevents the bronchoconstrictor response in subjects with exercise induced asthma [11e].

Heparin inhibits rat peritoneal mast cell degranulation in vitro. Heparin inhibits anti-immunoglobulin E induced histamine release from isolated human uterine mast cells. Heparin inhibits histamine release in vitro and prevents mast cell degranulation. Heparin's anti-allergic actions may be related to inhibition of mediator release from mast cells [13].

Heparin inhibits lymphocyte activation and limits the injurious effect of major basic protein [6]. Heparin can inhibit lymphocyte activation, trafficking and delayed hypersensitivity responses which are lymphocyte driven [7][12][10].

In vivo and in vitro studies show heparin acts as a chemoattractant for monocytes and neutrophils [13]. Heparin effects eosinophils and neutrophils [7]. Heparin inhibits neutrophil chemotaxis, smooth muscle growth and vascular tone [12].

The unfractionated heparin preparation multiparin, the low molecular weight heparin preparation Fragmin and the non-anticoagulant O-desulphated heparin inhibited the adhesion of polymorphonuclear leukocytes (PMNs) stimulated with formyl methionine leucyl phenylalanine (fMLP) to unstimulated human umbilical vein endothelial cells (HUVECs). Unfractionated heparin inhibits adhesion of radiolabelled polymorphonuclear leucocytes to human umbilical vein endothelial cells pre-stimulated with interleukin 1 $\beta$  [8].

Heparin in a polymorphonuclear leucocyte suspension reduced adhesion of these cells to activated HUVECs stimulated with cytokines and lipopolysaccharide. Co-incubation of heparin with stimulated endothelial cells inhibits adhesion [8].

Intravenous administration of various glycosaminoglycans including heparin causes an inhibition of leucocyte 'rolling' (a step essential for subsequent adhesion and extravasation) in the mesenteric vasculature of the rabbit; sulfation was deemed essential for the inhibition [8].

An O-desulphated derivative of heparin, which lacks anticoagulant activity, inhibits polymorphonuclear leucocyte adhesion suggests the inhibitory effect of glycosaminoglycans on cell adhesion is not related to the well know effect of heparin to inhibit the coagulation cascade [8].

Heparin and two related compounds can inhibit the adhesion of polymorphonuclear leukocytes (PMNs) to endothelial cells induced by a variety of stimuli. Heparin inhibits platelet activating factor induced airway hyperresponsiveness and eosinophil infiltration in guinea pigs [10].

Platelets factor 4 (PF4) and related member of the IL-8 supergene family, RANTES are chemoattractants for eosinophils. Heparin inhibits the actions of platelets factor 4, a cationic protein shown to be released following antigen challenge in sensitized rabbits and human asthmatics [10].

Aerosolized unfractionated heparin GM1060 significantly inhibits allergen induced eosinophil infiltration into the airways of guinea pigs. Glycosaminoglycans chondroitin sulphate A, chondroitin sulphate C and heparin sulphate influence the extent of allergen induced eosinophil infiltration into Broncho-Alveolar Lavage Fluid (BAL) [11d].

Heparin tends to reduce the number of epithelial cells recovered in bronchoalveolar lavage fluid after dry air challenge [13]. Heparin and some related glycosaminoglycans can inhibit allergen induced eosinophil infiltration when directly administered to airways [11d]. Heparin can inhibit tumour metastasis [3].

Several studies in the 1960s first reported subjective improvement in asthmatic symptoms with use of intravenous heparin. Bardana et al performed the first trial of inhaled heparin reporting subjective but not objective improvements [12].

Heparin decreased the response to vagally induced bronchoconstriction in sensitized, challenged animals [4]. Inhaled heparin attenuates antigen-induced bronchoconstriction in allergic sheep as well as prevents bronchoconstrictor response to exercise and antigen in asthmatic subjects [5].

Heparin attenuates allergic bronchoconstriction in sheep, inhibits anti-IgE mediated histamine release in isolated mast cells and prevents the bronchoconstrictor response in subjects with exercise induced asthma. Heparin offers greater protection against exercise induced asthma than cromolyn. Heparin prevents exercise induced asthma for up to 3 hours [11c].

Inhaled heparin attenuates acute bronchoconstrictor response to exercise. Heparin attenuates post exercise decrease of the specific airway resistance [5][7]. Heparin attenuates hyperventilation induced bronchoconstriction in asthmatics and acute antigen-induced bronchoconstriction in sheep without modifying the effects of histamine [13]. Inhaled low molecular weight heparin, enoxaparin attenuates exercise-induced bronchoconstriction in a dose dependent manner. Heparin completely, or partially inhibited exercise induced bronchoconstriction in 75% of subjects [5].

Heparin prevents exercise induced asthma but not histamine induced bronchoconstriction [7][11a][13]. Inhaled heparin inhibits early response to allergen and exercise induced bronchoconstriction in subjects with asthma [6]. Inhaled heparin inhibits hyperventilation induced bronchoconstriction in humans [13].

Heparin attenuates bronchoconstrictor response and immediate cutaneous reaction to antigen in allergic sheep [7]. Combination therapy of heparin and secretory leukocyte protease inhibitor largely eliminates late phase bronchoconstriction in sheep [9]. Heparin may act directly on smooth muscle [7].

Unfractionated heparin and the low molecular weight heparinoid Org 10172 can inhibit airway hyperresponsiveness induced by aerosolized platelet activating factor in neonatally immunized adult rabbits [10]. Inhaled heparin prevents allergic bronchoconstriction in sheep and inhibits anti-IgE-mediated release of histamine from mast cells in vivo [11a].

Heparin attenuates antigen-induced bronchoconstriction in sheep and humans with asthma and can inhibit histamine release from isolated human mast cells. Heparin attenuates antigen induced bronchoconstriction in sheep and human subjects with asthma and also inhibits histamine release from blood cells induced by antigen, trypsin and other proteases.

Inhaled heparin prevents post-exercise bronchoconstriction in subjects with exercise induced asthma. Heparin was more effective than cromolyn in preventing post exercise bronchoconstriction [11a]. Inhaled heparin preserves specific airway conductance (sGaw) in exercise induced asthma better than did 20 mg inhaled cromolyn. Inhaled heparin was superior in preserving specific airway conductance in exercise induced asthma when administered up to 3 hours prior to exercise [12]. Heparin inhibits bronchoconstriction by inhaled dust mite extract in allergic asthmatics [7].

In asthma, heparin in a single dose reduced early asthmatic response to inhaled allergen (house dust mite extract). Inhaled heparin reduces late asthmatic response in asthmatic subjects [11b].

Pre-treatment with 1000 U/kg heparin attenuated the increase in the lung resistance of previously sensitized sheep by 91% on allergen exposure [12]. Inhaled heparin attenuates antigen-induced bronchoconstriction and airway hyperresponsiveness in sheep. Heparin attenuates methacholine-induced bronchoconstriction in asthmatics [13]. O-desulphated heparin inhibits allergen induced eosinophil infiltration to the lungs of guinea pigs [8].

Heparin inhibits the acute cutaneous reaction and bronchoconstriction due to allergens in allergic subjects [7].

## Glycosaminoglycans

Glycosaminoglycans are made of disaccharide repeating units containing a derivative of an amino sugar, either glucosamine or galactosamine. At least one of the sugars in the repeating unit has a negatively charged carboxylate or sulfate group. Chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, dermatan sulfate, and hyaluronate are the major glycosaminoglycans [42].

Glycosaminoglycans can be anti-inflammatory [8]. The acetylated amino sugars N-acetyl-D-glucosamine and N-acetyl-D-Galactosamine are often encountered as components of glycoproteins. N-acetylneuraminic acid (sialic acid), is a characteristic component of glycoproteins. Other acidic monosaccharides such as D-glucuronic acid, D-galacturonic acid, and iduronic acid, are typical constituents of the glycosaminoglycans found in connective tissue [44].

Glycosaminoglycans are usually attached to proteins to form proteoglycans. Heparin is synthesized in a non-sulfated form, which is then deacetylated and sulfated. Incomplete modification leads to a mixture of variously sulphated sequences. Some of them act as anticoagulants by binding specifically to antithrombin, which accelerates its sequestration of thrombin. Heparan sulfate is like heparin except that it has fewer N- and O-sulfate groups and more acetyl groups [42].

Proteoglycans resemble polysaccharides more than proteins in as much as the carbohydrate makes up as much as 95% of the biomolecule by weight. Proteoglycans function as lubricants and structural components in connective tissue, mediate adhesion of cells to the extracellular matrix, and bind factors that stimulate cell proliferation [42].

Glycoproteins contain oligosaccharides attached to the protein either through O-glycosidic linkages with hydroxyl groups of side chains of serine, threonine, hydroxyproline, or hydroxylysine (O-linked) or via glycosylaminyl linkages to asparagine side chains (N-linked). The “core proteins” of the proteoglycans carry long polysaccharide chains, which are usually O-linked and are usually described as glycosaminoglycans [43].

Other glycosaminoglycans, including sulfate esters of chondroitin, dermatan, keratan, heparan, and heparin, grow at their nonreducing ends. Their synthesis is usually initiated by the hydroxyl group of serine or threonine side chains at special locations within several secreted proteins. These proteins are synthesized in the rough Endoplasmic Reticulum (ER) and then move to the Golgi [43].

Addition of the first sugar ring begins in the endoplasmic reticulum with transfer of single xylosyl residues to the initiating –OH groups. This reaction is catalyzed by the first of a group of special glycosyltransferases of high specificity that form the special terminal units, that anchor the alternating polysaccharide [43].

After transfer of the xylosyl residue from UDP-xylose to the –OH group in the protein, a second enzyme with proper specificity transfers a galactosyl group from UDP-galactose, joining it in  $\beta$ -1,4 linkage. A third enzyme transfers another galactosyl group onto the first one in  $\beta$ -1,3 linkage [43].

A fourth enzyme, with a specificity different from that used in creating the main chain, then transfers a glucuronosyl group from UDP-glucuronic acid onto the chain terminus to complete the terminal unit. Then two more enzymes transfer the alternating units in sequence to form the repeating polymer with lengths of up to 100 or more monosaccharide residues [43].

Subsequent modifications of the polymers involve extensive formation of O-sulfate esters, N-deacetylation and N-sulfation, and epimerization at C5. In some tissues almost all GluA is epimerized. The modifications are especially extensive in dermatan, heparan sulfates, and heparin. The modifications are not random and follow a defined order [43].

N-Deacetylation must precede N-sulfation, and O-sulfation is initiated only after N-sulfation of the entire chain is complete. The modifications occur within the Golgi but not all of the glycosyltransferases, PAPS (3'-phosphoadenosine 5'-phosphosulfate)-dependent sulfotransferases, and epimerases are present within a single compartment. Nevertheless, an entire glycosaminoglycan chain can be synthesized within 1–3 min [43].

The completed polymers are modified uniformly. One of the best known modifications forms the unique pentasaccharide sequence, which is essential to the anticoagulant activity of heparin. This sequence has been synthesized in the laboratory as have related longer heparin chains. A sequence about 17 residues in length containing an improved synthetic version of the unique pentasaccharide binds tightly to both thrombin and antithrombin [43].

Heparan sulfate chains are found on proteoglycans throughout the body, but the highly modified heparin does not circulate in the blood. It is largely sequestered in cytoplasmic granules within mast cells and is released as needed. Heparin binds to many different proteins. Among them is the glycoprotein selenoprotein P, which may impart antioxidant properties to the extracellular matrix [43].

Although glycosaminoglycans are most often attached to O-linked terminal units, chondroitin sulfate chains can also be synthesized with N-linked oligosaccharides attached to various glycoproteins serving as initiators. At least one form of keratan sulfate, found in the cornea, is linked to its initiator protein via GlcNAc-Man to N-linked oligosaccharides of the type present in many glycoproteins [43].

At least 25 different proteins that are secreted into the extracellular spaces of the mammalian body carry glycosaminoglycan chains. Most of these proteins can be described as small leucine-rich proteoglycans with 36- to 42-kDa protein cores and large modular proteoglycans whose protein cores have molecular masses of 40 to 500 kDa [43].

The most studied of the large modular proteoglycans is aggrecan, a major component of cartilage. This 220-kDa protein carries ~100 chondroitin chains, each averaging about 100 monosaccharide residues and ~100 negative charges from the carboxylate and sulfate groups. Aggrecan has three highly conserved globular domains near the N and C termini. The G1 domain near the N terminus is a lectin, which, together with a small link protein that is structurally similar to the G1 domain, binds to a decasaccharide unit of hyaluronan [43].

One hyaluronan molecule of 500- to 1000-kDa mass (~2500–5000 residues) may bind 100 aggrecan and link molecules to form an ~200,000-kDa particle. These enormous highly negatively charged molecules, together with associated counter-ions, draw in water and preserve osmotic balance. It is these molecules that keep our joints mobile and which deteriorate by proteolytic degradation in the common osteoarthritis [43].

The keratan sulfate content of cartilage varies with age, and the level in serum and in synovial fluid is increased in osteoarthritis. Keratan sulfate is also found in the cornea and the brain. Its content is dramatically decreased in the cerebral cortex of patients with Alzheimer disease. Other modular core proteins include versican of blood vessels and skin, neurocan and brevican of brain, perlecan of basement membranes, agrin of neuromuscular junctions, and testican of seminal fluid [43].

A number of these have a broader distribution. The sizes vary from 44 kDa for testican to greater than 400 kDa for perlecan. The numbers of glycosaminoglycan chains are smaller than for aggrecan, varying from 1 to 30. Another of the chondroitin sulfate-bearing core protein is appican, a protein found in brain and one of the splicing variants of the amyloid precursor protein that gives rise to amyloid deposits in Alzheimer disease [43].

The core proteins of the leucine-rich proteoglycans have characteristic horseshoe shapes and are constructed from ~ 28-residue repeats, each containing a  $\beta$ -turn and an  $\alpha$ -helix. A major function of these proteoglycans seems to be to interact with collagen fibrils, which have distinct proteoglycan-binding sites, and also with fibronectin [43].

The small leucine-rich proteoglycans have names such as biglycan, decorin, fibromodulin, lumican, keratoglycan, chondroadherin, osteoglycin, and osteoadherin. The distribution varies with the tissue and the stage of development. For example, biglycan may function in early bone formation; decorin, which has a high affinity for type I collagen, disappears from bone tissue as mineralization takes place. Osteoadherin is found in mature osteoblasts. Phosphocan, another brain proteoglycan, has an unusually high content (about one residue per mole) of L-isoaspartyl residues [43].

Proteoglycans bind to a variety of different proteins and polysaccharides. For example, the large extracellular matrix protein tenascin, which is important to adhesion, cell migration, and proliferation, binds to chondroitin sulfate proteoglycans such as neurocan. Syndecan, a transmembrane proteoglycan, carries both chondroitin and heparan sulfate chains, enabling it to interact with a variety of proteins that mediate cell-matrix adhesion [43].

The anti-inflammatory effects of glycosaminoglycans are as yet unexplained [8]. As mentioned, heparin is an endogenous glycosaminoglycan widely known for its anticoagulant properties [12].

Heparin is biosynthesized in mast cells as a proteoglycan consisting of a central core protein from which multiple glycosaminoglycan chains extend [9]. Two subpopulations of mast cells located in the mucosa and connective tissue have different morphological, histochemical, functional and pharmacological characteristics [18].

On isolation and purification from lung tissue, heparin is released from its protein core and isolated as a glycosaminoglycan. Glycosaminoglycan heparin is a polydisperse, highly sulphated, linear polysaccharide comprised of repeating 1-4 linked uronic acid and glucosamine residues with a molecular weight range of 5,000 - 40,000 and an average molecular weight of 14,000 [9].

Low molecular weight heparin has an average weight of 5,000 and is prepared from heparin by chemical or enzymatic treatment as a clinical antithrombotic agent [9]. Exoglycosidases such as  $\beta$  glucuronidase,  $\beta$  hexoaminidase,  $\beta$  galactosidase, arylsulfatase and neutral proteases disrupt the integrity of the mucous membrane through their specific actions on tissue ground substance, a ubiquitous glycosaminoglycan [18].

The granule proteoglycan of mucosal mast cells differs from classical heparin in containing glycosaminoglycan chains which are less sulphated (chondroitin sulphate di-B). The main neutral protease of human connective tissue is tryptase and the main proteoglycan is a smaller molecular weight species of heparin [18].

The activation of mast cells by IgE leads to the release of preformed mediators including histamine, exoglycosides, the neutral protease tryptase, and eosinophil and neutrophil chemotactic factors [18]. Heparin possesses multiple non-coagulant properties including anti-inflammatory and anti-complement activity, modulation of proteases and regulation of mast cell tryptase [7].

Numerous serine proteases, including trypsin-like enzymes called tryptases and chymotrypsin like chymases, are found within tissues in which they are stored in granules of mast cells, neutrophils, lymphocytes, and cytotoxic T cells [45].

Secretory granules of mast cells present in skin and other tissues contain high concentrations of tryptase and chymase precursors which may be released as part of an inflammatory response. Tryptase may be involved in asthma and other allergic responses. Many secreted proteins, as well as smaller peptide hormones, are acted upon in the endoplasmic reticulum by tryptases and other serine proteases. They often cut between pairs of basic residues such as KK, KR, or RR [45].

A range of glycosaminoglycans inhibit the trafficking of inflammatory cells into the airway in vivo induced by both Platelet Activating Factor (PAF) and antigen/antibody interactions. Heparin and partially desulphated derivatives of heparin non-activated neutrophil adhesion to endothelial cells stimulated by platelet activating factor or thrombin. Inhibition was observed only when glycosaminoglycans were present in the neutrophil suspension and not when added to the endothelial cells [8].

Glycosaminoglycans affect endothelial cell function and polymorphonuclear leucocyte function. Intravenous administration of various glycosaminoglycans including heparin causes an inhibition of leucocyte 'rolling' (a step essential for subsequent adhesion and extravasation) in the mesenteric vasculature of the rabbit. Sulfation was deemed essential for this inhibition [8].

Negative charge is not a requisite for the inhibitory effect of glycosaminoglycans on leucocyte 'rolling' as protamine sulphate, which is positively charged is also able to inhibit leucocyte 'rolling' [8].

Heparin and some related glycosaminoglycans can inhibit allergen induced eosinophil infiltration when directly administered to airways. Glycosaminoglycans chondroitin sulphate A, chondroitin sulphate C and heparin sulphate influence the extent of allergen induced eosinophil infiltration into Broncho-Alveolar Lavage Fluid (BALF) [11d].

## **Eczema**

The term 'eczema' derives from the Greek word for 'boiling', which reflects that the skin can become so acutely inflamed that fluid weeps out or vesicles appear. It is synonymous with the term dermatitis and the two words are interchangeable. In the developed world eczema accounts for a large proportion of skin disease, both in hospital and community-based populations [46].

It is estimated that 10% of people have some form of eczema at any one time, and up to 40% of the population will have an episode of eczema during their lifetime. All eczemas have some features in common and there is a spectrum of clinical presentation from acute through to chronic [46].

Eczema is nearly always itchy. In subacute eczema the skin can be erythematous, dry and flaky, oedematous, and crusted (especially if secondarily infected). Chronic persistent eczema is characterized by thickened or lichenified skin. Vesicles or bullae may appear in the acute stage if inflammation is intense [46].

Histologically 'eczematous change' refers to a collection of fluid in the epidermis between the keratinocytes ('spongiosis') and an upper dermal perivascular infiltrate of lymphohistiocytic cells. In more chronic disease there is marked thickening of the epidermis ('acanthosis') [46].

**Atopic eczema** This type of eczema (often called 'endogenous eczema') occurs in individuals who are 'atopic'. It is common, occurring in up to 5% of the UK population. It is commoner in early life, occurring at some stage during childhood in up to 10-20% of all children [46].

The exact pathophysiology is not fully understood but there is a selective activation of Th2-type CD4 lymphocytes in the skin which drives the inflammatory process. In at least 90% of cases there is a raised serum total IgE level [46].

Atopic eczema is a genetically complex, familial disease with a strong maternal influence. A positive family history of atopic disease is often present: there is a 90% concordance in monozygotic twins but only 20% in dizygotic twins [46].

If one parent has atopic disease the risk for a child of developing eczema is about 20-30%, and 50% if both parents are affected. Genetic studies in atopy have so far shown linkage to several different loci where pathologically relevant candidate genes exist: e.g. the  $\alpha$ -subunit of the high-affinity IgE receptor (11q13); Th2 cytokine genes (5q31-33); the  $\alpha$ -subunit of IL-4 (16q12); and RANTES (17q11). Both eczema and psoriasis have been linked to chromosome 1q21 and 17q25, suggesting common candidate genes controlling skin inflammation [46].

The disease is significantly influenced by environmental factors. Infection either in the skin or systemically can lead to an exacerbation, possibly by a superantigen effect. Paradoxically, lack of infection (in infancy) may cause the immune system to follow a Th-2 pathway and allow eczema to develop (the so-called 'hygiene hypothesis') [46].

Strong detergents, chemicals and even woollen clothes can be irritant and exacerbate eczema. Teething is another factor in young children. Severe anxiety or stress appear to exacerbate eczema in some individuals. Cat and dog fur can certainly make eczema worse, possibly by both allergic and irritant mechanisms. There is some evidence that food allergens may play a role in triggering atopic eczema and that dairy products may exacerbate eczema in some infants [46].

Atopic eczema can present as a number of distinct morphological variants. The commonest presentation is of itchy erythematous scaly patch especially in the flexures such as in front of the elbows and ankles, behind the knees and around the neck. In infants eczema often starts on the face before spreading to the body [46].

Very acute lesions may weep or exude and can show small vesicles. Scratching can produce excoriations, and repeated rubbing produces skin thickening (lichenification) with exaggerated skin markings. In patients with pigmented skin, eczema often shows a reverse pattern of extensor involvement. Also, the eczema may be papular or follicular in nature and lichenification is common. A final problem in pigmented skin is of post-inflammatory hyper- or hypopigmentation which is often very slow to fade after control of the eczema [46].

Involvement of the nail bed may produce pitting and ridging of the nails. In some atopic individuals the skin of the upper arms and thighs may feel roughened because of follicular hyperkeratosis ('keratosis pilaris'). The palms may show very prominent skin creases ('hyperlinear palms'). There may be an associated dry 'fish-like' scaling of the skin which is non-inflammatory and often prominent on the lower legs ('ichthyosis vulgaris') [46].

Broken skin commonly becomes secondarily infected by bacteria. This is usually due to *Staphylococcus aureus* although streptococci can colonize eczema, especially in macerated flexural areas such as the neck and groin. Clinically this infection may appear as crusted, weeping impetigo-like lesions. Occasionally *Pseudomonas* can be grown from skin swabs but this rarely causes a clinical problem [46].

Ocular complications of atopic eczema include conjunctival irritation and less commonly keratoconjunctivitis and cataract. Retarded growth may be seen in children with chronic severe eczema; it is due to the disease itself and not the use of topical steroids [46].

The diagnosis of atopic eczema is normally clinical. Atopy is characterized by high serum immunoglobulin E levels or high specific IgE levels to certain ingested or inhaled antigens. The latter can be tested by radio-immunoabsorbent assay (RAST tests) of blood, or indirectly by skin prick testing. A peripheral blood eosinophilia may also be evident [46].

The majority (90%) of children with early-onset atopic eczema will spontaneously improve and 'clear' before the teenage years, 50% being clear by the age of 6. A few will get a recurrence as adults, even if just as hand eczema. However, if the onset of eczema is late in childhood or in adulthood, the disorder follows a more chronic remitting/relapsing course [46].

General measures include avoiding known irritants (especially soaps or furry animals), wearing cotton clothes, and not getting too hot. Manipulating the diet (e.g. dairy-free diet). Any change in diet should be done under supervision, especially with growing children who may need supplements such as calcium [46].

Prostaglandins E1, E2, F1 $\alpha$  and F2 $\alpha$  were found in the inflamed skin of patients with allergic contact eczema. Perfusates from inflamed skin of allergic contact eczema contain nanogramme concentrations of prostaglandins. In contrast prostaglandins could not be detected by direct examination of perfusates from normal skin though ethyl acetate extraction revealed the presence of trace amounts [14].

It has been suggested that the increased concentrations of inflamed skin could be due to increased prostaglandin biosynthesis or alternatively prostaglandins could be released from cell membrane lipids after activation of tissue phospholipases. Intradermal injections of prostaglandin E1 in concentrations as low as 10ng/ml caused pronounced erythema of a sustained quality [14].

## Crohn's Disease

Two major forms of non-specific inflammatory bowel disease are recognized: Crohn's disease (Crohn's disease), which can affect any part of the GI tract, and ulcerative colitis (ulcerative colitis), which affects only the large bowel. There is overlap between these two conditions in their clinical features, histological and radiological abnormalities; in 10% of cases of colitis a definitive diagnosis of either ulcerative colitis or Crohn's disease is not possible [47].

It is deemed necessary to distinguish between these two conditions because of differences in their management. It is possible that these conditions represent two aspects of the same disease. Three additional forms of non-specific inflammatory bowel disease are also recognized, namely microscopic ulcerative, microscopic lymphocytic and microscopic collagenous colitis [47].

The incidence of Crohn's disease varies from country to country but is approximately 4-10 per 100 000 annually, with a prevalence of 27-106 per 100 000. The incidence of ulcerative colitis is stable at 6-15 per 100 000 annually, with a prevalence of 80-150 per 100 000. Both conditions have a world-wide distribution but are more common in the West. The incidence is lower in the non-white races. Jews are more prone to inflammatory bowel disease than non-Jews, and the Ashkenazi Jews have a higher risk than the Sephardic Jews [47].

Crohn's disease is slightly commoner in females (M : F = 1:1.2) and occurs at a younger age (mean 26 years) than ulcerative colitis (M : F = 1.2 : 1; mean 34 years). Although the aetiology of inflammatory bowel disease is unknown, although susceptibility involves, environment and host immune response, with the environmental factors representing both the local microenvironment (enteric microflora) and also the nutritional environment [47].

Both Crohn's disease (Crohn's disease) and ulcerative colitis (ulcerative colitis) are more common amongst relatives of patients than in the general population. Thus 6-10% of patients affected with Crohn's disease or ulcerative colitis have one or more relatives with the disease. The risk of Crohn's disease in first degree relatives of a Crohn's disease patient is 10-14 times higher than in the general population, with the risk of ulcerative colitis being about 8. In Crohn's disease, but not ulcerative colitis, affected patients are more likely to be siblings than first-degree relatives [47].

Good domestic hygiene has been shown to be a risk factor for Crohn's disease but not for ulcerative colitis - similarly *Helicobacter pylori* seroprevalence is reduced in Crohn's disease but not in ulcerative colitis. In a 'clean' environment the intestinal immune system may not be exposed to pathogenic or non-pathogenic microorganisms, particularly helminthic parasites, and therefore be 'un-trained' to confront minor infections without recruiting the full array of specific immune functions that lead to inflammation [47].

Helminth infections are associated with a type 2 helper T cell response (Th2), which would counterbalance the type-1 helper T cell response (Th1) that is characteristic of Crohn's disease. If such a mechanism is operative, it would explain why there is a frequent association of a recent intestinal infection with the first presentation and subsequent flare-ups of Crohn's disease [47].

Many foods and food components have been suggested to play a role in the aetiopathogenesis of inflammatory bowel disease. The results of numerous studies have been equivocal. The gut is colonized by 10 times more bacterial organisms than there are host cells, there being 300-400 distinct bacterial species. Inflammatory bowel disease is characterized by an overaggressive immune response to luminal bacterial antigens and other products [47].

There is an alteration in the bacterial flora, with an increase in anaerobic bacteria in Crohn's disease and an increase in aerobic bacteria in ulcerative colitis. Bacteria may exert their proinflammatory influence by producing toll-like receptor ligands such as peptidoglycan - polysaccharides (PG-PS), lipopolysaccharides (LPS), bacterial DNA motifs or formylated oligopeptides, e.g. N- formyl-methionyl leucylphenylalanine (FMLP), which interact in the normal intestine with surface toll-like receptors (TLR) [47].

The disruption in toll-like receptors signalling could prevent the mucosa withstanding bacterial insult. The intestinal wall in inflammatory bowel disease patients is contaminated by adherent and invading bacteria [47].

Cationic antimicrobial peptides normally protect the mucosa against adherent and invading bacteria. Evidence suggests a decrease in human P defensin- 1 (HBD-1) in both Crohn's disease and ulcerative colitis and lack of induction of HBD-2 and HBD-3 in Crohn's disease [47].

Impaired mucosal barrier function may explain the presence of unusual and potentially pathogenic bacteria, e.g. *Mycobacterium paratuberculosis* (MAP), *Listeria*, mucosal adherent *E. coli*. Their presence does not necessarily imply causation of the disease. However, *Mycobacterium paratuberculosis* has recently been found in the blood of patients with Crohn's disease and further studies are awaited [47].

Measles virus has been implicated as it was found in the vascular epithelium with associated vascular injury and focal enteritis. However, RT-PCR did not detect the virus from intestinal biopsies from patients. There may be antigen mimicry [47].

Butyrate Sulphate-producing bacteria increase luminal levels of hydrogen sulphide (H<sub>2</sub>S), which leads to a reduction of butyrate oxidation in colonic mucosa, producing an energy deficient state and leading to mucosal inflammation. H<sub>2</sub>S and methane-ethiol may produce the malodorous flatus that some patients complain of prior to a flare-up [47].

It is suggested that defective immunoregulation or barrier function/healing prevents the appropriate downregulation of immune (antigen-specific) or antigen-non-specific inflammatory responses to endogenous luminal antigens [47].

Specifically there is upregulation of macrophages and Th1 lymphocytes in Crohn's disease. This produces an excess of cytokines, interleukin-1/3 (IL-1/3), interleukin-1 receptor antagonist (IL-1RA), interleukin-6, the chemokine interleukin-8 and Tumour Necrosis Factor  $\alpha$  (TNF- $\alpha$ ). Ulcerative colitis is a modified Th2 response with interleukin-5 and interleukin-10 [47].

There is also activation of other cells (eosinophils, mast cells, neutrophils and fibroblasts) which leads to excess production of chemokines (lymphokines, arachidonic acid metabolites, neuropeptides and free oxygen radicals), all of which can lead to tissue damage [47].

Crohn's disease is a chronic inflammatory condition that may affect any part of the gastrointestinal tract from the mouth to the anus but has a particular tendency to affect the terminal ileum and ascending colon (ileocolonic disease). The disease can involve one small area of the gut such as the terminal ileum, or multiple areas with relatively normal bowel in between (skip lesions). It may also involve the whole of the colon (total colitis) sometimes without small bowel involvement [47].

Ulcerative colitis can affect the rectum alone (proctitis), can extend proximally to involve the sigmoid and descending colon (left-sided colitis), or may involve the whole colon (total colitis). In a few of these patients there is also inflammation of the distal terminal ileum (backwash ileitis) [47].

In Crohn's disease the involved small bowel is usually thickened and narrowed. There are deep ulcers and fissures in the mucosa, producing a cobblestone appearance. Fistulae and abscesses may be seen in the colon. An early feature is aphthoid ulceration, usually seen at colonoscopy. Later, larger and deeper ulcers appear in a patchy distribution, again producing a cobblestone appearance. In ulcerative colitis the mucosa looks reddened, inflamed and bleeds easily. In severe disease there is extensive ulceration with the adjacent mucosa appearing as inflammatory polyps [47].

In fulminant colonic disease of either type, most of the mucosa is lost, leaving a few islands of oedematous mucosa (mucosal islands), and toxic dilatation occurs. On healing, the mucosa can return to normal, although there is usually some residual glandular distortion [47].

In Crohn's disease the inflammation extends through all layers (transmural) of the bowel, whereas in ulcerative colitis a superficial inflammation is seen. In Crohn's disease there is an increase in chronic inflammatory cells and lymphoid hyperplasia, and in 50-60% of patients granulomas are present. These granulomas are noncaseating epithelioid cell aggregates with Langhans' giant cells. In ulcerative colitis the mucosa shows a chronic inflammatory cell infiltrate in the lamina propria. Crypt abscesses and goblet cell depletion are also seen [47].

The differentiation between these two diseases can usually be made not only on the basis of clinical and radiological data but also on the histological differences seen in the rectal and colonic mucosa obtained by biopsy. It is occasionally not possible to distinguish between the two disorders, particularly if biopsies are obtained in the acute phase, and such patients are considered to have an indeterminate inflammatory colitis. Serological testing may be of value in differentiating the two conditions [47].

Joint complications are the commonest extragastrointestinal manifestations and the peripheral arthropathies are now classified as type 1 (pauci-articular) and type 2 (polyarticular). Type 1 attacks are acute, self-limiting (< 10 weeks) and occur with inflammatory bowel disease relapses; they are associated with other extraintestinal manifestations of inflammatory bowel disease activity [47].

Type 2 arthropathy lasts longer (months to years), is independent of inflammatory bowel disease activity and usually associated with uveitis. This is an association HLA DRB1\*0103 with pauci-articular large joint arthritis in ulcerative colitis and Crohn's disease and small joint symmetrical arthritis with HLA-B44. HLA B27 is associated with sacroileitis [47].

The major symptoms are diarrhoea, abdominal pain and weight loss. Constitutional symptoms of malaise, lethargy, anorexia, nausea, vomiting and low-grade fever may be present and in 15% of these patients there are no gastrointestinal symptoms. Despite the recurrent nature of this condition, many patients remain well and have an almost normal lifestyle. However, patients with extensive disease often have frequent recurrences, necessitating multiple hospital admissions [47].

The clinical features are very variable and depend partly on the region of the bowel that is affected. The disease may present insidiously or acutely. The abdominal pain can be colicky, suggesting obstruction but it usually has no special characteristics and sometimes in colonic disease only minimal discomfort is present [47].

Diarrhoea is present in 80% of all cases and in colonic disease it usually contains blood, making it difficult to differentiate from ulcerative colitis. Steatorrhoea can be present in small bowel disease. Crohn's disease can present as an emergency with acute right iliac fossa pain mimicking appendicitis. If laparotomy is undertaken, an oedematous reddened terminal ileum is found. There are other causes of an acute ileitis (e.g. infections such as Yersinid) [47].

Up to 30% of patients presenting with acute ileitis turn out eventually to have Crohn's disease. Crohn's disease can be complicated by anal and perianal disease and this is the presenting feature in 25% of cases, often preceding colonic and small intestinal symptoms by many years. Enteric fistulae, e.g. to bladder or vagina, occur in 20-40% of cases, equally divided between internal and external fistulae; the latter usually occurring after surgery [47].

The major symptom in ulcerative colitis is diarrhoea with blood and mucus, sometimes accompanied by lower abdominal discomfort. General features include malaise, lethargy and anorexia. Aphthous ulceration in the mouth is seen. The disease can be mild, moderate or severe, and in most patients runs a course of remissions and exacerbations [47].

Ten per cent of patients have persistent chronic symptoms, while some patients may have only a single attack. When the disease is confined to the rectum (proctitis), blood mixed with the stool, urgency and tenesmus are common. There are normally few constitutional symptoms, but patients are nevertheless greatly inconvenienced by the frequency of defecation [47].

In an acute attack of ulcerative colitis, patients have bloody diarrhoea, passing up to 10-20 liquid stools per day. Diarrhoea also occurs at night, with urgency and incontinence that is severely disabling for the patient. Occasionally blood and mucus alone are passed. The patient is often very ill and needs urgent treatment in hospital [47].

In general there are no specific signs in ulcerative colitis. The abdomen may be slightly distended or tender to palpation. The anus is usually normal. Rectal examination will show the presence of blood [47].

Rigid sigmoidoscopy is usually abnormal, showing an inflamed, bleeding, friable mucosa. Very occasionally rectal sparing occurs, in which case sigmoidoscopy will be normal [47].

A third of patients with distal inflammatory proctitis due to ulcerative colitis will develop more proximal disease, with 5-10% developing total colitis. A third of patients with ulcerative colitis will have a single attack and the others will have a relapsing course. A third of patients with ulcerative colitis will undergo colectomy within 20 years of diagnosis [47].

There are three distinct forms of microscopic inflammatory colitis: microscopic ulcerative colitis; microscopic lymphocytic colitis; microscopic collagenous colitis. In microscopic ulcerative colitis, there is a chronic inflammatory cell infiltrate in the lamina propria, with deformed crypt architecture, and goblet cell depletion with or without crypt abscesses [47].

In microscopic lymphocytic colitis there is surface epithelial injury, prominent lymphocytic infiltration in the surface epithelium and increased lamina propria mononuclear cells. It affects males and females equally and is associated with a high prevalence of antibiotic use [47].

In microscopic collagenous colitis there is a thickened subepithelial collagen layer adjacent to the basal membrane with increased infiltration of the lamina propria with lymphocytes and plasma cells and surface epithelial cell damage. It is predominantly a disorder of middle-aged or elderly females, and is associated with a variety of autoimmune disorders (arthritis, thyroid disease, CREST syndrome and primary biliary cirrhosis) [47].

The prevalence of collagenous colitis has been shown to be 15.7 /10 5 population with an annual incidence of 1.8/105 population. There are a number of reports linking drugs to the development of collagenous colitis (non-steroidal anti-inflammatory drugs, simvastatin, H<sub>2</sub> - receptor antagonists) [47].

Prostaglandin synthesis in inflammatory bowel disease has been seen as the genesis of mucosal inflammation and a global increase in the synthesis of prostanoids. Several studies have shown increased synthesis of prostanoids such as prostaglandin E<sub>2</sub>, prostaglandin F<sub>2</sub> $\alpha$ , thromboxane B<sub>2</sub> and 6-keto prostaglandin F<sub>1</sub> $\alpha$  occurs during relapse of ulcerative colitis. Fundamental differences from normal have not been shown in remission [15].

Peripheral blood mononuclear cells are known to synthesize increased amounts of prostaglandin E<sub>2</sub> and thromboxane B<sub>2</sub> during relapse of Crohn's disease. The synthesis of prostaglandin E<sub>2</sub> by inflamed rectal mucosa is enhanced in Crohn's disease. There is also enhanced thromboxane synthesis by macrophages in Crohn's disease [15].

There is increased rectal mucosal release of cytokines and eicosanoids in ulcerative colitis, in proportion to disease activity. In vitro studies showed that mucosal release of interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), thromboxane B<sub>2</sub> (TxB<sub>2</sub>), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were significantly increased in active ulcerative colitis (p=0.001) and correlated directly with disease activity (p=0.02) [49].

Concentrations of all eicosanoids in inflammatory bowel disease patients were significantly increased. Leukotriene B<sub>4</sub> in ulcerative colitis: mean 73.2 pg/ml, in Crohn's disease: 96.4 pg/ml (both p<001 v controls). Prostaglandin E<sub>2</sub> in ulcerative colitis: 20.2 pg/ml, in Crohn's disease: 43.4 pg/ml (p<0 01). Thromboxane B<sub>2</sub> in ulcerative colitis: 719.3 pg/ml, in Crohn's disease: 180-6 pg/ml (both p<005) [50].

## Arthritis

Rheumatoid arthritis is an autoimmune disease in which the joints are chronically inflamed. In autoimmune diseases the immune system acts on the body's own tissues. For reasons that are not understood, specific lymphocytes are stimulated to produce antibodies, referred to as autoantibodies. These molecules bind to surface antigens on the patient's own cells as if they were foreign. Then the immune system attacks affected cells [48].

In rheumatoid arthritis, several types of white blood cells infiltrate joint tissue as part of the inflammatory process. The leakage of lysosomal enzymes from actively phagocytosing cells (neutrophils and macrophages) leads to further tissue damage. The inflammatory response is perpetuated by the release of eicosanoids by white blood cells. A variety of eicosanoids have been implicated. For example, macrophages are known to produce PGE<sub>2</sub>, TXA<sub>2</sub>, and several leukotrienes [48].

The eicosanoids are a diverse group of extremely powerful hormone-like molecules produced in most mammalian tissues. They mediate a wide variety of physiological processes. Examples include smooth muscle contraction, inflammation, pain perception, and blood flow regulation. Eicosanoids are also implicated in several diseases such as myocardial infarct and rheumatoid arthritis [48].

Rheumatoid arthritis is a chronic symmetrical polyarthritis of unexplained cause. It is a systemic disorder characterized by chronic inflammatory synovitis of mainly peripheral joints. Its course is extremely variable and it is associated with nonarticular features [51].

The chronic synovial inflammation may be caused by ongoing T cell activation. Alternatively it may be maintained by the local production of rheumatoid factors and continuous stimulation of macrophages via immunoglobulin G Fc receptors. Considering the extent of synovial inflammation and lymphocytic infiltration, there are only minimal amounts of factors normally produced by T cells (interferon and interleukin -2 and -4) [51].

Conversely, the cytokines (IL-1, IL-8, TNF- $\alpha$ , granulocyte-macrophage colony-stimulating factor) and chemokines produced by macrophages (macrophage inflammatory protein (MIP) and monocyte chemoattractant protein (MCP)) and fibroblasts (producing IL-6) are abundant [51].

Activated mast cells which release histamine and TNF- $\alpha$  may also play a role. CD4-specific antibodies, when used therapeutically, produce a specific helper T-cell lymphopenia but do not significantly alter the disease, raising the possibility that T cells play a lesser role [51].

Antibodies to TNF- $\alpha$ , IL-1 or specific blocking agents produce marked short-term improvement in synovitis, indicating the pivotal role of these cytokines in the chronic synovitis. They also reduce the malaise felt in active rheumatoid arthritis [51].

Synovial fibroblasts have high levels of the adhesion molecule, vascular cell adhesion molecule (VCAM-1), a molecule which supports B lymphocyte survival and differentiation, and of decay accelerating factor (DAF), a factor that prevents complement-induced cell lysis. These molecules may facilitate the formation of ectopic lymphoid tissue in synovium. High affinity antibodies are not a feature of rheumatoid arthritis, unlike other autoimmune diseases [51].

Bacterial or slow virus infections have been implicated but are unproven. It has been suggested that an immune response to any pathogen is to produce autoantibodies by B cell clonal expansion. In susceptible individuals such clones may persist [51].

Rheumatoid arthritis is typified by widespread persisting synovitis (inflammation of the synovial lining of joints, tendon sheaths or bursae). The cause of this is unclear, but the production of rheumatoid factors by plasma cells in the synovium and the local formation of immune complexes play a part [51].

In rheumatoid arthritis, the normal synovium becomes greatly thickened to the extent that it is palpable as a 'boggy' swelling around the joints and tendons. There is proliferation of the synovium into folds and fronds, and it is infiltrated by a variety of inflammatory cells, including polymorphs, which transit through the tissue into the joint fluid, and lymphocytes and plasma cells [51].

There are disorganized lymphoid follicles that are responsive to exogenous antigens. The normally sparse surface layer of lining cells becomes hyperplastic and thickened. There is marked vascular proliferation. Increased permeability of blood vessels and the synovial lining layer leads to joint effusions that contain lymphocytes and dying polymorphs [51].

The hyperplastic synovium spreads from the joint margins on to the cartilage surface. This 'pannus' of inflamed synovium damages the underlying cartilage by blocking its normal route for nutrition and by the direct effects of cytokines on the chondrocytes. The cartilage becomes thinned and the underlying bone exposed [51].

Local cytokine production and joint disuse combine to cause juxta-articular osteoporosis during active synovitis. Fibroblasts from the proliferating synovium also grow along the course of blood vessels between the synovial margins and the epiphyseal bone cavity and damage the bone. This is shown by MRI to occur in the first 3-6 months following onset of the arthritis, and before the diagnostic, ill-defined juxta-articular bony 'erosions' appear on X-ray [51].

Low-dose steroids delay and anti-TNF- $\alpha$  agents halt or even reverse erosion formation. Erosions lead to a variety of deformities and contribute to long-term disability. Rheumatoid factors These are circulating autoantibodies, which have the Fc portion of IgG as their antigen. The nature of the antigen means that they self-aggregate into immune complexes and thus activate complement and stimulate inflammation, causing chronic synovitis [51].

Transient production of rheumatoid factors is an essential part of the body's normal mechanism for removing immune complexes, but in rheumatoid arthritis they show a much higher affinity and their production is persistent and occurs in the joints. They may be of any immunoglobulin class (IgM, IgG or IgA), but the most common tests employed clinically detect IgM rheumatoid factor [51].

Around 70% of patients with polyarticular rheumatoid arthritis have IgM rheumatoid factor in the serum. The term seronegative rheumatoid arthritis is used for patients in whom the standard tests for IgM rheumatoid factor are persistently negative. They tend to have a more limited pattern of synovitis. IgM rheumatoid factor is not diagnostic of rheumatoid arthritis, nor does its absence rule the disease out; but it is a useful predictor of prognosis. A persistently high titre in early disease implies more persistently active synovitis, more joint damage and greater disability eventually [51].

The most typical presentation of rheumatoid arthritis (approximately 70% of cases) begins as a slowly progressive, symmetrical, peripheral polyarthritis, evolving over a period of a few weeks or months. Women are affected three times more often than are men [51].

The patient is usually in their thirties to fifties, but the disease can occur at any age. Less commonly (15%) a rapid onset can occur over a few days (or explosively overnight) with a severe symmetrical polyarticular involvement. These patients often have a better prognosis. A worse than average prognosis (with a predictive accuracy of about 80%) is indicated by being female, a gradual onset over a few months, and a positive IgM rheumatoid factor, and/or anaemia within 3 months of onset [51].

The majority of patients complain of pain and stiffness of the small joints of the hands (metacarpophalangeal, MCP), proximal and distal interphalangeal (PIP, DIP) and feet (metatarsophalangeal, MTP). The wrists, elbows, shoulders, knees and ankles are also affected [51].

In most cases many joints are involved, but 10% present with a monoarthritis of the knee or shoulder or with a carpal tunnel syndrome. The hips are rarely affected early in the disease. The patient feels tired and unwell and the pain and stiffness are significantly worse in the morning and may improve with gentle activity. Sleep is disturbed. The joints are usually warm and tender with some joint swelling [51].

There is limitation of movement and muscle wasting. Deformities develop as the disease progresses. Nonarticular features develop [51].

The presentation and progression of rheumatoid arthritis is variable. Relapses and remissions occur either spontaneously or in response to drug therapy. In some patients the disease remains active, producing progressive joint damage. Rarely the process may cease ('burnt-out rheumatoid arthritis') [51].

A seronegative, limited synovitis initially affects the wrists more often than the fingers and has a less symmetrical joint involvement. It has a better long-term prognosis, but some cases progress to severe disability. This form can be confused with psoriatic arthropathy, which has a similar distribution. There may be a family history of psoriasis or the patient may develop psoriasis later [51].

Palindromic rheumatism is unusual (5%) and consists of short lived (24 - 72 hours) episodes of acute monoarthritis. The joint becomes acutely painful, swollen and red, but resolves completely. Further attacks occur in the same or other joints. About 50% go on to develop typical chronic rheumatoid synovitis after a delay of months or years. The rest remit or continue to have acute episodic arthritis. The detection of IgM rheumatoid factor predicts conversion to chronic, destructive synovitis [51].

Rheumatoid arthritis is an inflammatory disorder which affects 1% of the adult population worldwide. Rheumatoid arthritis is characterized by systemic and local inflammation resulting in cartilage and bone destruction. Pharmacological evidence from rheumatoid arthritis trials and preclinical models of arthritis points to the importance of cox metabolites in the pathogenesis of the disease [16].

Synovial fluid from patients with rheumatoid arthritis shows increases in virtually all the prostanoids. Prostaglandin E2 is associated with some of the proinflammatory effects of rheumatoid arthritis specifically with the edema and erosion of cartilage and juxta-articular bone [16].

Evidence suggests that prostaglandin E2 contributes to the pathogenesis of rheumatoid arthritis. Non steroidal anti-inflammatory inhibitors of Prostaglandin E2 and other prostanoids are used to treat this disease [16].

Interleukin 6 deficient mice develop significantly less arthritis than do wild type controls. There is an association between prostaglandin E receptor EP4, interleukin 6, and arthritis [16].

## Hay Fever

Charles Blackley first described the association of nasal symptoms of sneezing, rhinorrhoea, and obstruction with seasonal changes in the pollen count and identified hay fever, seasonal allergic rhinitis or pollenosis. Pollenosis is the best clinical example of the type 1 or immediate hypersensitivity reaction. Soluble allergens derived from the pollen grains of trees, shrubs, grasses, and flowers can stimulate the systemic and mucosal synthesis of specific reaginic antibodies [18].

Mast cells are found in large numbers in the mucous membranes of the nose and conjunctivae. Experimental nasal provocation with allergens induces gross degranulation of mast cells both on the mucosal surface and in the submucosa [18].

The activation of mast cells by IgE leads to the release of preformed mediators including histamine, exoglycosides, the neutral protease tryptase, and eosinophil and neutrophil chemotactic factors. Immunoglobulin E binds via the C4 domain of its H chains with high affinity to Fc receptors on the surface of mast cells and basophils [18].

Chemotactic recruitment of secondary effector cells such as eosinophils, neutrophils, and basophils to the sites of mast cell degranulation, together with their activation, releases further mediators to compound the inflammatory response which leads to a state of chronic nasal hyperreactivity [18].

Cytological examination of nasal and conjunctival secretions of patients with active pollenosis shows many basophils and eosinophils. In patients with allergic rhinitis provocation by allergens evokes both early and late reactions characterized by the release of inflammatory mediators detectable in nasal lavage [18].

Histamine causes direct and reflex dilation of post capillary venules leading to nasal obstruction, it stimulates irritant receptors causing sneezing and also stimulates secretion from goblet cells and submucosal glands. Exoglycosidases such as  $\beta$  glucuronidase,  $\beta$  hexoaminidase,  $\beta$  galactosidase, arylsulfatase and neutral proteases disrupt the integrity of the mucous membrane through their specific actions on tissue ground substance [18].

Mast cell activation stimulates the generation and release of newly formed mediators, including prostaglandin D2 and the 5-lipoxygenase products leukotrienes C4 and D4 previously identified as slow acting substance of anaphylaxis [18].

Prostaglandin E was extracted from nasal secretions of individuals with hay fever and from nasal washings of normal subjects [17]. Prostaglandin D2 is a potent vasodilator causing vascular engorgement of the mucosa while leukotriene C4 increases capillary permeability causing exudation. Prostaglandin D2 and leukotrienes C4 and B4 are released during the early reaction in parallel with histamine and a proteolytic THAME esterase activity capable of generating bradykinin [18].

Histamine, leukotriene C4, and THAME esterase are also generated during the late reaction, but the absence of prostaglandin D2 (whose release is specific for mast cells) suggests that basophils rather than mast cells are responsible for the secretion of mediators [18].

## Anaphylactic Shock

Type I anaphylactic reactions are mediated by IgE (i.e., antibody-mediated), which binds to antibody receptors on basophils and mast cells. When cross-linked by antigens, IgE triggers basophils and mast cells to release their contents. Reaction occurs within minutes. Clinically, this type of reaction occurs in a wide spectrum ranging from rashes and wheal-and-flare reactions to anaphylactic shock [54].

Type I reactions are common. On first contact, the allergen internalized by B cells is presented to TH2 cells. The B cell then proliferates and differentiates into plasma cells, which release immunoglobulin E (IgE). The Fc fragment of IgE binds to mast cells and basophils [52].

On subsequent contact, the antigens bind to the already available IgE-linked mast cells. Due to the rapid release of mostly vasoactive mediators of inflammation such as histamine, leukotrienes and platelet-activating factor (PAF), an immediate reaction (anaphylaxis) occurs within seconds or minutes: immediate type hypersensitivity [52].

This is the mechanism by which allergens breathed into the lungs trigger hay fever and asthma attacks. The vasodilatory effect of a generalized type I reaction can lead to anaphylactic shock [52].

Anaphylactic shock is characterized by elevated immunoglobulin-E (IgE) antibodies that signal via the high affinity Fcε receptor (FcεRI) to release inflammatory mediators. A novel cytokine interleukin-33 (IL-33) potently induces anaphylactic shock in mice and is associated with the symptom in humans [53].

IL-33 is a new member of the IL-1 family and the ligand for the orphan receptor ST2. In humans, the levels of IL-33 are substantially elevated in the blood of atopic patients during anaphylactic shock, and in inflamed skin tissue of atopic dermatitis patients [53].

In murine experimental atopic models, IL-33 induced antigen-independent passive cutaneous and systemic anaphylaxis, in a T cell-independent, mast cell-dependent manner. In vitro, IL-33 directly induced degranulation, strong eicosanoid and cytokine production in IgE-sensitized mast cells [53].

The molecular mechanisms triggering these responses include the activation of phospholipase D1 and sphingosine kinase 1 to mediate calcium mobilization, Nuclear factor-κB activation, cytokine secretion, eicosanoid secretion, and degranulation [53].

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Bench-to-bedside review: The role of glycosaminoglycans in respiratory disease

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 Joanna M. Little,\* Mika Kurkela, † Julia Sonka,\* Sirkku Jäntti, † Raimo Ketola,  
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Increased urinary leukotriene E4 concentration in patients with eosinophilic pneumonia

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Glucuronidation of oxidized fatty acids and prostaglandins B1 and E2 by human hepatic and recombinant UDP-glucuronosyltransferases

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The Official Journal of the British Society for Allergy & Clinical Immunology

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Increased production of cysteinyl leukotrienes and prostaglandin D2 during human anaphylaxis

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