

## *Review*

# **Plasma Exudation and Asthma**

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**Abstract.** Several pieces of evidence support the view that exudation of plasma into the airway wall and into the airway lumen occurs in asthma. Vascular leakage of plasma results from inflammatory mediator-induced separation of endothelial cells in postcapillary venules belonging to the tracheobronchial circulation. Whereas proposed mediators of asthma induce reversible leakage, several antiasthma drugs exhibit antileakage effects in animals and humans. Potential consequences of plasma exudation are many. Mucosal/submucosal edema might contribute to airway hyperresponsiveness. Plasma exudate in the airway lumen in asthma may contribute to sloughing of epithelium, impairment of mucociliary transport, narrowing of small airways, and mucus plug formation. Exuded plasma may cause airway inflammation and constriction because of its content of powerful mediators, and chemoattractant factors and plasma proteins may condition the inflammatory cells abundant in asthmatic airways to release mediators in response to stimuli that otherwise would be innocuous to the cells. It is concluded that inflammatory stimulus-induced increase in macromolecular permeability of the tracheobronchial microvasculature and mucosa may be a significant pathogenetic mechanism in asthma and that the postcapillary venular endothelium and airway epithelium that regulate leakage of plasma are important effector cells in this disease.

**Key words:** Airway inflammation—Asthma pathology—Macromolecular leakage—Microvascular permeability—Mucosal permeability—Mediators—Antiasthma drugs.

## **Introduction**

Vascular leakage of plasma is a major sign of inflammation. This factor may deserve attention in asthma, a disease that has an important inflammatory component. However, the tracheobronchial venular endothelium, which regulates airway inflammatory plasma leakage, has not been considered an important effector cell of the lung. The occurrence of plasma leakage is nevertheless supported by findings of large amounts of plasma proteins in the sputum, mucus plugs, and in specific airway lavage fluid obtained from asthmatics [18, 38, 52, 55, 61, 75, 123]. In addition, it has been observed in experimental animals that exposure of tracheobronchial mucosa to inflammatory mediators causes a rapid movement of large plasma solutes not only into the airway wall but also into the lumen [43, 111, 115]. The physical and inflammatory effects of the plasma exudate and its content of protein-derived mediators would have a primary role in airway defense. Plasma leakage in airways may be linked with many facets of the pathophysiology of airway diseases such as asthma and rhinitis [38, 105].

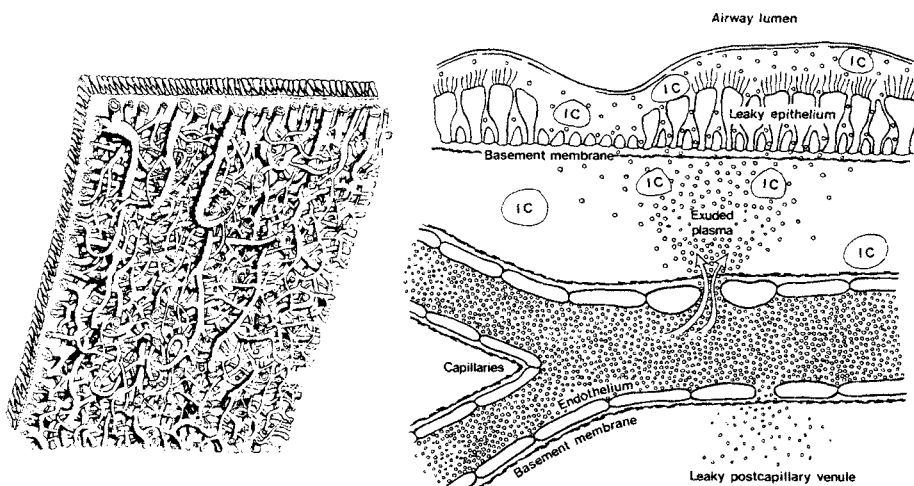
Vascular leakage of large molecules is an active process under physiological and pharmacologic control [24, 64, 78, 89, 102, 116, 134, 145, 153]. This leakage is generally referred to as increased vascular permeability. This review discusses mechanisms and potential consequences of increased tracheobronchial microvascular permeability.

## **Tracheobronchial Circulation**

Tracheal and bronchial arteries carrying systemic blood nourish the walls of the airways and their accompanying nerves and vessels. Species differences and variations within species exist for the origin and distribution of these arteries. Capillary and precapillary anastomosis between the bronchial and pulmonary circulation has been demonstrated and there are different views as to how far the bronchial arteries travel in peripheral airways, but terminal bronchioles and alveoli may be supplied by these vessels [9, 31, 81, 90, 109]. The bronchial circulation normally receives about 1% of the cardiac output [9] and a large fraction of bronchial blood flow may go to the airway mucosa/submucosa [103]. The bronchial veins from the first 2–3 generations of bronchi drain into the azygous veins and then into the right heart. The remaining bronchial blood drains into pulmonary capillaries and veins and enters the left heart [31, 90].

### *Capillary-Venular Plexuses*

Different workers have demonstrated the presence of an extensive microvasculature in the trachea and bronchi [72, 90, 100, 132] (Fig. 1). On either side of the bronchial muscle layer there is an abundance of capillary–venular plexuses coupled to a relatively sparse arterial supply. Both in the larger and the smaller



**Fig. 1.** To the left a profuse subepithelial network of microvessels [cf. 72, 90, 100, 132] is schematically illustrated. To the right the concept of a leaky postcapillary venule and a leaky epithelium of the inflamed airway are depicted. Inflammatory cells (IC) in the airway wall and lumen may be primed by the presence of exuded plasma proteins and their products.

bronchi the plexuses occupy the entire surface of the submucosal layer [72]. A rich continuous subepithelial network of microvessels would regulate clearance, and possibly distribution (from large to small airways), of inflammatory mediators and inhaled drugs [70, 72]. The profusion of the microvascular network of the airways may be illustrated by isolating the lung and perfusing it only through the pulmonary artery at normal pressures. The microvasculature of airways including the trachea will immediately be quite well perfused also, and this must have been accomplished by a retrograde microvascular flow along the airways [70].

The superficial bronchial microcirculation also has a role in the temperature and moisture conditioning of inhaled air [10, 132]. This function may be of particular relevance to asthmatic subjects who may respond with plasma exudation to inhalation of cold and dry air [107, 109]. A role for plasma exudation in “dry-air-induced asthma” may be hypothesized for 2 main reasons: (1) in inflamed airways it is vessel fluid that humidifies incoming air whereas other sources are used under normal conditions [25, 45]; (2) effective protection against this nonpharmacologic provocation is provided by drugs such as cromoglycate and glucocorticosteroids, which may have potent antileakage effects at airway endothelial–epithelial barriers [42, 107].

### *Hyperemia of Inflammation*

Alterations in bronchial blood flow will affect the delivery of plasma and white cells, the perfused surface area, and the microvascular hydrostatic pressure. The flow may be highly increased through tissue that is affected by inflamma-

tion [8] and the hydrostatic pressure increases in venules, from which exudation takes place in local hyperemia [153]. The hydrostatic microvascular pressure is dependent not only on the arterial and venous pressures but also on the ratio of post- to precapillary resistance. Vasodilation usually increases this ratio [82] and thus promotes plasma exudation.

It has long been recognized that once permeability is increased changes in blood flow may determine the degree of plasma exudation [64, 153]. However, pronounced synergistic effects as demonstrated in the skin between flow-increasing and permeability-increasing mediators [147] may not occur in airway mucosa/submucosa that has a high basal perfusion.

The neural, hormonal, and pharmacologic regulation of tracheobronchial blood flow has many of the general characteristics of a systemic circulation [9, 73, 103, 109, 112]. Many agents including histamine, bradykinin, acetylcholine, substance P, VIP, and prostaglandins have been demonstrated to increase tracheobronchial blood flow [9, 73, 107].

## **Inflammatory Leakage**

### *Venular Endothelial Effector Cells*

Under physiological conditions fluid equilibrium is maintained by a balance between the hydrostatic pressure in the capillary bed, which tends to drive fluid out of the vascular compartment, and a counteracting force of the osmotic pressure of plasma proteins. Inflammation brings about dramatic changes in the transmural colloid osmotic pressure gradient. After excluding a number of factors (changes in the blood, increased microvascular pressure, changes in the surrounding tissue, etc.), Julius Cohnheim [24], more than 100 years ago, concluded that the inflammatory extravasation of protein-rich fluid must be due to noxious stimuli acting directly on the microvascular wall to cause a molecular change resulting in increased permeability. Cohnheim reported that the inflammatory exudate is concentrated and that this is due largely to its proteinaceous nature (differing from high-pressure edema fluid that is protein-poor) [24]. Somewhat reluctantly he could then make his reasoning fit with previous publications by Julius Arnold, who had shown that injected dyes always passed through the vessel wall between endothelial cells [7, 24].

More recent ultrastructural, pathophysiological, and pharmacologic studies of systemic microvascular beds have shown that inflammatory mediator-induced leakage of protein-rich plasma occurs in postcapillary venules (diameter 10–50  $\mu\text{m}$ ) through large gaps (up to 1  $\mu\text{m}$ ) between endothelial cells [23, 62, 64, 78, 89, 116, 122, 134, 145, 153] (Fig. 2). In the delayed inflammatory response to mild thermal burns and some other types of injurious stimuli an inflammatory exudate may come both from capillaries and venules [27, 64, 87], but no mediator has yet been demonstrated to produce capillary leakage [64, 87].

The target cells for inflammatory mediators have thus been identified as venular endothelial cells. Plasma escapes through the mediator-induced interendothelial gaps and filters through the endothelial basement membrane, which offers little hindrance to diffusion of plasma proteins [153]. The concentration ratios of different proteins may be similar in blood plasma and in inflammatory exudate, indicating that there is a bulk flow of proteinaceous plasma out of leaky postcapillary venules [8, 12, 59].

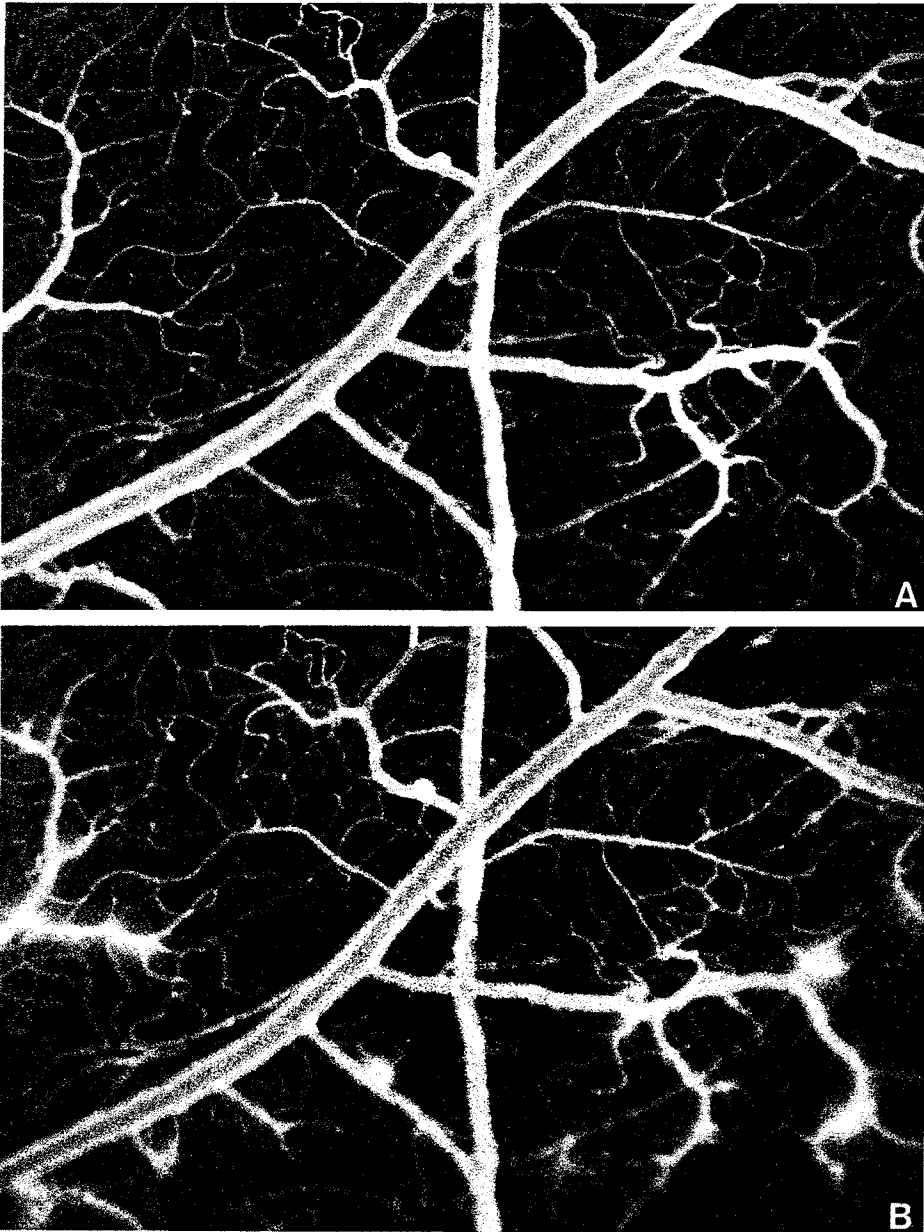
### *Mechanisms of Endothelial Cell Separation*

Contraction of venular endothelial cells has been a favored mechanism to explain mediator-induced macromolecular leakage [78]. This possibility is supported by the presence of actomyosin [13] and bundles of fibrils that could form an endothelial contractile machinery [64]. As in smooth muscle, endothelial contractility may be calcium-dependent. The contraction hypothesis is attractive because, as a corollary, the pronounced ability of endothelial cells to close mediator-induced gaps could be explained as a relaxation of these cells.

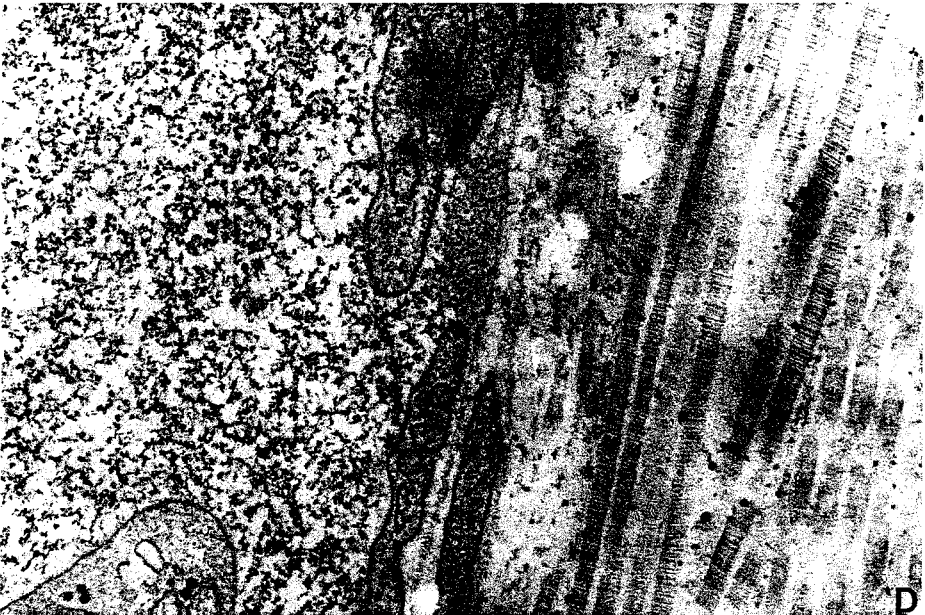
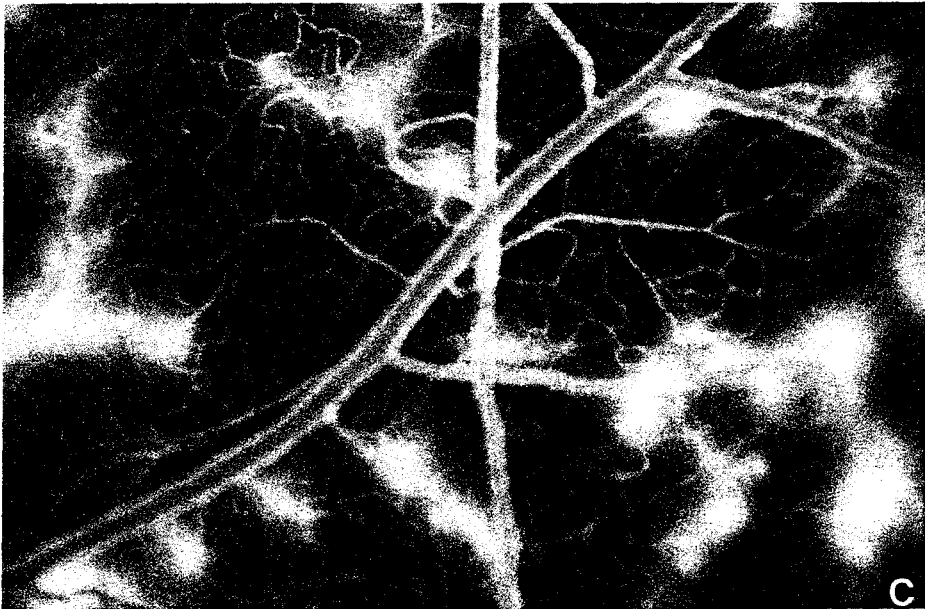
Another view on the mediator-induced deformation of endothelial cells has been discussed by Zweifach [152]. Inflammatory leaks may be produced by effects on elements interlocking endothelial cells. A weakening of these forces may change endothelial cell shape and cause increased permeability. The attachment of these cells to the basement membrane is reported to be particularly loose in collecting venules [152], which would facilitate deformation there. Also the surface material of the endothelial cells may be involved in macromolecular permeability [126]. At sites of inflammation there may also be changes in the configuration of the filamentous gel making up the basement membrane, and the ground substance may be transformed from a gel to a sol state (shown by rapid dispersion of an injected colloid, which otherwise forms a distinct bleb only) [153]. Zweifach [153] emphasized that in chronic diseases venules may be particularly sensitive due to defects in the collagenous and reticular fibers of the perivascular tissue that, together with the basement membrane, provide mechanical support for the vessel. Since plasma exudation in theory can be causally linked with several facets of asthma pathology, it would be of interest to examine whether such differences exist between normal and asthmatic airway microvessels. Recent ultrastructural examination of biopsies by Laitinen and Laitinen [71] has demonstrated that subepithelial postcapillary venules have endothelial gaps in asthmatic but not in normal airways.

### **Mediators**

New mediators are continually being discovered and characterized as factors of potential importance in asthma and other inflammatory diseases [5]. Irrespective of the chemical class of the mediator and whether it is applied extra- or intravascularly, the histologic and ultrastructural characteristics of the induced



**Fig. 2.** The 3 fluorescent graphs (magnification  $\times 34$ ) (A, B, C) illustrate the cheek pouch microcirculation in hamsters following iv injection of fluorescein-labeled dextran (FITC-dextran). The same area is shown before (A), 2 min (B), and 5 min (C) after topical application of bradykinin. It is evident that the mediator-induced macromolecular leakage occurs only from postcapillary venules of a diameter of 10–30  $\mu\text{m}$ . (D) In the electron micrograph of a leaky venular site in this microcircu-



lation the venular lumen is seen filled with dark precipitate (FITC-dextran). FITC-dextran macromolecules have been extravasated through a wide interendothelial gap and can be seen as dark spots among the collagen fibers to the right. (Erik Svensjö, Pharmacological Lab, Draco, Lund, Sweden, supplied the original prints. For details on experimental procedures, see [62, 136]).

acute macromolecular leakage appear identical [14, 64, 78, 116, 136]. However, different vascular beds may differ in their sensitivity to individual mediators. Not only are pulmonary microvessels quite resistant to histamine-type mediators [63, 64, 79, 114] but also some systemic beds such as microvessels of rat intestinal mucosa may be resistant [64]. The tracheobronchial microvasculature has generally responded in a sensitive way to leakage-inducing mediators [e.g., 109, 113].

#### *Effects on Bronchial Tone and Vascular Permeability*

Many proposed mediators of asthma are capable of inducing both bronchoconstriction and vascular leakage but may also differ in these 2 effects. Muscarinics are potent constrictors of airway smooth muscle but are without or almost without effects on microvascular permeability to macromolecules [112]. Histamine produces leakage in cat tracheal microvessels [41] although it may relax rather than contract cat large airways [3, 110]. Similarly, PAF-acether is a poor constrictor of guinea pig trachea but is effective in producing plasma exudate in this tissue [111, 115]. Due to their effect on vascular permeability, inflammatory mediators may in part produce bronchoconstriction through smooth muscle plasma-derived peptides.

#### *Effects of Allergen and Chemical Sensitizers*

The acute allergen-induced response in guinea pigs may be associated with airway edema [32]. Although edema could not be demonstrated, vascular leakage of macromolecules into the airway wall and lumen was pronounced in an acute IgE-driven anaphylactic reaction in the tracheal mucosa of anaesthetized guinea pigs [111, 113]. Extensive deposits of fibrin that would be secondary to a vascular leak were the most striking characteristic of IgE-dependent late phase reactions in human skin, whereas cellular infiltration was not a consistent finding [128].

Chemical sensitizers such as plicatic acid and isocyanates (e.g., toluenediisocyanate [TDI]) are important inducers of occupational asthma. Bronchial provocation with TDI causing both immediate and late phase responses is associated with significantly increased levels of albumin in bronchoalveolar lavage fluid [43]. TDI is also a potent inducer of very prolonged (>24 h) vascular and epithelial macromolecular permeability in guinea pig trachea (Erjefält and Persson, unpublished data). Airway bronchial lavage in patients with asthma due to exposure to Western red cedar (plicatic acid) showed a 10-fold higher albumin concentration in the lavage fluid than in normal subjects [75]. The lavage was performed 24–48 h after provocation, when symptoms had subsided. Preliminary observations with allergen and chemical sensitizers are thus compatible with the possibility that plasma exudate may contribute to late-phase and sustained airway reactions.



### *Time Course of Venular Leakage*

Leakage of macromolecules is evident within 10ths of seconds after application of a directly acting inflammatory agent on systemic microvascular beds (including the tracheobronchial circulation). A maximum effect of mediators such as histamine, bradykinin, tachykinins, and leukotrienes is usually established within 3–10 min [14, 84, 89, 111, 112, 116, 134, 136, 145, 151]. The response then declines quickly and normal low permeability is restored within a few minutes up to half an hour. During some time after the development of a response the affected tissue is partly refractory to further permeability effects [11, 20, 51, 137]. Tachyphylaxis or refractoriness is not absolute. Prolonged vascular leakiness may be due to sequential effects of different mediators or intermittent release of mediators that avoid tachyphylaxis. Since bradykinin did not exhibit tachyphylaxis in human skin responses [11, 51], it may participate in delayed inflammatory reactions [11]. Menkin [85] demonstrated that a cell-free plasma exudate injected into rabbit skin produced prominent vascular leakage of macromolecules. Due to their distribution and activity, plasma-derived mediators may exert positive feedback effects on the venular wall and be responsible in part for maintaining high vascular permeability. The inflammatory breakdown of blood and tissue proteins will not only produce mediators but also increase the number of molecules and, hence, increase interstitial osmotic pressure, thus promoting transudation [44].

Miles and co-workers [20, 29, 89] demonstrated interesting biphasic as well as sustained permeability responses to bacterial toxins injected into guinea pig skin. Hence many types of injury may cause an immediate phase of leakiness that often is short-lasting; after 1–2 h a late phase of increased microvascular permeability follows that is much more sustained [145]. We have recently observed that topical application of PAF-acether on guinea pig tracheal mucosa *in vivo* produced both an acute [111] and a late phase vascular leakiness 5 h after provocation [115], which has not as yet been seen with other mediators.

### *Plasma Leakage and Leukocytes*

Sticking of white cells to endothelium and their subsequent migration across the vascular wall characterize most inflammatory processes. As with protein leakage the leukocyte–endothelium interactions occur in postcapillary venules and the diapedesis is through endothelial intercellular junctions [64, 80, 134]. However, inflammatory extravasation of leukocytes and macromolecular solutes is induced via different mechanisms and can occur separately: white cells apparently have a protein-tight seal during migration, and mediator-induced leakage of plasma can occur without any cellular escape [44, 50, 64, 153].

Tissue leukocytosis may be associated with delayed responses and a causal relationship between leukocytes and leakage has been suggested [142]. However, neutropenia may not suppress the initial or the delayed permeability response [145, 148, 149], and mediators such as anaphylatoxins and PAF-

acether, which have been proposed to act through polymorphonuclear leukocytes, may have pronounced vascular leakage effects independent of leukocytes and platelets [14, 119]. Although participation of leukocytes is likely, their suggested pivotal role [142] in the development of a sustained microvascular permeability in inflammation has not been proven.

### *Supply of Mediators*

The mediators may come from many sources, the stationary and migrating inflammatory cells being the most widely explored. Airway epithelial cells may produce arachidonate mediators [97] and vascular endothelial cells may also generate several of the inflammatory mediators, including PAF acether and arachidonate products [e.g., 19]. Neuropeptides have been proposed as mediators of vascular permeability but substance P and other tachykinins may not be as effective as inflammatory agents in human airways as they are in the guinea pig [112]. Of particular relevance for the present discussion (i.e., the sequelae of microvascular leakage) must be the preformed and dormant protein mediators circulating in the bloodstream.

Huber and Koessler [61] included in their review the information that the serum of 1 patient dying of asthma "was very toxic for animals, 0.05 c.c. causing death of a guinea pig." Circulating kinins [1] and esterase activity [16] may be elevated and kininogen decreased [16] in asthma. Ciliary dyskinetic factors have been detected in asthmatic serum [150] as have indices for activation of complement [86]. Hence, the plasma of asthmatic subjects may be particularly noxious.

The plasma proteins leaking through the venular gaps may be activated by negative surface charges, proteases, and other factors during their transvascular passage and upon arrival in inflamed tissue [12, 48, 87, 88, 94, 120, 133, 141, 144]. Both in the airway wall and lumen proteins of the kinin, complement, clotting, and fibrinolysis systems may generate a variety of inflammatory, bronchoconstrictory, and chemoattractant mediators. Bradykinin, which is a powerful provocateur in asthma [56, 130], is but 1 of a plethora of plasma-derived mediators. Gerberick et al. [47] showed that rabbit alveolar macrophages were unable to release reactive oxygen intermediates unless they were conditioned by prolonged presence of plasma proteins. Exuded plasma may thus, by direct actions on a variety of target cells and by recruitment and conditioning of inflammatory cells, be an amplifying factor that escalates and sustains the inflammatory process in asthmatic airways [105].

### **Potential Effects of Exuded Plasma in the Airway Wall**

The result of plasma leakage is generally thought of as edema, and edema is accepted as a characteristic sign of asthmatic airways [38, 58, 65, 124]. This

view agrees with general descriptions of inflammation of mucosal membranes and is supported by histologic preparations that show "edema spaces" in airways obtained from patients dying of asthma [38, 58]. However, many workers who have done postmortem or biopsy examinations have not reported or been able to identify edema in asthmatic airways [4, 22, 30, 57, 60, 61, 85, 125, 139, 146]. Changes such as enlarged bronchial glands and increased thickness of the epithelial basement membrane and smooth muscle layer have been well documented [30, 39, 139], whereas edematous changes have not been easy to quantify. The relative lack of data on edema may be explained in part by movement of plasma exudate into the airway lumen. This possibility is supported by the abundant occurrence of plasma proteins in asthmatic airways [18, 38, 52, 55, 61, 75, 123] and by observations in experimental animals. Inflammatory mediators such as bradykinin, histamine, leukotrienes, tachykinins, PAF-acether, and allergen applied to the tracheal mucosa of anesthetized guinea pigs produced acute extravasation of plasma and tracer macromolecules. Edema could not be identified but extravasated large solutes were recovered in tracheal luminal fluid within a few minutes after provocation [43, 111–113, 115].

In contrast to tracheobronchial venules, the pulmonary microvessels are resistant to histamine-type mediators [63, 64, 79, 114]. This aspect, together with abundant microvascular connections between the bronchopulmonary circulations, has stimulated a debate as to the relative importance of bronchial microvessels to fluid and protein exchange in pulmonary inflammation. In adult respiratory distress syndrome (ARDS) pulmonary edema and increased permeability to macromolecules in the pulmonary vessels and the alveolar wall are present [59], but bronchial venules may also leak plasma [117]. Wheezing is one of the symptoms of ARDS, and, as in bronchial asthma, survivors of ARDS may exhibit increased airway responsiveness to methacholine inhalation challenges [131]. Hence, it cannot be excluded that bronchovascular plasma exudation is one of the factors contributing to asthmalike symptoms in pulmonary inflammatory diseases.

It has been calculated that small changes in mucosal thickness could have a profound influence on the tendency to airways closure as well as explain airway hyperresponsiveness to bronchoconstricting agents [65]. Slight edema of tissues between the bronchial muscle and the epithelium would thus only marginally reduce the baseline caliber of the airway lumen and be difficult to detect, but could cause abnormally large increases in resistance to airflow during bronchoconstriction. It has not yet been studied whether airway edema, similar to pulmonary edema [26, 66, 127], may lower the threshold for sensory receptor stimulation.

In 1900, Fraenkel [46] suggested that extensive epithelial shedding is a distinguishing characteristic of asthmatic airways. This proposal has been repeatedly substantiated [e.g., 37, 91] and Dunnill [37] has suggested that mucosal edema and transepithelial passage of plasma exudate cause the shedding of epithelium. However, a significant volume of proteinaceous plasma can rapidly traverse the epithelial barrier without causing shedding [115]. Sustained inflam-

mation and effects of potent and toxic cellular mediators such as eosinophil-derived proteins [49] may be required for significant shedding to occur. As discussed above several additional consequences of plasma exudation in the airways relate to the presence of plasma protein-derived mediators in the exudate that are capable of producing bronchoconstriction and inflammation.

### **Intercellular Transepithelial Passage of Plasma**

Albumin is a normal constituent of tracheobronchial luminal liquid [17, 75, 104, 111]. Stockley et al. [135] determined the relation between sputum/serum concentration ratios and Stokes radius for 5 selected proteins in chronic bronchitis. During stable noninfected conditions there was a significant negative correlation between the ratio and the protein size consistent with a passive diffusion of these proteins [135]. During infection the ratio was increased more than 10-fold [21]. This finding tallies with observations in upper airways: Nasal washings obtained during viral rhinitis contain much elevated levels of serum proteins and kinin activity [6, 33, 95, 121]. Also in many noninfectious types of acute inflammation the epithelial permeability to macromolecules may increase dramatically. Large amounts of charged macromolecules such as albumin and uncharged fluorescein-labeled dextran (MW 150,000 daltons) traversed vascular and epithelial barriers of guinea pig tracheas superfused with mediators or challenged with allergen [43, 111, 115]. This highly increased permeability to inflammatory stimuli reversed spontaneously and was significantly prevented by drugs [42, 43]. A pinocytotic transport mechanism would not suffice to bring about such a sudden transepithelial passage of a large volume of plasma [17, 78]. Perhaps intercellular junctions of tracheobronchial epithelium can be opened for macromolecular passage as in alveolar epithelium in high-permeability pulmonary edema [28, 59, 99]. An ultrastructural study of inflamed guinea pig trachea has demonstrated that intraluminal horseradish peroxidase penetrates the wall between epithelial cells [129].

A series of observations of nasal liquid composition in atopic subjects challenged with allergen agrees with the notion that inflammatory stimuli induce a rapid, transient bulk flow of plasma macromolecules (and activation of peptide mediators) extravascularly and across the nasal epithelial lining [12, 120]. It is of great interest that antiasthma drugs, notably glucocorticoids and xanthines, reduce this plasma leakage [96, 108, 118].

Mediator-induced increase in the permeability of the epithelial barrier can obviously be as dramatic as that across the venular endothelial lining. Experiments indicate that the inflammatory plasma leakage does not require tissue destruction. Instead, epithelial permeability to large molecules may be considered an active, reversible process that is under the control of mediators, hormones, and drugs. The subcellular epithelial mechanisms involved in these permeability changes and details of intercellular pathways for leaking macromolecules remain unexplored.

### *DTPA—Small Solute Permeability*

In recent years, airway mucosal or epithelial “permeability” has received widespread attention as a pathogenetic factor. What is usually measured in studies of this kind of “permeability,” in particular in human subjects, is the rate of transfer of inhaled <sup>99m</sup>Tc-labeled diethylenetriamine penta-acetate (DTPA) into the blood. DTPA is a relatively small molecule (MW 492 daltons) and its passage across airway epithelial–endothelial barriers may be regulated by mechanisms entirely different from those involved in leakage of large plasma proteins. Hence neither vascular nor mucosal permeability to plasma macromolecules may be reflected in studies using DTPA. Furthermore, lung clearance of inhaled DTPA may largely be across alveolar barriers. Respiratory mucosal permeability determined with DTPA was not increased in asthma [40] and, when it was increased, as in smokers, this did not correspond to increased airway reactivity [69]. These observations may not be taken as evidence against a role for plasma exudation in asthmatic airways.

### **Plasma Proteins in Asthmatic Airways**

#### *Sputum and Bronchial Lavage*

Many characteristics of asthmatic airways can be studied by analyzing the composition and pharmacologic effects of sputum [35]. Although their origin was not determined, anaphylatoxins have been identified in asthmatic sputa [98]. Asthmatic sputa have been demonstrated to produce smooth muscle contraction [36, 54] and inhibition of ciliary motility [150]. The factors responsible for these effects may well have come from the serum. They may be preformed mediators or mediators produced by activation of plasma proteins at exudation.

Using chemical analyses Menders et al. [83] confirmed the presence of plasma proteins in asthmatic sputa. Ryley and Brogan [123] showed that the solid phase of asthmatic sputa contained large amounts of albumin and that glucocorticosteroid treatment reduced the plasma protein content along with improvement in lung function [123]. In addition, they studied bronchitic sputa and concluded:

This would imply that the sputum of the asthmatic patient had more in common with an inflammatory exudate than that of the chronic bronchitic . . . this hypothesis is supported by the finding of a greater proportion of serum albumin and a greater variety of plasma proteins in the asthmatic as compared with the bronchitic sputum [123].

In a more quantitative study Brogan et al. [18] confirmed these findings and showed that levels of plasma proteins, but not secretory proteins, were elevated in asthmatic sputa. A high degree of plasma exudate in the airway lumen may also differentiate asthma from emphysema [52] and cystic fibrosis [18]. Heilpern and Rebuck [55] not only demonstrated high levels of plasma proteins

in asthmatic sputa, but also showed that cromoglycate normalized these levels. Cromoglycate seems to share with several other antiasthma drugs antileakage effects directly on airway endothelial–epithelial barriers [42, 43].

Bronchoalveolar lavage is frequently performed in the clinical evaluation of lung diseases. It is used also in asthma but a relatively large contribution of alveolar liquids to the lavage fluid makes this technique less suitable for the identification of bronchial liquids. This point is illustrated in recent work by Lam et al. [75]. They found no difference in albumin levels between normals and asthmatics in large-volume bronchoalveolar lavage liquids. However, using a small volume lavage in a large bronchus they could demonstrate a 10-fold increase of albumin in asthmatic airways compared with controls [75].

### *Potential Consequences of Entry of Plasma into the Airway Lumen*

Although many pieces of information are consistent with the pathophysiological importance of plasma and plasma-derived mediators in airway lumen, this subject has not been extensively reviewed. Lord Florey, who was experimentally acquainted with the possibility that a considerable amount of fluid may “come directly from the vessels” into the inflamed airway lumen [45], mentions only in passing, in his excellent chapter on inflammation of mucous membranes, that plasma exudate may seep through the mucosa [44]. In 1882 Julius Cohnheim [24] discussed how inflammation and plasma exudation might produce different results in different tissues and emphasized that in cavitary organs there may be a passage of exudate across the mucosal barriers. Diseases with protein leakage into the gut have received attention due to the ensuing large fall in blood levels of plasma proteins [67]. Plasma exudate may escape into the gut lumen through a deranged mucosa and through an apparently intact mucosa [67]. Plasma albumin loss due to bronchial diseases has been suggested to occur [15, 53]. For obvious reasons a luminal entry of plasma exudate would have rather more serious consequences for the lower airways than for the gastrointestinal tract.

A rapid passage of exudate may increase the depth of the fluid layer in which the cilia beat, and hence cause marked inhibition of mucociliary transport [140]. Plasma exudate most likely participates in the formation of mucus plugs: fibrin formation would make the mucus firm and obstructive [34, 61]; albumin may interact with mucin to form viscous complexes [76]; plasma proteins may impede normal hydration of mucin [2]. The plasma exudate may enter peripheral airways and compromise the surfactant activity, which in turn may lead to small airway narrowing [77].

### **Antiasthma Drugs**

It is intriguing that different antiasthma drugs may prevent the mediator-induced microvascular leakage. Drug-induced inhibition of vascular leakage can-

not be expected to show an immediate reversal of edema because the rate of resolution of interstitial fluid is dependent on lymphatic drainage, which is a relatively slow process. However, if obstructive bronchial tone is dependent on a continuous supply of activated plasma protein mediators from leaky microvessels, an antileakage action might reverse the airway obstruction. It is of interest that protease inhibitors may prevent bronchoconstriction induced by various challenges in asthmatic subjects [105].

### *Glucocorticosteroids*

In 1940 Menkin [84] found that adrenal cortex extract inhibited inflammatory vascular leakage. Thirty-three years later Leme and Wilhelm [74] demonstrated in rats that corticosteron prevents mediator-induced increase in vascular permeability and that this drug inhibits the enhanced venular responsiveness brought about by adrenalectomy. Current developments include attempts to identify proteins induced by glucocorticoids. Probably by binding with specific receptors followed by induction of anti-inflammatory proteins, glucocorticosteroids inhibit or reduce vascular leakage. In guinea pig airways glucocorticoids such as budesonide may reduce leakage of plasma across both endothelial and epithelial barriers [42]. Glucocorticoids have been shown to reduce plasma exudation in inflammatory airway diseases. Ryley and Brogan [123] found a relationship between a lowering of the albumin concentration in sputa with steroid therapy and clinical improvement in an asthmatic subject. Based on serial measurements of neuraminic acid concentration (indicator of bronchial secretion) in asthmatic sputa, Keal [68] inferred that the effect of steroid therapy "lies in the reduction of transudate rather than in any change in the bronchial mucosal gland secretion." Moretti et al. [92] studied patients with both reversible airways obstruction and bronchitis and showed that 2 weeks' treatment with methylprednisolone brought about a dramatic reduction in the sputum concentration of albumin. Stockley and associates [93, 143] examined the effects of about 1 week's therapy with prednisolone on sputum composition in patients with chronic obstructive bronchitis. The patients had no acute chest infections and had, therefore, relatively low levels of serum proteins in their sputa [135]. Still, after a few days of treatment a significant reduction was recorded in the ratio of sol-phase sputum concentration to serum concentration of albumin [143].

An antiexudative effect would also reduce the entry of plasma proteins that have protective functions in the airway. The values of  $\alpha_1$ -antitrypsin followed the same pattern as those of albumin with steroid treatment [143]. However, despite the reduced  $\alpha_1$ -antitrypsin levels the inhibitory capacity of the sputum (evaluated on porcine pancreatic elastase) was increased, suggesting that the overall effect of glucocorticosteroids on the airway liquid proteinase-antiprotease balance may be beneficial [93].

Findings in nasal washing experiments lend further support to the theory of an antiexudative action of glucocorticosteroids. Treatment for 2 days with

prednisolone significantly reduced clinical symptoms as well as amounts of albumin (kinins, TAME-esterase activity, and histamine) in washings performed during nasal late reaction following challenge with allergen in 13 allergic subjects [118]. This study also produced the interesting information that generation of arachidonate products such as leukotrienes and prostaglandin D was not affected by the steroid treatment [118].

Attenuation of plasma exudation by glucocorticoids may contribute to the general efficacy of these drugs in asthma and to their potency in inhibiting late-phase asthmatic responses and reducing airway hyperresponsiveness.

### *Xanthines and Cromoglycate*

Antiasthma xanthines may be subdivided into adenosine-blockers such as theophylline and adenosine-nonblockers such as enprofylline [106, 108]. These xanthines seem to share a number of potentially important pulmonary anti-inflammatory effects [see 106]. Included among the anti-inflammatory actions is a vascular and epithelial antileakage effect that has been demonstrated in guinea pig airways [42, 106, 112, 113]. It has also been shown that both enprofylline and theophylline prevent the development of pulmonary edema in guinea pigs inhaling histamine [106, 114]. Furthermore, antiasthmatic doses of theophylline reduced both symptoms and plasma exudation in human nasal mucosa provoked with different amounts of allergen [96, 108]. Cromoglycate also reduced macromolecular leakage across endothelial–epithelial barriers in guinea pigs. This action was not dependent on which mediator had induced the plasma leakage, nor was it due to a reduction of mucosal/submucosal blood flow [42, 107]. The animal data may explain observations in asthmatic subjects reported by Heilpern and Rebeck [55] 15 years ago. They [55] stated that their study

... does not attempt to explain why sodium cromoglycate is also effective in non-allergic asthma. However, the evidence points to a previously unrecognized action of the drug, that of lowering albumin concentration in sputum to levels found in non-asthmatic patients. The significance of this finding awaits further study.

The possibility that an anti-plasma-leakage action of cromoglycate is important and may compare favorably with other proposed mechanisms of action of the drug in asthma is discussed elsewhere [107].

### *Sympathomimetics*

It has been widely held that the anti-inflammatory effects of sympathomimetic drugs reflect vasoconstriction and diminished blood flow to inflamed tissues. In addition, it has now been demonstrated that these drugs have a vascular anti-permeability property. This is  $\beta_2$ -adrenoceptor mediated and more than outweighs the slightly proinflammatory blood flow increasing effect produced by the  $\beta_2$ -receptor agonists [113, 114, 116]. About 10 years ago we demonstrated in



guinea pigs that the  $\beta_2$ -receptor agonist terbutaline given systemically or by the inhaled route effectively prevented the development of pulmonary edema induced by subsequent exposure to histamine [114]. This protection might have been via effects on bronchial microvessels, because later studies have demonstrated that terbutaline reduces plasma leakage from the tracheobronchial microcirculation [113].  $\beta_2$ -receptor mediated antipermeability effects on airway mucosa and microvessels [42, 113] in asthma remain to be examined.

### *Mediator Antagonists*

A pharmacologic mediator antagonist is by definition effective with some specificity only against 1 type of mediator. Thus, antihistamines, leukotriene antagonists, PAF-acether antagonists, and tachykinin antagonists among others will specifically antagonize leakage produced by corresponding agonists. Since the leakage-regulating endothelial cells of postcapillary venules harbor specific receptors for a large variety of inflammatory mediators, it cannot be expected that a single mediator antagonist alone could produce an acceptable antileakage response in inflammation.

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