

Effects of luminal antigen on intestinal albumin and hyaluronan permeability and ion transport in atopic patients

Tina W. Knutson, MD,^a Ulf Bengtsson, MD,^b
Anders Dannaeus, MD,^a PhD, Staffan Ahlstedt, MD, PhD,^c and
Lars Knutson, MD, PhD^d Uppsala and Gothenburg, Sweden

Background: Increased luminal transport of proteins and fluid is part of the inflammatory reaction in inflammatory disease of the bowel and of the airways in allergic diseases and asthma. The objective of this study was to determine intestinal appearance rates of albumin and hyaluronan in vivo in atopic patients allergic to birch, as well as changes in net jejunal transport of monovalent ions and water induced by the antigen.

Methods: Secretion studies were performed with the use of a segmental jejunal perfusion system with a small two-balloon, six-channel tube. The intestinal mucosa was challenged with birch allergen in patients allergic to birch and in matched control subjects (n = 12 in both groups).

Results: In patients, but not in control subjects, the luminal antigen induced a net increase in albumin of 2689 ± 567 $\mu\text{g}/\text{cm}/\text{hr}$ and in hyaluronan of 2609 ± 737 $\text{ng}/\text{cm}/\text{hr}$ ($p < 0.01$ vs control subjects in both cases). Furthermore, basal net absorption of Cl^- ions, Na^+ ions, and water was converted to a net secretion after antigen challenge.

Conclusion: Exposure to antigen normally acting on the respiratory tract induced increased permeability of the gastrointestinal mucosa. This would suggest less organ specificity and more general allergic recognition shared by several immunocompetent tissues in the body, probably mediated by circulating IgE antibodies. (*J Allergy Clin Immunol* 1996;97:1225-32.)

Key words: Allergy, anaphylaxis, birch, food intolerance, intestinal permeability, ion transport, segmental intestinal perfusion

When the body's immune mechanisms show an exaggerated response to grains of birch pollen or to foods, the result may be unpleasant symptoms of allergy.¹ Proposed mechanisms of this heightened response include, first and foremost, immunologic reactions elicited by antigen-antibody complexes, as well as cell-mediated damage and changes in

Abbreviation used

PEG: Polyethylene glycol

From ^athe Department of Pediatrics, University Hospital, Uppsala; ^bthe Division of Allergology, Sahlgrens Hospital, Gothenburg; ^cPharmacia Diagnostics, Uppsala; and ^dthe Department of Surgery, University Hospital, Uppsala.

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Reprint requests: Lars Knutson, MD, Department of Surgery, University Hospital, S-751 85, Uppsala, Sweden.

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permeability with consequent mucosal edema.^{2,3} An inflammatory condition of the mucosa might increase the leakage from the capillary bed to the intestinal lumen.⁴ Food aversion, intolerance, and IgE-mediated allergy are difficult to distinguish in both children and adults. The available methods entail general exposure of a whole mucosa, such as inhalation provocations in patients with asthma, and are intended to provoke symptoms, which are sometimes systemic and always potentially dangerous.⁵

In previous perfusion studies in vivo, we found that patients allergic to birch and intolerant to cow's milk responded to intestinal antigen challenge with an increase in the release of inflammatory substances from mast cells and eosinophils.⁶ The release of histamine, prostaglandins, and eo-

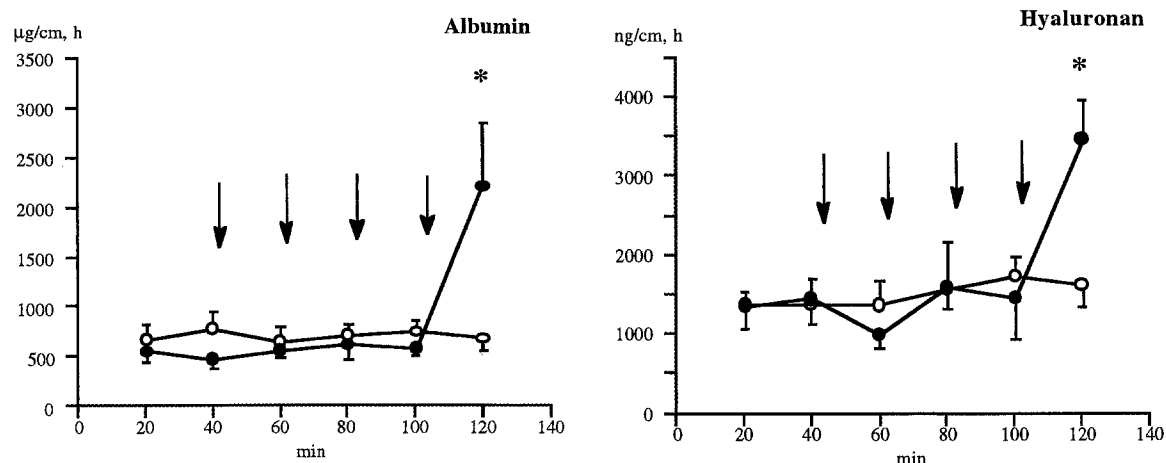


FIG. 1. Repeated intestinal provocation with increasing doses of birch allergen, starting with the weakest concentration (arrows). Only the highest concentration (1:100) resulted in a significant increase in permeation of albumin (left) and hyaluronan (right) onto the lumen in the patients (filled circles, $n = 6$) not followed by control subjects (unfilled circles, $n = 6$). Data are given as means $\pm$ SEM. * $p < 0.05$ versus control subjects.

sinophilic cationic protein into the intestinal lumen was enhanced. Another consistent finding was a substantial increase in the luminal permeation of albumin and hyaluronan (formerly called hyaluronic acid), a high molecular weight glycosaminoglycan likely of connective tissue origin. Similar observations have been made in Crohn's disease and after gliadin challenge in celiac disease, suggesting that the antigen challenge contributes to structural alterations such as subclinical lymph stasis and edema.^{7,8} In animals, antigen challenge resulted in specific transport abnormalities with a decrease in ion absorption.⁹ In fact, it was concluded that antigen-stimulated intestinal secretion was triggered by mucosal anaphylaxis.¹⁰

The aim of this study was to determine the effect of antigen challenge on intestinal appearance of substances from the plasma and/or lymph in patients allergic to birch as compared with healthy control subjects. Mucosal reactions likely play an important role in the pathophysiology of secretory changes associated with intestinal anaphylaxis and constitute a driving force for changes in active ion and water transport.¹¹ In a number of animal models, antigen stimulation has been associated with profound effects on luminal transport of Cl^- , Na^+ , and K^+ ions and on the function of the epithelium of the small intestine.⁹

METHODS

Patients and control subjects

Twelve study patients, four women and eight men, aged 19 to 31 years (median age, 24 years) were com-

pared with 12 sex- and age-matched healthy control subjects aged 21 to 29 years (median age, 26 years). The patients were seen at the Allergy Clinic of the Department of Pulmonary Medicine, University Hospital, Uppsala. Studies were performed in two groups of study patients with two matched groups of control subjects. The patients had proven allergy to birch pollen, and in all of them the result of a skin prick test performed at the time of the study, was positive. They had all reacted with asthmatic symptoms to inhalation of birch pollen during the pollen season, and they had positive RAST results. The studies on these patients were all carried out during the winter, when there is no exposure to birch. The patients and control subjects completed a standardized questionnaire regarding previous allergic reactions, and the study was approved by the Ethical Committee of the Medical Faculty of Uppsala University.

Intestinal perfusion procedures

The patients and control subjects were intubated orally with a polyvinylchloride tube with an outer diameter of 16F (5.3 mm). A detailed description of the technique is given elsewhere.^{6,12} The tube had six channels and was provided distally with two elongated latex balloons, 40 mm long, each separately connected to one of the smaller channels. The two wider channels in the center of the tube were for infusion into and aspiration of perfusate from the test segment, respectively. The two remaining peripheral, smaller channels were used for administration of marker substances or for drainage. A Teflon-coated guide wire was used for insertion of the tube, and the tip of the tube was provided with an 8 cm long weight made from short tungsten plates to facilitate positioning in the desired part of the small intestine, as verified fluoroscopically. Continuous gastric drainage

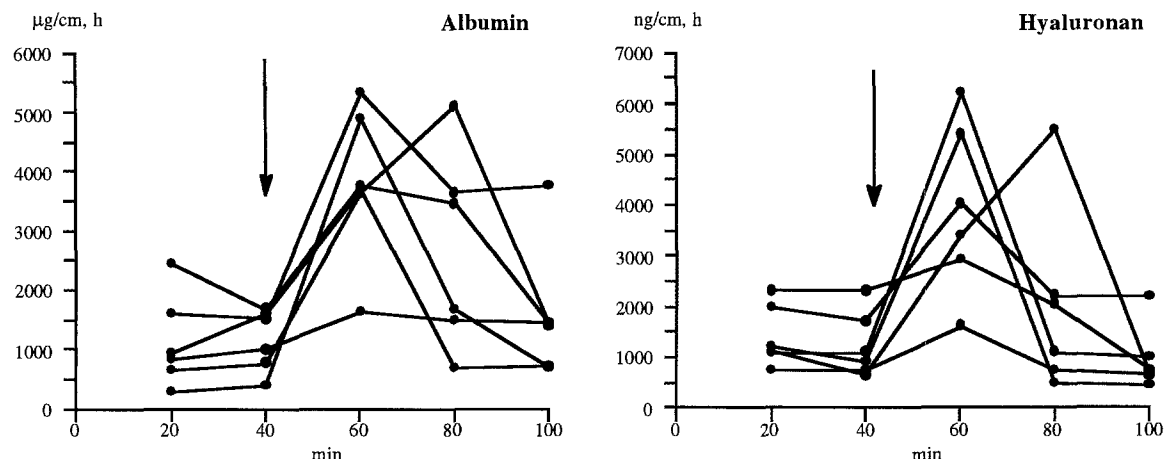


FIG. 2. Individual appearance rates of albumin (**left**) and hyaluronan (**right**) in the perfusate after the highest concentration of antigen had been given (*arrow*) in a new patient group ($n = 6$). In one of the patients the permeation rate of both albumin and hyaluronan increased further in the second 20-minute period after luminal challenge.

was achieved through a separate tube. The balloons were inflated with 30 to 34 ml of air, and the test segment was rinsed with 154 mmol/L NaCl, at 3 ml/min for 30 minutes, with a syringe pump. After the rinsing period, the segment was continuously perfused at 3 ml/min with a solution containing 10 mmol/L glucose, 5.4 mmol/L KCl, 120 mmol/L NaCl, 2 mmol/L Na_2HPO_4 , 1 gm/L polyethylene glycol (PEG) with a molecular weight of 4000 d, and 35 mmol/L mannitol. The osmolality of the perfusion fluid was 290 mosm/L. Carbon 14-labeled PEG (^{14}C PEG, molecular weight, 4000 d, 2.5 $\mu\text{Ci/L}$; Amersham Laboratories; Buckinghamshire, England) was added to the perfusion solution as a volume marker. To exclude contamination of the jejunal perfusate with gastric fluid, phenol red (50 mg/L in 154 mmol/L NaCl solution) was infused into the stomach through the drainage line of the gastric tube (1 ml/min) and was measured in the jejunal effluent. All solutions were infused at 37°C; they were collected from the stomach by active suction (Ameda Suction Pump Type 23; Ameda AG, Zug, Switzerland) and from the test segment by passive evacuation through gravity drainage. Samples of perfusate were collected on ice at 20-minute intervals and frozen at -70°C in plastic vials. Perfusions were performed for a total period of 100 to 140 minutes.

Allergen and experimental design

In the first experiments, dose-response curves were produced in six patients and six control subjects. Birch allergen (Pharmalgen Birch, 100,000 BE/ml; Pharmacia Diagnostics, Uppsala, Sweden) was serially diluted with the perfusion fluid mentioned previously to concentrations of 1:100,000, 1:10,000, 1:1000 and 1:100. After a 40-minute baseline period, these antigens were infused in amounts of 10 ml every 20 minutes in increasing concentrations, starting with the lowest concentration. In the remaining 12 subjects (6 patients and 6 control

subjects) only the effect of the highest concentration of antigen (1:100 BE/ml) was evaluated.

Analytic measurements

^{14}C -PEG was assayed in duplicate samples of the effluent by liquid scintillation counting (LKB Rackbeta II; Wallac OY., Turku, Finland) and phenol red was determined spectrophotometrically at 520 nm (model 101; Hitachi Ltd., Tokyo, Japan), after alkalization to pH 11, with Na_3PO_4 . Na^+ and K^+ ions in the effluent were measured by flame photometry (Flame Photometer FLM3; Radiometer, Copenhagen, Denmark), and Cl^- ions were measured by autotitration (ChlorideMeter 920 ml; Corning Ltd., Halstead, England).

The perfusate samples were analyzed in duplicate for their contents of hyaluronan and albumin. Hyaluronan and albumin were measured (50 μl sample) by radioimmunoassays according to the manufacturers' specifications (Pharmacia Diagnostics AB).¹³ The coefficients of variation for these methods were below 8%.

Calculations and statistics

Data are presented as means $\pm$ SEM of luminal appearance rates in 20-minute periods in Figs. 1 and 3 to 5 and as individual values in Fig. 2. The luminal appearance rate was calculated as: concentration in the effluent $\times$ perfusion rate $\div$ length of the jejunal segment corrected for changes in concentration of the volume marker ^{14}C -PEG. Net outputs of Na^+ , K^+ , and Cl^- ions and water were also calculated according to this formula and expressed as values of absorption or secretion. All results were analyzed individually, and the statistical significance of differences was determined by nonparametric tests with comparisons both between basal and peak values with each patient serving as his or her own control and between patients and control subjects, comparing net increases. The

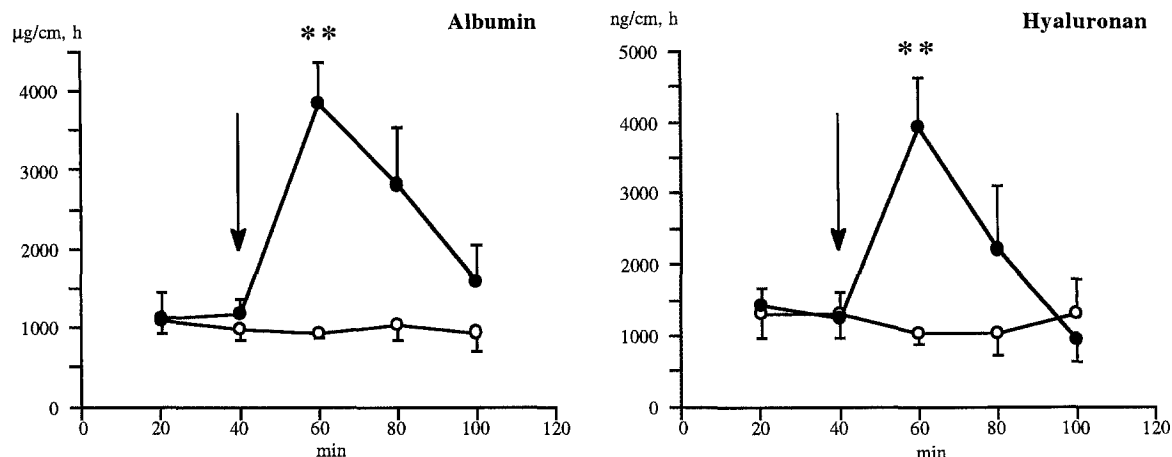


FIG. 3. Luminal permeation of albumin (**left**) and hyaluronan (**right**) in the same patient group as in Fig. 2 (filled circles) compared with that in a matched group of control subjects (unfilled circles, $n = 6$). Data are given as mean values $\pm$ SEM. (** $p < 0.01$ vs control subjects).

Mann-Whitney U test was used for unpaired values, and Wilcoxon's test was used for paired values. All experiments had been approved by the Ethical Committee of the Medical Faculty of Uppsala University, and both the patients and control subjects gave informed consent to participation in the study.

RESULTS

As in earlier studies, the perfusate instilled into the segment of the small intestine was effectively recovered by passive drainage (i.e., by gravity with no suction applied) and did not escape from the test segment to any appreciable extent.⁶ The 12 control subjects did not experience any discomfort or display any signs attributable to possible allergic reactions. Of the 12 patients allergic to birch, four experienced abdominal discomfort when the highest dose of antigen was given. In three of the patients, the perfusate leaving the segment became whitish and opalescent when the highest concentration of antigen was given. These patients also showed the highest concentrations of hyaluronan and albumin in the perfusate (Fig. 2).

The serum concentrations of albumin and hyaluronan were determined in six of the control subjects and four of the study patients before and after intestinal challenge. All values were within the normal reference range, with no sign of an increase after challenge (albumin, 36 to 48 gm/L; and hyaluronan, 10 to 100 μ g/L). The prechallenge ratio between the jejunal fluid and serum concentrations in the two groups was 0.65×10^{-3} for albumin and 1.72 for hyaluronan.

The basal appearance rates of hyaluronan and

albumin did not differ between the study patients and control subjects. Challenge with allergen at concentrations of 1:100,000 to 1:1000 only slightly increased these rates in the patient group. Only the highest concentration of allergen increased the appearance of hyaluronan and albumin in the patients, with no effect in the control subjects (Fig. 1). The recovered amount of hyaluronan in the patient group, when the dose-response design was used, increased from a mean basal value of 1395 ± 268 to 3467 ± 498 ng/cm/hr, and of albumin from 512 ± 45 to 2208 ± 646 μ g/cm/hr, when the highest dose of the antigen was given ($p < 0.05$ vs basal value for both substances). Although this design may seem somewhat cautious, the protocol was not changed during the first six experiments, because there was still a possibility that the provocation would evoke systemic effects. This design, however, did not permit an assessment of the duration of the increased permeability to hyaluronan and albumin.

The second part of the study was performed in another six patients and six matched control subjects by using only a concentration of birch allergen of 1:100 infused in an amount of 10 ml during the 20-minute perfusion period after 2 basal periods. In the 20-minute period after the administration of the antigen, the appearance rate of hyaluronan increased from a mean basal value of 1340 ± 255 to 3948 ± 683 ng/cm/hr, and that of albumin increased from 1157 ± 264 to 3845 ± 524 μ g/cm/hr ($p < 0.05$ vs basal value for both substances; Fig. 3).

The effects of birch pollen antigen on the net

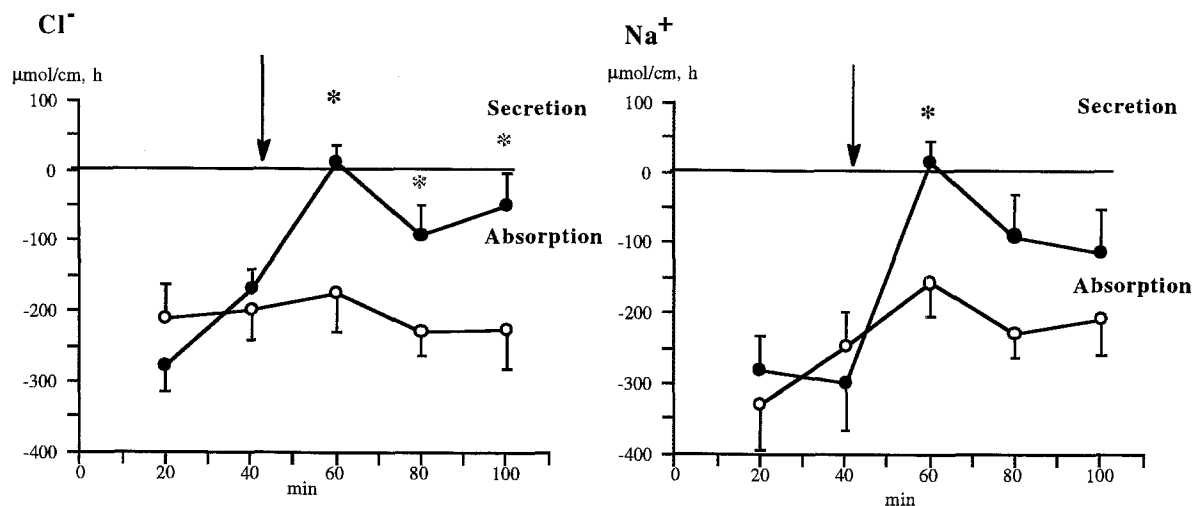


FIG. 4. A net chloride (left) and sodium (right) absorption was changed into a net secretion when the antigen was given (arrow). Filled circles, Patients ($n = 6$); unfilled circles, control subjects ($n = 6$). Data are given as means $\pm$ SEM. * $p < 0.05$ versus control subjects.

absorption or secretion of Na^+ , K^+ , and Cl^- ions and water in the luminal segments were also examined. Under baseline conditions, there was a net absorption of Na^+ , K^+ , and Cl^- ions and water from the proximal jejunum in both the study patients and the control subjects (Figs. 4 and 5). Challenge with luminal antigen in the study patients, however, converted the baseline absorption into a net secretion for Na^+ and Cl^- ions and water. Perfusate values for Na^+ ions changed from a net absorption (-290 ± 57) to a net secretion (16 ± 31) ($p < 0.05$); perfusate values for Cl^- ions also changed from a net absorption (-223 ± 33) to a net secretion (11 ± 27) ($p < 0.05$); water transport for the segmental perfusate also changed from a net absorption (-11 ± 5.9) to a net secretion (2 ± 2.7 ml/20 min) ($p < 0.05$). No significant change from baseline was observed for K^+ ions (-11 ± 2.0 to -3 ± 1.5 $\mu\text{mol}/\text{cm}/\text{hr}$) ($p > 0.05$).

DISCUSSION

Challenge of an isolated jejunal segment with birch pollen induced a more than fourfold increase in the appearance of albumin and a threefold increase in the appearance of hyaluronan in the intestinal lumen in atopic patients but not in control subjects. This response took place in the first 20 minutes after challenge, suggesting that it was not a late-phase type reaction. It is reasonable to attribute this reaction of the jejunum to a local antigen-induced reaction within the intestinal mucosa. These patients did not experience allergic symptoms of the usual target organs for birch pollen; that is, they did not have rhinitis, conjunc-

tivitis, or bronchospastic reactions during intestinal challenge.

In allergic diseases of the respiratory tract, such as asthma, bronchitis, and rhinitis, the severity of the condition is correlated with changes in mucosal exudation and entry of plasma proteins into the airway lumen.^{4, 14, 15} This exudation is proposed to be a first-line respiratory defense mechanism. This exudative "defense" mechanism, however, is not as well defined in the intestine. Studies with intraduodenal and intrainestinal allergens have shown both exudation and uptake of plasma tracer in the intestinal lumen in the rat.^{16, 17} Luminal antigens contribute to the pathogenesis of gastrointestinal disorders, such as cow's milk enteropathy, eosinophilic gastroenteritis, celiac disease, and perhaps inflammatory bowel disease.¹⁸⁻²⁰ In celiac disease, the small bowel mucosa of genetically predisposed individuals is damaged after ingestion of gluten and similar proteins in wheat, rye, and barley. The pathophysiologic event that leads to the characteristic morphologic changes in this disease is primarily immunologic, including both reactions elicited by antigen-antibody complexes and cell-mediated damage.²¹⁻²⁶ It has been reported that gliadin challenge may induce histologic changes of the mucosa as early as 2 hours after gliadin administration.²⁴⁻²⁶ Such inflammatory damage to the mucosa might influence exudation of plasma proteins into the bowel lumen. Exudation through the jejunal mucosa during challenge with gliadin has been demonstrated in patients with celiac disease.⁸ Increased appearance of hyaluronan has been detected in the intestinal lumen in patients with

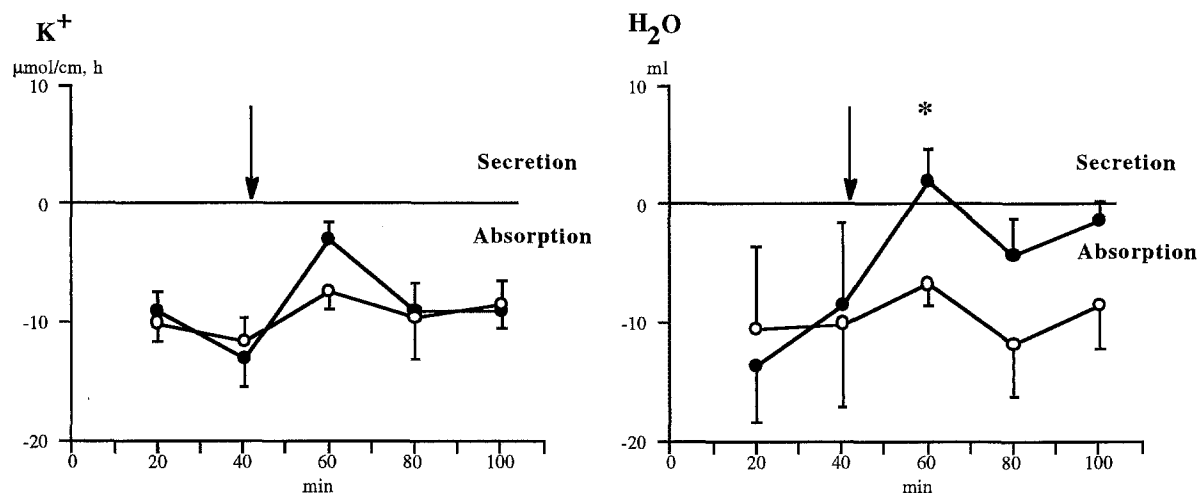


FIG. 5. Water (right) but not potassium absorption (left) was changed into net secretion after luminal exposure to antigen. Filled circles, Patients ($n = 6$); unfilled circles, control subjects ($n = 6$). Data are given as means $\pm$ SEM. * $p < 0.05$ versus control subjects.

Crohn's disease and was found to increase in line with disease severity.⁷ The results of several clinical studies also suggest an increased uptake of macromolecules across the mucosal barrier during inflammatory disorders in human beings.²⁷

In food-induced eczema and allergic eosinophilic gastroenteritis, the events taking place also involve a late-phase reaction with arrival of neutrophils, eosinophils, and mononuclear cells hours after the exposure to allergens.²⁸ Both these diseases and celiac disease, however, involve an element of repeated intestinal exposure to the disease-specific allergen, leading to the intermittent degranulation of mast cells, which may initiate the particular pathologic features seen.²⁹ In atopic patients allergic to birch allergen, there is no exposure of the gastrointestinal mucosa to this allergen in wintertime. The view that the gastrointestinal mucosa could react to topical challenge in a way that resembles the reaction in the upper airway is an intriguing concept.

In our study, albumin concentrations in jejunal perfusion fluids, measured under basal conditions both in patients and control subjects, averaged about 0.1% of that in serum, reflecting passive leakage of proteins from the plasma and interstitial fluid compartments. After intestinal challenge with antigen in study patients, the increase in the appearance rate of albumin reflected leakage of this protein from serum. The serum concentrations of hyaluronan, however, were close to the measured concentrations of hyaluronan in the jejunal perfusate. Thus a passive leakage from the plasma compartment cannot explain the high jejunal perfusate

concentrations of hyaluronan. A more likely source of hyaluronan is the lymph or interstitial fluid, because the concentration of hyaluronan is about 100 times higher in lymph than in plasma.³⁰ In contrast, albumin concentrations in lymph are close to those in serum. This would mean that the increase in the appearance rates of albumin and hyaluronan observed after intestinal challenge with birch allergen should reflect a lymph or interstitial fluid edema with enhanced leakage of interstitial constituents. This presumption of lymph leakage was further clinically supported by the observation that the perfusion fluid in three patients became opaque, almost like milk, after challenge with the highest concentration of antigen. These three patients also had the highest appearance rate of hyaluronan detected. The development of a local intestinal edema could also explain why more patients than control subjects experienced discomfort and somewhat colicky pain when challenged with the highest concentration of antigen.

Mucosal reactions constitute an important part of the pathophysiology of secretory changes associated with intestinal anaphylaxis, and such reactions induce changes in active ion and water transport. A variety of in vitro models of intestinal anaphylaxis have been developed. The experiments have mainly focused on responses to antigens added to the serosal side or on delayed responses to luminal antigens, but immediate changes in ion transport in response to luminal antigen have also been described.³¹⁻³³ Albumin and especially hyaluronan have unique water-binding properties and influence both water and ion trans-

port and osmotic activity.³⁴ In this in vivo model, stimulation with luminal antigen had a pronounced effect on luminal transport of both Cl^- and Na^+ ions and water. Basal absorption of ions and water was changed into a net secretion during the first 20 minutes after challenge, and Cl^- ion secretion was still significantly increased compared with that in control subjects during the next two 20-minute periods. Several different explanations for the increase in ion and water transport can be suggested. An osmotic drag can be induced by the increased luminal appearance of large macromolecules, such as albumin and hyaluronan, and this can cause an increase in luminal transport. Several mediators, such as prostaglandins and vasoactive intestinal peptide, are also known to stimulate active ion transport^{35, 36} and can be released during a local anaphylactic reaction. A direct action by mucosal anaphylaxis on epithelial pump mechanisms influencing ion transport has been demonstrated in vitro by Crowe et al.³⁷

In conclusion, we have shown that a very transient luminal challenge of the small bowel with birch allergen in atopic patients induced an increase in the appearance rates of albumin and hyaluronan, when compared with control subjects. The appearance of these substances in increasing intestinal concentrations after challenge is probably related to a lymphedema and enhanced leakage from the interstitial fluid or lymph. A basal net absorption of Cl^- and Na^+ ions and water was changed into a net secretion with a more sustained decrease in the absorption of Cl^- ions throughout the study. The proposed target organ of allergic stimuli in these patients is, however, the respiratory tract. The presence of an allergic predisposition of the gastrointestinal mucosa, as indicated in this study, would suggest a less organ-specific and more general allergic recognition shared by several immunocompetent tissues in the body, probably locally mediated by circulating IgE antibodies.

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