THE ROLE OF MUCUS AND ITS COMPONENTS IN PROTECTION AND REPAIR WITHIN THE ALIMENTARY TRACT MUCOSA: POLISH EXPERIENCE

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The Gastroenterology Research Laboratory at New York Medical College, New York City, NY, directed by Prof. Dr. George B. Jerzy Glass and after his retirement by Prof. Dr. Bronislaw L. Slomiany and Prof. Dr. Amalia Slomiany served as a lunching pad for successful careers in exploration of mucus for Dr. Andrzej Gindzienski and Dr. Krzysztof Zwierz and Janusz Badurski at the Medical School in Bialystok, Poland as well as Dr. Jerzy Sarosiek at Gastroenterology Research Laboratory, University of Virginia Health Sciences Center, Charlottesville, VA and currently, Gastroenterology Research Laboratory, Kansas University Medical Center, Kansas City, KS, USA. The dynamic and insightful research endeavors implemented at the Medical School of Bialystok revealed new information regarding enzymatic pathways of mucin synthesis especially its carbohydrate components such as hexosamines. These discoveries become instrumental in our understanding of the alimentary tract mucin synthesis and function in health and disease. Similarly innovative mucus research conducted across the Atlantic Ocean uncovered the novelty of mucin elaborated within the esophageal submucosal mucus glands in humans by demonstration that its chemical characteristics are different both from human salivary and gastric mucins. In addition, a novel method for the measurement of the thickness of the gastric mucus layer ex vivo in humans has also been developed. These pioneering works are continued at both mucus exploration centers attracting younger generation of investigators enticed by the mystery of the structure and function of the mucus barrier and its leading role in mucosal protection against injury as well as immediate and unequivocal contribution to mucosal repair and reconstitution process.

Key words: mucus, mucin, carbohydrates, fatty acids, phospholipids
INTRODUCTION

Mucus must be the most efficient protective mechanism considering the fact that an entire living world in oceans, seas, lakes and rivers is covered by mucus or mucus-like structures. No wonder that all systems in animals and humans, i.e. alimentary, respiratory, reproductive, and genitor-urinary that interact with the exogenous environment and are capable to maintain the right degree of hydration are also covered with the mucus layer.

It is relatively easy to imagine that considering the enormous intensity and variation in exogenous insults delivered intraluminally, the survival of the surface epithelium would have been highly unlikely without the protective quality of the mucus layer along the alimentary tract mucosa. The entire issue becomes even more perplexed considering sometimes the highly challenging components of the endogenous secretions, especially enormous concentration of the luminal hydrogen ion within the gastric lumen and plethora of proteolytic enzymes along the alimentary tract as well as iatrogenic impact of the ever growing list of medications.

No wonder, that during early ages of the exploration of the alimentary tract protective phenomenon, especially during contemplation of the phenomenon: "why the highly digestive gastric secretion does not digest its own mucosa", the mucus layer as a potential answer became a quite intellectually feasible explanation.

The beauty of the mucus layer is its preventive protective potential, its galaxy of components, extraordinarily dynamic nature, and quite powerful potential for preservation and restoration of the integrity of so delicate structures of the alimentary tract epithelium.

The efficacy of the mucus layer in its effective protective response to plethora of highly variable challenging factors is based on the multifactorial nature of its composition and sophisticated structure that is profoundly enhanced through an interaction with other mucus components, both originated from endocytoplasmic synthesis and released from the cell membranes during dynamic process of the living epithelial cell renewal.

Thus exploration of the mucus composition, chemical structure of its major and minor components, its physical properties during the luminal challenge with exogenous and endogenous injurious factors preoccupied minds of many investigators worldwide and Poland has been always at the center of this exploration.

Perspective from Bialystok Medical School, Bialystok, Poland
(by A. Gindzewski and K. Zwierz)

At the beginning of our work in Department of General Chemistry of Bialystok Medical Academy in early 60-ties, we were interested "why doesn't the stomach digest itself"(1) and the role of aminosugars metabolism in this protection. The human gastric mucosa was obtained from university and district hospitals as a result of surgical gastric resection, a mostly used medical treatment of gastric and duodenal ulcer on that time.
First, we found the presence of aminosugars in human gastric tissue and determined the amount of glucosamine and galactosamine in resected gastric specimens (2) and later, the influence of ethanol on its content in experiments in vitro (3). Than we demonstrate possibility of incorporation the radioactive glucose (3) and galactose (4) to human gastric glycoprotein, through glucosamine and galactosamine low molecular intermediates (5).

In the next step we found the activity of glucosamine isomerase, the first enzyme involved in hexosamine metabolism (6). Consequently, we started searching for another enzymes and metabolic intermediates of aminosugars. We found in human gastric extracts the presence of N-acetylglucosamine and its 1 and 6 phosphates, UDP-N-acetylhexosamines and N-acetylneuraminic acid (7). As a result of the presence of N-acetylglucosamine, we developed searching for its kinase (8). The enzyme was purified and its properties were determined (9). In the next years we determined the activity of glucosamine-6-phosphate acetyl transferase (10), described enzymatic degradation and epimerization of UDP-N-acetyl-D-glucosamine (11, 12), and degradation of glycoconjugates in human gastric mucosa (13).

In the human gastric mucosa we detected activity of lysosomal exoglycosidases acting on glycoconjugates: N-acetyl-β-glucosaminidase, α-fucosidase, β-galactosidase and α-mannosidase (13). Scheme of aminosugars metabolism in

![Scheme of aminosugars metabolism in human gastric mucosa](image)

*Fig. 1. Pathway of hexosamine constituents of gastric glycoprotein.*
human gastric tissue and our achievements in this area are presented on Fig. 1. The results obtained in our laboratory demonstrated intensive aminosugars metabolism in the human gastric mucous membrane and mucin biosynthesis.

Simultaneously with biosynthesis and degradation of gastric glycoconjugates, we were interested in stimulated and unstimulated gastric mucus secretion (14-16). Our investigations areas were strongly supported by dr GBJ Glass proposal of work in his New York Medical College GI laboratory, where we got one year fellowship positions (dr Zwierz in 1971-1972, see Picture 1, and dr Gindzienski in 1974-1975). Dr Zwierz's major research objective was the effect of stress in Ghosh-Lay rats on depletion of the mucous barrier and morphology of the gastric mucosa (17). Dr Gindzienski's major research objective in this laboratory was purification of gastrone, an inhibitor of gastric acid secretion, extracted from canine antral mucosa (18).

Our investigations in Bialystok, initiated in dr's Glass laboratory, targeted structure and function of gastric mucus. We prepared a few reviews on mucin biosynthesis, as well as mucus and mucin properties and structure (19-24). In our experiments, we started work with isolation and fractionation of gastric mucus from scrapings of human gastric mucosa. Lyophilized samples were difficult to solubilize, and we found that the best solubilizer was 1% SDS solution.

Additional improvement was obtained with mixture of SDS and 2-mercaptoethanol. After solubilization with both substances, using gel

*Picture 1. Prof. GBJ Glass with Dr. K. Zwierz during his fellowship at New York Medical College, New York City, NY.*
electrophoresis on mixed polyacrylamide-agarose gels, we found a high molecular weight glycoprotein and additional protein material released on that treatment (25). We also found, that in lyophilized samples, mucus irreversibly aggregates and only partial solubilization could be obtained. Supplementing samples with fresh mucus, demonstrated full mucus solubility in both SDS and 2-mercaptoethanol mixture, with decrease of mucin molecular weight and release above mentioned proteins (26). Released proteins created a problem of their participation in mucin structure.

Great progress in gastric ulcer medical treatment deprived us of human stomach specimens and we continue our work with the pig ones. Released proteins from SDS - 2-mercaptoethanol treated mucin samples inspire us to elaborate our

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Fig. 2. A diagram depicting the spatial dynamics of interaction between the basic mucin molecule and various non-covalently bound phospholipids, glycolipids, neutral lipids and fatty acids. A. Side view. B. Top view. (from 31).
own method for mucin purification (27). Results of our investigations on the
released proteins confirmed suggestions of other authors, that released material
originate from proteolytic cleavage of mucin particles, before or during
purification procedure. The proteins were still bound to glycoprotein with disulfide
bonds, which may be cleaved by 2-mercaptoethanol treatment. In our attitude to
this problem, we determined molecular weight of the proteins, released from pig
specimen at pH 7.0, as 100 and 140 kDa (28). At the present, we work with
transmembrane MUC1 mucin in cancer tissues, also in the GI tract.

The View from the Kansas University Medical Center, Kansas City, Kansas, US.
(by J. Sarosiek)

Although since 1986 I continuously explored the mucus enigma in various
university research settings in the United States, making inroads by using more and
more improved experimental tools, still my first encounter with the mucus theme
goes back to times of the early 1960-ties at the Medical School in Bialystok, Poland.

The Beginning at the Bialystok Medical School.

The first seeds of the mucus saga have been planted in my mind during my
student's life at the Medical School in Bialystok. As 1st year student I joined so
called Scientific Circle, a kind of scholarship at the Department of General
Chemistry under leadership of two great teachers: Dr. A. Gindzienski and Dr K.
Zwierz and a very colorful Chairman of the Department: Professor J. Popowicz.
During those years of early scholarship I felt a great chemistry of the general
chemistry around the measurements of chemical components of mucin, the major
component of mucus, such as galactose, glucosamine and galactosamine.

As the Medical School graduate I had a great privilege to join the faculty of
the Department of Internal Medicine under fascinating leadership of Prof. Beata
Bogdanikowa, whom I consider as Dr. Maria Sklodowska-Curie of Polish
Science of Serum Proteins. During those early years I had a pleasure to learn
some basics in the clinical laboratory research trenches from a very young, very
restless research wise and with great imagination, Dr. Janusz Badurski.

This encounter allowed us to explore for the first time in humans the impact
of systemic disease, rheumatoid arthritis on chemical composition and secretion
of gastric mucus components demonstrating profound impairment in its
protective quality (29). During those early years Dr. J. Badurski had a privilege
to receive a scholarship from the Gastroenterology Research Laboratory at New
York Medical College personally directed by world-wide leader in mucinology,
already established, Prof. George B. Jerzy Glass, MD, PhD. Dr. Janusz Badurski
conducted pioneering research that served as a nidus for his subsequent dynamic
research and teaching career.

The research scholarship and collaboration with Prof. G. B. J. Glass of two
other young, very dynamic researchers and great visionaries of the mucus
research arena, who co-author this chapter: Dr. Andrzej Gindzienski and Dr. Krzysztof Zwierz established the Bialystok Medical School as one of the most dynamic in the scientific world of exploration of mucus composition and its protective function. Furthermore, my collaboration with very insightful Dr. Wiktor Laszewicz, currently Professor and Chairmen of the Gastroenterology Clinic in Bialystok and its previous Chairmen, Prof. Antoni Gabryelewicz who had always open mind and desire to help young and restless investigators, resulted in significant progress in various mucus related research areas, including the clinical impact of histamine-2 receptor antagonist, ranitidine, on gastric mucus secretion stimulated by pentagastrin (30).

Subsequent Exploratory Research at the New York Medical College, New York.

I had a great privilege to receive twice an invitation from Prof. Bronislaw L. Slomiany, PhD., Director of Gastroenterology Research Laboratory at New York Medical College, New York City, NY, the most dynamic and talented mucus and mucin explorers in the United States, who continued the great tradition of mucus exploration originated by Prof. G.B.J. Glass.

The complete novelty and originality of the basic science approach to mucus and mucin research represented by Prof. Dr. B. L. Slomiany and his wife, Prof. Dr. Amalia Slomiany opened a new chapter in exploration of mucin chemistry, synthesis, and its physical properties. Prof. Dr. B.L.S. Slomiany and Prof. Dr. A. Slomiany for the first time demonstrated that mucin molecule contains within the structure of its basic molecule not only polypeptide core and covalently bound carbohydrates but also fatty acids (31). This was quite a revolution in our thinking about mucin and mucus composition. Furthermore, this excellent research team has also demonstrated that mucin molecule avidly interacts through non-covalent bonding with various classes of lipids originating from the mucous cell cytoplasm and cell membranes such as phospholipids, glycolipids, and neutral lipids (31). This interaction of the mucin molecule with various lipids is best illustrated by Fig. 2.

Of note, Prof. Dr. B.L.S. Slomiany and Prof. Dr. A. Slomiany had unprecedented approach to exploration of mucin, as they conducted parallel studies on gastrointestinal and salivary mucins in health and disease (32). It is impossible to overestimate the contribution of Prof. Dr. B.L.S. Slomiany and Prof. Dr. A. Slomiany to progress in mucin and mucus exploration, as this team published hundreds of papers in the best peer reviewed journals and to cover their entire contribution one would write a separate book. To adequately address scientific achievements of these great Polish scientists, a separate publication devoted to this cause hopefully will be written some time in the future.

My contribution during my research fellowship at Prof. Slomianys' laboratory relates to demonstration for the first time that both covalently bound fatty acids and non-covalently bound lipids, especially phospholipids, significantly enhance
the physical properties of gastric mucin and mucus, i.e. viscosity and permeability to hydrogen ion, (33 - 36). Of note, the viscosity and permeability or retardation of the hydrogen ion diffusion, are two the most pivotal factors determining protective quality of gastric mucin monomer and its polymerized tridimensional arrangements within the mucus layer (31, 32). Furthermore, we have also demonstrated that Helicobacter pylori degrades the mucin-lipid network and impairs its protective properties within the gastric mucosal barrier (37) and this is best outlined in Figs 3 and 4.

Continuation of Mucus and Mucin Exploration at the University of Virginia (UVA) Health Sciences Center, VA.

In 1989 I have started my independent research career as a Director of Gastroenterology Research Laboratory upon an invitation from Prof. Richard W. McCallum, Chairmen of the Division of Gastroenterology & Hepatology, at

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Fig 3. Cross-sectional view of the gastric mucosal barrier, with emphasis on the collapse of the mucin-lipid network under the impact of aggressive factors elaborated endogenously (acid, pepsin) or by Helicobacter pylori (protease, lipase, phospholipase A2). (Sarosiek J. et. al., 1988)
UVA, in Charlottesville, Virginia, great visionary in research related to the role of motility abnormalities in pathophysiology of the alimentary tract disorders.

This research at UVA has targeted salivary and esophageal mucin and mucus secretion in health and disease. We entirely pioneered the exploration of the esophageal mucus and mucin secretion in humans (38). To obtain human esophageal secretion we had to design and manufacture esophageal perfusion catheter that is illustrated by Fig. 5. Owing to this catheter, we have demonstrated for the first time in humans that human submucosal mucous glands elaborate and secrete mucin that is chemically distinct from both salivary and gastric mucin (38).

Furthermore, by simultaneous collection of saliva we demonstrated for the first time that exposure of the human esophageal mucosa to mechanical (catheter's balloons) and chemical (infusion of acid and pepsin) stimuli results not only in enhancement of the local mucosal secretion by submucosal mucous glands but also in vigorous secretory response from salivary glands (39 - 42), mediated by esophago-salivary reflex, displayed in Fig. 6. Therefore, in summary, the first line within the esophageal mucosal barrier is represented by the mucus layer with all

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**Fig. 4.** A diagram representing the impact of *Helicobacter pylori* colonization on the chemical and physical properties of the mucus layer and its implications in pathogenesis of mucosal injury.
its protective components contributed both by esophageal submucosal mucous glands and salivary gland secretions, displayed in Fig. 7.

This mucus layer thickness in human esophageal mucosa (95µm), although thinner than its counterpart within the gastroduodenal mucosa, still is capable to provide structural support for the mucus-buffer layer slowing down the rate of hydrogen ion back diffusion thus limiting its deleterious potential. Of note, the rate of esophageal mucin secretion in patients with gastroesophageal reflux disease (GERD) accompanied by severe reflux esophagitis is significantly impaired (43, 44). Furthermore, the mucosal exposure to HCl and pepsin impairs the protective quality of esophageal mucus as measured by its hydrophobicity, so important in retardation of hydrogen ion diffusion (45). Of note, significant changes in the rate of secretion of esophageal mucin under the impact of luminal HCl/pepsin has also been demonstrated in the cat model of esophageal perfusion. Interestingly, the cat esophageal mucosa resembles human in terms of its content of numerous submucosal mucous glands (46). Nitric oxide remains as one of the leading mediators of the esophageal mucin secretion in this animal model (47).
Administration of propulsid (Cisapride), a prokinetic agent, an agonist of serotonin receptor 5HT4, has also been demonstrated in our laboratory to exhibit stimulatory effect on salivary mucin secretion in asymptomatic volunteers, thus opening a new avenue of pharmacological intervention in patients with GERD who also may suffer from impairment in salivary and or esophageal mucus and mucin production (48).

Another pharmacological intervention targeting augmentation of the gastric mucus and mucin production and secretion has also been recently demonstrated by our group during administration of one of the newest and most potent proton pump inhibitors (PPI), rabeprazole (Aciphex) (49), as the best illustrated by Fig. 8.

Since Helicobacter pylori elaborates proteolytic and lipolytic enzymes in vitro, capable of degrading the mucus-lipid complex and its protective quality, we have developed a new method for the measurement of the mucus layer ex vivo in humans using biopsy specimens obtained during gastroscopy (50). These experiments revealed that colonization of the human gastric mucosa by Helicobacter pylori results in significant decline of the mucus layer thickness, thus potentially leading to compromised mucus-buffer layer which could

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facilitate the back-diffusion of hydrogen ion and accelerate injury to the gastric mucosa evoked both by hydrogen ion accompanied by pepsin and by various enzymes and toxins easily accessing the gastric epithelium (Fig. 9).

Considering the fact that administration of conventional non-steroidal anti-inflammatory drugs (NSAIDs), inhibiting both COX 1 and COX 2 pathways in animals and humans results in inhibition of gastric mucin and mucus production, co-administration of rabeprazole could potentially restore this mucus production impairment and prevent the subsequent mucosal injury. This hypothetical concept still requires to be confirmed in a double-blind, placebo-controlled, cross-over designed study protocol that is currently contemplated at our Gastroenterology Research Laboratory at KUMC.

I have been fortunate to receive tutoring and preceptorship from the best in the field of mucinology, and hopefully my work will help to better understand

both basic and clinical science as it relates to this so perplexed and so fascinating research arena.

My research would have not been possible to conduct and implement without so many young investigators, majority of them as visiting scholars from Poland who contributed profoundly to our progress in exploration of the role of mucus and its components in prevention of mucosal injury and repair.


**CONCLUDING REMARKS**

The authors of this chapter provided an outline of the most meaningful contributions to advancement of the mucus science during their research both at Medical School in Bialystok, Poland and in the United States that could be briefly listed as follows.

A. Analysis of composition/synthesis of carbohydrate component of humans gastric mucin (Prof. A. Gindzewski & Prof. K. Zwierz).
B. Detection of enzymatic pathways of aminosugars in human gastric mucosa.
C. Discovery of mucin aminosugers metabolism pathways in human gastric mucosa (Prof. A. Gindzewski & Prof. K. Zwierz).
D. Originality of insight into mucus secretion (Prof. A. Gindzewski & Prof. K. Zwierz).
E. Purification of gastrone, inhibitor of gastric acid secretion (Prof. A. Gindzewski & Prof. K. Zwierz).
F. Novelty solutions to purification and solubilization of isolated mucin (Prof. A. Gindzewski & Prof. K. Zwierz).
G. Discovery of the interrelationship between pentagastrin and histamine 2 receptor agonist in gastric mucus secretion in humans (Prof. J. Sarosiek).

Fig. 9. The novel method of assessment of the gastric mucus layer thickness (bright color) *ex vivo*: Top: the mucus layer of the normal gastric mucosa. Bottom: the mucus layer of patient colonized by *Helicobacter pylori*. (from 50)
H. A novelty of the chemical structure of gastric and salivary mucin enriched with covalently bound fatty acids (Prof. Dr. B.L. Slomiany & Prof. Dr A. Slomiany).

I. An originality of the concept of dynamic interaction between salivary and gastric mucin molecules and non-covalently bound lipids, especially phospholipids within mucus gel network (Prof. Dr. B.L. Slomiany & Prof. Dr A. Slomiany).

J. A profound impact of covalently bound fatty acids within the mucin molecule and mucus-associate lipids on physical properties (viscosity and permeability to hydrogen ion diffusion) of the mucus gel (Prof. Dr. B.L. Slomiany & Prof. Dr A. Slomiany and Prof. J. Sarosiek).

K. Detrimental impact of enzymes elaborated by H. pylori on mucus-lipid network in mucus gel layer (Prof. Dr. B.L. Slomiany & Prof. Dr A. Slomiany and Prof. J. Sarosiek).

L. Discovery and characterization of the human esophageal mucin, secreted by esophageal submucosal mucous glands (Prof. J. Sarosiek).

M. Stimulatory impact of propulsid, serotonin receptor HT4 agonist, on salivary mucin secretion in humans (Prof. J. Sarosiek).

N. Enhancement of the gastric mucin production and secretion by proton pump inhibitor, rabeprazole in humans (Prof. J. Sarosiek).

Post Scriptum

My research experience in exploration of the role of mucus and its mucin and non mucin components wouldn't have happened without my teachers who at the same time exhibited talents of great investigators. I could see far because I looked through the eyes of great visionaries. I could explore some visionary concepts because I experienced the intellectual strength of the medical research giants. I could sail through the uncharted waters of scientific seas because I felt safety with great captains at the helm of our ships. I am listing their names chronologically as I experienced their input in my research career: Prof. Dr. habil. med. Beat Bogdanikowa, Prof. Dr. habil. med. Andrzej Gindzienski, Prof. Dr. habil. med. Krzysztof Zwierz, Doc. Dr. habil. med. Janusz Badurski, Prof. Dr. habil. med. Antoni I Gabryelewicz from the Medical School, Bialystok, Poland and Prof. Bronislaw L. Slomiany, PhD, Prof. Amalia Slomiany, PhD, from the New York Medical College, NY, and later at the University of Medicine and Dentistry of New Jersey, NJ, Prof. Richard W. McCallum, MD, from the University of Virginia, VA and later at Kansas University Medical Center, KS, Prof. Barry J. Marshall, MD, and Prof. David A. Peura, MD, from the University of Virginia, VA, USA. I was fortunate to have tutoring from my early days of my research endeavors from all mentioned above opened minds that always looked and could see with their impressive imaginations farther than anybody could ever have seen.
It was my great privilege to experience their leadership and I am and I will always be very grateful.

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