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# Membrane-bound Atp-dependent Energy Systems and the Gastrointestinal Mucosal Damage and Protection

*Authored by Gyula Mozsik and Imre Szabo*

# MEMBRANE-BOUND ATP-DEPENDENT ENERGY SYSTEMS AND THE GASTROINTESTINAL MUCOSAL DAMAGE AND PROTECTION

Edited by **Gyula Mozsik** and **Imre Szabó**

**Membrane-bound Atp-dependent Energy Systems and the Gastrointestinal Mucosal Damage and Protection**
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Edited by Gyula Mozsik and Imre Szabo

**Contributors**

Gyula Mozsik, Imre Szabó

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# Meet the authors

Gyula Mozsik, MD, PhD, ScD (med):professor emeritus of medicine at First Department of Medicine, University of Pécs, Hungary. He was head of this Department from 1993 to 2003. His specialization: internist, gastroenterologists, clinical pharmacologists. His research fields are biochemical pharmacological studies in the gastrointestinal tract, and the role of capsaicin-sensitive afferention in human gastrointestinal tract. He published more 350 papers in peer-revieved journal ,19 monographs and edited 30 books.He received André Robert award from International Union of Pharmacology ,Gastrointestinal Section (2014).From his students, fourtheen full University Professors were appointed in Cuba, Egypt and Hungary.

Dr. Imre Szabó is a medical doctor holding Ph.D. (2004) and habilitation (2012) degrees in Gastroenterology from the University of Pécs (Hungary, 2004), where he studied the mechanisms of cytoprotection and mucosal healing processes in different regions of human gastrointestinal system. He currently works as an Associate Professor at the First Department of Medicine, University of Pécs and has 12 years of experience in teaching Internal Medicine and Gastroenterology at graduate and postgraduate level. His research in the United States included the study of ulcer healing, nonsteroidal gastroenteropathy in animal models and their molecular mechanisms in isolated cells. He has published his work in several peer reviewed journals and given oral and poster presentations at national and international conferences. In addition he has authored Hungarian grants and participated in grants from U.S. Veteran's Administration. His fields of special experience include gastrointestinal mucosal injury, defense and healing mechanisms.

# Contents

# Preface

This monograph deals with various aspects of peptic ulcer disease, like clinical pharmacology, nutrition, molecular biochemical pharmacology as well as clinical aspects, and especially with the evaluation of certain biochemical mechanisms in human gastric mucosa and in animal gastric tissues obtained from different ulcer models.

The authors have been working from the 1960s up till now in the fields of basic research involving molecular pharmacological studies, to understand the bases of gastrointestinal mucosa damage in animal models and in human gastrointestinal tissues, and of clinical research involving clinical pharmacology of prospective, randomized, multiclinical studies, biochemical molecular pharmacology and nutrition.

The authors reported first that duodenal ulcer can heal without any decrease of gastric acid secretion in patients with duodenal ulcer (1965), then prepared the $Na^+$–$K^+$-dependent ATPase and adenylate cyclase from the human gastric mucosa (1969–1970), and finally discovered the key role of intact vagal nerve in the development of prostacyclin-induced gastric cytoprotection (1982).

The mitochondrial ATP is the common substrate for both membrane-bound $Na^+$–$K^+$-dependent ATPase and adenylate cyclase in the presence of $Mg^{2+}$. The actual level of mitochondrial ATP is the consequence of the equilibrium between the ATP breakdown and ATP resynthesis (by oxidative phosphorylation).

The membrane-bound ATP-dependent energy systems are common meeting points for different physiological and pathological phenomena (gastric acid secretion, tissue hypoxia, tissue damage and gastrointestinal ulceration) as well as drug-induced intracellular targets.

The authors have focused on feedback mechanisms existing between the different drugs, hormones, and mediator-induced extra- and intracellular regulatory pathways in the human gastrointestinal resecates obtained from surgical interventions performed for treating peptic ulcer in patients and from various ulcer experimental animal models at the time of the development of gastrointestinal mucosal damage and prevention. It was clear that no hypoxemic damage can be proven biochemically in the ulcerated gastric mucosa and that a very complex intracellular feedback mechanism system exists between the $Na^+$–$K^+$-ATPase and adenylate cyclase enzymes under normal and ulcerated gastrointestinal mucosa regulated by different mediators, hormones and drugs. The results of biochemical examinations clearly indicated that several generally and internationally stated theories cannot be further accepted in the light of results obtained from human and animal gastric mucosal biochemistry described in this book. *Helicobacter pylori* bacteria (given in doses of $10^6$–$10^8$ germs/mL) can-

not produce cellular damage at the levels of cell membrane, mitochondrion and nucleic acids on freshly isolated rat gastric mucosal cells.

This book can be useful to physiologists; biochemists; pharmacologists, particularly molecular and biochemical pharmacologists; internists; gastroenterologists; biologists; surgeons and pharmacists.

**Gyula Mozsik, MD, PhD, ScD (med): professor emeritus,**
First Department of Medicine,
University of Pécs, Hungary

**Dr. Imre Szabó,**
Associate Professor,
First Department of Medicine,
University of Pécs, Hungary

Chapter 1

# General Medical Backgrounds

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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## 1.1. General medical introduction

The characteristics and epidemiology of the gastrointestinal diseases, including the peptic ulcer disease, have significantly changed during the past 40–50 years. Previously, physicians' diagnostic possibilities were limited to the patients' complaints, performance in physical examinations and basic X-ray examinations. Diagnosis was based mainly on the subjective (patients' complaints) and objective (physical observations) data obtained from the patients. However, the diagnostic modalities of the everyday medical practice have changed significantly during the past decades resulting in the usage of modern medical diagnostic instruments.

Peptic ulcers and gastrointestinal mucosal damage were accepted as the result of the unbalanced equilibrium between the aggressive (HCl and pepsin) and defensive (blood supply, mucus, bicarbonate, prostaglandins, biochemistry of target organs, etc.) factors. Consequently, the principal role of overproduction of gastric acid secretion and pepsin secretion or decreased defense against gastrointestinal damage (gastric, duodenal and jejunal ulcers) has been accepted as possible causes for the development of gastrointestinal mucosal damage. However, the details of these mechanisms and their key factors have changed over the past decades. The correction of unbalanced equilibrium between the aggressive and defensive factors in the gastrointestinal mucosa gave the basis of therapeutic possibilities in patients with peptic ulcers before the discovery of "gastric cytoprotection" by André Robert (1979). He indicated that prostaglandins prevent the gastric mucosal damage without the presence of gastric inhibitory actions in rats. Earlier, we observed that the duodenal ulcer in patients healed; however, the gastric secretory responses did not change during a prolonged atropine treatment (Mózsik et al., 1965 a, b). We observed this phenomenon in patients with duodenal ulcer, and we gave a clinical pharmacological explanation for the existence of this phenomenon (for details, see Chapter 2).

Conversely, we had very limited treatment options for patients, such as the application of alkaline mineral water, strict diets (which were not known in detail) and a small number of drugs to be applied in medical practice. Many changes have emerged in medical knowledge,

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history of patients, civilization, life styles, working and living conditions, nutrition, drug research and the introduction of various medications. We have gained new insight from the physiology, pathology and etiology of diseases and from drug development, nutrition, immunology, biochemistry and other instrumental studies. The genetic code of living cells led to a significant increase in molecular genetics research in medical sciences. Therefore, the correct diagnosis is increasingly becoming the key point of successful therapy.

### 1.2. Problem- orientated medicine

Since the plat 40–50 years, there has been a significant increase (advantage) in the diagnostic panel for bedside medical practice (endoscopy, ultrasonography, computer tomography, positive emission tomography, angiography, scintigraphy, special immunologically based scintigraphy, etc.). During the same time period, extremely significant advances were observed in the laboratory, such as immunological, histological, immunohistological and genetic diagnostic examinations in everyday medical practices. During this period (mainly during 1970–1980), the different diseases (and our medical activities) were approached by the citation of "problem-orientated medicine," keeping in mind that primary and correct diagnosis was the main aim of everyday medical practice.

The medical treatments including the medical (pharmacological), dietetic, psychological, surgical and others were offered to patients based on the correct diagnosis.

### 1.3. Evidence-based medicine

We have to note that the evaluation of applied medical therapy was based on the changes in patients' complaints, physicians' opinion, better general health conditions (appearance of the appetite, increase of body weight, better laboratory results, etc). During the past 50 years, the correct methodologies (clinical pharmacology, clinical dietetics, etc.) for critical and objective evaluation of the performed medical treatments were not available.

During the period 1960–1970, we only had the parasympatholytics (atropine, scopolamine and other quaternary ammonium compounds) and different antacids to be used in the medical treatment of peptic ulcer in patients. Furthermore, the new generation of various drugs (for inhibition of gastric acid secretion) – as histamine receptor-blocking agents (from the first to fourth generation), gastrin antagonists, proton pump inhibitors (PPI) and other compounds (prostaglandins, sucralfate, scavengers, etc.) – appeared in the field of medical treatment of patients with classical peptic ulcer disease. The increased gastrointestinal pharmacology led us to learn – in the clinical practice – the terminology of "evidence-based therapy." This terminology still exists; however, the applicability of this medical practice went over a significant change in the plast dcades. The "evidence-based therapy" suggested the existence of its development of human clinical pharmacology by the increase in the emergence of new families of drug compounds. The established clinical pharmacology (form human phase I to human phase IV) during 1960–1970 is given an objective method to crease the "evidence-based medicine." During the first period of clinical pharmacology, the experts analyzed the absorption, metabolization and excretion of different drugs from the gastrointestinal tract; thereafter, the identification of oral/parenteral dose rates was calculated in the case of same drugs, and following these observations, the efficiencies of therapeutic values of drug treatments were

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identified in small groups of patients, which extended many thousand patients in multiclinical, multicentric, multinational, randomized and prospective studies all over the world. The organizations of these large-scale studies, involvement (participation) of special patients, special clinical units and using the services of health experts (sisters, laboratory experts and physicians) represented extremely expensive financial requirements, which were covered by the involvement of other large pharmaceutical firms. The small countries (like Hungary) participated in these studies, however, together with others countries.

### 1.4. Changes in the etiologies and therapies in patients with peptic ulcer

During the past 50 years, the suggested etiological factors for the development of peptic ulcer diseases changed significantly and the medical terminology of peptic ulcer diseases changed as well ["genuine ulcer," secondary ulcer diseases that are associated with the presence of other chronic diseases (such as chronic pulmonary, liver diseases, stress, burn and stroke in the brain), and recently the increased drug consumption for reasons other than the gastrointestinal tract; however, these drugs cause gastrointestinal mucosal damage (e.g., nonsteroidal anti-inflammatory drugs (NSAIDs)) or overconsumption of alcohol]. In the 1980s, the terminology of "non-ulcer dyspepsia (NUD)" was introduced by Scandinavian researchers, which represented those patients who had typical complaints of peptic ulcer, but there was no possibility to detect any alterations (histology, endoscopy) in this group of patients. The gastric mucosal damage caused by different drugs was also introduced in the everyday medical practice.

The number of patients suffering from peptic ulcer diseases also changes over time, and the number of patients suffering from peptic ulcer diseases differs in different countries over the world. In our country (Hungary), the number of patients with peptic ulcer is about 10% of the total Hungarian population (inhabitants) since the 1960s.

We have to accept the changes in the terminology of "peptic ulcer disease." In the 1960s, only the "primary (or genuine)" and "secondary" peptic ulcer diseases were accepted in our medical practice. The patients with secondary peptic ulcer associated with other diseases (stress, sepsis, stroke, pulmonary and liver diseases, burn and trauma), whereas the presence of these factors in the cases of patients with "primary (or genuine)" peptic ulcer diseases was not detected. The "acid-related diseases" also came into medical practice (as diagnosis of patients).

The diagnosis of peptic ulcer disease is based on different objective factors, such as X-ray examination (only in the early period), endoscopic examination and histology.

The classical definition of peptic ulcer disease can be histologically accepted only by the presence of tissue mucosal damage that reaches the muscular mucosal layer. The correct histological definition is necessary for the diagnosis of patients with peptic ulcer diseases. Consequently, the presence of mucosal erosion cannot be considered as a "peptic ulcer disease" (independently from that, the treatment and prevention of these gastrointestinal mucosal injuries represent essential medical problems). After receiving the Nobel Prize in physiology (medicine) by Warren and Marshall (2005), the *Helicobacter pylori*-positive and -negative characterization appeared in patients with the above-mentioned clinical entities.

We have to note that various drugs (including the nonsteroidal anti-inflammatory drugs and others) can cause gastrointestinal diseases (increased complaints of pyrosis in the epigastric

region of the abdomen, vomiting, bleeding, etc.). The correct number of patients with these complaints is not known; however, the number of patients consuming NSAIDs is extremely high. These problems are very important in the our medical practices (looking for the etiology and later the treatment of disease) of medical practices; however, we have to emphasize very well and clearly that these patients are out from the patients with really peptic ulcer disease (because these mucosal damages are in present form out from the classical histological criterion of peptic ulcer disease in patients). These above-mentioned basic criteria of the histological events produced many offered (and gave) many misunderstanding information (results) to know and critically to evaluate thre of the different etiological factors, mechanisms and efficiencies of different medical treatments in patients with classical peptic ulcer diseases.

We have the details of the development of different gastrointestinal mucosal damages (injuries) and the possibilities to prevent these gastric (gastrointestinal) mucosal damages (injuries) through different experimental models in different animal spices.

### 1.5. Main lines of our research activities in field of peptic ulcer research in peptic ulcer in patients with peptic ulcer and in different animal models

The focus of our research is in accordance with the peptic ulcer research from the 1960s; in the beginning, we had many unresolved and unknown points to be addressed.

We started our research with the patients' problems, and later to other fields such as clinical pharmacology, general biochemistry, molecular pharmacology and biochemistry, oxygen free radicals and antioxidants, specific immunohistochemistry, clinical nutrition, experimental and clinical gastroenterology and nutrition as well as innovative drug research.

Our principal strategy was to understand the problems of patients. As we were not able to understand these problems, we started with animal observations (hoping that we receive correct answers to our questions). We tried answering different and concrete questions in the medical practice.

The research lines in our practice changed over time in the past decades (we started with patients' problems, followed by experimental observations and then back to patients' problems). This research philosophy has been kept in our life. We tried answering different and concrete questions of medical practice and followed this methodology later.

In the pertinent literature, the peptic ulcer disease appeared because of the unbalanced equilibrium between the aggressive and defensive factors in the gastrointestinal mucosa; however, the details of these factors remain to be unknown.

Recently, the possible mechanisms of gastric acid secretory responses have been widely studied in clinical gastroenterology. The extent of gastric basal acid secretory responses (basal acid output, BAO) and acid secretory responses (maximal acid output, MAO) produced by different doses of histamine (given subcutaneously in superliminal but submaximal or superliminal and supermaximal doses) was measured in different groups (gastric, duodenal and jejunal ulcers) of patients with peptic ulcer disease (PUD) (Semb and Myren, 1968). The real reason to study these gastric secretory responses in patients with PUD is that the increased gastric acid secretion has been suggested as one of the different factors, which is responsible for the increased aggressive factors in the gastrointestinal tract. The possible role of pepsin

secretion in the stomach has been suggested to be another aggressive factor for the development of PUD. The increased role of cholinergic dominance (by the increased activity of vagal nerve) is closely associated with the increased gastric acid secretory responses and pepsin production.

In these years, we have no concrete knowledge of the details of defensive factors. The presence of tissue hypoxia of gastroduodenal mucosa of patients with peptic ulcer has been indicated since many years in the ulcer development. Clinically, the presence of tissue hypoxia could be accepted in patients with massive bleeding, whereas no exact observations of gastric mucosal blood flow were carried out in patients during the development of ulcer. Many observations were carried out in animals under different conditions; however, it is interesting to note that the decreased gastric mucosal blood flow was not associated with the presence of tissue hypoxia in terms of biochemistry (increased level of lactate and decreased level of oxidative phosphorylation in the gastrointestinal mucosa).

After a careful survey of the pertinent literature, the research on the evaluation of the details of decreasing activity of vagal nerve in the everyday medical practice in patients with PUD (by parasympatholytic drugs at the beginning of our studies) was started.

During that time, only atropine and scopolamine (the only tertiary ammonium compounds) were used in the everyday medical practice.

Our primary aims of observations were as follows:

1. To establish the clinical pharmacological basis of anticholinergic agents in patients with PUD;
2. To measure the gastric secretory responses of patients with duodenal ulcer before and after (2–4 weeks treatment with atropine) healing of clinically detectable ulcer and
3. To detect the changes in complaints of patients during the medical treatment (especially at the beginning and at the end) (as in complaints of patients originated from the diseases and in the drug-induced side effects – tachycardia, mouth dryness and problems in visual functions).

These studies were carried out in patients with classical duodenal ulcer and were admitted to Second Department of Medicine, University of Debrecen, Hungary.

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 2

# Clinical Pharmacology of Drugs Used in the Treatment of Patients with Peptic Ulcer

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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## 2.1. Primary (genuine) and secondary (associated with stress, sepsis, stroke, pulmonary and liver diseases, burn, trauma) Peptic Ulcer Diseases (PUD)

The criteria of diagnosis and treatment of peptic ulcer disease (PUD) have been changed significantly in the past 40–50 years. At the beginning of this period, peptic ulcer disease was only diagnosed by X-ray examination. Currently, gastrofiberoscopy has also become a common practice in medical diagnosis.

Clinically, the PUD was divided into two groups: "primary" and "secondary". The diseases in the "secondary group" associated with a chronic liver, lung, brain damage or burn; however, no obvious reasons for the PUD could be provided as the etiological factor and these forms of the diseases were named as "genuine diseases."

The presence of two main factors was emphasized in the development of gastroduodenal ulceration: the hyperacidity and hypoxemic damage of gastroduodenal mucosa.

There was a significant contradiction between these mentioned factors, namely the gastric hyperacidity associated with the increased gastric mucosal blood flow (GMBF) and ulcer development, and it was practically impossible to understand the development of gastroduodenal mucosal damage based on the increased gastroduodenal mucosal blood flow (Bowen and Fairchild., 1984).

## 2.2. Medical treatment with anticholinergic agents in patients with gastric and duodenal ulcers

Our scientific attention focused on the medical treatment of patients with peptic ulcer diseases from 1960 onwards.

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Our original aim was to create a complete scientific methodology for the evaluation of efficiencies of different drugs (previously, parasympatholytic agents, antacids) in the field of gastroenterology. We have to emphasize that during 1960–1970, no clinical pharmacology existed.

We wanted to introduce into the conventional human clinical pharmacological approaches using that we were established in our group at Debrecen, Hungary, in the field of gastroenterology (in the period of 1962–1970).

Our interest focused on changes of gastric secretory responses in patients with duodenal ulcer (DU) during chronic atropine treatment. In the earlier days, only atropine and scopolamine were used for treating DU patients in whom we studied the presence of DU using X-ray examination (fiberoptic endoscopy did not exist earlier).

We studied (at present) the changes of gastric acid secretory responses in patients with duodenal ulcer before and after a classical medical treatment. We measured the gastric basal acid output (BAO) and secretory responses to superluminal (but submaximal) dose of histamine.

We were surprised to find that the patients with DU were cured by a chronic (2–4 weeks) treatment with atropine. However, the gastric secretory responses in patients were not decreased (Mózsik et al., 1965c; Mózsik and Jávor, 1966 a, b; Mózsik et al., 2011) (see Table 1).

| **Examined Parameters** | **Before** | | **After** | |
|---|---|---|---|---|
| | **A chronic atropine treatment** | | | |
| | A | B | A | B |
| Volume of gastric juice (mL/h) | 180±32 | 175±37 | 178±44 | 181±28 |
| $H^+$ output (mEq/h) | 0.97±0.02 | 1.49±0.2 | 0.8±0.02 | 1.61±0.11 |

**Table 1.** Gastric secretory responses before and after (3 × 0.3 mg orally given for 2–4 weeks) a chronic atropine treatment without and with histamine (A and B, respectively) (0.5 mg subcutaneously injected). The results were expressed as means ± SEM in ten patients. *P*-values between the identical parameters before and after chronic atropine treatments are not significant. [Mózsik et al., 1965c; 1966b (with kind permission).]

These results showed that duodenal protection could be obtained by independent effects on acid secretion: a phenomenon that defied the earlier well-established view, "no acid, no ulcer" originally pioneered by Lester Dragstedt in 1943 (Gustafson and Welling, 2010).

Following these observations, the acute inhibitory effect of atropine was tested on the gastric acid secretion before and after a chronic atropine treatment. Here, we observed that the extent of acute inhibitory effect of atropine on patients' acid secretion decreased significantly after a chronic treatment (Mózsik et al., 1965c; Mózsik and Jávor 1966b; Mózsik et al., 2011).

| Examined Parameters | Before | | After | |
|---|---|---|---|---|
| | A chronic atropine treatment | | | |
| | A | B | A | B |
| Volume of gastric juice (mL/h) | 125±10 | 137±10 | 189±8 | 109±10 |
| $H^+$ output (mEq/h) | 0.25±0.08 | 0.4±0.1 | 0.49±0.08 | 0.68±0.09 |

**Table 2.** Changes in the gastric secretory responses before and after (3×0.3 mg orally given for 2–weeks) a chronic atropine treatment without and with histamine (A and B, respectively) (0.5 mg subcutaneously injected) administration in eight patients. The results were expressed as means ± SEM. *P*-values between the identical parameters before and after a chronic atropine treatment: $P < 0.05$. [Mózsik et al., 1965c; 1966b (with kind permission).]

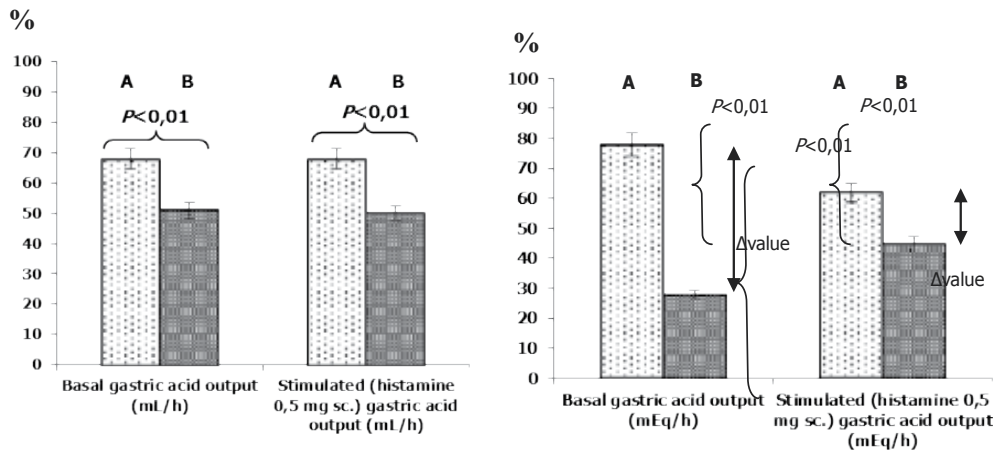


**Figure 1.** Decreased inhibitory effect of atropine (1 mg sc.) before (A) and after (B) chronic atropine treatment in patients with peptic ulcer disease ($n = 8$; means ± SEM).The results are expressed in percentage of their control. [Mózsik, Szabó, Czimmer: Curr. Pharm. Des. 17: 1556–1572, 2011 (with kind permission).]

## 2.3. Clinical Pharmacology of anticholinergic agents in patients with peptic ulcer disease (measurements of anticholinergic agents in the sera, urine in patients with duodenal ulcer)

It was hard to understand our previously demonstrated results obtained with application of atropine treatment in patients with duodenal ulcer because these results contradict the international experts' opinions. We assumed that:

1. The atropine will not absorb from the gastrointestinal tract owing to some reasons (e.g., some pharmaceutical industrial problem(s) appeared during the drug pharmaceutical production );
2. Even if the atropine absorbs well, some other unknown pharmacological event (such as tolerance) will appear during a chronic (classical) atropine treatment.

To evaluate these questions, we have to establish the real basis of clinical pharmacology. Therefore, we have to provide a good and scientific possibility to bring about different answers for our questions.

In the first step, the concentration of anticholinergic agents was measured by sera and urine bioassay (using isolated guinea-pig ileum for biological titration of parasympatholytic drugs in Magnus vessel) of patients. The absorption, metabolism and urinary excretion of parasympatholytics were studied after chronically applied parasympatholytic treatments (Győrffy et al., 1964; Jávor et al., 1965; 1967; Mozsik, 1969a; Mózsik et al., 1965 a, b; 1967d; Mozsik and Jávor, 1966 a, b, c; 1968a).

The atropine absorbed well from the gastrointestinal tract (during 4–5 hours), and it was proved by titration of atropine in the sera and urine of patients. These observations were carried out in the same patients when the same doses of atropine were given orally or subcutaneously injected. It was clear that the absorbed quantities of atropine were the same (given in the same doses), indicating that the oral/parenteral ration of atropine absorption is equal to 1.0. Furthermore, when we carried out these observations, we found the same results (with respect to the absorption and excretion of atropine) in the treated patients.

Since we had classical clinical pharmacological methods (e.g., detection of small doses of atropine in the serum and urine of treated patients), we were able to exclude any changes of absorption in gastrointestinal tract, metabolism and urinary excretion of atropine as a consequence of the effects of chronic atropine treatment (Mózsik et al., 1966 a, b, c; 1968; 1969 a, b, c, d, e, f; Mózsik, 1969a).

Later, we observed the development of tolerance along with the development of "pharmacological denervation supersensitivity" (e.g., the efficiencies of drugs used in the treatment of patients decreased significantly). These phenomena exist together (Mózsik et al., 1967a; Mózsik and Jávor, 1968 a, b) in the background of unchanged gastric acid secretion during a chronic atropine treatment in DU patients. It was also interesting to note the development of tolerance to atropine and the disappearance of pharmacological denervation phenomena in 6–10 days after cessation of atropine treatment (Mózsik et al., 1965b; Mózsik and Jávor, 1968b; 1969; Mózsik, 1969a). The development of tolerance to atropine does not represent a specific phenomenon related to atropine (during a chronic atropine treatment) because the development of tolerance was found with other parasympatholytics, which were not used in the medical treatment (Mózsik and Jávor, 1969).

The results of the clinical pharmacological studies proved the following main points:

1. No change was obtained in the gastric acid secretion during a chronic anticholinergic treatment;
2. The gastroduodenal ulceration healed during this time period;
3. The results of the clinical pharmacology could not provide an obvious explanation of how the gastroduodenal ulceration was healed without any changes in gastric secretory responses (Mózsik et al., 1965b; 1967a; Mózsik and Jávor, 1966b; 1968 a, b; 1969; Mózsik, 1969a).

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This clinical pharmacological methodology was applied in the medical evaluation of different anticholinergic agents (drugs) before their clinical applications.

Recently, the pharmacological basic research offered a possibility to introduce new anticholinergic agents into the medical practice. The experts working in the field of experimental pharmacology showed that the actions of these anticholinergic agents can be enhanced on the autonomic nervous system by the changes in their chemical structures (perhaps by the production of quaternary ammonium compounds instead of only tertiary quaternary ammonium compounds) (Gyermek, 1951; 1953; Gyermek and Nádor, 1952; 1953 a, b; György et al., 1961).

The Gastropin® is one of the most representative quaternary ammonium compounds, which was introduced into the medical treatment in the 1960s. We were clearly able to prove that the Gastropin® does not absorb from the gastrointestinal tract. We were not able to detect any drug levels in the serum and urine of patients when the patients were orally given 1000 pills; however, these parameters were well detectable after it was injected in the same patients.

The established clinical pharmacological methods were widely used in the evaluation of different anticholinergic agents (Mózsik, 1969a).

Many clinical pharmacological studies (from human phase I to III) were carried out by our work team who were working on different drugs [(tertiary amine, oxyphen cylamine), muscarinic receptor blocking (gastrozepin), histamine$_2$ receptor blocking (first to fourth generation), proton pump inhibitors and other components] for patients with peptic ulcer (Mózsik et al., 1965b; Mózsik, 1969a; Jávor et al., 1967; Garamszegi et al., 1997; Nagy et al., 1997; Tárnok et al., 1979, 1997).

### 2.4. Comparative clinical pharmacology of anticholinergic drugs

Different clinical pharmacological studies were carried out in patients with peptic ulcer to compare the detectable concentrations in the sera, excretion in the urine and bile, inhibitory actions on the salivary secretion, gastric acid secretion, necessary dose rate to decrease glandular secretion and gastric motility, detectability of drug to proteins and the excretion time. The results are summarized in Table 3.

| | **Atropine** | **Novatropine** | **Isopropamide** |
|---|---|---|---|
| Oral – parenteral dose rate | 1 : 1 | 6-8 : 1 | 4-6 : 1 |
| Detectable in serum | + | + | - |
| Detectable in urine | + | + | + |
| Detectable in saliva | - | - | + |
| Detectable in bile | - | - | + |
| Necessary dose rate to decreased glandular secretion and the gastric motility | 1 : 2 | 1 : 2 | 1 : 2 |

| | Atropine | Novatropine | Isopropamide |
|---|---|---|---|
| Ihibitory effect on salivary secretition | + | ++ | - |
| Inhibitory effect of gastric secretion | + | ++ | ++ |
| Inhibitory effect of gastric emtying in decreasing dose of glandular secretion | - | - | + |
| Duration of inhibitory effect on the salivary secretion | 3-4 hours | 6-8 hours | 10-15 hours |
| Excretion in urine up | 3-4 hours | 6-8 hours | 10-15 hours |
| detectable protein binding of the drug in serum | | | |

**Table 3.** Comparative demonstration of the clinical pharmacological properties of parasympatholytics. [Mózsik, Vizi, Jávor, Recent Advances in Gastroenterology. The 3rd World Congress of Gastroenterology, Tokyo, 1967, 681–683 (with kind permission).]

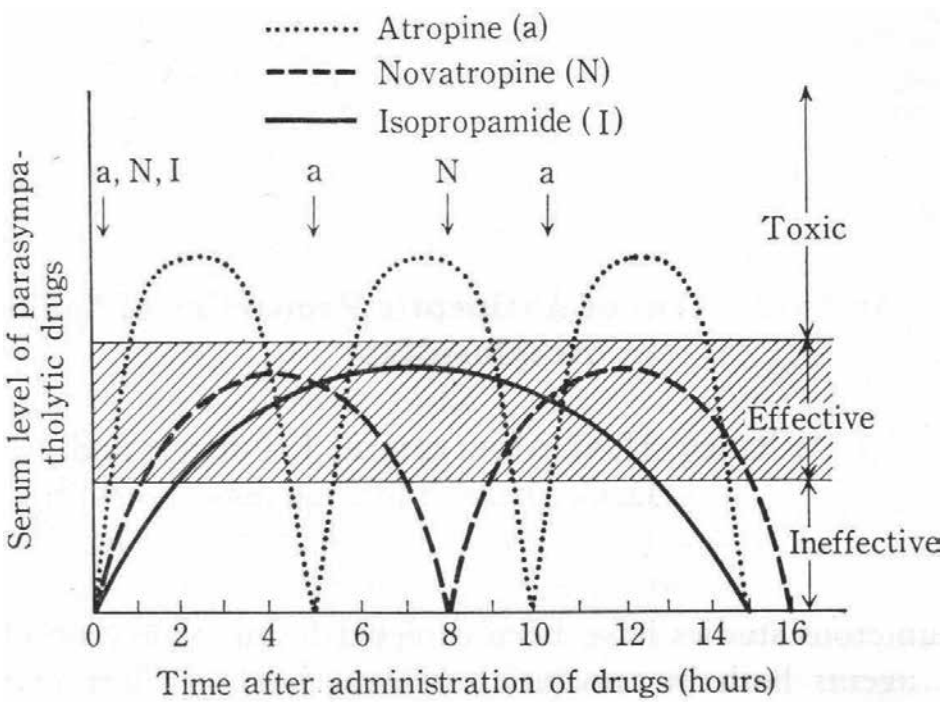


**Figure 2.** The schematic presentation of the measured serum levels of different parasympatholytic drugs in peptic ulcer patients. The ordinate shows the level of parasympatholytic drugs (toxic, effective and ineffective); the abscisse demonstrates the time after administration of these drugs. a: atropine; N: Novatropine; I: isopropamide. [Mózsik, Vizi, Jávor: Recent Advances in Gastroenterology. The 3rd World Congress of Gastroenterology, Tokyo, 1967, 681–683 (with kind permission).]

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### 2.5. Clinical evidence of the existence of gastrointestinal protection differs from the decrease of gastric acid secretion (Gastric cytoprotection)

The results of our observation of chronic atropine treatment in DU patients were given in detail previously.

In 1978, we found that the GI anti-ulcer effects of atropine, cimetidine and carbenoxolone were superior to placebo in a multiclinical, randomized, prospective and comparative study in DU patients. However, no significant difference was obtained in the beneficial effects of atropine versus cimetidine versus carbenoxolone (Tárnok et al., 1979). Since carbenoxolone has no inhibitory action on the gastric acid secretion in DU patients, the ulcer-healing effects (due to stimulation of mucus) could be considered independent of any effects on gastric acid secretion.

Thus, these clinical observations during 1960–1970 were performed before the classical concept of "gastric cytoprotection" was formulated and published by André Robert (Robert et al., 1979).

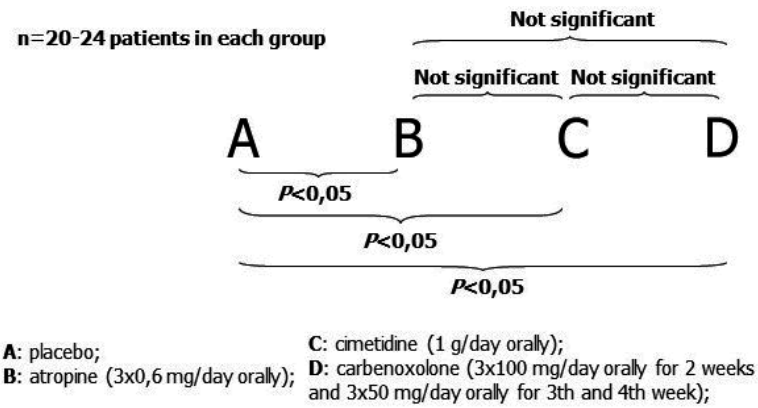


**Figure 3.** A prospective multiclinical study comparing the effects of placebo, carbenoxolone, atropine and cimetidine in patients with duodenal ulcer (4 weeks' study). [Tárnok et al., Drugs Exp. Clin. Res. 5: 157–166, 1979 and Mózsik, Szabó, Czimmer, Curr. Pharm. Des. 17: 1556–1572, 2011 (with kind permission).]

Our clinical pharmacological studies (1965) (Mózsik et al., 1965b; 1967 a, b, c, d; Mózsik and Jávor, 1968 a, b; Mózsik, 1969a; Tárnok et al., 1979) as well as randomized, prospective, multiclinical and comparative studies (1978) are clear even now in retrospect that we had been observing acid-independent gastroduodenal protection with atropine and other drugs before André Robert had defined the existence of "gastric cytoprotection" (Mózsik, 2010; Mózsik et al., 2011).

During 1965–1970, the existence of "gastric cytoprotection" was not reported; conversely, the clinical importance of gastric acid secretion had only been emphasized. Chaudhury and Jacobson (1978) were the first to indicate that the gastric mucosal damage could be prevented without inhibition of gastric secretion.

The existence of "gastric cytoprotection" has been widely accepted after the work of Robert et al. (1979) in animal experiments. The clinical applicability of gastric cytoprotection was later

accepted for patients with gastroduodenal ulcer (with the exception of our previously carried out observations in DU patients).

### 2.6. Randomized, multiclinical, prospective and multicentric study of treatment of chronic gastric ulcer patients with vitamin A

Later, we demonstrated in multiclinical, multicentric, prospective and randomized study the ulcer-healing effect of vitamin A (as scavenger component) in patients with gastric ulcer (Patty et al., 1982; 1983). However, no gastric acid inhibitory action of the vitamin A exists in humans (Jávor et al., 1983 a, b; Mózsik et al., 1986; 2001; 2005; 2007; Rumi et al., 2001 a, b).

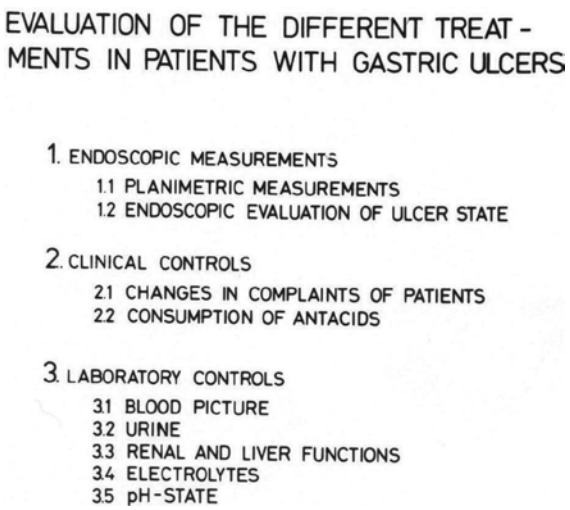


**Figure 4.** Evaluation of the different treatments of efficiency of vitamin A in patients with chronic gastric ulcer (4 weeks of study). The endoscopic and laboratory parameters were carried out at the beginning and during 2 and 4 weeks after the beginning of the treatment; meanwhile, the changes of complaints and consumption of antacids in patients were registered every day and these values were summarized with the similar points in this clinical study. [Patty et al., Lancet II, 872, 1982; Int. J. Tiss. React. 5: 301–307, 1983 (with kind permission).]

Fifty-six patients with chronic gastric ulcer were examined for this multiclinical, multicentric, randomized and prospective study (Patty et al., 1982; 1983).

The patients were divided into three groups: group A (16 patients) received only antacids; group B (18 patients) were treated with antacids plus vitamin A (3 × 50,000 IU orally) and group C (22 patients) received antacids, vitamin A (3 × 50,000 IU orally) and cyproheptadine (Peritol®).The observations were carried out in 4 weeks' time.

Endoscopic measurements, and clinical and laboratory parameters were carried out at the beginning and at the end ($2^{nd}$ and $4^{th}$ week) of the treatment of these patients (Figure 4).

The parameters in Figure 4 help us to evaluate the different objective (planimetric measurement of ulcer sizes, presence or absence of gastric ulcer – at the beginning and end of different treatments – and states of ulcer) and subjective (changes in complaints of patients and

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consumption of antacids) events of the process of ulcer healing (including the possible roles of drug actions). The obtained results are summarized in Figures 5–8.

The dynamism of the ulcer healing can be scientifically studied by the changes of ulcer sizes at 2 and 4 weeks after beginning the treatment (Figure 8). It was surprising to note that the healing rate differed significantly at 2 weeks; however, this value was the same at 4 weeks.

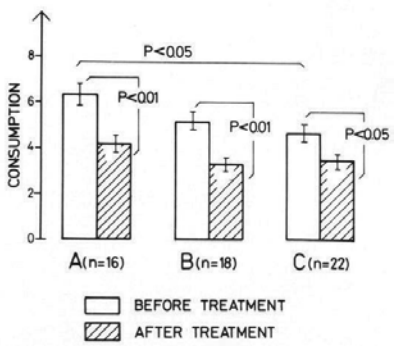



**Figure 5.** Changes in the antacid consumption in the different groups of patients with chronic gastric ulcer. [Patty et al., Lancet II, 872, 1982; Int. J. Tiss. React. 5: 301–307, 1983 (with kind permission).]

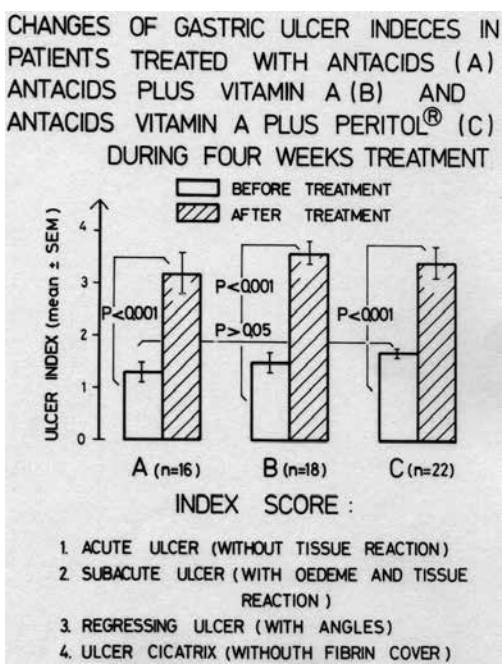
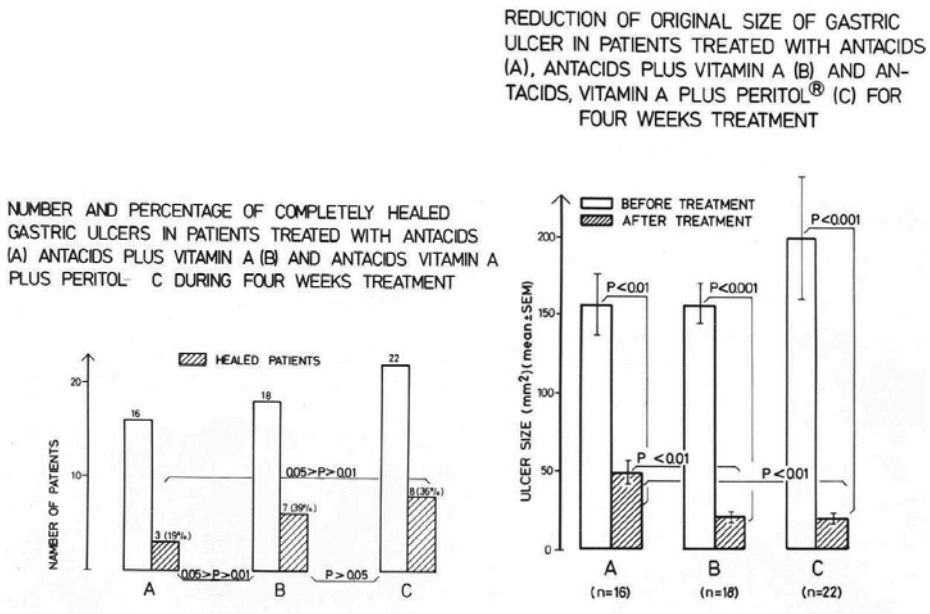



**Figure 6.** Changes of gastric ulcer indices in patients treated with antacids (A); antacids plus vitamin A (B) and antacids, vitamin A and Peritol® (C) during 4 weeks of treatment. [Patty et al., Lancet II, 872, 1982; Patty et al., Int. J. Tiss. React. 5: 301–307, 1983 (with kind permission).]




(A) (B)

**Figure 7.** A. Changes in number (and percentage) of completely healed gastric ulcers in patients treated with antacids (A); antacid plus vitamin A (B) and antacid, vitamin A and Peritol® (C) during 4 weeks of treatment (with kind permission). B. Reduction of original planimetric size of gastric ulcers in patients treated with antacids (A); antacids plus vitamin A (B) and antacids, vitamin A plus Peritol® (C) before and after 4 weeks of treatment. [Patty et al., Lancet II, 872, 1982; Patty et al., Int. J. Tiss. React. 5: 301–307, 1983 (with kind permission).]

## 2.7. Comparative clinical pharmacology of "cytoprotective" and "anti-secretory" drugs (multiclinical, randomized, prospective and multicentric studies) in patients with chronic gastric and duodenal ulcers

Our work team participated in the clinical pharmacological studies of peptic ulcer patients (including different international studies as well as ours). After our previously performed studies, our attention focused on the results of clinical pharmacological studies with "cytoprotective" and "anti-secretory" drugs in patients with chronic gastric and duodenal ulcers.

In this subchapter, we summarized the results of clinical pharmacological studies of 441 chronic gastric and duodenal ulcers. The patients treated with nonsteroidal, anti-inflammatory drugs, steroids, antihypertensive drugs (e.g., Reserpine) and those who underwent some stress, burns or trauma were excluded from this comparative study. These patients were divided into different groups and were treated with different drugs: placebo (calcium carbonicum, magnesium trisilicate and sodium bicarbonate in equal portion), atropine (1.0 mg/os), cimetidine (1000 mg/day orally), ranitidine (300 mg/day orally), famotidine (80 mg/day orally), pirenzepine (150 mg/day orally), sucralfate (1000 mg/day orally), vitamin A (3 × 50,000 IU/day orally) alone or in combination with cyproheptadinum chloratum (3 × 4 mg/

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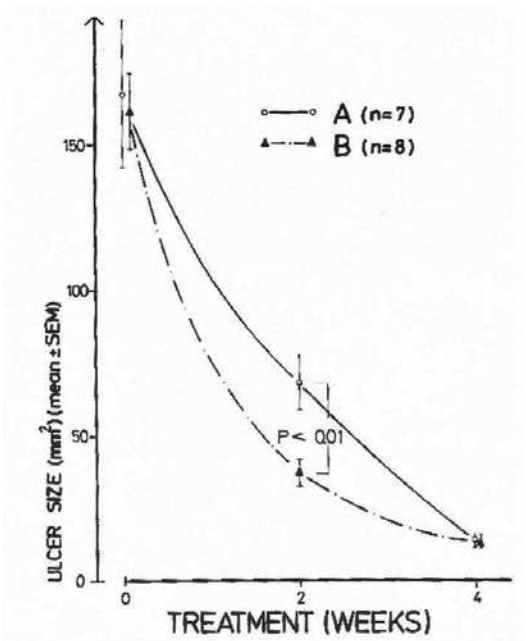


**Figure 8.** Dynamism of ulcer-healing effect of vitamin A in patients with chronic gastric ulcer, indicating the changes of ulcer size in unhealed patients (in 4 weeks of treatment). [Patty et al., Lancet II, 872, 1982; Patty et al., Int. J. Tiss. React. 5: 301–307, 1983 (with kind permission).]

day orally), DE-NOL (3 × 5 mL/day orally) and Tisacid® (Al–Mg–antacidum 3 × 1 g/day orally). The antacids in composition mentioned above were applied with the so-called active compounds. Twenty patients were allocated into each group. The treatments were carried out for 4 or 6 weeks. (These results are reused with the permission of Wiley publisher.)

Endoscopy (estimation of ulcer size), laboratory measurements (blood count, urine, kidney and liver functions), pH status, medical examinations, summed pain score (expressed in percentage of original values) and antacid consumption were evaluated at the beginning, during 2nd, 4th and 6th week (if the treatment extends) and after the treatment.

The aims of this study are as follows:

1. To compare the efficiencies of ulcer treatments by cytoprotective (Tisacid®, sucralfate, DE-NOL and vitamin A) and anti-secretory (atropine, pirenzepine, cimetidine, ranitidine, famotidine) drugs in a short-term study (4–6 weeks);
2. To evaluate the dynamism of ulcer healing in unhealed gastric and duodenal patients;
3. To evaluate the summed pain score and antacid consumption in GU and DU patients;
4. To compare the different results (ulcer healing, laboratory parameters, summed pain score and antacids) obtained in healed and unhealed GU and DU patients.

| Treatment | Number of Patients | Number of Unhealed Patients (%) | P-value |
|---|---|---|---|
| Antacid | 40 | 30(75) | - |
| Al-Mg-hydroxy- Carbonate (Tisacid®) | 15 | 10(66.7) | NS |
| Vitamin A | 18 | 11(61.1) | <0.05 |
| Vitamin A and cyproheptadine | 22 | 14(63.9) | <0.05 |
| DE-NOL | 16 | 5 ( 31.2) | <0.01 |
| Sucralfate | 19 | 14(73.7) | NS |
| Gastrozepine | 16 | 9 ( 56.2) | < 0.0.5 |
| Cimetidine | 30 | 8 ( 26.7) | < 0.01 |

**Table 4.** Changes in the incidence of gastric ulcer (GU) healing during 4 weeks of treatment with different groups of patients compared with antacid. The numbers in parentheses are calculated in percentage. NS: not significant [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

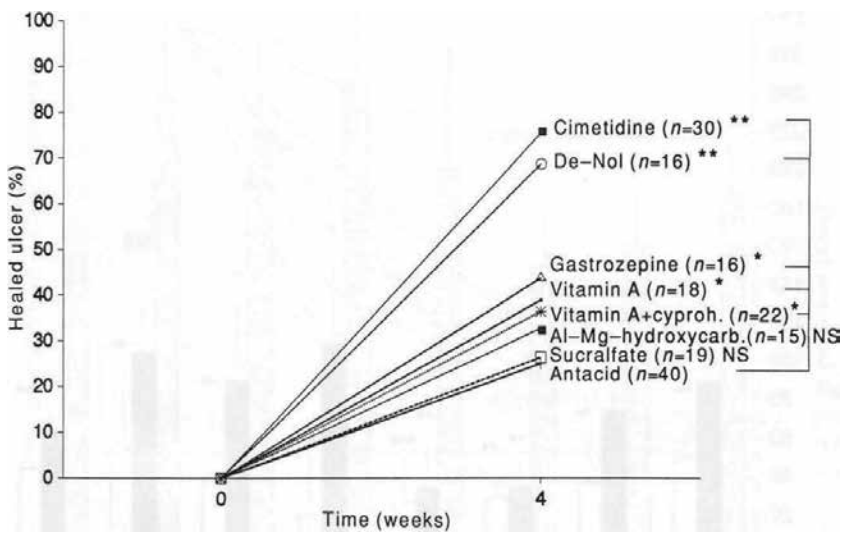


**Figure 9.** Changes in ulcer-healing rate during the different therapies in patients with chronic gastric ulcer (4 weeks). The results are expressed as means; *n* indicates the number of patients. *P*-values between the groups treated with antacids (placebo) and different groups. +: $P < 00.5$; ++: $P < 00.1$; NS: not significant. [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

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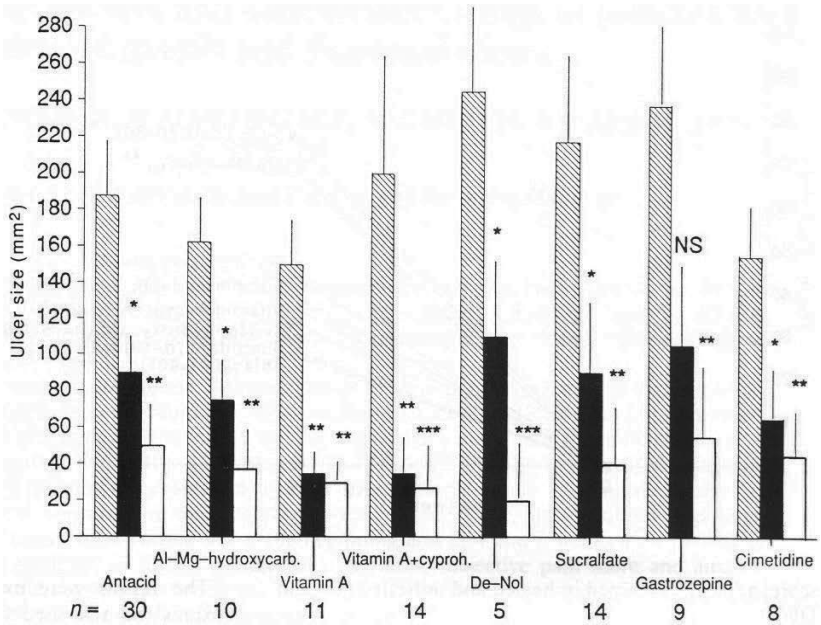


**Figure 10.** Changes in the ulcer size of patients with incompletely healed gastric ulcer during 4 weeks of treatment. The ulcer size was perceived at the beginning (first bar), 2 weeks (middle bar) and 4 weeks (right bar) of the treatment (*n*, the number of incompletely healed patients). *P*-values are based on the ulcer size at the beginning and at 2 and 4 weeks; *: $P < 0.0.5$; **: $P < 0.01$; ***: $P < 0.00.1$; NS: not significant. [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

| Treatment | Number of Patients | Unhealed Patients (%) | P values |
|---|---|---|---|
| Antacid | 22 | 14 (63.6) | |
| Al-Mghydroxy- carbonate (Tisacid®) | 39 | 13 (33.3) | < 0.0.5 |
| Atropine and Cyproheptadine | 22 | 5 (22.7) | < 0.0.1 |
| Atropine and Cimetidine | 25 | 9 (36) | < 0.0.5 |
| Cimetidine | 25 | 9 (36) | < 0.0.5 |
| Ranitidine | 25 | 9 (36) | < 0.0.5 |
| Famotidine | 33 | 9 (27.3) | < 0.0.1 |
| Carbenoxolone | 19 | 8 (42.1) | < 0.0.5 |
| Sucralfate | 31 | 15 (45.4) | < 0.0.5 |
| Gastroozepine | 24 | 10 (41.7) | < 0.0.5 |

**Table 5.** Changes in the incidence of duodenal ulcer healing during 4 weeks of treatment in different groups of patients compared with antacid. The numbers in parentheses are calculated in percentage. [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

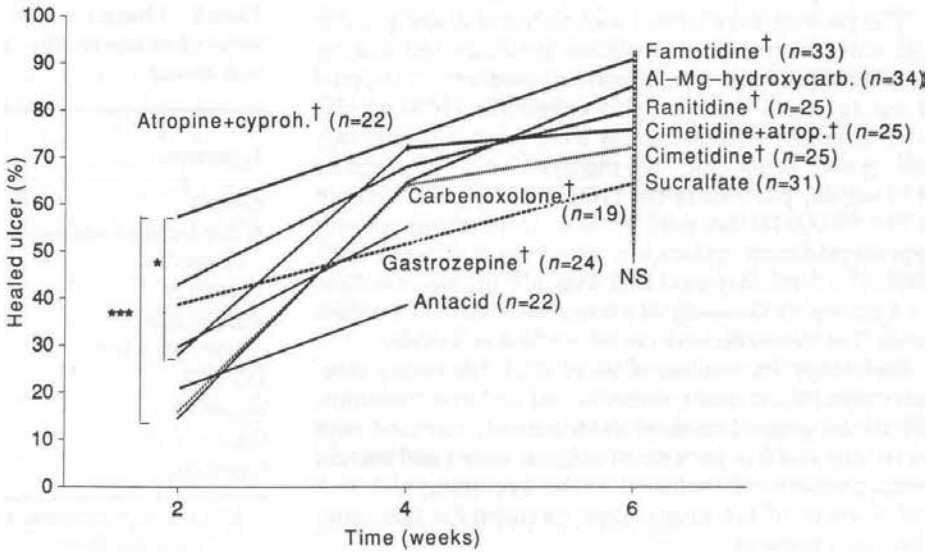


**Figure 11.** Changes in ulcer-healing rate in chronic duodenal ulcer patients treated with different drugs. *P*-values are between antacid (placebo) and treated groups at 4 and 6 weeks. *: $P < 0.05$; ***: $P < 0.001$; NS: not significant. [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

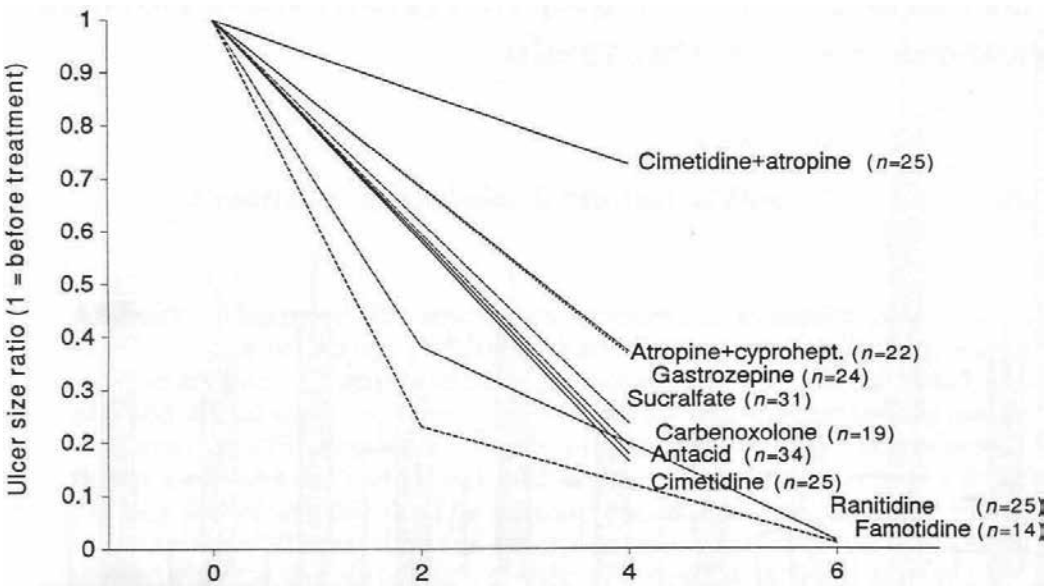


**Figure 12.** Changes in ulcer size in patients with incompletely healed duodenal ulcer after 4 weeks of treatment. Ulcer size before treatment = 1. [Mózsik et al., J. Gastroenterol. Hepatol 9: S88–S92, 1994 (with kind permission).]

### 2.8. General conclusion(s) of clinical pharmacological studies in patients with peptic ulcer

Returning the results of our earlier clinical pharmacological studies in patients with duodenal ulcer (see before), our final conclusion was that we have no any correct knowledge on the principle biochemical cellular and extracellular changes (mechanisms) are present in the keeping the normal homeostasis of the GI mucosa, and in time of development of GI mucosal damage and protection neither in animal experiments nor in humans (patients).

In addition to these, the discovery of "gastric cytoprotection" represented a new challenge to us in answer to the scientific problem mentioned above.

The results of these observations helped us to start a new scale of examinations in the field of peptic ulcer disease. We wanted to start with biochemical examinations in this field, but, unfortunately, nobody worked on it. Consequently, we have to learn the principal points of the general biochemical research to introduce it into the field of peptic ulcer research.

We have to emphasize that scientific problems and their knowledge have been changing over the past few decades, and many new scientific results are published.

Four well-known scientists were awarded the Nobel Prize [Jens Christian Skou (Department of Physiology, Aarhus University, Denmark) in chemistry, 1997; Earl W. Sutherland (Department of Physiology, Vanderbilt University, Nashville, TN, USA) in physiology (medicine), 1971; and Barry James Marshall and J. Robin Warren (Royal Perth Hospital, Australia) in physiology (medicine), 2005]. Their results inspired es to ttudy these fields in our peptic ulcer research in animals and humans.

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 3

# Theoretical Backgrounds of the Necessarity for the Changes of Paradigm in Our Ulcer Research

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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After the active participation in the human clinical pharmacology of patients with peptic ulcer, we were not able to understand how the ulcer actually heals in the patients. Our problems pointed out the following results:

1. The gastric acid secretion [as basal secretion and stimulated secretion produced (provoked) by superluminal – but submaximal – dose of histamine] was unchanged during the medical treatment; however, the ulcer healed. We were not able to explain these contradictory results;

2. The results of clinical pharmacological studies (wide scale of anticholinergic agents) gave us a lot of new insight from the clinical pharmacological parameters (e.g., their absorption, metabolism, excretion) of clinically used drugs. We learned well that different therapeutically applied drugs a priori (e.g., Troparin, Gastropin®) will not absorb from the GI tract during the treatment; consequently, we were not able to find the beneficial effects of these drugs during the medical treatment;

3. The development of tolerance to atropine together with development of "pharmacological denervation phenomenon" has been proven in the background of unchanged gastric acid secretion in patients with duodenal ulcer, during a chronic atropine treatment (see chapter 2.2.). This newer clinical pharmacological explanation has been applied for "existence of gastric cytoprotection." It is true that this phenomenon was observed in patients with duodenal ulcer in terms of the existence of "cytoprotection phenomenon" (not in animal experiments but in patients with duodenal ulcer);

4. When the existence of gastric mucosal protective effect was proved in animal experiments (Jávor et al., 1983), it was clear that vitamin A does not inhibit the gastric acid secretion.

Similar results were obtained in patients with vitamin A, for example, it does not inhibit the gastric basal acid (BAO) and maximal (MAO) acid outputs in healthy subjects (Morón et al.,

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1984); however, it prevented the indomethacin-induced gastric mucosal damage in healthy human subjects (Mózsik et al., 1986).

The results of our randomized, prospective, multiclinical and multicentered studies in patients with chronic gastric ulcer clearly proved that the chronic gastric ulcer really heals without any decrease of gastric acid secretion (Patty et al., 1982; 1983). In other words, we also proved the existence of gastric "cytoprotection" in patients with chronic gastric ulcer.

The vitamin A is a typical scavenger and nutritional molecule, and we used the name of "nutritional gastrointestinal cytoprotection" for animals, healthy human subjects and in patients with different gastrointestinal disorders (Mózsik et al., 2013). These results are absolutely offered to exclude the privileged role of gastric acid secretion in the development of peptic ulcer disease in patients with gastric and duodenal ulcers;

5. To give a correct explanation for the together and simultaneously presence of ulcer with hyperacidity. It was clear at that time that the gastric acid secretion is a result of the very active metabolism; meanwhile, the development of ulcer was suggested as a consequence of impaired metabolism in the gastrointestinal mucosa. It was absolutely an unexplainable fact that both the gastric acid secretion (as an increased metabolic process) and the gastric ulcer (as a consequence of the impaired tissue metabolism) occur together and at the same time in the same organ;

6. In these years, we applied only anticholinergic drugs (together antacids) in the everyday medical treatment. If we suggest that these drugs are able to decrease the gastric acid secretion and if we apply the anticholinergic agent in the medical treatment, we can find a decrease in the metabolic process in the fundus of stomach. If we accept the presence of these decreased metabolic processes in the gastrointestinal tract, we can explain the healing process of ulcerated tissue and that really no hypoxemic presence exists in the ulcerated tissues around the gastric or duodenal ulcer in patients. If the last suggestion is true, than we contradict the generally suggested etiological theories published by the international experts;

7. We had no concrete information (knowledge) on the metabolism of gastrointestinal mucosa produced therapeutically (especially we had no information in this regard to humans).

We discussed very deeply our clinical pharmacological results obtained in patients with classical peptic ulcer disease. Our conclusion is that we do something (treatment) in the medical treatment; however, we have no concrete knowledge on the presence of the different biological events existing in the gastrointestinal tract.

We have to emphasize that we are in the second part of the 1960s–1970s. We had no knowledge of histamine-2 receptor antagonists, gastrin antagonist and, of course, proton pump inhibitors. The established clinical pharmacology helps us to select different compounds (from the medical practices), which were failures both clinically and pharmacologically (e.g., these agents are not absorbed from the gastrointestinal tract). These results offered clear pharmacological evidence to explain that the tertiary ammonium compounds absorb well from the

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human GI tract, meanwhile the absorptions of the quaternary ammonium compounds failed. The aims of pharmacologists were the production of new quaternary ammonium compounds:

1. To produce new compounds with longer and stronger inhibitory actions on the gastric acid secretion in comparison to actions of tertiary ammonium compounds (and these were proved clearly in animal experiments);

2. The tertiary ammonium compounds are able to enter into the circulation of brain (going through the hemato-encephalic barrier), with the quaternary ammonium compounds not entering into thain circulation (through hemato-encephalic barrier).

The results of the established clinical pharmacology clearly indicated that the results of animal experiments can be accepted only with a significant criticism in the application of medical practice. It was also indicated clearly that the existence of clinical pharmacology is absolutely necessary in the pathways of new drug productions versus their clinical applications. The clinical pharmacology was established by us (Jávor et. al., 1965; Mózsik et al., 1965 a, b;,1967 a, b;,1969a;,Mózsik and Jávor, 1965 a, b;,1966 a, b, c;,1968 a, b; Mózsik, 1969a; Jávor, 1968) at Debrecen, Hungary (iuring tie period of 1–-197 year.

Of course, there was a heated debate between the basic researchers and clinical pharmacologists, and the clinical pharmacologists prevailed over the basic researchers.

The classical pharmacology has been studied at our Department of Medicine of Universities (in Debrecen and Pécs, Hungary) up to now (2014) (Hunyady, 1997; Garamszegi et al., 1997; Mózsik et al., 1984d; Mózsik et al., 1985; 1986; 1997a; 1979c; 1981g; 1985; 1992e; Nagy et al., 1997; Patty et al., 1982; 1983; 1987; Tárnok et al., 1979; 1997).

Independently from the establishment of clinical pharmacology in patients with peptic ulcer, we wanted to know more and more on the nature of peptic ulcer disease. We tried to investigate and to know the most important possibilities of ulcer research:

1. The possibilities to approaches of neural effects in human patients with peptic ulcer were very complicated and their methodologies of clinicians were very limited. The increased activity of vagus nerve has been emphasized in the development of peptic ulcer disease. Clinically, our therapeutic possibility was only to apply the anticholinergic agents, which was the reason for starting with the clinical pharmacological evaluation of atropine treatment in patients with peptic ulcer;

2. The surgical intervention, as medical treatment, was frequently used in the 1970s. If the indication of surgery was based on the common consultations between the internists (later gastroenterologists) and surgeons (later gastrointestinal surgeons), then the "medical treatment" (generally for 4 weeks) was carried out as a primary therapeutic possibility, and this treatment was accepted as failed therapeutic process (the clinical pharmacology significantly decreased the number of patients with peptic ulcer disease who had to go over the surgical intervention).

The surgical tradition of Hungarian surgeons was based on the classical German surgical schools, and Billroth II surgical method was generally accepted by them. Only in the 1980s

surgical vagotomy appeared in the practice of Hungarian surgeons (Ihász, 1980). Consequently, we had no possibility to study and to follow these patients;

**3.** The possibility of the new therapeutic compounds was zero to clinicians;

**4.** There was little experimental ulcer research conducted in that time all over the world. Furthermore, experimental ulcer research was not effective in Hungary (these types of observations were done at First Department of Medicine, University of Szeged, Hungary, and were followed at Second Department of Medicine, University of Debrecen, Hungary, up to 1968 and thereafter at First Department of Medicine, University of Pécs, Hungary).

After a critical evaluation of our results obtained from our clinical pharmacology, we started in an absolutely onsure direction, namely the establishment of the biochemical approaches to gastrointestinal mucosa in the field of peptic ulcer research. (One of the authors, GyM, was the esy person.). Ths established biochemical research line was the first trend in peptic ulcer research inalover the World. We have to tethat the applicatin of biochemistry in the ulcer research wasgave a hard scientillaenginge to us (becauseno simends were existed in the Worwhere).

Of course, we tried to do some animal experiments (observations) to receive some practice in this field. Besides, we have to emphasize clearly that we wanted to study the different biochemical mechanisms in the gastrointestinal tract (obtained from the preparations at surgical intervention of patients with peptic ulcer).

Our principal aims were to obtain and to understand the reasons behind biochemical events in the gastrointestinal tract (during the ulcer development and at the time of gastric surgical interventions).

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

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Chapter 4

# General Biochemical Methods to be Used in Gastrointestinal Mucosa in Animal Experiments and in Human Observations Done on Gastrointestinal Resecates (after Surgical Interventions)

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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## 4.1. Introduction

General aims of these observations were to biochemically examine all the gastrointestinal tissues in both animals and humans. The outstanding point was that from 1965 to date, we have been unable to know the exact details of different regulatory mechanisms (by neural, hormonal, pharmacological, immunological and nutritional pathways) under normal (non-ulcerated) and damaged conditions. When we undertook clinical pharmacological studies, we had to face different hard-to-understand medical facts:

1. We had no scientific knowledge on the possible correlation between drug actions and biochemisms in the GI mucosa of animals and humans;
2. We only had different suggestions for the development of hypoxia-induced mucosal damage in the GI mucosa at the time of ulcer development; however, we had no evidence for the existence of this situation;
3. The regulatory mechanisms at the levels of out of gastrointestinal tract and of only in the gastrointestinal tract are absolutely indicated a gap between them;
4. Though the classical, histological definition of peptic ulcer is unchanged, many contradictory "actual discoveries" (new hormones, new growth factors, many chemical compounds, new trends in the medical and surgical treatments, *Helicobacter pylori*, etc.) appeared in the etiology of ulcer disease;
5. Our principal scientific question was in the period 1965–1970: How was the peptic ulcer healed in the human gastrointestinal tract without any decrease of gastric acid secretion?

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> We suggested an answer to this question by applying different and precise biochemical methods in the study of human gastrointestinal tract (with and without the presence of the classical ulcer). Unfortunately, we had no methodology to answer this question.

These observations maintained the main trends of clinical pharmacology (e.g., time period with drugs, to keep the time period after cessation of treatment); however, we introduced the biochemical methodology to the pharmacology.

We tried to approach the biochemical events in the whole tissue by simultaneously using more parallel biochemical measurements (using the same tissue samples, with the measurements carried out at the same time).

Before the human biochemical examinations, we learned the biochemical methodology in animal experiments.

### 4.2. Methodologies of experimental models and clinical studies

The observations were carried out in CFY (Sprague-Dawley) (Gödöllő, Hungary) strain rats, weighing 180–210 g, and on the resecates of stomach and small intestine of patients who underwent gastric surgery because of unhealed ulcer disease (during 1970–1980).

The patients suffered from classical peptic ulcer diseases (PUD) with clinical symptoms (decreased appetite, feeling of dullness and pain in the epigastric region of the abdomen, pyrosis, impaired gastric emptying and retention syndrome). These patients presented one month before the surgical intervention. The presence of gastroduodenal ulcers was endoscopically diagnosed, and thereafter these patients received medical treatments (anticholinergic agents, late $H_2$ receptor antagonist and antacids for one month). A possibility of surgical interventions was evaluated for those patients who were not healed after the treatment.

The indication of gastric surgery was done by physicians [consultations between internists (gastroenterologists) and surgeons] independently from us]. The resecates of stomach and small intestine (according to the method of Billroth II). A small group of patients underwent classical partial gastrectomy (according to the method of Billroth II), and jejunal ulcer was developed. These patients were also medically (pharmacologically) treated during 1970–1980.

During surgical intervention, the stomach and small intestine were removed immediately and these were cut into two parts. One part was given for histological evaluation of resected tissues and the other part was immersed (after separation of mucosa and muscular layer) in liquid nitrogen and used for biochemical examinations. The mucosa specimens were also separated from each other (depending on the distance of ulcer edge). Both the biochemical measurements from the mucosa specimens and muscular layers (independently from the number of tissue specimens), obtained from one patient, and the surgical intervention were carried out at the same time.

The animal observations were carried out in both sexes of CFY-strain rats.

The following experimental models were used:

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1. Pylorus ligation was carried out according to the method of Shay et al., (1945) (using different experimental time periods after pyloric ligation: 1, 4, 7, 24 hours);
2. Pylorus ligation plus surgical vagotomy (using different experimental time periods after surgical intervention);
3. Gastric mucosal damage was caused by aspirin given intragastrically according to the method of Guth et al., (1979);
4. Gastric mucosal preventive actions of atropine, vitamin A and β-carotene in 4-hour pylorus-ligated, aspirin-treated rats;
5. Indomethacin model (4 hours);
6. Gastric mucosal preventive effects of atropine, cimetidine, vitamin A and β-carotene in 4-hour indomethacin-treated rats;
7. Stress ulcer in rats was caused by 4 hours of immobilization (Nagy et al., 1982; 1983);
8. The stress ulcer in rats was caused due to 5 hours of swimming. In some animals, the stress ulcer provocation (swimming) was combined with pyloric ligation during the beginning of stress (Nagy et al., 1982; 1983);
9. Reserpine was subcutaneously injected in animals (Mózsik et al., 1983a);
10. Gastric fundic mucosal damage was caused by topical application of 1 mL from 25 v/v 0.2 M NaOH, 0.6 M HCl and 96 v/v ethanol (Robert et al., 1979);
11. Epinephrine model was completed according to Sethbakdi et al., (1970 a, b, and Pfeiffer, 1971; Pfeiffer and Sethbakdi, 1971). The epinephrine (Tonogén, Budapest) was injected in different doses at different times after pyloric ligation;
12. All biochemical examinations were carried out in the control (non-ulcerated) in the ulcerated (mucosa up to 2 cm around the ulcer) antral, duodenal, jejunal mucosa or from the corpus (fundic), antral, duodenal and jejunal mucosa and from the tissues located below the mucosa (muscular layer). All patients with jejunal ulcers previously underwent a gastric partial resection because of duodenal ulcer. No direct provocative agents such as drugs or primary diseases (renal, endocrine, hematological, liver, pulmonary diseases) could be detected in the background of peptic ulceration ("genuine ulcer"). The tissue specimens were obtained during the surgery.

The following measurements were carried out in different observations:

1. Determination of gastric acid output. The gastric basal acid output (BAO) and maximal acid output (MAO) were determined in patients;
2. The extent of experimental gastric ulcer (except in Shay rats) was scored in the following way: score 0: no ulceration; score 1: the erosions were less than 1 mm; score 2: the erosions were between 2 and 4 mm; score 4: the erosions were greater than 4 mm; and score 5: represents the mucosal damage in all part of fundus. The values of scores were summarized for every stomach, and the average ± SEM was given (Mózsik et al., 1983 a, b);

3. The number of ulcers was calculated;

4. The tissue levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were measured enzymatically (Boehringer, Ingelheim; Germany);

5. The tissue level of cyclic 3′,5′-adenosine monophosphate (cAMP) was measured by RIA (Beckton Dikinson, Orengeburg; USA) (Mózsik et al., 1970 a, b);

6. The tissue level of lactate was enzymatically measured (Ingelheim, Boehringer; Germany);

7. The separation and measurements of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were completed according to the methods published earlier (Mózsik et al., 1967 b, c; 1969 c; 1976 a, b, c, d; 1978 a, b; 1979 a, b, c, d, e; Mózsik and Vizi, 1976 a, b);

8. The separation of membrane ATPase was carried out by the treatment with NaJ and differential centrifugation (Mózsik and Øye, 1969; Mózsik et al., 1974 a, b, c, d; 1979 a, b, c, d, g, e; Schmidt and Tannhauser, 1945);

9. The ATPase activity was measured *in vitro* system by liberation of inorganic phosphorus, followed by the ATP transformation into ADP in the presence of $Mg^{2+}$ (Mg dependent) and $Mg^{2+}$, $Na^{+}$ and $K^{+}$ (total) or $Mg^{2+}$-, $Na^{+}$-, $K^{+}$-dependent ATPase. The $Na^{+}$- and $K^{+}$-dependent ATPase were calculated by the difference between the ATPase activities obtained in the presence of $Mg^{2+}$, $Na^{+}$ and $K^{+}$ and $Mg^{2+}$ ($Mg^{2+}$-dependent part) (Mózsik and Øye, 1969; Mózsik, 1969 a, b; Mózsik et al., 1974 a, b, c, d);

10. The tissue levels of ATP, ADP, AMP, cAMP and lactate were calculated in accordance with 1.0 mg protein, as per the method of Lowry et al. (1951), or with 1.0 mg DNA (in human observations).

The enzyme activity was expressed as micromoles of Pi/mg membrane protein/hour. The results were given as means ± SEM (Mozsik and Øye, 1969; Mózsik, 1969 a, b).The Student "t" test was used for the statistical analysis of the parametric results and by Mann and Whitney's method for the severity of erosions.

We used the rats as experimental animals in these observations to approach the changes in the cellular energy systems and their regulation in different experimental conditions.

The rat's stomach is divided into two parts, namely glandular (fundic) and membranous (rumen). These parts can be separated well and clearly.

The following biochemical measurements were carried out from both parts of the rat's stomach: acid-soluble inorganic phosphates, acid-soluble organic phosphates, lipids, ribonucleic acids (RNA) and deoxyribonucleic acid (DNA) (see the scheme of these measurements in Table 6).

The measurements of these biochemical parameters generally represented the main components of the cells: lipids (as cell membrane), acid-soluble inorganic and organic phosphates (mitochondrion), RNA (partly the cytoplasm as well as nucleus) and DNA (nucleus). In other

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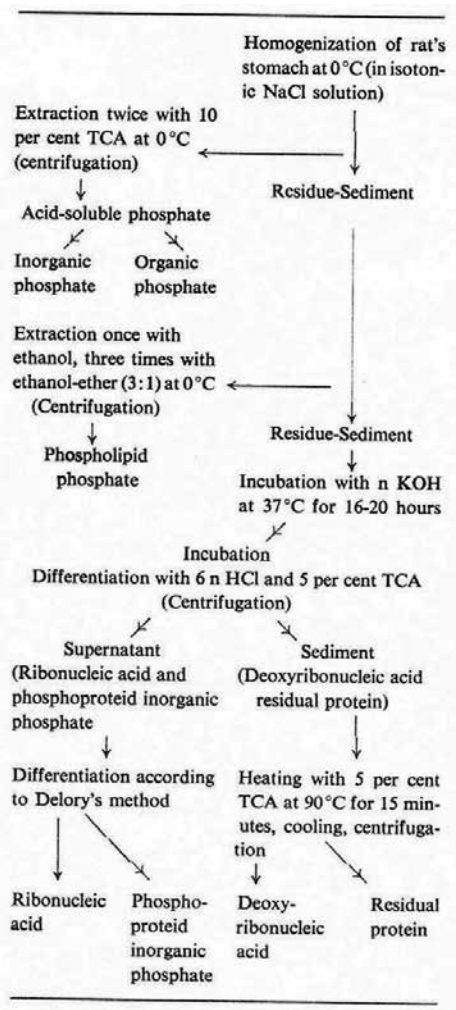


**Table 6.** Summarizing steps of the biochemical procedures for separation of phosphate fractions and nucleic acids of the rat's stomach and of resecates of human gastrointestinal tracts of patients with chronic gastric, duodenal and jejunal ulcer, who underwent surgical intervention because of peptic ulcer diseases. [Mózsik, Szabó, Krausz, Jávor: Scand. J. Gastroenterol. 2: 321–325, 1967 (with kind permission).]

words, we tried to observe different compartments of cells. The measurements of amounts of acid-soluble inorganic phosphates in the different tissues are widely used to approach the dephosphorylation [i.e., these compounds originated from the splitting of adenosine triphosphate (ATP) independently from its different pathways]. The components of the acid-soluble organic phosphates were not known at that time; meanwhile, the presence of adenosine triphosphate (ATP), adenosine diphosphate (ADP), cyclic 3′,5′-adenosine monophosphate (cAMP), adenosine monophosphate (AMP) and adenine and adenosine were incorporated in this tissue extract. Naturally, these methodologies were updated later by direct measurements of these compounds using thin-layer chromatographic and enzymatic methods.

### 4.3. The pharmacological–biochemical studies of animals in acute and chronic experimental conditions

*4.3.1. General biochemistry of glandular (fundic) part and of forestomach (rumen) in 24-hour pylorus-ligated rats*

The necessity of the biochemical analysis of the stomach (gastroduodenal) mucosa was suggested for a better understanding. The underline mechanisms involved in development of mucosal damage and prevention (1962–1964) (Gheorghui, 1975; Mózsik et al., 1967 a, b; 1969 a, b, c, d; Mózsik et al., 1970 a, b).

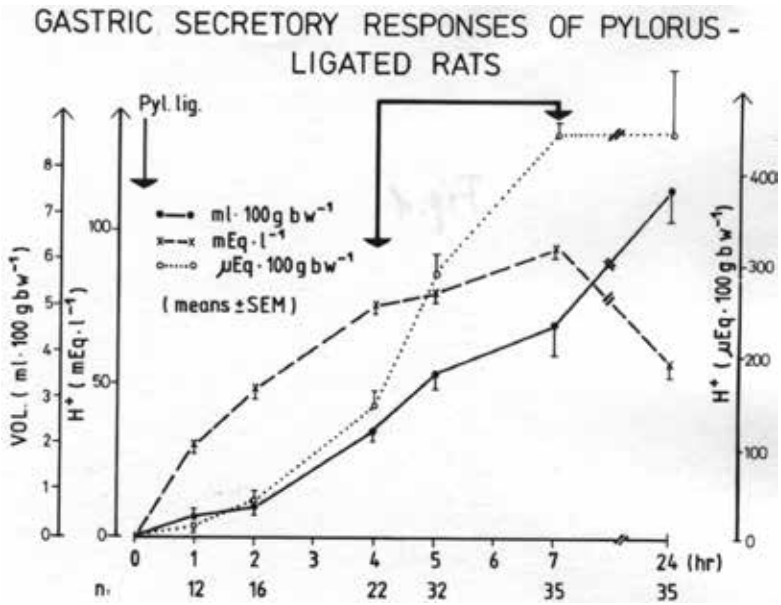


**Figure 13.** The pattern of gastric secretory responses in 24-hour pylorus-ligated rats. The volume of gastric secretory responses (mL/100 body weight), $H^+$ concentration (mEq/L) and $H^+$ output (μEq/100 b.w.) were measured and their results were expressed as means ± SEM, indicating the number of animals. The time period between 4 and 7 hours after pyloric ligation represents the optimal time period to study the actions of drugs and hormones and their biochemical changes in the different parts of the stomach. The peak of maximal acid output can be obtained in 7 hours; meanwhile, the gastric ulceration appears after 7 hours in pylorus-ligated rats (means ± SEM). [Mózsik et al., Scand. J. Gastroenterol 4:633–640; 1969 and Acta Physiol. Scand. Spec. Suppl. 1978a (with kind permission).]

In the first period, the acid-soluble organic and inorganic phosphates and phospholipids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were extracted from the gastric mucosa, and their quantities were measured with (Mózsik et al., 1969 a, b, c, d) and without surgical vagotomy (Mózsik et al., 1967 a, b) (Figures 13–17). Later adenosine triphosphate (ATP) and adenosine diphosphate (ADP) were measured during atropine treatment (Mózsik et al., 1970 a, b). The biochemical parameters were chosen as a suitable methodology to give a "biochemical cross-section" of gastroduodenal mucosa under different experimental conditions.

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These results stimulated us for doing further biochemical observations in the animal stomach on dependence of increased and decreased vagal activity.

GENERAL BIOCHEMICAL MECHANISMS OF DEVELOPMENT OF GASTRIC HYPERSECRETION AND ULCER DEVELOPMENT IN PYLORUS-LIGATED RATS

| | GLANDULAR STOMACH | | | FORESTOMACH | | |
|---|---|---|---|---|---|---|
| | SHAM-OPERATED | 7 HR | 24 HR | SHAM-OPERATED | 7HR | 24HR |
| secretory vol.(ml) | – | 5.85±0.06 | 15.76±0.8 | – | – | – |
| $H^+$ output (μEq) | – | 1145±35 $^{+++}$ | 1260±64 $^{+++}$ | – | – | – |
| ulcer number | – | – | – | – | – | 60/10 |
| wet weight (g) | 0.68±0.02 | 0.81±0.02 $^{+++}$ | 0.80±0.04 $^{++}$ | 0.36±0.01 | 0.48±0.01 $^{+++}$ | 0.56±0.02 $^{+++}$ |
| Acid soluble inorg. P (μg) | 241±11 | 184±17 $^{+++}$ | 260±13 $^{++}$ | 115±18 | 87±16 $^{NS}$ | 214±22 $^{+++}$ |
| org. P (μg) | 156±18 | 245±18 $^{++}$ | 49±22 $^{+++}$ | 32±10 | 131±14 $^{+++}$ | 0±0 $^{+++}$ |
| Lipid-P (μg) | 255±22 | 365±42 $^{+++}$ | 749±123 $^{+++}$ | 178±28 | 291±30 $^{+}$ | 611±111 $^{+++}$ |
| RNA (μg) | 2886±237 | 3147±137 $^{NS}$ | 2766±78 $^{NS}$ | 905±71 | 1104±58 $^{+}$ | 1932±264 $^{++}$ |
| DNA (μg) | 4400±131 | 5335±336 $^{NS}$ | 5610±215 $^{++}$ | 650±48 | 1720±392 $^{+}$ | 1825±208 |

NS=not significant +=0.05>P>0.01 ++=0.01>P>0.001 +++=P<0.001
(means ± SEM) (n=10)

**Figure 14.** The changes in the chemical composition of glandular stomach (fundus) and forestomach (rumen) in pylorus-ligated rats. The following parameters were measured: gastric secretory volume (mL), $H^+$ output, number of ulcers, wet tissue (g), acid-soluble inorganic ($P_i$) and organic ($P_i$) phosphates, lipid phosphates (μg), ribonucleic acid (RNA) (μg) and deoxyribonucleic (DNA) (μg) acids. The results were expressed as percentage values of sham-operated (=100%) animals. The statistical analysis was carried out between the sham-operated rats and 7- and 24-hour pylorus-ligated rats (means ± SEM). [Mózsik et al., Scand J Gastroenterol 4: 633–640; 1969 (with kind permission).]

There were many criticisms for this experimental ulcer model because the ulceration appeared in the forestomach (not in the glandular part of the animal stomach); however, different typical events of experimentally developed ulcer can be detected using this model:

**a.** The gastric hypersecretion can be obtained before the ulcer development in accordance with the time (after surgical intervention);

**b.** The peak of gastric acid hypersecretion can be obtained in this model in 7 hours after the surgical intervention (see Figure 2), and its value does not change from 7 to 24 hours after pyloric ligation;

**c.** The time period between 4 and 7 hours offers excellent good possibility to study the stimulatory or inhibitory actions of different compounds on the gastric acid secretion in rats;

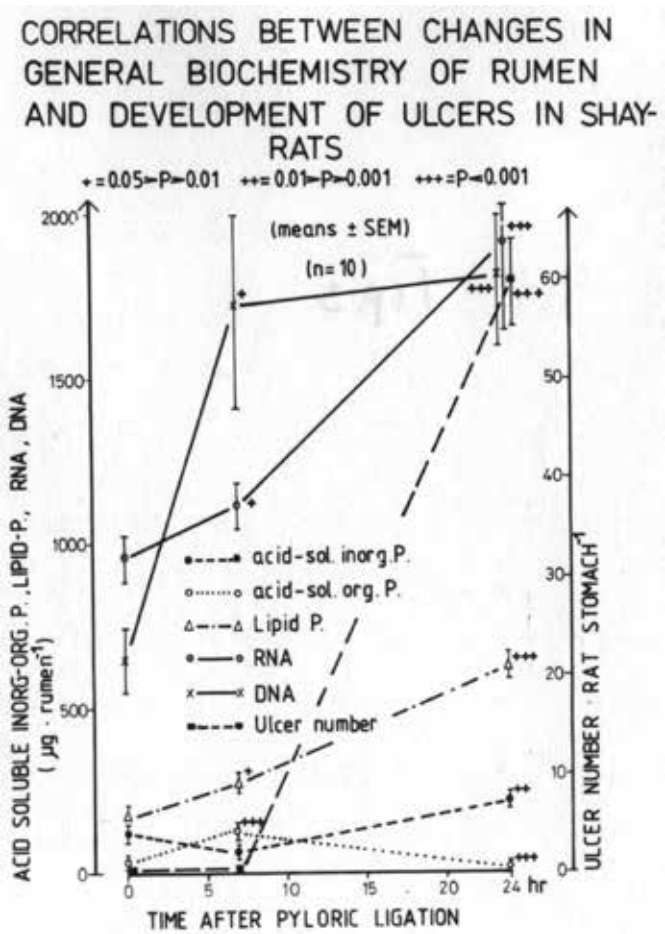


**Figure 15.** Correlation between the changes in the general biochemistry of ulcer development in forestomach (rumen) of the 24-hour pylorus-ligated rats depending on time after pyloric ligation. [Mózsik et al., Scand. J. Gastroenterol. 4: 633–640; 1969 (with kind permission).]

**d.** We can very well study the possible correlations between gastric acid secretory responses and development of gastric ulcer.

Bearing these conclusions in mind, we started with the "general biochemical" approach – the main biochemical events during the development of gastric acid hypersecretion and ulcer (of course, respecting the actual level of international research). We have to emphasize that no general biochemical examinations were given in the gastrointestinal research for animals and patients earlier. So, these types of observations internationally opened a new avenue ("biochemistry") in the gastrointestinal research.

We tried to select the different biochemically measured parameters for providing nearest approach to the cell functions (e.g., membrane, mitochondrion, ribonucleic acid and deoxyribonucleic acid).

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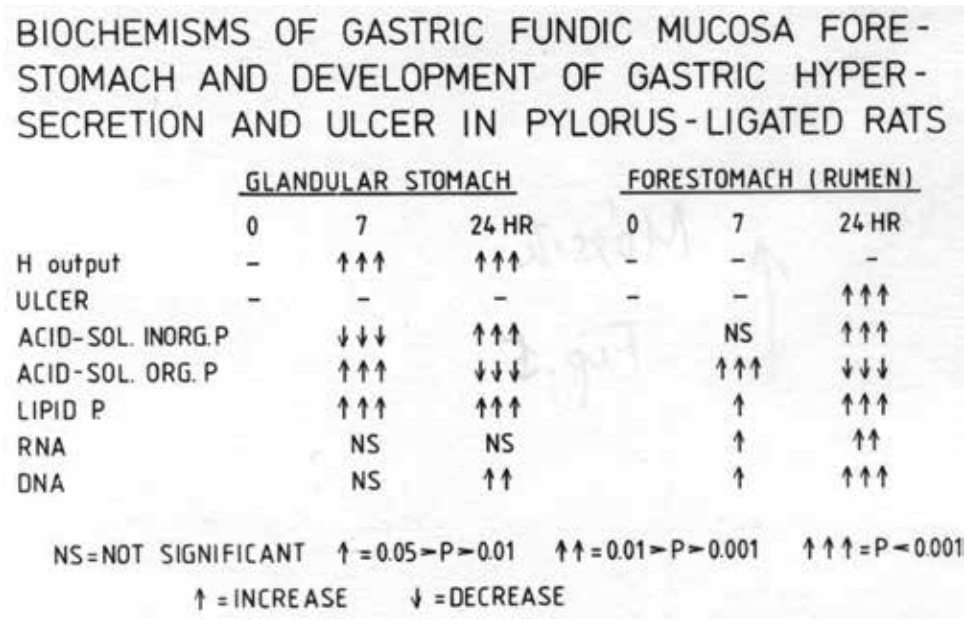


**Figure 16.** Comparative changes in the biochemistry (acid-soluble inorganic and organic phosphates, lipid phosphates, RNA and DNA) of glandular stomach and forestomach of 24-hour pylorus-ligated rats. The means ± SEM values of sham-operated rats (=100%) are expressed in percentage. *P* values: $P < 0.05$; $P < 0.01$; $P < 0.001$. [Mózsik et al., Scand. J. Gastroenterol. 4: 633–640; 1969 (with kind permission).]

The functions of the organs are specific events (gastric secretion, ulcer development); meanwhile, the biochemical mechanisms obtained in the target organs are extremely complicated. The biochemical extractums (e.g., acid-soluble inorganic and organic phosphates, lipids, ribonucleic acid and deoxyribonucleic acid) from the gastric tissues dominantly represent the cell membranes (lipids), mitochondrion (lipid-soluble organic and inorganic phosphates, partly ribonucleic acid) and nucleus (deoxyribonucleic acid).

At that time, the measurements of acid-soluble inorganic phosphate represent the cumulative effect of breakdown of adenosine triphosphate (ATP) (by different pathways) from the effector organs; meanwhile, the compounds of the acid-soluble organic phosphates (when these observations were carried out) were unknown. Now, we know that the acid-soluble organic phosphates contain the adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic 3′,5′-adenosine monophosphate (cAMP) and adenosines and adenines.

We have to emphasize that we tried to approach only the main biochemical lines in the stomach during the development of gastric acid hypersecretion and ulcer after surgical intervention.

The obtained results of our present observations clearly indicated to us:

**a.** All biochemical changes (in acid-soluble inorganic and organic phosphates, lipids, RNA and DNA) – depending on time – are similar to each other;

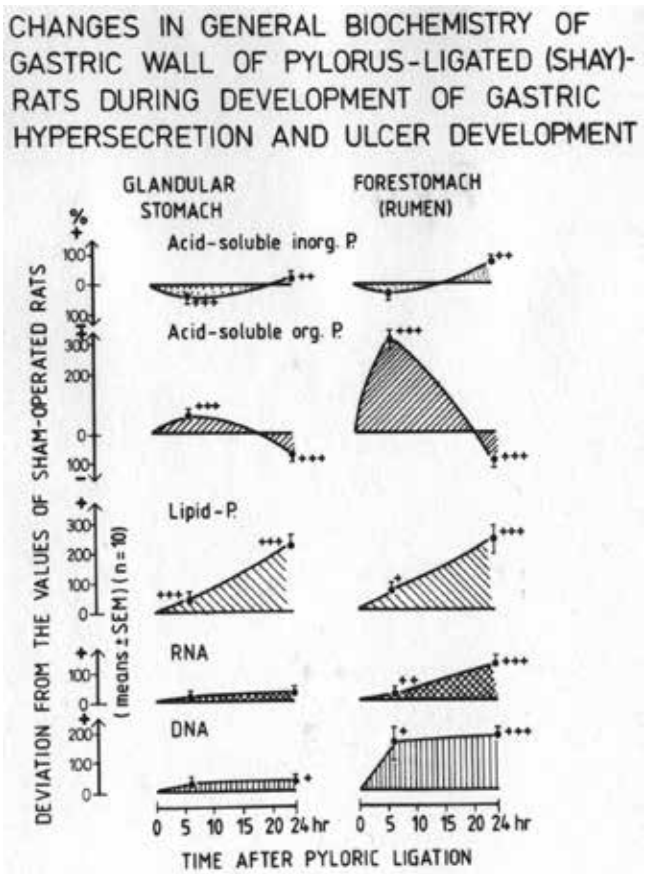


**Figure 17.** Schematic summary of the changes in gastric hypersecretion, ulcer development (forestomach) and biochemical compositions (acid-soluble inorganic and organic phosphates, lipid phosphates, RNA and DNA) observed in 24-hour pylorus-ligated rats. [Mózsik et al., Scand. J. Gastroenterol. 4:633–640; 1969 (with kind permission).]

**b.** The biochemical changes in both parts of stomach (glandular stomach and forestomach) appeared before the development of gastric acid hypersecretion and ulcer development;

**c.** Because gastric acid hypersecretion is the result of a consequence of very active metabolic processes in the glandular part of stomach and because the same biochemical changes were obtained from the forestomach, it can be concluded that the gastric ulceration appears after increased biochemism in the forestomach;

**d.** The decrease of acid-soluble inorganic phosphates (earlier) along with the increase of acid-soluble organic phosphates clearly indicates the increased biochemical metabolism in both parts of the stomach;

**e.** The mentioned changes in the acid-soluble inorganic and organic phosphates led to the significant changes of ATP breakdown and ATP resynthesis [in other words changes in the dephosphorylation and oxidative phosphorylation pATP synthesis)].

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The critical evaluation of these experimental results called our attention to reconsider our previously created knowledge on the development of gastric acid hypersecretion and ulcer development in pylorus-ligated rats.

*4.3.2. General biochemistry of glandular (fundic) part and forestomach (rumen) after acute "chemical" and "surgical" vagotomy in rats (using different anticholinergic compounds)*

The effects of acute administrations of parasympatholytics [(atropine, isopropamide 2,2-diphenyl-4-diisopropylamino-methyl iodide) and Gastrixon (methyl-tropinium-bromide-xanthene-9-carboxylate)] and bilateral surgical vagotomy on the biochemism of stomach [glandular part and membranaceous (forestomach, rumen)] in 7-hour pyloric-ligation rats were studied.

Atropine is a tertiary ammonium amine, while the isopropamide and Gastrixon are quaternary ammonium components. The chemical diameter of quaternary ammonium structure of Gastrixon is greater than isopropamide (Gyermek, 1951, 1953; Gyermek and Nádor, 1952, 1953 a, b; György et al., 1961; De Jongh et al.,J1955; Proosdij-Harzeme,et al., J955). The blocking effects of parasympatholytics increase with the increasing diameter of tertiary and quaternary ammonium molecules on the autonomic nerve systems (Fehér, 1960; Grenell and Mullins, 1956).

Our aims were (1) to study the effects of different parasympatholytics and surgical vagotomy on the biochemical parameters of stomach and (2) to compare the changes in the gastric mucosal biochemical parameters produced by "chemical" and "surgical" vagotomy.

| | Glandular stomach wall | | Membranaceous stomach wall | |
|---|---|---|---|---|
| Experimental groups | n | quantities (*g*) | n | quantities (*g*) |
| 7 hours after laparotomy + pyloric ligation | 10 | 0.81±0.02 ( 1) | 10 | 0.48±0.01 ( 2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 0.89±0.04 ( 3) | 10 | 0.61±0.03 ( 4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c ) | 8 | 0.85±0.04 ( 5) | 8 | 0.56±0.03 ( 6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c ) | 8 | 0.80±0.02 ( 7) | 8 | 0.48±0.01 ( 8) |
| 24 hours after laparotomy + pyloric ligation | 10 | 0.80±0.04 ( 9) | 10 | 0.56±0.01 (10) |
| 24 hours after laparotomy + pyloric ligation + vagotomy | 8 | 0.70±0.02 (11) | 8 | 0.51±0.03 (12) |

*Significances :*

| | | | |
|---|---|---|---|
| 1 – 3 : P = 0.05 | 3 – 5 : P > 0.05 | 2 – 4 : P < 0.001 | 4 – 6 : P > 0.05 |
| 1 – 5 : P > 0.05 | 5 – 9 : P > 0.05 | 2 – 6 : P = 0.02 | 6 – 8 : P = 0.02 |
| 1 – 7 : P > 0.05 | 3 – 9 : P > 0.05 | 2 – 8 : P > 0.05 | 4 – 8 : P < 0.001 |
| 9 – 11 : P = 0.02 | | 10 – 12 : P > 0.05 | |

**Table 7.** Changes of stomach weight in Shay rats after administration of parasympatholytics and bilateral surgical vagotomy. The results are expressed as means ± standard error of means (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651 1969c (with kind permission).]

| Experimental groups | n | Volume of gastric secretion (ml) | Acidity (free/total) | Frequency of ulcer (number of ulcers/examined animals) | |
|---|---|---|---|---|---|
| | | | | Glandular stomach wall | Membranaceous stomach wall |
| 7 hours after laparotomy + pyloric ligation | 10 | 5.85±0.06 | 59/89 | 0/10 | 0/10 |
| 7 hours after laparotomy + pyrolic lig. + Atropine (1.0 mg s. c. ) | 13 | 0.60±0.002 | 0/5 | 0/13 | 0/13 |
| 7 hours after laparotomy + pyrolic lig. + Isopropamide (1.0 mg s. c.) | 8 | 0.30±0.001 | 0/10 | 0/8 | 0/8 |
| 7 hours after laparotomy + pyloric lig. + Gastrixone (1.0 mg s. c.) | 8 | 0.60±0.002 | 0/8 | 0/8 | 0/8 |
| 24 hours after laparotomy + pyloric ligation | 10 | 15.76±0.80 | 80/100 | 0/10 | 12/10 |
| 24 hours after laparotomy + pyloric lig. + bilateral surgical vagotomy | 8 | 1.40±0.06 | 32/64 | 0/8 | 0/8 |

**Table 8.** Changes of gastric secretion, acidity and frequency of ulcer in Shay rats after administration of parasympatholytics and surgical vagotomy. The results are expressed as means ± standard error of means (mL, mEq/L), and total number of ulcers in one rat's stomach. (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

| Experimental groups | Glandular stomach wall | | Membranaceous stomach wall | |
|---|---|---|---|---|
| | n | quantities (*g*) | n | quantities (*g*) |
| 7 hours after laparotomy + pyloric ligation | 10 | 0.81±0.02 ( 1) | 10 | 0.48±0.01 ( 2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 0.89±0.04 ( 3) | 10 | 0.61±0.03 ( 4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c ) | 8 | 0.85±0.04 ( 5) | 8 | 0.56±0.03 ( 6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c ) | 8 | 0.80±0.02 ( 7) | 8 | 0.48±0.01 ( 8) |
| 24 hours after laparotomy + pyloric ligation | 10 | 0.80±0.04 ( 9) | 10 | 0.56±0.01 (10) |
| 24 hours after laparotomy + pyloric ligation + vagotomy | 8 | 0.70±0.02 (11) | 8 | 0.51±0.03 (12) |

*Significances :*

1 − 3 : P = 0.05 3 − 5 : P > 0.05 2 − 4 : P < 0.001 4 − 6 : P > 0.05
1 − 5 : P > 0.05 5 − 9 : P > 0.05 2 − 6 : P = 0.02 6 − 8 : P = 0.02
1 − 7 : P > 0.05 3 − 9 : P > 0.05 2 − 8 : P > 0.05 4 − 8 : P < 0.001
9 − 11 : P = 0.02 10 − 12 : P > 0.05

**Table 9.** Changes of stomach weight in Shay rats after administration of parasympatholytics and bilateral surgical vagotomy. The results are expressed as means ± standard error of means (mL, mEq/L), and total number of ulcers in one rat's stomach (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

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| Experimental groups | n | Acid-soluble inorganic phosphates quantities | n | Acid-soluble organic phosphates quantities |
|---|---|---|---|---|
| 7 hours after laparotomy + pyloric ligation | 10 | 185 ± 17 (1) | 10 | 245 ± 18 (2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 405 ± 52 (3) | 13 | 119 ± 34 (4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c.) | 8 | 232 ± 9 (5) | 8 | 208 ± 20 (6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c.) | 8 | 219 ± 17 (7) | 8 | 281 ± 17 (8) |

*Significances*

| | | | |
|---|---|---|---|
| 1–3: $P < 0.001$ | 3–5: $0.01 > P > 0.001$ | 2–4: $0.01 > P > 0.001$ | 4–6: $P = 0.04$ |
| 1–5: $P = 0.02$ | 3–7: $0.01 > P > 0.001$ | 2–6: $P > 0.05$ | 4–8: $0.01 > P > 0.001$ |
| 1–7: $P > 0.05$ (=0.08) | 5–7: $P > 0.05$ | 2–8: $P > 0.05$ | 6–8: $0.01 > P > 0.001$ |

**Table 10.** Changes of the acid-soluble phosphates in Shay rats' glandular stomach wall after administration of parasympatholytics. The results are expressed as means ± standard error of means in μg phosphate per total wet glandular stomach wall (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

| Experimental groups | n | Acid-soluble inorganic phosphates quantities | n | Acid-soluble organic phosphates quantities |
|---|---|---|---|---|
| 7 hours after laparotomy + pyloric ligation | 10 | 87 ± 16 (1) | 10 | 131 ± 14 (2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 116 ± 6 (3) | 13 | 26 ± 10 (4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c.) | 8 | 133 ± 6 (5) | 8 | 81 ± 10 (6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c.) | 8 | 88 ± 4 (7) | 8 | 87 ± 5 (8) |

*Significances*

| | | | |
|---|---|---|---|
| 1–3: $P > 0.05$ (= 0.06) | 3–5: $P = 0.06$ | 2–4: $P < 0.001$ | 4–6: $P = 0.001$ |
| 1–5: $0.01 > P > 0.001$ | 3–7: $P < 0.001$ | 2–6: $0.01 > P > 0.001$ | 4–8: $P < 0.001$ |
| 1–7: $P > 0.05$ | 5–7: $P < 0.001$ | 2–8: $0.01 > P > 0.001$ | 6–8: $P < 0.05$ |

**Table 11.** Changes of the acid-soluble phosphates in Shay rats' membranaceous stomach (rumen) wall after administration parasympatholytics. The results are expressed as means ± standard error of means in μg phosphate per total wet membranaceous stomach wall (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

| Experimental groups | Phospholipid phosphates | | | |
|---|---|---|---|---|
| | Glandular stomach wall | | Membranaceous stomach wall | |
| | n | quantities | n | quantities |
| 7 hours after laparotomy + pyloric ligation | 10 | 365 ± 42 (1) | 10 | 291 ± 30 (2) |
| 7 hours after laparotomy + pyloric ligation + Atropine (1.0 mg s. c.) | 13 | 229 ± 27 (3) | 13 | 137 ± 18 (4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c.) | 8 | 168 ± 21 (5) | 8 | 107 ± 13 (6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c.) | 8 | 265 ± 20 (7) | 8 | 128 ± 9 (8) |

*Significances*

| | | | |
|---|---|---|---|
| 1–3: $P = 0.02$ | 3–5: $P > 0.05$ | 2–4: $P < 0.001$ | 4–6: $P > 0.05$ |
| 1–5: $P < 0.001$ | 3–7: $P > 0.05$ | 2–6: $P < 0.001$ | 4–8: $P > 0.05$ |
| 1–7: $P = 0.05$ | 5–7: $0.01 > P > 0.001$ | 2–8: $P < 0.001$ | 6–8: $P > 0.05$ |

**Table 12.** Changes of phospholipids (phosphates) in Shay rats' glandular and membranaceous stomach wall after administration of parasympatholytics. The results are presented as means ± standard error of means in μg phosphate per total wet glandular and membranaceous stomach wall (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

| Experimental groups | Ribonucleic acid | | | |
|---|---|---|---|---|
| | Glandular stomach wall | | Membranaceous stomach wall | |
| | n | quantities | n | quantities |
| 7 hours after laparotomy + pyloric ligation | 10 | 3147 ± 157 (1) | 10 | 1104 ± 58 (2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 2818 ± 340 (3) | 13 | 685 ± 66 (4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c.) | 8 | 3400 ± 198 (5) | 8 | 1270 ± 95 (6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c.) | 8 | 3538 ± 189 (7) | 8 | 1048 ± 53 (8) |

*Significances*

| | | | |
|---|---|---|---|
| 1–3: $P > 0.05$ | 3–5: $P > 0.05$ | 2–4: $P < 0.001$ | 4–6: $P < 0.001$ |
| 1–5: $P > 0.05$ | 3–7: $P > 0.05$ | 2–6: $P > 0.05$ | 4–8: $P < 0.001$ |
| 1–7: $P > 0.05$ | 5–7: $P > 0.05$ | 2–8: $P > 0.05$ | 6–8: $P > 0.05$ |

**Table 13.** Changes of ribonucleic acids in Shay rats' glandular and membranaceous stomach wall after administration of parasympatholytics. The results are presented as means ± standard error of means in μg nucleic acid per total wet glandular and membranaceous stomach wall (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

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| Experimental groups | Deoxyribonucleic acid: Glandular stomach wall n | Glandular stomach wall quantities | Membranaceous stomach wall n | Membranaceous stomach wall quantities |
|---|---|---|---|---|
| 7 hours after laparotomy + pyloric ligation | 10 | 5335 ± 366 (1) | 10 | 1720 ± 392 (2) |
| 7 hours after laparotomy + pyloric ligation + atropine (1.0 mg s. c.) | 13 | 3058 ± 394 (3) | 13 | 1273 ± 237 (4) |
| 7 hours after laparotomy + pyloric ligation + Isopropamide (1.0 mg s. c.) | 8 | 5834 ± 458 (5) | 8 | 1577 ± 119 (6) |
| 7 hours after laparotomy + pyloric ligation + Gastrixone (1.0 mg s. c.) | 8 | 5815 ± 263 (7) | 8 | 1247 ± 103 (8) |

*Significances*

| | | | |
|---|---|---|---|
| 1–3: $P < 0.001$ | 3–5: $P < 0.001$ | 2–4: $P > 0.05$ | 4–6: $P > 0.05$ |
| 1–5: $P > 0.05$ | 3–7: $P < 0.001$ | 2–6: $P > 0.05$ | 4–8: $P > 0.05$ |
| 1–7: $P > 0.05$ | 5–7: $P > 0.05$ | 2–8: $P > 0.05$ | 6–8: $P = 0.02$ |

**Table 14.** Changes of deoxyribonucleic acids in Shay rats' glandular and membranaceous stomach wall after administration of parasympatholytics. The results are presented as means ± standard error of means in μg deoxyribonucleic acid per total wet glandular and membranaceous stomach wall (*n* indicates the number of animals). [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

| Examined experimental parameters | Parts of stomach wall: Glandular stomach wall, Experimental groups: Control | After Atropine | After Isopropamide | After Gastrixone | Membranaceous stomach wall, Experimental groups: Control | After Atropine | After Isopropamide | After Gastrixone |
|---|---|---|---|---|---|---|---|---|
| Gastric secretion | 100.0 | 12.0 | 6.0 | 12.0 | – | – | – | – |
| Acidity (free/total) | 100/100 | 0/5 | 0/12 | 0/10 | – | – | – | – |
| Acid soluble inorganic phosphates | 100.0 | 225.0 | 128.0 | 121.0 | 100.0 | 145.0 | 166.0 | 100.0 |
| Acid-soluble organic phosphates | 100.0 | 49.0 | 86.0 | 117.0 | 100.0 | 20.0 | 62.0 | 66.0 |
| Phospholipid phosphates | 100.0 | 63.0 | 46.0 | 73.0 | 100.0 | 47.0 | 36.0 | 44.0 |
| Ribonucleic acid | 100.0 | 90.0 | 109.0 | 114.0 | 100.0 | 66.0 | 115.0 | 91.0 |
| Deoxyribonucleic acid | 100.0 | 57.0 | 110.0 | 109.0 | 100.0 | 80.0 | 93.0 | 73.0 |

**Table 15.** The changes in the percentage of examined parameters of Shay rats' stomach wall induced by parasympatholytics in percentage of control ($n = 100$) values 7 hours after pyloric ligation. [Mózsik et al., Scand. J. Gastroenterol., 4: 641–651, 1969c (with kind permission).]

Increasing inhibitory effects of parasympatholytics – depending on the extent of chemical diameters – did not run closely parallel with increasing diameter of tertiary and quaternary ammonium molecules, and we also perceived biochemical changes of gastric mucosal biochemistry. After bilateral surgical vagotomy, the quantities of acid-soluble inorganic

| Examined experimental parameters | Parts of stomach wall | | | |
|---|---|---|---|---|
| | Glandular stomach wall | | Membranaceous stomach wall | |
| | Experimental groups | | Experimental groups | |
| | Control | After vagotomy | Control | After vagotomy |
| Gastric secretion | 100.0 | 9.3 | – | – |
| Acidity (free/total) | 100/100 | 40/65 | – | – |
| Acid-soluble inorganic phosphates | 100.0 | 85.0 | 100.0 | 55.0 |
| Acid-soluble organic phosphates | 100.0 | 278.0 | 0.0 | |
| Phospholipid phosphates | 100.0 | 31.0 | 100.0 | 19.0 |
| Ribonucleic acid | 100.0 | 57.0 | 100.0 | 29.0 |
| Deoxyribonucleic acid | 100.0 | 115.0 | 100.0 | 74.0 |

**Table 16.** The changes in percentage of examined parameters of Shay rats' stomach wall induced by surgical vagotomy of control ($n$ = 100) values 24 hours after pyloric ligation. [Mózsik et al., Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

phosphates decreased significantly in both glandular and membranaceous (forestomach and rumen) stomach wall. The alterations of acid-soluble inorganic and organic phosphates in the stomach wall showed contradictory trends of surgical vagotomy to those after administration of parasympatholytics ("chemical" vagotomy), the effects of surgical vagotomy on the nucleic acid metabolism being greater than the effects of different parasympatholytics.

A biochemical–cellular–morphological explanation of parasympatholytics and surgical vagotomy has been suggested (Figure 18). According to this explanation, the nucleic acids are in the center of Figure 18, and phospholipids, acid-soluble inorganic and organic phosphates are in periphery of a "hypothetic cell." The products of cells (HCl secretion) are presented in an outer part of the figure. After a large alteration of periphery, the center will change to a small degree and *vice versa.*

GENERAL FUNCTIONS OF ORGANS
↓↑
BIOCHEMICAL REACTIONS OF TISSUES
(acid-soluble phosphates, phospholipids)
↓↑
PROTEINS, ENZYMES
↓↑
RIBONUCLEIC ACIDS
↓↑
DEOXYRIBONUCLEIC ACIDS

**Table 17.** Main steps of regulatory levels of cells in the living organs. The most stable regulatory steps are located at the level of DNA and the most instable regulatory steps are at the level of functions of organs.

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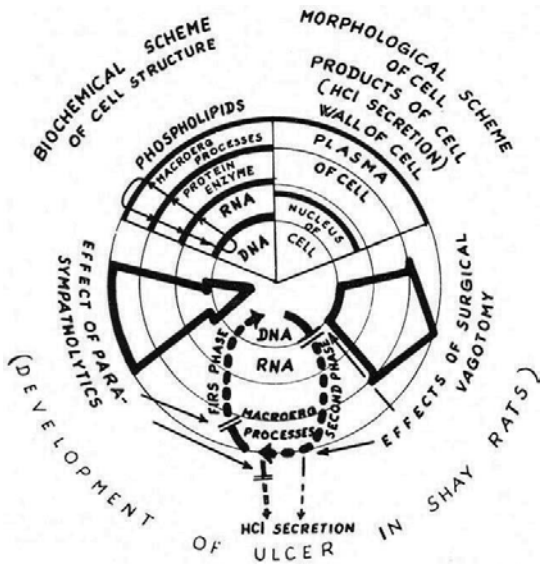


**Figure 18.** A schematic and possible biochemical–cellular–morphological explanation of parasympatholytics and surgical vagotomy. [Mózsik, Kiss, Krausz, Jávor: Scand. J. Gastroenterol. 4: 641–651, 1969c (with kind permission).]

### *4.3.3. Pharmacological and biochemical studies in rats after chronic "chemical" and "surgical" vagotomy and cholinesterase inhibitor treatment*

Biochemical observations were carried out to study the changes in these biochemical parameters of rat's stomach after chronic "chemical vagotomy" (2 × 1.0 mg i.p. atropine for 25 days) and cholinesterase inhibitor treatment (2 × 0.25 mg of neostigmine i.p for three weeks).

The biochemical examinations in the drug-treated groups of animals were also divided into two different groups. The biochemical observations of the first group were carried out immediately after the end of the drug treatment, whereas the observations of the second group were carried out after cessation of drug treatments (10 days after the cessation of atropine treatment and three weeks after cessation of cholinesterase inhibitor treatment).

To compare the changes in the stomach after a chronic "chemical" vagotomy (atropine treatment), the surgical vagotomy was carried out in a group of animals (without any other treatment), and the biochemical measurements were done one month after surgical vagotomy.

The control animals were treated with saline solution for 25 days. It has been suggested that the results of these animal observations will give a biochemical explanation for the effects of increased cholinergic activity (produced by cholinesterase inhibitor), for "chemical" vagotomy and "surgical" vagotomy ("use" vs. "disuse" of vagal nerve on the metabolism of gastric tissues). The results of the biochemical examinations are presented in cases of chronic atropine treatment and chronic neostigmine (see the forthcoming tables). The biochemical results after one month of surgical vagotomy are presented only in comparison with the changes in the biochemical parameters obtained in rats treated chronically with atropine and neostigmine.

| | Controls (treated with physiological saline) | | Animals treated with atropine for 25 days | | 10 days after cessation of atropine treatment | |
|---|---|---|---|---|---|---|
| | *n* | quantities | *n* | quantities | *n* | quantities |
| Body weight (g) | 11 | 227 ± 10 (1) | 10 | 189 ± 8 (2) | 6 | 207 ± 4 (3) |
| Weight of the glandular (pyloric) part of the stomach (g) | 11 | 0.99 ± 0.06 (4) | 10 | 0.91 ± 0.05 (5) | 6 | 0.88 ± 0.07 (6) |
| Weight of the membranous (rumenal) part of the stomach (g) | 11 | 0.65 ± 0.001 (7) | 10 | 0.71 ± 0.05 (8) | 6 | 0.63 ± 0.01 (9) |

Statistical values:
Between 1 and 2: $0.01 > P > 0.001$, between 2 and 3 : $P > 0.05$, between 1 and 3 : $P > 0.05$.
Between 4 and 5 : $P > 0.05$, between 5 and 6 : $P > 0.05$, between 4 and 6 : $P > 0.05$ (= 0.06).
Between 7 and 8 : $P > 0.05$, between 8 and 9 : $P > 0.05$, between 7 and 9 : $P > 0.05$.

**Table 18.** Changes of body weight and weight of the stomach wall in animals treated with atropine. The results are expressed as mean values ± standard error (n indicates the number of animals). [Mózsik et al., Eur. J. Pharmacol 7: 66–72, 1969c (with kind permission).]

| | Controls (animals treated with physiological saline for 25 days) | | Animals treated with atropine for 25 days | | 10 days after cessation of atropine treatment | |
|---|---|---|---|---|---|---|
| | *n* | quantities | *n* | quantities | *n* | quantities |
| Acid-soluble inorganic phosphates | 11 | 308 ± 15 (1) | 10 | 259 ± 12 (2) | 6 | 257 ± 8 (3) |
| Acid-soluble organic phosphates | 11 | 374 ± 21 (4) | 10 | 169 ± 39 (5) | 6 | 284 ± 10 (6) |
| Phospholipid phosphates | 11 | 499 ± 61 (7) | 10 | 219 ± 19 (8) | 6 | 338 ± 6 (9) |
| Ribonucleic acid | 11 | 4237 ± 467 (10) | 10 | 3405 ± 217 (11) | 6 | 3009 ± 81 (12) |
| Deoxyribonucleic acid | 11 | 6181 ± 457 (13) | 10 | 5504 ± 303 (14) | 6 | 5228 ± 115 (15) |

Statistical values:
between 1 and 2 : $P = 0.02$ between 2 and 3 : $P > 0.05$ between 1 and 3 : $0.01 > P > 0.001$
between 4 and 5 : $P < 0.001$ between 5 and 6 : $P = 0.02$ between 4 and 6 : $0.01 > P > 0.001$
between 7 and 8 : $P < 0.001$ between 8 and 9 : $P < 0.001$ between 7 and 9 : $P = 0.02$
between 10 and 11 : $P = 0.03$ between 11 and 12 : $P > 0.05$ between 10 and 12 : $P < 0.001$
between 13 and 14 : $P > 0.05$ between 14 and 15 : $P > 0.05$ between 13 and 15 : $P > 0.05$ (= 0.06).

**Table 19.** Changes of phosphate and nucleic acid content in glandular (pyloric) part of the stomach wall in rats treated with atropine for 25 days. The results are expressed as means (μg per total body weight of the glandular part of the stomach wall) ± standard error (*n* indicates the number of animals). [Mózsik et al., Eur. J. Pharmacol 7: 66–72, 1969c (with kind permission).]

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| | Controls (animals treated with physiological saline for 25 days) | | Animals treated with atropine for 25 days | | 10 days after cessation of atropine treatment | |
|---|---|---|---|---|---|---|
| | *n* | quantities | *n* | quantities | *n* | quantities |
| Acid-soluble inorganic phosphates | 11 | 151 ± 5 (1) | 10 | 169 ± 10 (2) | 6 | 162 ± 15 (3) |
| Acid-soluble organic phosphates | 11 | 158 ± 12 (4) | 10 | 145 ± 19 (5) | 6 | 98 ± 18 (6) |
| Phospholipid phosphates | 11 | 236 ± 14 (7) | 10 | 221 ± 23 (8) | 6 | 160 ± 30 (9) |
| Ribonucleic acid | 11 | 1611 ± 38 (10) | 10 | 1788 ± 73 (11) | 6 | 1301 ± 115 (12) |
| Deoxyribonucleic acid | 11 | 1757 ± 147 (13) | 10 | 915 ± 273 (14) | 6 | 1415 ± 188 (15) |

Statistical values:
between 1 and 2 : $P > 0.05$ — between 2 and 3 : $P > 0.05$ — between 1 and 3 : $P > 0.05$
between 4 and 5 : $P > 0.05$ — between 5 and 6 : $P > 0.05$ — between 4 and 6 : $0.01 > P > 0.001$
between 7 and 8 : $P > 0.05$ — between 8 and 9 : $P > 0.05$ — between 7 and 9 : $P = 0.04$
between 10 and 11 : $P > 0.05$ — between 11 and 12 : $P = 0.02$ — between 10 and 12 : $P = 0.02$
between 13 and 14 : $0.01 > P > 0.001$ — between 14 and 15 : $P > 0.05$ — between 13 and 15 : $P > 0.05$.

**Table 20.** Changes of phosphate and nucleic acid content in membranaceous (rumenal) part of the stomach wall in rats treated with atropine for 25 days. The results of mean values (μg per total body weight of the membranaceous part of the stomach wall) are calculated as means ± standard error (*n* indicates the number of animals). [Mózsik et al., Eur. J. Pharmacol 7: 66–72, 1969c (with kind permission).]

| Parts of the stomach wall | Controls | | At the end of neostigmine treatment | | One month after cessation of neostigmine treatment | |
|---|---|---|---|---|---|---|
| | n | quantities | n | quantities | n | quantities |
| Glandular stomach wall | 10 | 0.92±0.04 (1) | 10 | 0.93±0.03 (2) | 7 | 0.88±0.07 (3) |
| Membranaceous stomach wall | 10 | 0.71±0.06 (4) | 10 | 0.45±0.02 (5) | 7 | 0.43±0.02 (6) |
| Whole stomach wall | 10 | 1.63±0.06 (7) | 10 | 1.37±0.05 (8) | 7 | 1.31±0.08 (9) |

Significance:

1–2: $P > 0.05$ — 2–3: $P > 0.05$ — 1–3: $P > 0.05$
4–5: $P < 0.001$ — 5–6: $P > 0.05$ — 4–6: $P < 0.001$
7–8: $P = 0.001$ — 8–9: $P > 0.05$ — 7–9: $0{,}01 > P > 0.001$

**Table 21.** Changes in the weight of the stomach wall in animals treated with high doses of neostigmine. The results are presented as means ± SEM in grams (*n* indicates the number of animals). [Mózsik, Kiss, Jávor, Krausz, Tóth, Pharmacology 2: 45–59, 1969d (with kind permission).]

The treatment's effects on the body weight and weight of the glandular and membranaceous (forestomach) parts are shown in Table 18: there was a significant decrease in body weight ($0.01 > P > 0.001$), but no significant change in the weight of the parts of the stomach.

| Fractions | Controls | | At the end of neostigmine treatment | | One month after cessation of neostigmine treatment | |
|---|---|---|---|---|---|---|
| | n | quantities | n | quantities | n | quantities |
| Acid-soluble inorganic phosphates | 10 | 305±16 (1) | 10 | 217±15 (2) | 7 | 223±21 (3) |
| Acid-soluble organic phosphates | 10 | 284±19 (4) | 10 | 355±20 (5) | 7 | 264±22 (6) |
| Phospholipid phosphates | 10 | 443±40 (7) | 10 | 386±44 (8) | 7 | 283±34 (9) |
| Total phosphates in incubate | 10 | 747±24 (10) | 10 | 720±20 (11) | 7 | 651±21 (12) |
| RNA – P. | 10 | 397±35 (13) | 10 | 290±19 (14) | 7 | 270±19 (15) |
| Ribonucleic acid (according to *Brown*) | 10 | 3999±359 (16) | 10 | 2884±195 (17) | 7 | 2660±149 (18) |
| DNA – P. | 10 | 395±26 (19) | 10 | 430±20 (20) | 7 | 395±13 (21) |
| Deoxyribonucleic acid (according to *Seibert*) | 10 | 3812±278 (22) | 10 | 4259±194 (23) | 7 | 3915±272 (24) |

Significance:

| | | |
|---|---|---|
| 1–2 : $P<0.001$ | 2–3: $P>0.05$ | 1–3: $0.01>P>0.001$ |
| 4–5: $P = 0.03$ | 5–6: $0.01>P>0.001$ | 4–6: $P>0.05$ |
| 7–8: $P>0.05$ | 8–9: $P>0.05$ | 7–9: $0.01>P>0.001$ |
| 10–11: $P>0.05$ | 11–12: $P = 0.03$ | 10–12: $0.01>P>0.001$ |
| 13–14: $P = 0.04$ | 14–15: $P>0.05$ | 13–15: $0.01>P>0.001$ |
| 16–17: $0.01>P>0.001$ | 17–18: $P>0.05$ | 16–18: $0.01>P>0.001$ |
| 19–20: $P>0.05$ | 20–21: $P>0.05$ | 19–21: $P>0.05$ |
| 22–23: $P>0.05$ | 23–24: $P>0.05$ | 22–24: $P>0.05$ |

**Table 22.** Changes in quantities of phosphate fractions and nucleic acids in the glandular stomach wall of animals treated with high doses of neostigmine. The results are presented as means ± SEM in μg phosphate or nucleic acid per total weight (*n* indicates the number of animals). [Mózsik, Kiss, Jávor, Krausz, Tóth, Pharmacology 2:45–59, 1969d (with kind permission).]

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After prolonged atropine, there was a decrease in acid-soluble inorganic phosphate ($P = 0.02$), acid-soluble organic phosphates ($P < 0.001$), phospholipids phosphates ($P < 0.001$), ribonucleic acid ($P = 0.03$) and deoxyribonucleic acid ($P < 0.05$) in the glandular part. Ten days after cessation of atropine treatment, levels of acid-soluble organic phosphates ($P = 0.02$) and phospholipid phosphates ($P< 0.001$) improved but levels of ribonucleic acid and deoxyribonucleic acid did not.

| Fractions | Controls | | At the end of neostigmine treatment | | One month after cessation of neostigmine treatment | |
|---|---|---|---|---|---|---|
| | n | quantities | n | quantities | n | quantities |
| Acid-soluble inorganic phosphates | 10 | 163±15 (1) | 10 | 81±7 (2) | 7 | 114±14 (3) |
| Acid-soluble organic phosphates | 10 | 110±14 (4) | 10 | 69±13 (5) | 7 | 69±17 (6) |
| Phospholipid phosphates | 10 | 190±13 (7) | 10 | 91±14 (8) | 7 | 59±16 (9) |
| Total phosphates in incubate | 10 | 210±7 (10) | 10 | 170±20 (11) | 7 | 211±19 (12) |
| RNA – P. | 10 | 136±15 (13) | 10 | 110±25 (14) | 7 | 81±17 (15) |
| Ribonucleic acid (according to *Brown*) | 10 | 1382±151 (16) | 10 | 1110±287 (17) | 7 | 789±177 (18) |
| DNA – P. | 10 | 71±9 (19) | 10 | 55±10 (20) | 7 | 126±21 (21) |
| Deoxyribonucleic acid (according to *Seibert*) | 10 | 758±89 (22) | 10 | 527±118 (23) | 7 | 1260±264 (24) |

Significance:

| | | |
|---|---|---|
| 1–2: P<0.001 | 2–3: P = 0.04 | 1–3: P = 0.03 |
| 4–5: P = 0.04 | 5–6: P>0.05 | 4–6: P = 0.06 |
| 7–8: P<0.001 | 8–9: P>0.05 | 7–9: P>0.05 |
| 10–11: P>0.05 | 11–12: P>0.05 | 10–11: P>0.05 |
| 13–14: P>0.05 | 14–15: P>0.05 | 13–15: P = 0.02 |
| 16–17: P>0.05 | 17–18: P>0.05 | 16–18: P = 0.05 |
| 19–20: P>0.05 | 20–21: 0.01>P>0.001 | 19–21: P = 0.04 |
| 22–23: P>0.05 | 23–24: P = 0.02 | 22–24: P>0.05 |

**Table 23.** Changes in quantities of phosphate fractions and nucleic acids in the membranaceous stomach wall (forestomach) of animals treated with high doses of neostigmine. The results are presented as means ± SEM in μg phosphate or nucleic acid per total weight (*n* indicates the number of animals). [Mózsik, Kiss, Jávor, Krausz, Tóth, Pharmacology 2: 45–59, 1969d (with kind permission).]

Prolonged atropine treatment did not alter the acid-soluble inorganic and organic, phospholipids phosphate and ribonucleic acid, but there was a decrease in deoxyribonucleic acid ($0.01 > P > 0.001$). Ten days after cessation of atropine treatment, there was a further reduction of ribonucleic acid ($P = 0.02$) and increase of deoxyribonucleic acid ($P > 0.05$).

| Fractions | Controls | | At the end of neostigmine treatment | | One month after cessation of neostigmine treatment | |
|---|---|---|---|---|---|---|
| | n | quantities | n | quantities | n | quantities |
| Acid-soluble inorganic phosphates | 10 | 332±13 (1) | 10 | 239±13 (2) | 7 | 302±21 (3) |
| Acid-soluble organic phosphates | 10 | 296±15 (4) | 10 | 393±44 (5) | 7 | 328±39 (6) |
| Phospholipid phosphates | 10 | 461±44 (7) | 10 | 430±47 (8) | 7 | 382±39 (9) |
| Total phosphates in incubate | 10 | 830±26 (10) | 10 | 787±28 (11) | 7 | 845±63 (12) |
| RNA – P. | 10 | 409±17 (13) | 10 | 315±26 (14) | 7 | 320±8 (15) |
| Ribonucleic acid (according to *Brown*) | 10 | 4333±349 (16) | 10 | 3155±250 (17) | 7 | 3228±96 (18) |
| DNA – P. | 10 | 398±17 (19) | 10 | 470±20 (20) | 7 | 515±25 (21) |
| Deoxyribonucleic acid (according to *Seibert*) | 10 | 4128±187 (22) | 10 | 4777±252 (23) | 7 | 5159±259 (24) |

Significance:

| | | |
|---|---|---|
| 1–2: $P<0.001$ | 2–3: $P = 0.02$ | 1–3: $P>0.05$ |
| 4–5: $P = 0.06$ | 5–6: $P>0.05$ | 4–6: $P>0.05$ |
| 7–8: $P>0.05$ | 8–9: $P>0.05$ | 7–9: $P>0.05$ |
| 10–11: $P>0.05$ | 11–12: $P>0.05$ | 10–12: $P>0.05$ |
| 13–14: $0.01>P>0.001$ | 14–15: $P>0.05$ | 13–15: $P<0.001$ |
| 16–17: $0.01>P>0.001$ | 17–18: $P>0.05$ | 16–18: $P = 0.001$ |
| 19–20: $P = 0.02$ | 20–21: $P>0.05$ | 19–21: $P = 0.001$ |
| 22–23: $P = 0.05$ | 23–24: $P>0.05$ | 22–24: $0.01>P>0.001$ |

**Table 24.** Changes in quantities of phosphate fractions and nucleic acids in the glandular stomach wall of animals treated with high doses of neostigmine. The results are presented as means ± SEM in μg phosphate or nucleic acid per 1 gram of fresh gastric tissue (*n* indicates the number of animals). [Mózsik, Kiss, Jávor, Krausz, Tóth, Pharmacology 2: 45–59, 1969d (with kind permission).]

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| Fractions | Controls | | At the end of neostigmine treatment | | One month after cessation of neostigmine treatment | |
|---|---|---|---|---|---|---|
| | n | quantities | n | quantities | n | quantities |
| Acid-soluble inorganic phosphates | 10 | 224±31 (1) | 10 | 177±14 (2) | 7 | 261±14 (3) |
| Acid-soluble organic phosphates | 10 | 147±18 (4) | 10 | 153±35 (5) | 7 | 154±23 (6) |
| Phospholipid phosphates | 10 | 270±22 (7) | 10 | 207±35 (8) | 7 | 161±23 (9) |
| Total phosphates in incubate | 10 | 320±7 (10) | 10 | 330±26 (11) | 7 | 390±28 (12) |
| RNA – P. | 10 | 190±10 (13) | 10 | 230±30 (14) | 7 | 110±30 (15) |
| Ribonucleic acid (according to *Brown*) | 10 | 1922±72 (16) | 10 | 2390±350 (17) | 7 | 1180±352 (18) |
| DNA – P. | 10 | 101±10 (19) | 10 | 110±21 (20) | 7 | 290±40 (21) |
| Deoxyribonucleic acid (according to *Seibert*) | 10 | 1073±113 (22) | 10 | 1139±217 (23) | 7 | 2880±492 (24) |

Significance:

| | | |
|---|---|---|
| 1–2: $P \times 0.05$ | 2–3: $P < 0.001$ | 1–3: $P > 0.05$ |
| 4–5: $P > 0.05$ | 5–6: $P > 0.05$ | 4–6: $P > 0.05$ |
| 7–8: $P > 0.05$ | 8–9: $P > 0.05$ | 7–9: $0.01 > P > 0.001$ |
| 10–11: $P > 0.05$ | 11–12: $P > 0.05$ | 10–12: $P = 0.06$ |
| 13–14: $P > 0.05$ | 14–15: $P = 0.02$ | 13–15: $P = 0.03$ |
| 16–17: $P > 0.05$ | 17–18: $P = 0.03$ | 16–18: $P > 0.05$ |
| 19–20: $P > 0.05$ | 20–21: $P = 0.001$ | 19–21: $P < 0.001$ |
| 22–23: $P > 0.05$ | 23–24: $0.01 > P > 0.001$ | 22–24: $0.01 > P > 0.001$ |

**Table 25.** Changes in quantities of phosphate fractions and nucleic acids in the membranaceous gastric wall (forestomach) of animals treated with high doses of neostigmine. The results are presented as means ± SEM in μg phosphate or nucleic acid per 1 gram of fresh gastric tissue (*n* indicates the number of animals). [Mózsik, Kiss, Jávor, Krausz, Tóth, Pharmacology 2: 45–59, 1969d (with kind permission).]

The results obtained from chronic neostigmine treatment provided the following conclusions:

1. The cholinergic dominance involves the decrease of the weight and biochemical constituents of the membranaceous (forestomach). This is the effect of cholinergic dominance on the membranaceous stomach wall.

2. Examined biochemical constituents of the glandular stomach behave differently during the existence of the cholinergic dominance due to the decrease of acid-soluble inorganic phosphates, phospholipid phosphates and ribonucleic acid and the increase of acid-soluble organic phosphates during neostigmine treatment.

3. We had observed "short-term" and "long-term" biochemical changes in the glandular stomach wall after neostigmine treatment. The "short-term" biochemical change (acid-soluble organic phosphates) was a reversible process lasting up to one month after cessation of neostigmine treatment. At the same time, the "long-term" biochemical changes (acid-soluble inorganic phosphate, phospholipids phosphate and ribonucleic acid) were observed as irreversible processes. It is interesting to note that the stomach can "remember" to the neostigmine treatment one month after cessation of treatment.

In the 1970s, there was a famous topic on physiology to approach the possible backgrounds of "use" and "disuse" of the neural regulation (especially after denervation of muscles) (Graff et al., 1965 a, b, c; Gregory, 1962). The surgical ablation of nerves was used extensively in these types of observations. There was a general note that the denervated organ became to b seniitive to mediators than that inavated organ. Emmelin and Rosenblueth (1951), Emmelin and Muren (1951 a, b; 1952), and Elin (1952, 1961) observed that the efficenciees of drugs and mediators changes after a prolonged reatment (including the atropine). In these observations, no surgical manipulation was done with the nerves of different organs, however, they dichronic drunt was done to inhibit the neural functions at the levels of synapses or at the levels of tra to organs. This phenomenon was named as "pharmacological denervation" and was associated with the supersensitivity (Emmelin, 1952, 1961). We were the first authors, who demonstrated the existence of supersensitivity of "pharmacologic denervation" phenomenon, together along with opment of tolerance to drugs used in the treatment and cross-tolerance to the pharmacologicallypharmarugs, but that are not used in the trea, under classical medical treatment with parasympatholytics in patients with peptic ulcer (see chapters of Sections 2.2-.2.3— 2.4)

There was an important note that the efficacy of atropine decreased during a chronic atropine treatment in patients with peptic ulcer, however, the decrease effect of atropine returned in time 0 days after cessation of atropine treatment.

These human observations called our attention to carry out different biochemical observations in the rat's stomach after cessation of atropine and neostigmine treatment.

We tried to approach the biochemical backgrounds of the "use" and "disuse" of the gastric tissues in rats (under experimental conditions). The changes in gastric mucosal constituents were presented in percentage values of "sham treated" (with physiological saline solution) (=100%) after chronic "chemical" and "surgical" vagotomy and neostigmine treatments. The

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comparative results are presented in the glandular stomach wall (Figure 19) and membranaceous stomach wall (forestomach) (Figure 20).

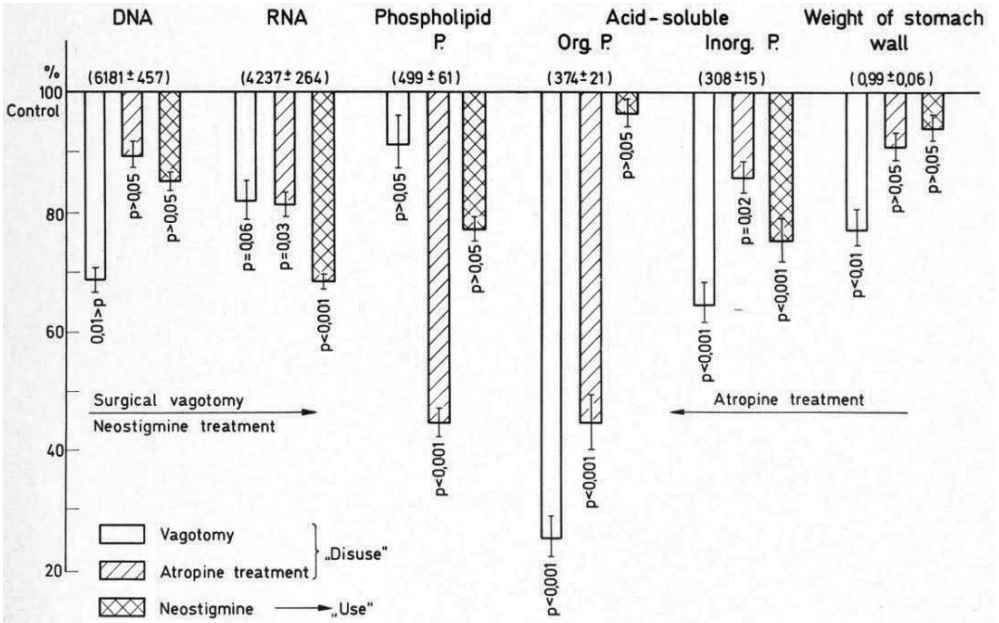


**Figure 19.** Biochemical backgrounds of "use" and "disuse" of cholinergic innervations on gastric tissue in the glandular stomach wall (in one month's treatment or surgical vagotomy). The results are expressed as means ± SEM in percentage values of control (sham-treated) rats. [Mózsik, Kiss, Jávor, Krausz, In: Gregor O., Riedl O. (eds) Modern Gastroenterology, Schattauer Verlag, Stuttgard, New York, 561–563, 1968 (with kind permission).]

The comparative biochemical results of "use" and "disuse" on cholinergic function of vagus nerve offered us the following conclusions:

1. The behavior of membranaceous and glandular stomach wall is not the same from the point of view of cholinergic innervation;
2. The "surgical," "chemical" vagotomy ("disuse") and neostigmine treatment ("use") can induce alterations in the stomach at the levels of general functions, biochemical reactions, ribonucleic and deoxyribonucleic acids;
3. The effects of "surgical" and "chemical" vagotomy are different at biochemical levels in the stomach wall. We assumed that the "surgical" vagotomy induces the alterations of DNA and RNA at first and that the alterations of other biochemical constituents follow these. On the contrary, DNA and RNA alternate only after the "chemical" vagotomy changes the metabolism of phosphorus components. The neostigmine can change the biochemism of the stomach wall similarly to the "surgical" vagotomy;
4. The "use" and "disuse" can involve the same biochemical alterations in the biochemism of the stomach in rats.

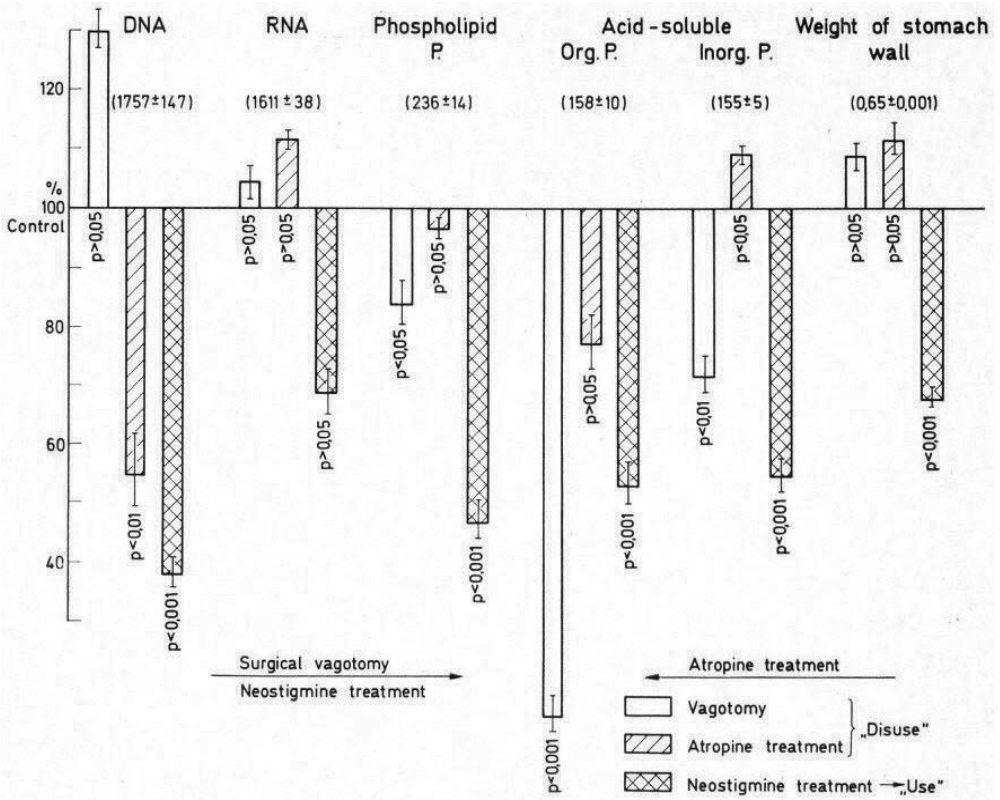


**Figure 20.** Biochemical backgrounds of "use" and "disuse" of cholinergic innervations at the biochemical level on gastric tissue in the membranaceous stomach wall (forestomach) (in one month's treatment or surgical vagotomy). The results are expressed as means ± SEM in percentage values of control (sham-treated) rats. [Mózsik, Kiss, Jávor, Krausz, In: Gregor O., Riedl O. (eds) Modern Gastroenterology, Schattauer Verlag, Stuttgard, New York, 561–563, 1968 (with kind permission).]

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

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Chapter 5

# Membrane-Bound Atp-Dependent Energy System (A Short Historic View from Their Discoveries up to Now)

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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This chapter is important and hence we felt that some general information has to be given globally on the physiological research, as we could not do a detailed study being interns in the medical department of the medical services.

We met the presentations of these new results at different times in our life, which modified our aims and possibilities in the research. On the other hand, our lifes gave to us a possibility to our scientific and methological konowledges to incorporate these new fields in our research field.

We have to learn the following:

1. Being clinicians, our main work is related to the medical service of patients' problems; however, we wanted to learn the main biochemical events in the gastrointestinal tract (in terms of development of mucosal damage and prevention);
2. We started with the biochemical examinations of gastric tissues from 1960s, and these new observations offered us to do the forthcoming steps in our research work;
3. We had time periods in our lifes when we had contamining (infecting) with basic and fruitful international research lines , when we had opportunities to participate and to work in research of different foreign countries (GyM in Norway in 1968–1969 and USA in 1985; IL Szabo in USA in 1999–2001);
4. We learned new research methods while working with foreign countries, and we tried to apply the new knowledge in researches that we did in our country (Hungary);
5. It was very clear that there is a big gap between the problems (results) of a basic research and clinical practice; however, we wanted to understand the main common problems in the basic research and in clinical science;

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**6.** We had many ethical and medical problems in doing biochemical observations in the gastrointestinal resecates of human GI tract in patients with peptic ulcer who underwent surgical interventions owing to their primary disease;

**7.** We participated in the different international forums of GI research (as established persons, future leaders, organizers and responsible persons).

Our main lines of research series of international conferences are as follows:

**a.** An international series of international conferences [International Conference of Experimental Ulcer (from 1970 to 1994), International Conference on Ulcer Research (from 1994 to 2000) and International Conference on Gastrointestinal Research (from 2000)] has been internationally established at Copenhagen (Denmark) – in connection with Fourth World Congress of Gastroenterology – in 1970. Thereafter, different international conferences were organized in different countries of different continents (Copenhagen, Denmark, 1970; Cologne, Germany, 1972; Parádfürdő, Hungary, 1976; Tokyo, Japan, 1980; Boston, USA, 1985; Jerusalem, Israel, 1988; Berlin, Germany, 1991; Kyoto, Japan, 1994; Hong Kong, China, 1997; Budapest-Pécs, Hungary, 2000; Dubrovnik, Croatia, 2003; Osaka, Japan, 2006; Split, Croatia, 2009; Tokyo, Japan, 2012) (for details, see Mózsik, 2006b);

**b.** Another series of international symposiums was established in connection with main World Congresses of the International Union of Pharmacology (from 1984). The gastrointestinal section of International Union of Pharmacology was established at Montreal (Canada) in 1994 (IUPHAR GI Section); however, this series of the international symposiums started earlier (Pécs, Hungary, 1984; Pécs, Hungary, 1990; Pécs, Hungary, 1995; Sperlonga, Italy, 1996; Pécs, Hungary, 1998; Honolulu, USA, 2002; Kyoto, Japan, 2004; Osaka, Japan, 2006; Honolulu, Hawaii, USA, 2009; Tokyo, Japan, 2012) (for details, see Mózsik et al., 2006);

**c.** International Symposia on "Cell/Tissue Injury and Cytoprotection/Organoprotection" [Heidelberg, Germany, 1986; Boston, USA, 1989; Long Beach, CA, USA, 2000; 2006; Yalta (Crimea), Ukraine, 2008; Saint Petersburg, Russia, 2011; Honolulu, USA, 2012; Budapest, Hungary, 2014];

**d.** International Symposia on Gastrointestinal Cytoprotection (Pécs, Hungary, 1983; 1987; 1991; 1995, 2000);

**e.** International Symposia of the "International Brain-Gut Society" (Lake Arrowhead, Los Angeles, USA, 1988; Pécs, Hungary, 1990; Florence, Italy, 1993; Pécs, Hungary, 1995);

**f.** Interscience World Conferences on Inflammation [Antirheumatics, Analgesics, Immunomodulators (Venice, Italy, 1984; Monte Carlo, France, 1986; 1989; Geneva, Switzerland, 1995; 1998)].

**8.** This part of the book has been tried to give a short information on the different membrane-bound ATP-dependent energy systems with emphasizing the main problems in these

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fields, however in briefly up to now (we had no these summaries of these studies in our mind during the time of our observations done in GI tract).

We have to emphasize that we have not done a very detailed study on the membrane-bound ATP-dependent energy systems; however, the results of our study (namely the study of the details of ATP splitting by different membrane-bound enzymes, $Na^+$–$K^+$-dependent ATPase and adenylate cyclase, represent different pathways of energy-liberating cellular events) offered us a very successful research possibility in our field. As ATP splitting results in energy liberation of cells, and the circumstances of the ATP resynthesis (by oxidative phosphorylation) confirmed the presence of tissue hypoxia in the GI mucosa under different (e.g., in the ulcerated or the damaged mucosal tissues) experimental and clinical circumstances.

The main lines of the studies on membrane-bound ATP-dependent energy systems were carried dominantly from GI tract, except for $H^+$–$K^+$-ATPase system (we did not participate in these studies), and hence we started with these studies in our field. Furthermore, these studies offered good possibility to understand the presence of tissue hypoxia (anoxia) during the development of mucosal damage, which was suggested as one of the main reasons (or consequences) of peptic ulcer.

Before the demonstrations of our biochemical results, we will give a very short summary of the main membrane-bound ATP-dependent energy systems (of course, not in the gastric mucosa).

### 5.1. $Na^+$-$K^+$dependent ATP-ase

This enzyme (other names: sodium–potassium adenosine triphosphatase, $Na^+/K^+$ pump, sodium–potassium pump, sodium pump) is located in the plasma membrane of all animal cells. The $Na^+$–$K^+$-ATPase enzyme pumps sodium out of the cells, while pumping potassium into the cells.

Active transport is responsible for cells containing relatively high concentrations of potassium ions but low concentrations of sodium ions. The mechanism responsible for this sodium-potassium pump moves these two ions in opposite directions across the plasma membrane. This was investigated by following the passage of radioactive ions across the plasma membrane of certain cells. It was found that the concentrations of sodium and potassium ions on the two sides of the membrane are independent, which suggests that the same carrier transports both ions. It is now known that the carrier is an ATPase and it pumps three sodium ions of the cell for every two potassium ions pumped in.

The sodium–potassium pump was discovered in the 1950s (Skou, 1957) by Jens Christian Skou (Department of Physiology, Aarhus University, Denmark), who was awarded Nobel Prize in chemistry in 1997 (http://nobelprize.org/chemistry/laureates/1997/index.html). It marked an important step forward in our understanding of how ions get into and out of cells, and it is particularly significant for excitable cells such as nervous cells, which depend on this pump for responding to stimuli and transmitting impulses (Skou, 1965).

### *5.1.1. Schematic morphological bases of sodium-potassium pump ($Na^+$-$K^+$-dependent adenosine triphosphatase enzyme)*

The $Na^+$–$K^+$-ATPases belong to the $P_2$ class and specifically to the $P_{2c}$ subclass of ATPases. These ATPases are composed of two subunits ($\alpha$ and $\beta$). The $\alpha$-subunit (about 113 kD) binds ATP and both $Na^+$ and $K^+$ ions and contains the phosphorylation sites typical of the P-type ATPases. The autophosphorylation site is the P domain. The P-type ATPases are also subject to additional phosphorylation events via other kinases. The smaller $\beta$-subunit (about 35-kD glycoprotein) is absolutely necessary for activity of the complex. It appears to be critical in facilitating the plasma membrane localization and activation of the $\alpha$-subunit.

Several isoforms of both $\alpha$- and $\beta$-subunits have been identified that exhibit different kinetic parameters and tissue distribution. There are four $\alpha$-subunit genes and three $\beta$-subunit genes in humans. The $\alpha_1$ isoform is the predominant form and is ubiquitously expressed. The $\alpha_2$ isoform is primarily expressed in muscle tissues (skeletal, smooth and cardiac) as well as in adipose tissue, brain and lungs. The $\alpha_3$ isoform is expressed primarily in the heart and neurons. The $\alpha_4$ isoform is only expressed in the testes. The $\beta_1$ isoform is ubiquitously expressed and is associated with the $\alpha$-subunit in the ubiquitously expressed $\alpha_1$ $\beta_1$ $Na^+$–$K^+$-ATPase complex. There are many more detailed parts and interactions of $Na^+$–$K^+$-ATPase discovered and proved in the last decades (http://themedicalbiochemistrypage.org/membranes.php; Morth et al., www.nature.com/reviews/molcellbio 12:60–70, 2011).

### *5.1.2. Main functions of $Na^+$-$K^+$-ATPase*

The $Na^+$–$K^+$-ATPases help to maintain resting potential and regulate cellular volume (Hall and Guyton, 2006). It also functions as signal transducer/integrator to regulate the mitogen-activated protein kinase (MARK) pathway, mitochondrial reactive oxygen species (ROS) as well as intracellular calcium. In most animal cells, the $Na^+$–$K^+$-ATPase is responsible for about 1/5 of the cell's energy expenditure, meanwhile, for neurons, the $Na^+$–$K^+$-ATPase can be responsible for up to 2/3 of the energy expenditure (Howard et al., 2012).

#### *5.1.2.1. Resting potential*

In order to maintain the cell membrane potential, cells keep a low concentration of sodium ions and high levels of potassium within the cell (intracellular). The sodium–potassium pump moves three sodium ions out and moves two potassium ions in, thus in total removing one positive-charge carrier from the intracellular space.

#### *5.1.2.2. Transport*

Export of sodium from the cell provides the driving force for several secondary active transporters, membrane transport proteins, which import glucose, amino acids and other nutrients into the cell by the use of sodium gradient.

Another important task of the $Na^+$–$K^+$-pump is to provide a $Na^+$ gradient that is used by certain carrier processes. In the gut, sodium is transported out of the reabsorbing cell on the blood

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(intestinal fluid) side via the $Na^+$–$K^+$-pump, whereas, on the reabsorbing (luminal) side, the $Na^+$-glucose symporter uses the created $Na^+$ gradient as a source of energy to import both $Na^+$ and glucose, which is far more efficient than simple diffusion. Similar processes can be found and located in the renal tubular system.

*5.1.2.3. Controlling cell volume*

Failure of the $Na^+$–$K^+$ pumps can result in swelling of the cell. A cell's osmolarity is the sum of the concentrations of the various ion species and many proteins and other organic compounds inside the cell. When this is higher than the osmolarity outside of the cell, water flows into the cell through osmosis. This can cause the cell to swell up and lyze. The $Na^+$–$K^+$ pump helps to maintain the right concentrations of ions. Furthermore, when the cell begins to swell, this automatically activates the $Na^+$–$K^+$ pump.

Within the last decade, many independent laboratories have demonstrated that, in addition to classical ion transporting, this membrane protein can relay extracellular ouabain-binding signaling into the cell through regulation of protein tyrosine phosphorylation. The downstream signals through ouabain-triggered protein phosphorylation events include activation of the mitogen-activated protein kinase (MARK) signal cascades, mitochondrial reactive oxidative species (ROS) production, as well as activation of phospholipase C (PLC) and inositol triphosphate (IP3) receptor (IP3R) in different intracellular compartments (Yuan et al., 2005).

Protein–protein interactions play a very important role in $Na^+$–$K^+$ pump-mediated signal transduction (e.g., $Na^+$–$K^+$ pump interacts directly with Scr, a non-receptor tyrosine kinase) to form a signaling receptor complex (Tian et al., 2006). Scr kinase is inhibited by $Na^+$–$K^+$ pump, while, upon ouabain binding, the Scr kinase domain will be released and then activated. Based on this scenario, NaKtide, a peptide Scr inhibitor derived from the $Na^+$–$K^+$ pump, was developed as a functional ouabain-$Na^+$–$K^+$ pump-mediated signal transduction (Li et al., 2009; Forrest et al., 2012; Cannon, 2004; Calderon et al., 2011; Young et al., 2008).

*5.1.3. Main steps of mechanism*

The following main steps are involved in the mechanism:

- The pump, while binding ATP, binds three intracellular $Na^+$ ions (Hall and Guyton, 2006);
- ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP;
- A conformational change in the pump exposes the $Na^+$ ions to the outside. The phosphorylated form of the pump has a low affinity for $Na^+$ ions, so they are released;
- The pump binds two extracellular K+ ions. This causes the phosphorylation of the pump, reverting it to its previous state, transporting the K+ ions into the cells;
- The unphosphorylated form of the pump has a higher affinity for $Na^+$ ions than $K^+$ ions, so two bound $K^+$ ions are released. ATP binds and the process starts again.

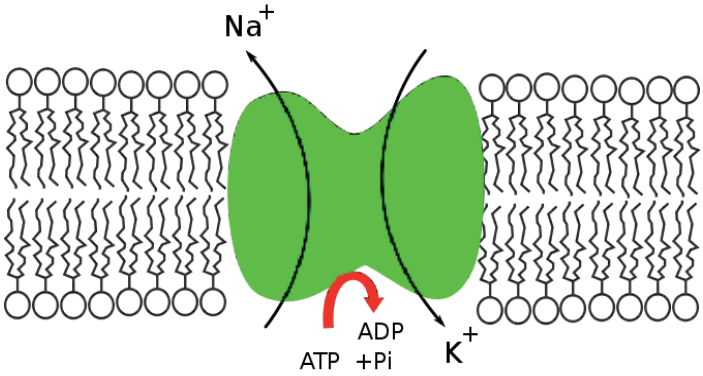


**Figure 21.** Schematic representation of the sodium pump ($Na^+$–$K^+$ ATPase): flow of ions (http://enzyme.expasy.org/EC/3.6.3.9).

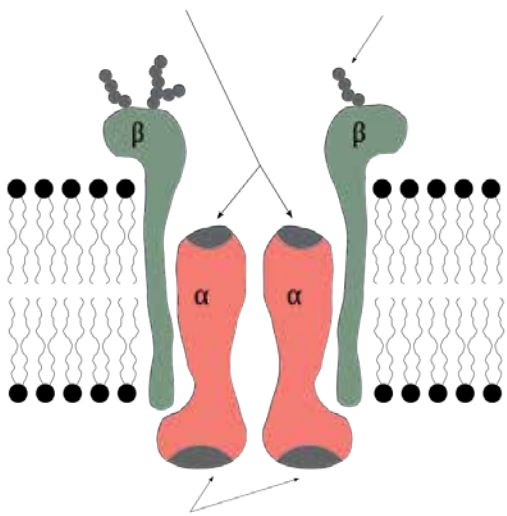


**Figure 22.** Schematic representation of the sodium pump ($Na^+$–$K^+$ ATPase): alpha and beta units (http://enzyme.expasy.org/EC/3.6.3.9).

The function of Na+–K+ pump can be regulated endogenously and exogenously.

The $Na^+$–$K^+$ pump is upregulated by cyclic adenosine monophosphate (cAMP) (Bunnier 2008). Thus, substances causing an increase in cAMP level upregulate the $Na^+$–$K^+$ ATPase. These include the ligands of the $G_s$-coupled GPCRs. In contrast, the substances causing a decrease in cAMP downregulate the $Na^+$–$K^+$-ase. These include the ligands of $G_i$-coupled GPCRs.

The ouabain (as cardiac glycoside) inhibits the $Na^+$–$K^+$ ATPase activity. The prove the presence of ouabain inhibition of different preparates of plasma membranes was one of the most criteria to indicate presence a good $Na^+$-$K^+$-dependent ATPase.

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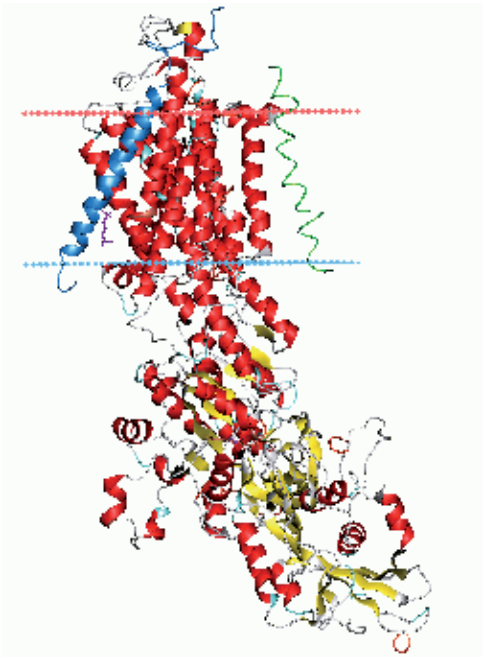

**Figure 23.** Schematic representation of the sodium pump ($Na^+$–$K^+$ ATPase), E2-$P_i$ state. Calculated hydrocarbon boundaries of the lipid bilayer are shown as blue (intracellular) and red (extracellular) planes (http://enzyme.expasy.org /EC/3.6.3.9).

### 5.2. The gastric $H^+$- $K^+$-ATP-ASE

The gastric hydrogen potassium ATPase or $H^+/K^+$-ATPase is the proton pump of the stomach and hence it is the enzyme primarily responsible for the acidification of the stomach (acid secretion) (Potassium Hydrogen ATPase (https://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode= &term=Potassium +Hydrogen+ ATPase).

The $H^+$–$K^+$- ATPase is found in the parietal cells, which are highly specialized epithelial cells located in the inner cell lining of the stomach called gastric mucosa. Parietal cells process an extensive secretory membrane system and the $H^+$–$K^+$-ATPase is the major protein constituent of these membranes.

The enzyme that keeps the stomach at pH 0.6:

- The parietal cells of the gastric mucosa (lining of the stomach) have an external pH of 7.4;
- $H^+$–$K^+$-ATPase pumps protons from these cells into the stomach (using energy of ATP) to maintain a pH difference across a single plasma membrane of 6.6;
- This is the largest known transmembrane gradient in eukaryotic cells.

#### *5.2.1. The gastric $H^+$-$K^+$-ATPase can be characterized by the followings*

- $H^+$–$K^+$-ATPase is similar in many respects to $Na^+$–$K^+$-ATPase and $Ca^{2+}$-ATPase;

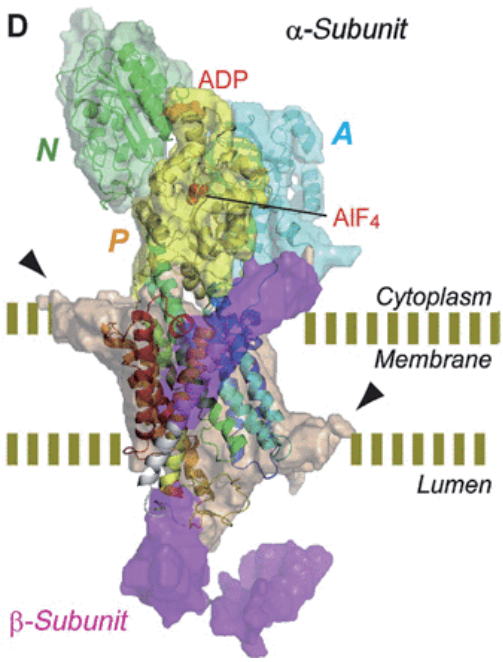


**Figure 24.** Schematic surface representation of gastric $H^+$–$K^+$-ATPase αβ-protomer with the fit homology model in ribbon representation. Color code of the density map: N domain, green; A domain, cyan; P domain, yellow; TM domain of the α-subunit, wheat; β-subunit, magenta. Color code of the homology model: N, A and P domains have the same color as the density map; TM helices M1–M10 of the α-subunit, gradual change from M1 (blue) to M10 (red); TM helix of β-subunit, white. The dotted lines indicate the probable position of the lipid head group and result in total thickness of approximately 35 Å, which is based on the densities protruding perpendicular from the TM domain (arrowheads). The bound ADP and $AlF_4$ molecules are shown as red sphere.

- All three enzymes form covalent E–P intermediates (P-type pumps);
- All three have similar sequences for the large (α) subunit;
- All three are involved in active transport.

The $H^+$–$K^+$-ATPase is a heterodimeric protein, the product of two genes. The gene ATP4A encodes the $H^+$–$K^+$-ATPase α-subunit and is about 1000 amino acid protein that contains the catalytic sites of enzyme and forms the pore through the cell membrane that allows the transport of ions.

The gene ATP4B encodes the β-subunit of the $H^+$–$K^+$-ATPase, which is about 300-amino acid protein with a 36 –amino acid N-terminal cytoplastic domain, a single transmembrane domain and a highly glycosylated extracellular domain. The $H^+$–$K^+$-ATPase β-subunit stabilizes the $H^+$–$K^+$-ATPase α-subunit and is required for function of the enzyme. It also appears to contain signals that direct the heterodimer to membrane destinations within the cell, although some of these signals are subordinate to signals found in $H^+$–$K^+$-ATPase α-subunit.

The $H^+$–$K^+$-ATPase is a member of the P-type ATPase superfamily, a large family of related proteins that transport ions, usually cations, across biological membranes of nearly all species.

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The $H^+$–$K^+$-ATPase transports one hydrogen ion ($H^+$) from the cytoplasm of the parietal cell in exchange for potassium ($K^+$) retrieved from the gastric lumen. As an ion pump, the $H^+$–$K^+$-ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transformed from adenosine triphosphate (ATP) to the $H^+$–$K^+$-ATPase during transport cycle. This phosphate transfer powers a conformational change in the enzyme that helps drive ion transport (Yao and Forte, 2003; Kühlbrandt, 2004; Dunbar and Caplan, 2001; Sachs et al., 1995) (http://www.mlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Potassium+Hydrogen+ATPase).

The $Na^+$–$K^+$-ATPase and $H^+$–$K^+$-ATPase indicate some similarities in the structural building of these enzymes; however, they can be separated from each other by using immunological methods (Stoll and Berglinch, 1987; Sachs, 1987; Chang et al., 1977; Faller et al., 1982; Ganser and Forte, 1973; Lee et al., 1974; 2001; Maclennan et al., 1985; Saccomani et al., 1979 a, b; Sachs, 1977; Sachs et al., 1972; 1980; 1995; Shull et al., 1985; Koenderink et al., 1999; Asano et al., 2003).

### 5.3. Adenylate cyclase

#### *5.3.1. Short historic background*

Sutherland (1951) studied how the hormone epinephrine (adrenaline) signals to regulate the degradation of glycogen to glucose in the liver, thereby increasing glucose output into the blood, so that an organism can respond to stress. He worked alongside future Nobel Prize winners Edmond Fishers and Edwin Krebs, who demonstrated that adenosine triphosphate (ATP) and magnesium were required for phosphorylase activation. Sutherland (1953) agreed to be the chairman of the pharmacology department of Case Western Reserve University in Cleveland and was joined by Theodore R. Rall, "marking the beginning of a long and fruitful period of collaboration between us" [Sutherland EW. Studies on the mechanism of hormone. Science 177: 401–408, 1972 (Lecture delivered 11 December 1971 when he received the Nobel Prize in Physiology or Medicine)]. Sutherland and Rall discovered that epinephrine works by stimulating another chemical messenger, the enzyme glycogen phosphorylase, to begin the sugar-releasing process in the cells (Henion and Sutherland, 1955). This stimulation occurs by means of an intermediary that Sutherland called it the "second messenger," which he identified as a nucleotide and named cyclic AMP (Sutherland et al., 1957; Sutherland and Rall, 1957; Rall and Sutherland, 1957; 1958; Butcher and Sutherland, 1962; 1965; Klainer et al., 1962; Murad et al., 1962). As early as 1960, Sutherland suggested that cyclic AMP acts as a "second messenger" for other hormones as well (Sutherland and Rall, 1958) (for other summaries, see Butcher, 1966; Sutherland, 1962; Sutherland and Rall, 1960; Sutherland et al., 1962; Sutherland and Robison, 1966; Andrási, 1997).

Practically at the same time, the discovery of cyclic 3′-5′-AMP appeared in the organ pharmacology (Sutherland et al., 1965; 1968).

The adenylate cyclase is an enzyme with key regulatory roles in essentially all cells. It is the most polyphyletic known enzyme: six district classes have been described, all catalyzing the same reaction, but representing unrelated gene families with no known sequence or structural homology (Class I AC, Class II AC, Class III AC, Class IV AC, Class V AC and Class VI AC).

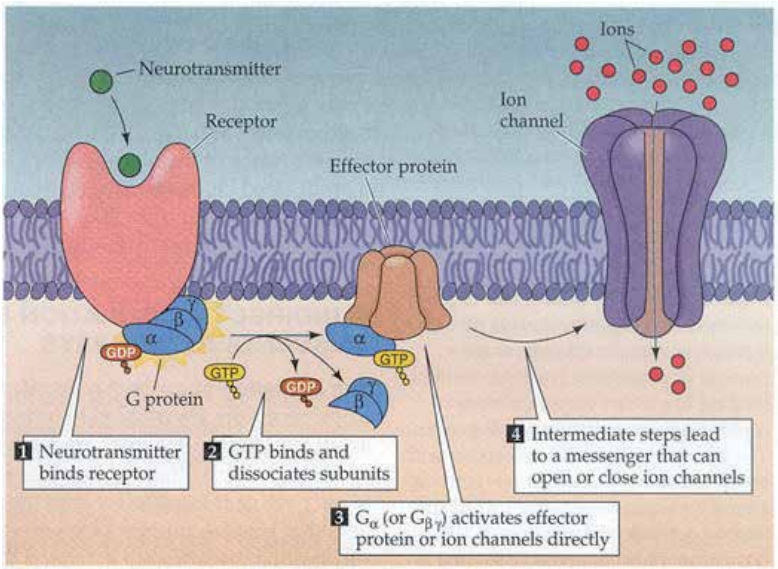


**Figure 25.** Schematic representation of adenylate cyclase location in the plasma membrane and main steps of intracellular regulatory pathways (http://www.cs.stedwards.edu/chem/Chemistry/ CHem43/).

The Classes AC I (*E. coli*), II (Bacillus anthracis and Bordetella pertussis), IV (Aeromonas hydrophyla, Yersinia pestis), V and VI (Prevotella ruminicola and Rhizobium etli) are present in different bacteria. The best-known AC class is class III or AC-III (Roman numerals are used for classes). The AC-III occurs widely in eukaryotes and has important roles in many human tissues.

All classes of AC catalyze the conversion of ATP to 3′,5′-cyclic adenosine monophosphate (cAMP) and pyrophosphate. Divalent cations (usually $Mg^{2+}$) are generally present in the enzymatic mechanism. The cAMP produced by AC then serves as regulatory signal via specific cAMP-binding proteins, either transcription factors or other enzymes (e.g., cAMP-dependent kinases).

Most AC-IIIs are integral membrane proteins involved in transducing extracellular signals into intracellular responses. A Nobel Prize in physiology (medicine) was awarded to Earl W. Sutherland (Department of Physiology, Vanderbilt University, Nashville, TN, USA) in 1971 for discovering the key role of AC-III in human liver, where adrenaline directly stimulates AC to mobilize stored energy in the "fight or flight" response (Robinson GA, Butcher RW, Sutherland EW: Cyclic AMP. Academic Press, New York and London, 1971) (two papers published by myself – Mozsik G.: Eur. J. Pharmacol 7: 319, 1969 and in 9: 107, 1970 – were cited by the authors in this book).

The effect of adrenaline is via a G protein-signaling cascade, which transmits chemical signals from outside the cell across the membrane to the inside of the cell (cytoplasm). The outside signal (in this case, adrenaline) binds to a receptor, which transmits a signal to the G protein,

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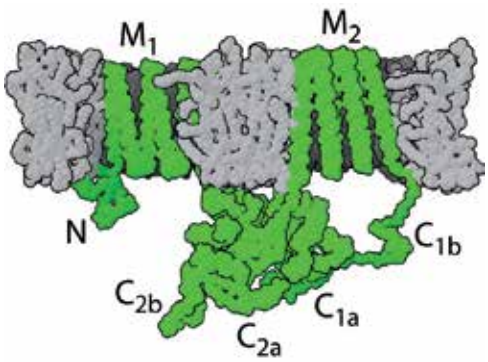


**Figure 26.** Schematic representation of the structure of adenylate cyclase enzyme (http://enzyme. expasy.org/EC/4.6.1.1).

which transmits a signal to adenylate cyclase, which transmits a signal by converting adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Cyclic AMP is known as the so-called "second messenger" (Reece and Campbell, 2002).

Adenylate cyclases are often activated or inhibited by G proteins, which are coupled to membrane receptors, and thus can respond to hormonal or other stimuli. Following the activation of adenylate cyclase, the resulting cAMP acts as a second messenger by interacting with and regulating other proteins, such as protein kinase A and cyclic nucleotide-gated ions channels.

**AC-III structure:**

Most class-III adenylate cyclases are transmembrane proteins with 12 transmembrane segments. The protein is organized with six transmembrane segments, the C1 cytoplasmic domain, another six membrane segments and then a second cytoplasmic domain called C1 and C2 regions.

The C1a and C2a sub-domains are homologous and form an intracellular "dimmer" that forms the active site.

There are ten known isoforms of adenylate cyclases in mammals: ADCY1, ADCY2, ADCY3, ADCY4, ADCY5, ADCY6, ADCY7, ADCY8, ADCY9 and ADCY10. These are sometimes called as AC1, AC2, etc., and sometimes Roman numerals are used for these isoforms that all belong to the overall AC class III. They differ mainly in how they are regulated, and are differentially expressed in various tissues throughout mammalian development.

**Adenylate cyclase AC-III regulation:**

Adenylate cyclase is dually regulated by G proteins (Gs stimulating activity and Gi inhibiting activity) and forskolin, as well as other isoform-specific effectors:

Isoforms III, V and VIII are also stimulated by $Ca^{2+}$ /calmodulin;

Isoforms I and VI are inhibited by Ca2+ in a calmodulin-independent manner;

Isoforms II, IV and IX are stimulated by beta–gamma-subunits of the G protein;

Isoforms I, V and VI are most clearly inhibited by Gi, while other isoforms show less dual regulation by the inhibitory G protein.

Soluble AC (sAC) is not a transmembrane form and is not regulated by G proteins or forskolin, instead it acts as a bicarbonate/pH sensor. It is anchored at various locations within the cell and, with phosphodiesterases, forms local cAMP-signaling domain

[(http://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Adenylate +cyclase) (http://proteopedia.org/wiki/index.php/Adenylyl_cyclase)].

### 5.4. Sodium-pump and second messenger system in the gastrointestinal mucosa

#### *5.4.1. Short personal background*

After taking short reviews on these biochemical problems in the nature of living cells, we faced a unique situation, when one of us (GyM) received a fellowship possibility in the Department of Pharmacology, University of Blindern, Oslo, in 1968–1969. We encountered these problems in this Department. Furthermore, I worked together with associate professor Ivar Ǿye, who spent 4 years in the Vanderbilt University (Department of Physiology) and worked together with Professor Sutherland on the problems of cyclic AMP.

After returning home (Norway) from USA, Ivar Øye wanted to do his research in the field of cardiac tissues. He wanted to obtain "plasma membrane" materials, and he wanted to use the "membrane materials" for the study of pharmacological regulation of cyclic AMP transformation in the heart muscles. I was moved to this Department at that time, and I had to receive the critically good evaluation of action of these drugs, all of those were able to inhibit the preparations of "membrane materials" from the heart.

I had some methodological knowledge gained from our earlier biochemical studies of the stomach. However, my new scientific work offered a new challenge in the gastrointestinal mucosal research.

Furthermore, I had some other affinities to enter into this research field, which are as follows:

**a.** We earlier mentioned that we were not able to understand and explain the results of clinical pharmacology in patients with peptic ulcer. This is because most of the researchers emphasized the key role of vagus nerve (by the increase of gastric acid secretion); thereby the application of various inhibitory drugs was thought to be reasonable in terms of decrease of gastric acid production. However, if we evaluated critically the actions of these drugs, we had to learn that these drugs are able to inhibit the active metabolism in the gastric tissues. Consequently, we can explain the beneficial effects of these drugs on the healing of peptic ulcer (which was explained by impaired tissue metabolism).

We concluded that we have to do some change in paradigm of the peptic ulcer research. These facts and suggestions led us to study the biochemisms in the gastrointestinal mucosa;

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**b.** Many neural, hormonal (and pharmacological) factors, discovered absolutely new mechanisms; later, the immunological events could be collected in participation of peptic ulcer disease. Practically, the researchers studied the influences (action) of one or two factors (especially HCl and pepsin secretion, mucus secretion, bicarbonate secretion, prostaglandins, scavengers and antacids, and their different actions) on the stomach (gastrointestinal tract) in respect to development and protection (healing) of peptic ulcer disease. The results of these observations reflect only indirectly to the gastrointestinal tract.

Besides these results, we were uninformed on the biochemical changes in the gastrointestinal tract in time of gastric acid secretion and mucosal damage versus mucosal protection;

**c.** We wanted to study the biochemism of gastrointestinal tract, and we suggested that the final results (and summary) of different etiological factors can modify the functions of cells in the gastrointestinal tract. The blood supply was a remarkable extracellular factor; however, the changes in the blood flow also produce significant cellular biochemical changes in the target organ;

**d.** A general biochemical approach to study the biochemisms was built up in animal experiments from 1967. Our biochemical methodologies gave absolutely new insight into the biochemism of the gastrointestinal tract.

These biochemical observations were done dominantly on pylorus-ligated rats (as in an acute animal model). One of the most surprising results was to note that similar biochemical changes were detected in both the rumen and glandular parts (Mózsik et al., 1967 a, b). These results led us to study the changes in both the parts of the rat stomach after chronic "surgical vagotomy," chronic atropine treatment and cholinesterase (neostigmine) inhibitor treatments (for details, refer sections 4.3.1.–4.3.3 of Chapter 4). From the evaluation of the results of clinical pharmacological studies, we conducted biochemical observations in rats after cessation of drug (atropine, cholinesterase inhibitor) treatments.

The evaluation of these biochemical results in rats led us to understand some main lines of cellular biochemistry (cytoplasm, nucleic acids):

**a.** They were reversible in rat gastric tissues;

**b.** They were irreversible also.

The first one accepted the metabolic changes in biochemistry and the second one accepted the metabolic changes in genetic field (nucleic acids). These reversible and irreversible lines in the biochemical changes especially can be seen during the time of chronic "use" and "disuse" of vagus nerve. In some observations, we studied the changes of tissue levels of ATP, ADP (Mózsik et al., 1970a) and base alterations of nucleic acids in the stomach wall after a chronic atropine treatment (Mózsik et al., 1969b).

The results of these observations opened our eyes to start new observations with the main cellular functions of cells;

**c.** The international discoveries with membrane-bound ATP-dependent energy systems left a great impression and offered new possibilities to understand the regulations of ATP splitting and its resynthesis in the cells of the gastrointestinal tract;

**d.** There was no question that energy liberation exists in all of the functions of membrane-bound ATP-dependent enzymes ($Na^+$–$K^+$-dependent ATPase, $H^+$–$K^+$-ATPase, adenylate cyclase). However, the presence of these enzymes was not known in 1968;

**e.** In my works (in the Department of Pharmacology of Oslo University, Norway), the biochemical presence of $Na^+$–$K^+$-ATPase was used in my works to prove clearly that we really work with "membrane preparates," and these preparates were used to study the functions of adenylate cyclase. It's true that my boos wanted to see and to study these steps in cardiac muscles; however, I used parallelly the gastric mucosa (earlier obtained in rats, later in human gastric resecates) (Mózsik and Øye, 1969), and we wanted to study the actions of different drugs (epinephrine, ouabain, NaF ) on the adenylate cyclase. The NaF was used as the most powerful compound to stimulate the adenylase cyclase, because it inhibits the activity of all membrane ATPase activity (this fact is well known in the pertinent literature).

Therefore, consequently, I realized two important things during my study tour in Oslo (Norway), namely to prepare the adenylate cyclase from the crude "membrane materials" (from heart muscles and gastric mucosa) and the presence of $Na^+$–$K^+$-ATPase was used as a chemical marker for the plasma membrane.

We have to emphasize that the presence of neither Na+–K+-ATPase nor adenylate cyclase was known in animals and especially in humans;

**f.** I suggested that the functions of these enzymes can be modified by different drugs (not by only hormones) under different experimental conditions (including the animal experiments and human medical problems).

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 6

# Actual Positions in the Research of Membrane-bound ATP-dependent ATP-ase Systems in the World in the Different Tissues and Gastrointestinal Mucosa (in 1968-69)

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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During the close of the 1960s, two different workgroups dealt with the problems of active movements of cations across the cell membrane:

**a.** Skou (Skou, 1965; Albers, 1967; Askari, 1974; Schwartz et al., 1975; Glynn, 1964) indicated the existence of sodium–potassium pump located in the plasma membrane, which is responsible for the active movements of sodium and potassium. He clearly stated that: (a). this enzyme is located in the plasma membrane; (b) the activity of the enzyme located in plasma membrane can be enhanced by the combined application of sodium and potassium; (c) this enzyme splits the ATP located in the mitochondria into ADP in the presence of magnesium ion and (d) the activity of this enzyme can be specifically inhibited by the application of ouabain (Skou, 1965). The preparation of the membrane enzyme (sodium–potassium-dependent ATPase) was tried to prepare from the amphibian; frog-, rat-, rabbit- gastric mucosa without any success (Kasbekar and Durbin, 1965; Post and Albright, 1961; Sachs et al., 1972).

We used a special treatment for the preparation of plasma membrane and separation or centrifugation, which resulted in a "typical" sodium–potassium-dependent ATPase from the rat and human gastric mucosa (Mózsik and Øye, 1969).

**b.** Practically at the same time as the years up to the preparation of sodium–potassium-dependent ATPase (Butcher and Sutherland, 1962; Øye et al., 1964; Øye and Sutherland, 1966; Øye and Butcher, 1967 a, b) discovered another enzyme located in the plasma membrane, which spilled the ATP into 3′, 5′-cyclic AMP in the presence of magnesium

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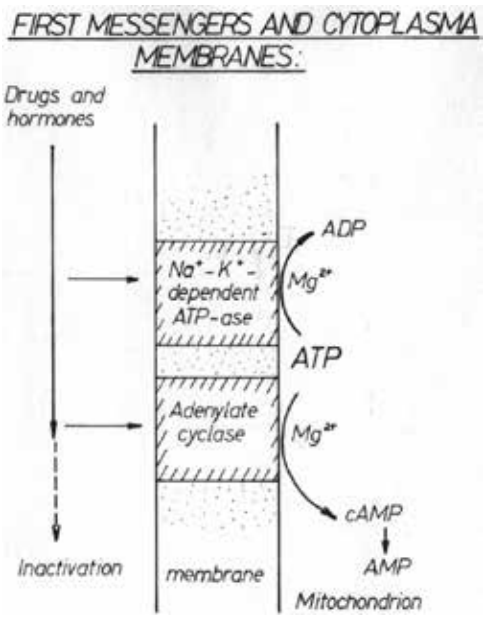


**Figure 27.** First (drugs, hormones) and second messenger systems located in the plasma membrane of cells. [Mózsik et al., Acta Medica Acad Sci Hung 36, 1–29, 1979 (with kind permission).]

ions (Butcher and Sutherland, l962; Øye and Sutherland, l966; Robinson et al., 1971; Sutherland and Rall, 1957; Butcher, 1966).

We were successful in preparing this enzyme from the human gastric mucosa (Mózsik and Øye, 1969).

It was exciting when we were able to detect the changes in different adenosine phosphates (adenosine triphosphate – ATP, adenosine diphosphate – ADP, adenosine monophosphate – AMP, cyclic 3′, 5′-adenosine monophosphate – cAMP) together with the changes in sodium–potassium-dependent ATPase and in adenylate cyclase activities (Figure 27).

We planned to carry out these types of observations under different experimental conditions (with and without drug administration, acute and chronic surgical and chemical vagotomy and with and without application of different necrotizing agents) in rats and patients with different gastric basal acid outputs (BAO) and maximal acid outputs (MAO), and the gastric antral, duodenal and jejunal mucosa around ulcer and the mucosal tissue samples without ulcer.

The energy liberation through the membrane-bound ATP-dependent energy systems (sodium–potassium pump and adenylate cyclase) can be detected well. The ATP–ADP transformation by sodium–potassium–ATPase enzyme releases 7.3 kcal/mol, while the ATP–cAMP transformation by adenylate cyclase results in 2 × 7.3 kcal/mL. Both the ADP–AMP and cAMP–AMP transformations release 7.3 kcal/mol (Figure 28).

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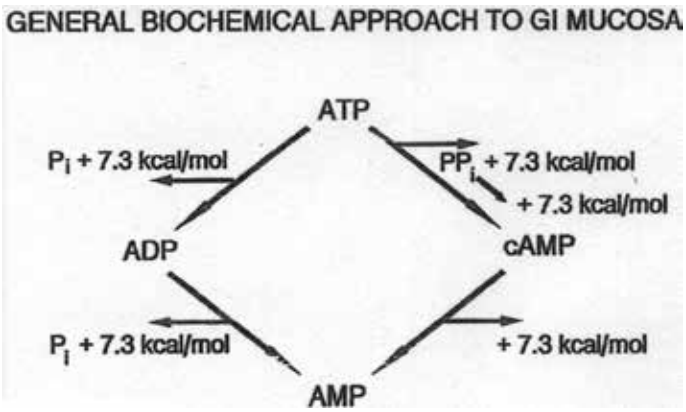


**Figure 28.** Pathways of energy liberation from ATP to ADP by membrane ATPase and from ATP to cAMP by adenylate cyclase located in the plasma membrane. The ATP–ADP transformation releases 7.3 kcal/mol, while ATP–cAMP transformation results in 2 × 7.3 kcal/mol. On the other hand, ADP–AMP and cAMP–AMP transformations produce 7.3 kcal/mol energy liberation. [Mózsik, Abdel Salam, Király, Morón, Nagy, Sütő, Tárnok, Jávor: in: Mózsik Gy., Nagy L., Király Á. (eds): Twenty Five Years of Peptic Ulcer Research in Hungary: From Basic Science to Clinical Practice.Akadémiai Kiadó, Budapest, pp. 159–170, 1997 (with kind permission).]

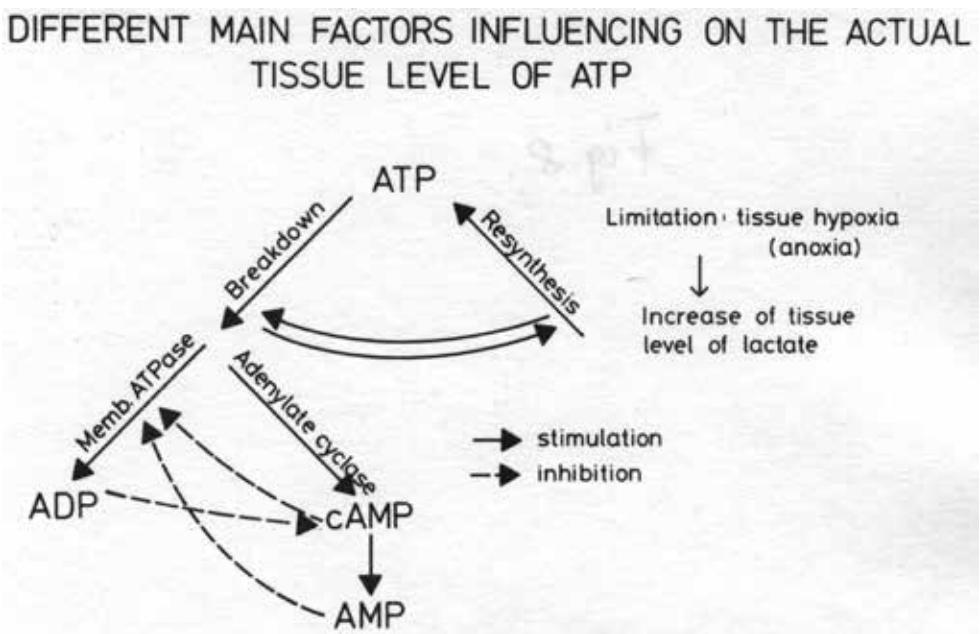


**Figure 29.** Different regulatory steps in the determination of actual levels of tissues of ATP. The tissue level of ATP is a consequence of ATP breakdown and ATP resynthesis. The limiting factor of ATP resynthesis by the pathway of oxidative phosphorylation is the presence of tissue hypoxia. The presence of tissue hypoxia can be proven biochemically by the increase of lactate levels in the tissue.

### 6.1. Materials and methods of experiments and human observations

The observations were carried out in CFY (Sprague-Dawley) (Gödöllő, Hungary) strain rats, weighing 180–210 g body weight and on the resecates of stomach and small intestine of patients who underwent gastric surgery because of unhealed ulcer disease (during 1970–1980).

The patients suffered from classical peptic ulcer disease (PUD) together with classical clinical symptoms (decreased appetite, feeling of dullness and pain in epigastrial region of the abdomen, pyrosis and impaired gastric emptying and retention syndrome). These patients presented in about one month before the surgical intervention. The presence of gastroduodenal ulcers were endoscopically diagnosed, and thereafter these patients received medical treatments (anticholinergic agents, later $H_2$ receptor antagonist and antacids for one month time period). The patients who did not heal during this time period were evaluated with the possibility of surgical interventions.

The indication of gastric surgery was given by physicians [with consultations between internists (gastroenterologists vs. surgeons) independently from us] on the resecates of stomach and small intestine (according to the method of Billroth II). A small group of patients underwent classical partial gastrectomy (according to the method of Billroth II) and jejunal ulcer was identified. The patients in this group were also treated medically (pharmacologically) for the same time period as mentioned earlier.

During the time of surgical intervention, the removals of stomach and small intestine were obtained immediately and these were cut into two parts from which one part was given for histological evaluation of resected tissues and the other part was immersed (after separation of mucosa and muscular layers) in liquid nitrogen and used for biochemical examinations. The mucosa specimens were also further separated from each other (depending on the distance of ulcer edge). The biochemical measurements from the mucosa specimens and muscular layers (independently from the number of tissue specimens) obtained from one patient were done (carried out) simultaneously dominantly on the same the as the surgical intervention was carried out.

The animal observations were carried out in both sexes of CFY-strain rats (the used methods are detailed in Section 4.2).

### 6.2. Membrane-bound ATP-dependent energy systems in the rat and human gastric mucosa

#### *6.2.1. General characterization of membrane ATPase prepared from the rat and human gastric fundic mucosa*

The method for preparation of membrane ATPase from the human gastric mucosa was published in 1969 (Mózsik and Øye, 1969; Mózsik et al., 1971 a, b; 1973 a, b; 1974 a, b, c, d; 1975 a, b; 1976 a, b). Figure 30 shows the typical characterization of membrane ATPase from the human gastric fundic mucosa. The membrane ATPase split the ATP into ADP *in vitro* incubation system in the presence of $Mg^{2+}$ ($Mg^{2+}$-dependent), which can be enhanced by the combined application of $Na^+$ and $K^+$ ($Mg^{2+}$–$Na^+$–$K^+$-dependent ATPase), in liberation of inorganic phosphorus followed by the ATP splitting process. The total ATPase can be inhibited by the application of ouabain (g-strophanthin). The difference between the total ($Mg^{2+}$–$Na^+$–K

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$^{+}$-dependent) versus only $Mg^{2+}$-dependent ATPase is given as $Na^{+}$–$K^{+}$-dependent ATPase (Mózsik et al., 1978 a, b, c). This $Na^{+}$–$K^{+}$-dependent ATPase has been associated with the active movements of $Na^{+}$ and $K^{+}$ (Skou, 1967; Albers, 1967; Askari, 1974; Glynn, 1964).

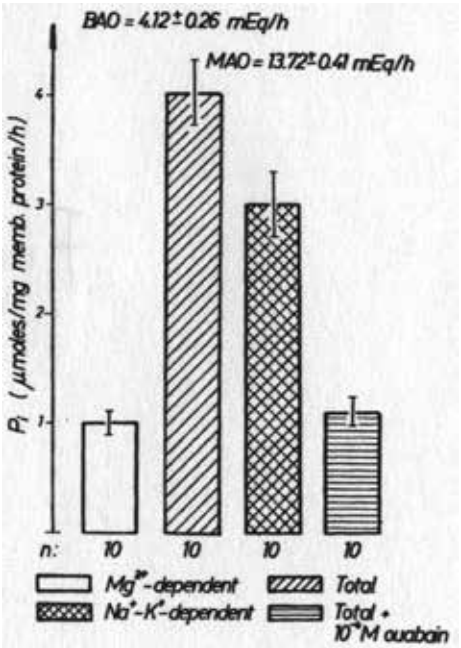


**Figure 30.** Biochemical characterization of membrane ATPase prepared from human gastric fundic mucosa. The membrane ATPase activity was measured by the liberation of inorganic phosphate from ATP produced by membrane ATPase *in vitro* incubation system in the presence of $Mg^{2+}$, $Mg^{-2+}$, $Na^{+}$ and $K^{+}$ (total ATPase). The difference between the total and only magnesium-dependent ATPase activity is equal to $Na^{+}$–$K^{+}$-dependent ATPase activity. The g-strophanthin (ouabain) is able to inhibit the $Na^{+}$–$K^{+}$-dependent ATPase (means ± SEM). [Mózsik et al.: Acta Physiol Scand Spec Suppl. 199–208; 1978 (with kind permission).]

There was a question whether there is any correlation between the neural regulation Andana $^{+}$– $K^{+}$-dependent ATPase. A log-concentration dose–response curve for acetylcholine, prepared from the human gastric fundic mucosa, was identified (Csáky, 1969; Mózsik et al., 1978 a, b, c) (Figure 31).

### *6.2.2. Determination of affinity and intrinsic activity curves for the dugs modifying the $Na^{+}$-$K^{+}$-dependent ATPase activity obtained from rat and human gastrointestinal mucosa*

The affinity and intrinsic activity curves were determined and calculated according to the method of Csáky (Csáky, 1969). The intrinsic activity ($\alpha$) of ouabain was taken to be equal to 1.00. The values of $pD_2$ (dose necessary to produce 50% inhibition in affinity curves) and $pA_2$ (the dose necessary to produce 50% inhibition in the intrinsic curves) were calculated from affinity and intrinsic activity curves.

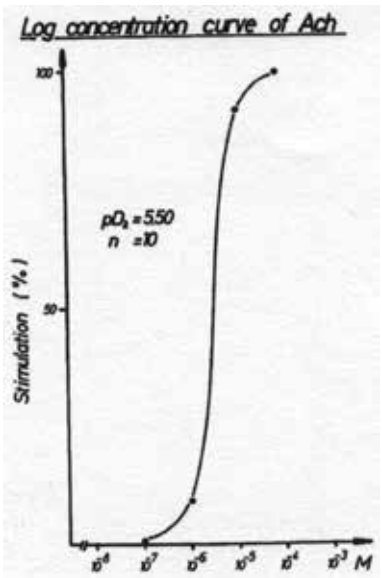


**Figure 31.** Log-concentration curve of acetylcholine (ACh) on the membrane ATPase prepared from the human gastric fundic mucosa. [Mózsik et al.: Acta Physiol Scand Spec Suppl 199–208; 1978 (with kind permission).]

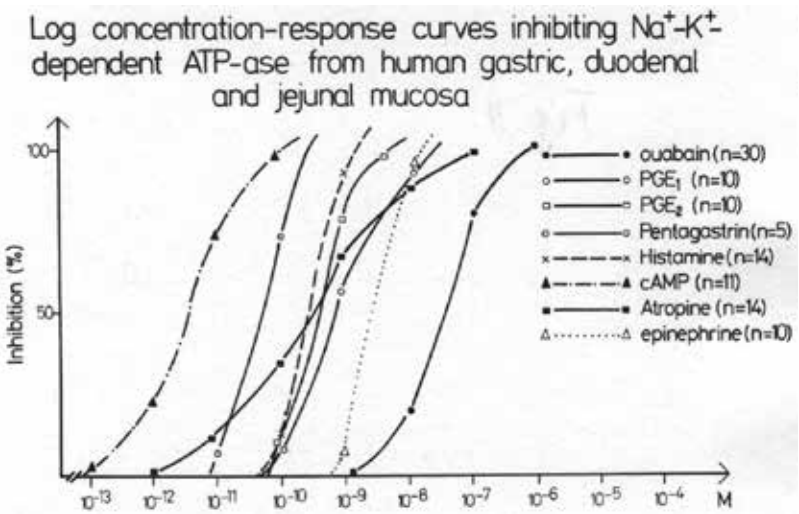


**Figure 32.** Dose–response curves for the drugs on the sodium–potassium-dependent ATPase prepared from human gastric, duodenal and jejunal mucosa (affinity curves). [Mózsik, Kutas, Nagy, Tárnok: Acta Medica Acad Sci Hung 36:459–466; 1979c (with kind permission).]

The dose–response curves (affinity curves) of the drugs were analyzed on the $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic, duodenal and jejunal mucosa (Mózsik et al., 1979 a, b, c, d, e) (Figures 32–34).

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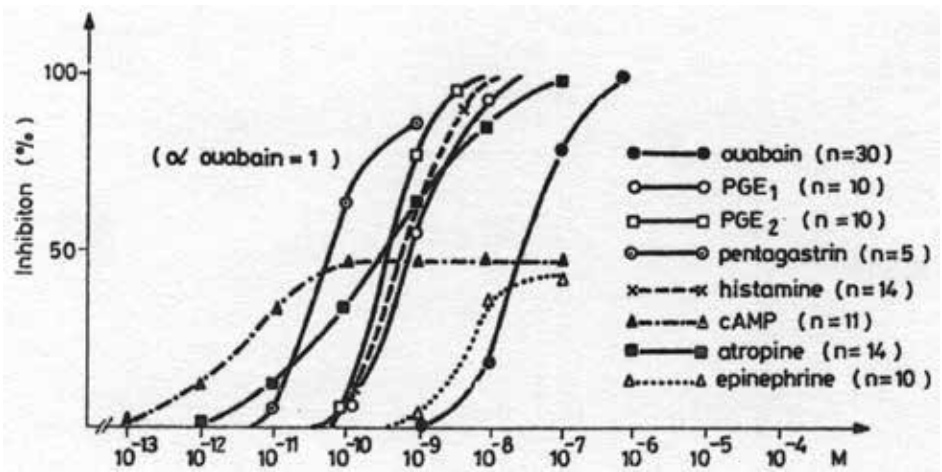


**Figure 33.** Dose–response curves for drugs inhibiting the $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic mucosa. The intrinsic activity ($\alpha$) of ouabain to $Na^+$–$K^+$-dependent ATPase system was taken to be equal to 1.00 and the effects of different drugs were compared with its effect; *n* indicates the number of patients. The values of intrinsic activities of drugs to $Na^+$–$K^+$-dependent ATPase system were calculated from the curves and presented in Table 3. [Mózsik, Kutas, Nagy, Tárnok: Acta Medica Acad Sci Hung 36:459–466, l979c (with kind permission).]

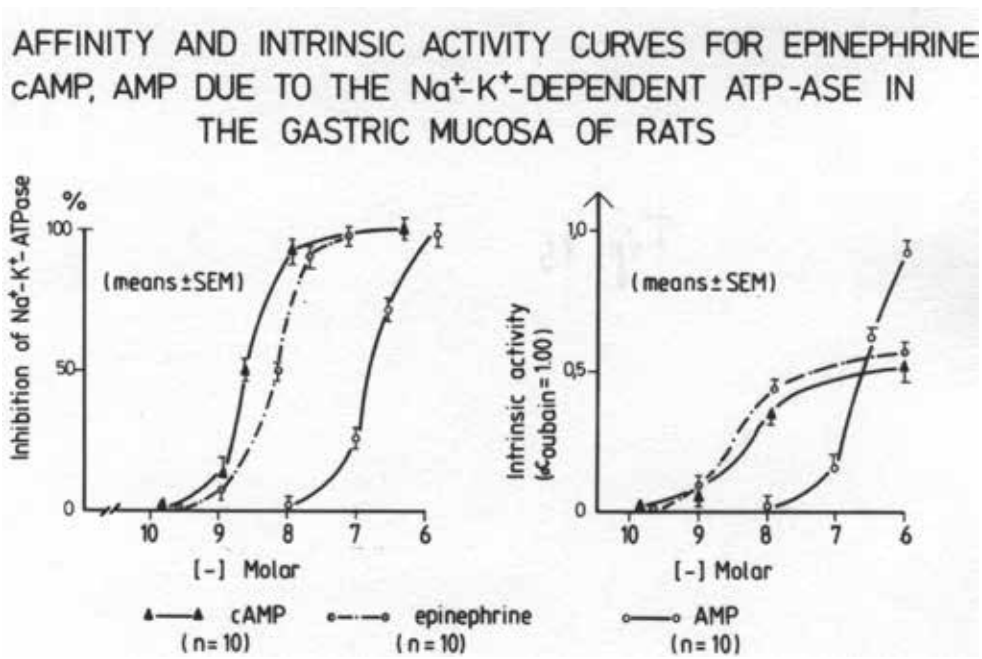


**Figure 34.** Affinity and intrinsic activity curves of epinephrine, cAMP and AMP on $Na^+$–$K^+$-dependent ATPase prepared from the rat gastric fundic mucosa. [Mózsik Gy, Garamszegi M, Jávor T, Nagy L, Patty I, Sütő G, Vincze Á: Ann N Y Acad Sci 597:264–281, 1990a (with kind permission).]

| Drugs | Affinities ($pD_2$) | Intrinsic activities ($\alpha$)** | ($pA_2$) |
|---|---|---|---|
| Atropine | 9.50 | 1.00 | 9.50 |
| Epinephrine | 8.60 | 0.41 | 8.60 |
| cAMP | 11.30 | 0.48 | 11.30 |
| $PGE_1$ | 9.30 | 1.00 | 8.30 |
| $PGE_2$ | 9.45 | 1.00 | 9.45 |
| Pentagastrin | 10.55 | 0.87 | 10.55 |
| Histamine | 9.70 | 1.00 | 9.70 |
| Ouabain | 7.50 | 1.00 | 7.50 |

**Table 26.** Characterization of the actions of drugs inhibiting $Na^+$–$K^+$-dependent ATPases prepared from human fundic mucosa.* expressed in [- ], $^{**}\alpha_{oubain}$=1.00

The values of $pD_2$ and $pA_2$ were calculated from the measured affinity and calculated intrinsic activity curves (Table 26).

### 6.3. Adenylate cyclase preparation from the rat and human gastric fundic mucosa

The adenylate cyclase was originally and first prepared from the human gastric fundic mucosa (Mózsik, 1969b; Mózsik et al., 1970b). Similar observations and results were done and obtained from the rat fundic mucosa (Ruoff and Sewing, 1974; Salganik and Bersimbaev, 1976; Thompson et al.; 1977 a, b).

The fresh gastric mucosal tissues of rats and humans were put into 20 mL of ice-cold 0.25 M sucrose solution, and it was homogenized in the room temperature of 4°C, then centrifugation was done at 0°C with 2000 g for 20 minutes. The supernatant material was removed and the sediment was resuspended in 25 mL ice-cold glycil–glycin buffer solution (264 mg of glycil–glycin and 24.65 mg of $MgSO_4$ were immersed in 100 mL of distilled water, and its pH value was fixed at 7.8). The rehomogenization was done, and thereafter centrifugation was carried out with 2000 g value for 20 minutes at 0°C. The supernatant material was removed after centrifugation, and the sediment was resuspended in 20 mL glycil–glycin buffer solution. Two-milliliter samples from this sediment solution were put in other tubes and centrifuged with 2000 g for 10 minutes at 0°C. After this centrifugation, the supernatant material was removed and the sediment was resuspended in 150-μL glycin–glycin buffer solution and it was used for measurements of adenylate cyclase.

The incubation solution (150 μL of resuspended enzyme, 50 μL of tested compounds: $H_2O$, epinephrine, NaF, atropine, acetylcholine, 150 μL Tris–buffer, 50 μL $C^{14}$–ATP – 20–25 mC/mmol dissolved in Mg solution) was used for 10 minutes at 30°C temperature before the measurements of enzyme activity of adenylate cyclase. The enzyme activity was stopped after boiling for 3 minutes; however, before the heat treatment 20 μL $H^3$-cyclic 3′, 5′-adenosine monophosphate was added into incubated samples.

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The ATP, ADP, cyclic AMP and AMP were separated from the 300 μL of incubated suspension by Dowex 50 ion exchange column (Krishna et al., 1968), and three peaks were obtained. The first peak contained the ATP and ADP, the second one the cyclic AMP and the third one the 5′-AMP. The other components (such as nucleotides, inorganic phosphate and cyclic 3′, 5′-AMP) were then precipitated by the treatment of $ZnSO_4$–$Ba(0H)_2$. After centrifugation of these samples, the supernatants were evaporated and thereafter the residue was immersed in 10 mLLof Bay's solution. The radioactivity of cyclic AMP was detected by liquid scintillation equicment (H. Pacard) of $H^3$ and $C^{14}$ labelld cyclic 3′, 5′-AMP. The extent of the ATP–-AMP transformation was given as ounts of $C^{14}$ ounts of 100 $H^3$ (see Table 7) .

| Examined compounds | Incubation time (min) | $C^{14}$ / 100 $H^3$ |
|---|---|---|
| 1. $H_20$ | 0 | 12.36 |
| 2. $H_20$ | 10 (control) | 23.96 |
| 2. Epinephrine ($10^{-4}$ M ) | 10 | 28.43 |
| 4. NaF ($10^{-2}$ M) | 10 | 35.84 |
| 5. Atropine ($10^{-4}$ M) | 10 | 29.60 |
| 6. Acetylcholine ($10^{-4}$ M) | 10 | 21.52 |

**Table 27.** Testing of the effects of epinephrine, NaF, atropine and acetylcholine on the transformation of ATP into cyclic AMP by adenylate cyclase prepared from the human gastric mucosa (Mózsik, 1969a: PhD Dissertation of Pécs University, Hungary)

### 6.4. Feedback mechanism systems between the membrane-bound ATP-dependent energy systems in the gastrointestinal mucosa in rats and humans

#### *6.4.1. Pharmacological regulatory mechanisms between the membrane ATPase (ATP – ADP transformation) and adenylate cyclase (ATP – cAMP transformation) prepared from the rat and human gastric fundic mucosa*

The origin of the suggestion to existence of feedback system between the $Na^+$–$K^+$-dependent ATP and adenylate cyclase was created by me (GyM) based on the results of personal observations and after studying the actions of different drugs on these enzymes.

It was interesting to compare the effects of drugs on $Na^+$–$K^+$-dependent ATPase and adenylate cyclase activity from different "membrane fractions" under *in vitro* conditions (Table 28). The $Na^+$–$K^+$-dependent ATPase activity was inhibited by epinephrine, NaF and atropine (Mózsik,

| Drugs | $Na^+$-$K^+$-dependent ATPase activity<br>Effects | Tissue specimens |
|---|---|---|
| Adrenaline | Inhibition<br>($10^{-10}$ to $10^{-4}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa |
| NaF | Inhibition<br>($10^{-3}$ to $10^{-2}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa |
| Atropine | Inhibiton<br>($10^{-10}$ to $10^{-4}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa |
| Acetylcholine | No effect<br>($10^{-10}$ to $10^{-4}$ M ) | rat heart<br>rat gastric mucosa<br>human gastric mucosa |

| | Adenylate cyclase activity<br>Effecs | Tissue specimens | References to adenylate Cyclase |
|---|---|---|---|
| Adrenaline | Stimulation<br>($10^{-7}$ to $10^{-4}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa | Murad et al., 1962<br>Mózsik, 1969a,b |
| NaF | Stimulation<br>($10^{-3}$ to $10^{-2}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa<br>erythrocyte | Sutherland et al., 1962<br>Mózsik, 1969a, b<br>Øye and Sutherland, 1966 |
| Atropine | Stimulation<br>($10^{-5}$ to $10^{-4}$ M) | rat heart<br>rat gastric mucosa<br>human gastric mucosa | Murad et al., 1962a, b<br>Mózsik, 1969 |
| Acetylcholine | Inhibition<br>($10^{-5}$ to $10^{-4}$ M)<br>(30 %) | rat heart | Murad et al., 1962 |

**Table 28.** Effects of drugs used on $Na^+$–$K^+$-dependent ATPase and adenylate cyclase activity by different "membrane preparates" prepared from rat heart, rat and human gastric mucosa under *in vitro* conditions (Mózsik, 1969 a, b).

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1969b), whereas the adenylate cyclase activity was stimulated by adrenaline (epinephrine) (Murad et al., 1962; KlainerCet al., 1962; Øye and Sutherland, 1966), NaF (Sutherland, t al., 1962; Øye and Sutherland, 1966) and by tropine ( 5 t 20 % aplied in $10^{-5}$ and $10^{-4}$ cocentrations) ( Murd, Chal., 1962). The adenylate cyclase activity was inhibited by acetylcholine and acetyl-β-methylcholine (Murad, Chi, R 1962). Acetylcholine had no effect on $Na^+$–-$K^+$-dependent APase activity (consequently, the ATP transformation into ADP is workeding wiout hinnceany trouble duringcholine's effect on $Na^+$–-$K^+$-dependent ATPae).

When adenylate cyclase activity is studied in course particle preparations having high ATPase activity, the local concentration of ATP at the catalytic site of adenylate cyclase might be rate limiting for the reaction. An inhibition of $Na^+$–$K^+$-dependent ATPase by adrenaline, NaF and atropine might therefore apparently stimulate the adenylate cyclase activity (by the increase of ATP concentration). It was particularly interesting that NaF not only inhibited $Na^+$–$K^+$-ATPase activity, but also stimulated the adenylate cyclase activity more than other drugs. The inhibition of adenylate cyclase activity by acetylcholine is due to a decrease in local concentration of ATP caused by $Na^+$–$K^+$-dependent ATPase activity at the catalytic site of adenylate cyclase. The inhibitory effect of acetylcholine on adenylate cyclase activity was prevented by adding atropine in a concentration of $10^{-7}$ M leading to an inhibitory effect on $Na^+$–$K^+$-dependent ATPase; there was no effect of this concentration of atropine alone on the adenylate cyclase (Murad et al., 1962).

The inhibition caused by adrenaline and atropine was present in concentrations from $10^{-9}$ to $10^{-4}$ M (Mózsik, 1969 a, b), whereas the stimulation by adrenaline and atropine on adenylate cyclase activity was obtained by concentrations from $10^{-6}$ to $10^{-5}$ M (Klainer et al., 1962; Murad et al., 1962; Mózsik, 1969 a, b). Thus, *in vitro* $Na^+$–$K^+$-dependent (active transport) ATPase system was 100–1000 times more sensitive to drugs than the adenylate cyclase system.

The difference in sensitivity between the $Na^+$–$K^+$-dependent (transport) ATPase and adenylate cyclase activity, and the contradictory effects of drugs on them indicated the following:

**1.** The stimulatory effects by these drugs on adenylate cyclase activity is associated with blocking of $Na^+$–$K^+$-dependent ATPase activity;

**2.** The $Na^+$–$K^+$-dependent ATPase (transport) system and adenylate cyclase system can be separated.

A direct inhibitory effect of cyclic AMP and adenosine 5′-monophosphate (5′-AMP) of $Na^+$–$K^+$-dependent ATPase activity from human gastric mucosa was observed in a wide range of concentrations (Mózsik, 1970).The cyclic 3′, 5′-AMP and 5′-AMP are present in the cells as products of adenylate cyclase activity. The direct inhibition of $Na^+$–$K^+$-dependent (active transport) ATPase by cyclic 3′, 5′-AMP and 5′-AMP showed an antagonistic interrelationship between the adenylate cyclase system and the active transport ATPase system (Figure 35).

In the forthcoming years and thereafter, the tissue levels of ATP, ADP, cyclic AMP, AMP (adenine–adenosine) were measured *in vivo* conditions (in different human gastrointestinal diseases and under different animal experiments) directly from the gastrointestinal mucosal

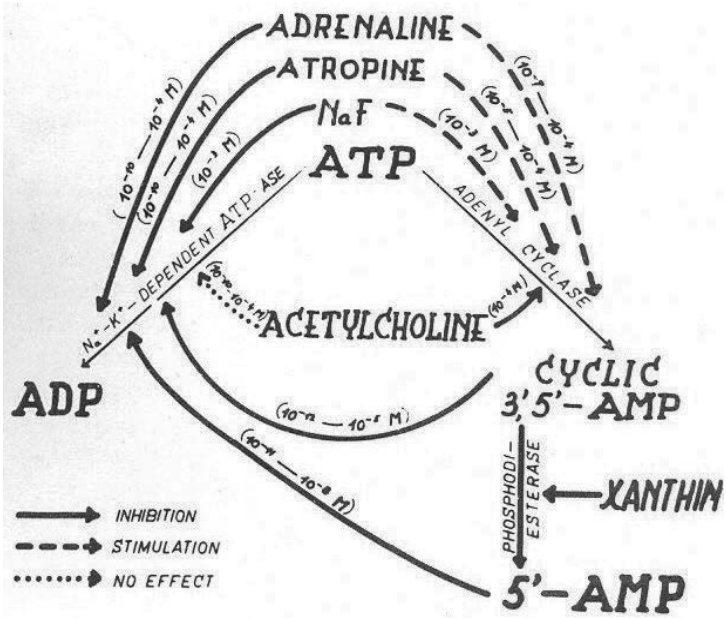


**Figure 35.** The summary of some feedback mechanisms of drugs with interrelationship between the active transport ($Na^+$–$K^+$-dependent ATPase) system and the adenylate cyclase prepared from rat and human gastric mucosa (Mózsik, 1969b ). These types of observations were carried out under *in vitro* conditions (with kind permission).

tissues. However, the details of the above entioned hypothesis iere taken into account for the evaluation of the forthcoming observations.

These pharmacological regulatory functions were opposite ones on the $Na^+$–$K^+$-dependent ATPase and adenylate cyclase (Tables 28, 29). Generally, different drugs were able to modify the $Na^+$–$K^+$-dependent ATPase in lower molar concentrations than those in the adenylate cyclase concentrations. These results a priori suggested the existence of a complex feedback system, which could be modified by mediators, hormones and drugs.

*6.4.2. Regulatory mechanisms between the tissues levels of ATP, ADP, cAMP and AMP in the intact rat gastric mucosa (in vivo studies)*

The tissue levels of ATP, ADP and AMP were enzymatically (Boehringer Ingelheim, Germany); while the cAMP by RIA (Beckton, Dikinson, Orengeburg, USA) was measured. The protein content was measured by the method of Lowry et al. (1951) in the tissue concentration of ATP, ADP and AMP (except for cAMP which was expressed as pmol/mg) in nmol/mg protein. The ratio of ATP/ADP and the values of adenylate pool (ATP+ADP+AMP) were calculated. From the ATP, ADP, AMP and adenylate pool, the values of "energy charge"[(ATP +0.5 ADP)/(ATP+ADP+AMP)] were calculated according to the method of Atkinson's formula (1968). This value is theoretically 1 when all adenosine compounds are in phosphorylated form, and its value is 0 when all adenosine nucleotides are in dephosphorylated form.

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| | ATP-ADP transformation | | ATP-cAMP transformation | |
|---|---|---|---|---|
| Drugs | Effects | Doses (M) | Effects | Doses (M) |
| Acetylcholine | Stimulation | $10^{-7}$ M → | Inhibition | $10^{-4}$ M → |
| Parasympatholytics: | | | | |
| Atropine | Inhibition | $10^{-11}$ M → | Stimulation | $10^{-8}$ M → |
| Isopropamide | Inhibition | $10^{-8}$ M → | Stimulation | $10^{-5}$ M → |
| Gastrixon | Inhibition | $10^{-7}$ M → | Stimulation | $10^{-4}$ M → |
| Epinephrine | Inhibition | $10^{-9}$ M → | Stimulation | $10^{-7}$ M → |
| β-blocker (Visken) | Stimulation | $10^{-4}$ M → | Inhibition | $10^{-5}$ M → |
| Histamine | Inhibition | $10^{-11}$ M → | Stimulation | $10^{-8}$ M → |
| Cimetidine | Stimulation(?) | $10^{-4}$ M → | Inhibition | $10^{-6}$ M → |
| Pentagastrin | Inhibition | $10^{-11}$ M → | Stimulation | $10^{-9}$ M → |
| $PGE_1$ Inhibition | $10^{-11}$ M | → Stimulation | $10^{-9}$ M | → |
| $PGE_2$ Inhibition | $10^{-11}$ M | → Stimulation | $10^{-9}$ M | → |
| Ouabain | Inhibition | $10^{-8}$ M → | Stimulation | $10^{-4}$ M → |
| cAMP Inhibition | $10^{-13}$ M | → | | |
| AMP Inhibition | $10^{-8}$ M | → | | |

*Direct effects of the membrane-bound ATP-splitting enzyme activities.

**Table 29.** Pharmacological effects on the transformation of ATP into ADP by membrane ATPase and the ATP–cAMP transformation by adenylate cyclase from rat and human gastric, fundic, antral, duodenal and jejunal mucosa. [Mózsik and Jávor: Dig. Dis. Sci 33:92–105, 1988* (with kind permission).]

Some general aspects of the biochemical approach need to be emphasized for the establishment of the theoretical base of the feedback systems between the tissue levels of ATP, ADP, AMP and cAMP:

**1.** Simultaneous determinations of the tissue ATP, ADP, AMP and cAMP and lactate may be used for a correct evaluation of the role of membrane-bound ATP-dependent energy systems in both development and prevention of ulcer. No such conclusions can be derived from the determination of ATP or cAMP alone;

**2.** The tissue hypoxia produced by two characteristic biochemical properties: (a) a significant increase in the level of lactate in tissue and (b) failure of the ATP resynthesis due to impaired oxidative phosphorylation by tissue hypoxia. A significant decrease in tissue ATP level was observed in many experimental models, but without an increase in the lactate level (Mózsik et al., 1976 a, b, c, d; Mózsik and Vizi, 1976 a, b; Mózsik et al., 1979 a, b, c, d, e, f, g; Mózsik et al., 1981 a, b, c, d, e; Nagy et al., 1983). The simultaneous measurements of ATP–membrane, ATPase–ADP and ATP–adenylate cyclase–cAMP systems offer useful information on the energy turnover. If there was increased activity of ATP-splitting enzymes together with increased concentration of ADP and cAMP, it would be indicative of increased ATP breakdown in one or another direction, or in both pathways (Mózsik and Vizi, 1976 a, b; Mózsik et al., 1978c; Mózsik et al., a, b, c, d, e, f, g; Mózsik et

al., 1983 a, b, c). If the ATP level had been found at a significantly higher level, this would indicate an increased extent of oxidative phosphorylation. These findings however do not indicate the presence of tissue hypoxia in the gastrointestinal mucosa;

**3.** The balance between the membrane-bound ATP-dependent energy systems can be modified by hormones, mediators and drugs, under physiological and pathophysiological conditions (Mózsik et al., 1979 a, b, c, d, e, f, g; Mózsik et al., 1983 a, b, c). The major effects produced by these agents can be evaluated as steps in the injury to the gastrointestinal mucosa. It is important to note that the ATP–ADP and ATP–cAMP energy systems are affected by different dose–response curves of drugs. For example, the doses required to produce 50% inhibition of ATP–ADP and ATP–cAMP transformation from affinity curves ($pD_2$) and those required to express intrinsic activities ($pA_2$) using ouabain as having an activity ($\alpha = 1.00$) differ significantly in both systems (Tables 26, 29);

**4.** Data are scarce for the two ATP-dependent energy systems (Robinson et al., 1957; Schwartz et al., 1975; Sutherland and Rall, 1975, 1960; Butcher and Sutherland, 1971). Their correlations were evaluated by responses to various hormones, mediators and drugs by the gastric mucosa in rats and human subjects. These responses show that feedback mechanisms exist between the membrane-bound energy systems (Mózsik et al., 1979 a, b, d, e, f, g; Mózsik, 1969 a, b);

**5.** The system is characterized mainly by the following:

**a.** ATP is a common substrate for both $Na^+$–$K^+$-dependent membrane ATPase and adenylate cyclase (Mózsik, 1969 a, b);

**b.** the drugs which stimulate membrane ATPase activity, inhibit the transformation of ATP into cAMP by adenylate cyclase (Mózsik, 1969 a, b );

**c.** drugs and hormones inhibiting the membrane ATPase activity stimulate the adenylate cyclase activity directly and vice versa (Mózsik et al., 1973 a, b; Mózsik et al., 1974 a, b, c; Mózsik et al., 1979 a, b, c, d, e, f ; Mózsik et al., 1981 a, b, c);

**d.** the increased transformation of ATP into ADP indirectly inhibits the extent of ATP–cAMP transformation (Mózsik et al., 1983b; Morón et al., 1983; Mózsik et al., 1983 b, c);

**e.** the decrease in transformation of ATP into ADP by various agents leads to the indirect stimulation of the transformation of ATP into cAMP (Mózsik et al., 1979 a);

**f.** the increase in ATP transformation into cAMP leads to an indirect inhibition of ATP–ADP transformation (Mózsik et al., 1979 a, b, c, d, e, f, g, h);

**g.** AMP and cAMP directly inhibit the transformation of ATP into AMP by direct inhibition of membrane ATPase activity (Mózsik, 1969a, 1970);

**h.** the inhibition in the transformation of ATP into cAMP is the result of the indirect stimulation in the transformation of ATP into ADP (Mózsik et al., 1987 a, b);

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**i.** the extent of cAMP transformation through regulation of phosphodiesterase by drugs may be regulated by both ATP–ADP and ATP–cAMP transformation.

**6.** The extent of phosphorylation and/or dephosphorylation can be estimated by the Atkinson's formula (1968).

The critical evaluation of the results of these observations offers a significant possibility for the biochemical background of gastrointestinal tract.

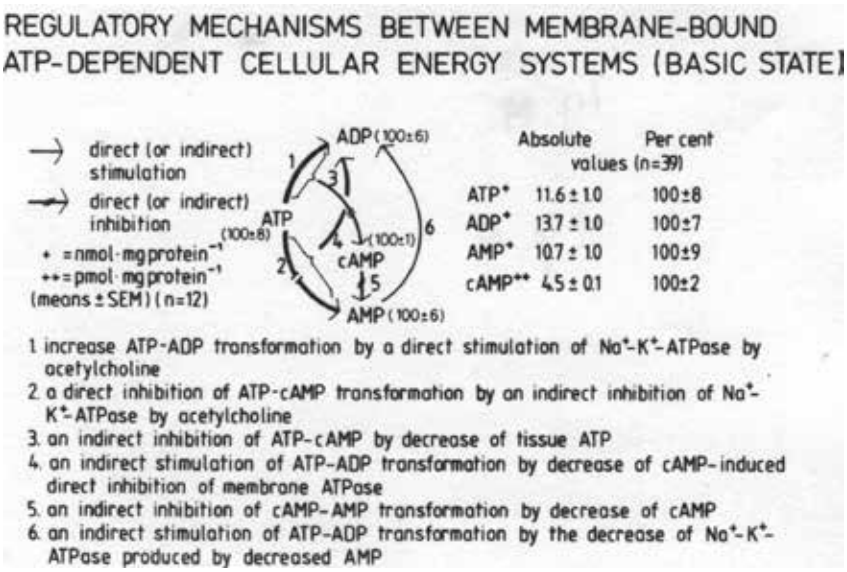


**Figure 36.** Main regulatory steps to membrane-bound ATP-dependent energy systems in rats under the physiological conditions. [Mózsik et al., Ann N Y Acac Sci. 597: 264–281, l990a (with kind permission).]

*6.4.3. Cholinergic and adrenergic influences on the regulatory mechanisms between the tissue levels of ATP, ADP, cAMP and AMP in the intact rat stomach*

Under the physiological conditions, the ATP–ADP transformation is the first-line regulation in the active movements of cations ($Na^+$, $K^+$) across the cell membrane, and this ATP decrease in the membrane ATPase results in an indirect inhibition on the ATP–cAMP transformation. Furthermore, the acetylcholine (ACh) alone directly inhibits the adenylate cyclase activity (Mózsik, 1969 a, b). The decrease in cellular level of cAMP relatively enhances the extent of the ATP–ADP transformation, and in the meantime further decreases the extent of ATP–cAMP transformation (Figure 38).

Epinephrine directly enhances the adenylate cyclase activity and it directly inhibits the membrane ATPase activity in smaller doses (Mózsik, 1970). During the epinephrine effect, the extent of ATP–cAMP transformation will increase, and in the meanwhile the ATP–ADP transformation will decrease (Figures 16, 17).

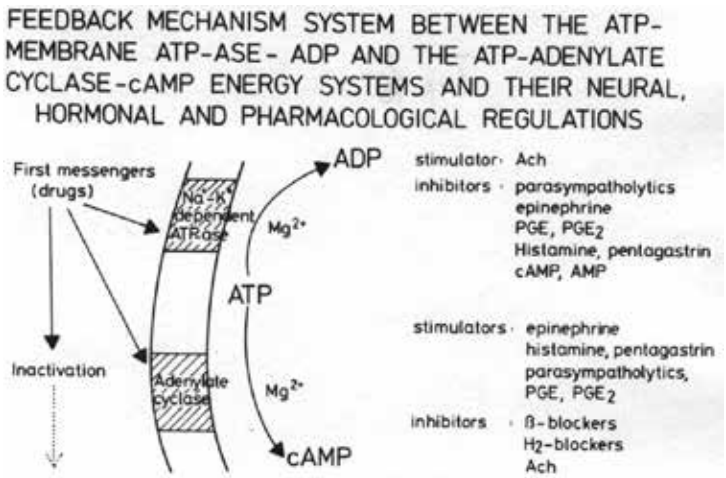


**Figure 37.** Pharmacological regulations (first messengers) of membrane-bound ATP-dependent energy systems (second messengers) in the rat and human gastric mucosa under the physiological conditions. [Mózsik et al., Acta Medica Acad Sci Hung 36, 1–29, 1979 (with kind permission).]

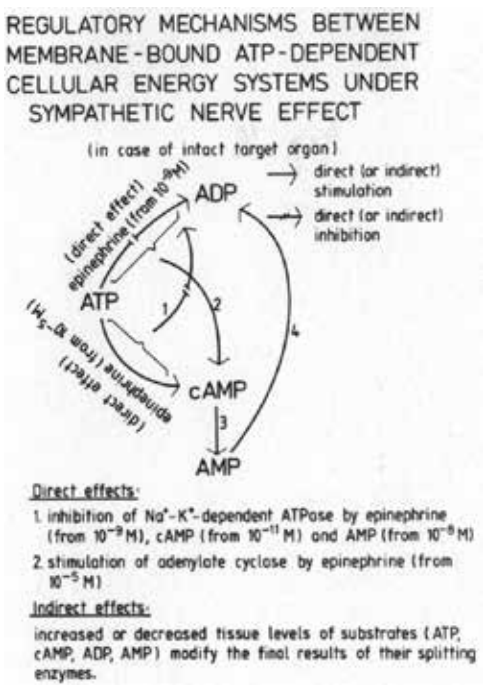


**Figure 38.** Epinephrine-induced direct and indirect regulatory mechanisms on the transformation of ATP–ADP by membrane ATPase and of ATP–cAMP by adenylate cyclase and of ADP–AMP and cAMP–AMP transformation in the gastric mucosa in intact rats. [Mózsik et al., Ann N Y Acad Sci 579, 264–281, l990a (with kind permission).]

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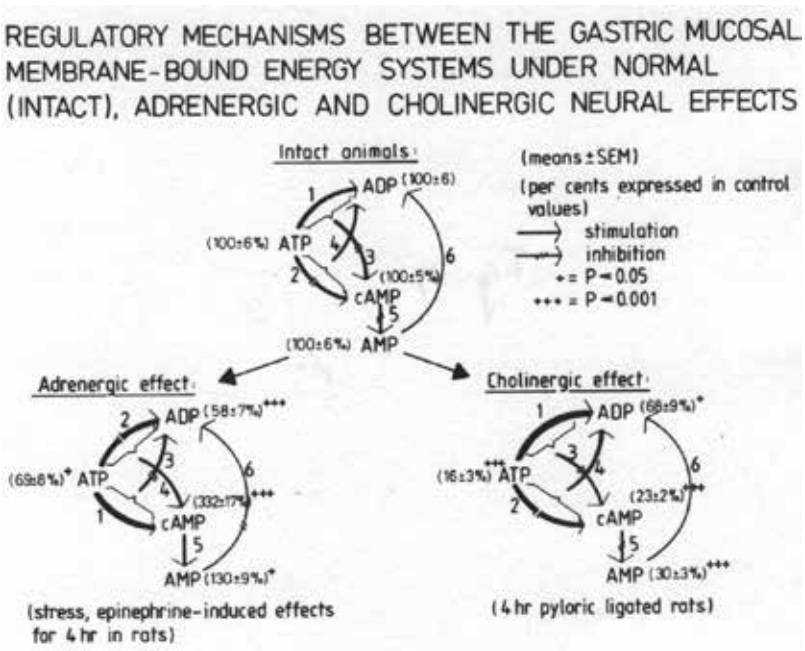


**Figure 39.** Regulatory mechanisms between the cellular ATP, ADP, cAMP and AMP in the rat gastric mucosa under normal (intact), adrenergic and cholinergic neural effects.

*6.4.4. The effects of "surgical" and "chemical" vagotomy on the energy metabolism of gastric mucosa in intact rats*

The aims of these studies were as follows:

**a.** To evaluate the effects of acute "chemical" and "surgical" vagotomy on the gastric mucosal biochemistry in rats;

**b.** To find correlations between these tissue biochemical parameters after "surgical" and "chemical" vagotomy (without the application of any damaging agents) in intact rats.

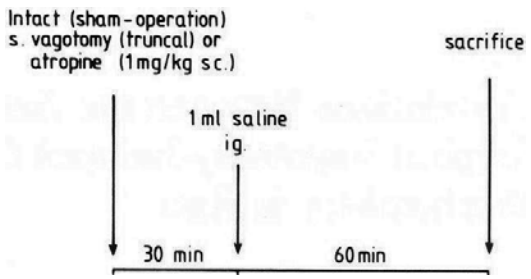


**Figure 40.** Experimental design of these experiments. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol S135–S139, 1992b (with kind permission).]

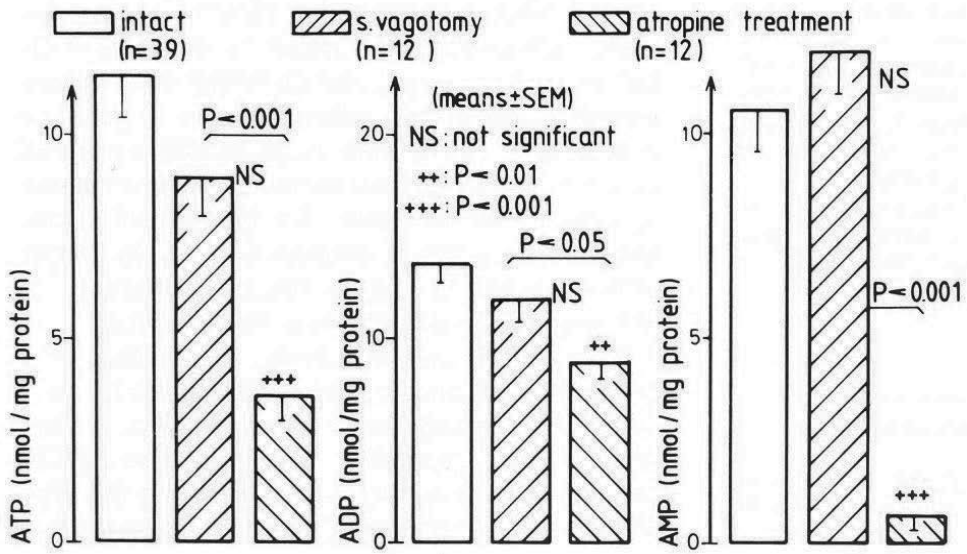


**Figure 41.** Surgical and chemical vagotomy-induced changes in the gastric mucosal levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in rats (without giving any necrotizing agents). *P* values are between the intact (sham-operated) versus surgical and chemical vagotomized rats. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2):S135–S139, 1992b (with kind permission).]

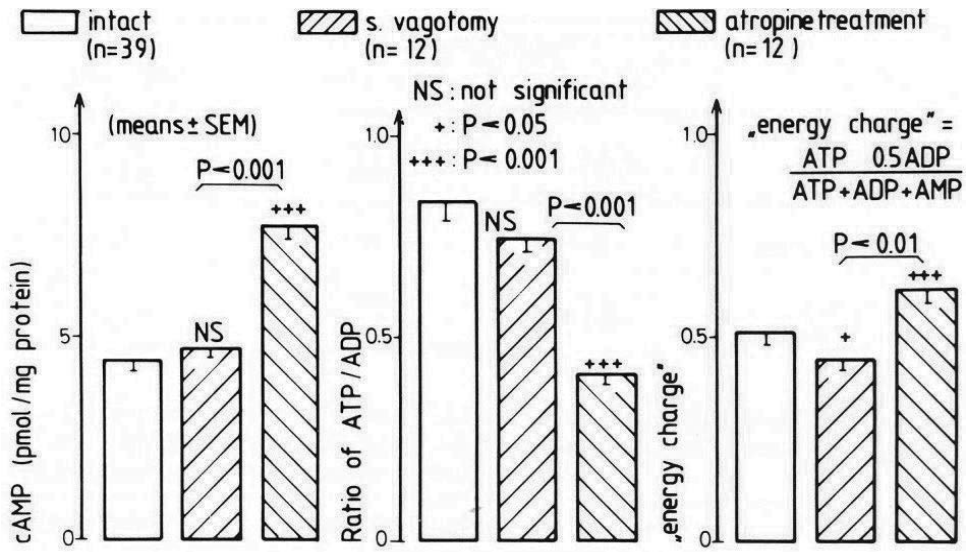


**Figure 42.** "Surgical" and "chemical" vagotomy-induced changes in the tissue levels of cyclic adenosine monophosphate (cAMP), ratio of ATP/ADP and "energy charge" in the gastric fundic mucosa of rats (without application of any necrotizing agents). For further explanations, see Figure 41. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135–S139, 1992b (with kind permission).]

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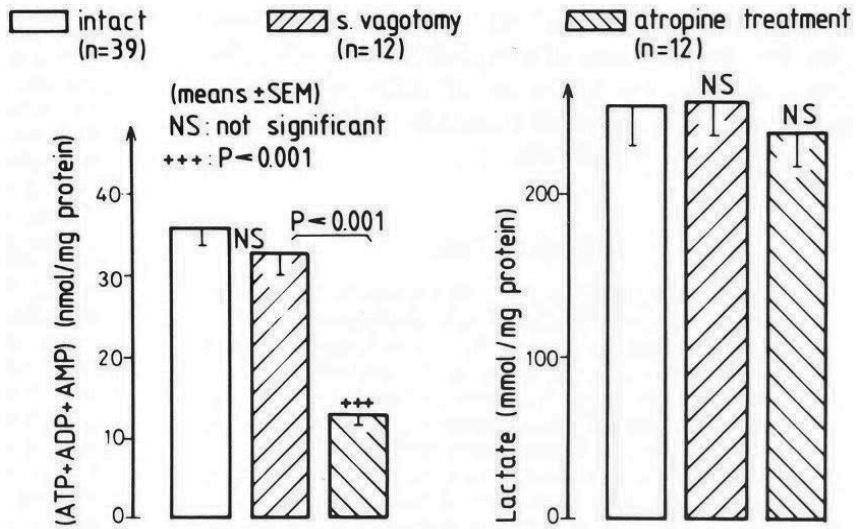


**Figure 43.** "Surgical" and "chemical" vagotomy-induced changes in the gastric mucosal levels of the adenylate pool (ATP+ADP+AMP) and lactate. For further explanation, see Figure 41. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135–S139, 1992b (with kind permission).]

| | Transformations of | | |
|---|---|---|---|
| | ATP – ADP | ATP - cAMP | cAMP - AMP |
| Intact vagus | ↑↑↑ | ↓↓↓ | ↓↓↓ |
| Surgical vagotomy | 0 | 0 | 0 |
| | P<0.05 | P<0.001 | P<0.001 |
| Pharmacological vagotomy | ↓↓ | ↑↑↑ | ↓↓↓ |

**Figure 44.** Surgical" and "chemical" vagotomy-induced changes in the gastric mucosal membrane-bound ATP-dependent energy systems of rats (without application of any necrotizing agents). *P* values are presented between intact (sham-operated) versus "surgical" and "chemical" vagotomized groups: ↑ , increase; ↓ , decrease. Abbreviations: 0, not significant; ↓ ↓ , $P < 0.01$; ↑ ↑ ↑ ( ↓ ↓ ↓ ) : $P < 0.001$. (Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135-S139, 1992b) (with kind permission).

The results in this chapter clearly indicate that the acute "surgical" and "chemical" (acute administration of atropine) vagotomy produce significantly different changes in the gastric mucosal energy systems (similar conclusions were obtained from our biochemical observations carried out earlier).

| Membrane-bound ATP-dependent (ATP-splitting) systems | | | |
|---|---|---|---|
| | $Mg^{2+}$-$Na^{+}$-$K^{+}$-dependent ATPase | | |
| Compounds | $Mg^{2+}$-dependent | $Na^{+}K^{+}$-dependent | Adenylate cyclase |
| Acetylcholine | No effect | Stimulation | Inhibition |
| Atropine | No effect | Inhibition | Stimulation |
| cAMP | No effect | Inhibtion | No effect |
| AMP | Stimulation | Inhibition | No effect |

**Table 30.** Effect of compounds on membrane- bound ATP-dependent systems. [Mózsik, Jávor: Dig. Dis. Sci. 33:92–105, 1988; Mózsik, Nagy, Tárnok, Vizi: Acta Med. Acad. Sci. Hung. 36: 1–29, 1979) (with kind permission).]

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 7

# Biochemical Examination of the Human Gastrointestinal Tract

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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The forthcoming biochemical observations were from the resecates of the patients who underwent surgical intervention because of peptic ulcer disease. These observations were done during 1969–1976, when only the parasympatholytics were used in the medical treatment of patients with peptic ulcer.

The resecates of the gastrointestinal tract were divided into two parts: one was used for classical histopathological examinations (with the permission from Professor György Romhányi, Head of the Department of Pathology and of Professor Tihamer Gy. Karlinger, Head of First Department of Surgery, Pécs University, Hungary),, awhile the other part was used for biochemical examinations. Different parts from the mucosa and musculature were separated from each other immediately after the surgical resection of the GI tract, and these parts were put immediately into liquid nitrogen.

All biochemical parameters of the GI tissue samples (normal and ulcerated antral, duodenal and jejunal mucosa and three gastric fundic mucosa and musculature, if we received fundic tissues) were maintained in the same time in all the received tissue samples.

It is very important to emphasize that, on the one hand, all biochemical parameters maintained from the same tissue samples, and on the other hand, all tissue samples were biochemically examined. It is also important to note that the weight of these tissue samples was about 0.25–0.5 g wet tissue (these examinations cannot be carried out from biopsy materials).

The methodologies were detailed in the original published papers.

These patients had typical ulcer histories and endoscopic pictures. These patients received medical treatment (by dominant internists) for about 4 weeks before surgery. The patients who were suspected to have malignant ulcers did not receive any tissue samples from the resecates (because of necessary histopathological examinations).

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### 7.1. Correlation between the gastric fundic mucosal $Na^+$-$K^+$-dependent ATPase activity versus gastric basal acid output (BAO) in humans

In the gastric basal acid output (BAO) and maximal acid output (MAO), there are different associations with cations and protein secretion from the serosa to the mucosa: the $H^+$, chloride, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and albumin increase significantly, while the Na indicates a decrease (Myren, 1968; Semb and Myren, 1968; Wright and Hirschowitz, 1976). These called for attention to study the correlations between the classical membrane-bound ATP-dependent energy systems and gastric BAO and MAO.

There was no doubt that after the administration of pentagastrin or histamine (given in doses) to produce gastric MAO values, the changes in the contents of the gastric juice cations became much higher than those in gastric BAO. These data also suggested that there is some correlation between the membrane-bound ATP-dependent energy systems and the gastric BAO and MAO values.

In 45 patients with peptic ulcer (20 patients with gastric and 25 patients with duodenal ulcer), the gastric $H^+$ was measured without the administration of any drug (basal acid output, BAO), and its value was expressed in mEq/L. These patients underwent resection of the stomach for peptic ulcer. During the surgery, a piece was cut from the fundic part of the stomach. The gastric mucosa and the muscular layer were separated from each other, and the membrane ATPase was prepared from fundic gastric mucosa with differential centrifugation (20.000 × g and 40.000 × g) and treatment with 2.0 M NaI solution as per our method (Mózsik and Øye, 1969). The membrane ATPase activity was measured in an incubation system at 37 °C by the liberation of inorganic phosphorus (Mózsik, 1969b). The $Na^+$–$K^+$-dependent ATPase activity was calculated as the difference between the total (obtained in the presence of $Mg^{2+}$, $Na^+$ and $K^+$) and $Mg^{2+}$-dependent system (obtained in the presence of $Mg^{2+}$) (Figure 45).

The $Na^+$–$K^+$-dependent ATPase differs from the $H^+$–$K^+$-dependent ATPase, independently from the similarities of protein structures (see Sections 5.1, 5.2), and the mitochondrial ATP is a common substrate for both these enzymes. Our enzyme was prepared from the whole gastric fundic mucosa; $H^+$–$K^+$–ATPase is located only in the parietal cells, which are highly specialized epithelial cells in the inner cell lining of the stomach. $H^+$–$K^+$–ATPase can be separated from the $Na^+$–$K^+$-pump enzyme based on specific immunological studies (Saccomani et al., 1979b; Yao and Forte, 2004; Dunbar and Caplan, 2001; Sachs et al., 1995; https.//www.nlm.nil.gov/cgi/mesh/2011/MB_cgi?&term=Potassium+Hydrogen+ATPase).

There is no doubt that both the active transport of $Na^+$ and $K^+$ (Na pump) and the gastric acid secretion are energy-dependent processes (obtained from the ATP transformation into ADP); however, the sodium pump is a general function of all cells, while the $H^+$–$K^+$–ATPase is responsible only for gastric acid secretion.

We never participated in the study of $H^+$–$K^+$–ATPase; however, it is true that the presence of $Na^+$–$K^+$–ATPase was proved earlier in the rat and human gastrointestinal (gastric) mucosa (Mózsik and Øye, 1969).

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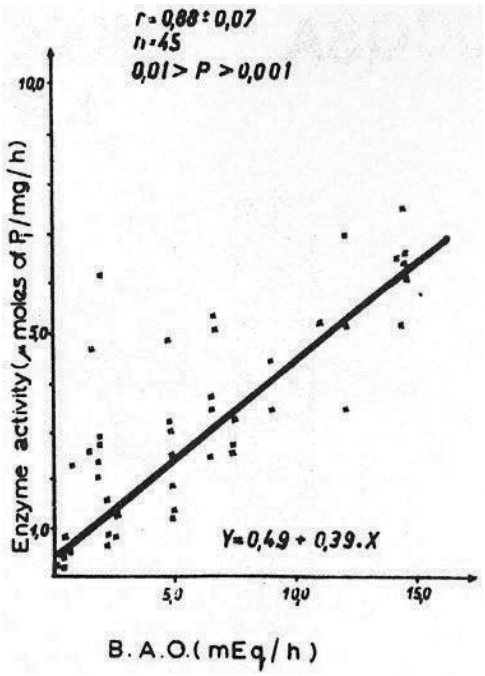


**Figure 45.** Correlation between the $Na^+$–$K^+$-dependent ATPase activity from human fundic mucosa (ordinate) and basal acid output (BAO) (abscissa) in 45 patients with peptic ulcer. The enzyme activities are expressed as $P_i$ (μMol/mg protein/hour). [Mózsik, Nagy, Tárnok, Vizi F, Kutas J: Experientia (Basel) 30: 1024–1025, 1974 (with kind permission).]

We used the $Na^+$–$K^+$-dependent ATPase system as a key enzyme participating in the regulation of cell functions, of course, in the gastric mucosal cells. The function of $Na^+$–$K^+$–ATPase represents only one side of the ATP splitting process (as energy source), while the actual level of tissue ATP indicates the other side of energy source system (namely, the ATP resynthesis). These processes occur together in all cells and tissues. Both $Na^+$ and $K^+$ are involved in both the classical sodium pump and the gastric acid secretion.

Our results presented here are related dominantly to the function of the classical sodium pump in the human gastric mucosa. It is interesting and important to note that the activity of $Na^+$–$K^+$-dependent ATPase is closely associated with the gastric acid secretion.

### 7.2. Interaction of cholinergic function with $Mg^{2+}$-$Na^+$-$K^+$-dependent ATPase system of cells in the human fundic gastric mucosa

This section deals with the effects of some cholinergic and anticholinergic drugs on the membrane-bound ATPase enzyme (Mózsik et al., 1974c).

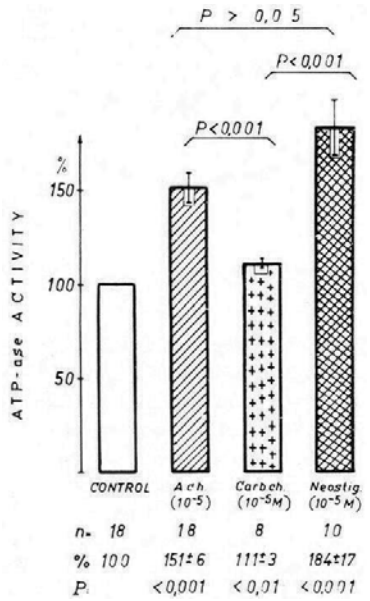


**Figure 46.** Effects of acetylcholine (Ach), carbamylcholine (Carb.ch.) and neostigmine (Neostig.) on the total ATPase activity in the human gastric fundic mucosa. The enzyme activity is expressed in percentage of total ATPase activity (=100%) without any drug [Mózsik, Nagy, Kutas, Tárnok: Scand. J. Gastroenterol. 9: 741–745, 1974 (with kind permission).]

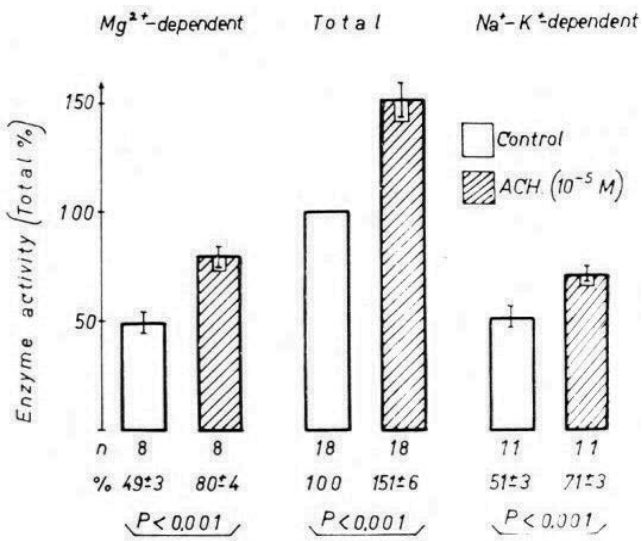


**Figure 47.** Effects of acetylcholine on $Mg^{2+}$-dependent, total and $Na^{+}$–$K^{+}$-dependent ATPase prepared from the human gastric fundic mucosa. The results are presented in percentage of total ATPase (=100%) activity without the administration of any drugs. [Mózsik, Nagy, Kutas, Tárnok: Scand. J. Gastroenterol. 9: 741–745, 1974 (with kind permission).]

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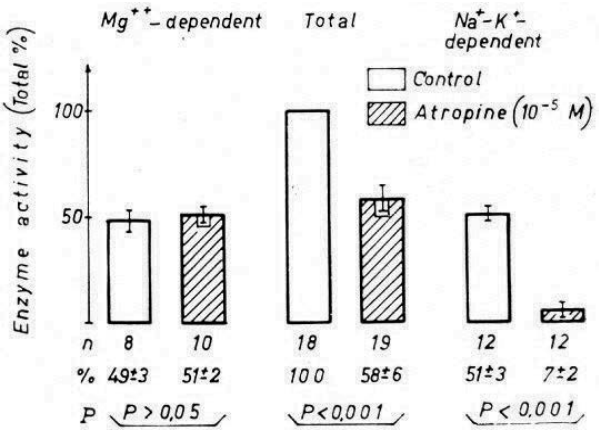


**Figure 48.** Effects of atropine on $Mg^{2+}$-dependent, total and $Na^{+}$–$K^{+}$-dependent ATPase prepared from the human gastric fundic mucosa. The results are presented in percentage of total ATPase (=100%) activity without the administration of any drugs. [Mózsik, Nagy, Kutas, Tárnok: Scand. J. Gastroenterol. 9: 741–745, 1974 (with kind permission).]

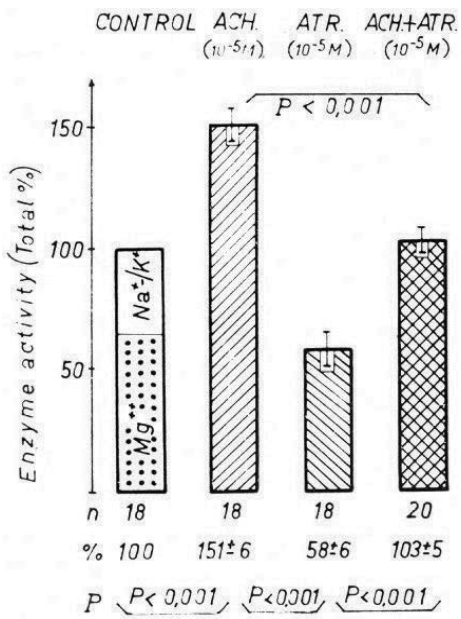


**Figure 49.** Comparative inhibitory effect of different parasympatholytics on the total membrane ATPase prepared from the human gastric fundic mucosa. The results are presented in percentage of total ATPase (=100%) activity without the administration of any drugs. [Mózsik, Nagy, Kutas, Tárnok: Scand. J. Gastroenterol. 9: 741–745, 1974 (with kind permission).]

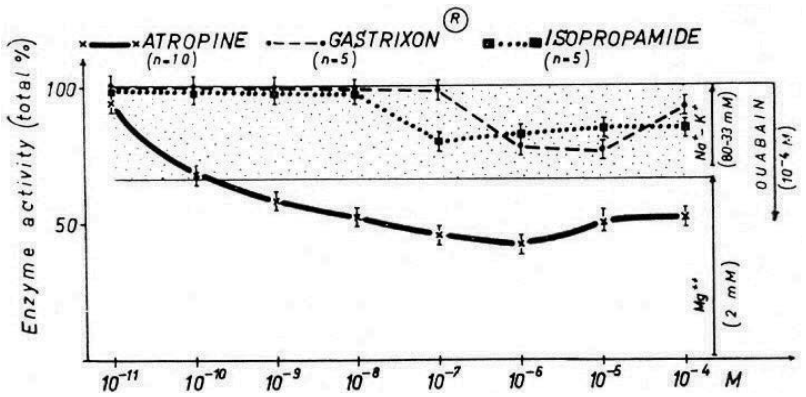


**Figure 50.** Comparative inhibitory effect of different parasympatholytics on the total membrane ATPase activity prepared from the human gastric fundic mucosa. The results are presented in per cent of total ATPase (= 100 per cent) activity without application of any drugs. (Mózsik, Nagy, Kutas, Tárnok: Scand. J. Gastroenterol. 9: 741-745, 1974) (with kind permission).

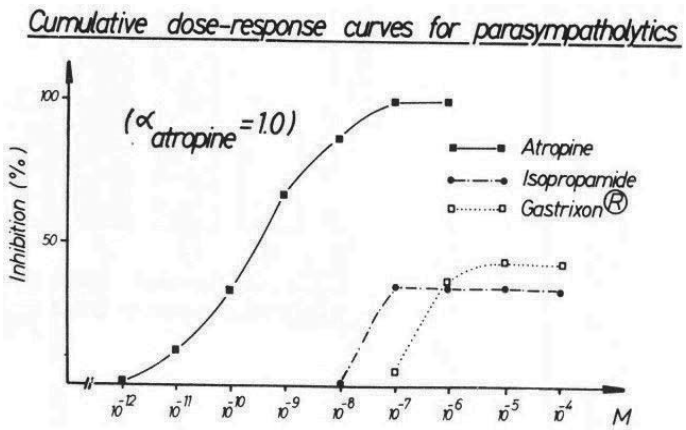


**Figure 51.** Cumulative dose–response curves for parasympatholytics inhibiting the $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic mucosa. The intrinsic activity ($\alpha$) of atropine was found to be equal to 1.0 on $Na^+$–$K^+$-dependent ATPase activity. Each point represents the average of 10 measurements (chemical structures: isopropamide, 2,2-diphenyl-4-diiso-propylamino-methyliodide; Gastrixon®, methyl-tropinium-bromide-xanthene-9-carboxylate). [Mózsik, Kutas, Nagy, Tárnok, Vizi: Acta Physiol. Sand. Special Suppl. 199–208, 1978 (with kind permission).]

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| Parasympatholytics | Affinity ($pD_2$) | Intrinsic activity ($\alpha$) |
|---|---|---|
| Atropine | 9.50 | 1.00 |
| Isopropamide | 7.50 | 0.36 |
| Gastrixon$^R$ | 6.40 | 0.43 |

**Table 31.** Values of affinity ($pD_2$) and intrinsic activities ($\alpha$) for parasympatholytics inhibiting the $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic mucosa. The intrinsic activity of atropine was found to be equal to 1.0.

The results presented above indicate clearly the following:

1. During the cholinergic activation, the enzyme system of sodium pump (the transformation of ATP into ADP by membrane ATPase) is in working state (acetylcholine, carbamylcholine, neostigmine);
2. The parasympatholytics inhibit the function of membrane ATPase prepared from the human gastric fundic mucosa;
3. The different parasympatholytics produced different extents on the inhibition of membrane ATPase activity.

### 7.3. Inhibitory effects of histamine, pentagastrin, $PGE_1$ and $PGE_2$ on $Na^+$-$K^+$-dependent ATPase prepared from human gastric mucosa

The gastric acid secretory responses are characterized by the so-called basal acid secretory responses (e.g., without the administration of any drug to stimulate the gastric acid secretion) (basal acid output, BAO) and maximal gastric acid secretory responses (maximal acid output, MAO). The MAO can be produced by the administration of histamine (0.04 mg/kg body weight, given subcutaneously) (Kay's test) or pentagastrin (6 μg/kg given subcutaneously).

The effect of histamine was studied on the typical membrane ATPase prepared from the gastric fundic mucosa of patients with gastric (8 patients) and duodenal (6 patients) ulcer, before resection of their stomach: BAO value = 3.86 ± 1.14 mEg/h and MAO = 16.30 ± 2.25 mEg/h.

Histamine was used in $10^{-7}$ M concentration to test its effect only on $Mg^{2+}$-dependent part and $Na^+$–$K^+$-dependent part (Figure 52).

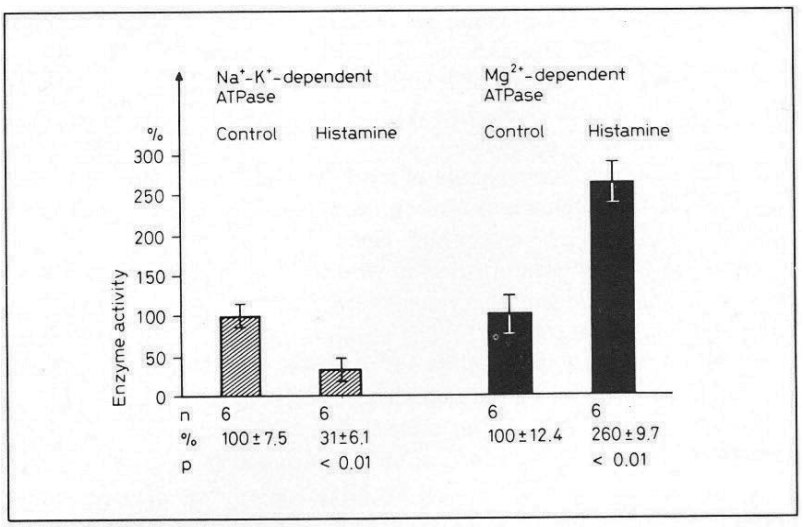


**Figure 52.** Histamine ($10^{-7}$ M) effects on only $Mg^{2+}$-dependent ATPase and on $Na^{+}$-$K^{+}$-dependent ATPase activity. The results (means±SEM) are presented as per cent of control values. (Mózsik, Nagy, Tárnok, Jávor, Kutas: Pharmacology 12: 193-200, 1974) (with kind permission).

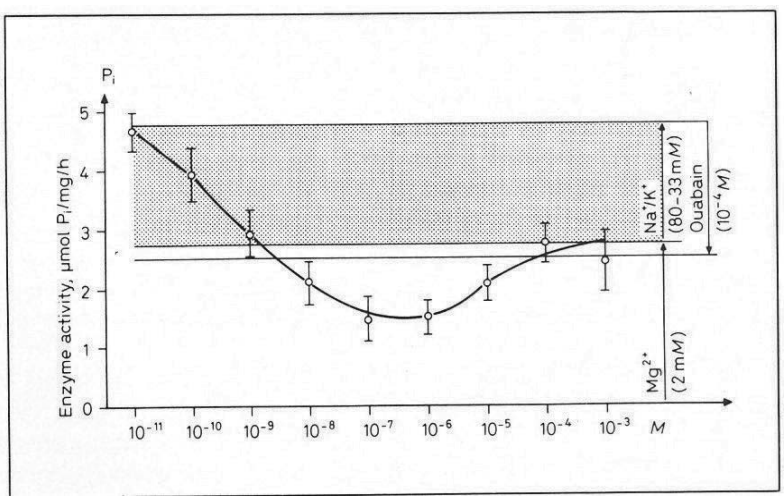


**Figure 53.** Inhibition of total ATPase from the human gastric fundic mucosa by histamine. The results of 14 patients are expressed in means ± SEM. The left ordinate shows the enzyme activity (µmol $P_i$ /mg membrane protein/h, while the abscissa indicates the histamine concentration (M). The right side of the figure demonstrates the "chemical marker behavior" of this membrane fraction taken from 14 patients' gastric fundic mucosa. [Mózsik, Nagy, Tárnok, Jávor, Kutas: Pharmacology 12: 193–200, 1974 (with kind permission).]

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The inhibition of total membrane ATPase activity was caused by histamine in a concentration of $10^{-7}$ to $10^{-3}$ M.

Similar observations were noted in pentagastrin, and the same type of inhibition of membrane ATPase was obtained as that of histamine.

The effects of prostaglandin $E_1$ and $E_2$ were studied on the $Mg^{2+}$- dependent and $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic mucosa (Figures 54, 55), and significant inhibitory actions were produced by $PGE_1$ and $PGE_2$ in concentrations of $10^{-10}$ to $10^{-6}$ M.

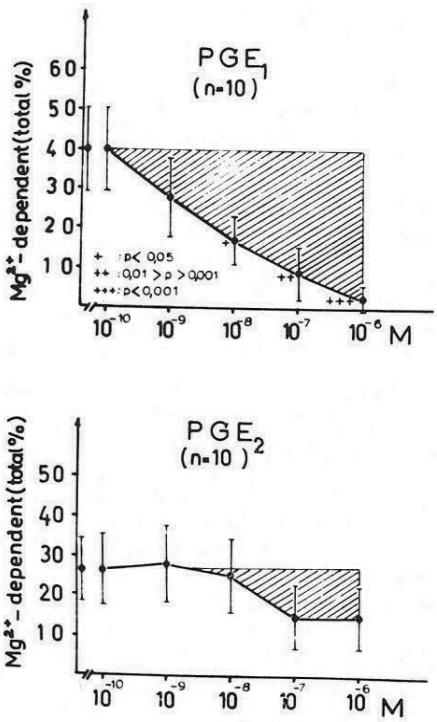


**Figure 54.** Inhibitory effects of prostaglandin $E_1$ (top) and $E_2$ (bottom) on $Mg^{2+}$-dependent ATPase prepared from the human gastric fundic mucosa. The results are expressed as percentage value of total ATPase activity (=100%). The abscissa indicates the concentrations of prostaglandins (M). Each point represents the means ± SEM of 10 observations. [Mózsik, Kutas, Nagy, Németh: Eur. J. Pharmacol. 29: 133–137, 1974 (with kind permission).]

### 7.4. Correlations between the magnitudes of drug actions versus magnitudes of $Na^+$-$K^+$-dependent ATPase prepared from the human gastric fundic mucosa and from the small intestinal mucosa

It was clear to conclude that the magnitudes of drugs actions depend on the membrane enzyme activity. Because the membrane ATPase activity significantly changes on the activity of target organ, the drug actions depend on the activity of the target organ (Nagy et al., 1976, 1981 a, b).

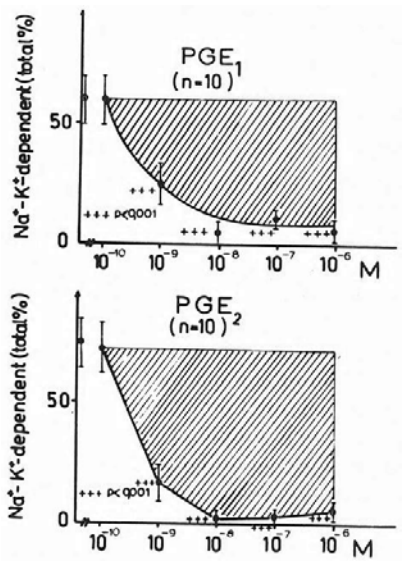


**Figure 55.** Inhibitory effects of prostaglandin $E_1$ (top) and $E_2$ (bottom) on $Na^+$–$K^+$-dependent ATPase prepared from the human gastric fundic mucosa. The results are expressed as percentage value of total ATPase activity (=100%). The abscissa indicates the concentrations of prostaglandins (M). Each point represents the means ± SEM of 10 observations. [Mózsik, Kutas, Nagy, Németh: Eur. J. Pharmacol. 29: 133–137, 1974 (with kind permission).]

| Drug: | Drug effect: | Tested dose: | r: | Regression line: | p value: | n: |
|---|---|---|---|---|---|---|
| AcH | stimulation | $10^{-4}$ M | 0.98 | Y=0.5X−0.8 | <0.001 | 7 |
| Atropine | inhibition | $10^{-7}$ M | 0.97 | Y=1.35X+0.49 | <0.001 | 19 |
| cAMP | inhibition | $10^{-9}$ M | 0.78 | Y=1.34X−1.63 | <0.05 | 8 |
| Epinephrine | inhibition | $10^{-7}$ M | 0.68 | Y=1.10X+0.12 | <0.05 | 10 |
| Histamine | inhibition | $10^{-7}$ M | 0.87 | Y=0.96X+0.88 | <0.01 | 9 |
| Ouabain | inhibition | $10^{-7}$ M | 0.93 | Y=1.03X+0.20 | <0.001 | 45 |
| | inhibition | $10^{-4}$ M | 0.93 | Y=1.20X+1.03 | <0.001 | 45 |
| Pentagastrin | inhibition | $10^{-7}$ M | 0.94 | Y=0.85X+0.11 | <0.02 | 10 |
| Affinity ($pD_2$) values: | | Ach=5.50 ; Atr.=9.50; cAMP=11.30 ; Epinephr.=8.60 ; Hist.=9.70 ; Pentagast.=10.55 ; Ouabain=7.50 (9.20–6.50) | | | | |

**Table 32.** Correlations between the magnitudes of drug actions and the magnitudes of $Na^+$–$K^+$–ATPase prepared from the human gastric fundic mucosa.

## 7.5. Correlations between the magnitudes $Na^+$-$K^+$-dependent ATPase, tissue levels of ATP, ADP in the human gastric fundic mucosa versus values of gastric basal acid ouput (BAO) in humans

We prepared the membrane ATPase from the human gastric fundic mucosa and, simultaneously, directly measured the tissue level of ATP, ADP, lipid phosphates, ribonucleic acid and deoxyribonucleic acid. The tissue levels of ATP, ADP, lipid phosphates and ribonucleic acid

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were expressed in accordance to 1.0 mg deoxyribonucleic acid (DNA). The membrane ATPase activity was assayed (Mózsik and Øye, 1969) by *in vitro* method (Figures 56, 58). The different correlations were expressed in consequence of mathematical analyses: BAO versus $Na^+$–$K^+$-dependent ATPase ($r = 0.88$, $n = 45$; $P<0.001$); BAO versus tissue level of ATP ($r = 0.53$, $n = 28$, $P<0.01$); ATP versus $Na^+$–$K^+$–ATPase ($r = 0.55$, $n = 32$, $P<0.001$ ); ATP versus ADP ($r = 0.99$, $n = 32$, $P <0.001$); BAO versus ADP ($r = 0.59$, $n = 28$, $P<0.001$) (Mózsik et al., 1979 a, b, 1981d, 1978b) (Figure 56).

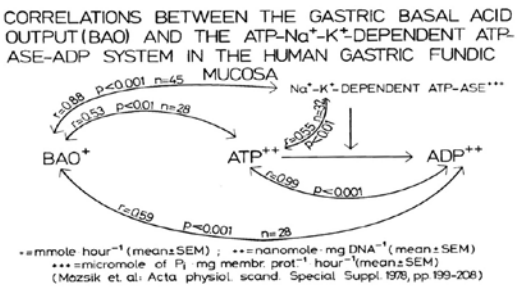


**Figure 56.** Biochemical regulatory pathways between $Na^+$–$K^+$-dependent ATPase, tissue levels of ATP, ADP in the human gastric fundic mucosa dependent on the gastric BAO values (means ± SEM). [Mózsik, Tárnok, Kutas (1981) in Gáti, Szollár, Ungváry (eds.) Advances in Physiological Sciences Vol. 12. Nutrition, Digestion, Metabolism. pp. 117–128. 1981 (with kind permission).]

## 7.6. Correlation between gastric basal acid output (BAO) versus gastric maximal acid ouput (MAO) in humans

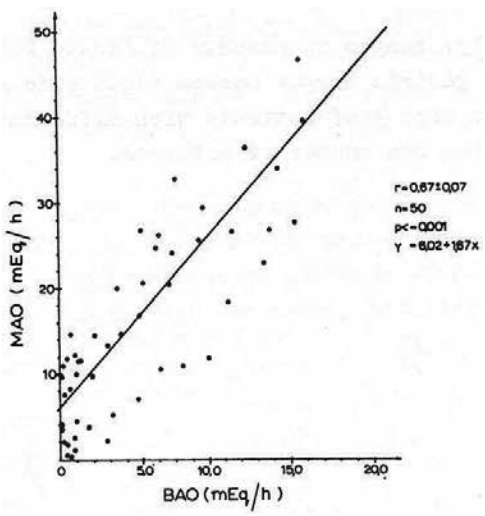


**Figure 57.** Correlation between the gastric basal acid output (BAO) versus gastric maximal acid output (MAO) in patients examined biochemically. [Mózsik, Vizi, Nagy, Bero, Tárnok, Kutas (1976): $Na^+$–$K^+$-dependent ATPase system and the $H^+$ secretion by the human gastric mucosa. In: Mozsik Gy., Javor T. (eds). Progress in Peptic Ulcer. Budapest, Akadémiai Kiadó, pp. 37–72 (with kind permission).]

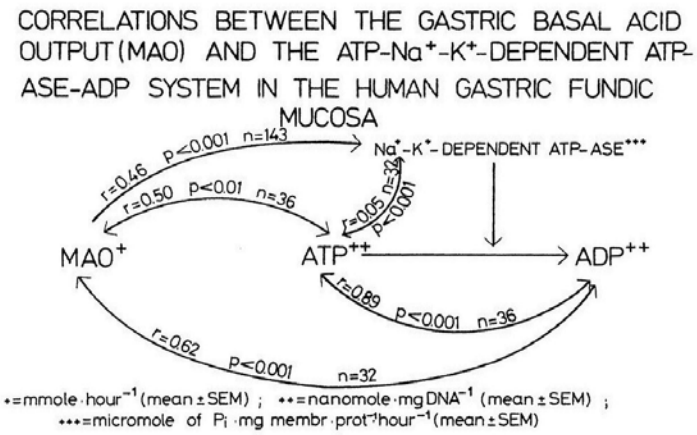


**Figure 58.** Biochemical regulatory pathways between $Na^+$–$K^+$-dependent ATPase, tissue levels of ATP, ADP in the human gastric fundic mucosa dependent on the gastric maximal acid output (MAO) values. For further explanation, see Figure 56 (with kind permission).

### 7.7. Correlations between $Na^+$-$K^+$-dependent ATPase activity, tissue level of ATP, ADP in the human gastric fundic mucosa versus gastric maximal acid output (MAO) in humans

Figure 58 indicates the results of different correlation calculations versus MAO values.

We found positive and significant correlations between the following parameters:

**a.** MAO versus $Na^+$–$K^+$-dependent ATPase;

**b.** MAO versus tissue level of ATP;

**c.** $Na^+$–$K^+$-dependent ATPase versus ATP;

**d.** ATP versus ADP;

**e.** MAO versus ADP. (Andrási, 1997; Bódis et al., 1977 a, b; Levine, 1971; Mózsik et al., 1978b).

### 7.8. Affinity and intrinsic activity curves of acetylcholine, histamine and pentagastrin on the $Na^+$-$K^+$-dependent ATPase prepared and on the adenylate cyclase prepared from human gastric mucosa

The contradictory effects of drugs on $Na^+$–$K^+$-dependent ATPase and adenylate cyclase were demonstrated earlier (Mózsik, 1969 a, b, 1970, 1974 a, b, 1979 b, c, e).

Table 33 indicates affinity ($pD_2$) and intrinsic activity ($pA_2$) curves for the actions of acetylcholine, histamine and pentagastrin. The table also indicates the contradictory actions of these agents on $Na^+$–$K^+$-dependent and adenyl cyclase systems.

We hypothesized a feedback system between the membrane ATPase and adenylate cyclase in the development of gastric BAO and MAO (Figure 59).

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| Mediators | Actions | Affinity values ($pD_2$) | Intrinsic activities ($\alpha$) | ($pA_2$) |
|---|---|---|---|---|
| **ATP – membrane ATP-ase – ADP** | | | | |
| Ach | Stimulation | 5.50 | $1.00_{Ach}$ | 5.50 |
| Histamine | Inhibibion | 9.70 | $1.00_{Ouabain}$ | 9.70 |
| Pentagastrin | Inhibition | 10.55 | $0.87_{Ouabain}$ | 10.55 |
| **ATP – adenylate cyclase – cAMP** | | | | |
| Ach | Inhibition | 5.30 | $-0.70_{Pentagastrin}$ | 5.30 |
| Histamine | Stimulation | 9.30 | $1.00_{Pentagastrin}$ | 9.30 |
| Pentagastrin | Stimulation | 9.40 | 1.00 | 9.40 |

**Table 33.** Actions of acetylcholine, histamine and pentagastrin in human beings.

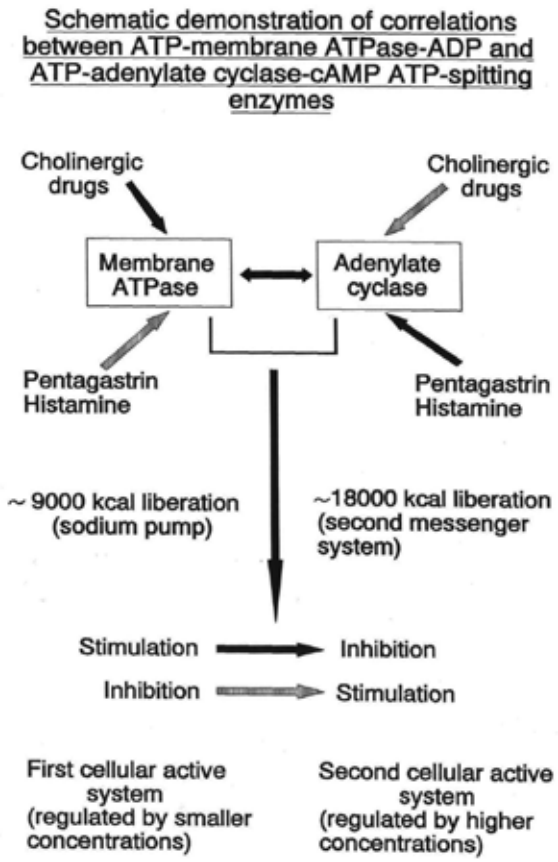


**Figure 59.** Our suggestion to demonstrate the correlations between the ATP-dependent membrane-bound energy systems and the gastric BAO and MAO values regulated by acetylcholine, pentagastrin and histamine. [Mózsik, Debreceni, Juricskay, Karádi, Nagy (1997) In: Gaginella T.S., MózsikGy., Rainsford K.D. (eds) Biochemical Pharmacology as an Approach to Gastrointestinal Disorders, Kluwer Academic Publishers, Dordrecht, Boston, London. pp. 199–222 (with kind permission).]

## 7.9. Energetical and biochemical gradients between the gastric fundic, antral and duodenal mucosa in dependence of gastric BAO values in peptic ulcer patients

### *7.9.1. Energetical and biochemical gradients in the gastric fundic mucosa and musculature in peptic ulcer patients in dependence of gastric basal secretory responses*

The biochemical examinations were carried out in resecates of stomach (fundus, antrum) and small intestine (duodenum, jejunum) in patients with chronic peptic ulcer, who underwent surgical intervention.

The gastric BAO and MAO (subcutaneously given 6 μg/kg body weight of pentagastrin) were measured before the surgical intervention.

The examined patients were divided into three groups, according to the BAO values (Table 34). These patients underwent resection of stomach (and some part from duodenum; in patients with jejunal ulcer, who underwent surgery previously, Billroth II-type gastric resection was carried out, and jejunal ulcer appeared later). Immediately after resection, different tissue samples were separated from the obtained tissues, and they were put into liquid nitrogen.

| Groups of patients | Basal acid outputs (BAO) | Maximal acid outputs (MAO) |
|---|---|---|
| BAO < 2.0 mEq/ h | 0.27 ± 0.0.1<br>(n = 12) | 7.95 ± 1.38<br>(n = 12 ) |
| BAO 2.00 to 4.0 mEq/h | 2.81 ± 0.10<br>(n = 9 ) | 15.69 ± 0.76<br>(n=9) |
| BAO > 4.0 mEq/h | 5.33 ± 0.24<br>(n = 11) | 18.76 ± 2.15<br>(n=11 ) |

**Table 34.** Gastric secretory responses in patients in whom the biochemical examinations were carried out. The gastric acid secretory responses are presented in mEq/h (means ± SEM), *n* indicates the number of patients. [Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976 (with kind permission).]

The following biochemical examinations were carried out from different tissue samples:

1. Determination of membrane ($Mg^{2+}$–$Na^{+}$–$K^{+}$-dependent) ATPase;
2. Separation and determination of adenine–adenosine, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP);
3. Separation and determination of lipid phosphates;
4. Separation and determination of nucleic acids.

The details of these methodological problems are presented in a paper (Mózsik et al., 1976b).

It is important to note and emphasize the following:

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1. The number of biochemically evaluated tissue samples in different figures is different (e.g., in case of gastric fundic mucosa vs. musculature), which is dependent on the *in situ* situation at the time of surgical intervention;

2. We had no possibility for direct measurement of cyclic AMP from these tissue samples in these series of observations;

3. All biochemical examinations (including the preparative works) were carried out in all tissue samples obtained in one patient.

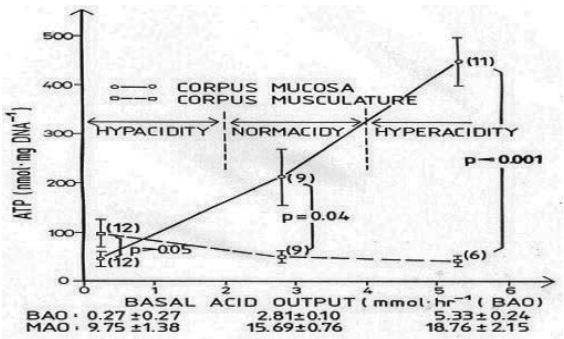


**Figure 60.** Tissue levels of adenosine triphosphate (ATP) in the gastric fundic mucosa and musculature prepared from patients with peptic ulcer depending on the gastric acid secretory responses. The results are presented in nmol/mg DNA (means ± SEM). The results are based on the results published in a paper (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976) (with some modification).

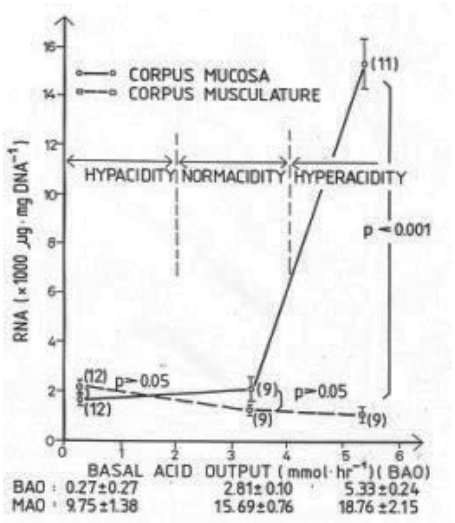


**Figure 61.** Tissue levels of ribonucleic acid (RNA) in the gastric fundic mucosa and musculature prepared from patients with peptic ulcer depending on the gastric acid secretory responses. The results are presented in mg RNA/mg DNA (means ± SEM).The results are based on the results published in a paper (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976) (with some modification). The numbers in parenthesis indicate the number of patients.

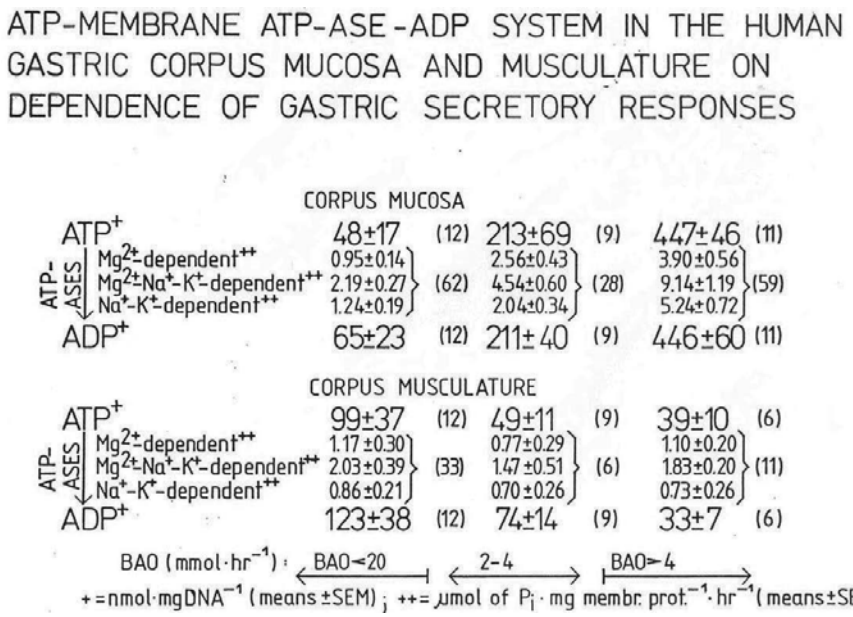


**Figure 62.** ATP–membrane ATPase–ADP system in the human gastric fundic mucosa and musculature prepared in the stomach resecates obtained from patients with peptic ulcer. The results are based on the results published in a paper (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976 and Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977) (with some modification). The numbers in parenthesis indicate the number of patients.

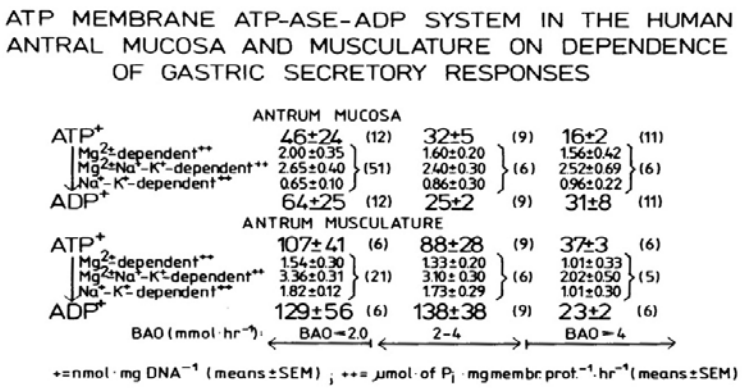


**Figure 63.** Correlations between the membrane ATPase activity, tissue levels of ATP and ADP in the human gastric fundic mucosa and musculature in patients with different gastric (hypacid, normacid and hyperacid) secretory responses depending on the gastric basal acid output (BAO) (means ± SEM). The results are based on the results published in a paper (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976 and Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977) (with some modification).

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MAIN CHANGES IN METABOLISM OF GASTRIC MUCOSA (A) AND MUSCULATURE (B) IN PATIENTS WITH DIFFERENT GASTRIC SECRETORY RESPONSES (BAO)
(means±SEM)

| BAO | ⩽2 (n=12) | | 2-4 (n=9) | | ⩾4 (n=11) | |
|---|---|---|---|---|---|---|
| (mmol·hr$^{-1}$) | A | B | A | B | A | B |
| | | | CORPUS | | | |
| [a] ATP·ADP$^{-1}$ | 0.74±0.05 | 0.80±0.06 | 1.01±0.07 | 0.66±0.05 | 1.00±0.02 | 1.18±0.07 |
| [b] Lipid-P·DNA$^{-1}$ | 0.36±0.04 | 0.43±0.04 | 4.04±0.10 | 0.33±0.04 | 13.58±0.70 | 1.40±0.30 |
| [b] RNA·DNA$^{-1}$ | 1.74±0.08 | 2.03±0.15 | 2.00±0.24 | 1.24±0.06 | 15.28±1.00 | 7.14±0.84 |
| [a] „energy charge" | 0.43±0.04 | 0.46±0.03 | 0.33±0.04 | 0.42±0.03 | 0.03±0.01 | 0.14±0.02 |
| | | | ANTRUM | | | |
| [a] ATP·ADP$^{-1}$ | 0.72±0.06 | 0.83±0.06 | 1.29±0.08 | 0.64±0.05 | 0.52±0.04 | 1.60±0.10 |
| [b] Lipid-P·DNA$^{-1}$ | 0.16±0.05 | 0.85±0.08 | 0.26±0.06 | 0.31±0.04 | 0.43±0.03 | 0.34±0.04 |
| [b] RNA·DNA$^{-1}$ | 1.39±0.10 | 1.75±0.11 | 1.05±0.05 | 2.96±0.02 | 2.03±0.15 | 1.24±0.06 |
| [a] „energy charge" | 0.45±0.04 | 0.51±0.04 | 0.39±0.03 | 0.46±0.07 | 0.07±0.01 | 0.27±0.03 |

a=values are in nmoles·mg DNA$^{-1}$ ; b=values are in mg·mg DNA$^{-1}$
„energy charge"=(ATP+0.5 ADP)·(ATP+ADP+AMP)$^{-1}$

**Figure 64.** The changes in the biochemistry of gastric fundic mucosa and musculature in patients with different gastric BAO values. The results are expressed as means ± SEM/1.0 mg deoxyribonucleic acid (DNA) (means ± SEM). The results are based on the results published in a paper (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 11: 205–211, 1976 and Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977) (with some modification). The numbers in parenthesis indicate the number of patients.

### *7.9.2. Energetical and biochemical gradients in the gastric antral and duodenal mucosa and musculature in patients with peptic ulcer in dependence of gastric basal acid secretory responses*

There was an energy gradient in the corpus, antrum and duodenum mucosa depending on the gastric basal and maximal acid secretory activities (Mózsik et al., 1976 a, b, c, 1979 b, 1981a) (Figures 65–66; Tables 35–37).

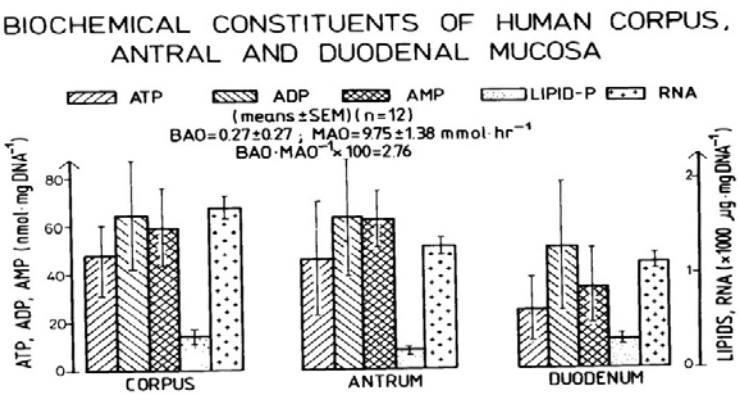


**Figure 65.** Biochemical parameters in the corpus, antrum and duodenum in patients with gastric hypacidity (BAO, MAO) (means ± SEM) (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1976) (with some modification).

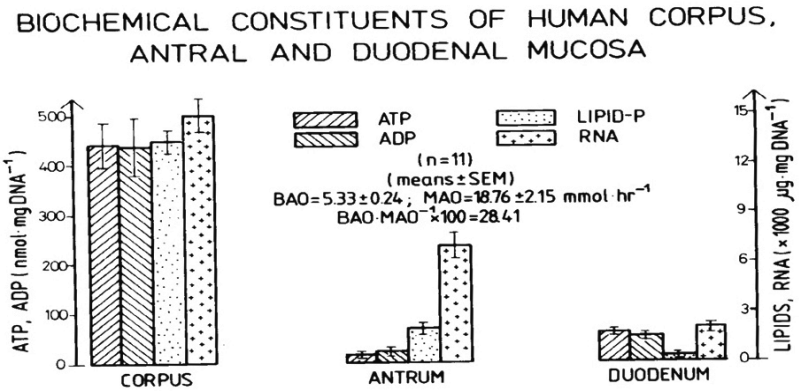


**Figure 66.** Biochemical gradients in the gastric fundic, antral and duodenal mucosa in patients with hyperacidity (means ± SEM). (Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1976) (with some modification).

| | Corpus (12)[b] | Antrum (12)[b] | Duodenum (12)[b] | p¹ [c] | p² [c] | p³ [c] |
|---|---|---|---|---|---|---|
| ATP[d] | 48±17 | 46±24 | 24±14 | NS[e] | NS | NS |
| ADP[d] | 65±23 | 64±25 | 51±28 | NS | NS | NS |
| ATP/ADP | 0.74±0.05 | 0.72±0.06 | 0.47±0.06 | NS | <0.01 | <0.01 |
| AMP[d] | 60±17 | 63±12 | 34±16 | NS | NS | NS |
| ATP+ADP+AMP[d] | 173±25 | 173±18 | 109±12 | NS | <0.01 | <0.02 |
| Energy charge[f] | 0.43±0.04 | 0.45±0.04 | 0.45±0.08 | NS | NS | NS |
| Adenine-adenosine[d] | 718±179 | 549±229 | 435±207 | NS | NS | NS |
| Lipid-P[g] | 358±46 | 163±50 | 306±43 | <0.02 | NS | NS |
| RNA[g] | 1741±81 | 1397±107 | 1133±41 | =0.02 | =0.04 | <0.001 |

[a] ATP,adenosine triphosphate; ADP,adenosine diphosphate; AMP,adenosine monophosphate; lipid-P, lipid phosphates; RNA,ribonucleic acid.
[b] Numbers in parentheses indicate numbers of patients.
[c] p¹,corpus versus antrum; p²,antrum versus duodenum; p³,corpus versus duodenum.
[d] Values=nanomoles of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.).
[e] Not significant.
[f] Adenylate pool energy charge (ATP+0.5 ADP/ATP+ADP+AMP).
[g] Values=micrograms of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.).

**Table 35.** Substrate levels in the gastric mucosa of the corpus, antrum and duodenum of patients with BAO values <2.0 mEq/h (BAO = 0.27 ± 0.27; MAO = 9.75 ± 1.38)[a]. [Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977 (with kind permission).]

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| | Corpus (9)[b] | Antrum (9)[b] | Duodenum (9)[b] | p[1] [c] | p[2] [c] | p[3] [c] |
|---|---|---|---|---|---|---|
| ATP[d] | 213±69 | 32±5 | 25±3 | =0.02 | NS[e] | =0.02 |
| ADP[d] | 211±40 | 25±2 | 29±6 | <0.001 | NS | <0.001 |
| ATP/ADP | 1.01±0.07 | 1.29±0.08 | 0.86±0.07 | NS | <0.001 | NS |
| AMP[d] | 545±75 | 57±15 | 60±7 | <0.001 | NS | <0.001 |
| ATP+ADP+AMP[d] | 969±100 | 114±18 | 114±11 | <0.001 | NS | <0.001 |
| Energy charge[f] | 0.33±0.04 | 0.39±0.03 | 0.43±0.11 | NS | NS | NS |
| Adenine-adenosine[d] | 4327±1908 | 353±39 | 326±48 | <0.001 | NS | <0.001 |
| Lipid-P[g] | 4047±108 | 269±60 | 261±50 | <0.001 | NS | <0.001 |
| RNA[g] | 2008±543 | 1050±53 | 933±32 | NS | NS | =0.06 |

[a] ATP,adenosine triphosphate; ADP,adenosine diphosphate; AMP,adenosine monophosphate; lipid-P, lipid phosphates; RNA, ribonucleic acid.
[b] Numbers in parentheses indicate numbers of patients.
[c] p[1],corpus versus antrum; p[2],antrum versus duodenum; p[3],corpus versus duodenum.
[d] Values=nanomoles of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.).
[e] Not significant.
[f] Adenylate pool energy charge (ATP+0.5 ADP/ATP+ADP+AMP).
[g] Values=micrograms of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.).

**Table 36.** Substrate levels in the gastric mucosa of the corpus, antrum and duodenum of patients with 2.0<BAO<4.0 mEq/h (BAO = 2.81 ± 0.10; MAO = 15.69 ± 0.76)[a]. [Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977 (with kind permission).]

| | Corpus (11)[b] | Antrum (11)[b] | Duodenum (11)[b] | p[1] [c] | p[2] [c] | p[3] [c] |
|---|---|---|---|---|---|---|
| ATP[d] | 447±46 | 16±4 | 60±2 | <0.001 | <0.001 | <0.001 |
| ADP[d] | 446±60 | 31±8 | 63±6 | <0.001 | <0.001 | <0.001 |
| ATP/ADP | 1.00±0.02 | 0.52±0.04 | 0.95±0.10 | <0.001 | <0.001 | NS[e] |
| AMP[d] | 19606±720 | 381±70 | 38±6 | <0.001 | <0.001 | <0.001 |
| ATP+ADP+AMP[d] | 20449±200 | 428±38 | 224±21 | <0.001 | <0.001 | <0.001 |
| Energy charge[f] | 0.03±0.01 | 0.07±0.01 | 0.40±0.07 | NS | <0.001 | <0.001 |
| Adenine-adenosine[d] | 13394±569 | 3212±1311 | 370±27 | <0.001 | =0.05 | <0.001 |
| Lipid-P[g] | 13588±708 | 1406±300 | 91±18 | <0.001 | <0.001 | <0.001 |
| RNA[g] | 15289±1000 | 7141±847 | 1961±21 | <0.001 | <0.001 | <0.001 |

[a] ATP,adenosine triphosphate; ADP,adenosine diphosphate; AMP,adenosine monophosphate; lipid-P, lipid phosphates; RNA,ribonucleic acid.
[b] Numbers in parentheses indicate numbers of patients.
[c] p[1],corpus versus antrum; p[2],antrum versus duodenum; p[3],corpus versus duodenum.
[d] Values=nanomoles of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.).
[e] Not significant.
[f] Adenylate pool energy charge (ATP+0.5 ADP/ATP+ADP+AMP)).
[g] Values=micrograms of substrates per 1.0 mg deoxyribonucleic acid (means±S.E.M.)

**Table 37.** Substrate levels in the gastric mucosa of the corpus, antrum and duodenum of patients with BAO values >4.0 mEq/h (BAO = 5.33 ± 0.24; MAO = 18.76 ± 2.15)[a]. [(Mózsik, Vizi, Kutas: Scand. J. Gastroenterol. 12: 461–464, 1977 (with kind permission).]

## 7.10. Comparative biochemistry of ulcerated and non-ulcerated mucosal tissues in peptic (antral, duodenal, jejunal) ulcer patients

The membrane ATPase activity and tissue levels of ATP and ADP were measured around the gastric antral, duodenal and jejunal ulcer patients (after partial gastrectomy).

The ATPase activity and tissue levels of ATP and ADP were significantly higher around the gastric antral, duodenal and jejunal mucosa than those obtained in the non-ulcerated (control) mucosa (Mózsik et al., 1976 d, f, 1981 a, c, 1987 a, b, 2000) (Figures 65, 66; Tables 35–37).

*7.10.1. Mucosal biochemistry of chronic ulcerated and non-ulcerated antral mucosa in patients with chronic antral ulcer*

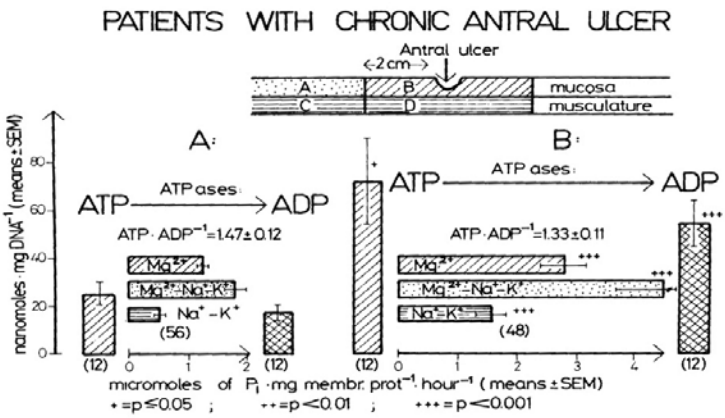


**Figure 67.** Changes in the extents of ATP–ADP transformation in the antral mucosa of patients with chronic antral ulcer in the ulcerated and non-ulcerated (control) mucosa (means ± SEM). [Mózsik, Kutas, Nagy, Tárnok, 1979; Mózsik, Kutas, Nagy, Tárnok, Acta Medica Acad. Sci. Hung. 36: 1–29, 1979 and Acta Medica Acad. Sci. Hung. 38: 129–134, 1984 (with kind permission).]

*7.10.2. Mucosal biochemistry of ulcerated and non-ulcerated duodenal mucosa in patients with chronic duodenal ulcer*

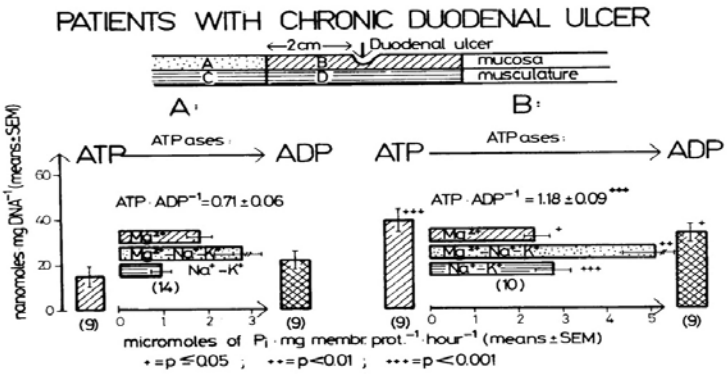


**Figure 68.** Changes in the extent of ATP–ADP transformation in the duodenal mucosa of patients with chronic duodenal ulcer, in the ulcerated and non-ulcerated duodenal mucosa (means ± SEM). [Mózsik, Kutas, Nagy, Tárnok: Acta Medica Acad. Sci. Hung. 37: 39–49, 1979 (with kind permission).]

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*7.10.3. Mucosal biochemistry of ulcerated and non-ulcerated jejunal mucosa in patients with chronic jejunal ulcer*

It was clear to conclude that the magnitudes of drugs actions depend on the membrane enzyme activity. Because the membrane ATPase activity significantly changes on the activity of target organ, the drug actions depend on the activity of the target organ (Nagy et al.,1976, 1981 a, b).

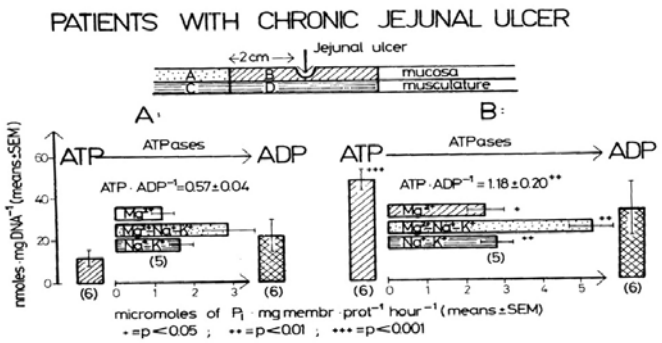


**Figure 69.** Changes in the extent of ATP–ADP transformation in the jejunal mucosa of patients who underwent partial gastrectomy (according to the method of Billroth II) around the jejunal ulcerated mucosa and non-ulcerated jejunal mucosa (means ± SEM). [Mózsik, Nagy, Tárnok, Kutas: Acta Medica Acad. Sci. Hung. 38, 129–134, 1981 (with kind permission).]

*7.10.4. Exclusion of tissue hypoxia in the tissues around the chronic gastric, duodenal and jejunal mucosa in patients*

Figures 70 and 71 clearly indicate that the tissue levels of mucosal levels of ATP and ADP are significantly higher in the ulcerated antral, duodenal and jejunal mucosa than those obtained in the control (non-ulcerated) mucosa specimen (the measurements were done simultaneously in patients).

As we indicated earlier, the membrane ATPase activity was also significantly higher in these ulcerated mucosa specimens than that in the control (non-ulcerated) mucosa. Against these biochemical changes, the values of "energy charge" remained the same (Figure 72).

These results demonstrated earlier in Figures 70–72 clearly indicate the following:

**1.** The extent of ATP–ADP breakdown is significantly higher in the ulcerated antral, duodenal and jejunal mucosa specimens than that in the control (non-ulcerated) mucosa specimens. This fact can be proven by the increased membrane ATPase activity and by increased level of ADP;

**2.** No impaired phosphorylation can be found in the ulcerated mucosa specimens, which can be proven by increased tissue levels of ATP in time when the ATP–ADP breakdown was significantly increased (significantly higher membrane ATPase activity and increased level of ADP);

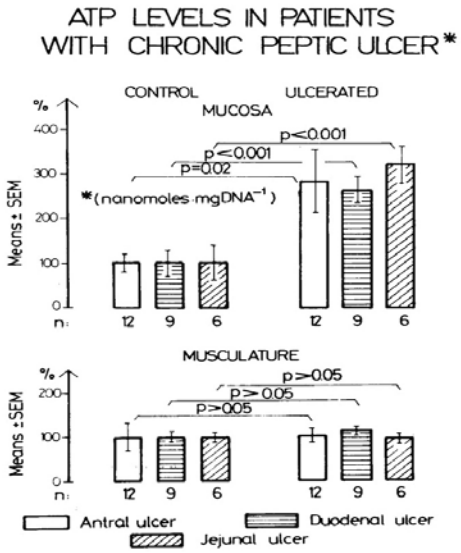


**Figure 70.** Comparative demonstration in the changes of the tissue levels of ATP in the ulcerated versus non-ulcerated antral, duodenal and jejunal mucosa (the musculature is located under the examined mucosa tissues) (means ± SEM). [Mózsik el al., in Mózsik, Hänninen, Jávor (eds.) Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism, Pergamon Press, Oxford-Akadémiai Kiadó, Budapest. pp. 213–288, 1981 (with kind permission).]

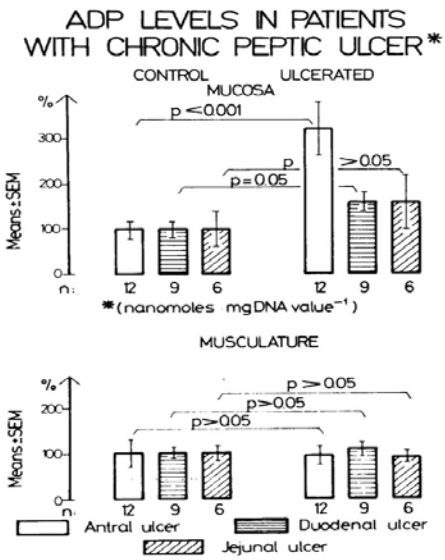


**Figure 71.** Comparative demonstration in the changes of tissue levels of ADP in the ulcerated and non-ulcerated antral, duodenal and jejunal mucosa (the musculature is located under the examined mucosa tissues). (Mózsik et al., In: Mózsik, Hänninen, Jávor (eds.) Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism, Pergamon Press, Oxford- Akadémiai Kiadó, Budapest. pp. 213–288, 1981). For further explanation, see Figure 70.

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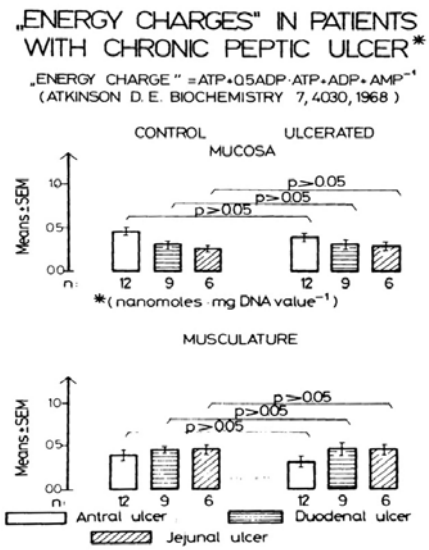


**Figure 72.** Critical evaluation of the "energy charge" in the mucosa around the chronic antral, duodenal and jejunal mucosa versus Non-ulcerated mucosa (the musculature located under the studies mucosa tissues). [Mózsik el al., in Mózsik, Hänninen, Jávor (eds.) Advances in Physiological Sciences. Vol.29. Gastrointestinal Defence Mechanism, Pergamon Press, Oxford- Akadémiai Kiadó, Budapest. pp. 213–288, 1981 (with kind permission).] For further explanation, see Figure 70.

3. The extent of ATP–cAMP transformation was significantly higher in the ulcerated antral, duodenal and jejunal mucosa around the chronic ulcer;

4. The tissue levels of ATP were significantly higher in the mucosa – around the chronic antral, duodenal and jejunal ulcer – that those in the control (non-ulcerated) mucosa, while the extents of both ATP–ADP and ATP–cAMP transformations were increased in the ulcerated antral mucosa specimens;

5. The higher ATP tissue levels (in time when the ATP breakdown was increased in both directions) can be obtained by the intact oxidative phosphorylation pathway;

6. The biochemical components of gastric mucosal tissue were expressed in accordance to 1.0 mg DNA, which represents the same number of cells (Figure 297). The values of adenine–adenosine, ATP, ADP and AMP were increased in the gastric fundic mucosa in patients with increased gastric secretory responses (BAO, MAO) and in the mucosa around chronic antral, duodenal and jejunal ulcers.

No physiological data are available in the literature to prove the presence of decreased GMBF in the gastric fundic mucosa in patients with gastric hyperacidity, and nobody found an increased tissue level of lactate. All experts accept the increased energy turnover (increased extents of ATP–ADP and ATP–cAMP transformation) in these gastric fundic mucosa specimens.

The results of animal experiments clearly indicated that the biochemical components differ significantly in the glandular stomach in comparison with the values in the forestomach. When we analyzed the time -sequence of biochemical changes, development of gastric hyperacidity and ulcer development, we first obtained the gastric hyperacidity and then the ulcer development. The same tendency was obtained in the changes of gastric mucosal biochemistry in both parts of the stomach, and these changes appeared before the development of gastric hyperacidity in 24-hour pylorus-ligated rats (Mózsik and Vizi, 1976 a, b).

A significant biochemical gradient was biochemically proved in the gastric fundic, antral, duodenal and jejunal mucosa depending on the gastric secretory responses (BAO, MAO).

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 8

# Animal Models Used to Study the Different Mechanisms Involved in the Gastric Mucosal Damage and in the Protection

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60505

## 8.1. 24-hour pylorus-ligated rats

### *8.1.1. 24-hour pylorus-ligated rats alone*

The peak of the membrane ATPase appears earlier than the gastric hypersecretion (Figure 73). When we prepared the membrane ATPase from the forestomach (rumen), we also found its peak earlier than the ulcer development (Shay et al., 1945) (Figure 74).

It is interesting to note that the changes in the membrane ATPase reached its peak at the same time in both stomach parts after pylorus-ligation (4 hours after the pyloric ligation). When we compared the sequence of biochemistry, gastric hypersecretion and ulcer development, we found a similar sequence to that in patients with antral, duodenal and jejunal ulcers.

The membrane ATPase can be specifically inhibited by ouabain application. This principal argument offers an approach to the changes in the gastric acid output and concentration) and ulcer development (Figures 75, 76).

The tissue level of cAMP decreased significantly in both parts of the rat stomach (Mózsik et al., 1978 a, c; Mózsik et al., 1979 a, b, c, d, e, f).

### *8.1.2. 24-hour pylorus-ligated rats after bilateral surgical vagotomy*

After a 24-hour bilateral surgical vagotomy, the gastric acid secretory responses and gastric ulcer significantly decreased (Table 38), meanwhile the ATP–-ADP transformation significantly decreased in both glandular part and the forestomach (Figures 77, 78).

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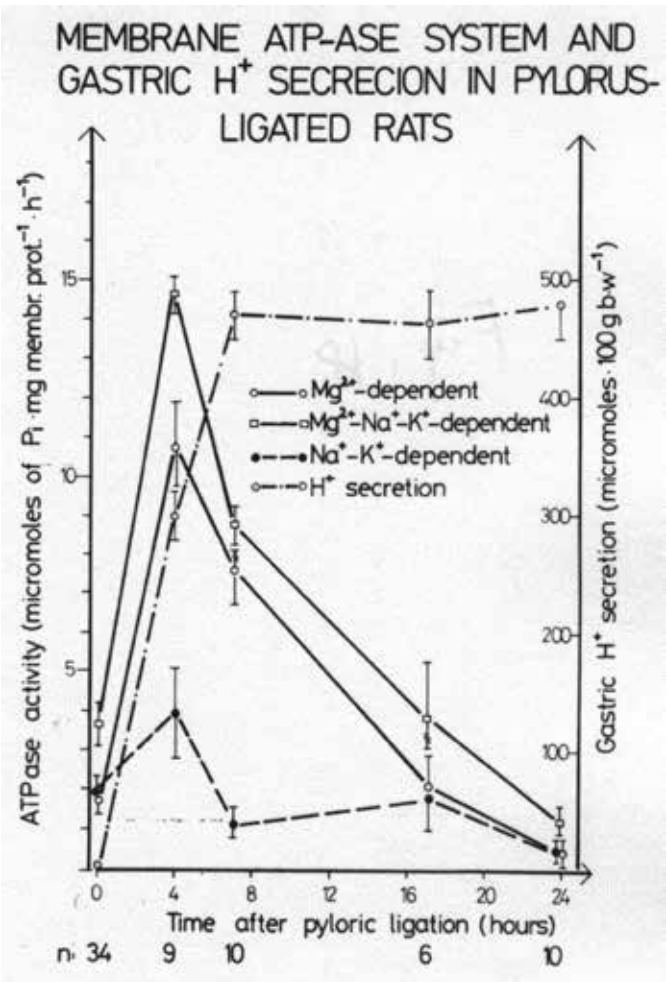


**Figure 73.** Correlations between the changes in membrane ATPase activity and $H^+$ output in 24-hour pylorus-ligated rats (means ± SEM) [Mózsik and Vizi: Amer. J. Dig. Dis. 21:449-454, 1976a) (with kind permission)].

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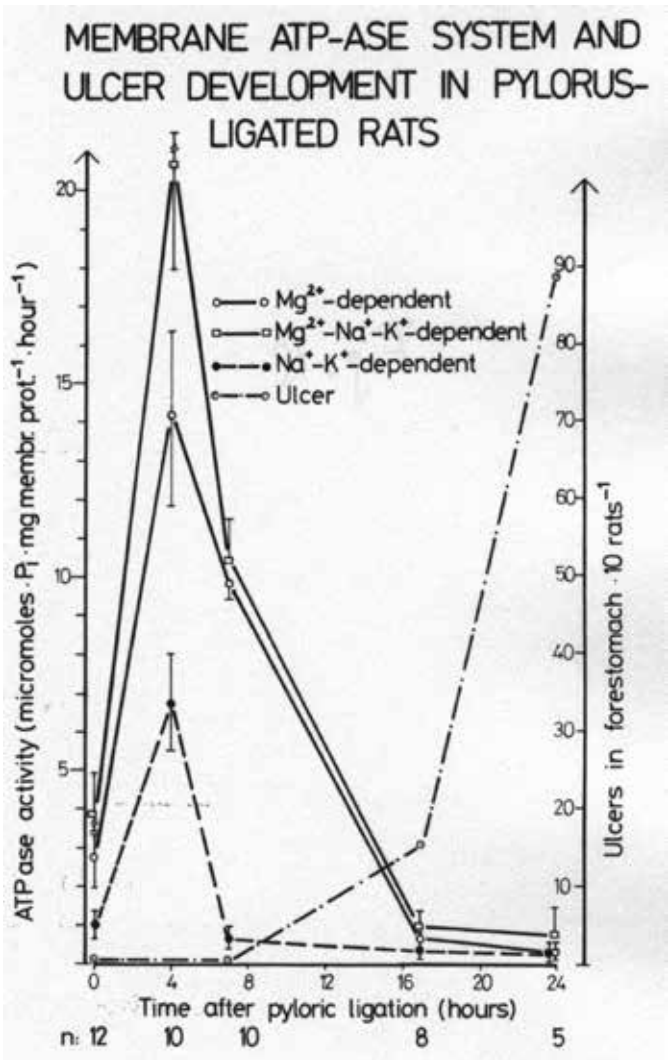


**Figure 74.** Correlation between the changes in membrane ATPase prepared from the forestomach and ulcer development in 24-hour pyloru-ligated rats (means ± SEM) [Mózsik Gy. and Vizi: Amer. J. Dig. Dis. 21:449-454, 1976a)(with kind permission)].

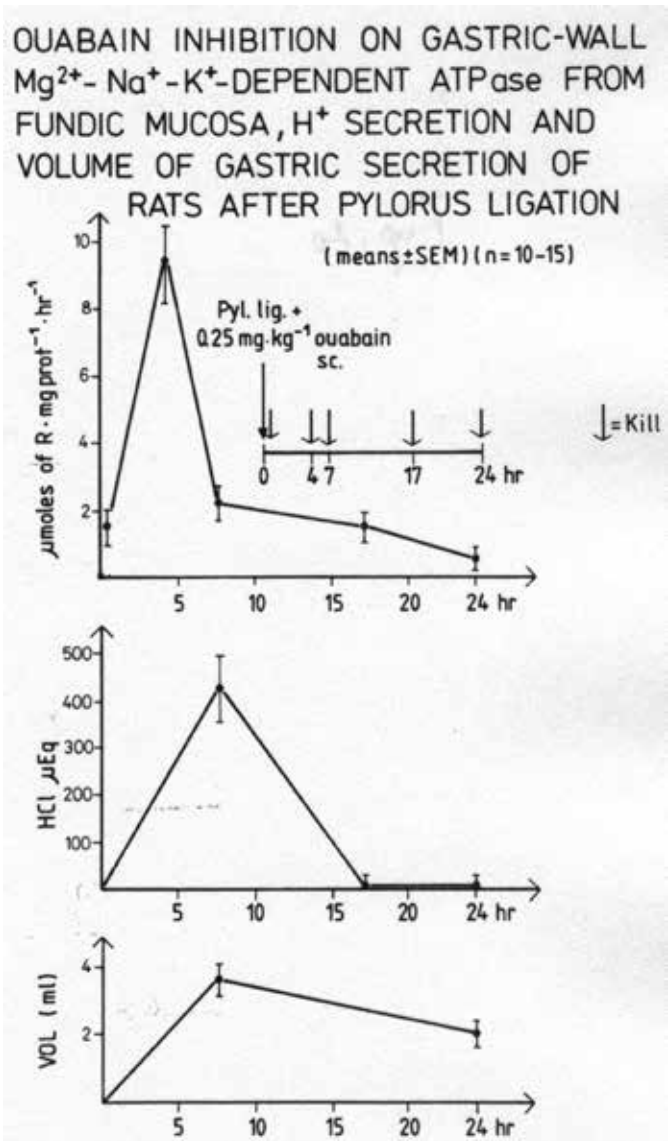


**Figure 75.** Ouabain-induced inhibition of the membrane ATPase prepared from gastric fundic mucosa, volume of gastric secretion and $H^+$ output in 24-hour pylorus-ligated rats dependent upon time after pyloric ligation (means ± SEM). [Mózsik and Vizi: Amer. J. Dig. Dis. 21:449–454, 1976a (with kind permission).]

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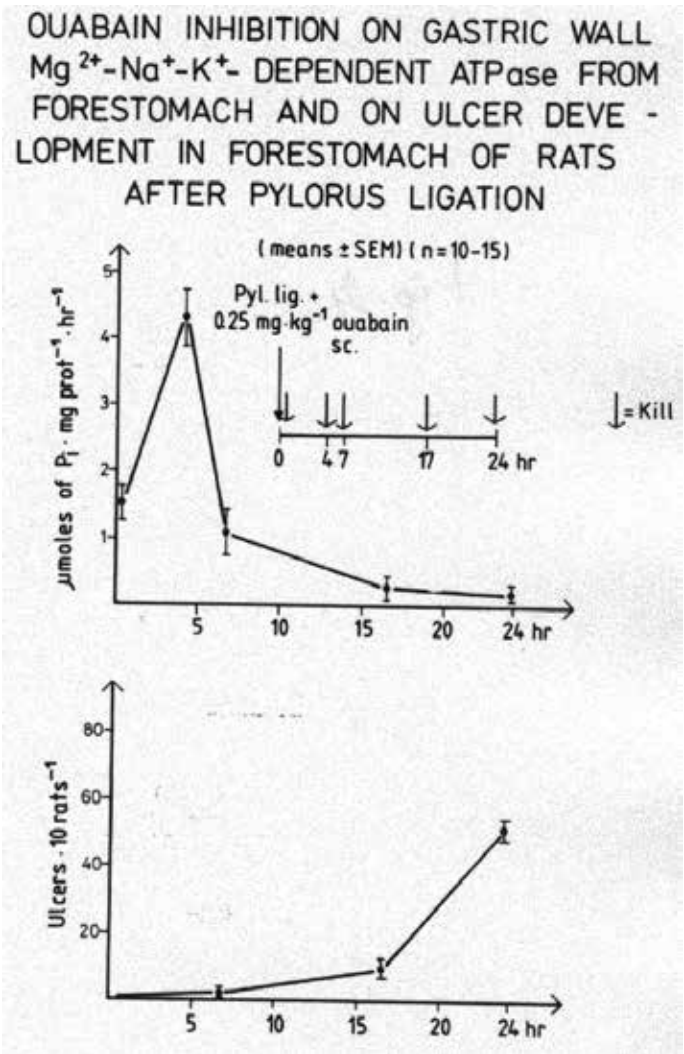


**Figure 76.** Ouabain-induced inhibition of the membrane ATPase prepared from the forestomach and ulcer development in 24-hour pylorus-ligated rats (means ± SEM) [Mózsik and Vizi: Amer. J. Dig. Dis. 21: 449–454, 1976a (with kind permission).]The tissue level of cAMP decreased significantly in both parts of the rat stomach (Mózsik et al., 1978 a, c; Mózsik et al., 1979 a, b, c, d, e, f).

| Experimal parameters | Pylorus ligation alone | Pylorus ligation + bilateral surgical vagotomy |
|---|---|---|
| Volume of gastric secretion (mL/24 h) | 15.4 ± 2.1 | 2.0 ± 0.3* |
| $H^+$ output (μEq/ 24) | 867 ± 50 | 130 ± 20* |
| $H^+$ concentration (mEq/L) | 55.2 ± 2.6 | 65 ± 10 |
| Total number of ulcer in the forestomach/ 10 rats | 86 | 0 |

**Table 38.** Gastric secretory numbers (means ± SEM) and ulcer development (total ulcer/10 rats) in pylorus-ligated rats with and without bilateral surgical vagotomy. Abbreviation: * : $P < 0.001$. [Mózsik and Vizi: Dig. Dis. Sci. 22: 1072–1075, 1976b (with kind permission).]

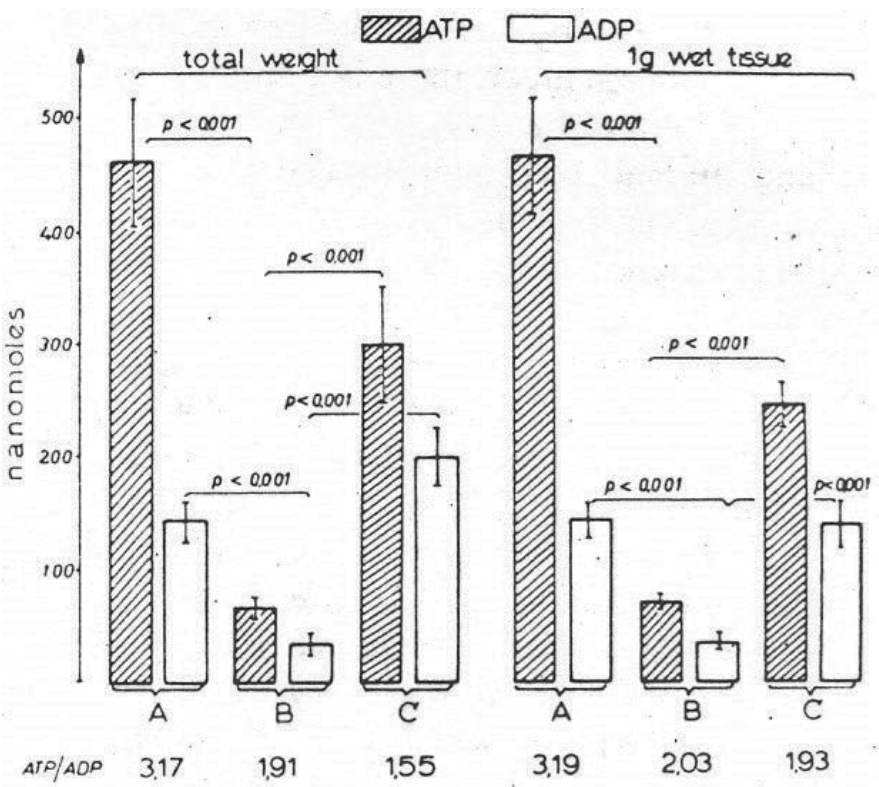


**Figure 77.** Changes in stomach wall ATP and ADP in the corpus of pylorus-ligated rats 24 hours after pylorus ligation, with and without bilateral surgical vagotomy. The results are expressed in nanomoles (means ± SEM of 10 rats). Abbreviations: A, sham-operated rats; B, pylorus-ligated rats; C, pylorus-ligated and surgically vagotomized rats. The ATP/ADP for A is significantly greater than that for B: $P < 0.001$. [Mózsik and Vizi: Dig. Dis. 22: 1072–1075, 1976b (with kind permission).]

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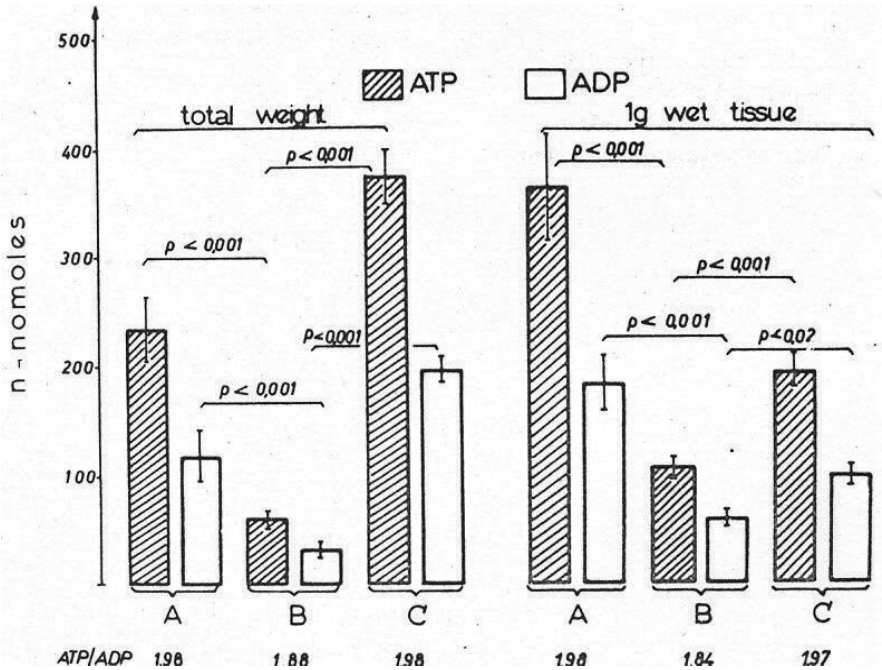


**Figure 78.** Changes in stomach wall ATP and ADP in the forestomach of rats 24 hours after pylorus ligation, with and without bilateral surgical vagotomy. The results are expressed in nanomoles (means ± SEM of 10 rats). Abbreviations: A, sham-operated rats; B, pylorus-ligated rats; C, pylorus-ligated and surgically vagotomized rats. The ATP/ADP for A is not significantly different from that for B. [Mózsik and Vizi: Dig. Dis. 22:1072–1075, 1976b (with kind permission).]

The stomach wall ATP and ADP have been studied in rats, with and without bilateral surgical vagotomy, 24 hours after pylorus ligation. It was observed that the amounts and concentrations significantly decreased in both parts (corpus + antrum and forestomach) in pylorus-ligated rats, whereas the amounts and concentrations remained significantly higher in pylorus-ligated + vagotomized rats. Changes in amounts and concentrations of ADP paralleled those of ATP. It has been concluded that a significant decrease of cellular ATP is necessary to produce gastric hypersecretion in ulceration in pylorus-ligated rats. This significant decrease of cellular ATP, in corpus + antrum and forestomach, can be prevented by bilateral surgical vagotomy (Mózsik and Vizi, 1976b).

### 8.2. Dynamic evaluation the changes of extra- and intracellular regulatory mechanisms of gastric cellular energy systems in pylorus-ligated rats without application of any drugs

As we mentioned earlier, the time period of 4 hours after pylorus ligation offers an ideal time to study the stimulatory or inhibitory actions of drugs, hormones and mediators given immediately after pylorus ligation (Figure 73). During this time period, gastric ulceration upon gastric hyperacidity never appears.

In the study, the fundus and forestomach in the rats were biochemically analyzed in depth to understand the drugs, hormones and mediators.

BIOCHEMICAL CONSTITUENTS IN THE GASTRIC FUNDIC MUCOSA OF UNTREATED RATS

| BIOCHEMICAL CONSTITUENTS | MEANS ± SEM | N |
|---|---|---|
| ATP* | 11.58 ± 1 | 39 |
| ADP* | 13.75 ± 1 | 39 |
| ATP·ADP$^{-1}$ | 0.84 ± 0.06 | 39 |
| AMP* | 10.73 ± 2 | 39 |
| ATP+ADP+AMP* | 36.06 ± 2 | 39 |
| $\frac{ATP+0.5\,ADP}{ATP+ADP+AMP}$ | 0.51 ± 0.02 | 39 |
| cAMP** | 4.51 ± 0.10 | 39 |
| LACTATE*** | 277 ±20 | 39 |

* nmoles·mg mucosal protein$^{-1}$ (means ± SEM)
** pmoles·mg mucosal protein$^{-1}$ (means ± SEM)
*** μmoles·mg mucosal protein$^{-1}$ (means ± SEM)

**Table 39.** Biochemical constituents in the gastric fundic mucosa of intact rats.

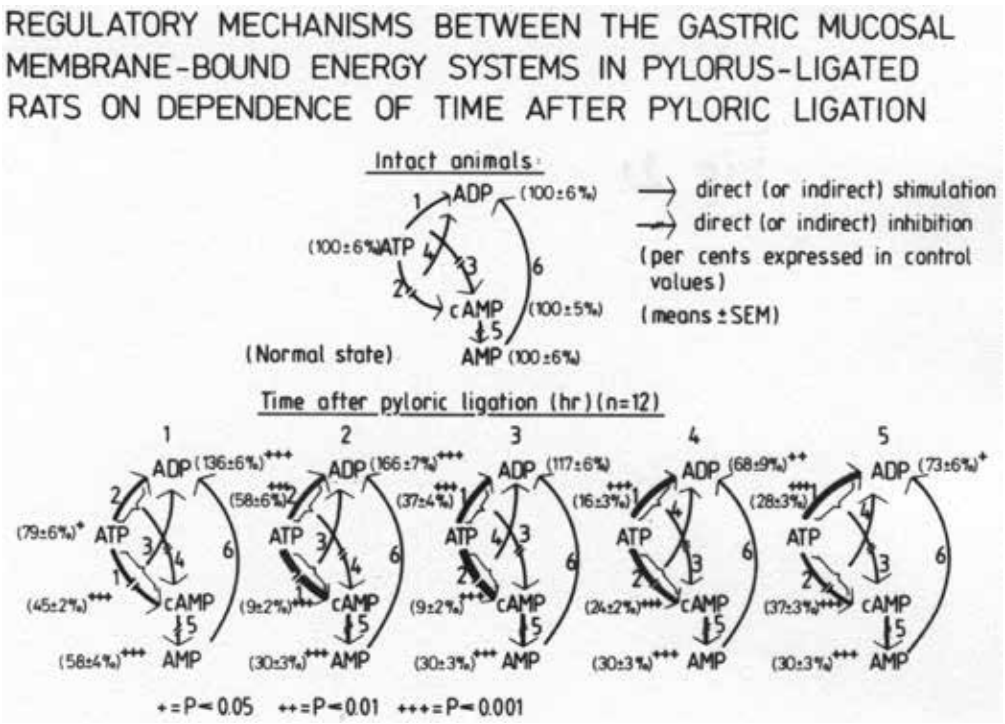


**Figure 79.** Changes in the cellular ATP, ADP, cAMP and AMP in the time period of first 5 hours dependent upon time after pyloric ligation. The observations were carried out on gastric fundic mucosa. The results obtained in sham-operated rats (normal state) were taken to be equal to 100% (means ± SEM). [Mozsik (2006) Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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The tissue levels of ATP, ADP, cAMP and AMP and the regulatory mechanisms between them were measured at 1, 2, 3, 4 and 5 hours after pylorus ligation (Figure 79, Table 39).

*8.2.1. Biochemical backgrounds of effect of atropine in 4 hours pylorus-ligated rats*

The atropine dose-dependently decreases the gastric $H^+$ output (Figure 80). In these observations, the ADP was decreased dose-dependently, whereas the cAMP and AMP were increased in the rat gastric fundic mucosa (Figure 81).

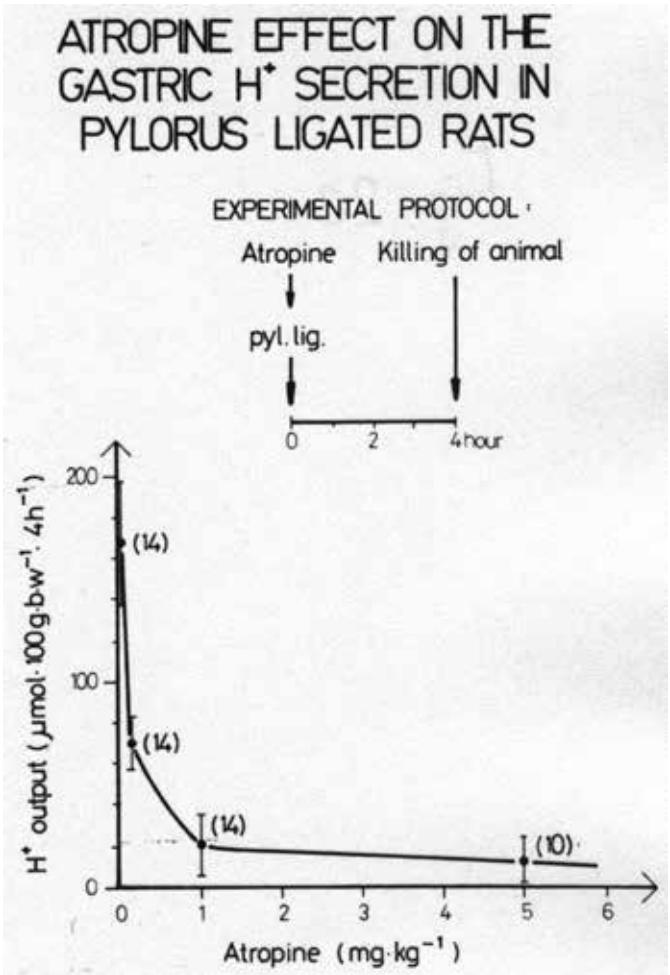


**Figure 80.** Dose-dependent inhibitory effect of atropine on the gastric acid secretion in 4-hour pylorus-ligated rats (means ± SEM). [Mózsik, Figler, Nagy, Patty, Tárnok (1981): Gastric and small intestinal energy metabolism in mucosal damage. In: Mózsik Gy., Hänninen O., Jávor T. (Eds.). Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest. pp. 213–288 (with kind permission).]

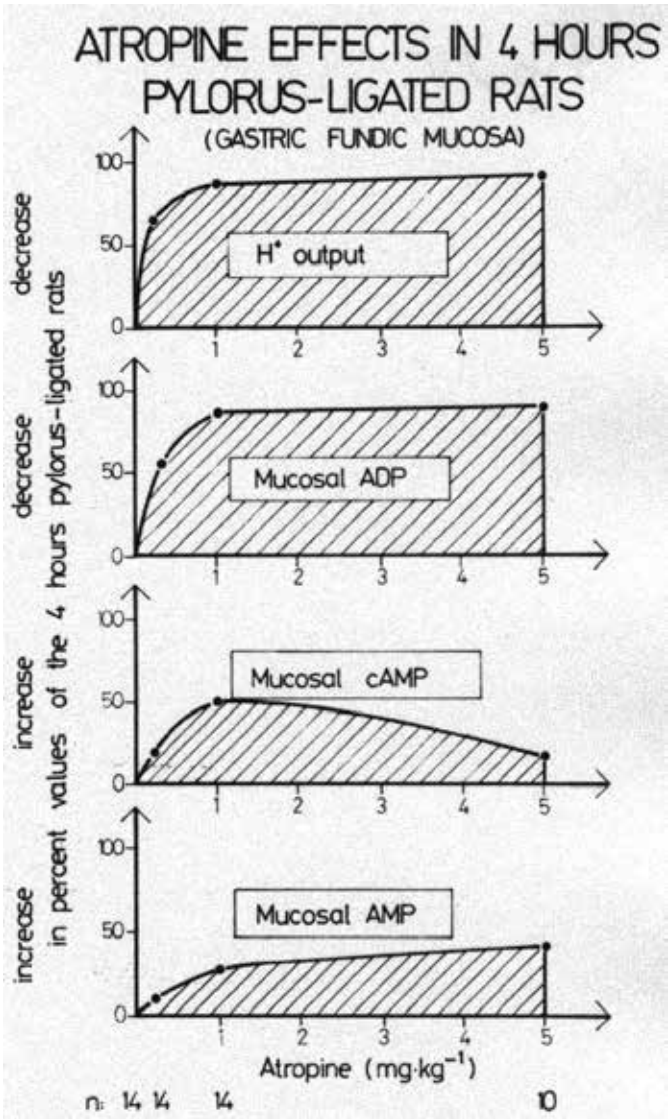


**Figure 81.** Atropine-induced changes in gastric $H^+$ output, gastric fundic mucosal levels of ATP, ADP and AMP in 4-hour pylorus-ligated rats. The results were expressed as percent values obtained immediately after pylorus ligation (=100%), except for gastric $H^+$ output, which was also expressed in percent values, but obtained at 4 hours after pylorus ligation (means ± SEM). [Mózsik, Figler, Nagy, Patty, Tárnok (1981): Gastric and small intestinal energy metabolism in mucosal damage. In: Mózsik Gy., Hänninen O., Jávor T. (Eds.). Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest. pp. 213–288 (with kind permission).]

Similar changes were found in the forestomach during atropine treatment.

Schematic summary of atropine treatment-induced (given in different doses) gastric fundic mucosal damage in 4-hour pylorus-ligated rats (Figure 82).

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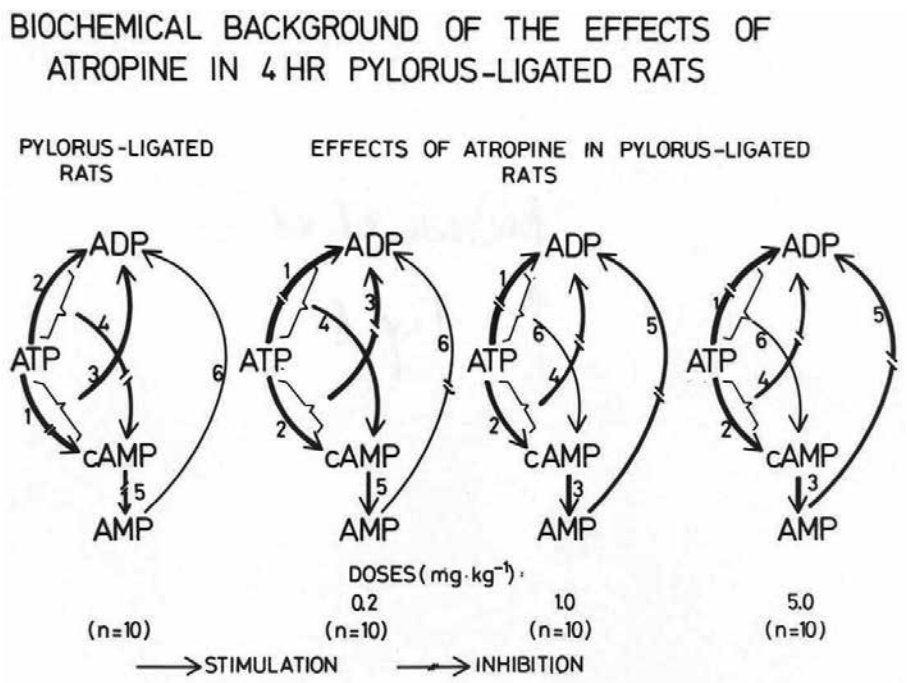


**Figure 82.** Biochemical changes in the regulatory steps of cellular energy systems in the gastric fundic mucosa produced by different (cytoprotective and antisecretory) doses of atropine in 4-hour pylorus-ligated rats.

*8.2.2. Biochemical backgrounds of epinephrine in hours pylorus-ligated rats*

Epinephrine dose-dependently decreased the gastric $H^+$ output and gastric fundic mucosal level of ADP, whereas the cAMP level was increased (Shay et al., 1945) (Figures 83–85).

Similar changes were found in the forestomach after epinephrine administration to those in the rat gastric fundic mucosa.

Figure 85 indicates the main steps of epinephrine-induced regulatory pathways in the tissue levels of ATP, ADP, cAMP and AMP in the gastric fundic mucosa of 4-hour pylorus-ligated rats.

## 8.3. Biochemistry of aspirin-induced mucosal damage and its prevention by different compounds

*8.3.1. Biochemistry of aspirin-induced mucosal damage in 4 hours pylorus-ligated rats*

Aspirin is a specific inhibitor of cyclooxygenase (COX-1; Mózsik et al., 2003).

Earlier, Davernport and coworkers proved that aspirin breaks the gastric mucosal barrier, which increases the $H^+$ backdiffusion.

We observed later (during the 1980s) that the transformation of ATP into ADP could be inhibited by aspirin administration (Figler et al., 1985, 1986, 1999).

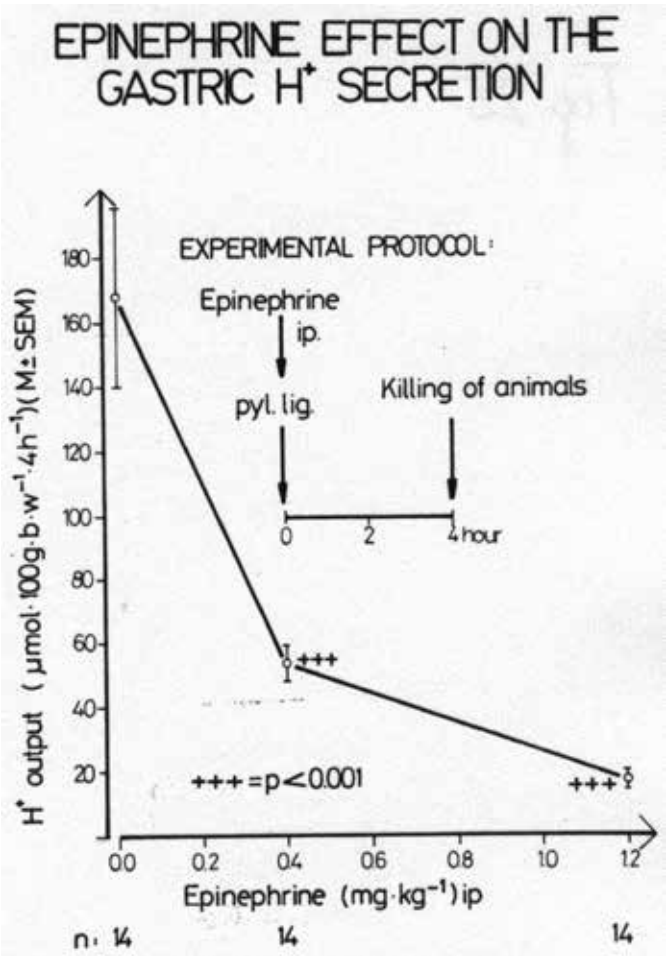


**Figure 83.** Dose-dependent inhibitory effect of epinephrine on the gastric acid secretion in 4-hour pylorus-ligated rats (means ± SEM). [Mózsik, Figler, Nagy, Patty, Tárnok (1981): Gastric and small intestinal energy metabolism in mucosal damage. In: Mózsik Gy., Hänninen O., Jávor T. (Eds.). Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest. pp. 213–288 (with kind permission).]

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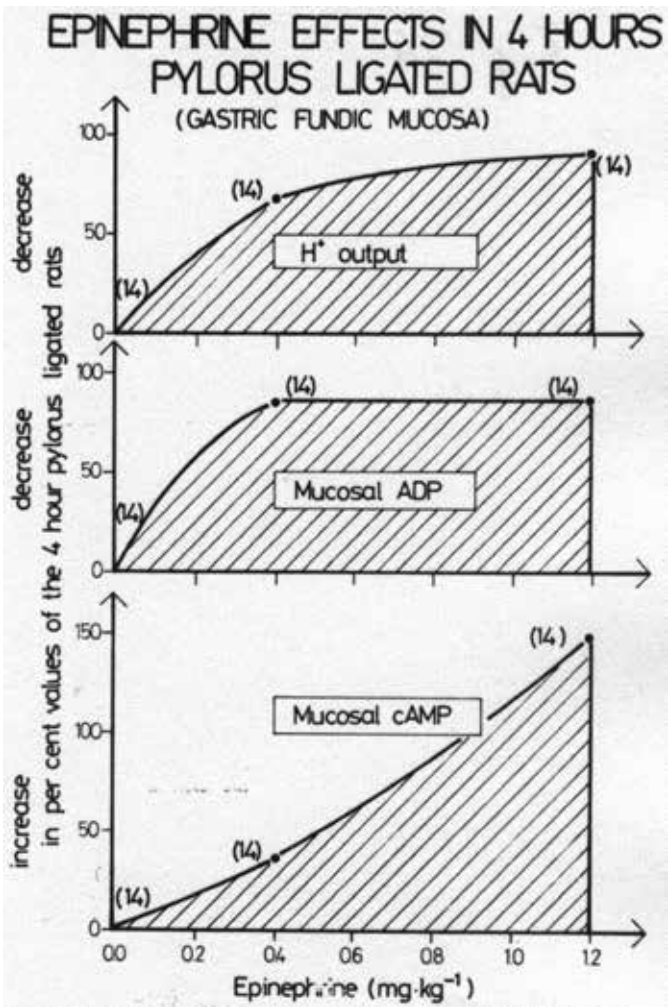


**Figure 84.** Epinephrine-induced changes in the fundic gastric mucosal levels of ADP and cAMP in comparison with the inhibition of gastric acid secretion (means ± SEM). [Mózsik, Figler, Nagy, Patty, Tárnok (1981): Gastric and small intestinal energy metabolism in mucosal damage. In: Mózsik Gy., Hänninen O., Jávor T. (Eds.). Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanism. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest. pp. 213–288 (with kind permission).] For further explanation, see Figure 83.

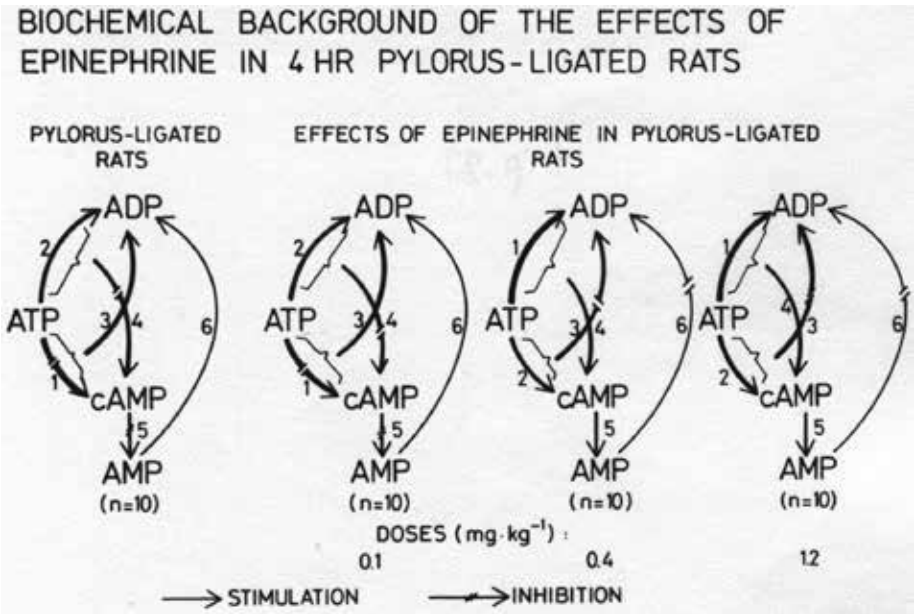


**Figure 85.** Epinephrine-induced changes in the regulatory mechanisms between the cellular ATP, ADP and AMP by different doses of epinephrine in 4-hour pylorus-ligated rats. The different doses of epinephrine were subcutaneously applied immediately after pyloric ligation (means ± SEM). For further explanation, see Figure 83.

In these studies, we analyzed the changes in the gastric $H^+$ output and changes in the rat gastric fundic mucosa after aspirin administration (200 mg/kg dissolved in 2 mL of 150 mmoles of $H^+$) given immediately after pylorus ligation.

Figure 83 demonstrates the effects of aspirin on 4-hour pylorus-ligated rats. The gastric $H^+$ output, obtained at 0, 1 and 3 hours, was less than the quantity given immediately after pyloric ligation. The difference between the given acid and the measured acid output was taken as a marker of gastric $H^+$ backdiffusion (Figure 86). This figure clearly indicates that the active gastric $H^+$ secretion starts only from the second hour (Figler et al., 1985, 1986, 1999) (Figures 87, 88).

The results presented in Figure 84 demonstrate the absolute values of volume (mL/100 g body weight/4 hours), $H^+$ output (μEq/100 b.w./4 hours) and glandular ulcer in 4-hour pylorus-ligated (A) and 4-hour pylorus-ligated plus aspirin (B) rats. The values of groups A and B are presented in Figure 87. The forthcoming figure (Figure 30) demonstrates the main changes in the rat gastric fundic mucosal biochemistry without (group A) and with (group B) aspirin administration (Figure 88).

Figure 89 demonstrates the correlations between the changes in the rat gastric fundic mucosal biochemistry without (group A) and with (group B) aspirin treatment. The values expressed

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the differences between the pylorus-ligated versus pylorus-ligated plus aspirin-treated rats (Figures 88, 89).

We tried to summarize the changes in the regulatory pathways between the tissue levels of ATP, ADP, cAMP and AMP in intact (untreated), 4-hour pylorus-ligated and 4-hour pylorus-ligated plus aspirin-treated rats (Figure 90).

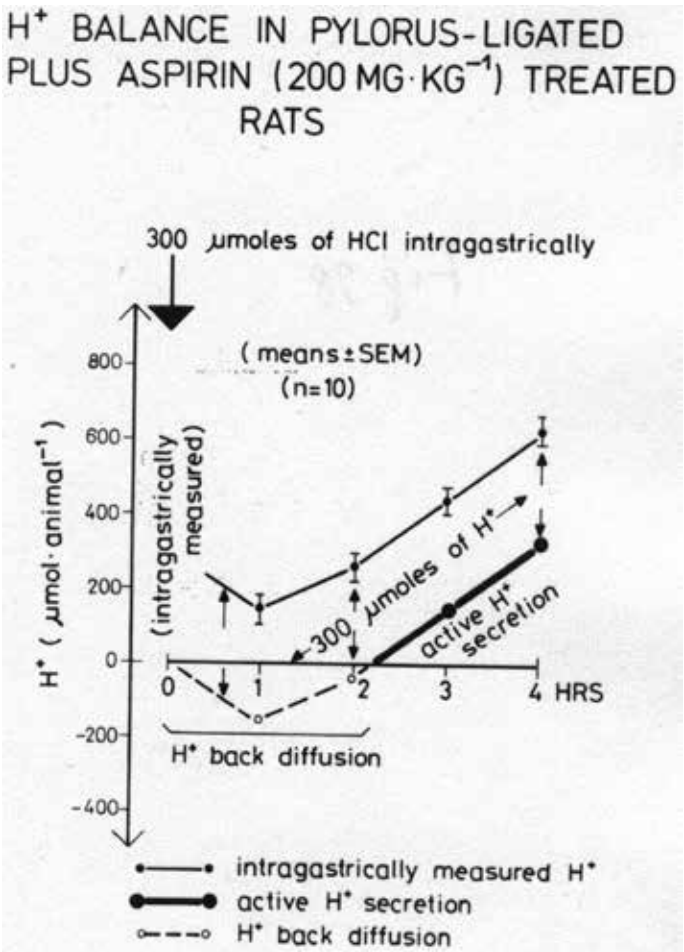


**Figure 86.** The balance between the aspirin-induced gastric $H^+$ backdiffusion and active $H^+$ secretion in 4-hour pylorus-ligated plus aspirin (200 mg/kg ig. in 2 mL = 300 μmoles of $H^+$)-treated animals (means ± SEM). [Figler, Jávor, Nagy, Patty, Mózsik 1986. Int. J. Tiss. React. 8: 15–22 (with kind permission).]

CHANGES IN GASTRIC SECRETORY RESPONSES AND DEVELOPMENT OF GASTRIC MUCOSAL LESIONS IN 4 HR PYLORUS-LIGATED (B) AND PYLORUS-LIGATED PLUS ASPIRIN-TREATED (B) RATS

| | A (n=11) | B (n=10) | Δ(A-B) |
|---|---|---|---|
| Volume ($ml \cdot 100g^{-1} \cdot 4hr^{-1}$) | 1.9 ± 0.1 | $3.0 \pm 0.17^{++}$ | + 1.1 ± 0.1 |
| $H^+$ output | 150 ± 10 | $110 \pm 10^{+}$ | - 40 ± 10 |
| ($\mu Eq \cdot 100g^{-1} \cdot 4hr^{-1}$) | 0 ± 0 | $141 \pm 2.1^{+++}$ | + 14.1 ± 2.1 |
| ULCER NUMBER | 0 ± 0 | $27.2 \pm 2.1^{+++}$ | + 27.2 ± 2.1 |

**Figure 87.** Changes in the volume (mL/100 g b.w./4 hours), acid output (μEq/100 g b.w./4 hours) in the 4-hour pylorus-ligated (A) versus. 4-hour pylorus-ligated plus aspirin-treated (B) rats. The Δ values expressed as difference between the groups of B versus A (means ± SEM). [(Figler, Jávor, Nagy, Patty, Mózsik 1986. Int. J. Tiss. React. 8: 15–22 (with kind permission).]

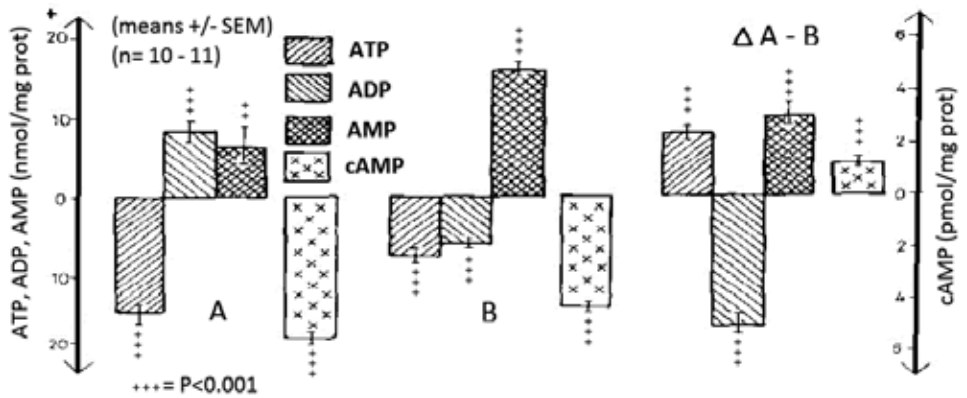


**Figure 88.** The changes in the gastric fundic mucosal levels of ATP, ADP, AMP and cAMP in 4-hour pylorus-ligated (A) and 4-hour pylorus-ligated plus aspirin-treated (B) rats expressed as absolute values. The values of groups A versus B were given as Δ values (means ± SEM). [Figler, Jávor, Nagy, Patty, Mózsik 1986. Int. J. Tiss. React. 8: 15–22 (with kind permission).]

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CORRELATIONS BETWEEN CHANGES IN BIOCHEMISM OF GASTRIC MUCOSA IN 4 HR PYLORUS-LIGATED (A) AND PYLORUS-LIGATED PLUS ASPIRIN TREATED (B) RATS

| | A | B | Δ A-B |
|---|---|---|---|
| ULCER NUMBER | - | ↑↑↑ | ↓↓↓ |
| ULCER SEVERITY | - | ↑↑↑ | ↓↓↓ |
| SECRETORY VOLUME | ↑↑↑ | ↑↑↑ | ↓↓ |
| $H^+$ output | ↑↑↑ | ↑↑↑ | ↓ |
| ATP | ↓↓↓ | ↓↓↓ | ↑↑↑ |
| ADP | ↑↑↑ | ↓↓↓ | ↓↓ |
| ATP:$ADP^{-1}$ | ↓↓↓ | NS | ↑↑↑ |
| AMP | ↑↑ | ↑↑↑ | ↑↑↑ |
| ADENYLATE POOL | NS | NS | NS |
| cAMP | ↓↓↓ | ↓↓↓ | ↑↑↑ |
| „ENERGY CHARGE" LACTATE | ↓↓ | ↓↓ | NS |

NS = NOT SIGNIFICANT
↓ = 0.05 > P > 0.01 ↓↓ = 0.01 > P > 0.001 ↓↓↓ = P < 0.001
↑ = INCREASE ↓ = DECREASE

**Figure 89.** The summary of the changes in the rat gastric fundic mucosa and ulcer development in 4-hour pylorus-ligated and 4-hour pylorus-ligated plus aspirin-treated animals.

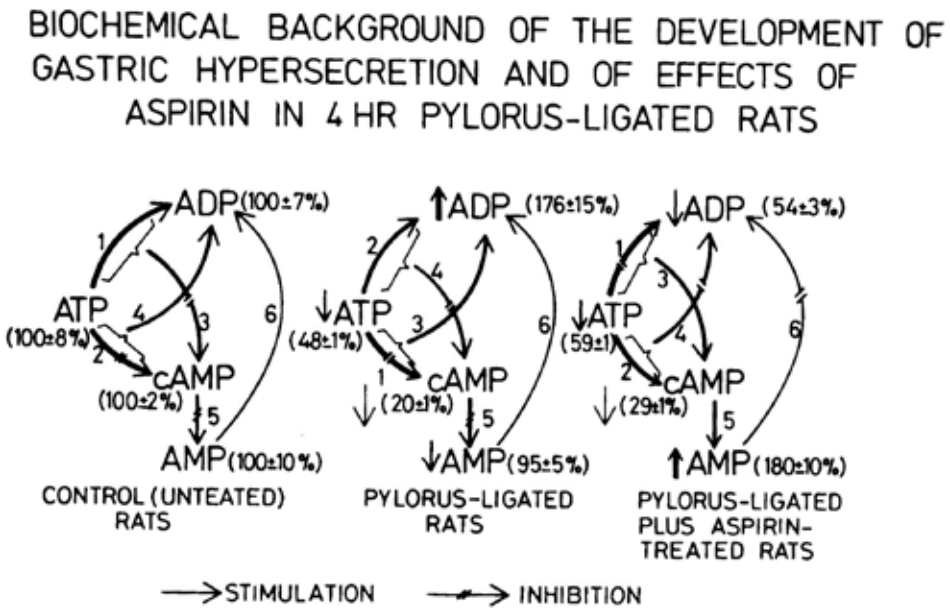


**Figure 90.** The details of the regulatory pathways in intact (left figure), in 4-hour pylorus-ligated (middle figure) and in 4-hour pylorus-ligated plus aspirin-treated rats (right figure) (means ± SEM).

*8.3.2. Gastric mucosal-protective effect of atropine and its changes in biochemistry of gastric mucosa in 4-hour pylorus-ligated plus aspirin-treated rats*

Following the observations presented before (as a section of 5.5.), the mucosal-protective effect of atropine was studied under the same experimental conditions. The gastric mucosal damage was produced after intragastric administration of 200 mg/kg aspirin (dissolved in 2 mL of 150 mmol HCl) immediately after pyloric ligation. The different doses of atropine (0.1, 0.5 and 1.0 mg/kg s.c.) given at the same time as that of aspirin in 4-hour pylorus-ligated rats (Figure 91).

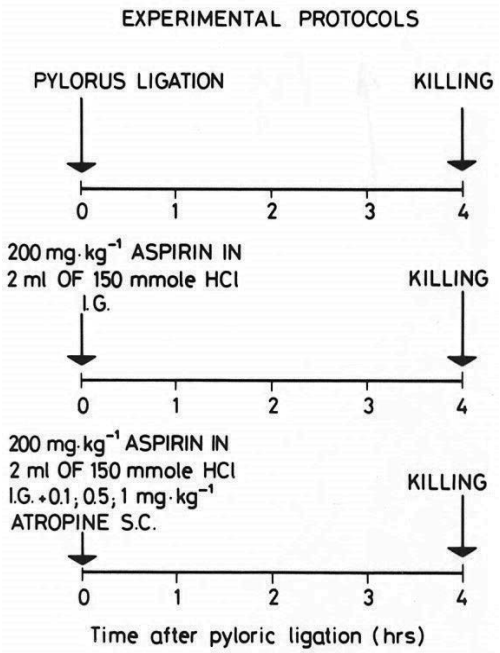


**Figure 91.** Experimental protocol for studies examined the mucosal-protective effect of atropine (given in doses of 0.1, 0.5 and 1.0 mg/kg subcutaneously immediately after pyloric ligation plus aspirin administration). [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

The volume, concentration of $H^+$, gastric acid output (Figure 92) and the number and sum of ulcers (Figure 93) could be decreased dose-dependently by the application of atropine (Figure 93).

The results of biochemical measurements [tissue levels of ATP, ADP and AMP (Figure 94)], adenylate pool, "energy charge", ratio of ATP/ADP (Figure 95), tissue level of cAMP (Figure 96) and lactate (Figure 97) are provided.

The results clearly indicate that the ATP transformation is inhibited by atropine; however, its value decreases by the increase of atropine doses. In the background, the ATP transformation pathway will lead to an increase in ATP–cAMP transformation, whereas the ratio of ATP/ADP and "energy charge" increases; however, the adenylate pool and tissue level of lactate remained unchanged (Figure 98).

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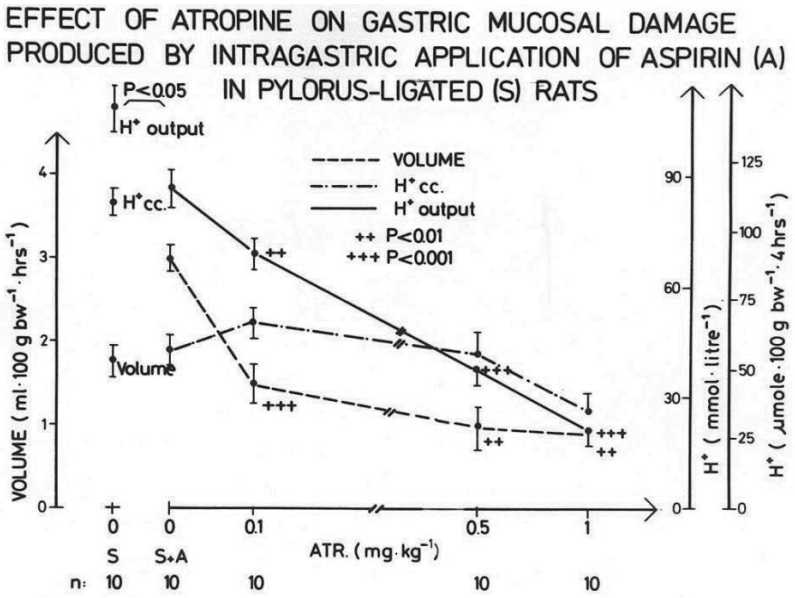


**Figure 92.** Changes in gastric secretory responses in 4-hour pylorus-ligated plus aspirin-treated rats after application of different doses of atropine. The results are expressed as means ± SEM. Abbreviations: S, surgical interventions; S + A, surgical intervention plus aspirin treatment, *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

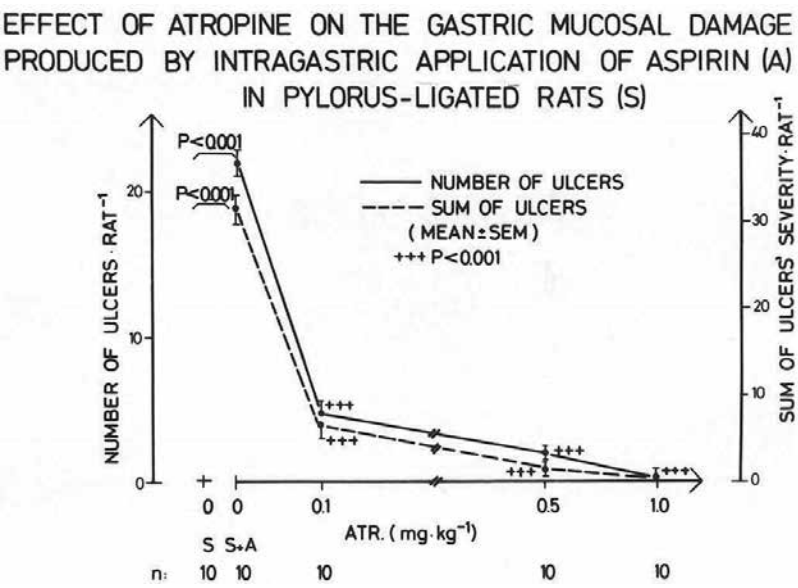


**Figure 93.** Dose-dependent gastric mucosal-preventive effects of different doses of atropine in 4-hour pylorus-ligated plus aspirin-treated rats. The results are presented as means ± SEM. Abbreviations: S, surgical intervention; S + A, surgical intervention plus aspirin treatment; *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

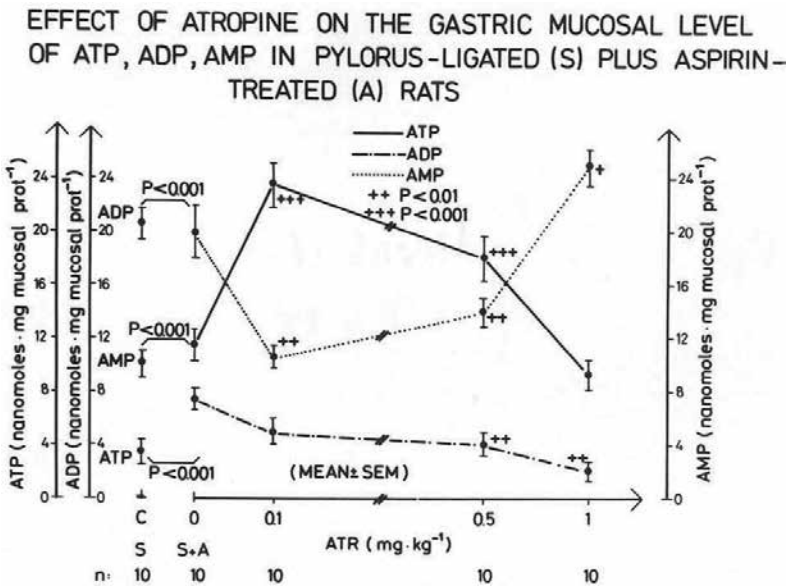


**Figure 94.** Atropine treatment-induced changes in adenylate pool, ratio of ATP/ADP and "energy charge" in the gastric mucosal tissues in 4-hour pylorus-ligated plus aspirin-treated rats. The results are expressed as means ± SEM. Abbreviations: S, surgical intervention; S + A, surgical intervention plus aspirin treatment; *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

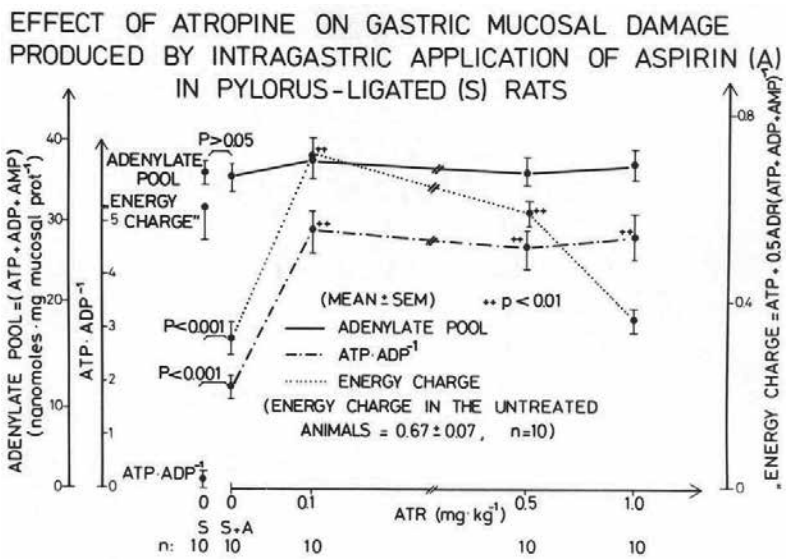


**Figure 95.** Atropine treatment-induced changes in adenylate pool, ratio of ATP/ADP and "energy charge" in the gastric mucosal tissues in 4-hour pylorus-ligated plus aspirin-treated rats. The results are expressed as means ± SEM. Abbreviations: S, surgical intervention; S + A, surgical intervention plus aspirin treatment; *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

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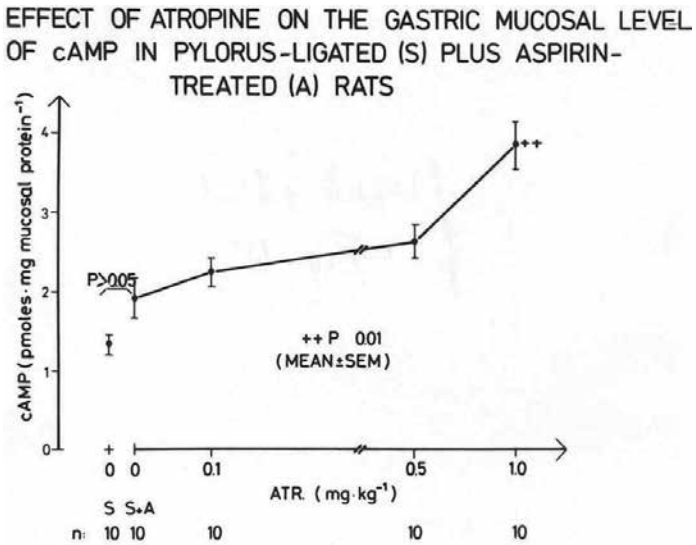


**Figure 96.** Atropine-induced changes in tissue level of cAMP in the gastric mucosa in 4-hour pylorus-ligated plus aspirin-treated rats. The results are expressed as means ± SEM. Abbreviations: S, surgical intervention; S + A, surgical intervention plus aspirin treatment; *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

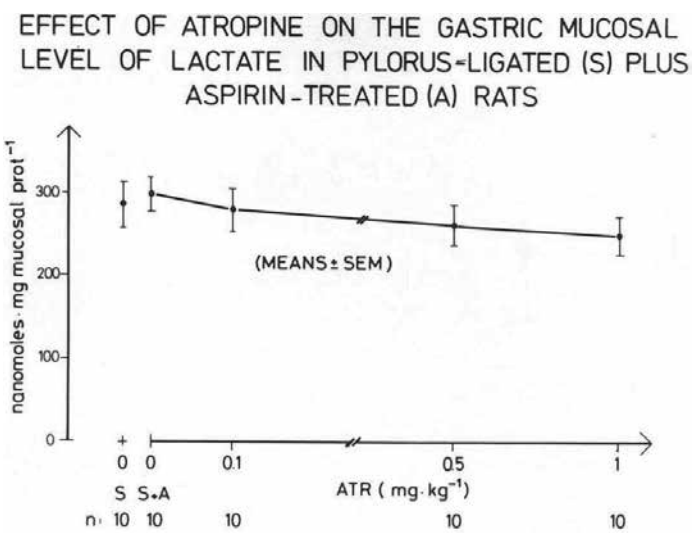


**Figure 97.** Effect of atropine treatment on the tissue level of lactate in the gastric mucosa of 4-hour pylorus-ligated plus aspirin-treated rats. The results are expressed as means ± SEM. Abbreviations: S, surgical intervention; S + A, surgical intervention plus atropine treatment; *n*, indicates the number of animals. [Figler et al., 1985, Digestion 31, 145–146 (with kind permission).]

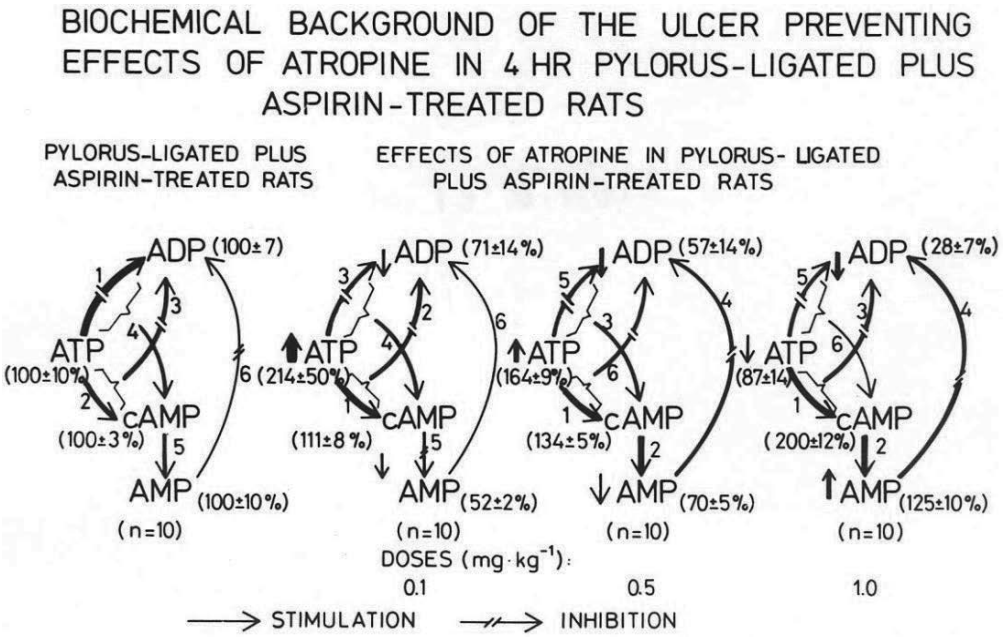


**Figure 98.** Schematic representation of changes in the extra- and intercellular feedback mechanisms, which exist after application of different doses of atropine. The different steps in the regulatory mechanisms were expressed by the results based on the changes in perpetual values of tissue levels of ATP, ADP, AMP and cAMP of the pathological (pylorus-ligated plus aspirin-treated) values (and these were taken as 100%).

Consequently, the intracellular regulatory mechanism between the energy systems also significantly changes in the gastric mucosa during the development of gastric mucosal protection by atropine.

*8.3.3. Gastric mucosal-protective effects of vitamin A and β-carotene and their biochemical backgrounds in 4-hour pylorus-ligated plus aspirin-treated rats*

In the previous sections (5.5. and 5.5.1.), the biochemical changes in the gastric mucosa and gastric secretory responses were studied in the 4-hour pylorus-ligated plus aspirin-treated rats during the time of development of gastric mucosal damage (5.5.) and prevention by atropine (5.5.1).

In this chapter, the 4-hour pylorus-ligated plus aspirin-treated ulcer model was further used; however, the actions of mechanisms of two scavengers (namely vitamin A and β-carotene) were studied. As indicated earlier, these compounds have no gastric secretory inhibitory actions neither in animals (Jávor et al., 1983) nor in human beings (Mózsik et al., 1986). Consequently, the scavenger action versus cytoprotection phenomenon were together studied in the aspirin animal model.

The experimental protocol and different measurements (gastric secretory responses, gastric mucosal lesions and biochemical measurements) are presented in Figure 99.

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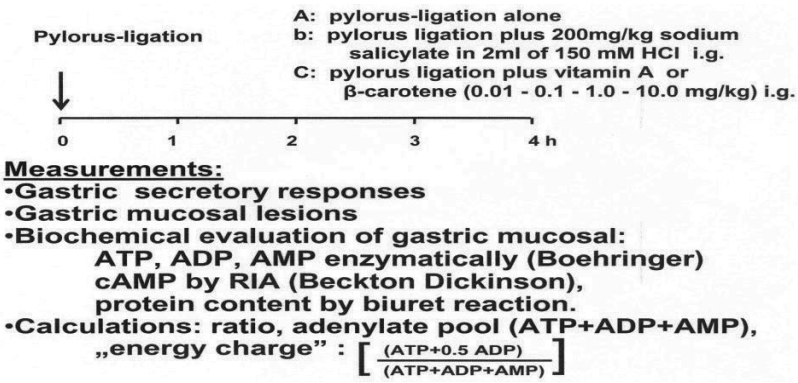


**Figure 99.** Experimental protocol (design) for the studies of actions of mechanisms of gastric mucosal protection of orally administered vitamin A and β-carotene (vitamin A and β-carotene were dissolved in oleum helianthi and given intragastrically in doses of 0.01, 0.1, 1.0 and 10.0 mg/kg by a flexible nasogastric tube). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

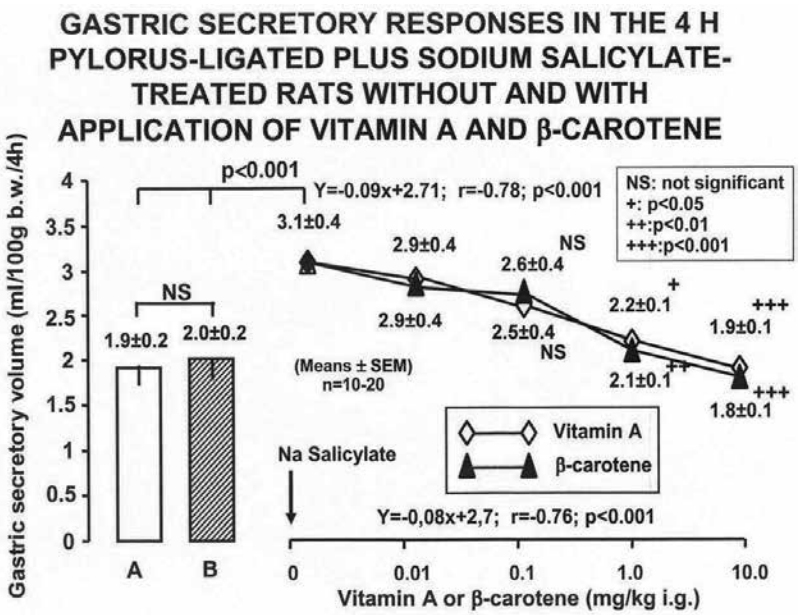


**Figure 100.** Changes in the volume of gastric secretion in 4-hour pylorus-ligated (A) and aspirin-treated (B) rats with and without vitamin A and β-carotene treatment (right side of the figure). The detailed results are presented in the figure. [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

The volume of gastric secretion and acid output did not change in 4-hour pylorus-ligated plus aspirin-treated rats (B) versus pylorus-ligated rats alone (A), the volume decreased after the application of higher doses (1.0 and 10.0 mg/kg) (Figure 100); however, no changes were found in the gastric acid secretion (Figure 101).

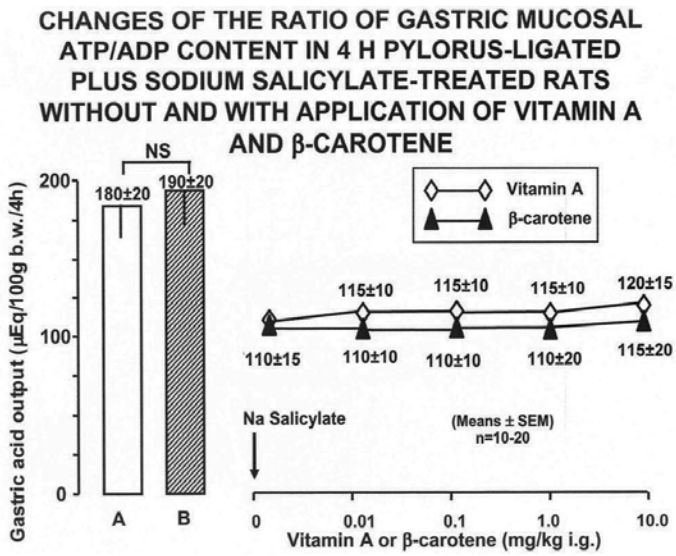


**Figure 101.** No change in the gastric acid secretion in 4-hour pylorus-ligated ligated plus aspirin-treated rats after intragastrically given vitamin A and β-carotene. [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

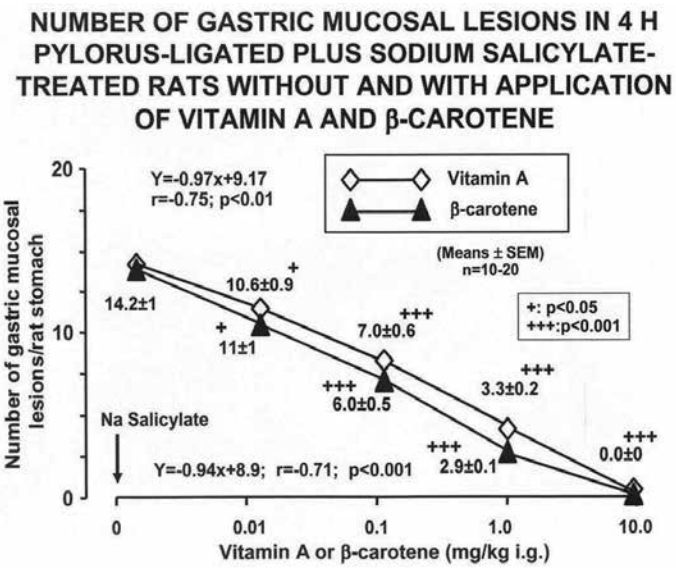


**Figure 102.** Gastric mucosal-preventive effect (on number of lesions) of vitamin A and β- carotene on 4-hour pylorus-ligated plus aspirin-treated rats. [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

Both vitamin A and β-carotene dose-dependently prevented the number (Figure 102) and severity (Figure 103), which differ from the decrease of gastric acid secretion (Figure 101).

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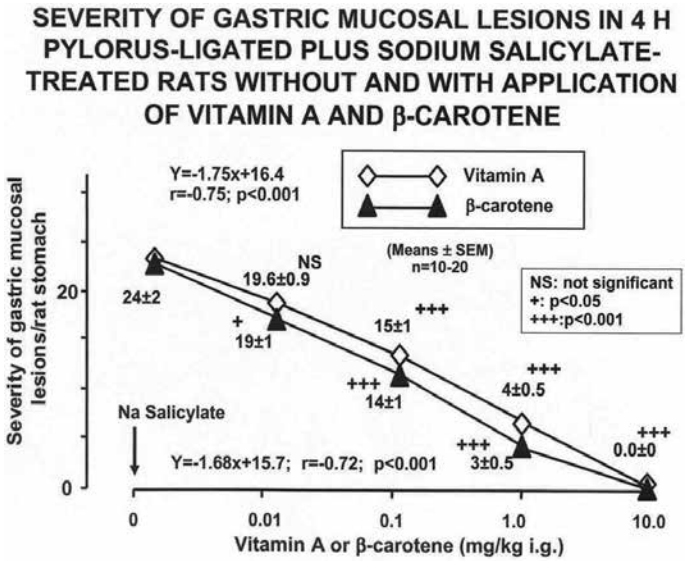


**Figure 103.** Gastric mucosal (severity)-preventive effects of vitamin A and β-carotene on 4-hour pylorus-ligated plus aspirin-treated rats. [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

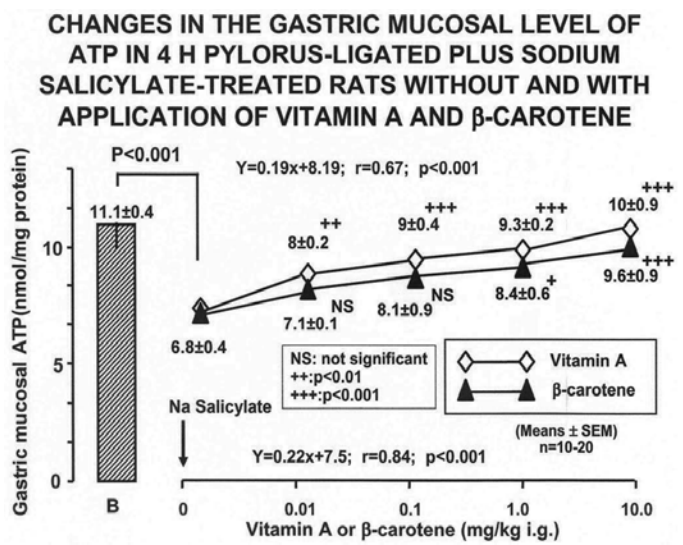


**Figure 104.** Changes in the gastric mucosal ATP in 4-hour pylorus-ligated and aspirin-treated rats after intragastric administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

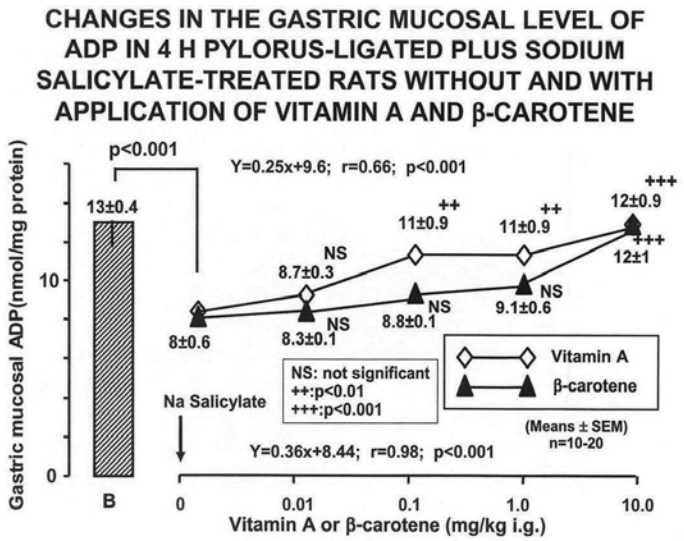


**Figure 105.** Changes in the gastric mucosal ADP in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

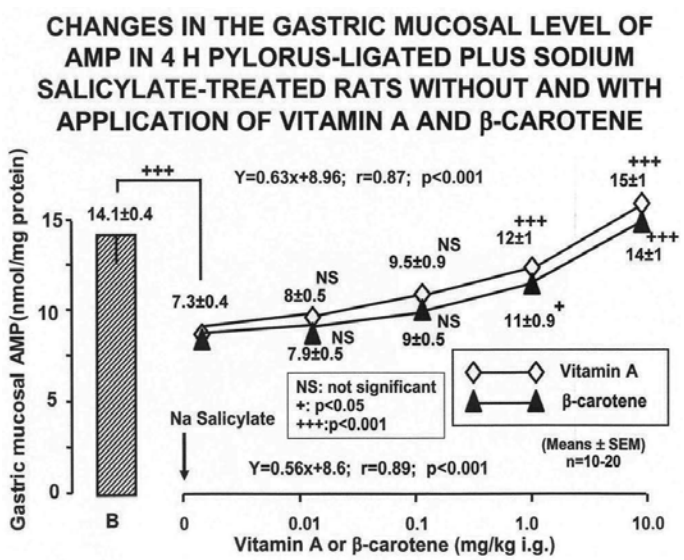


**Figure 106.** Changes in the gastric mucosal AMP in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

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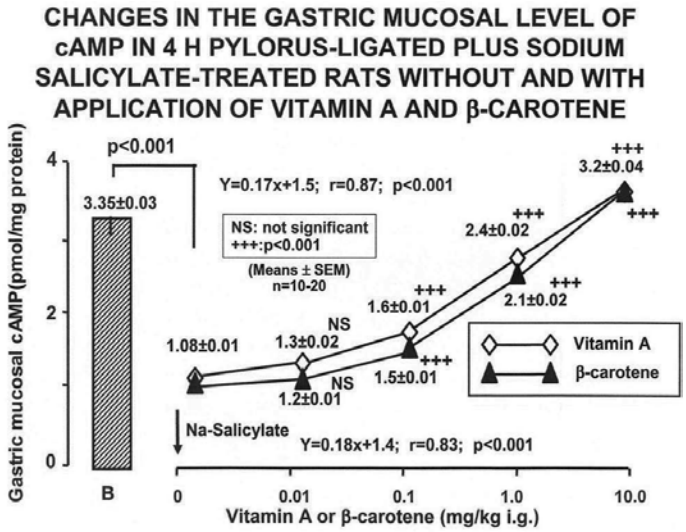


**Figure 107.** Changes in the gastric mucosal cAMP in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560-562, 2003 (with kind permission).]

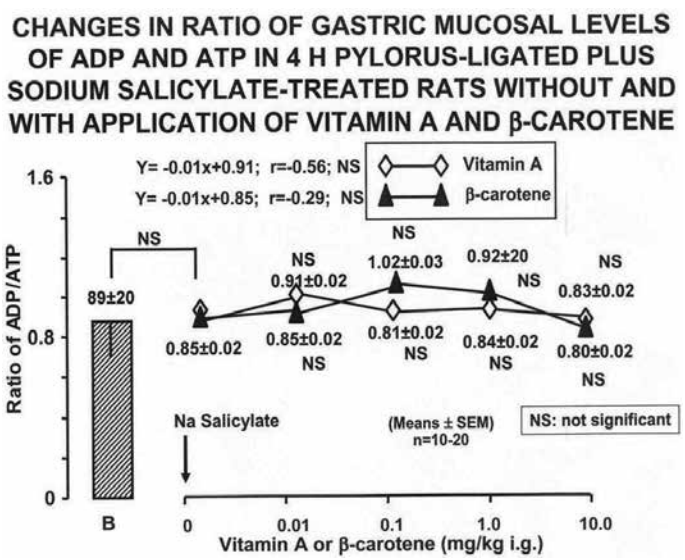


**Figure 108.** ATP/ADP ratio in the gastric mucosa in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

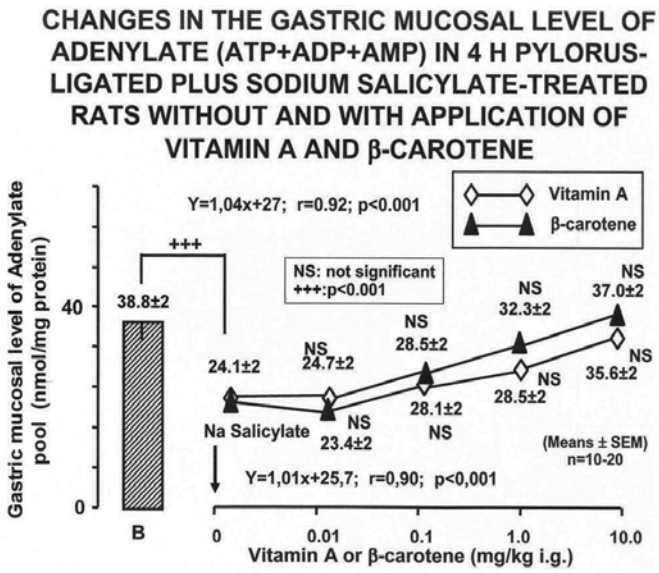


**Figure 109.** Changes in the gastric mucosal levels of adenylate pool (ATP + ADP + AMP) in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission)].

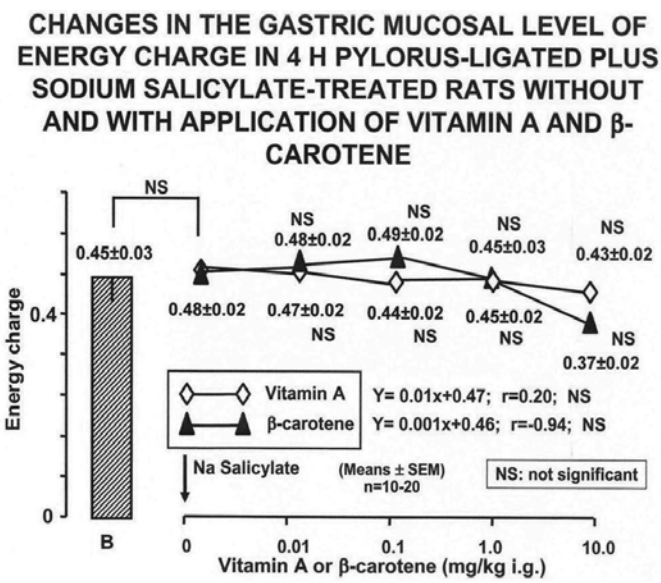


**Figure 110.** Changes in the gastric mucosal level of "energy charge" in 4-hour pylorus-ligated plus aspirin-treated rats after administration of vitamin A and β-carotene (given intragastrically in different doses). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

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The tissue level of ATP (Figure 104), ADP (Figure 105), AMP (Figure 106) and cAMP (Figure 107) dose-dependently increased by intragastric administration of vitamin A and β-carotene in the animal model, whereas the ratio of ATP/ADP (Figure 108), adenylate pool (ATP+ADP +AMP) (Figure 109) and "energy charge" (Figure 110) did not change.

The actions of vitamin A and β-carotene on the gastric secretory responses, mucosal damage and biochemical parameters of gastric mucosal tissues produced by intragastric administration (in doses of 0.01, 0.1, 1.0 and 10.0 mg/kg) are summarized in Table 40.

| Parameters | Vitamin (A) | | | β-carotene (B) | | |
|---|---|---|---|---|---|---|
| | Regressio | R | P value | Regressio | R | P value |
| Gastric secretory volume | Y=-0.09x+2.71 | -0.78 | P<0.001 | Y=-0,08x+2,7 | -0.76 | P<0.001 |
| Gastric acid secretion | No significant action | | | No significant action | | |
| Number of gastric ulcers | Y=-0.97x+9.17 | -0.75 | P<0.001 | Y=-0.9x+8.9 | -0.71 | P<0.001 |
| Severity of gastric ulcer | Y=-1.75x+16.4 | -0.75 | P<0.001 | Y=-1.68x+15.7 | -0.72 | P<0.001 |
| ATP | Y=0.19x+8.19 | 0.76 | P<0.001 | Y=0.22x+7.5 | 0.84 | P<0.001 |
| ADP | Y=-0.25x+9.6 | 0.66 | P<0.001 | Y=0.36x+8.44 | 0.98 | P<0.001 |
| ATP/ADP | Y=-0.01x+0.913 | 0,56 | P<0.01 | Y=0,1x+0.84 | 0.46 | P<0.001 |
| AMP | Y=0.63x+8.96 | 0.87 | P<0.001 | Y=0.56x+8.6 | 0.89 | P<0.001 |
| Adenylate pool | No significant action | | | No significant action | | |
| cAMP | Y=1.17x+1.5 | 0.87 | P<0.001 | Y=0.18x+1.4 | 0.83 | P<0.001 |
| Energy charge | No significant action | | | No significant action | | |

**Table 40.** Correlation between gastric mucosal-preventive effects of vitamin A and β-carotene (given in doses of 0.01, 0.1, 1.0 and 10.0 mg/kg i.g.) versus the changes in gastric secretory responses and biochemical parameters in the gastric mucosa of rats treated with sodium salicylate (200 mg/kg dissolved in 2 mL of 150 mmol/L i.g.) in 4-h pylorus-ligated rats). [Mozsik et al. Inflammopharmacology 11:560–562, 2003 (with kind permission).]

### 8.4. Effect of indomethacin (IND) in 4-hour rats

#### *8.4.1. Biochemical mechanisms of indomethacin-induced gastric mucosal damage in rats (4 hours)*

The value of $ED_{5O}$ was identified for indomethacin (Djahanguiri, 1969; Karádi and Mózsik, 2000) and their value was obtained in 20 mg/kg. When IND (20 mg/kg s.c.) was given immediately after pylorus ligation, no significant decrease was obtained neither in gastric volume nor in gastric $H^+$ output. The tissue levels of ATP, AMP and cAMP decreased significantly (Király et al., 1992 a, b; Morón et al., 1982, 1983, 1984 a, b, c; Mózsik et al., 1992 b, f; Rumi et al., 2001 a, b) (Figures 111–114).

The number of ulcers in the glandular stomach was 15 ± 2, and the severity was 16 ± 2 in the indomethacin-treated animals.

The following changes in the cellular ATP-dependent energy systems were obtained in the gastric fundic mucosa of 4-hour indomethacin-treated rats: significant decrease of ATP ($P < 0.001$) and AMP ($P < 0.05$) and cAMP ($P < 0.001$), ratio of ATP/ADP ($P < 0.001$), adenylate pool ($P < 0.05$), ADP increased ($P < 0.0.5$) and changes were found in "energy charge" and tissue level of lactate. The correlation between the decrease of cAMP and indomethacin-induced gastric mucosal damage will be detailed in Section 8.4.4.

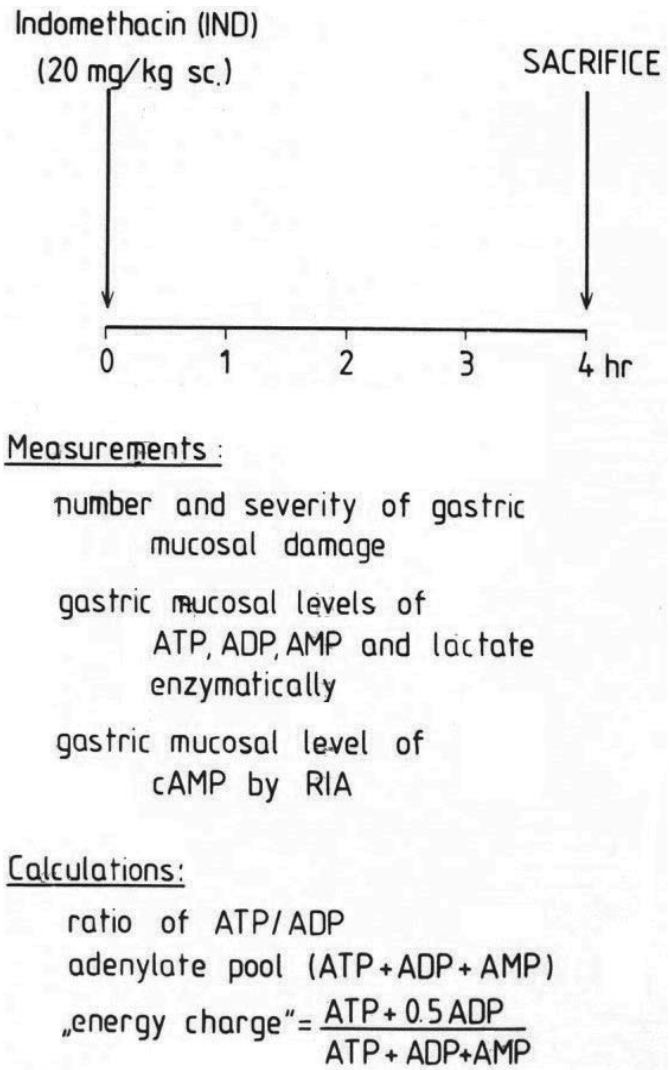


**Figure 111.** Experimental design of the study with 4-hour indomethacin-treated rats.

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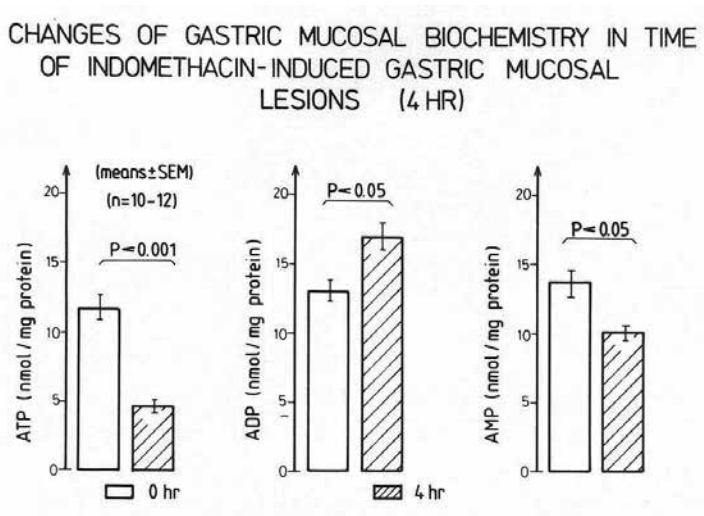


**Figure 112.** Changes in tissue levels of gastric mucosal ATP, ADP and AMP in 4-hour indomethacin-treated rats.

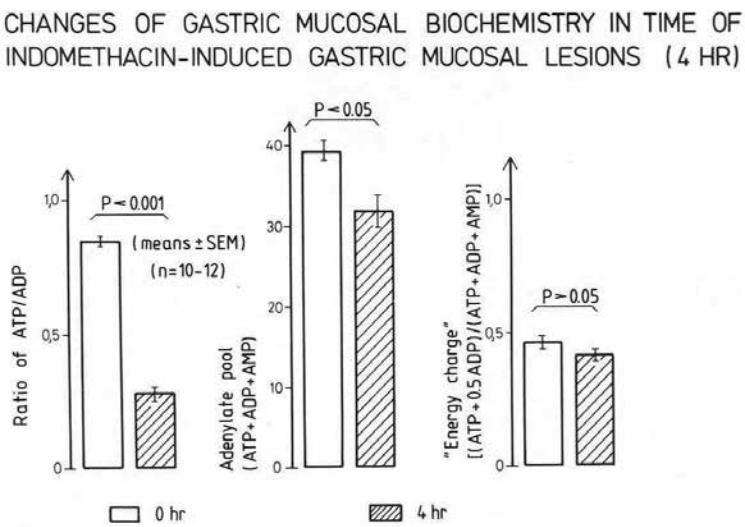


**Figure 113.** Changes in ratio of ATP/ADP, adenylate pool (ATP + ADP + AMP) and "energy charge" [(ATP + 0.5 ADP)/(ATP + ADP + AMP)] in gastric fundic mucosa of 4-hour indomethacin-treated rats.

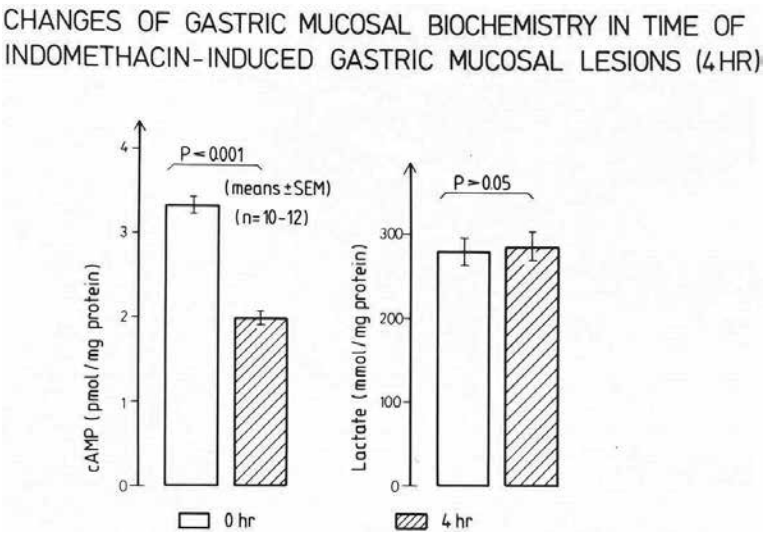


**Figure 114.** Changes in tissue levels of cAMP and lactate in the gastric fundic mucosa of 4-hour indomethacin-treated rats.

*8.4.2. Comparative biochemical studies between the gastric-protective effects and biochemical mechanisms (tissue levels of ATP, ADP, cAMP and AMP) of atropine and cimetidine in the gastric mucosa of rats treated with indomethacin*

Both atropine and cimetidine decreased dose-dependently and significantly the number (Figure 115) and severity (Figure 116).

It was interesting to note that atropine dose-dependently increased, whereas cimetidine decreased the tissue level of ATP (Figure 117), and the tissue levels of ADP could be decreased by atropine and increased by cimetidine (Figure 118). Consequently, these atropine- and cimetidine-induced changes in the tissue levels of ATP and ADP significantly modified the ratio of ATP/ADP (Figure 119), the AMP increased dose-dependently by atropine, whereas its value dose-dependently decreased (Mózsik et al., 1992f) (Figure 120).

The values of adenylate pool (ATP + ADP + AMP) were unchanged (Figure 121). The "energy charge" also did not change because of treatment with atropine or cimetidine (Figure 122). The tissue level of cAMP dose-dependently increased by atropine, whereas its value decreased by cimetidine (Figure 123).

When we measured the tissue level of lactate, we found no significant changes in case of atropine or in cimetidine (Figure 124). In the gastric juice, we could not demonstrate the presence of parenterally applied Evans blue (Figure 125).

Figures 126 (a, b) demonstrate the difference in actions of atropine and cimetidine in 4-hours rats. Although both drugs inhibit the IND-induced gastric mucosal damage, however, their biochemical effects completely differed from each other's.

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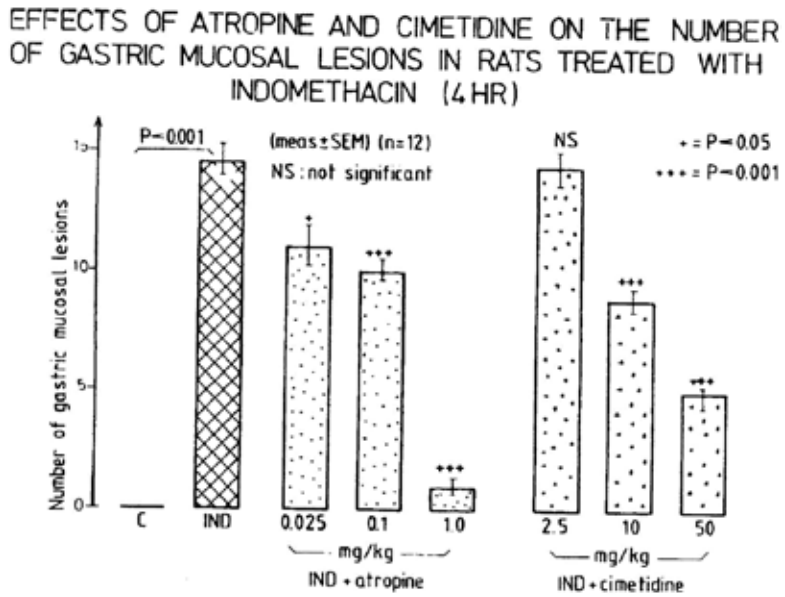


**Figure 115.** Effects of atropine and cimetidine on the number of gastric mucosal lesions in 4-hour indomethacin (IND)-treated rats. The small doses of atropine (0.025 mg/kg) and cimetidine (2.5 mg/kg) represent the cytoprotective dose of both drugs. C: indicates the results obtained from the saline-treated rats, IND: indicates the groups of animals treated with IND (20 mg/kg s.c.) alone or with atropine and cimetidine. *P* values are between the IND-treated versus IND plus atropine or IND-treated plus cimetidine-treated groups (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).]

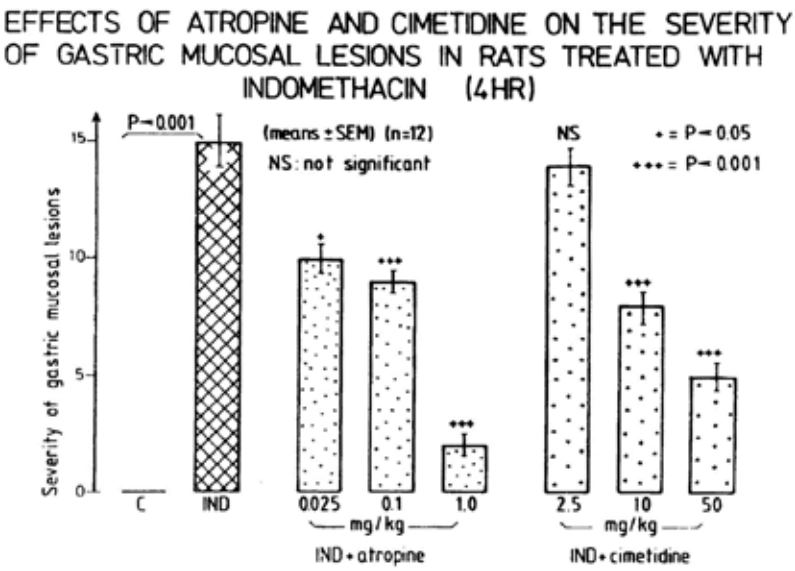


**Figure 116.** Effects of atropine or cimetidine on the severity of gastric mucosal damage produced by IND (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).]

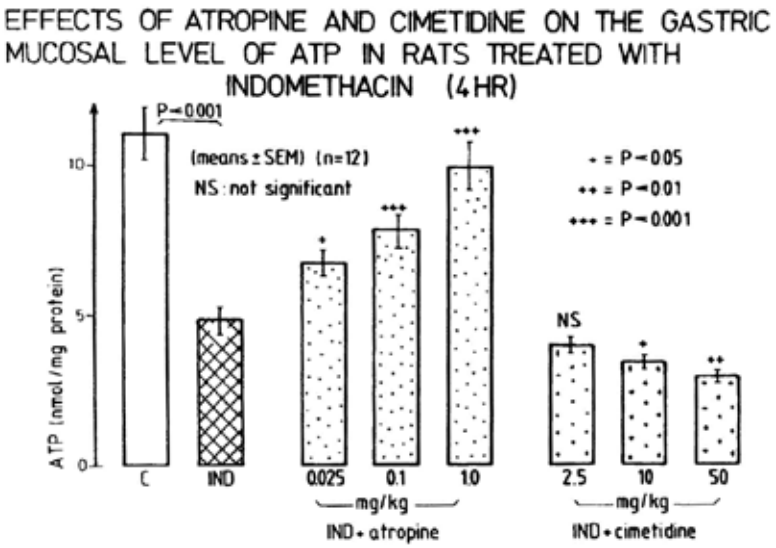


**Figure 117.** Changes in the gastric mucosal level of ATP produced by atropine and cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

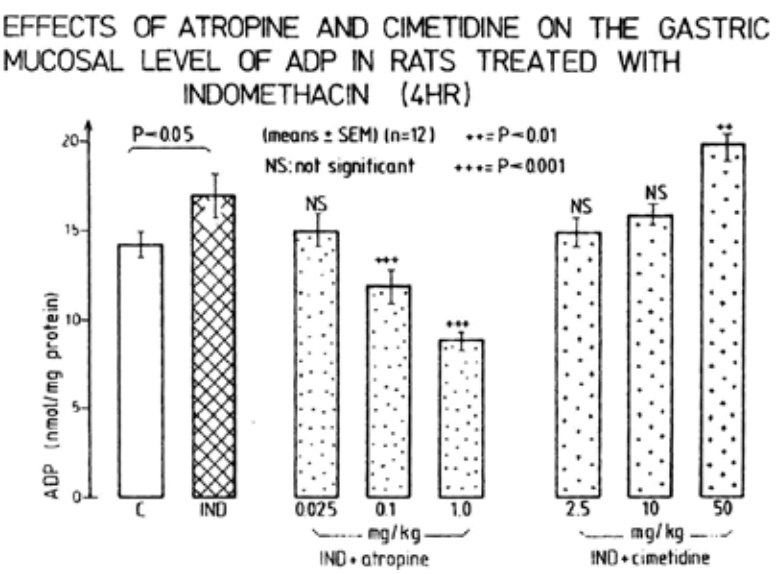


**Figure 118.** Changes in the gastric mucosal levels of ADP produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

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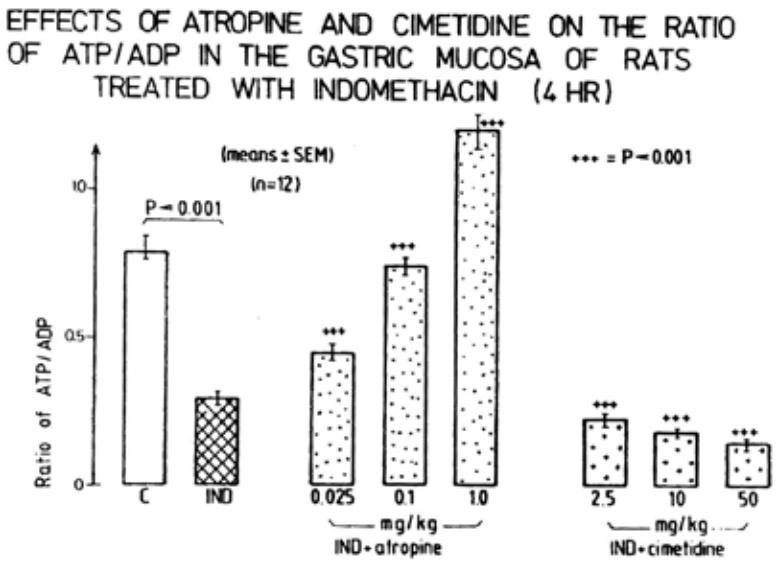


**Figure 119.** Changes in the ratio of ATP/ADP of gastric mucosa in 4-hour IND-treated rats produced by atropine or cimetidine (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

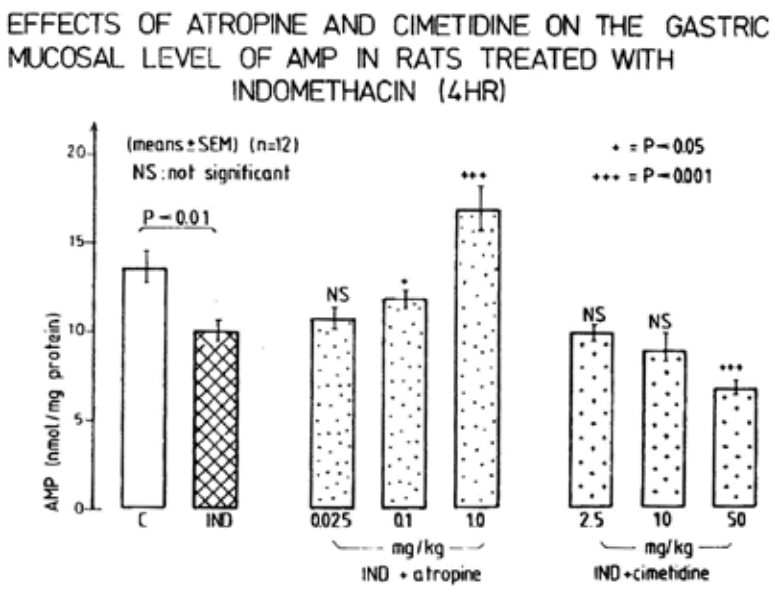


**Figure 120.** Changes in the gastric mucosal level of AMP produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

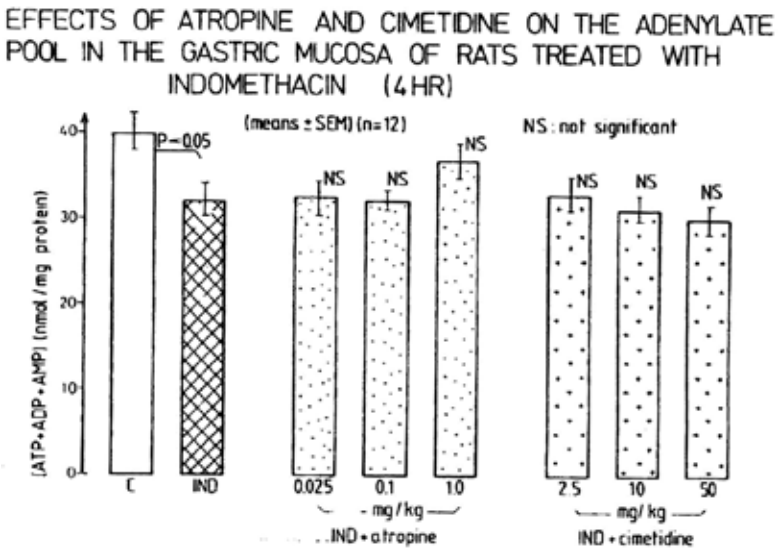


**Figure 121.** Changes in the gastric mucosal level of adenylate pool (ATP + ADP + AMP) produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

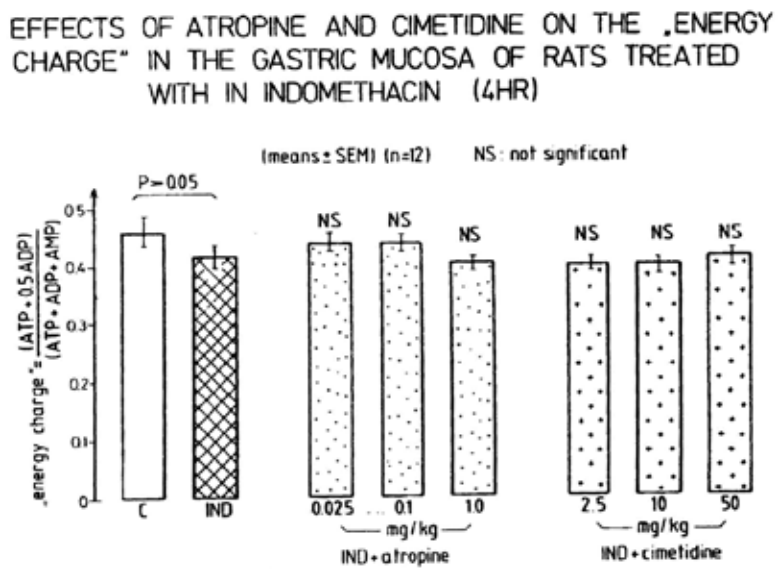


**Figure 122.** Changes in the values of "energy charge" produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

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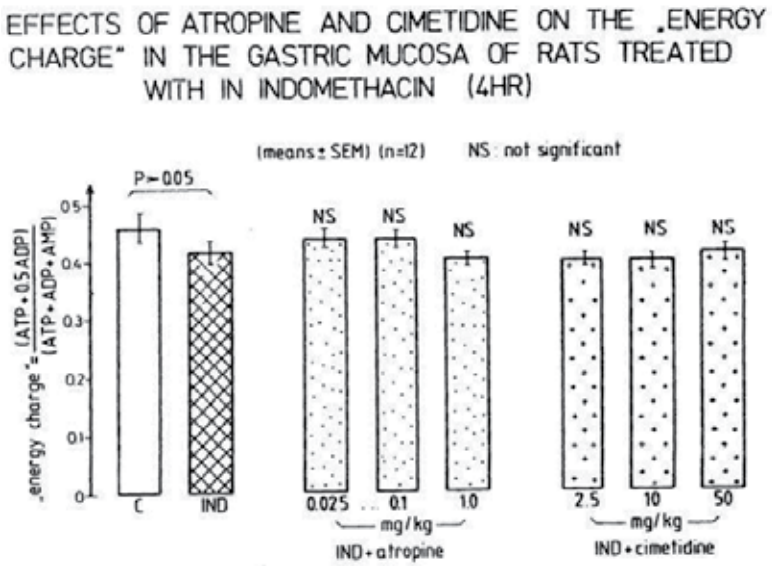


**Figure 123.** Changes in the gastric mucosal level of cAMP produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 126 a, b.

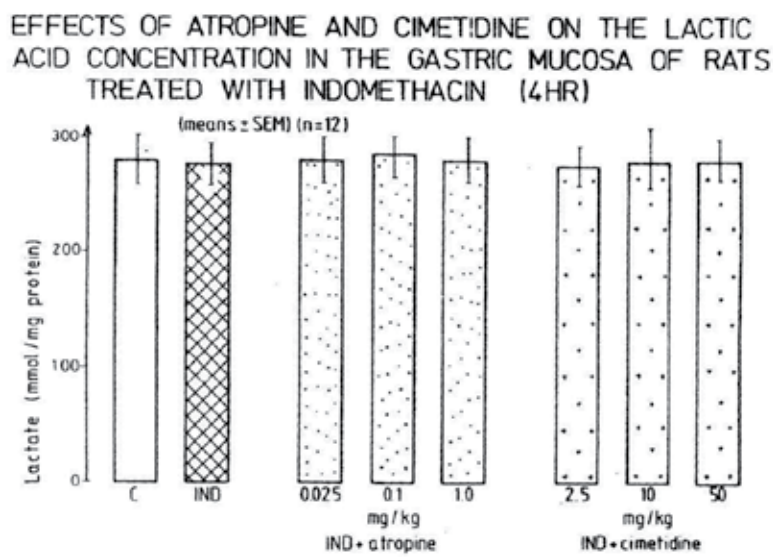


**Figure 124.** Changes in the gastric mucosal levels of lactate produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).]

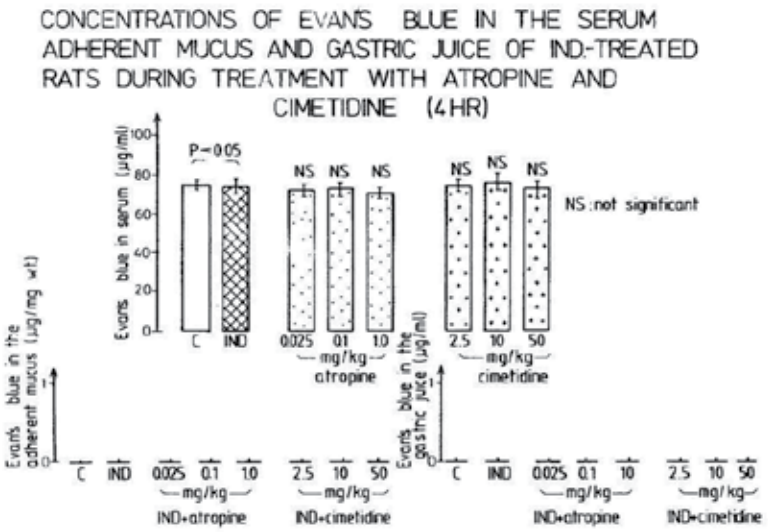


**Figure 125.** Concentration of Evan's blue in the serum, adherent gastric mucus and gastric juice produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115.

P values untreated vs. IND. and IND vs. IND plus atropine or cimetidine

| | IND | IND + atropine (mg/kg) | | | IND + cimetidine (mg/kg) | | |
|---|---|---|---|---|---|---|---|
| | | 0.025 | 0.1 | 1.0 | 2.5 | 10 | 50 |
| ulcer number | ↑↑↑ | ↓ | ↓↓↓ | ↓↓↓ | NS | ↓↓↓ | ↓↓↓ |
| ulcer severity | ↑↑↑ | ↓ | ↓↓↓ | ↓↓↓ | NS | ↓↓↓ | ↓↓↓ |
| ATP | ↓↓↓ | ↑ | ↑↑↑ | ↑↑↑ | NS | ↓ | ↓↓ |
| ADP | ↑ | NS | ↓↓↓ | ↓↓↓ | NS | NS | ↑↑ |
| ATP/ADP | ↓↓↓ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| AMP | ↓↓ | NS | ↑ | ↑↑↑ | NS | NS | ↓↓↓ |
| ATP+ADP+AMP | ↓ | NS | NS | NS | NS | NS | NS |
| „energy charge" | NS | NS | NS | NS | NS | NS | NS |
| cAMP | ↓↓↓ | NS | ↑↑↑ | ↑↑↑ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| Lactate | NS | NS | NS | NS | NS | NS | NS |

NS: not significant ↑: increase ↓: decrease
↑(↓): P < 0.05 ↑↑(↓↓): P < 0.01 ↑↑↑(↓↓↓): P < 0.001

**Figure 126.** (a) Summary of the biochemical results obtained in the gastric fundic mucosa produced by atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).] For further explanation, see Figure 115. (b) Feedback mechanism between the membrane-bound ATP-dependent energy systems produced by cytoprotective and antisecretory doses of atropine or cimetidine in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Exp. Clin. Gastroenterol. 3, 205–215, l992a (with kind permission).]

These results clearly proved that the changes in the biochemical parameters in gastric fundic mucosa are significantly opposite in case of administration of atropine and cimetidine in 4-hour indomethacin-treated rats.

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*8.4.3. Biochemical backgrounds of gastric mucosal-protective effect of vitamin A in 4-hour indomethacin-treated rats*

Vitamin A, β-carotene and other retinoids are not able to decrease the gastric acid outputs neither in pylorus-ligated rats nor in healthy human subjects. It was interesting to study the effects of vitamin A in 4-hour pylorus-ligated plus indomethacin-treated rats. In these studies, the tissue levels of ATP, ADP, cAMP and AMP were measured besides the inhibitory effects on the number and severity of IND-induced gastric mucosal damage (Figures 127,128).

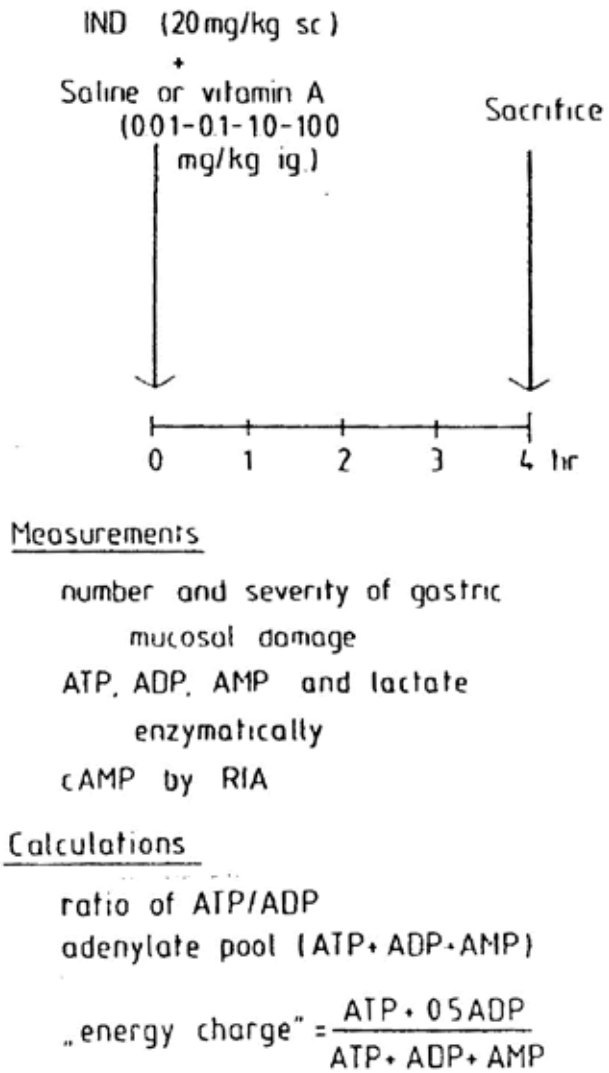


**Figure 127.** Experimental protocol for the study of gastroprotective and metabolic effects of vitamin A on 4-hour IND-treated rats. [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

Vitamin A dose-dependently decreased in both the number and severity of IND-induced gastric mucosal damage (Figure 128). During the development of gastric mucosal effect, the tissue levels of ATP significantly decreased in association with the significant increase of tissue ADP (Mózsik and Jávor, 1991; Mózsik et al., 1989c, 1996a) (Figure 129).

No change was obtained in the adenylate pool (Figure 130). It was interesting to note that the dose-dependent increase of cAMP (Figure 131) also increased slightly the "energy charge" and the ratio of ATP/ADP (Figure 132).

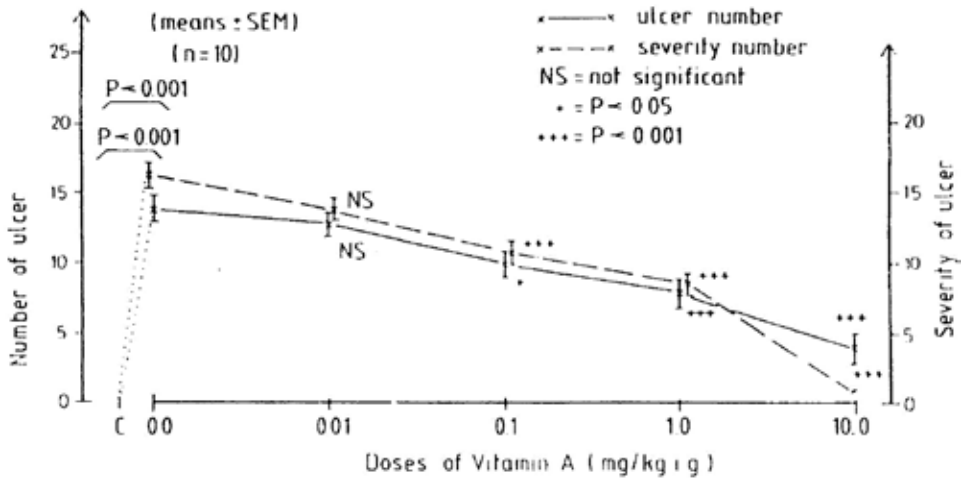


**Figure 128.** Changes in the number and severity of gastric mucosal lesions prevented by the application of different doses of vitamin A in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

When the changes in tissue levels of ATP, ADP, AMP and cAMP and the number of gastric mucosal damage were simultaneously compared, similar changes were obtained as those by atropine (Figure 133).

### *8.4.4. Gastroprotective effect of β-carotene differs from the prostaglandin system in 4-hour indomethacin-treated rats*

The details of the gastric mucosal-protective effect of vitamin A were studied – in a dose-dependent manner – in the previous Section (8.4.3). In this section, the correlations between the gastric mucosal-protective effect of β-carotene (as a provitamin for vitamin A), gastric mucosal levels of $PGE_2$ and cAMP were analyzed depending on the application of different doses of β-carotene and on time (the examinations were carried out at the 1, 2, 3 and 4 hours after indomethacin application) in indomethacin-treated rats. Earlier, it was proved that the carotenoids (including the β-carotene) prevent the chemically induced gastric mucosal damage (Jávor et al., 1983) by cytoprotection (without decreasing the gastric acid secretion).

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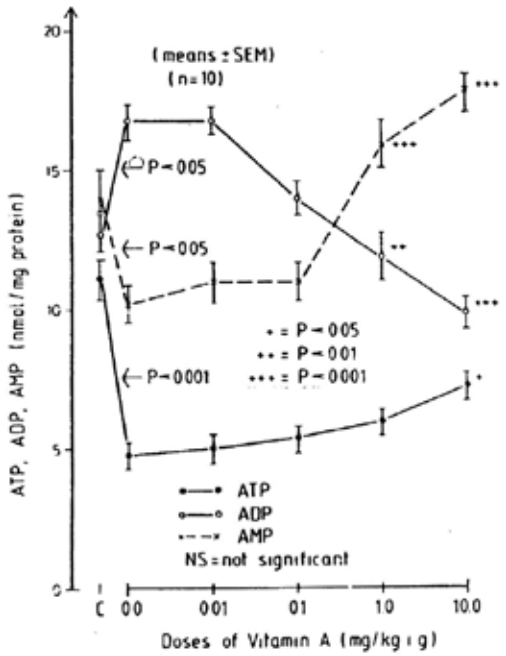


**Figure 129.** Changes in the gastric mucosal levels of ATP, ADP, AMP produced by vitamin A in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

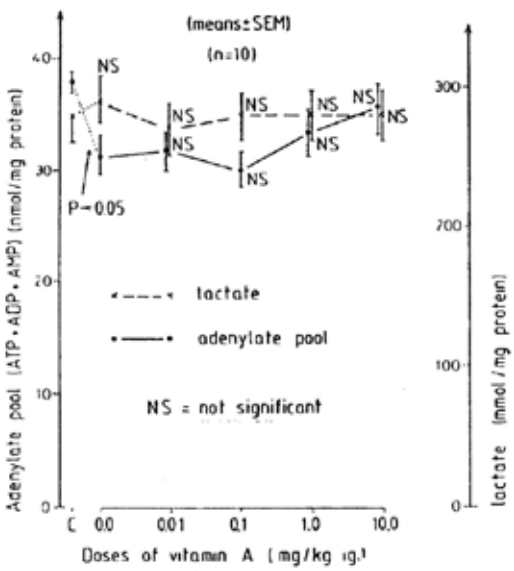


**Figure 130.** Changes in the gastric mucosal levels of adenylate pool and lactate produced by vitamin A in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

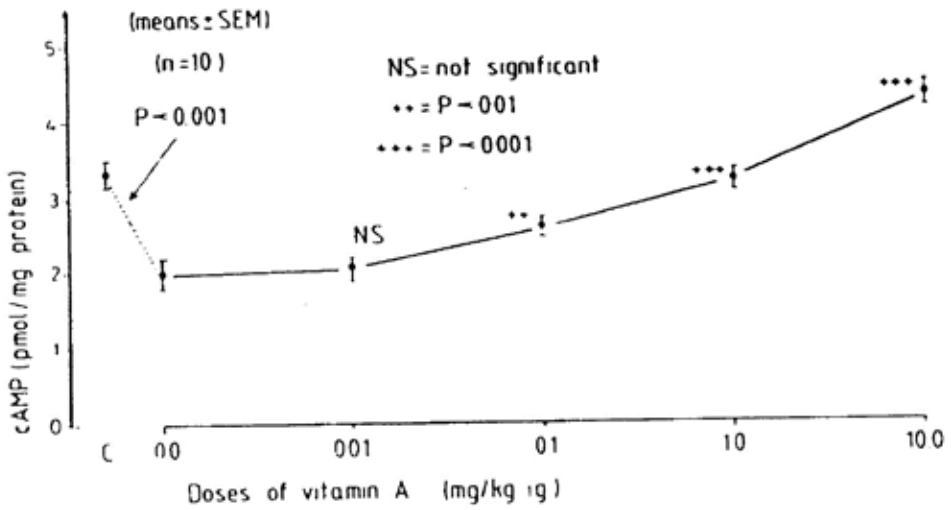


**Figure 131.** Changes in the gastric mucosal level of cAMP produced by vitamin A in 4-hour IND-treated rats (means ± SEM). [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

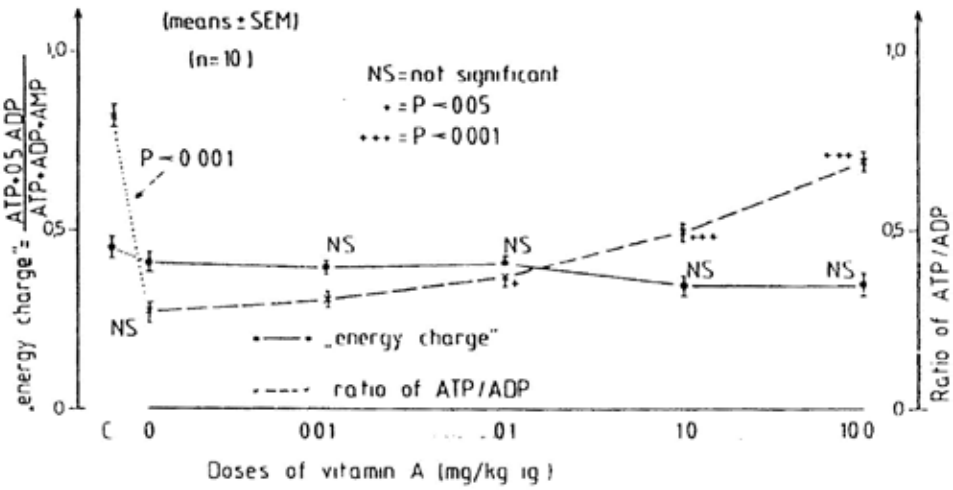


**Figure 132.** Changes in the gastric mucosal "energy charge" and ratio of ATP/ADP produced by vitamin A in 4-hour IND-treated rats (means ± SEM). [Mózsik et al. Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

The aims of these observations were as follows:

1. To evaluate the details of β-carotene-induced gastric mucosal prevention in rat model experiment;
2. Indomethacin produces inhibitory effect of the prostaglandin synthesis (and we proved this in rats in Section 8.4.3.) in 4-hour indomethacin-treated rats;

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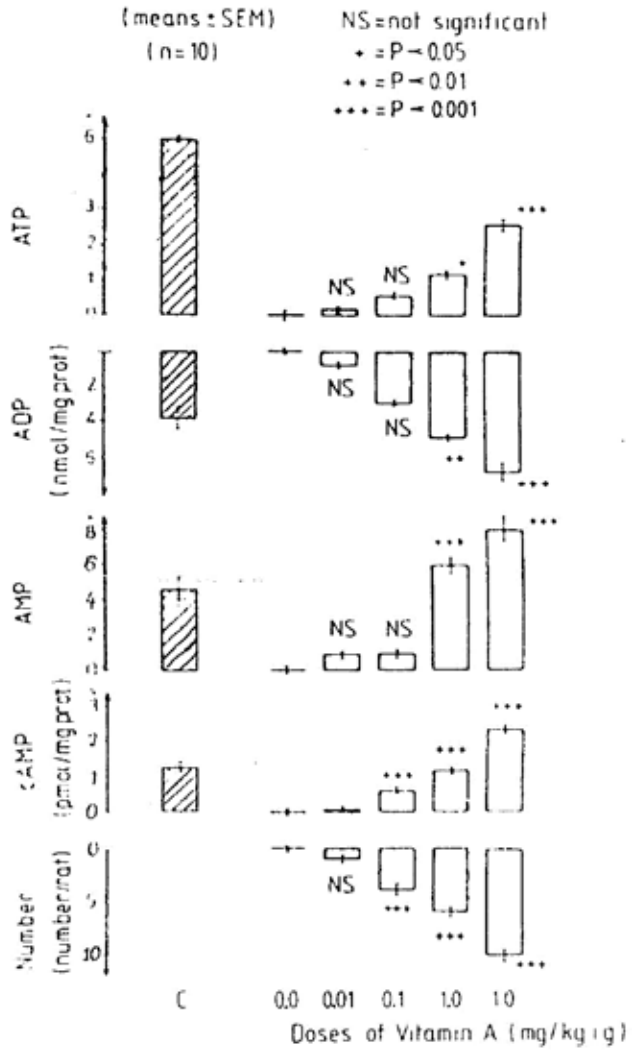


**Figure 133.** Regulatory pathways between the IND-induced gastric mucosal damage and vitamin A-induced changes in the 4-hour indomethacin-treated rat gastric fundic mucosa (means ± SEM). [Mózsik et al.: Int. J. Tiss. Reac. 11, 65–71, 1989c (with kind permission).]

3. To evaluate the possible correlations between the changes (decrease vs. development of indomethacin ulcer and increase vs. mucosal protection) of tissue levels of $PGE_2$ in indomethacin-treated rats;

4. To find (and to prove) the existence of β-carotene-induced mucosal protection versus changes (increase) in the gastric mucosal level of $PGE_2$ system; and

5. Is it really a well-established scientific fact that β-carotene produces only the scavenger pathway?

The experimental protocols (Figure 134) and the main results are presented in Figure 135.

Figure 136 indicated that a close correlation exists between the gastric fundic mucosal level of cAMP at 4 hours of experiments. We also indicated in many experiments that the responsible biochemical changes appeared earlier than the macroscopic appearance of gastric mucosal damage. So it was suggested that a similar correlation exists between the cAMP level in the gastric fundic mucosa versus clinical appearance of gastric mucosal damage, therefore the correlation between the tissue level of cAMP in the gastric fundic mucosa at 1 hour versus macroscopic images (number and severity) at 4 hours after indomethacin administration. Figures 137 and 138 clearly proved these correlations were clearly positive between these parameters.

When β-carotene (given intragastrically at doses of 0.1, 1.0 and 10 mg/kg dissolved in oleum helianthi) was given, then both the number (Figure 139) and severity (Figure 140) decreased dose-dependently. Interestingly, β-carotene produced a dose-dependent increase in the gastric fundic mucosal level of cAMP at 1 hour and at 4 hours after indomethacin administration (Figure 141).

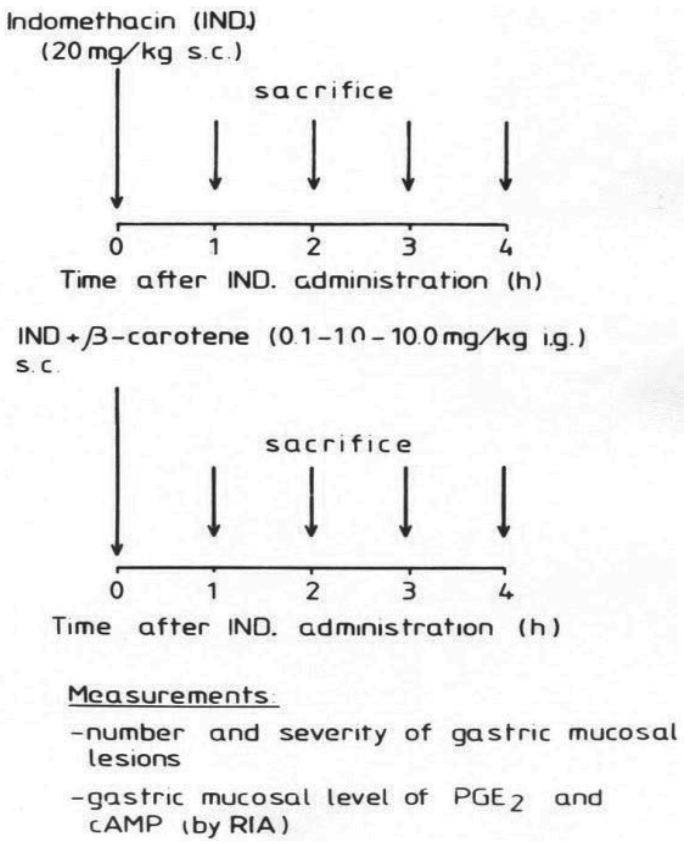


**Figure 134.** Design of the animal experiments in rats. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

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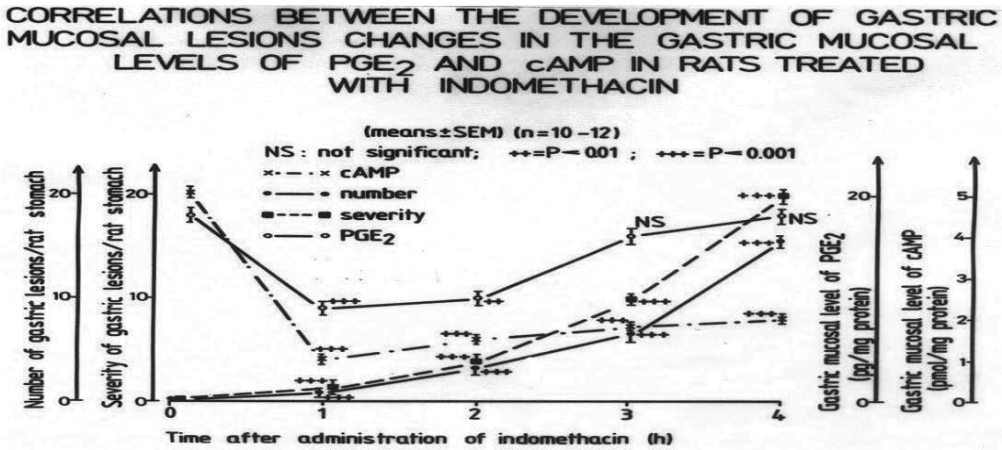


**Figure 135.** Correlations between the development of gastric mucosal damage (number and severity), changes in the tissue levels of $PGE_2$ and cAMP in the gastric fundic mucosa of 4-hour indomethacin-treated rats (at 0, 1, 2, 3 and 4 hours after administration of indomethacin). [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

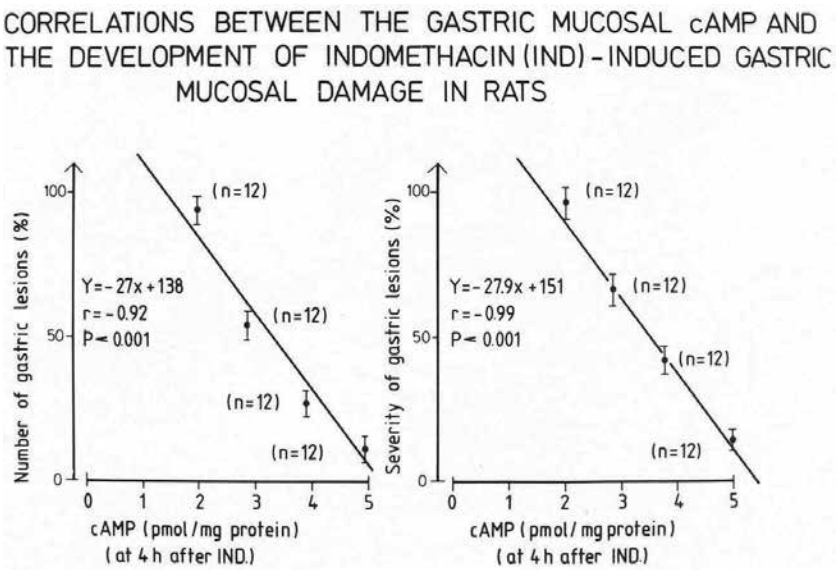


**Figure 136.** Correlation between the decrease of gastric mucosal level of cAMP (at 4 hours) versus number (and severity) (at 4 hours) in the gastric fundic mucosal damage in 4-hour indomethacin-treated rats. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

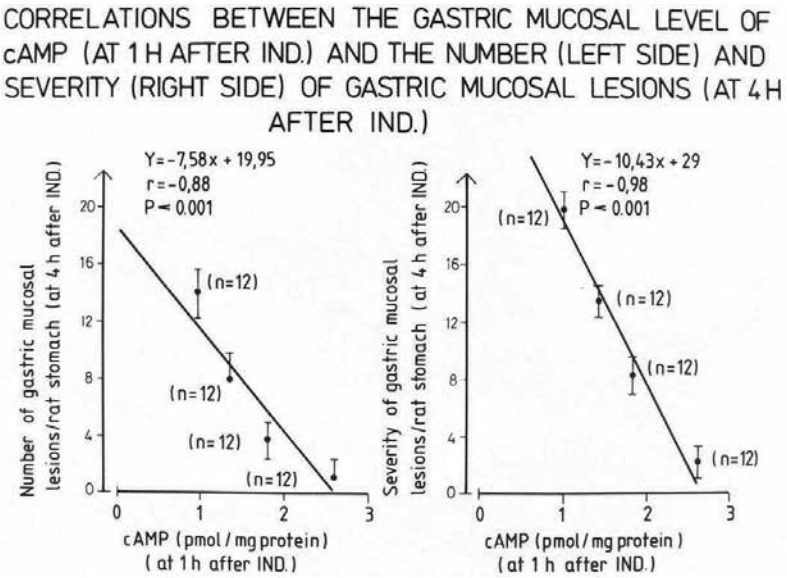


**Figure 137.** Correlations between the decrease of gastric fundic mucosal level of cAMP at 1 hour versus gastric mucosal damage (number and severity) at 4 hours in 4-hour indomethacin-treated rats. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

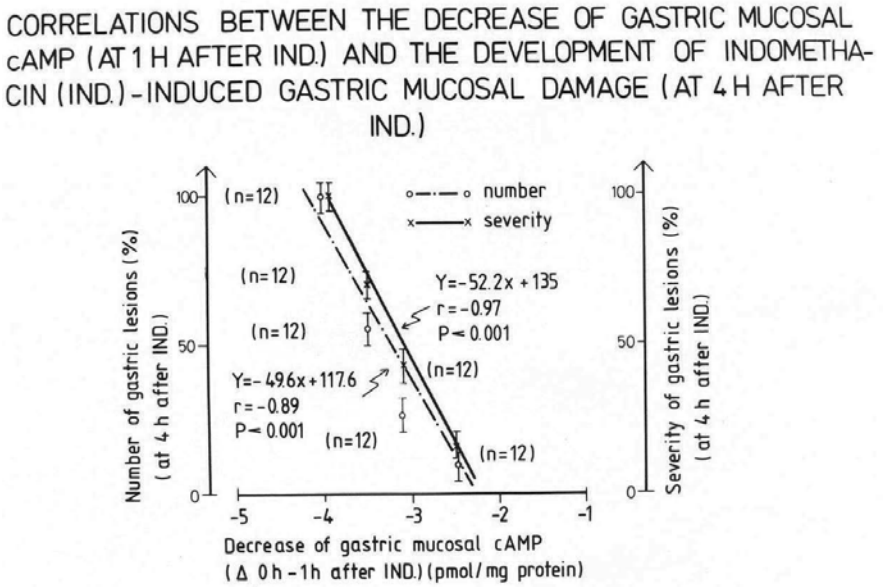


**Figure 138.** Similar results were obtained between the Δ values of decrease in gastric fundic mucosal level of cAMP (Δ 0–1 hour after IND) versus number and severity (expressed in percent values) (at 4 hours after IND) in 4-hour indomethacin-treated rats. This figure indicates that the existence of this correlation is practically the same in cases of both development of number and severity of gastric mucosal damage in 4-hour indomethacin-treated rats. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

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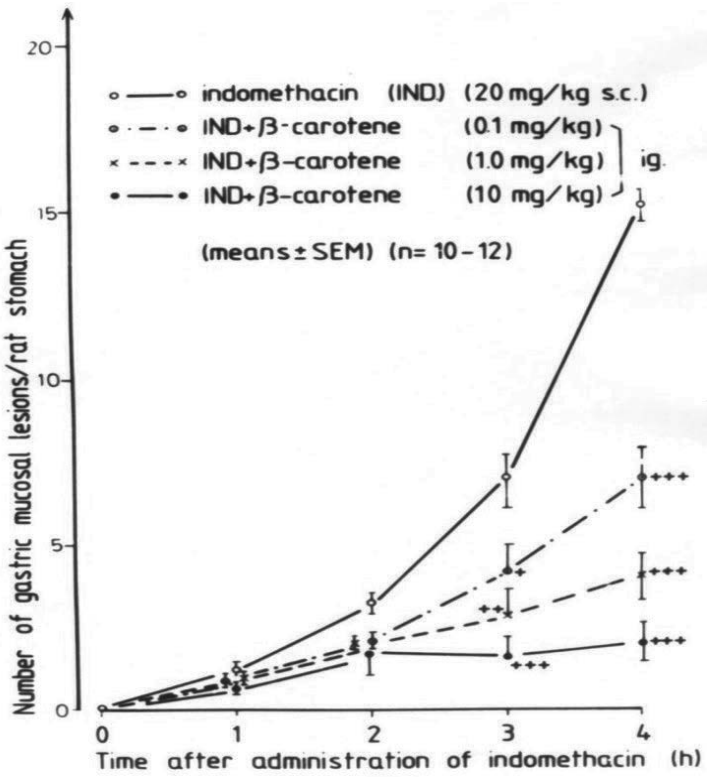


**Figure 139.** β-carotene-induced gastric fundic mucosal protection in 4-hour indomethacin-treated rats. The ordinate indicates the number of lesions, while the abscissa shows the time (in hours) after indomethacin administration. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

No similar correlations were obtained between the changes in the gastric fundic mucosal $PGE_2$ level versus development of the gastric mucosal damage (and/or protection). The conclusions are as follows:

**1.** The decrease of cAMP in the gastric fundic mucosa appears at 1 hour after administration of indomethacin, and its level shows a moderate increase in the time period from 1 to 4 hours in 4-hour indomethacin-treated rats;

**2.** The extent of decrease of tissue levels of $PGE_2$ and cAMP differ significantly (including the different time periods) in 4-hour indomethacin-treated rats;

**3.** β-carotene, in a dose-dependent manner, prevents the indomethacin-induced gastric mucosal damage in association with dose- and time-dependent increase of gastric fundic mucosal cAMP level; however, no increase in gastric fundic mucosal $PGE_2$ was obtained during β-carotene-induced gastric mucosal damage;

**4.** β-carotene-induced gastric mucosal protection (in indomethacin model) differs from the gastric mucosal level of $PGE_2$;

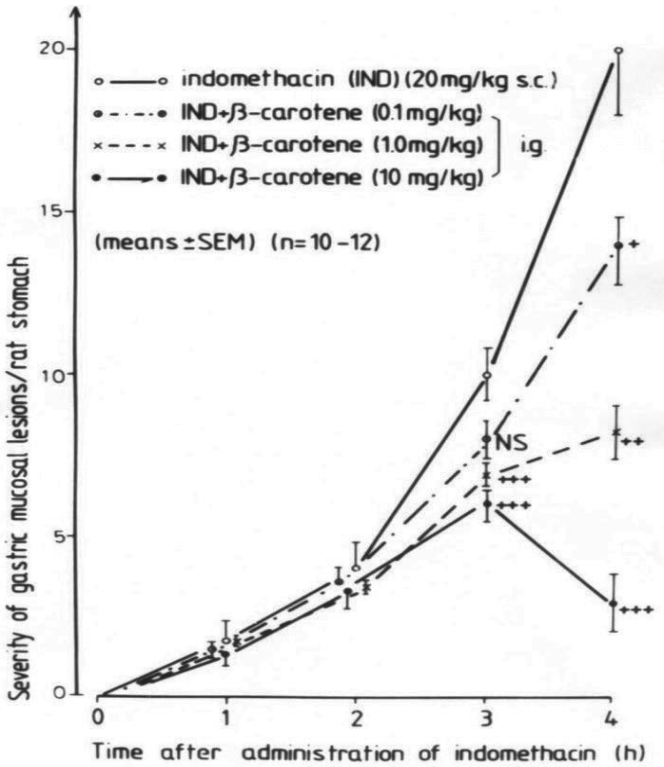


**Figure 140.** β-carotene-induced gastric fundic mucosal protection in 4-hour indomethacin-treated rats. The ordinate indicates the severity of lesions, while the abscissa shows the time (in hours) after indomethacin administration. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

**5.** The decrease in gastric fundic mucosal cAMP is a responsible intracellular signal for the development of indomethacin-induced gastric mucosal damage, and the increase of cAMP by β-carotene is responsible for the development of β-carotene-induced gastric mucosal protection;

**6.** The treatment with vitamin A and β-carotene produces gastric mucosal protection against indomethacin-induced mucosal damage by a very complex extra- and intracellularly existing regulatory systems of the membrane-bound ATP-dependent energy systems in the rat gastric mucosa (Mózsik et al., 1990 a, b, c).

### 8.5. Changes in the gastric mucosal energy systems during 5-hour stress in rats

The aims of this study were as follows:

**1.** To evaluate the changes in the membrane-bound ATP-dependent energy systems during the development of stress-induced gastric mucosal damage;

**2.** To prove (or to exclude) the presence of tissue hypoxia in the rat gastric mucosa during development of gastric mucosal damage;

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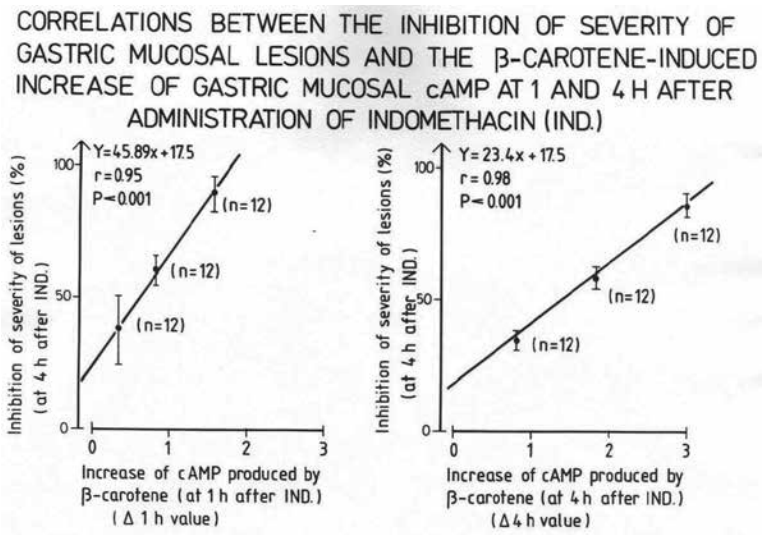


**Figure 141.** Correlations between the Δ values of gastric fundic mucosal level of cAMP at 1 hour (left figure) and at 4 hours (right figure) versus mucosal-protective effect of β-carotene on the severity of gastric mucosal damage (expressed in percent values) in 4-hour indomethacin-treated rats. [Mózsik, Bódis, Karádi, Király, Nagy, Rumi, Sütő, Szabó (1998). Inflammopharmacology 6: 27–40 (with kind permission).]

3. To present further evidence of a feedback mechanism existing between ATP-ADP and ATP-cAMP transformations;

4. To analyze the different biochemical changes in the gastric mucosa before and after macroscopic appearance of gastric mucosal damage; and

5. To study the direct effect of epinephrine, cAMP and AMP on $Na^+$-$K^+$-dependent ATPase prepared from the rat gastric fundic mucosa.

The animals were forced to swim in water below the body temperature (24 °C) for maximally 5 hours. They were sacrificed at 0, 1, 2, 3, 4 and 5 hours after the introduction of stress. The stress produced gastric mucosal lesions in the glandular portion of the stomach (its incidence is 100%). After sacrificing the animals (at the different times after the introduction of stress) (Figure 142), the number (and severity) of gastric mucosal lesions was registered and different biochemical examinations were carried out from the rat gastric fundic mucosa as the following:

1. The measurements of tissue levels of ATP, ADP, AMP, lactate and cAMP (and different calculations of the obtained results were performed between the results obtained in the measurements);

2. The preparation of membrane ($Mg^{2+}$-dependent and $Na^+$–$K^+$-dependent) ATPase was prepared and its activity was measured;

3. The effects of epinephrine, cAMP and AMP were measured on the $Na^+$–$K^+$-dependent ATPase activity prepared from the rat gastric fundic mucosa (for details, see Mózsik et al., 1990a).

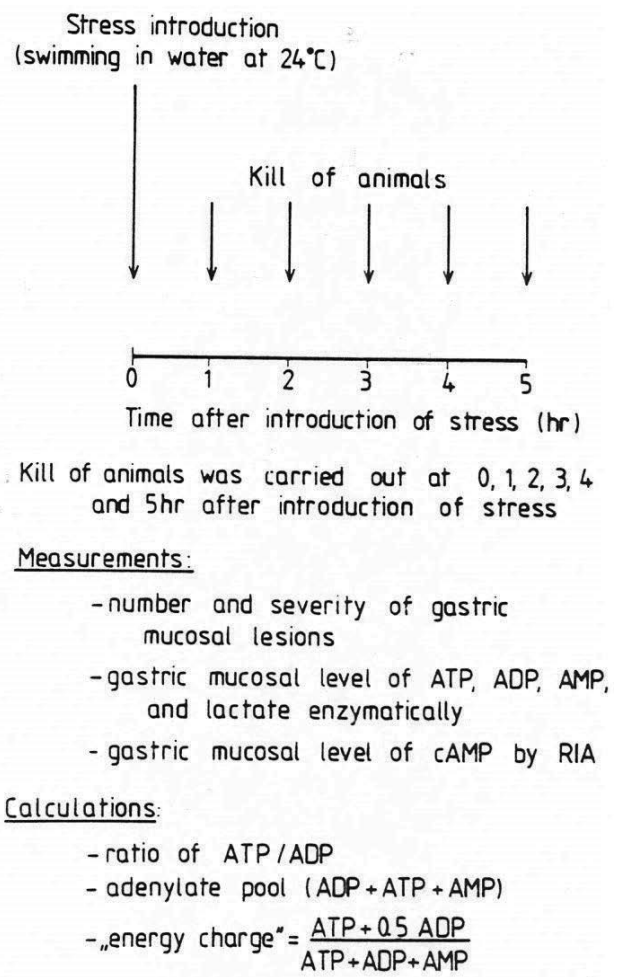


**Figure 142.** Experimental protocol of study of stress-induced gastric ulcer in rats [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

The macroscopic appearance of gastric fundic mucosal lesions could be detected at the 2nd hour after the introduction of stress (Nagy et al., 1982, 1983; Mózsik et al., 1990a) (Figure 143). The results of biochemical examinations are presented in Figures 114–156 and Table 41.

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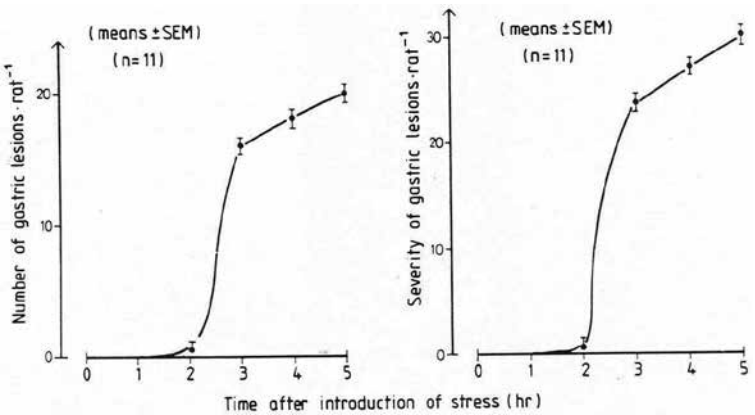


**Figure 143.** Macroscopic appearance of gastric mucosal lesions (number and severity) after the introduction of stress (means ± SEM) [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

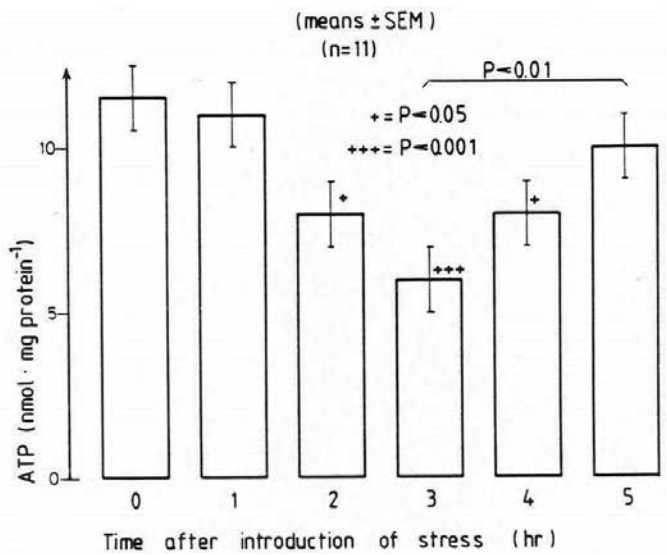


**Figure 144.** Changes in gastric mucosal level of adenosine triphosphate (ATP) during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

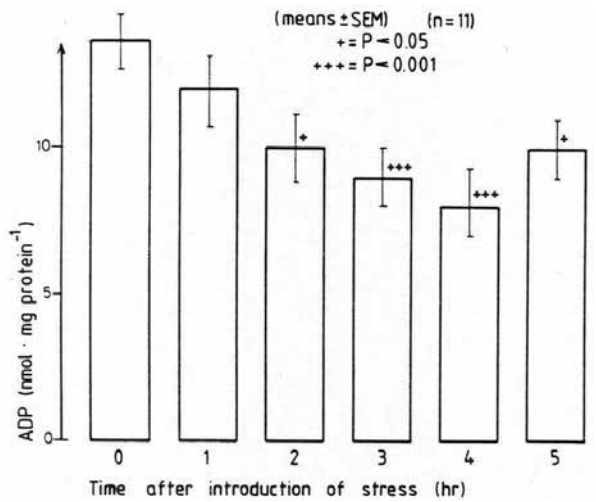


**Figure 145.** Changes in gastric mucosal level of adenosine diphosphate (ADP) during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

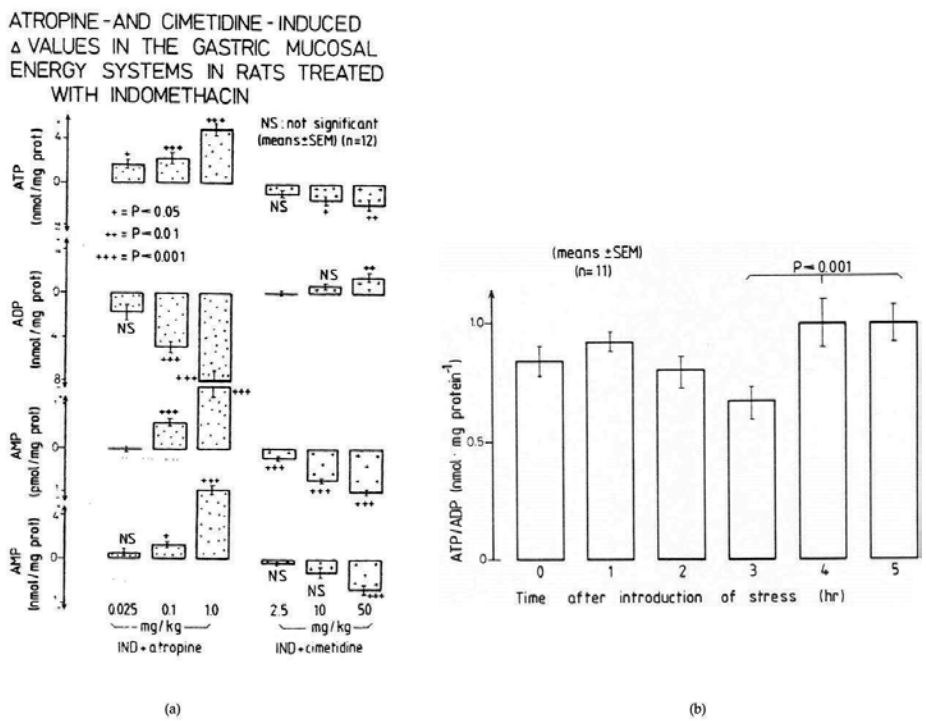


**Figure 146.** Stress-induced changes in the ratio of ATP–ADP (ATP/ADP) in the gastric mucosa of rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

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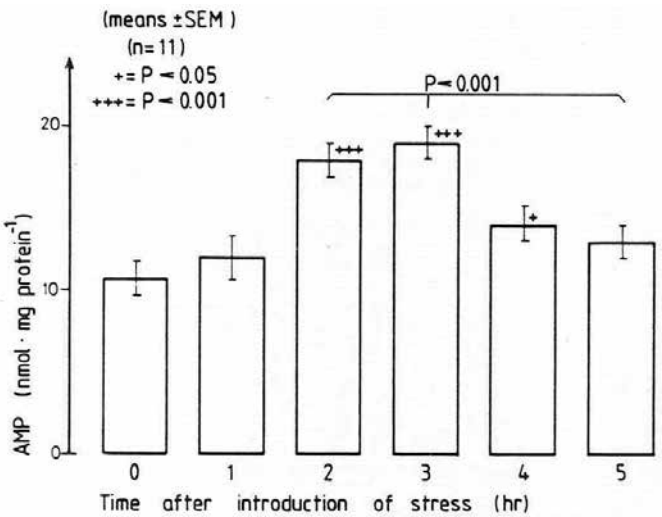


**Figure 147.** Changes in gastric mucosal level of adenosine monophosphate (AMP) during the development of stress-induced gastric mucosal lesions in rats. (Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

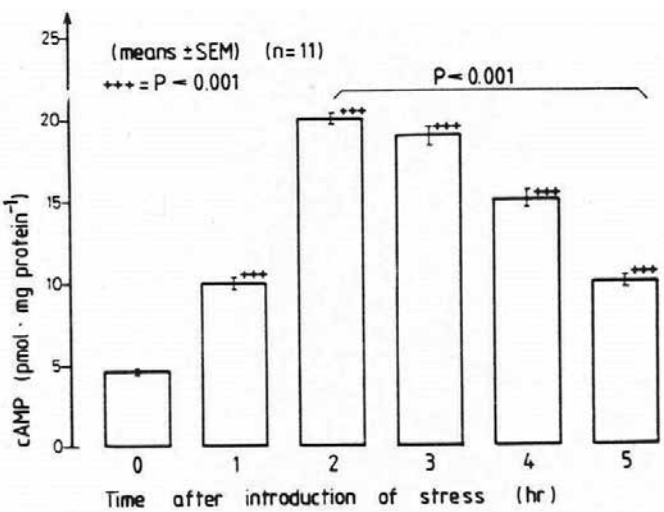


**Figure 148.** Changes in gastric mucosal level of cyclic adenosine monophosphate (cAMP) during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

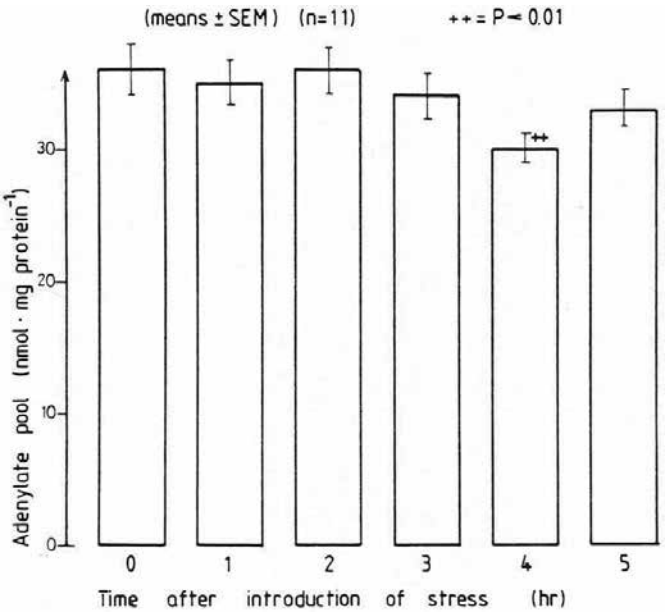


**Figure 149.** Tissue levels of adenylate pool (ATP + ADP + AMP) in the gastric mucosa during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

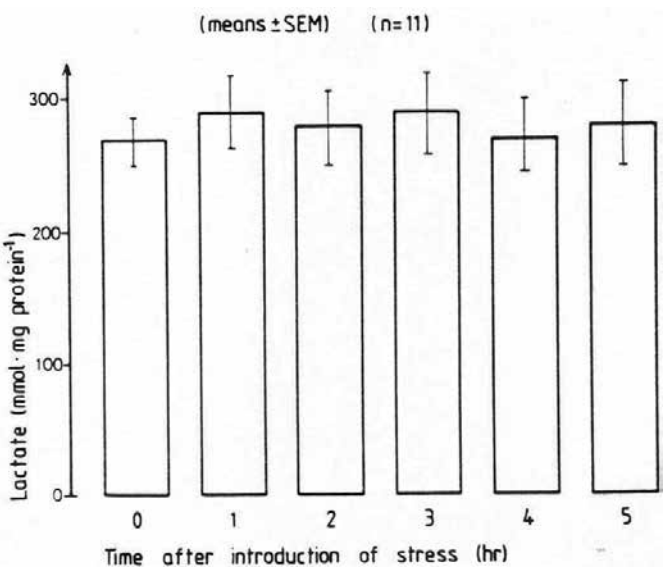


**Figure 150.** Gastric mucosal level of lactate during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

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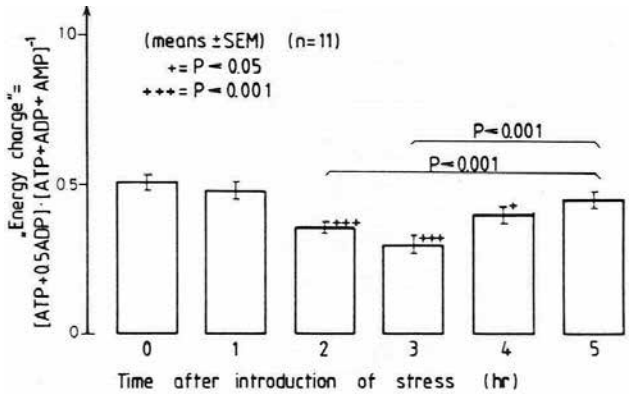


**Figure 151.** Changes in the gastric mucosal level of "energy charge" [(ATP + 0.5 ADP)/(ATP + ADP + AMP)] during the development of stress-induced gastric mucosal lesions in rats. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

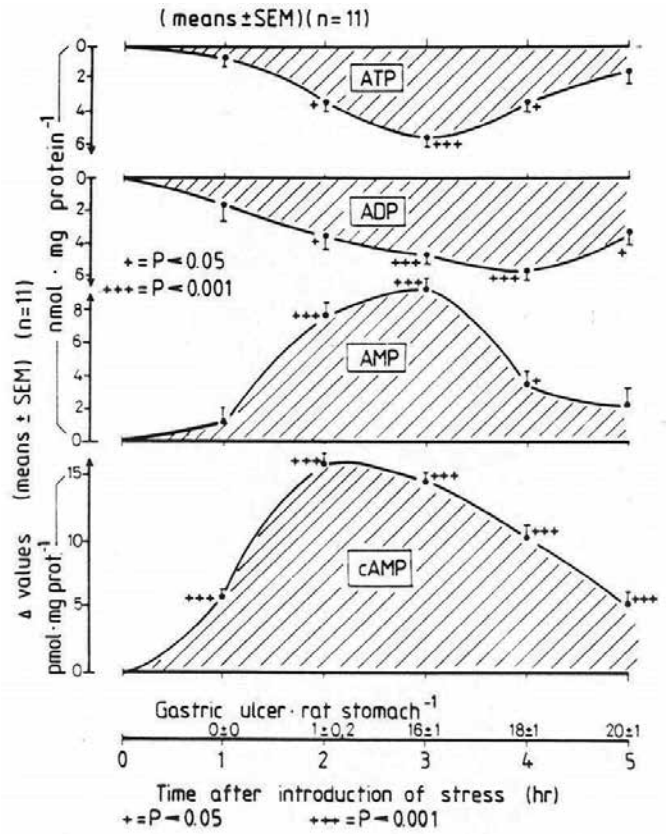


**Figure 152.** Correlations between changes in energy systems and development of gastric mucosal lesions in rats. Changes in the gastric mucosal levels of ATP, ADP, AMP and cAMP are expressed as Δ values. [Mózsik et al., Ann N Y Acad Sci 597: 264–281, 1990a (with kind permission).]

It was interesting to note that cAMP decreased significantly in 24 and 4 hours, with the increased level of cAMP being associated with gastric mucosal prevention in many models (pylorus-ligated plus IND-treated animals and the effects of atropine and vitamin A) (Mózsik et al., 1978c; 1979 a, b; 1988a, b; 1992 a, b, c, d, e; 1996 a, b; 1997 b, c; 1998).

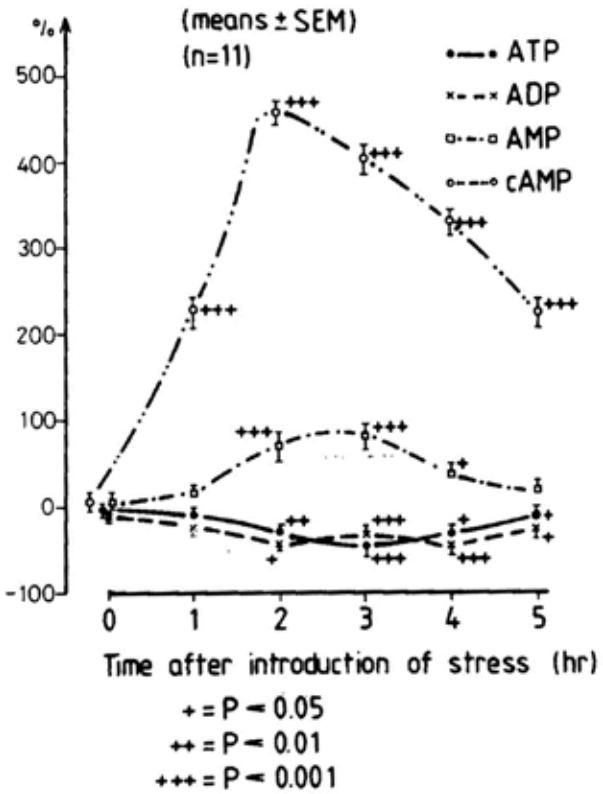


**Figure 153.** Regulatory mechanisms between the tissue levels of ATP, ADP, AMP and cAMP during stress-induced gastric mucosal biochemistry (means ± SEM). [Mózsik et al., Ann N Y Acad Sci 597, 264–281, l990 (with kind permission).]

| Drugs | $pD_2$ | $pA_2$ | $\alpha$ |
|---|---|---|---|
| Epinephrine | 8.60 | 8.40 | 0.41 |
| cAMP | 11.80 | 10.00 | 0.48 |
| AMP | 8.80 | 8.70 | 0.90 |
| Oabain | 5.90 | 5.80 | 1.00 |

**Table 41.** Values of affinities ($pD_2$) and intrinsic activities ($pA_2$) ($\alpha_{atropine} = 1.00$) for epinephrine, cAMP, AMP and ouabain on $Na^+$–$K^+$-dependent ATPase activity prepared from the rat gastric fundic mucosa. The necessary dose to produce 50% action of affinity ($pD_2$) and intrinsic activity ($pA_2$) is expressed in [−] molar values. [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

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We followed the changes in the ratio of ATP/ADP and "energy charge" (Figure 154). No significant change in ATP/ADP was found.

MEAN STEPS OF THE BIOCHEMICAL BACKGROUNDS OF THE DEVELOPMENT OF STRESS-INDUCED GASTRIC MUCOSAL DAMAGE IN RATS

(UNTREATED RATS)

$ATP \cdot ADP^{-1}$: 0.84 ± 0.06

„Energy charge" = 0.51 ± 0.02

(n=11)

Time after introduction of stess (hr)

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $ATP \cdot ADP^{-1}$ | 0.92 ± 0.09 | 0.80 ± 0.08 | 0.67 ± 0.08 | 1.00 ± 0.10 | 1.00 ± 0.08 |
| „energy charge" | 0.48 ± 0.03 | 0.36 ± 0.01$^{+++}$ | 0.30 ± 0.02$^{+++}$ | 0.40 ± 0.02$^{+}$ | 0.45 ± 0.02 |
| | (n=11) | (n=11) | (n=11) | (n=11) | (n=11) |

+ = P < 0.05

+++ = P < 0.001

**Figure 154.** Changes in the ratio of ATP/ADP and in "energy charge" dependent upon time after the introduction of stress (means ± SEM). [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

CHANGES IN THE RAT GASTRIC MUCOSAL BIOCHEMISTRY DURING DEVELOPMENT OF STRESS ULCER PRODUCED BY SWIMMING IN THE WATER

Time after introduction of streess (hr)

| | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Ulcer number | | | | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| Ulcer severity | | | | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| ATP | | | ↓ | ↓↓↓ | ↓ | |
| ADP | | | ↓ | ↓↓↓ | ↓↓↓ | ↓ |
| $ATP \cdot ADP^{-1}$ | | | | | | |
| AMP | | | ↑↑↑ | ↑↑↑ | ↑ | |
| ATP+ADP+AMP | | | | | ↓ | |
| „energy charge" | | | ↓↓↓ | ↓↓↓ | ↓ | |
| cAMP | | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| Lactate | | | | | | |

↑ = increase ↓ = decrease

↑(↓) = P < 0.05 ↑↑↑(↓↓↓) = P < 0.001

**Figure 155.** Time-sequence changes between the macroscopic development of gastric mucosal damage and biochemical parameters in the gastric fundic mucosa dependent upon time after the introduction of stress (means ± SEM). [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

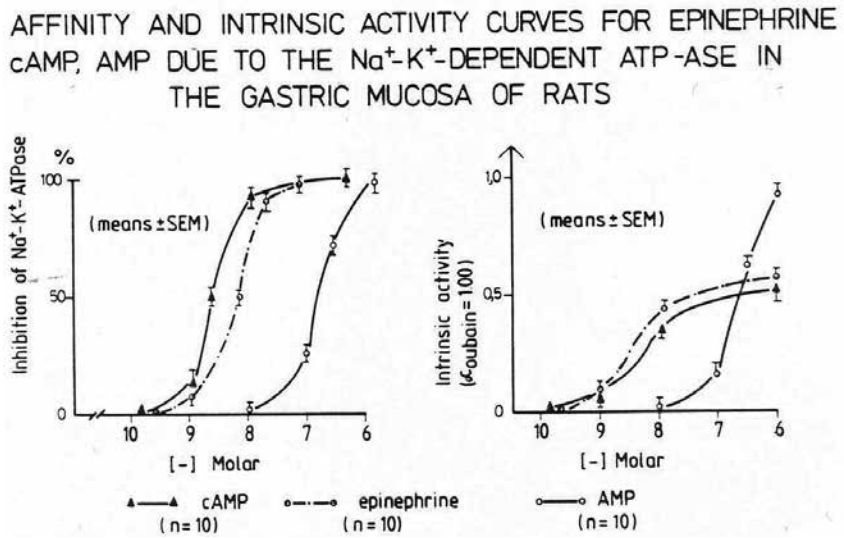


**Figure 156.** Affinity and intrinsic activity curves for inhibition by epinephrine, cAMP, AMP and ouabain of $Na^+$–$K^+$-dependent ATPase prepared from the rat gastric fundic mucosa. [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

The results presented in this chapter are as follows:

1. Gastric mucosal lesions appeared and increased gradually 3 hours after the introduction of stress;
2. The extent of ATP–cAMP and cAMP–AMP transformations was increased considerably during the development of stress ulcer;
3. The extent of ATP–ADP transformation was completely inhibited;
4. The activity of $Na^+$–$K^+$-dependent ATPase from the rat gastric fundic mucosa could be inhibited by epinephrine, cAMP and AMP;
5. The ratio of ATP/ADP was unchanged during the first time period (from 0 to 3 hours), after its value increased;
6. The value of "energy charge" (e.g., the extent of phosphorylation and/or dephosphorylation) of cells was decreased at 2 and 3 hours, after which its value returned to a normal level;
7. There was no increase in the tissue level of lactate;
8. Several biochemical changes (decrease of ATP, ADP and "energy charge", increase of cAMP and AMP) preceded the macroscopic appearance of stress ulcer.

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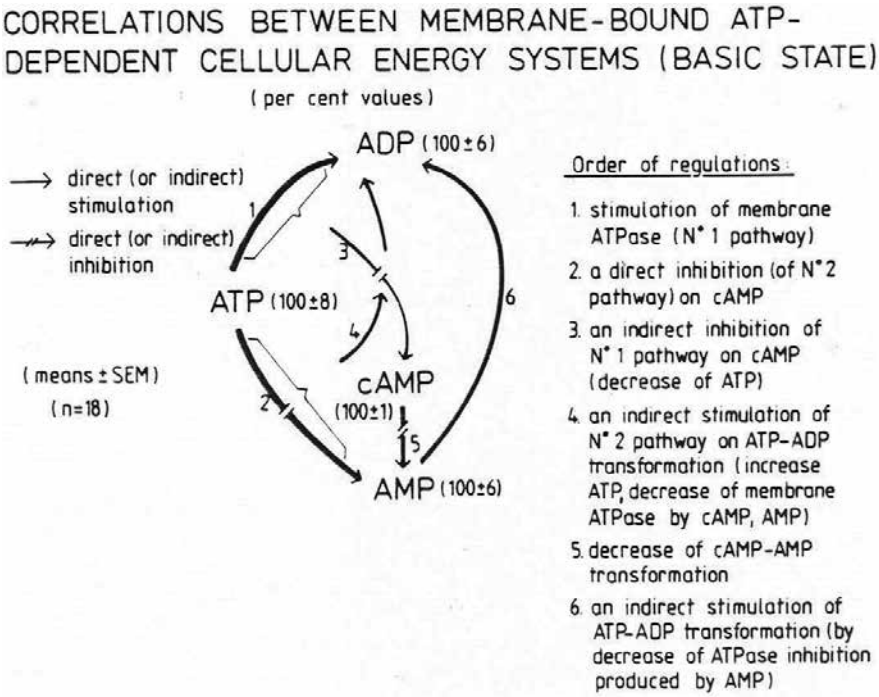


**Figure 157.** Suggested extra- and intracellular regulatory steps existing between the $Na^+$–$K^+$-dependent ATPase (transformation of ATP into ADP) and adenylate cyclase (transformation of ATP into cAMP) under physiological (basic) state of the target organ. It is important to emphasize that the different drugs, hormones and mediators ("first messengers") are able to modify the in smaller concentrations the $Na^+$–$K^+$-dependent ATPase system, and only in higher doses the adenylate cyclase, and the ATP as a common substrate cellular components for the function of both enzymes in presence of $Mg^{2+}$. [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

For a better understanding, the existence of feedback extra- and intracellular regulatory mechanism system between the different membrane-bound ATP-dependent energy systems (under physiological and pathological conditions existing in the gastric fundic tissues) and the different (suggested) regulatory steps are demonstrated in Figures 157–160.

### 8.6. Reserpine ulcer and gastric mucosal biochemistry in rats

Reserpine liberates epinephrine and norepinephrine in the rats. If the animals receive reserpine (5 mg/kg) after 6 hours, visible ulceration will appear in the glandular stomach (Mózsik et al., 1983c) (Figure 161).

It was interesting to note that significant changes could be detected before the ulcer development; the peaks of the biochemical measurements were obtained at 6–12 hours after reserpine administration (Figures 162–164), whereas the peak of ulceration was obtained at 24 hours after reserpine administration.

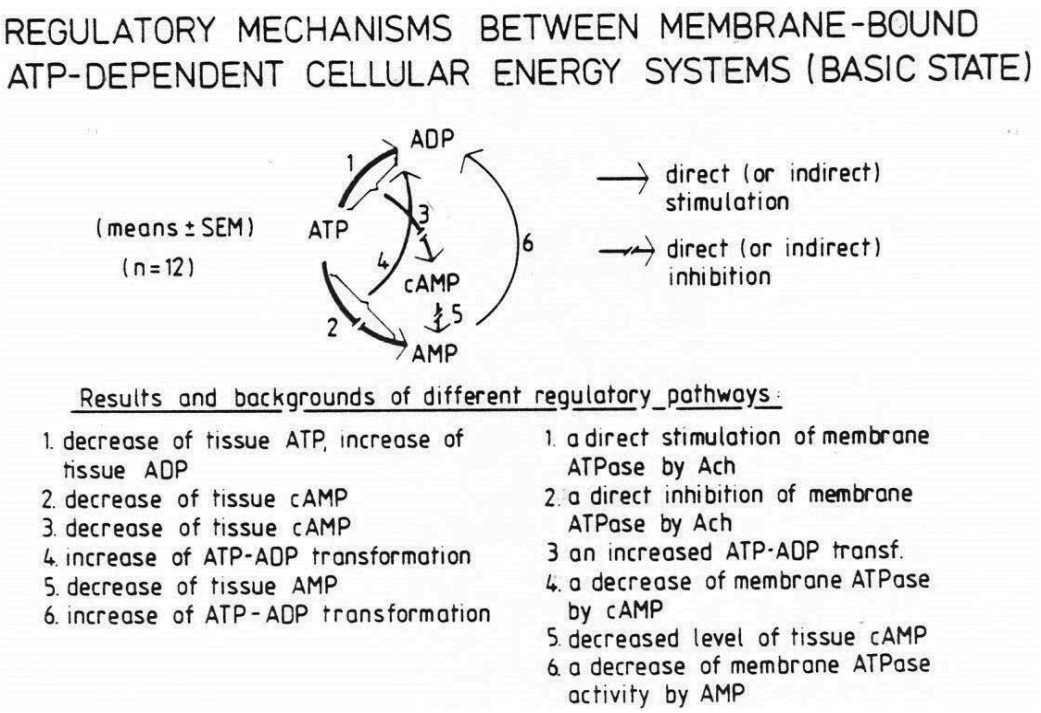


**Figure 158.** A detailed summary of the existence of the extra- and intracellular regulatory steps between the $Na^+$–$K^+$-dependent ATPase (ATP transformation of ATP into ADP) and adenylate cyclase (ATP transformation into cAMP) enzymes in the gastric fundic mucosa under physiological (basic) state. [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

### 8.7. NaOH-, NaCl-, HCl- and ethanol-induced gastric mucosal damage in rats (1 hour): different chemically induced gastric mucosal damage versus same changes in the gastric mucosal biochemical backgrounds

The gastric fundic mucosal damage was first produced by Chaudhury and Jacobson (1978) and Robert and coworkers (1979), when they clearly indicated that the gastric mucosal damage was prevented by small doses of prostaglandins (PGs) without the presence of any inhibitory effect on the gastric acid secretion in rats. This event was the first evidence for that the gastric mucosal prevention can be separated from the importance of gastric acid secretion (that was the old dogma in the treatment of PUD). This step in the gastroenterological research offered a better understanding of patients' problems to the existing principal basic (experimental) research. Furthermore, these types of experimental approaches provide new results, which were responsible, and simple methods (Morón et al., 1983, 1984c; Mózsik et al, 1980; 1981a; 1983b; 1984 a, b, c, d; 1989 a, b, c, d, e; 1990b).

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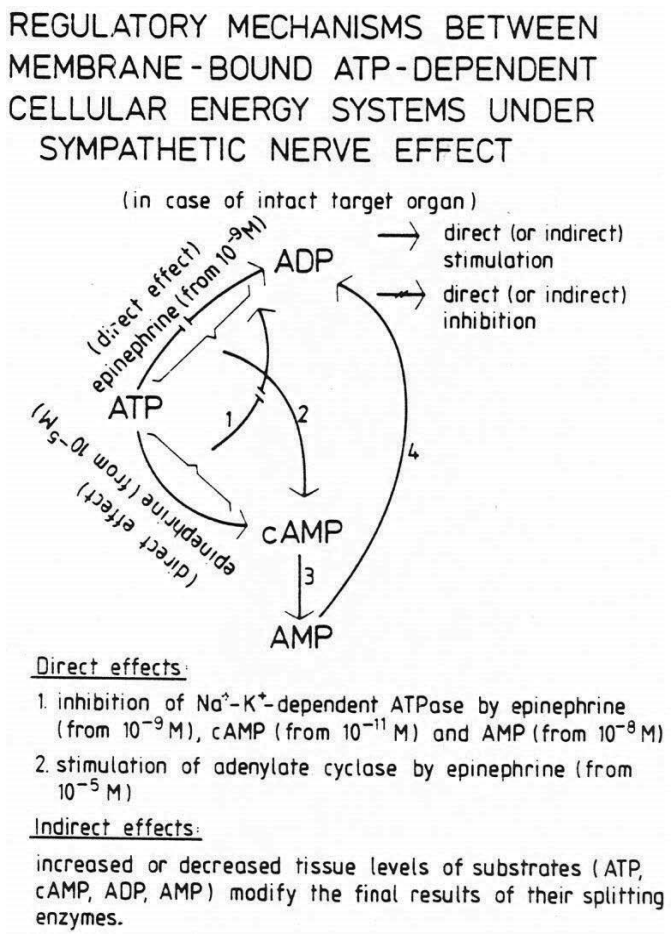


**Figure 159.** The epinephrine-induced changes in $Na^+$–$K^+$-dependent ATPase, adenylate cyclase prepared from the rat gastric fundic mucosa and in the tissue levels of ATP, ADP, AMP and cAMP in the rat gastric fundic mucosa. [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

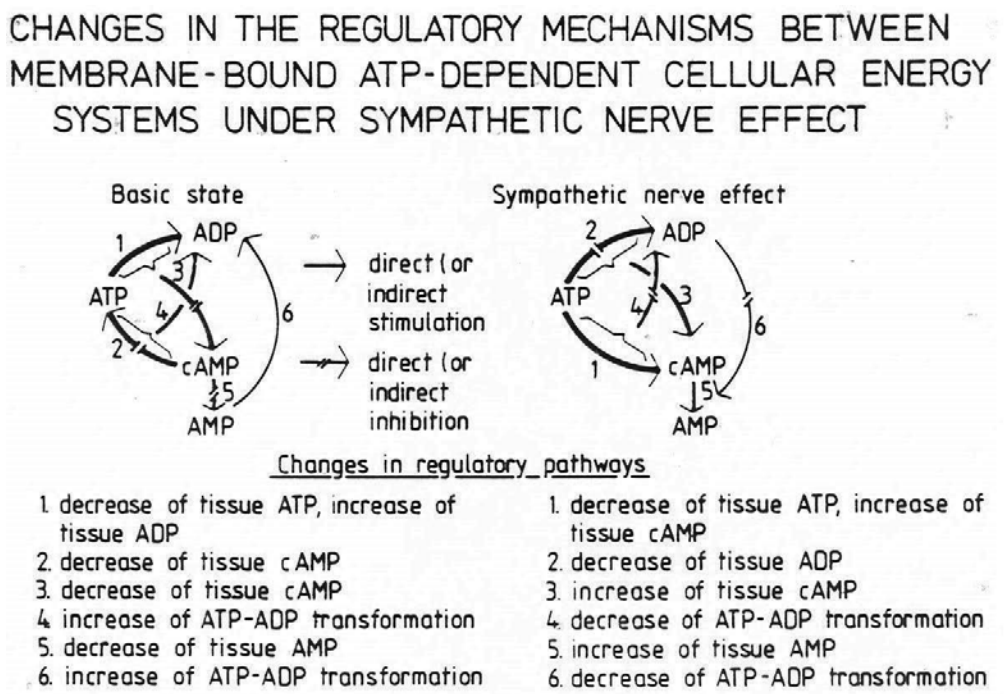


**Figure 160.** Summary of the changes in the feedback mechanism system during stress-induced gastric ulcer in rats (in comparison with each step to the existing physiological state). [Mózsik et al., Ann N Y Acad Sci 59, 264–281, 1990a (with kind permission).]

*8.7.1. A model study of ethanol-induced injury to gastric mucosa in rats*

Rats were given 96% ethanol intragastrically, and they were sacrificed at 0, 1, 5, 15, 30 and 60 minutes later. At each time period, the number and severity of mucosal lesions were recorded. At the same time intervals, the tissue levels of ATP, ADP, AMP and lactate were determined in homogenates of gastric mucosa by enzymatic methods. Tissue content of cAMP was determined by RIA (Becton Dickinson, Orangeburg, USA). The protein content was measured by the method of Lowry et al. (1951). The tissue levels of ATP, ADP, AMP and lactate are expressed as nmoles/mg protein, except for cAMP, which is expressed as pmoles mucosal protein. The ratio of ATP/ADP, values of adenylate pool (ATP + ADP + ANP) and "energy charge" [(ATP + 0.5 ADP)/(ATP + ADP + AMP)] were calculated from the individual values (Figures 165–173, Table 42).

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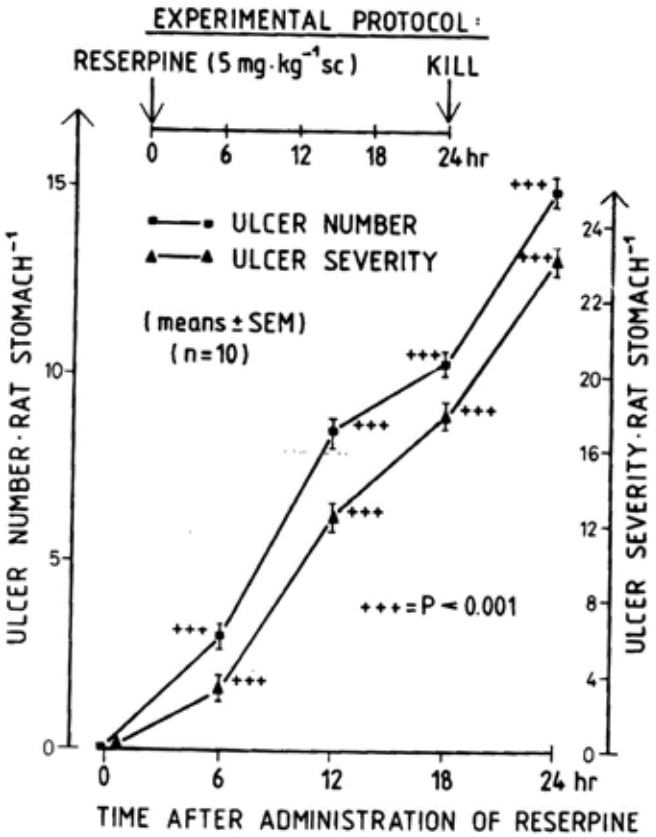


**Figure 161.** Ulcer (number and severity) development in reserpine (5 mg/kg s.c.)-induced gastric fundic mucosal lesions dependent upon time after reserpine administration (means ± SEM). [Mózsik et al., Acta Physiol Hung 62, 107–112, 1983c (with kind permission).]

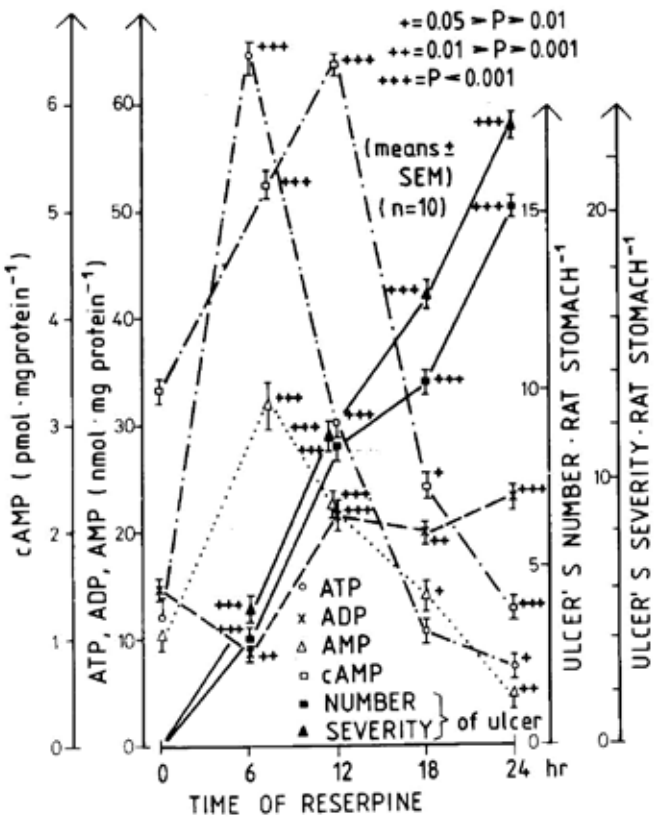


**Figure 162.** The time sequence between the biochemical changes (tissue levels of ATP, ADP, AMP and cAMP) versus gastric fundic mucosal damage ulcer (number and severity) in reserpine-treated rats (means ± SEM). [Mózsik et al., Acta Physiol Hung 62, 107–112, 1983c (with kind permission).]

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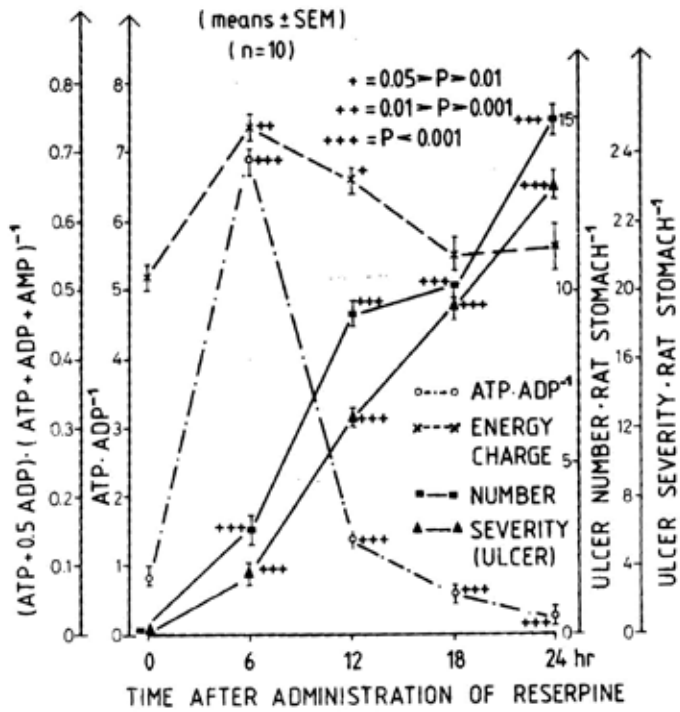


**Figure 163.** Correlations between the changes in "energy charge" and ratio of ATP/ADP versus gastric fundic mucosal damage (number and severity) produced by reserpine administration dependent on time after reserpine administration (means ± SEM). [Mózsik et al., Acta Physiol Hung 62, 107–112, 1983a (with kind permission).]

| | **Time periods after ethanol administration (min)** | | | |
|---|---|---|---|---|
| | 0 to 5 | 5 to 15 (Δ 10 min) | 15 to 30 (Δ 15 min) | 30 to 60 (Δ 30 min) |
| **Number of gastric mucosal lesions** | 5.85 ± 0.60 (5.85)* | 3.67 ± 1.00 (1.82)* | 1.30 ± 0.40 (0.32)* | 3.00 ± 0.80 (0.50)* |
| **Severity of gastric mucosal esions** | 28 ± 2 (28)* | 9 ± 1 ( 4.5)* | 13 ± 1 (4.3)* | 12 ± 2 (2)* |

**Table 42.** Changes in macroscopically appearing gastric mucosal damage produced by intragastric administration of ethanol and time dependence after administration (means ± SEM) ($n$=12).* = average value per 5 minutes corresponding to different time periods after ethanol administration. [Mózsik and Javor: Dig. Dis. Sci. 33:92–105, 1988 (with kind permission).]

CORRELATIONS BETWEEN CHANGES IN BIOCHEMISM OF GASTRIC MUCOSA AND DEVELOPMENT OF RESERPINE INDUCED GASTRIC MUCOSAL LESIONS IN RATS

RESERPINE (5 mg·kg$^{-1}$ sc.) — KILL

0 6 12 18 24 hr

TIME (HRS) AFTER ADMINISTRATION OF RESERPINE

| | 6 hr | 12 hr | 18 hr | 24 hr |
|---|---|---|---|---|
| ULCER NUMBER | ↑ | ↑↑ | ↑↑↑ | ↑↑↑ |
| ULCER SEVERITY | ↑ | ↑↑ | ↑↑↑ | ↑↑↑ |
| ATP | ↑↑↑ | ↑↑↑ | NS | ↓ |
| ADP | ↓↓ | ↑↑↑ | ↑↑ | ↑↑↑ |
| ATP·ADP$^{-1}$ | ↑↑↑ | ↑↑↑ | ↓↓↓ | ↓↓↓ |
| AMP | ↑↑↑ | ↑↑↑ | I | ↓↓ |
| ADENYLATE POOL | ↑↑↑ | ↑↑ | NS | NS |
| cAMP | ↑↑↑ | ↑↑↑ | ↓ | ↓↓↓ |
| „ENERGY CHARGE | ↑↑ | ↑ | NS | NS |

NS = NOT SIGNIFICANT ↑ = 0.05 > P > 0.01 ↑↑ = 0.01 > P > 0.001 ↑↑↑ = P < 0.001

↓ = DECREASE ↑ = INCREASE

**Figure 164.** The correlations between the changes in the tissue levels of ATP, ADP, AMP, adenylate pool, ratio of ATP/ADP, cAMP versus ulcer (number and severity) development dependent on time after reserpine administration (means ± SEM). [Mózsik et al., Acta Physiol Hung 62, 107–112, 1983a (with kind permission).]

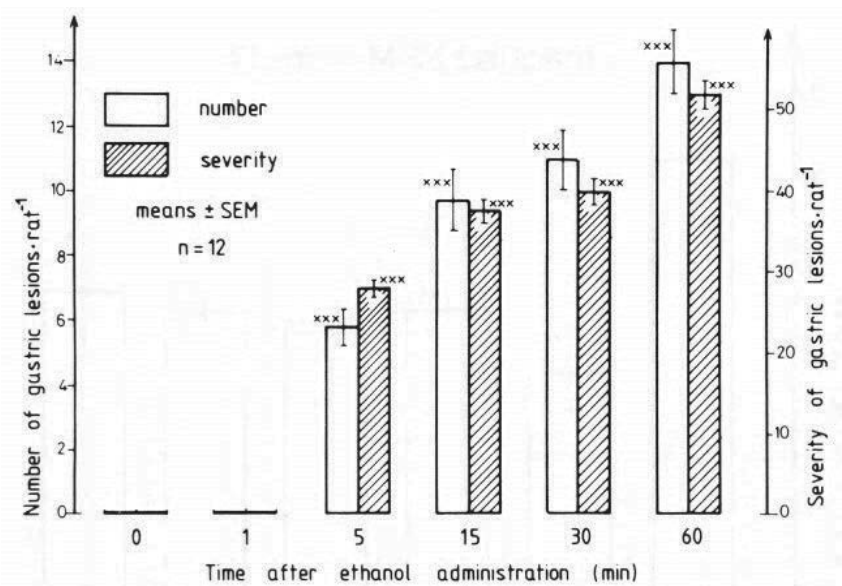


**Figure 165.** Macroscopic appearance of gastric mucosal damage in rats produced by intragastric administration of 96% ethanol. Time dependence after administration of ethanol. [Mózsik and Javor: Dig. Dis. Sci. 33:92–105, 1988 (with kind permission).]

http://dx.doi.org/10.5772/60505

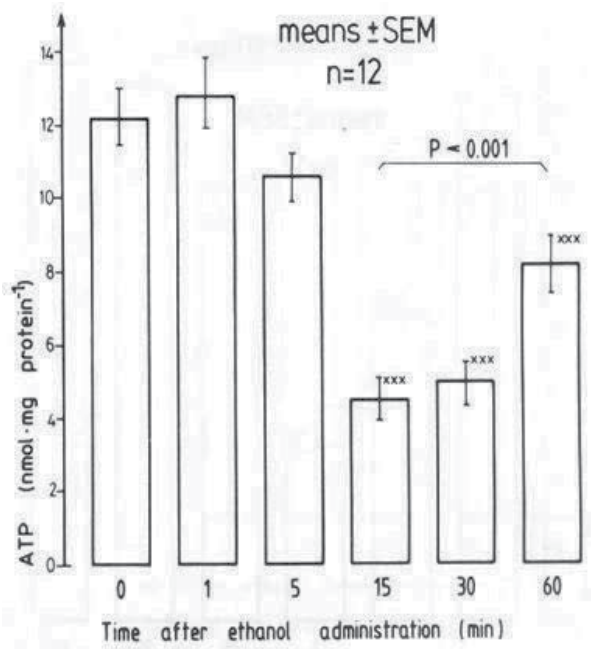


**Figure 166.** Changes in gastric mucosal level of ATP in rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

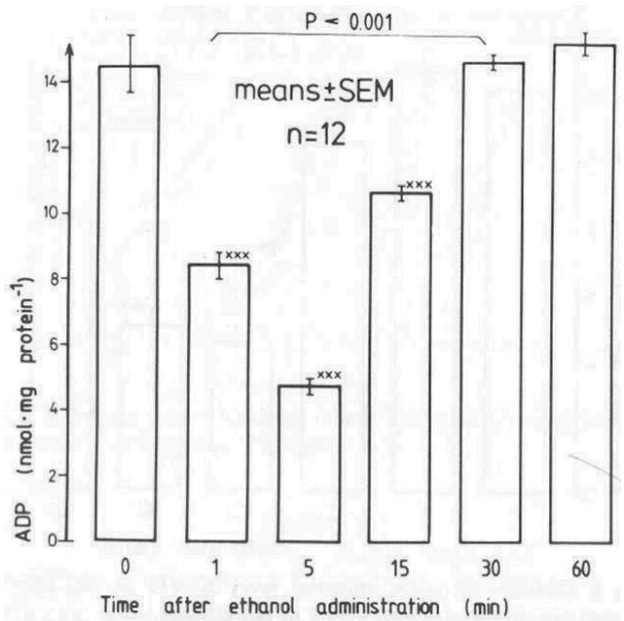


**Figure 167.** Changes in gastric mucosal level of ADP in rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

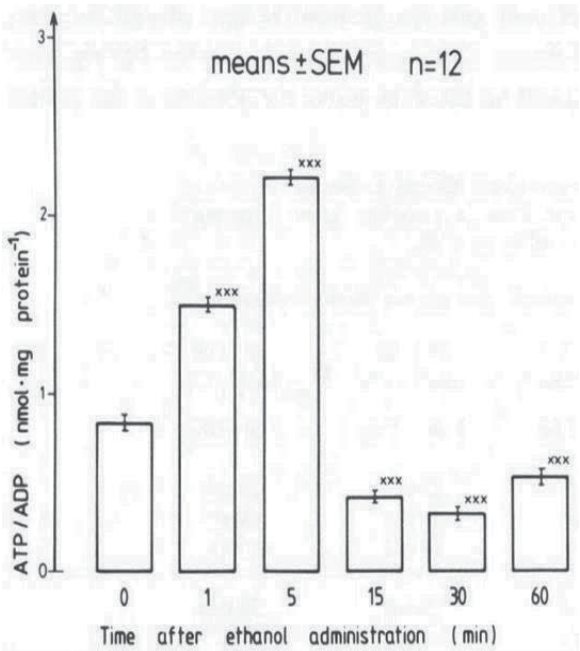


**Figure 168.** Changes in ratio of ATP/ADP in the gastric mucosa of rats after administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

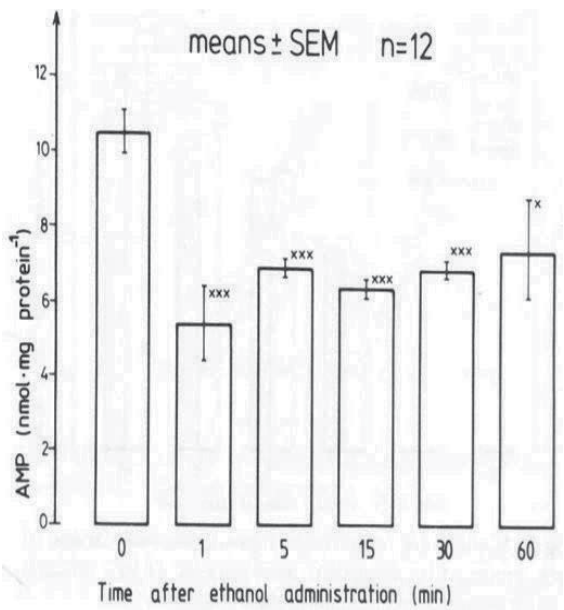


**Figure 169.** Changes in gastric mucosal level of AMP in rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

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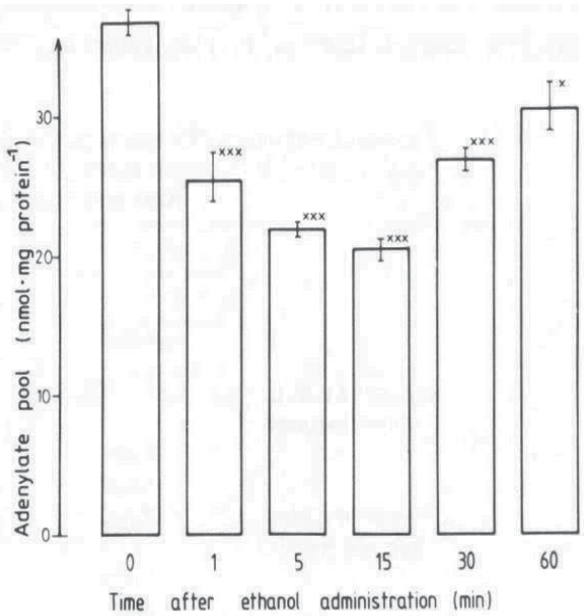


**Figure 170.** Changes in gastric mucosal level of adenylate pool of rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

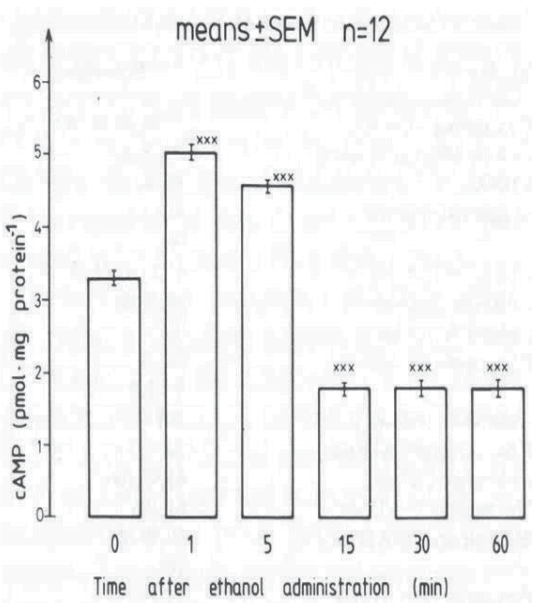


**Figure 171.** Changes in gastric mucosal level of cAMP of rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

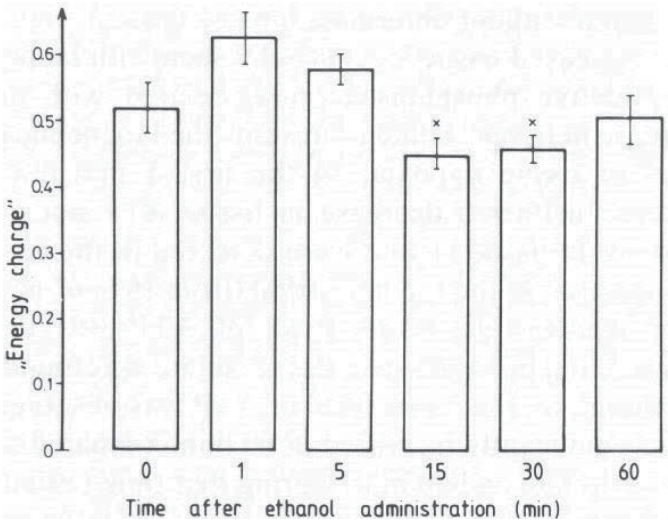


**Figure 172.** Changes in "energy charge" [(ATP + 0.5 ADP)/(ATP + ADP + AMP)] in the gastric mucosa after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

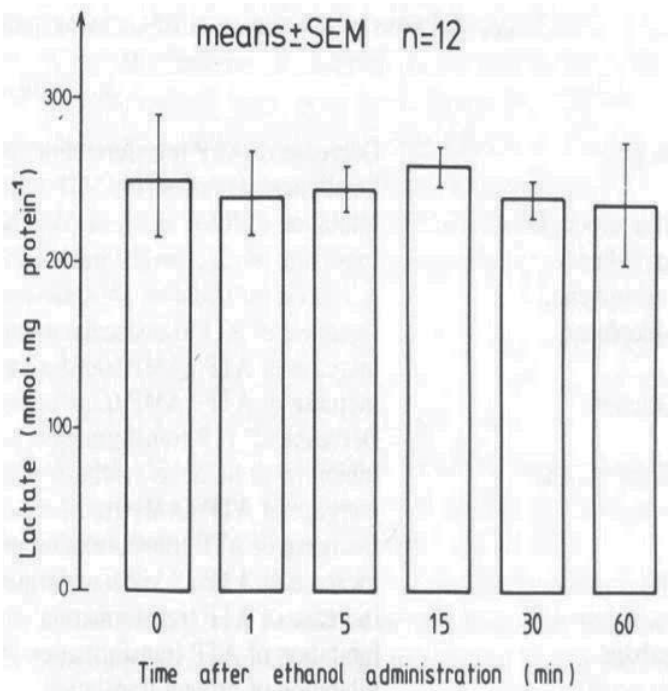


**Figure 173.** Gastric mucosal level of lactate in rats after intragastric administration of 96% ethanol (1 mL). [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

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The tissue level of ATP did not change during the first 5 min. It decreased from 15 to 60 minutes after ethanol administration, but then increased again at 60 minutes compared with levels at 15 and 30 minutes after ethanol administration. The tissue level of ADP decreased during the first 15 minutes and then returned to pretreatment values at 30 and 60 minutes after ethanol administration. The ratio of ATP/ADP increased over the first 5 minutes and then decreased. The tissue level of AMP was decreased during the first hour. The value of adenylate pool was decreased at all time intervals. The tissue level of cAMP increased at 1 and 5 minutes after ethanol administration and decreased thereafter. The extent of phosphorylation and/or dephosphorylation as estimated by Atkinson's formula was decreased at 15 and 30 minutes after ethanol administration. Surprisingly, there was no change in the tissue level of lactate independently of the presence of macroscopic mucosal injury.

Gastric mucosal damage appeared macroscopically after 5 min. It occurred to half the extent of that present after 1 hour following the exposure to ethanol. The tissue level of ATP did not decrease, whereas the ratio of ATP/ADP and tissue level of cAMP increased. There was no increase in the levels of ADP, AMP or lactate during the first 5 minutes after ethanol administration. It can be concluded that the presence of mucosal hypoxia before and at the time of appearance of macroscopic mucosal damage after ethanol administration can be excluded.

### *8.7.2. Extra- and intracellular regulatory mechanism system in the gastric mucosa during the development of ethanol-induced gastric mucosal damage in rats*

If the changes in tissue levels of ATP, ADP, AMP and cAMP are expressed in percent values at 0 minute (intact animals) dependent upon time after the administration of ethanol, then two different characteristic changes can be found in the gastric mucosa: the cAMP level significantly increased together with of the inhibition of ATP transformation into ADP at 5 minutes; thereafter (from 5 to 60 minutes), the tissue level of cAMP decreased in association with increase in tissue level of ADP and gradually increasing tissue level of ATP by intact oxidative phosphorylation. The critical evaluation of these changes in the membrane-bound ATP-dependent energy systems offers us to demonstrate the existence of feedback systems between the ATP–ADP and ATP–cAMP transformations in the gastric mucosa during the development of ethanol-induced macroscopic damage (*in vivo* examinations) (Figure 174).

### *8.7.3. Comparison of development of gastric mucosal damage and gastric mucosal biochemistry in acid-dependent (HCl) and nonacid-dependent (ETOH) experimental models in rats*

The time dependence of gastric fundic mucosal damage (number and severity) appeared in time order after the application of HCl or ethanol (ETOH). The extent of gastric fundic mucosal damage reaches its value in about 50% at 5 minutes after the administration of necrotizing agents (Figures 175, 176).

The extent of ATP–ADP transformation significantly increased at 0–5 minutes after the administration of necrotizing agents (Figures 177, 178), whereas the ATP–cAMP transformation decreased significantly (Figures 179). During this time period, no elevation was obtained in the tissue level of lactate (Figure 180). The different necrotizing agents were applied (presently HCl and ETOH); however, the biochemical changes in the rat gastric mucosa were found to be the same (Figure 181).

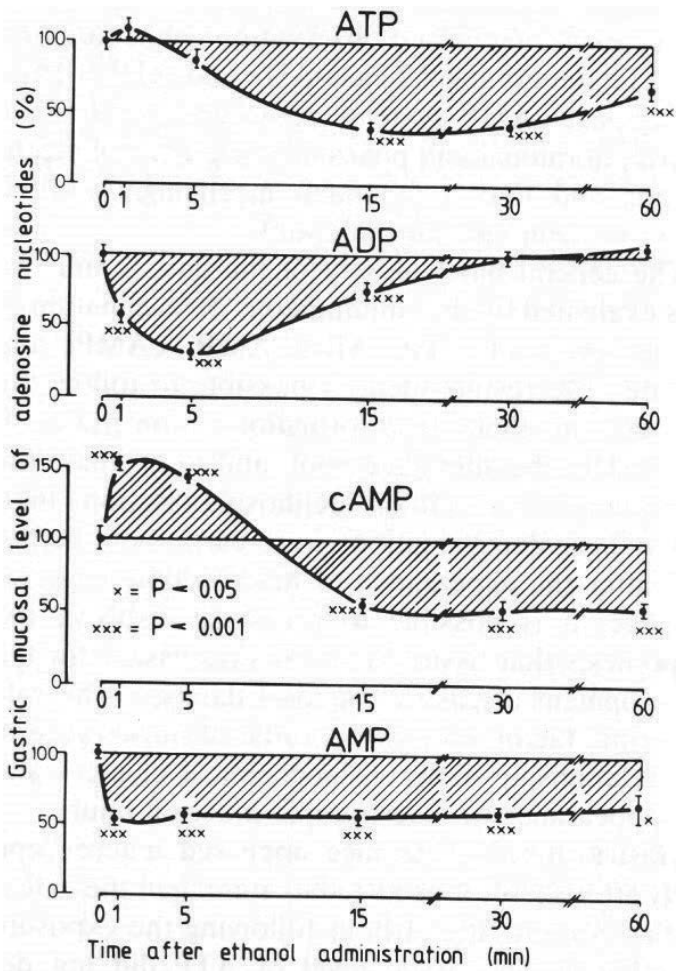


**Figure 174.** Feedback mechanisms between ATP–ADP, ATP–cAMP systems in the rat gastric mucosa after intragastric administration of 96% (1 mL) ethanol. [Mózsik and Javor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

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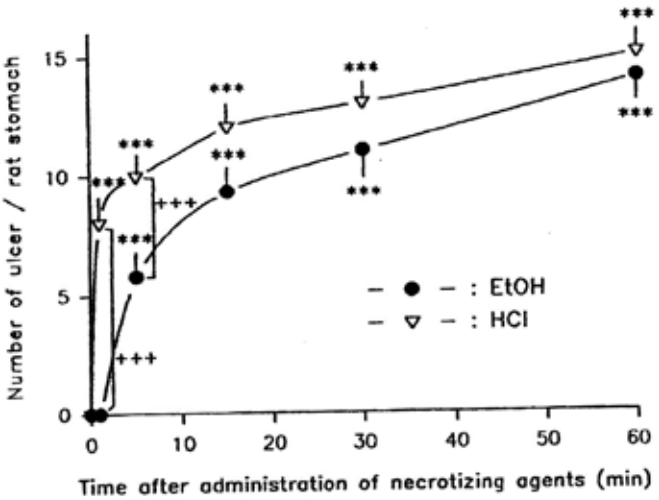


**Figure 175.** Macroscopic appearance of the number of gastric mucosal damage in rats treated with ethanol (ETOH) (1 mL of 96 v/v and HCl (1 mL, 0.6 M HCl i.g.) dependent on time after administration of necrotizing agents. The results were expressed as means ± SEM of 12 animals. *P* values were calculated as results between 0 versus different times (*) and results obtained at the same time in ETOH and HCl models (+) (means ± SEM; $P < 0.5$; $P < 0.001$; $P < 0.100$). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

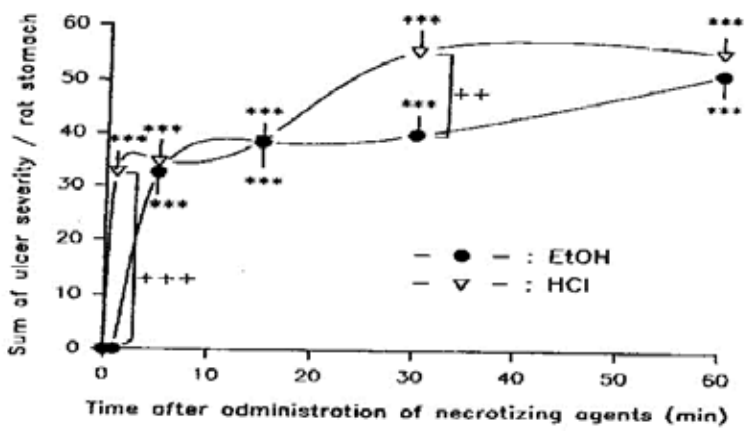


**Figure 176.** Macroscopic appearance of sum of gastric mucosal damage in rats with ETOH and HCl models (means ± SEM). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

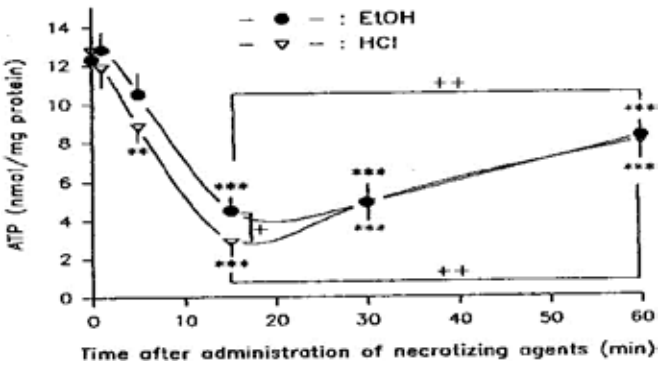


**Figure 177.** Changes in the gastric mucosal level of ATP in rats with ETOH- and HCl-treated rats after application of necrotizing agents (means ± SEM). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

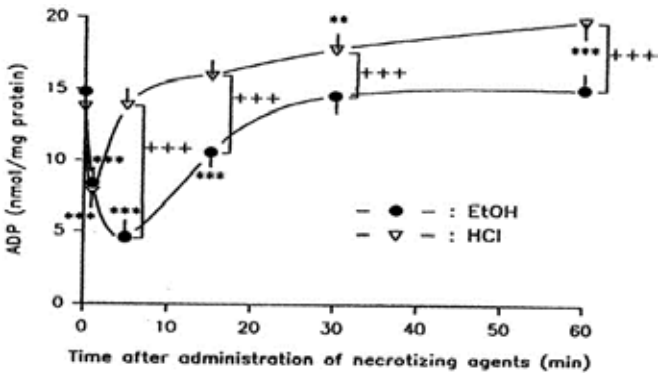


**Figure 178.** Changes in the gastric mucosal level of ADP in ETOH- and HCl-treated rats dependent upon time after application of necrotizing agents (means ± SEM). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

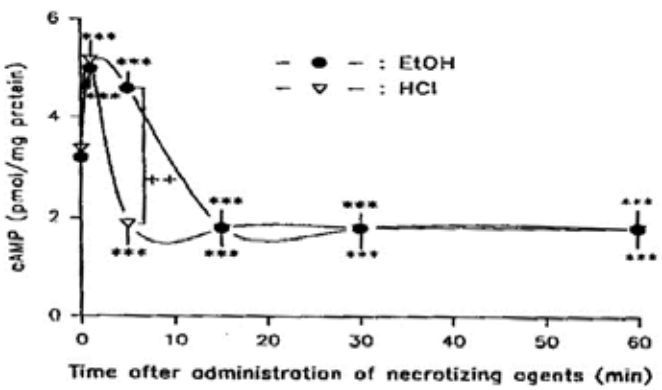


**Figure 179.** Changes in gastric mucosal cAMP of rats treated with ETOH or HCl dependent on time after administration of necrotizing agents (means ± SEM). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

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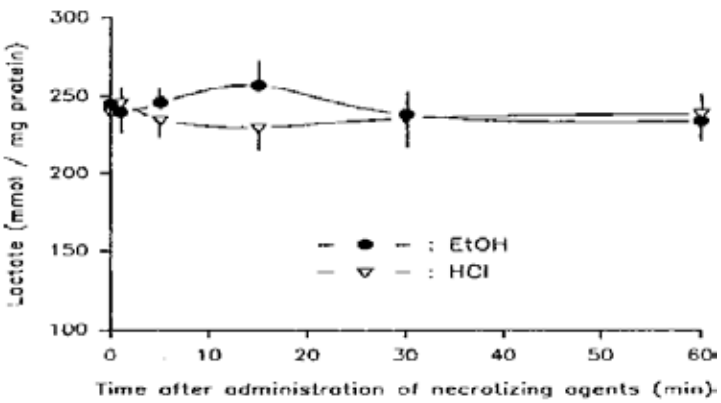



**Figure 180.** Gastric mucosal level of lactate in rats treated with ETOH and HCl dependent upon time after application of necrotizing agents (means ± SEM). For further explanation, see Figure 175. [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

The biochemical measurements were carried out in the HCl-, NaOH-, NaCl- and ethanol-induced models, and the summary of these results is given in Figure 182. Although the necrotizing agents differ significantly, the biochemical changes in the rat gastric mucosa are quite similar (Figure 182). Very similar changes were obtained in the ratio of ATP/ADP and "energy charge" (Figure 183).

| Examined parameters | 1 | | 5 | | 15 | | 30 | | 60 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | min after administration of HCl (A) and EtOH (B) | | | | | | | | | |
| | A | B | A | B | A | B | A | B | A | B |
| Number of ulcers | ↑↑↑ | NS | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| Severity of ulcers | ↑↑↑ | NS | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| ATP | NS | NS | ↓↓ | NS | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| ADP | ↓↓↓ | ↓↓↓ | NS | ↓↓↓ | NS | ↓↓↓ | ↑↑ | NS | ↑↑ | NS |
| cAMP | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| Lactate | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

NS: not significant

↑↑↑/↓↓↓ (increase/decrease): $p<0.001$
↑↑/↓↓: $p<0.01$

**Figure 181.** Changes in the gastric fundic mucosal levels of biochemistry, gastric mucosal damage (number and severity) in ETOH or HCl models (means ± SEM). [Gasztonyi et al., Inflammopharmacology 4, 351–360, 1996 (with kind permission).]

CORRELATIONS BETWEEN CHANGES IN BIOCHEMISM OF GASTRIC MUCOSA AND DEVELOPMENT OF NECROTIZING AGENTS-INDUCED GASTRIC MUCOSAL LESIONS IN RATS

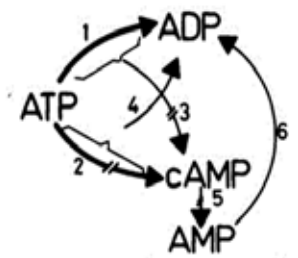
| | 96% ETHANOL-INDUCED | 0.6 M HCl-GASTRIC | 0.2 M NaOH-MUCOSAL | 25% NaCl-LESIONS |
|---|---|---|---|---|
| ULCER NUMBER | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| ULCER SEVERITY | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| ATP | ↓↓ | ↓↓ | ↓↓↓ | ↓↓↓ |
| ADP | NS | ↑ | ↑ | ↑ |
| ATP·ADP$^{-1}$ | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| cAMP | ↓↓↓ | ↓↓↓ | ↓↓↓ | ↓↓↓ |
| AMP | ↓ | ↓↓↓ | NS | ↓↓ |
| ADENYLATE POOL | NS | NS | NS | NS |
| „ENERGY CHARGE" | NS | NS | ↓↓ | NS |
| LACTATE | NS | NS | NS | NS |

NS = NOT SIGNIFICANT ↓ = 0.05 ⩾ P ⩾ 0.01 ↓↓ = 0.01 ⩾ P ⩾ 0.001 ↓↓↓ = P ⩽ 0.001

↑ = INCREASE ↓ = DECREASE

**Figure 182.** The schematic representation of regulatory pathways between the tissue levels of ATP, ADP, AMP and cAMP, values of ATP/ADP, "adenylate pool" and "energy charge" as well as the development of gastric mucosal lesions in chemicals [(1 mL from 96 v/v, 1 mL from 0.2 M NaOH, 1 mL from 25 v/v NaCl and 1 mL 0.6 M HCl)]. [Mózsik (2006): Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

SCHEMATIC PRESENTATION OF THE INTERRELATIONSHIPS BETWEEN THE CELLULAR ENERGY SYSTEMS DURING THE DEVELOPMENT OF GASTRIC MUCOSAL DAMAGE BY 0.2 M NaOH (I.), 25% NaCl (II.), 0.6 M HCl (III.), AND 96% ETHANOL (IV)




| | C | I. | II. | III. | IV. |
|---|---|---|---|---|---|
| ATP·ADP$^{-1}$ | 0.84 ± 0.06 | 0.16 ± 0.01*** | 0.27 ± 0.02*** | 0.44 ± 0.03*** | 0.53 ± 0.04*** |
| ENERGY CHARGE | 0.52 ± 0.04 | 0.38 ± 0.03** | 0.45 ± 0.02 | 0.53 ± 0.04 | 0.51 ± 0.04 |

STIMULATION → ← INHIBITION

C = Control (untreated) ** = P<0.01 *** = P<0.001

**Figure 183.** The changes in the regulatory pathways between the tissue level of ATP, ADP, AMP and cAMP in the intact (untreated) and in HCl models. The same results were obtained in the HCl model, 25% NaCl and 96% ETOH (means ± SEM). [Mózsik (2006): Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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*8.7.4. Dynamism of extra- and intracellular regulatory mechanisms in the ethanol-induced gastric mucosal damage in 1-hour experiments dependent on time after intragastric application of ethanol*

The changes in the regulatory pathways between the tissue levels of ATP, ADP, cAMP and AMP are indicated in Figure 184 dependent upon time after the administration of ethanol. This figure demonstrates well that the changes in the cellular energy systems significantly dynamically change from time to time in the rat gastric fundic mucosa; however, the ATP resynthesis worked well in this tissue.

The changes in the membrane-bound ATP-dependent energy systems in the gastric fundic mucosa were the same in the other models, independent from the kinds of intragastric application of 0.2 M NaOH, 25% NaCl, 0.6 M HCl and 96% ethanol.

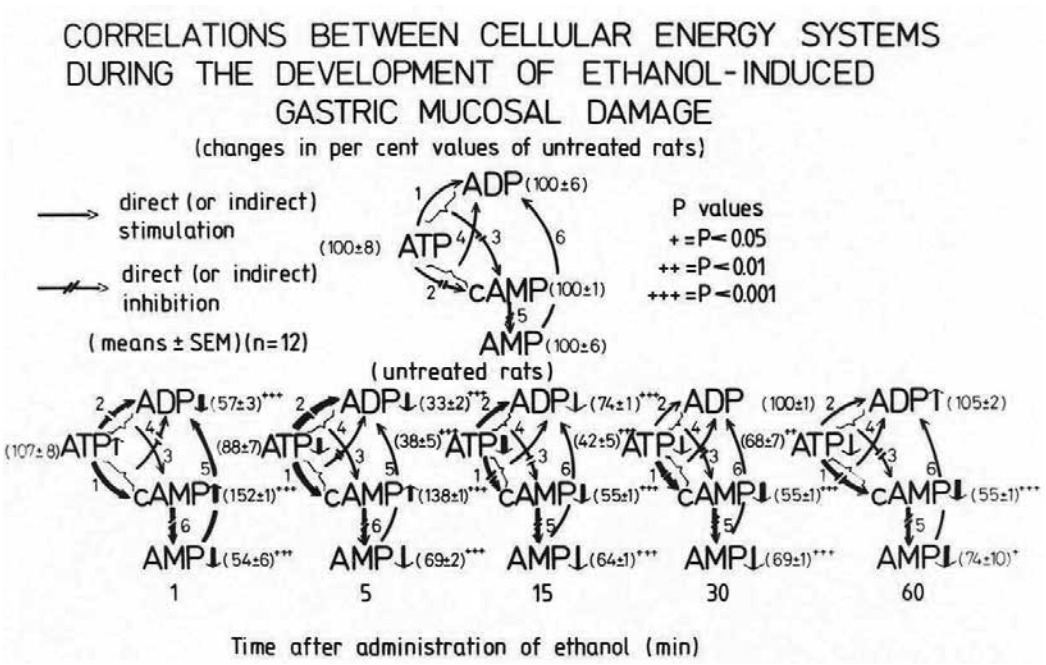


**Figure 184.** Changes in the cellular regulatory mechanisms of energy systems in ethanol (ETOH) induced in the rat gastric fundic mucosa dependent on time after intragastric administration of ETOH (1 mL from 96%). [Mózsik (2006): Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

*8.7.5. Biochemical backgrounds of gastric mucosal-protective effects of cytoprotective and antisecretory doses of atropine and cimetidine in acid-dependent model in rats (1-hour model)*

After the classical cytoprotective agents ($PGE_2$ and $PGI_2$), the gastric mucosal-protective effect of different antisecretory drugs was studied (given in different doses) in animal experiments. It was suggested that all the drugs having antisecretory properties are able to produce gastric

mucosal protection without the presence of any antisecretory activities. We studied these problems in cases of atropine and cimetidine (Figure 185).

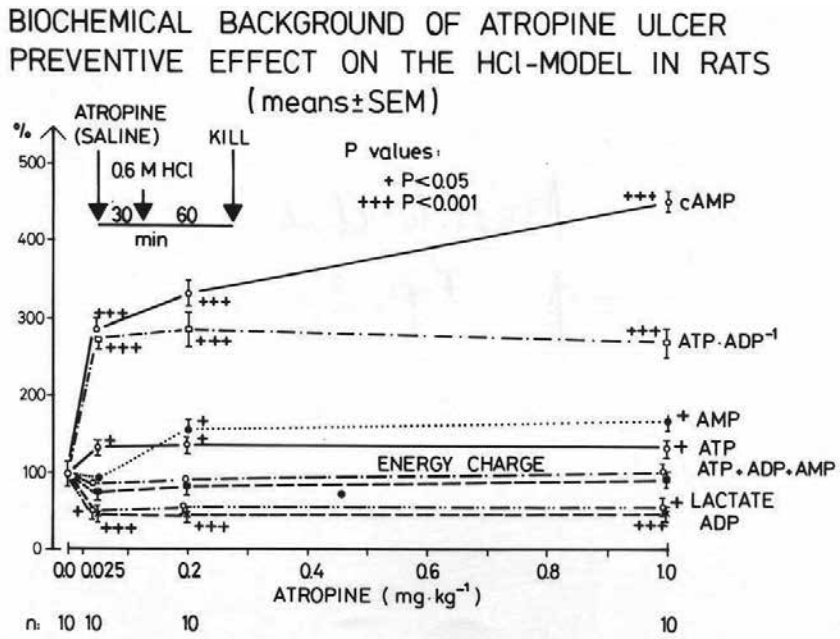


**Figure 185.** Changes in biochemical parameters of cellular energy systems and lactate in the gastric fundic mucosa in HCl model of rats. [Mózsik (2006): Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

We studied the gastric mucosal-preventive effect of atropine (given in doses of 0.025, 0.2 and 1.0 mg/kg s.c.). The 0.025-mg/kg atropine indicated mucosal protection in the HCl model, together with significant changes in the gastric fundic mucosa, without the presence of inhibitory effect on gastric acid secretion. It was interesting to note that the gastric mucosal protection and the biochemical changes in the gastric fundic mucosa differ only in their extents.

The evaluation of quantitative changes produced by "cytoprotective" doses of atropine and cimetidine is presented in Figures 186 and 187.

These figures indicate that very complex intracellular mechanisms exist in the gastric fundic mucosa of rats produced by the gastric mucosal-protective effects of cytoprotective and antisecretory doses of atropine and cimetidine; however, it is clear that the original cellular regulatory mechanisms by $PGI_2$ (5 μg/kg), atropine (0.025 mg/kg) and cimetidine (2.5 mg/kg) also differ from each other (Figure 188).

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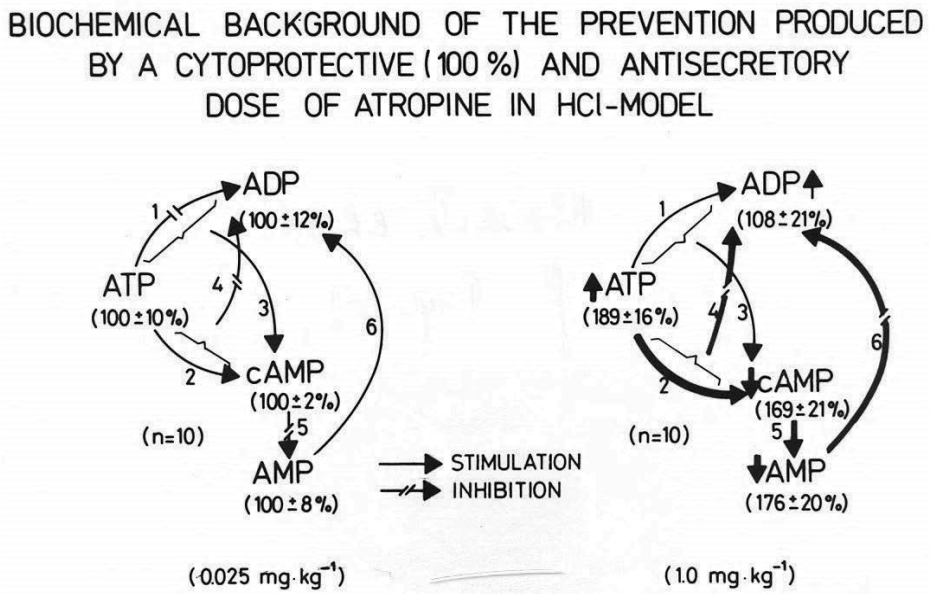


**Figure 186.** Comparison between changes in cellular energy systems of gastric fundic mucosa of rats produced by cytoprotective (0.025 mg/kg) and antisecretory (1.0 mg/kg) doses of atropine. The changes in the antisecretory dose of atropine are expressed in percent of cytoprotective dose (= 100%).

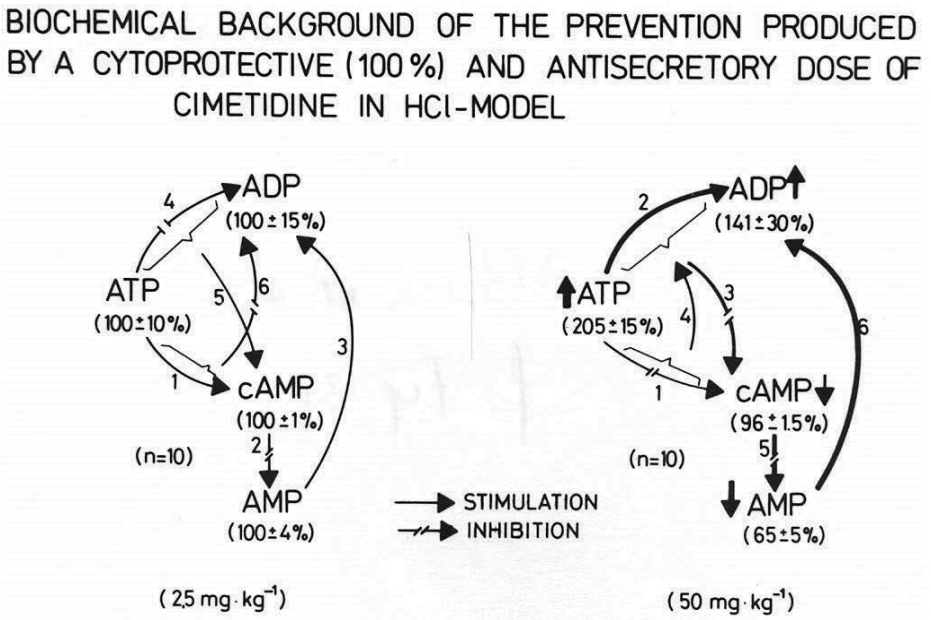


**Figure 187.** Comparison between changes in cellular energy systems of gastric fundic mucosa of rats produced by cytoprotective (2.5 mg/kg) and antisecretory (50 mg/kg) doses of cimetidine. The changes of antisecretory dose of atropine are expressed in percent of cytoprotective dose (= 100%).

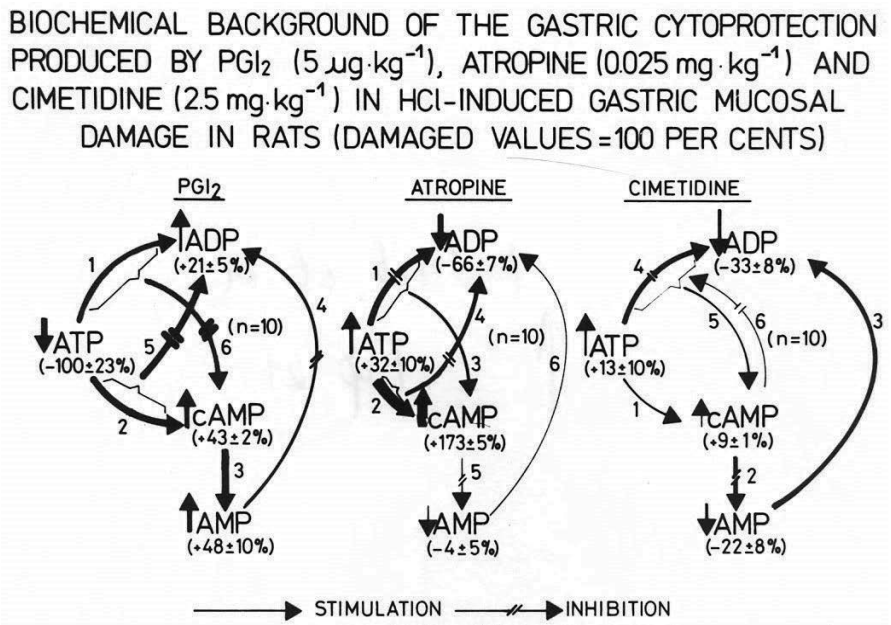


**Figure 188.** Comparison of the cellular regulatory mechanisms (steps) in the gastric fundic mucosa of rats produced by cytoprotective doses (5 μg/kg), atropine (0.025 mg/kg) and cimetidine (2.5 mg/kg). The changes in the different parameters of the cellular energy systems are presented in percent values of control (untreated) rats (100% values).

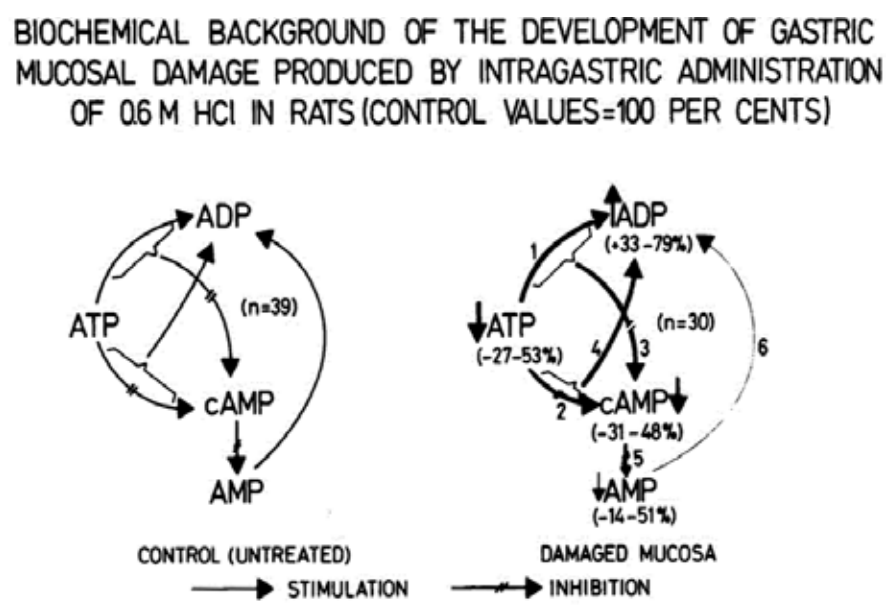


**Figure 189.** The extra- and intracellular regulatory mechanism system in intact (untreated) and in HCl-treated rats. The results obtained from the untreated rats were taken to be equal to 100%, and the changes in the HCl-treated rats expressed as changes in percent values in comparison with the normal (100) values.

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Bilateral surgical vagotomy completely inhibited the volume and gastric $H^+$ output together with complete prevention of gastric ulcer (Mózsik and Vizi, 1976 a, b). It was surprising that the tissue level of ATP after surgical vagotomy remained (Mózsik and Vizi, 1976 a, b) neither in the glandular stomach nor in the forestomach. These results suggested that the tissue level of ATP must break down for the energy liberation and development of gastric secretory responses and ulcer development (Mózsik et al., 1993 a, b, 2001 a, b).

When the chemical vagotomy (i.e. atropine administration) was carried out, the tissue levels of ATP decreased significantly and the tissue levels of ADP and AMP were increased (Figures 80, 81). The tissue levels of cAMP were found to be significantly higher after chemical vagotomy than after bilateral surgical vagotomy (Karádi and Mózsik, 2000; Mózsik et al., 1981a, b; 1987a; 1996b; Vincze et al., 1992, 1993a) (Figures 81, 190).

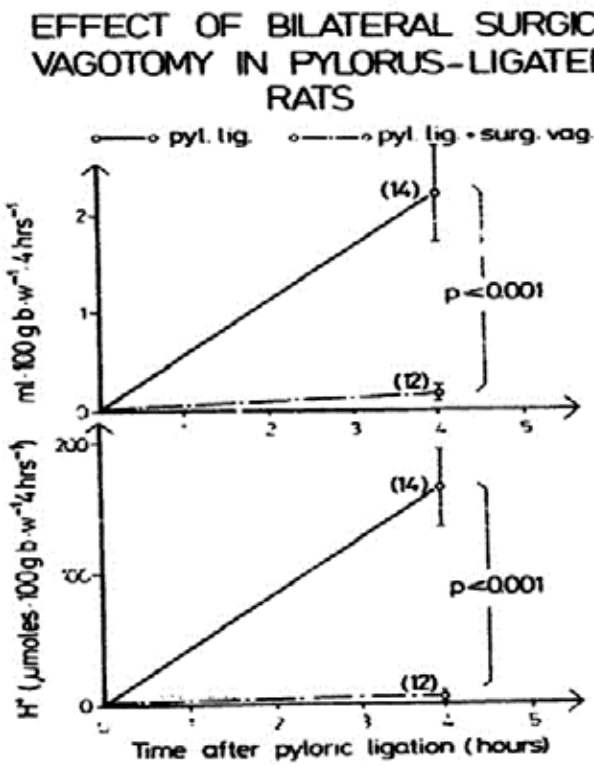


**Figure 190.** The effect of bilateral surgical vagotomy in the gastric secretory responses (volume and $H^+$ output) in 4-hour pylorus-ligated rats (means ± SEM). [Mózsik and Vizi, Am J Digest Dis 22, 1072–1075, 1976 (with kind permission).]

The best conclusions of these observations were as follows:

**1.** The ATP breakdown in the glandular stomach and forestomach is basically necessary to obtain gastric hypersecretion as well gastric ulceration;

2. Represents an intracellular regulatory mechanism to obtain a gastric hypersecretion;
3. In case of chemical vagotomy, the significant increase of mucosal cAMP probably represents an important regulatory step against the development of gastric hypersecretion (Karádi and Mózsik, 2000).

Previously, gastric ulceration was considered to appear as a result of extremely decreased tissue ATP produced by the impaired oxidative phosphorylation. However, we first demonstrated that the gastric ulceration in the forestomach appears as the consequence of active biochemical mechanisms of the gastric tissues (Mózsik et al., 1967 a, b; 1969 b, c).

These results also demonstrated that the intracellular mechanism systems differ significantly in the rat stomach after surgical and chemical vagotomy.

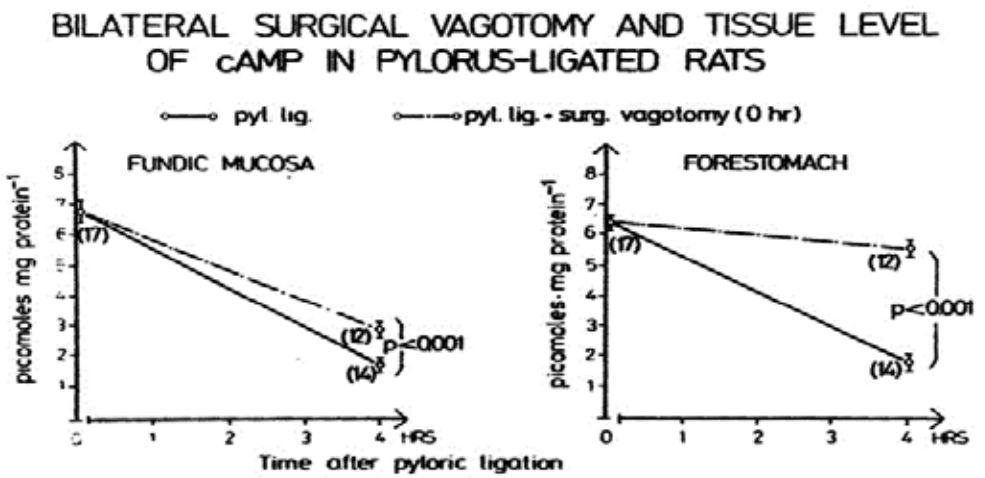


**Figure 191.** The effect of bilateral surgical vagotomy on the gastric tissue levels of cAMP in pylorus-ligated rats (means ± SEM). [Mózsik and Vizi, Amer J Digest Dis 22, 1072–1075, 1976 (with kind permission).]

### 8.8. "Surgical" and "chemical" vagotomy on the biochemistry of gastric mucosa in 1-hour rats

The results of these observations are presented in Section 4.3.2.

#### *8.8.1. "Surgical" vagotomy inhibits the $PGI_2$ –induced gastric mucosal-protective effect in rats*

The surgical vagotomy alone cannot produce any ulcer development in the rat stomach; however, Sikiric and coworkers found the appearance of ulcer in the rat stomach (Mózsik et al., 1981a; 1990 b, c; Karádi and Mózsik, 2000).

After bilateral surgical vagotomy, the extent of IND-induced gastric mucosal lesion was found to be increased, whereas the chemical vagotomy significantly decreased the gastric mucosal damage to chemicals.

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The interpretation of these results led to the following conclusions:

**a.** The intact vagal nerve is basically necessary for the mechanisms involved in different chemically induced gastric mucosal damage;

**b.** The gastric mucosal-protective effects significantly differ from each other against chemically induced gastric mucosal damage.

These results were presented in a Satellite Symposium of the Congress of International Union of Physiological Sciences (IUPS) (Budapest, Hungary, 1980) [Mózsik, Hänninen and Jávor (1981) (eds.) Advances in the Physiological Sciences. Vol. 29, Gastrointestinal Mucosal Defence. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest] (Figure 192).

These type of observations were published in the *Journal of Prostaglandins, Leucotrienes and Medicine* (Mózsik, Moron, Jávor, 9: 71–84, 1982), in which we also emphasized on the essential role of the intact vagal nerve for the development of gastric mucosal-preventive effect of prostacyclin against the chemically induced mucosal damage, e.g., this protective effect of prostacyclin was not present in these animal models. We concluded from the results of these observations that the intact vagal nerve is basically necessary for the gastric mucosal protection.

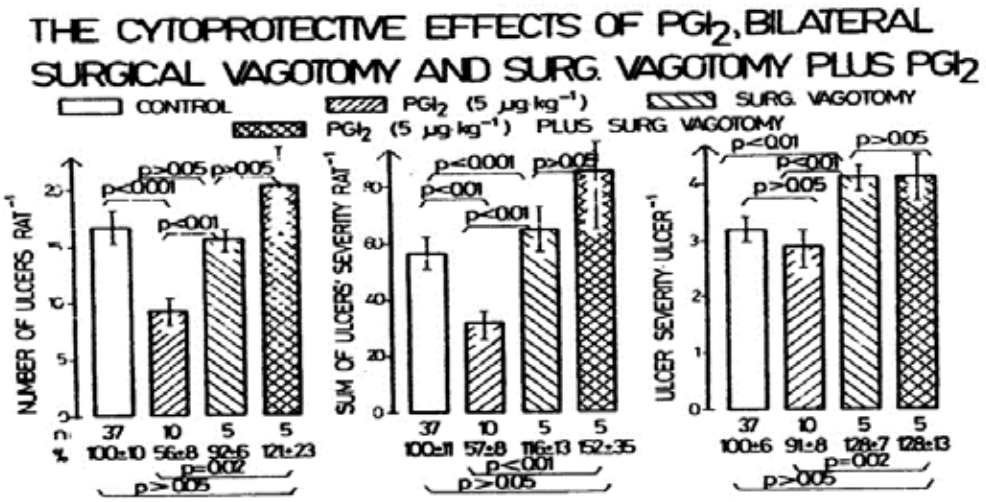


**Figure 192.** The presentation (first in the world) of $PGI_2$ gastric-protective effect disappears after bilateral surgical vagotomy in rats treated with ETOH (96 v/v) (means ± SEM). [Jávor et al., (1981) Gastric mucosal resistance to physical and chemical stress. In: Mózsik Gy., Hänninen O., Jávor T. (Eds.) Advances in the Physiological Sciences. Vol. 29. Gastrointestinal Mucosal Defence. Pergamon Press, Oxford – Akadémiai Kiadó, Budapest. pp. 141–159 (with kind permission).]

Similar observations were published by Miller (Amer J Physiol. 245: 601–623, 1983), and Henagen, Seidel and Miller cited our results in their forthcoming paper (Gastroenterology 84: 1186, 1983); however, from time to time, our priority has been the key role of the intact vagal

nerve in the gastric mucosal defense mechanisms (Karádi and Mózsik, 2001; Király et al., 1992b; Mózsik, 2006 a, b; Mózsik et al., 1990a, 1991a, 2001b; Vincze et al., 1992, 1993a, 1997).

However, it is important to emphasize that the "chemical vagotomy" (atropine treatment) does not clinically inhibit the appearance of prostaglandin- and prostacyclin-induced gastric mucosal protection against the chemically induced mucosal damage in animals.

We need to emphasize well that surgical vagotomy is widely used in the clinical practice of treatment of patients with peptic ulcer. We never accepted the application of surgical vagotomy in the medical practice and we emphasized the application of "chemical vagotomy" in the medical treatment of patients with peptic ulcer (see Chapter 2).

*8.9. Biochemical backgrounds of the $PGI_2$–induced gastric mucosal protection against by different chemicals in rats*

When small doses (5 and 50 μg/kg) of $PG_2$ were given, both the number (Figure 193) and severity (Figure 194) dose-dependently decreased and surprisingly the tissue level of ATP practically decreased to a value of zero (Figure 195). The tissue level of ADP (Figure 196), cAMP (Figure 197) and AMP (Figure 198) increased, and the values of ATP/ADP were practically zero (Mózsik et al., 1983b) (Figure 199).

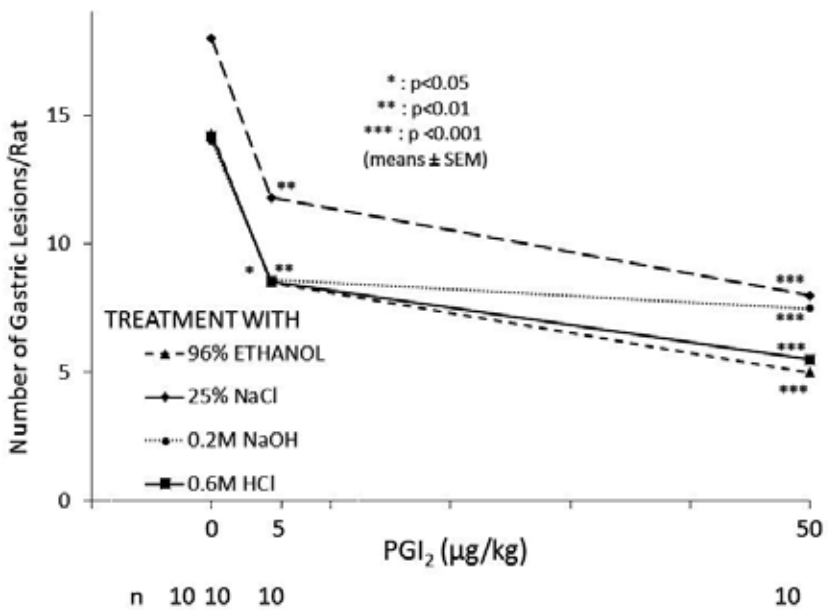


**Figure 193.** Preventive effects of $PGI_2$ on the number of gastric lesions produced by topical application of 0.2 M NaOH, 25%, 0.6 M HCl and 96% ethanol (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

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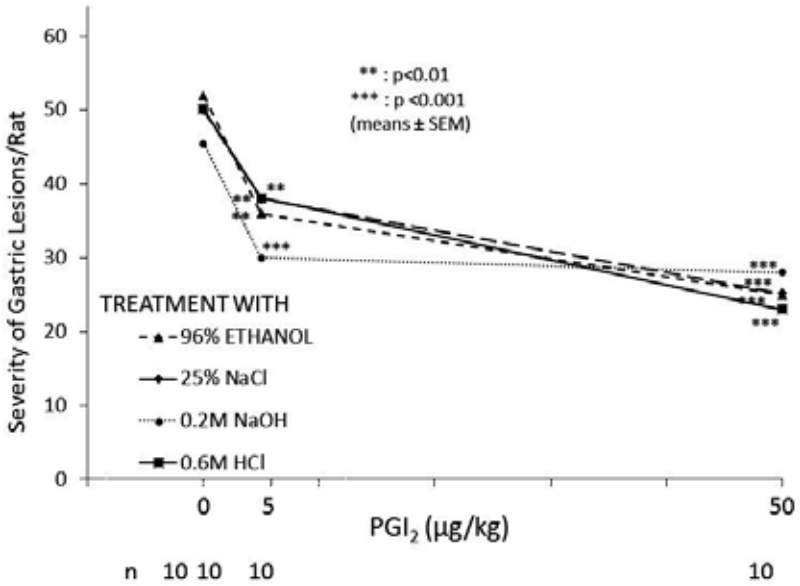


**Figure 194.** Gastric-preventive effects of $PGI_2$ on the severity in 0.2 M NaOH, 0.6 M HCl, 25% NaCl and 96% ethanol (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

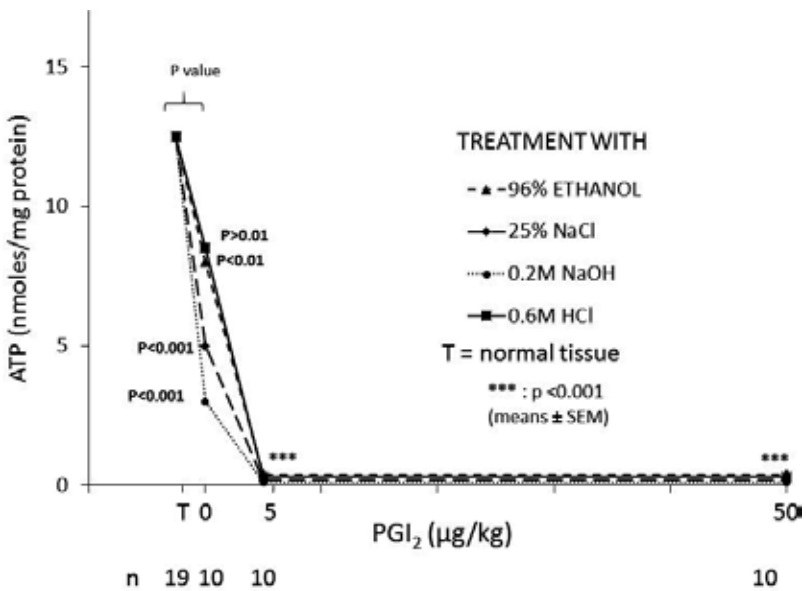


**Figure 195.** Changes in the gastric fundic mucosal level of ATP during development of gastric mucosal damage produced by different necrotizing agents (left side of the figure) and of different doses of $PGI_2$ (right side of figure). The differences in the results obtained in the normal pathological state (O) indicate the changes in the levels of ATP during the development of gastric mucosal damage (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

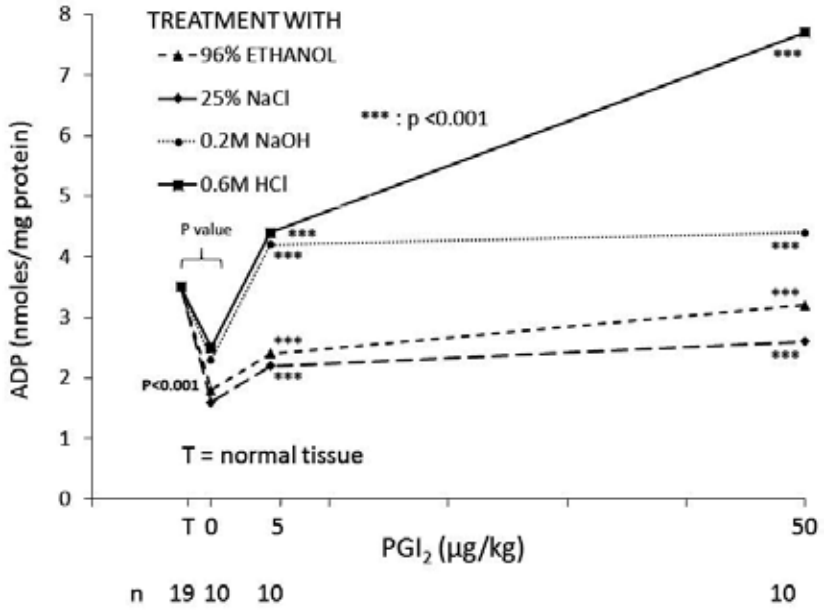


**Figure 196.** Changes in the gastric fundic mucosal level of ADP during mucosal damage produced by different necrotizing agents (left side) and of $PGI_2$-induced gastric mucosal protection (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

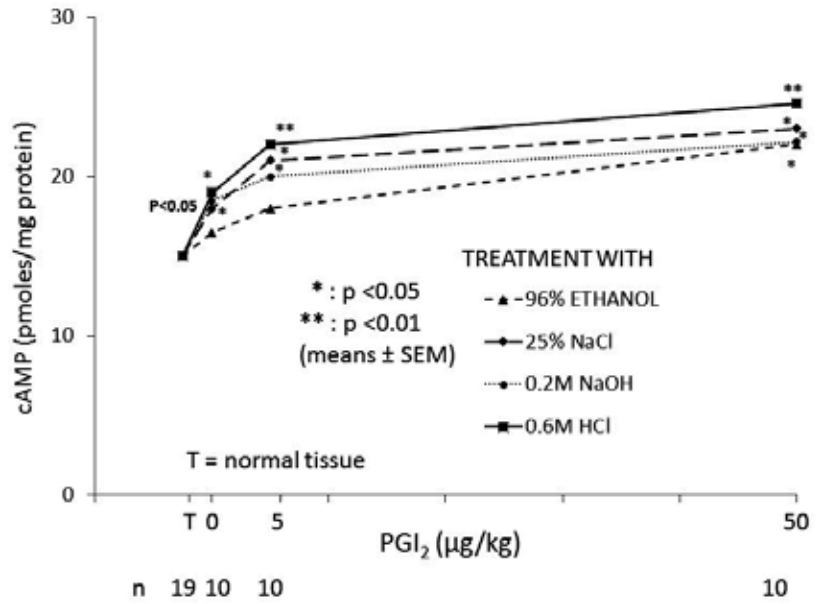


**Figure 197.** Changes in the gastric fundic mucosal level of cAMP during the development of gastric mucosal damage and of $PGI_2$-induced gastric cytoprotection (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

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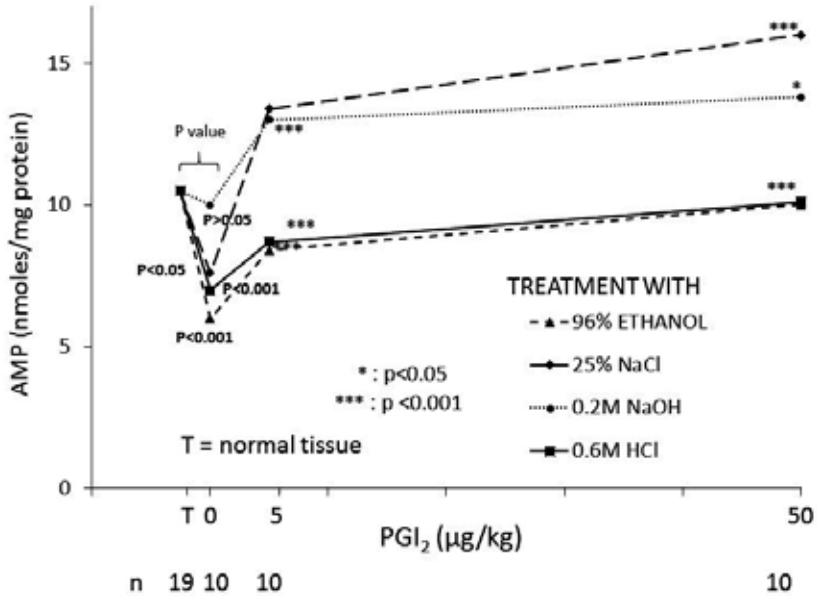


**Figure 198.** Changes in the gastric mucosal level of AMP during the development of gastric mucosal damage produced by different necrotizing agents and of $PGI_2$-induced gastric cytoprotection (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med. 12, 423–436, 1983 (with kind permission).]

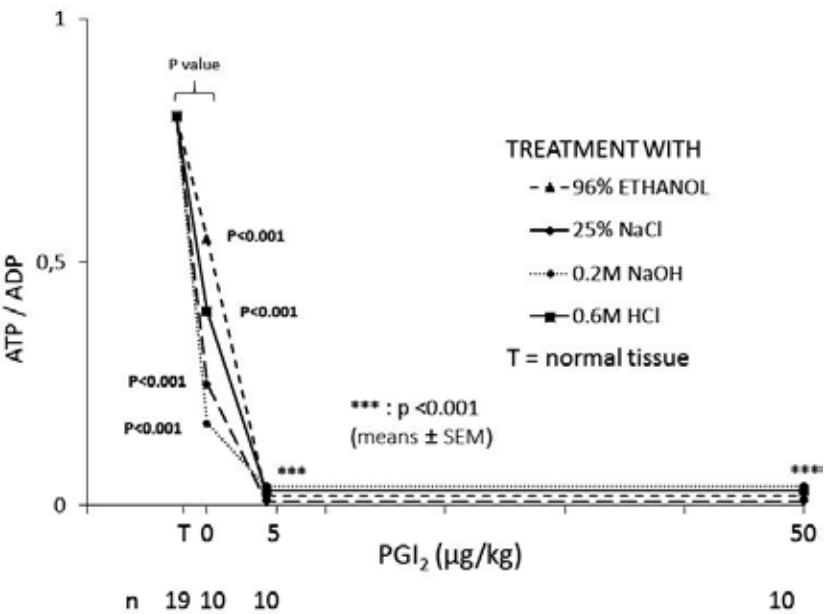


**Figure 199.** Changes in the ratio of ATP/ADP in the rat gastric fundic mucosa during the development of gastric mucosal damage produced by different necrotizing agents and of $PGI_2$-induced gastric cytoprotection (means ± SEM). [Mózsik et al., Prostagland. Leucot. Med 12, 423–436, 1983 (with kind permission).]

The results of these observations indicated the following:

1. The different chemically induced mucosal acute gastric mucosal damages are practically the same (during 1-hour time period) in relation to the time dependence of mucosal damage and its biochemical backgrounds (in membrane-bound ATP-dependent energy systems);

2. The prostacyclin-induced gastric mucosal-preventive effects are the same in different experimental models (produced by 0.6 M HCl, 0.2 M NaOH, 25% NaCl solution and 96% ethanol) in relation to time dependence of macroscopic detectable patterns of defensive actions and to changes in the membrane-bound ATP-dependent energy systems in the rat gastric mucosa;

3. It was very surprising to note that the tissue levels of ATP in the rat gastric mucosa practically reached the value of zero in the rat gastric mucosa, when the prostacyclin-induced mucosal-protective effects are present in the different animal models;

4. The prostacyclin (and other prostaglandins) inhibits the extent of ATP transformation into ADP (*in vitro* conditions) in smaller concentrations, whereas they stimulate the ATP transformation into cAMP. These steps of the regulatory steps of prostacyclin are present in different chemically induced experimental models (under *in vivo* conditions);

5. The results indicated similar changes in the regulatory feedback mechanisms of different chemical agents (drugs) to those we received in other experimental models.

### 8.10. Similarities and differences of $PGI_2$- and ß-carotene-induced gastric mucosal protections and their biochemical actions in an acid-dependent (HCl) and in a non-acid dependent (ETOH) experimental model in rats

#### *8.10.1. Gastric mucosal protective effect of $PGI_2$ versus changes in the gastric mucosal energy systems in HCl model in rat*

The experimental protocol and the obtained results are presented in Figure 200. The 5 μg/kg $PGI_2$ was found as $ED_{50}$ value (Figure 201). The $PGI_2$ prevented the HCl-induced gastric mucosal damage (Figures 202, 203), and this inhibition appeared in the early period after the administration of HCl (Figures 203–211). In all biochemical parameters of the gastric mucosal biochemistry, the $PGI_2$ produced dose-dependent actions (Mózsik et al., 1983b; 1984a; 1989 a, b; 1990 b, c; 1998; Sütő et al., 1989; Vincze et al., 1993a, 1997; Garamszegi et al., 1989; Gasztonyi et al., 1996).

#### *8.10.2. Gastric mucosal protective effect of $PGI_2$ versus changes in the gastric mucosal enery systems in ethanol (ETOH) model in rat*

The dose of 5 μg/kg was also found as the value of ED5O in the ETOH model (Figure 213). The actions of PGI2 were found to be dose-dependent (Figures 214–222), except for the tissue level of lactate because its level was unchanged (Figure 222).

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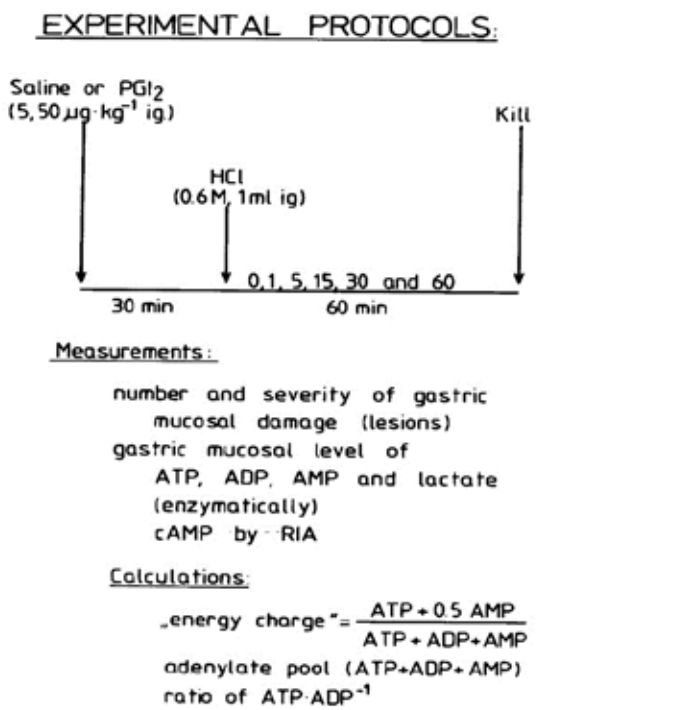


**Figure 200.** Experimental protocol for study of $PGI_2$-induced gastric mucosal-preventive effects and their biochemical changes in HCl model. [Mózsik Gy. (2006) Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

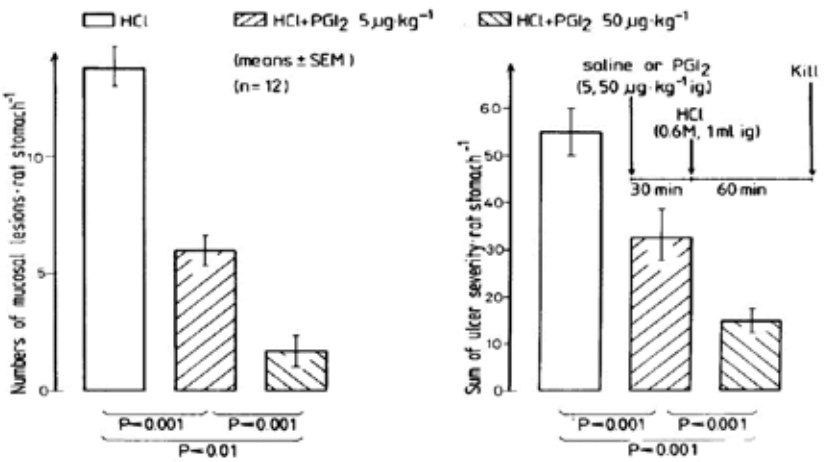


**Figure 201.** Determination of $ED_{50}$ value for $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

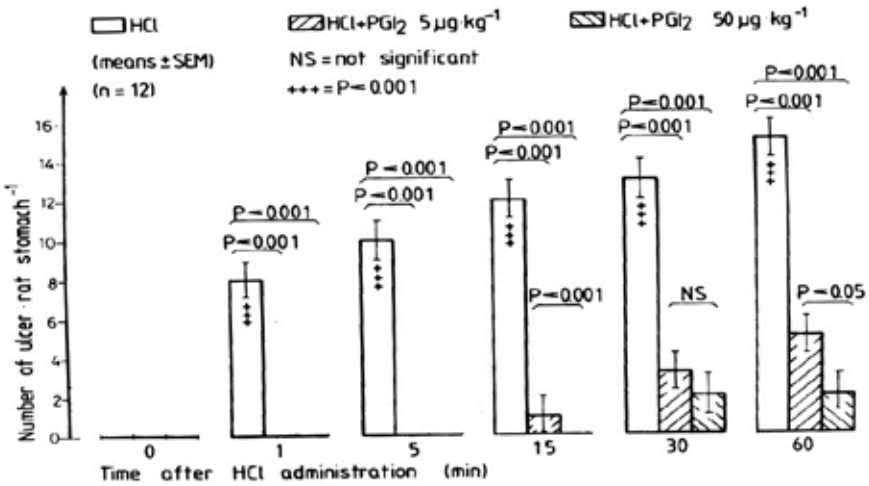


**Figure 202.** Inhibitory effect of $PGI_2$ on the number of gastric mucosal damage (means ± SEM) in the HCl model. [Mózsik Gy. (2006).Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

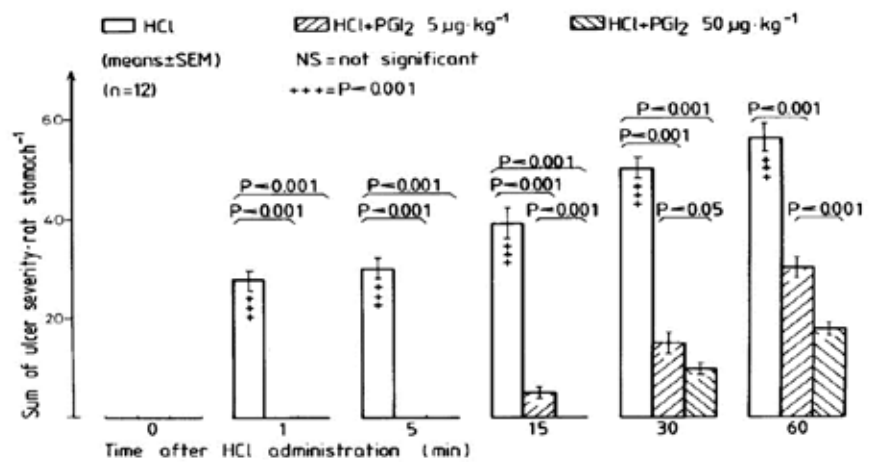


**Figure 203.** Inhibitory effect of $PGI_2$ on the sum of the ulcer severity in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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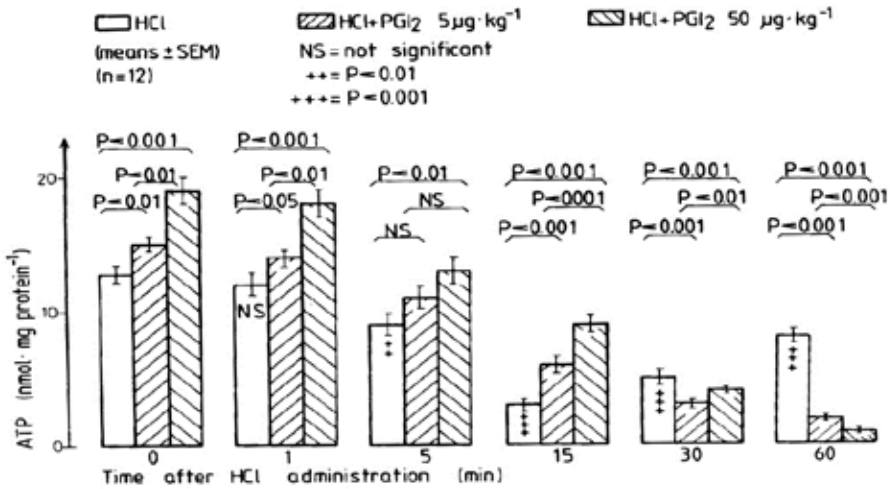


**Figure 204.** Dose–response changes produced by $PGI_2$ in the rat gastric fundic mucosal level of ATP (means ± SEM in HCl model). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

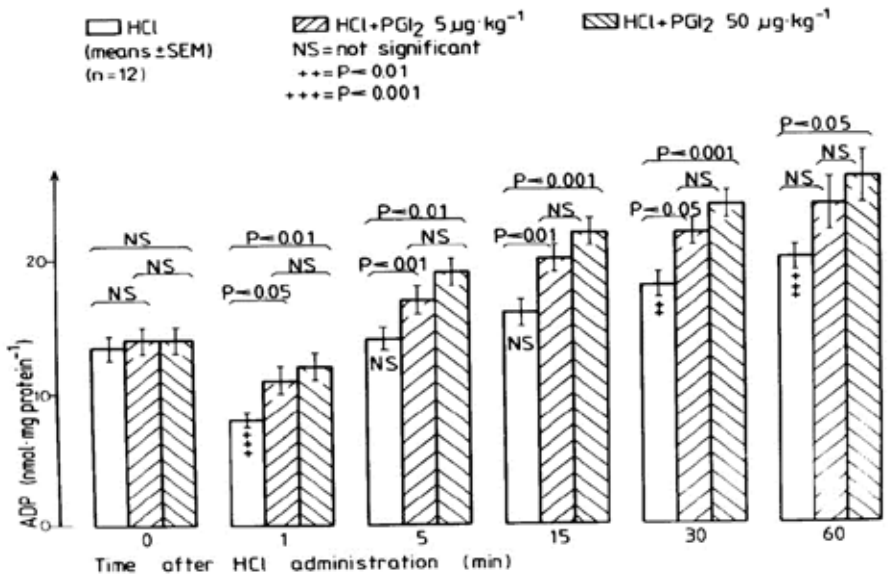


**Figure 205.** Dose–response changes produced by $PGI_2$ in the rat gastric fundic mucosal level of ADP in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

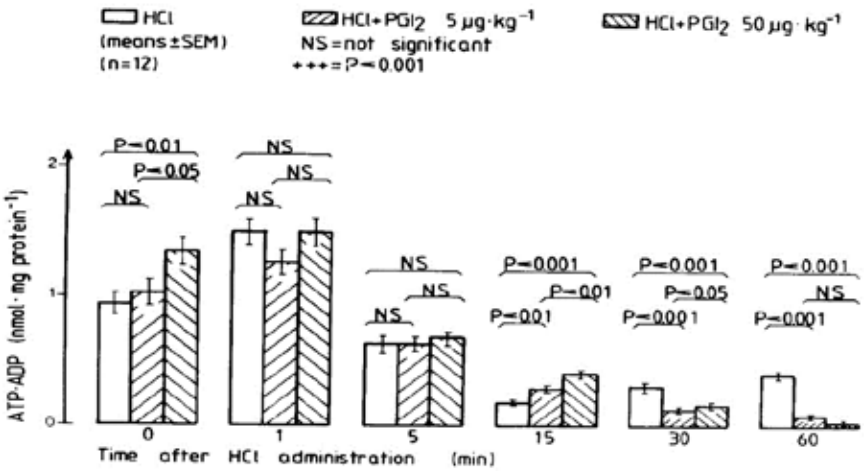


**Figure 206.** Changes in the values of ATP/ADP produced by $PGI_2$ in the rat gastric mucosa in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

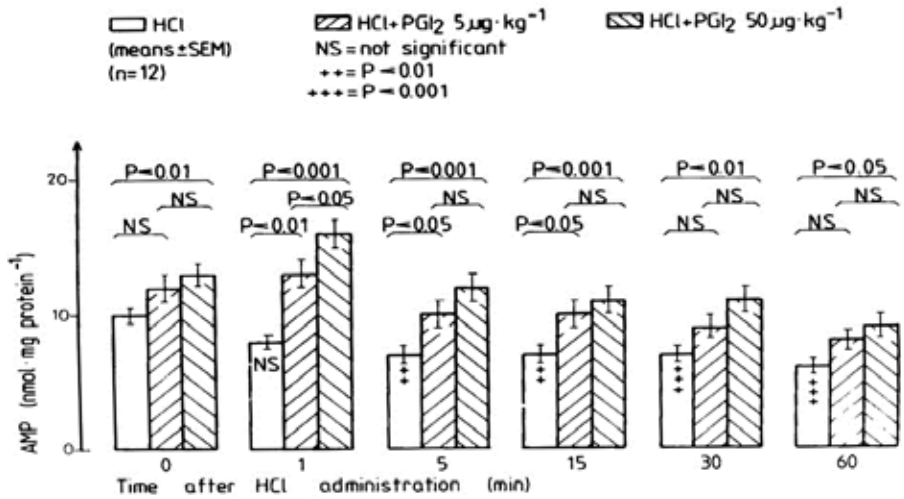


**Figure 207.** Dose–response changes in the rat gastric fundic mucosal level of AMP produced by $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005) Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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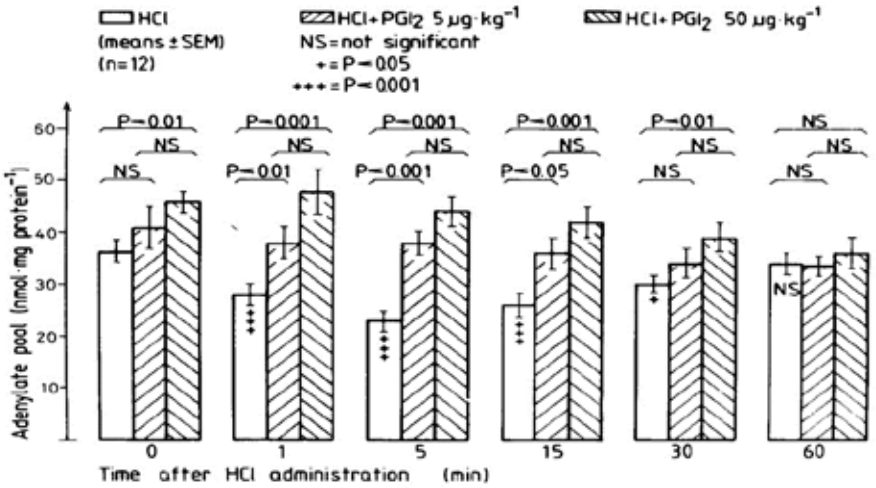


**Figure 208.** Dose–response changes in the rat gastric fundic mucosal levels of adenylate pool produced by $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

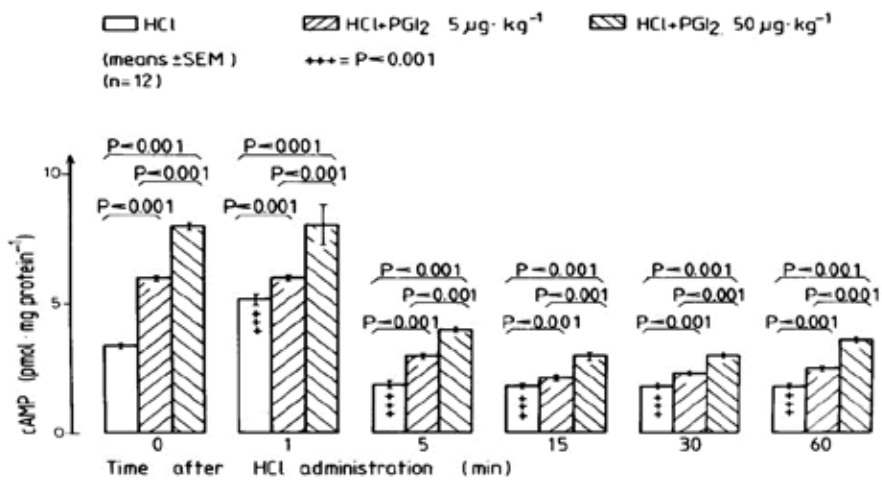


**Figure 209.** Dose–response changes in the rat gastric fundic mucosal levels of cAMP produced by $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

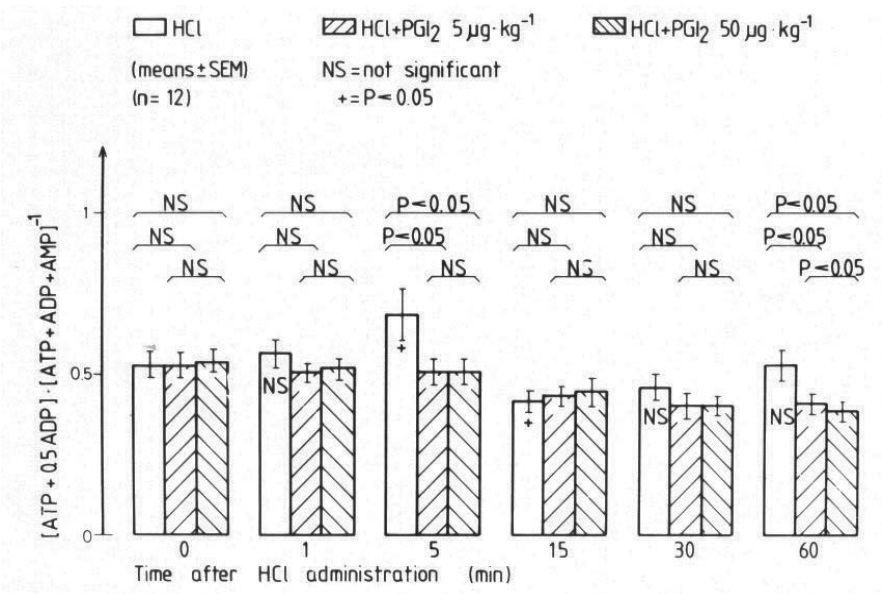


**Figure 210.** Changes in the rat gastric fundic mucosal level of "energy charge" produced by $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

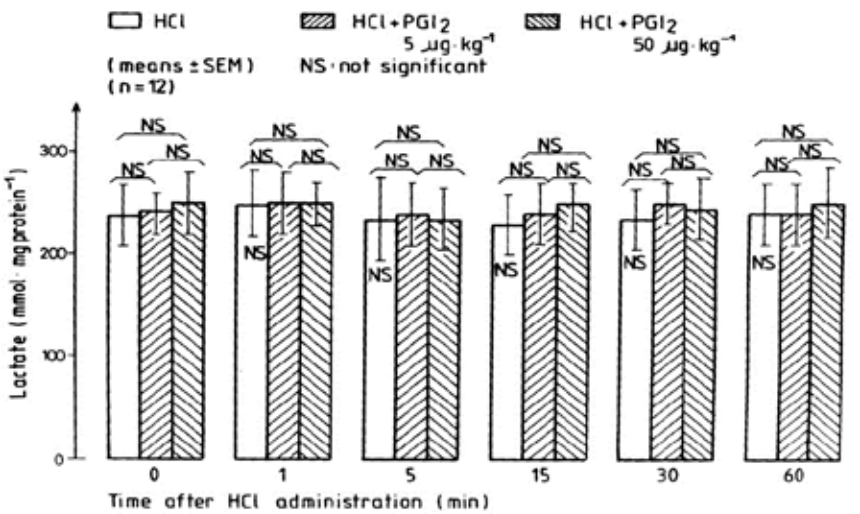


**Figure 211.** $PGI_2$-induced effects on the rat gastric fundic mucosal levels of lactate produced by $PGI_2$ in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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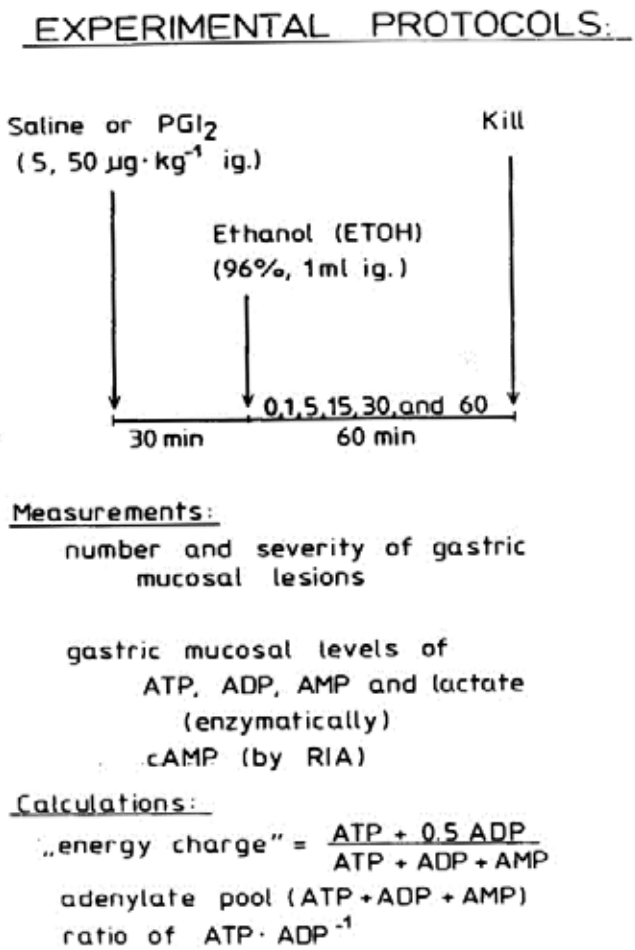


**Figure 212.** Experimental protocol for the study of gastric fundic mucosal-preventive effect of $PGI_2$ and its biochemical background in ethanol (ETOH) (nonacid-dependent) model. [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

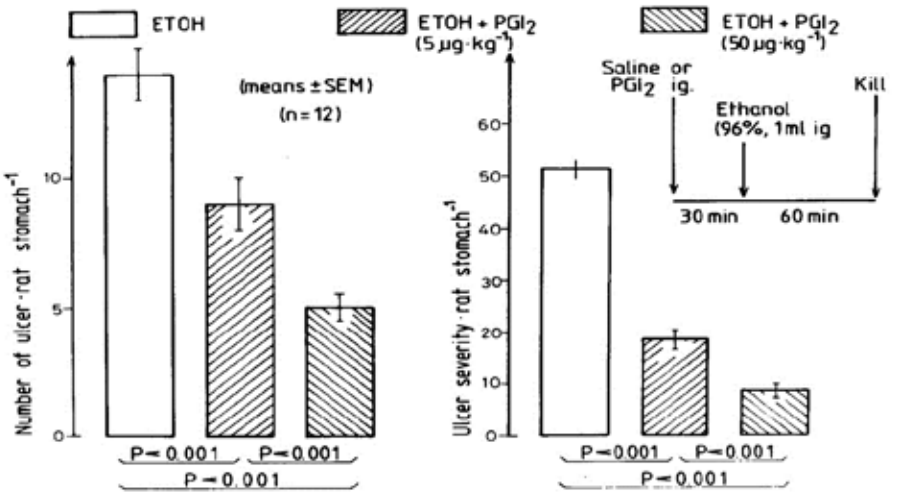


**Figure 213.** Determination of $ED_{50}$ value for $PGI_2$ in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

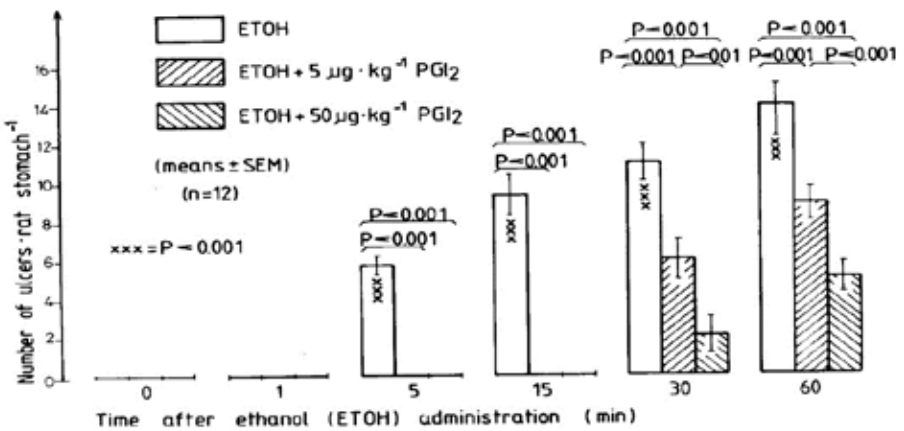


**Figure 214.** $PGI_2$-induced gastric mucosal-preventive effect (number) in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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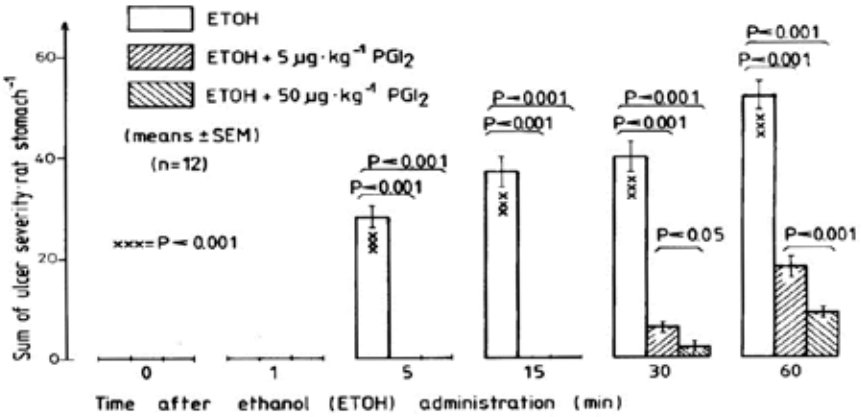


**Figure 215.** $PGI_2$-induced gastric mucosal-preventive effect on the severity in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

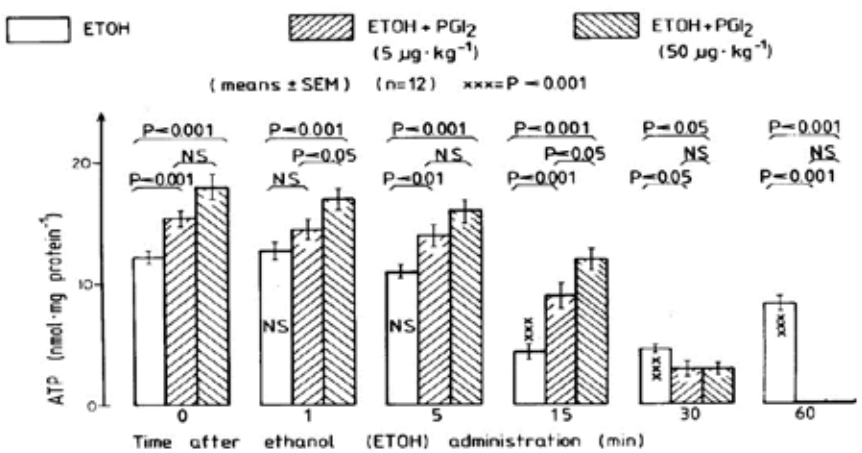


**Figure 216.** Dose-dependent changes produced by $PGI_2$ in the tissue levels of ATP of the rat gastric fundic mucosa in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

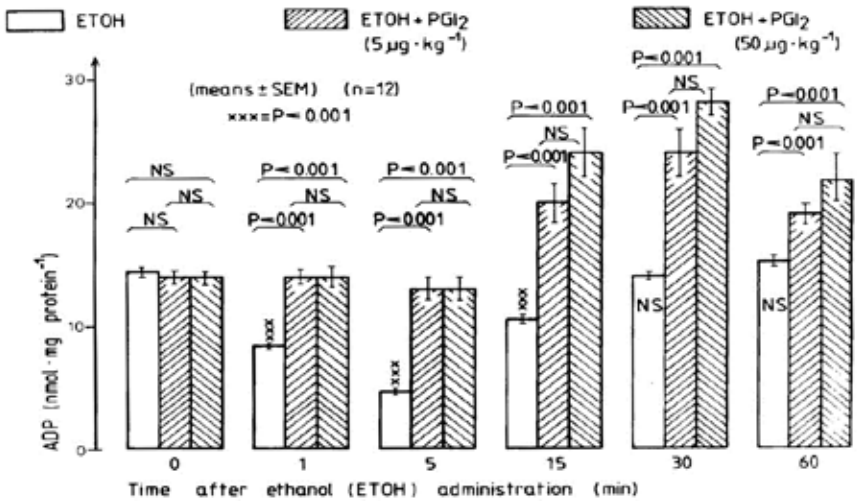


**Figure 217.** Dose-dependent changes produced by $PGI_2$ in the gastric fundic mucosal level of ADP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

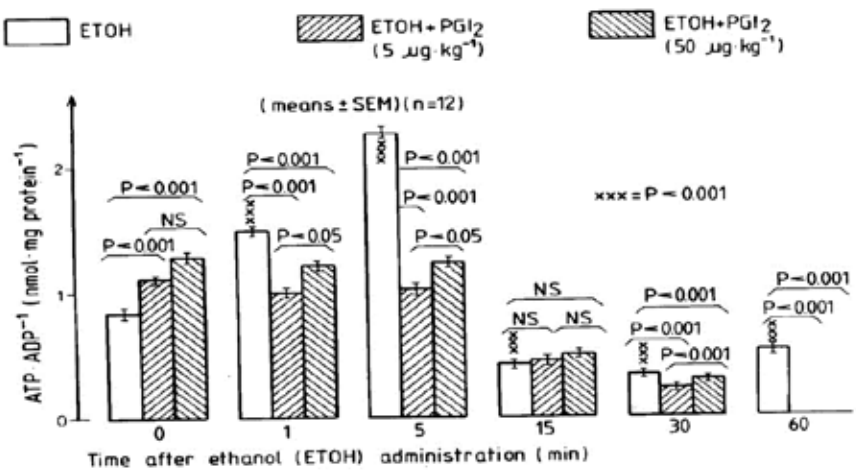


**Figure 218.** $PGI_2$-induced changes in the gastric fundic mucosal level of ATP/ADP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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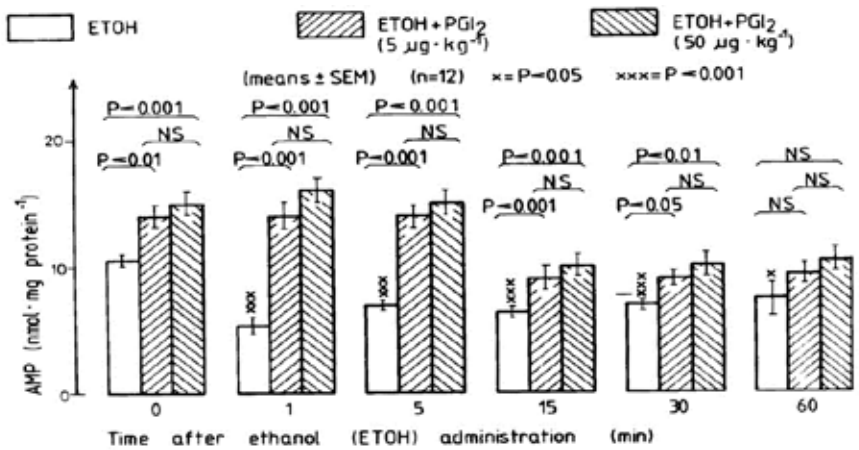


**Figure 219.** Dose-dependent changes in the rat gastric fundic mucosal level of AMP produced by $PGI_2$ in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

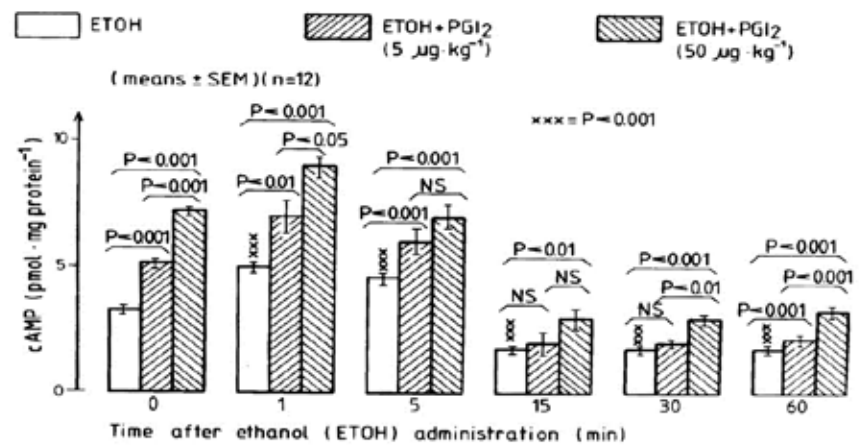


**Figure 220.** Dose-dependent changes in the rat gastric fundic mucosal level of cAMP produced by $PGI_2$ in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

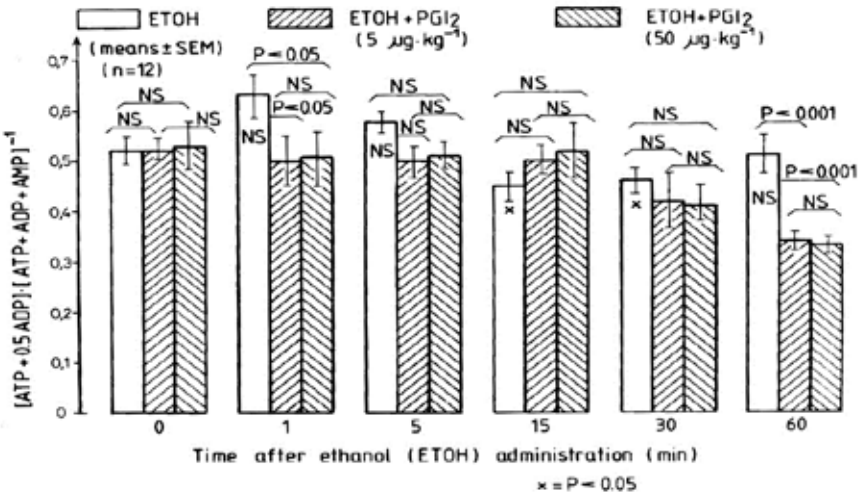


**Figure 221.** Changes in the rat gastric fundic mucosal level of "energy charge" produced by $PGI_2$ in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

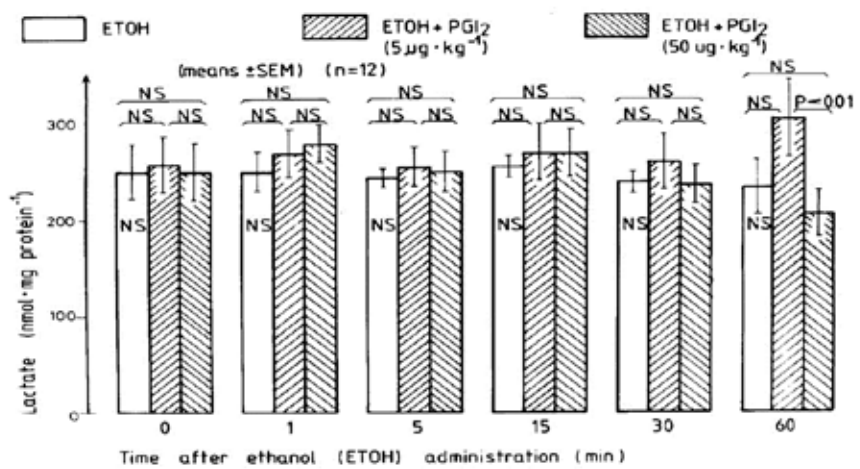


**Figure 222.** $PGI_2$-induced changes in the rat gastric fundic mucosal level of lactate in ETOH model (means ±SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

It was interesting to note that the $PGI_2$-induced gastric mucosal-preventive effect appears in the first (0–15 min) time period as that obtained by $PGI_2$ in the HCl model.

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The biochemically obtained results indicated dose-dependent actions on the tissue levels of ATP, ADP, AMP and cAMP and changes in the values of ratio of ATP/ADP, and the adenylate pool was found to be about the same.

*8.10.3. Gastric mucosal-preventive effect of β-carotene versus changes in the gastric mucosal energy systems in HCl model in rats*

β-carotene (given in 1 and 10 mg/kg doses i.g.) led to significant and dose-dependent prevention of the gastric mucosal damage (Figures 222, 223). The 1 mg/kg β-carotene produced the $ED_{50}$ value in the HCl model (Figure 223). It was also interesting to note that β-carotene-induced gastric mucosal prevention appears later (from 15 to 60 min) (Figures 225, 226). The ATP–ADP transformation was decreased, whereas the ATP–cAMP transformation was increased (Figures 227–230). No significant changes were obtained in the adenylate pool (Figure 231) and the tissue level of lactate (Figure 232).

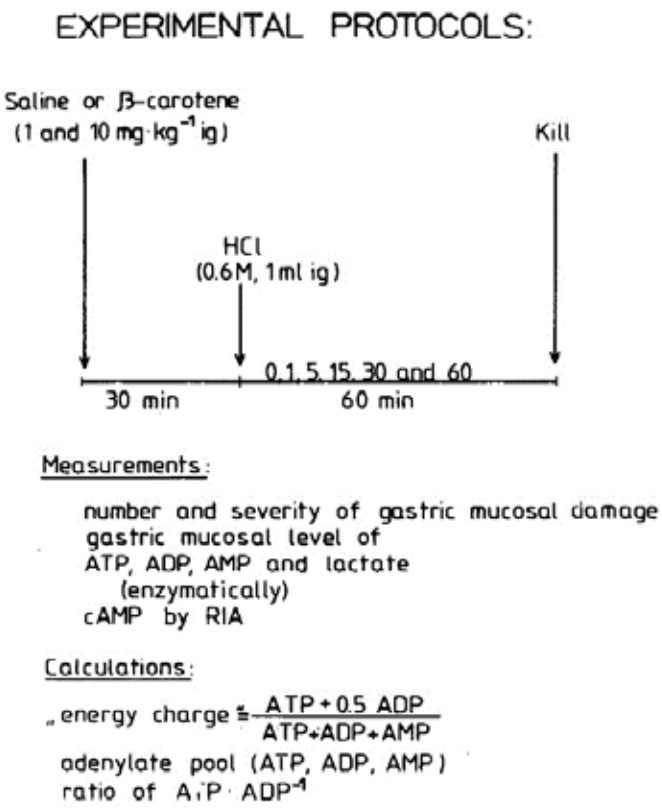



**Figure 223.** Experimental protocol for the study of gastric mucosal-preventive effects and their biochemistry produced by β-carotene in HCl model. [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

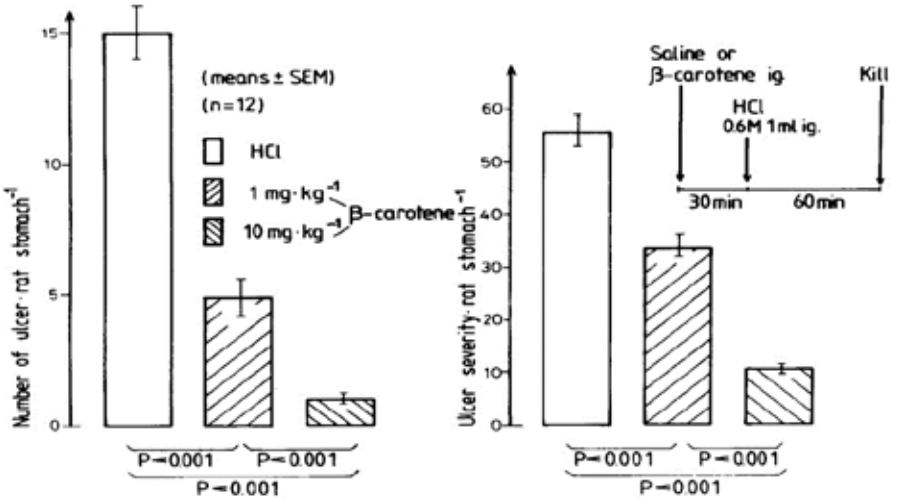
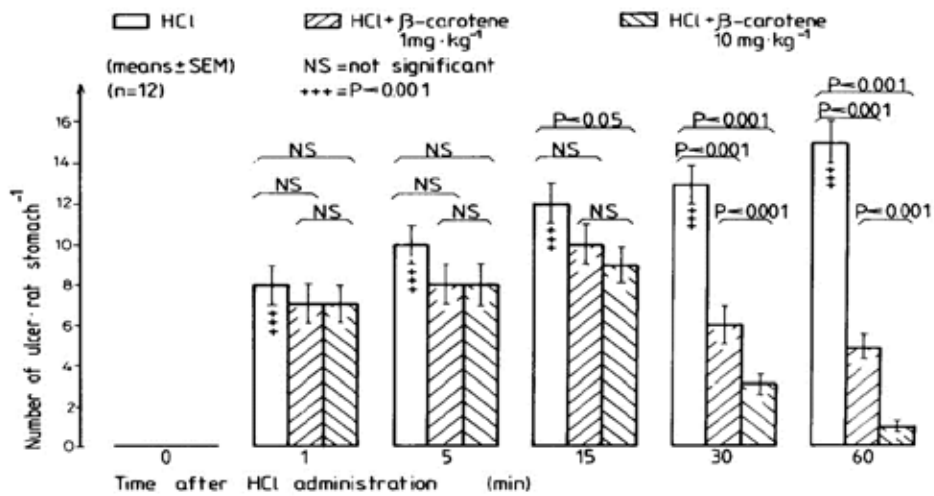



**Figure 224.** Determination of $ED_{50}$ values for gastric fundic mucosal-preventive effects of β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]




Dose-dependent gastric mucosal-preventive effect (number) of β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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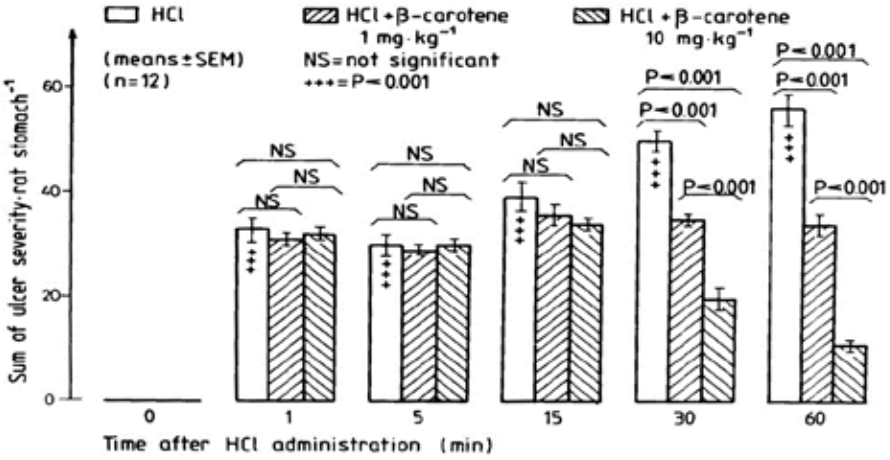


**Figure 226.** Dose-dependent gastric mucosal-protective effect (severity) of β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

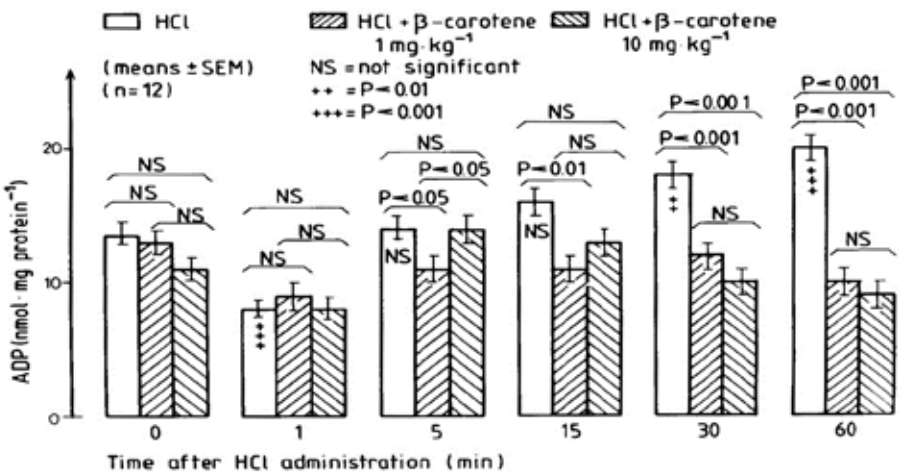


**Figure 227.** Dose-dependent changes in the rat gastric fundic mucosal level of ADP produced by β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

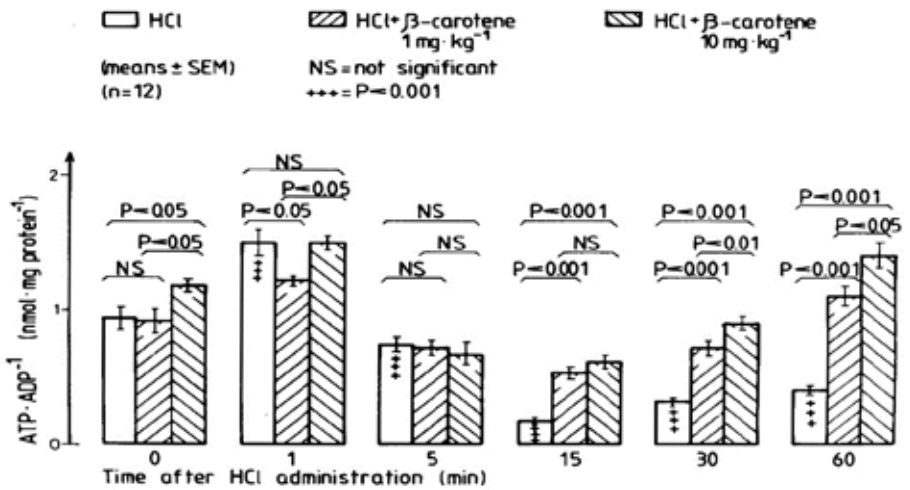


**Figure 228.** Changes in the gastric fundic mucosal level of ATP/ADP produced by β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

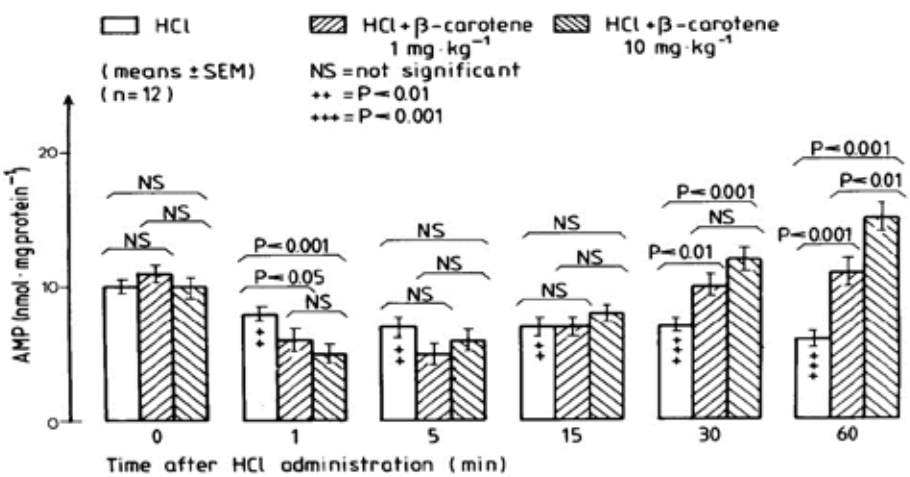


**Figure 229.** Changes in the rat gastric fundic mucosal level of AMP produced by β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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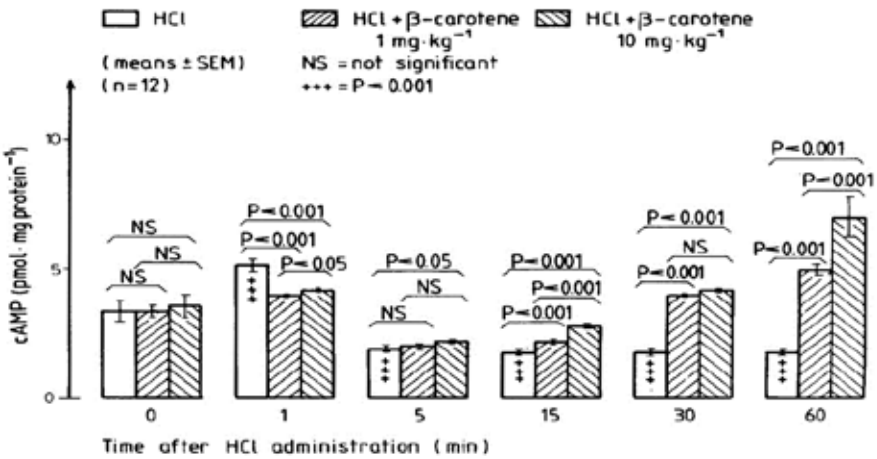


**Figure 230.** Changes in the rat gastric fundic mucosal level of cAMP produced by β-carotene in HCl model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

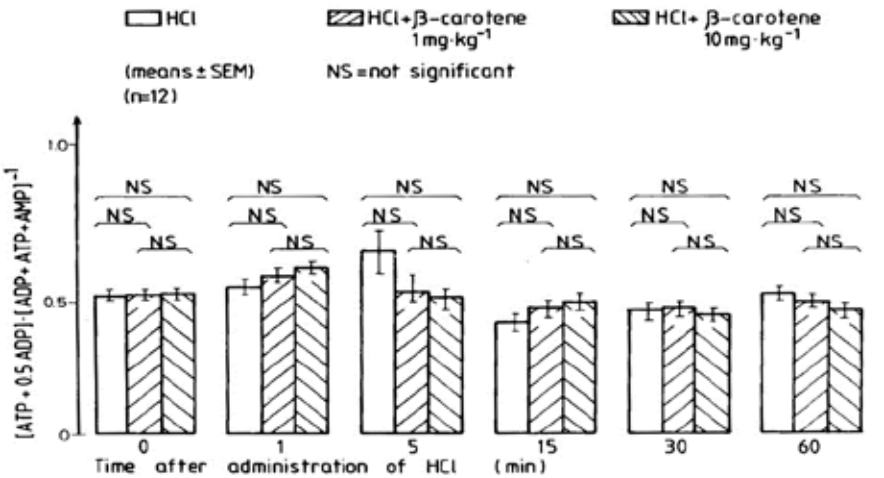


**Figure 231.** "Energy charge" in the rat gastric fundic mucosal level in HCl model before and after β-carotene treatment (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

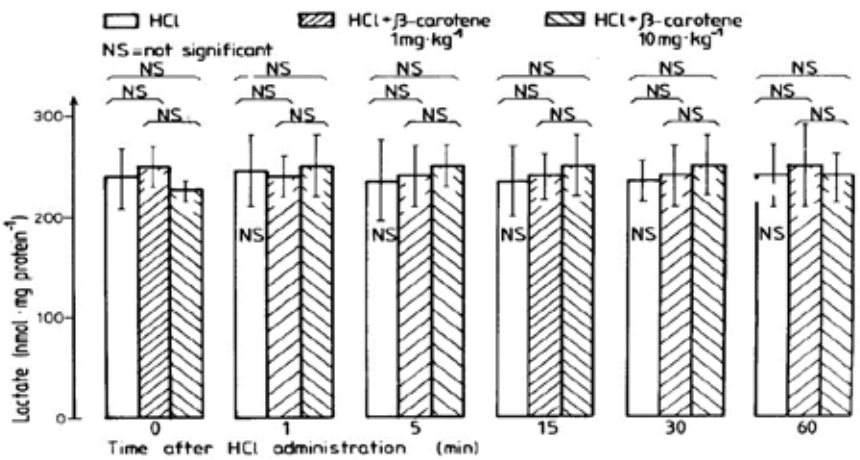


**Figure 232.** Tissue levels of lactate in the rat gastric fundic mucosa in HCl model before and after β-carotene treatment (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

### *8.10.4. Mucosal-preventive effect of β-carotene versus changes in the gastric mucosal energy systems in ETOH model in rats*

β-carotene showed gastric mucosal-preventive effect in ETOH model (Jávor et al., 1983; Mózsik and Jávor, 1981) (Figures 232–243).

The $ED_{5O}$ was also found for 1 mg/kg of β-carotene (i.g. given) (Figure 234). The gastric mucosal effect of β-carotene was also found in the later (from 15 to 60 min) time in order to number and severity of gastric mucosal damage (Figures 235–236). The ATP–ADP transformation was decreased, whereas the ATP–cAMP transformation was increased (Figures 237–241). No significant changes were obtained in the adenylate pool, "energy charge" and lactate (Figures 242, 243).

The results of our observations obtained from HCl- and ethanol-induced gastric mucosal damage and their gastric mucosal-preventive effects produced by $PGI_2$ and β-carotene (including the changes in the membrane-bound ATP-dependent energy systems) allowed us to evaluate the details of their mechanisms in the development of gastric mucosal damage (HCl and ethanol) and $PGI_2$ and β-carotene-induced gastric mucosal prevention (under animal experiments):

**1.** HCl and ethanol (as mucosal-damaging chemical agents with different chemical properties) produce the same macroscopic appearance of gastric mucosal damage after their applications;

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2. The macroscopic appearance of gastric mucosal damage produced by HCl and ethanol indicated the same patterns of time sequences (in order of number and severity) of the development of gastric mucosal tissues;

3. $PGI_2$ and β-carotene exert gastric mucosal protection against HCl- and ethanol-induced gastric mucosal damage; however, their gastroprotective effects differ (from each other) in time after the administration of necrotizing agents: $PGI_2$ acts earlier (from 0 to 15 min), whereas β-carotene is gastroprotective and it can be detected later (from 15 to 60 min) after the administration of necrotizing agents;

4. The extent of ATP transformation into ADP is increased during the development of gastric mucosal damage produced by HCl and ethanol in the gastric fundic mucosa (in association with a small increase of gastric mucosal cAMP level) in both models;

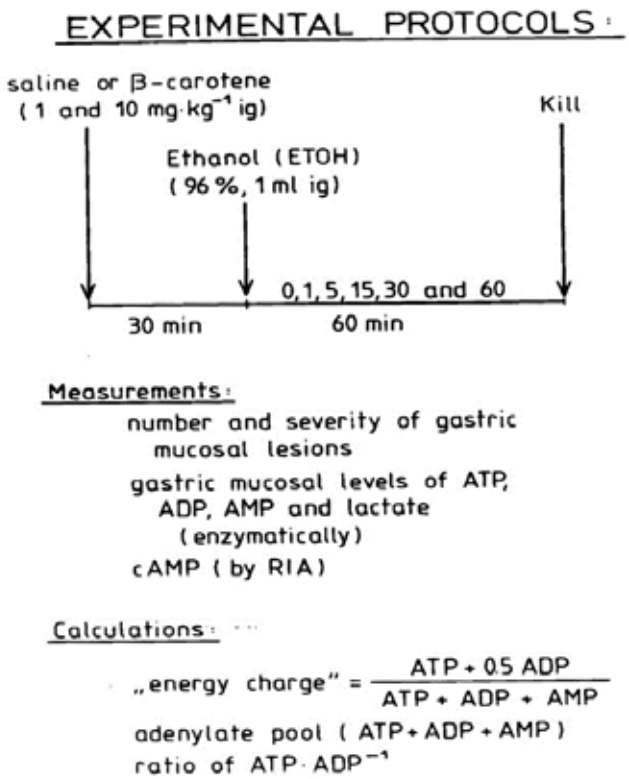


**Figure 233.** Experimental protocol for the study of gastric mucosal-protective effects and their biochemistry of β-carotene in ETOH model. [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

5. The gastric mucosal-preventive effects of $PGI_2$ and β-carotene associate with the decrease of ATP transformation into ADP and increase of ATP transformation into cAMP in the rat gastric fundic mucosa;

6. $PGI_2$ and β-carotene produce dose-dependent biochemical responses in the rat gastric mucosa (ATP, ADP, AMP, cAMP and adenylate pool) during the development of gastric mucosal-preventive protection against the chemically induced gastric mucosal damage;

7. The biochemical mechanisms (changes in the membrane-bound ATP-dependent energy systems) of gastric mucosal-protective effects of $PGI_2$ and β-carotene are the same in the HCl- and ethanol-induced mucosa;

8. Biochemically, no presence of gastric hypoxemia was proved in the rat gastric fundic mucosa during the development of both gastric mucosal damage (by HCl and ethanol) and gastric mucosal protection of $PGI_2$ and β-carotene against HCl and ethanol.

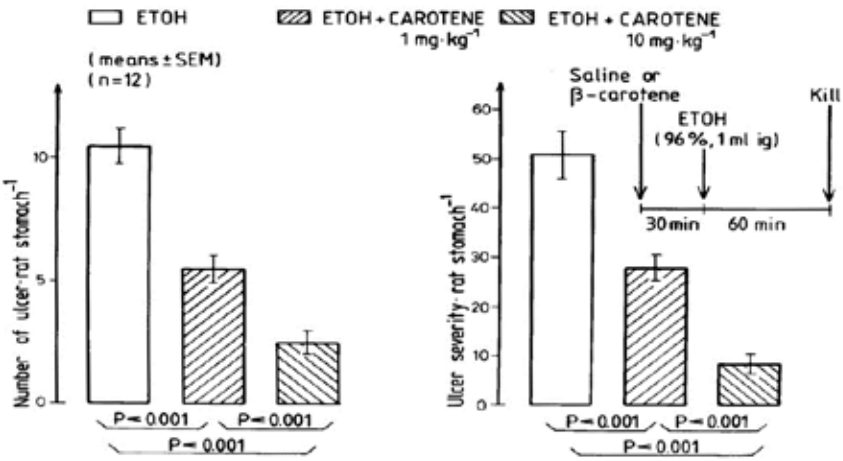


**Figure 234.** Determination of $ED_{50}$ value for the gastric mucosal-protective effect of β-carotene in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission)].

### 8.11. Interrelationships between the changes in parameters of oxygen free radical systems versus cellular energy systems in the gastric mucosa in acid-dependent (HCl) and nonacid-dependent (ETOH) experimental models during the time of development of gastric mucosal damage and gastric mucosal prevention produced by $PGI_2$ and β-carotene

Many observations indicated clearly that the maintenance of good equilibrium (between the aggressive and defensive mechanisms) is an extremely complicated summary (result) in the living organs. We are able to learn and recognize different aspects of this extremely complicated equilibrium in the living organs, and our knowledge has changed over time.

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The researchers are able to approach only some small piece from the whole, meanwhile many-many processes (regulatory pathways) are running beside each others. We are willing to hope that the selected piece(s) (to be used in the studies) is (are) an important field(s) for the whole screening method to understand the key point(s) in the keeping of this equilibrium between the aggressive and defensive factors.

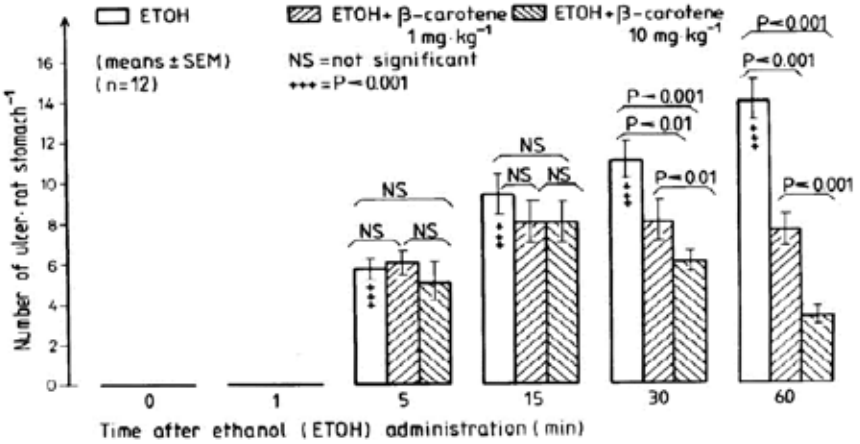


**Figure 235.** β-carotene-induced gastric mucosal-preventive effects on the rat gastric fundic mucosa (number) in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

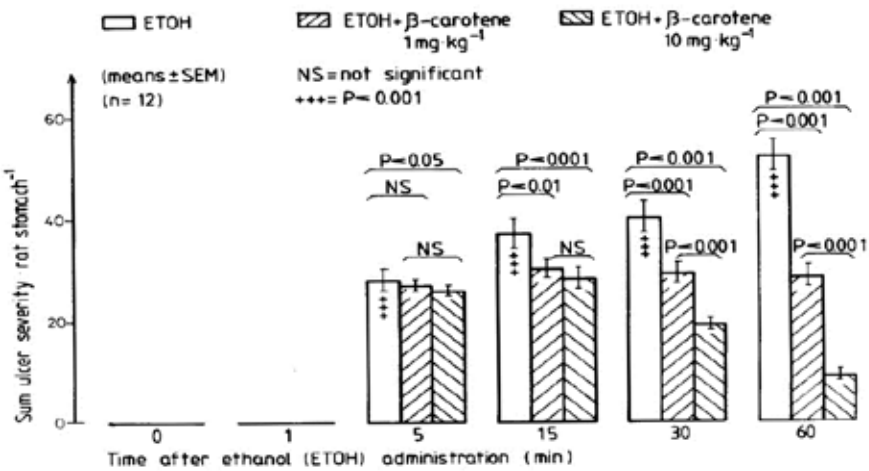


**Figure 236.** β-carotene-induced gastric fundic mucosal-preventive effect (on the severity) in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

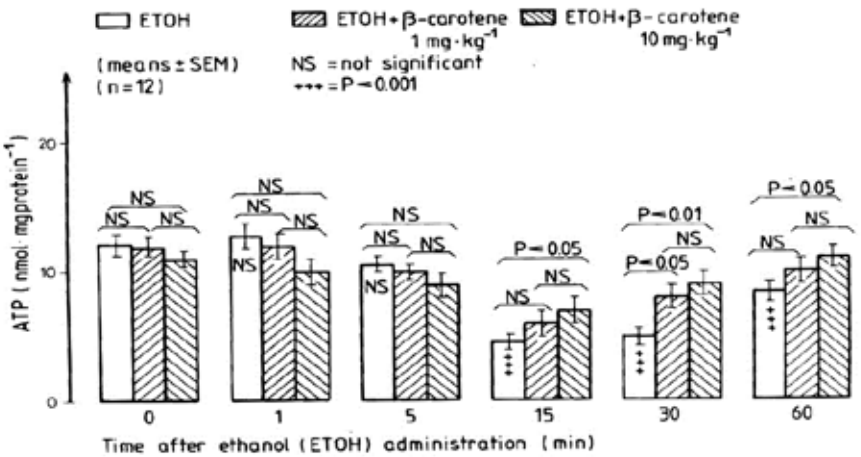


**Figure 237.** β-carotene-induced changes in the rat gastric fundic mucosal level of ATP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

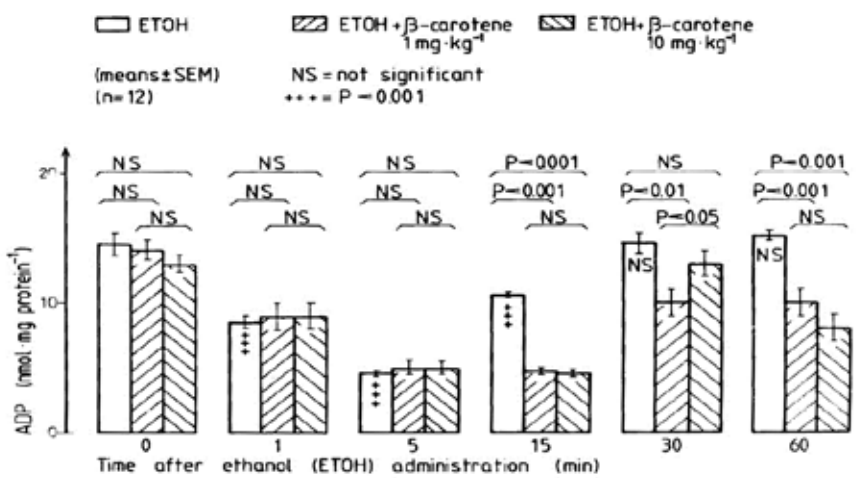


**Figure 238.** β-carotene-induced changes in the rat gastric fundic mucosal levels of ADP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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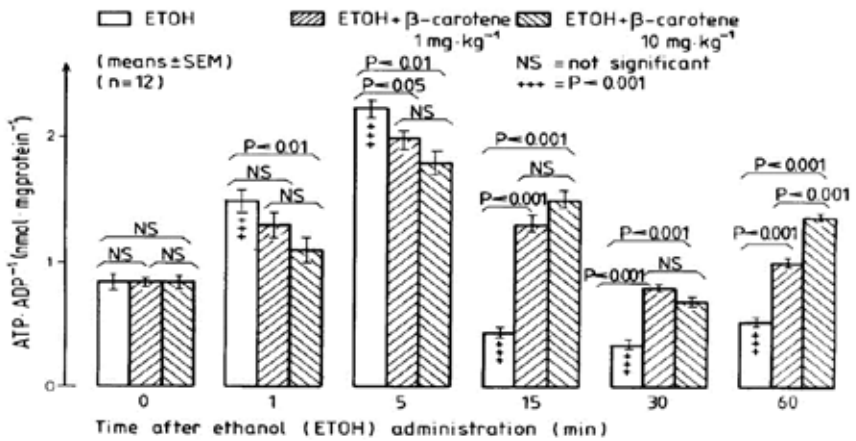


**Figure 239.** β-carotene-induced changes in ratio of ATP/ADP of the rat gastric fundic mucosa in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

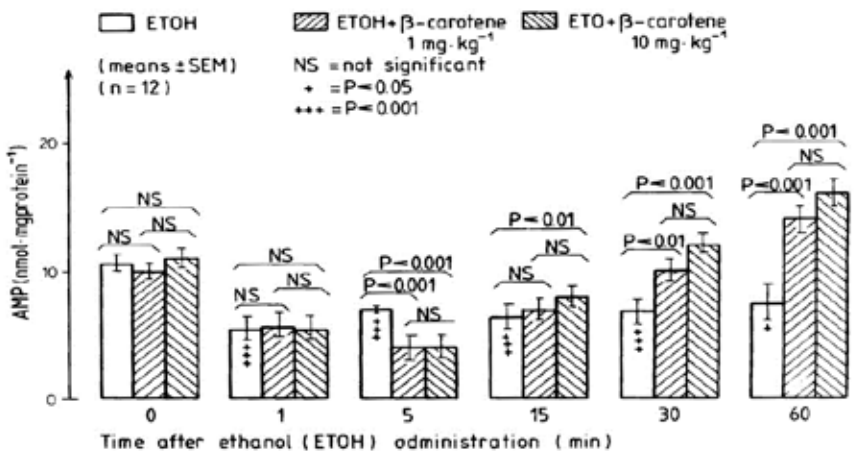


**Figure 240.** β-carotene-induced changes in the rat gastric mucosal levels of AMP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

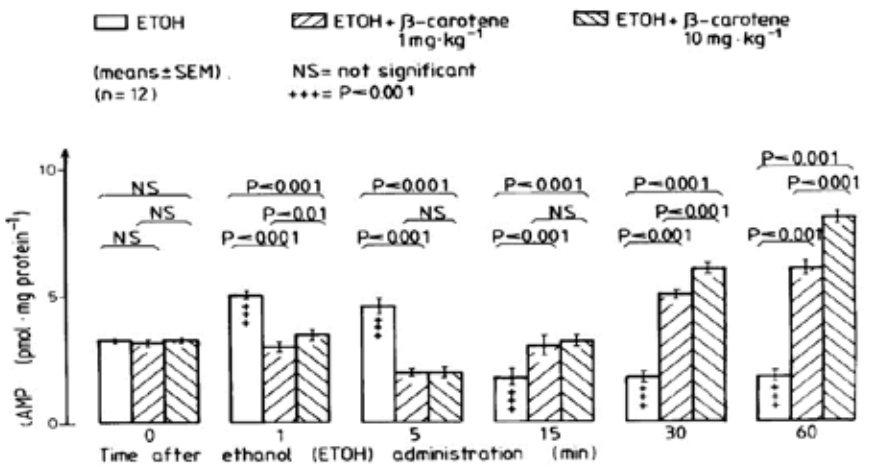


**Figure 241.** β-carotene-induced changes in the rat gastric fundic mucosal levels of cAMP in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

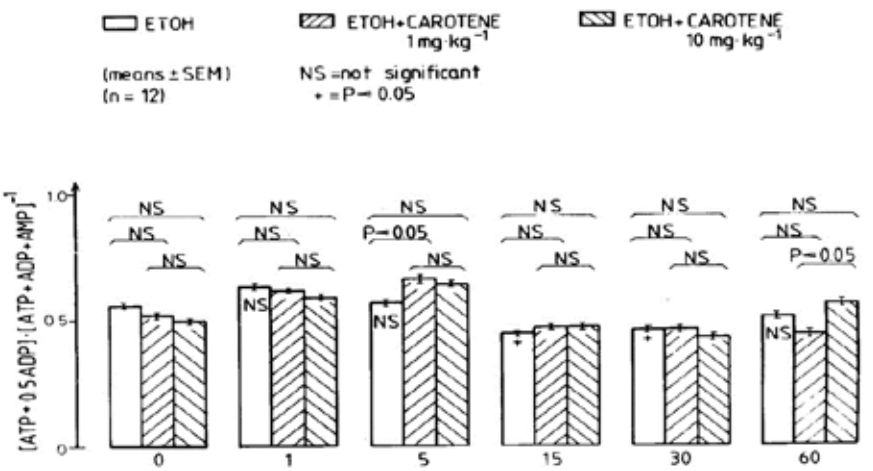


**Figure 242.** "Energy charge" in the rat gastric fundic mucosa with and without application of β-carotene in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

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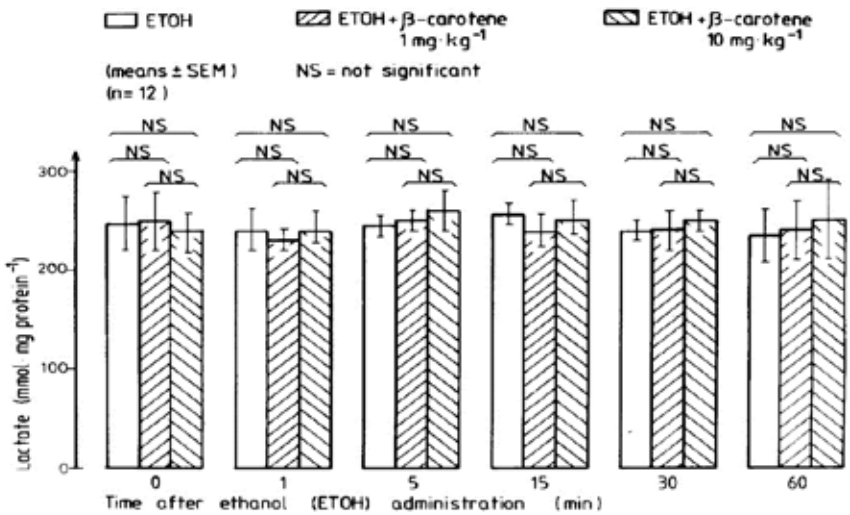


**Figure 243.** Lactate levels in the rat gastric fundic mucosa produced by β-carotene in ETOH model (means ± SEM). [Mózsik Gy. (2006). Molecular pharmacology and biochemistry of gastroduodenal mucosal damage and protection. In: Mózsik Gy. (Ed.) Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó, Budapest. pp. 139–224 (with kind permission).]

One of these fields is the oxygen free radical system, which incorporates different endogen enzyme systems (catalase, peroxidase, reduced glutathione peroxidase and superoxide dismutase) and many internal (vitamin C, vitamins A and E) and other external (chemical) components.

The production of oxygen free radicals and the compensation with different antioxidant compounds (scavengers) came into the focus of research. An excellent handbook was published (Laher, 2014), in which these problems were overviewed in the different organs (including the cellular, organ and body levels of living organisms).

We were also involved in this field of the studies (Bódis et al., 1995 a, b; 1996, 1997 a, b; 1998; 2000; Mózsik et al., 2001a; Szabó et al., 1996, 1997 a, b, c; 2000, 2012, 2014).

In these observations, systematic observations were carried out in the experimental models as those detailed in Section 8.10.

In the forthcoming sections, we demonstrate the tendencies of changes of oxygen free radical systems in comparison with the changes in the cellular energy systems in the gastrointestinal tract.

The superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972), which was modified by Matkovics et al. (1977). The glutathione peroxidase (GSH-px) activity was determined by the loss of reduced added to the supernatant and expressed as μmol GSH oxidized per minute (Flohe and Güzlei, 1984). The catalase (CAT) activity was measured using the method of Beers and Sizer (1952) and the glutathione (GSH) content was determined using Ellman's method (1959). The tissue level of malondial-

dehyde (MDA) was measured using a modification (Zsoldos et al., 1983) of the original method described by Fong et al. (1973). The protein content was assayed by the method described by Lowry et al. (1951). The biochemical results were expressed in accordance to 1.0-mg protein (means ± SEM).

These parameters were measured from the gastric mucosal tissues (and not from the sera). Furthermore, these studies were carried out in an acid-dependent (HCl) and nonacid-dependent (ETOH) ulcer model in rats. Additionally we applied to prevent the gastric mucosal damage an endogenous compound ($PGI_2$) (which has no scavenger property) and a scavenger compound (β-carotene). The main results of these observations are summarized briefly in the forthcoming sections.

*8.11.1. Relationships between the changes in parameters of oxygen free radical systems versus cellular energy systems in the gastric mucosa of rats treated by intragastrically given HCl and ETOH*

The observations were carried out in the morning. The gastric lesions were produced by intragastric administration of 1 mL of 96% ethanol or 1 mL of 0.6 M HCl. The animals were sacrificed at 0, 1, 5, 15, 30 and 60 minutes after giving the necrotizing agents.

The number and severity, parameters of the oxygen free radicals and the biochemical constituents of cellular energy systems were measured from the gastric fundic mucosa (the details of these measurements).

It is important to note that all measurements were carried out from the same tissue samples.

The detailed results are expressed in case of changes in the gastric fundic mucosa of rats, after the application of necrotizing agents on time sequence (Mózsik7et al., 1991 a).

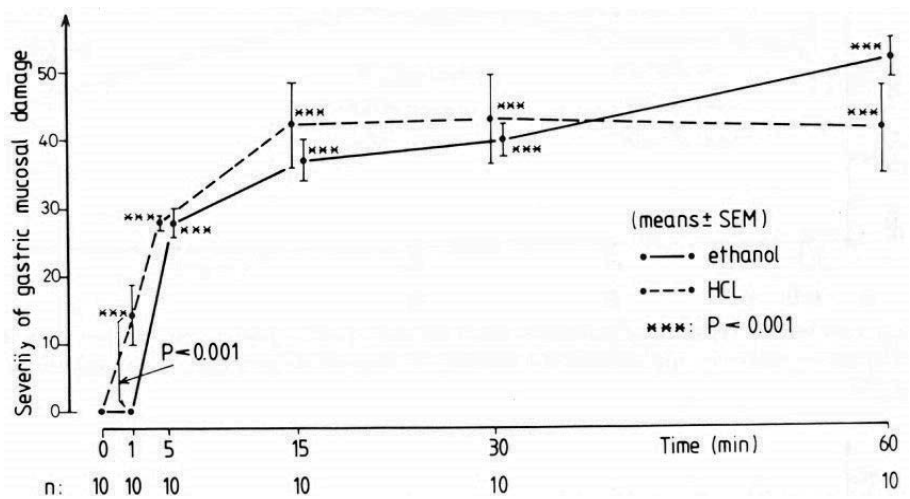


**Figure 244.** Changes in the severity of gastric mucosal damage of rats, produced by intragastrically applied ethanol (ETOH) and HCl, dependent on time after administration of necrotizing agents. [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission).]

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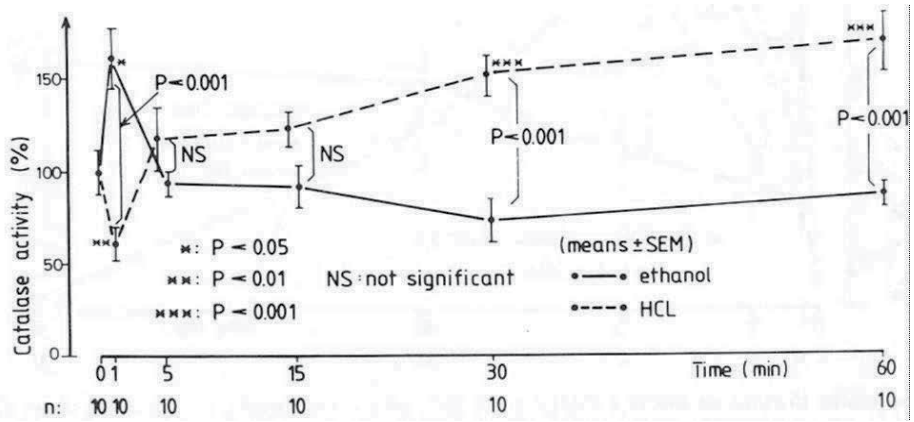


**Figure 245.** Changes in the catalase activity in the gastric fundic mucosa of rats, during the development of gastric mucosal damage produced by intragastrically applied ethanol or HCl, dependent on time after administration of necrotizing agents (100 = 0.60 Bergmeyer unit/mg protein). [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission).]

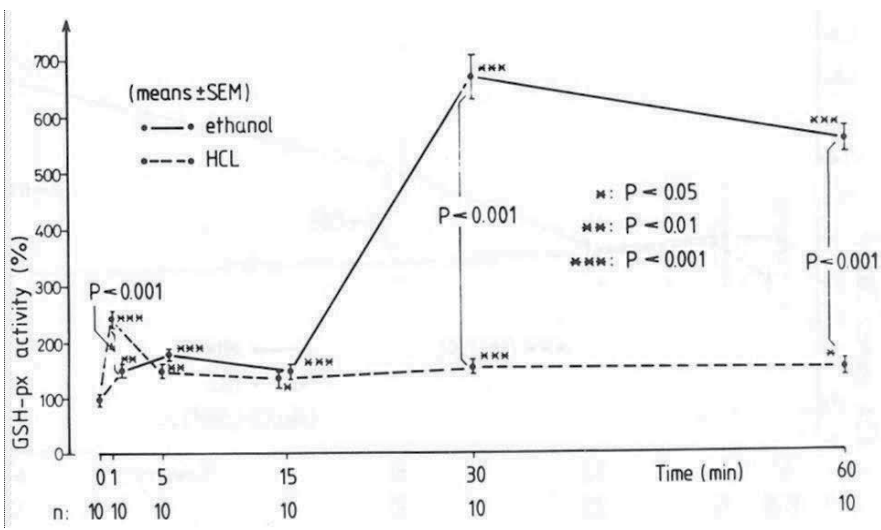


**Figure 246.** Changes in the glutathione peroxidase (GSH-px) activity in the gastric fundic mucosa of rats, during the development of gastric mucosal damage produced by intragastrically applied ethanol or HCl, dependent on time after administration of necrotizing agents (100 = 0.60 Bergmeyer unit/mg protein). [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission).]

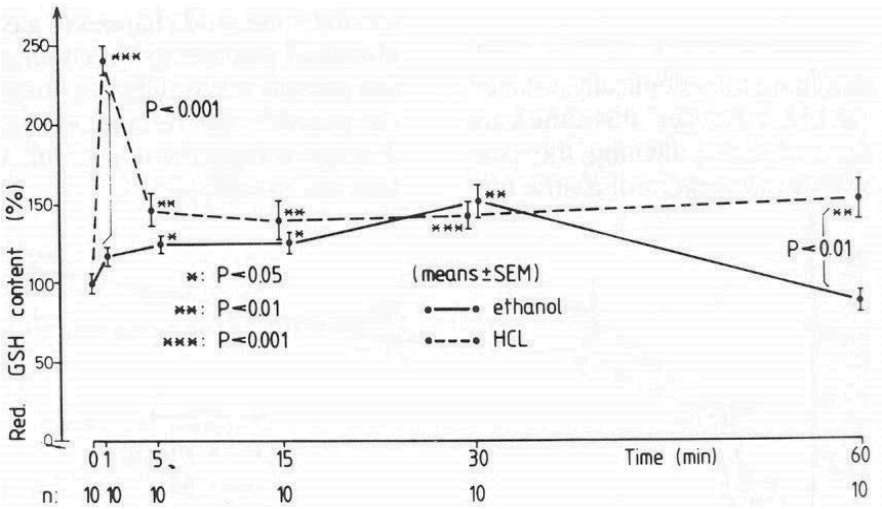


**Figure 247.** Changes in the tissue contents of reduced glutathione (GSH) from the gastric fundic mucosa of rats, during the development of gastric mucosal damage produced by intragastrically applied ethanol or HCl, dependent on time after administration of necrotizing agents (100 = 0.60 Bergmeyer unit/mg protein). [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission).]

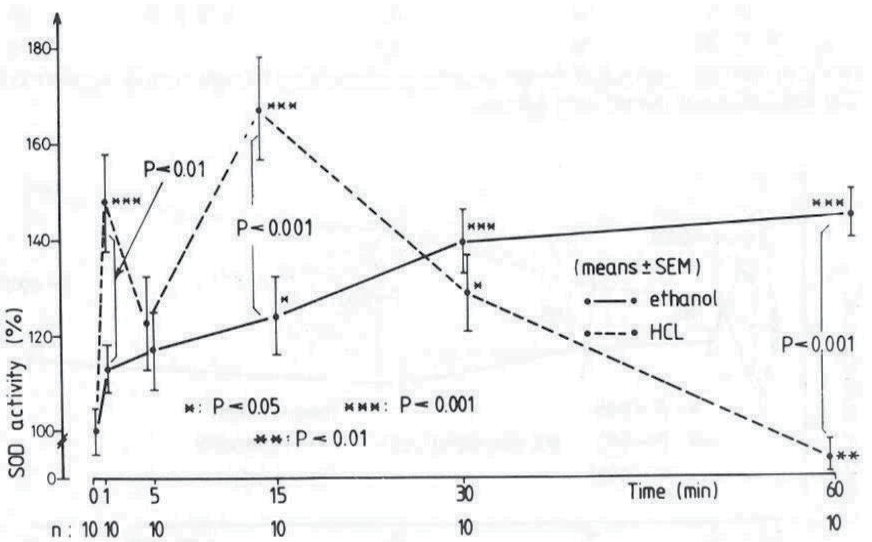


**Figure 248.** Changes in the superoxide dismutase (SOD) activity in the gastric fundic mucosa of rats, during the development of gastric mucosal damage produced by intragastrically applied ethanol or HCl, dependent on time after administration of necrotizing agents (100% = 20.0 U/mg protein). [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission)].

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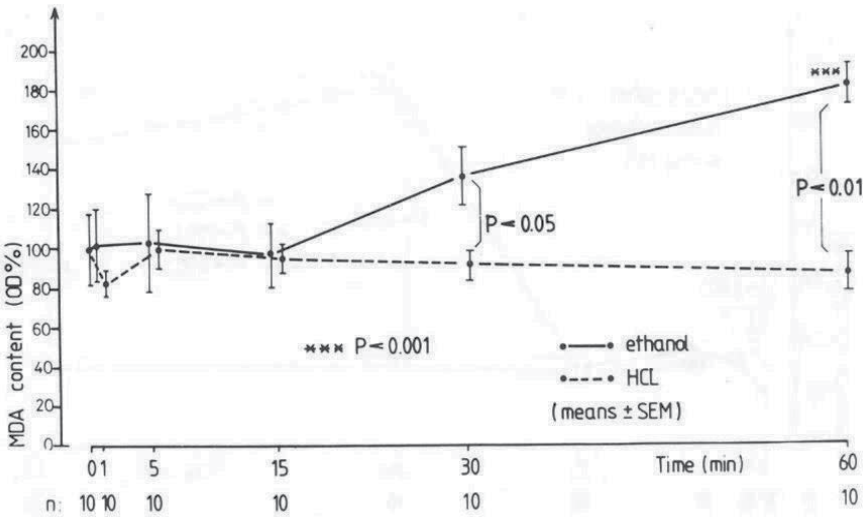


**Figure 249.** Changes in the tissue content of malondialdehyde (MDA) in the rat gastric fundic mucosa, during the development of gastric mucosal damage produced by intragastrically applied ethanol and HCl, dependent on time after administration of necrotizing agents (100% = 0.316 optical density/mg protein). [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission)].

| | Ethanol-model | | | | | HCL-model | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | 1 | 5 | 15 | 30 | 60 | 1 | 5 | 15 | 30 | 60 |
| Number of gastric lesions | | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| Severity of gastric lesions | | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ |
| Gastric mucosal Catalase | ↑ | | | | | ↓↓ | | | ↑↑↑ | ↑↑↑ |
| Gastric mucosal GSH-px | ↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | ↑↑ | ↑ | ↑↑↑ | ↑↑ |
| Gastric mucosal GSH | | ↑ | ↑ | ↑↑ | | ↑↑↑ | ↑↑ | ↑↑ | ↑↑↑ | ↑↑ |
| Gastric mucosal SOD | | | ↑ | ↑↑↑ | ↑↑↑ | ↑↑↑ | | ↑↑↑ | ↑ | |
| Gastric mucosal MDA | | | | | ↑↑↑ | | | | | |

↑ = increase ↓ = decrease

↑(↓): P < 0.05 ; ↑↑(↓↓): P < 0.01 ; ↑↑↑(↓↓↓): P < 0.001

**Figure 250.** Summary of the changes in oxygen free radicals (catalase; glutathione peroxidase, GSH; reduced glutathione, GSH; superoxide dismutase, SOD and malondialdehyde, MDA) in the gastric fundic mucosa of rats treated with ethanol or HCl during the development of gastric mucosal damage dependent on time after administration of necrotizing agents. [Mózsik, Sütő, Garamszegi, Jávor, Nagy, Vincze, Zsoldos: Eur. J. Gastroenterol. Hepatol. 3: 757–761, 1991 (with kind permission).]

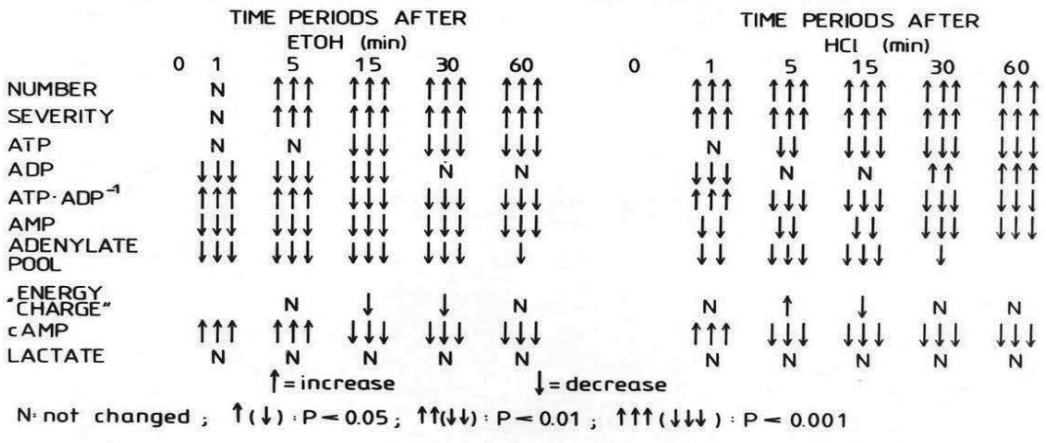


**Figure 251.** Summary of the changes in the biochemical parameters of cellular energy systems in the rat gastric fundic mucosa after intragastrically applied ethanol and HCl, dependent on time after administration of necrotizing agents (details are presented earlier). [Mózsik, Figler, Garamszegi, Jávor, Nagy, Sütő, Tárnok, Zsoldos, 1987c (with kind permission).]

The critical analyses of these results allowed us to conclude that:

**1.** The different changes in the experimentally measurable parameters (SOD, GSH-px, GSH, CAT and MDA) are present in gastric fundic mucosa of rats during the development of gastric mucosal damage produced by necrotizing agents, ETOH and HCl), however, these are not in a time-dependent manner;

**2.** The changes in the oxygen free radicals differ in the gastric fundic mucosa of rats treated with intragastrically applied necrotizing agents during the development of gastric mucosal damage, depending upon the extent of gastric mucosal damage and time after administration of necrotizing agents;

**3.** The measurable parameters of cellular energy systems are also detectable in the rat gastric fundic mucosa during the development of gastric mucosal damage produced by ETOH and HCl, dependent on time after necrotizing agents;

**4.** The changes in the parameters of cellular energy systems indicate in the same directions, however, there are differ from each other, on dependence of time after administration of necrotizing agents;

**5.** No close (which mathematically can be proven) the existing correlations are present in changes on experimentally measurable parameters of oxygen free radicals and of cellular energy systems in the gastric fundic mucosa of rats, treated by intragastrically applied ethanol and HCl on dependence severity (number) of gastric mucosa and on dependence of time after administrations of necrotizing agents (Mózsik et al., 1987c; Szabó et al., 2014).

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*8.10.2. Correlations between the oxygen free radicals and cellular energy systems in the gastric fundic mucosa of rats, during the development of gastric mucosal protection by prostacyclin ($PGI_2$) and β-carotene, on dependence of applied doses of protecting compounds, extent of mucosal protective effects and time after administration of EtOH and HCl*

These observations were carried out in the gastric fundic mucosa of rats during the development of gastric mucosal protection produced by $PGI_2$ and β-carotene in which the gastric mucosal damage induced by intragastrically applied 1 mL of 0.6 M HCl and 1.0 of 96% EtOH in 1-hour observations. The results are presented in the forthcoming schematic summaries of Figures 252–255.

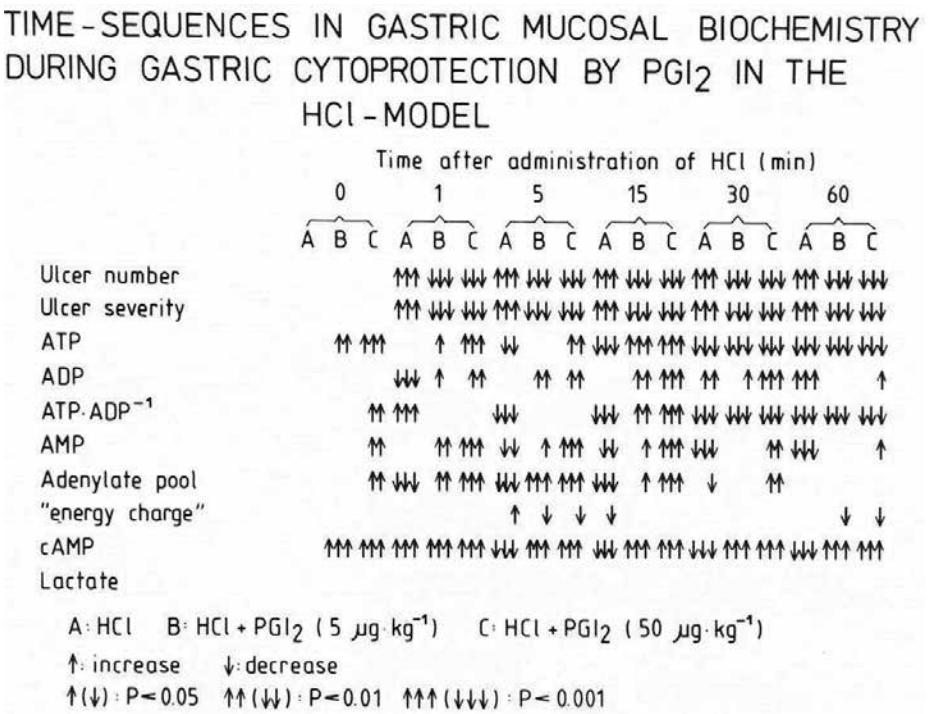


**Figure 252.** Summary of the changes in the parameters of cellular energy systems from the gastric fundic mucosa during the development of gastric mucosal protection of $PGI_2$ in rats, in which the gastric mucosal damage was produced by intragastric application of 1 mL of 0.6 M HCl (HCl model), depending upon the extents of mucosal protection, doses of $PGI_2$ dependent on time after administration of necrotizing and protecting agents. (Note that the $PGI_2$ was given 30 minutes before the HCl.) [(Mózsik, Garamszegi, Jávor, Sütő, Vincze, Tóth, Zsoldos: Mechanisms of gastric mucosal protection. In: Tsuchiya et al., 1988, Free Radical in Digestive Diseases. Elsevier Science Publishers, pp. 111–116 (with kind permission).]

Similar observations were carried out with the oxygen free radicals and cellular energy systems in the gastric fundic mucosa of rats, during the development of gastric mucosal protection of $PGI_2$ and β-carotene in rats, in which the gastric mucosal damage was produced by 1 mL of 96% ethanol given intragastrically in 1-hour time period. $PGI_2$ and β-carotene were applied 30 minutes before the application of ETOH.

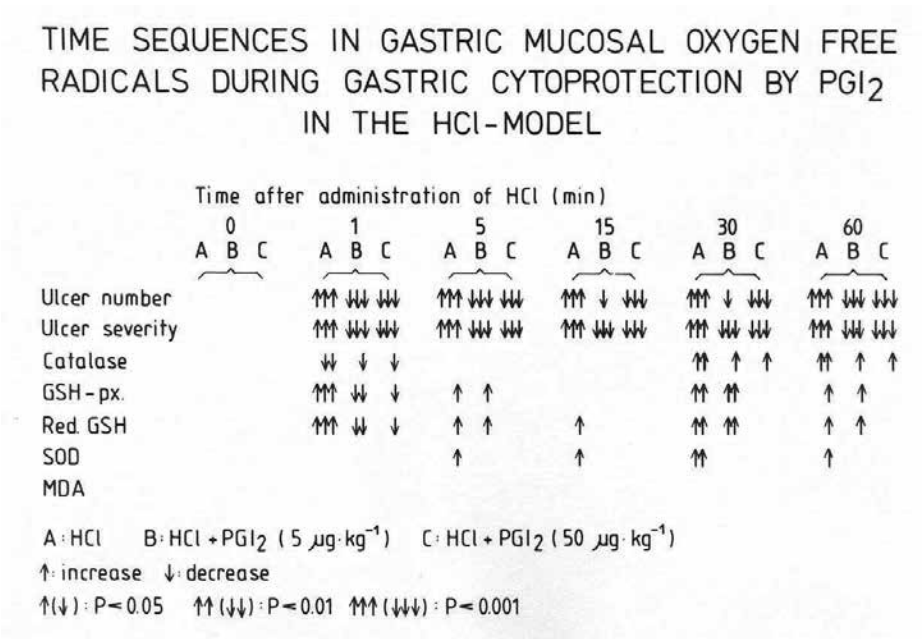


**Figure 253.** Summary of the changes in the parameters of oxygen free radicals in the gastric fundic mucosa during the development of gastric mucosal protection of $PGI_2$ in rats, in which the gastric mucosal damage was produced by intragastric application of 1 mL of 0.6 M HCl (HCl model), depending on the extents of mucosal protection, doses of $PGI_2$ and dependent on the time after administration of necrotizing and protecting agents. (Note that the $PGI_2$ was given 30 minutes before the HCl.) [Mózsik, Garamszegi, Jávor, Sütő, Vincze, Tóth, Zsoldos: Mechanisms of gastric mucosal protection. In: Tsuchiya et al., 1988, Free Radical in Digestive Diseases. Elsevier Science Publishers, pp. 111–116 (with kind permission).]

The results and correlations between the results in these experimental parameters were in ETOH model the same as those in HCl model with $PGI_2$ and β-carotene (these results are not presented in figures).

The following conclusions have been made from these observations:

**1.** The parameters of oxygen free radicals are involved in the gastric mucosa during the development of gastric mucosal-preventive effects of $PGI_2$ and β-carotene both in the HCl- and ETOH models in rats; however, there were no correlations depending on the doses of preventive drugs and on time after the application of necrotizing agents;

**2.** The changes in the parameters of cellular energy systems in the gastric fundic mucosa in HCl- and ETOH models, during the development of gastric mucosal-protective effects, are dose-dependent in the case of both $PGI_2$ and β-carotene depending on the extent of their mucosal-protective effects related to their characteristic to times of their different appearances, after the application of necrotizing agents;

**3.** The changes in both the parameters of oxygen free radicals and cellular energy systems from the rat gastric fundic mucosa, during the development of gastric mucosal damage – produced by HCl and ETOH – and the development of gastric mucosal prevention by

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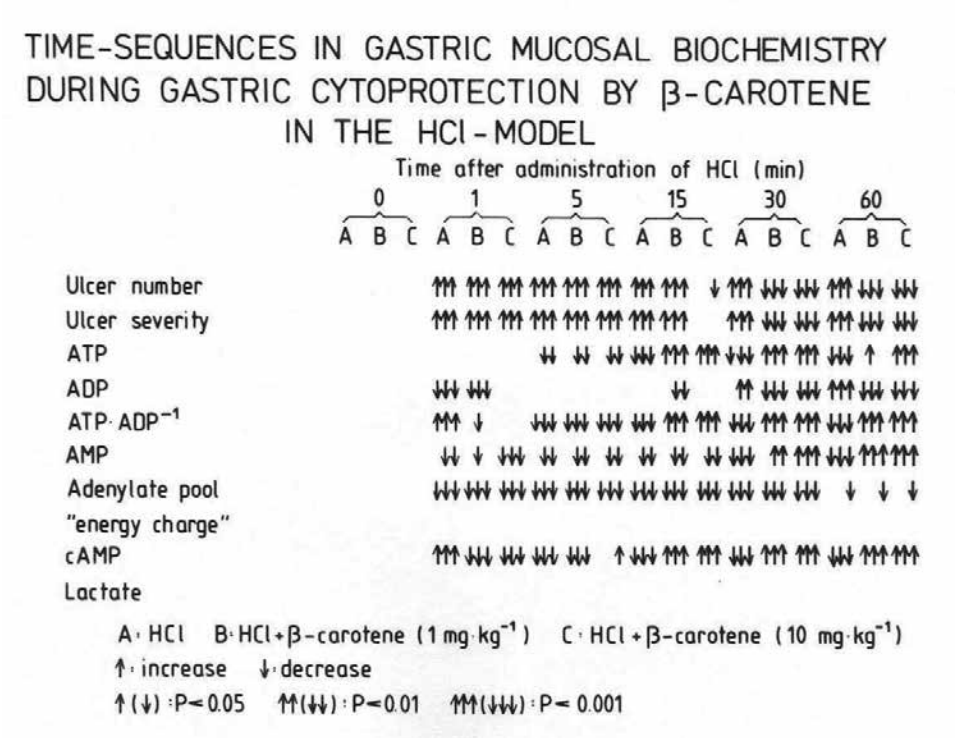


**Figure 254.** Summary of the changes in the parameters of cellular energy systems in the gastric fundic mucosa during the development of gastric mucosal protection of β-carotene in rats, in which the gastric mucosal damage was produced by intragastric application of 1 mL of 0.6 M HCl (HCl model), depending on the extents of mucosal protection, doses of $PGI_2$ and dependent on the time after administration of necrotizing and protecting agents. (Note that the $PGI_2$ was given 30 minutes before the HCl.) [Mózsik, Garamszegi, Jávor, Sütő, Vincze, Tóth, Zsoldos: Mechanisms of gastric mucosal protection. In: Tsuchiya et al., 1988, Free Radical in Digestive Diseases. Elsevier Science Publishers, pp. 111–116 (with kind permission).]

$PGI_2$ and β-carotene, are to be present; however, dose–response correlations are presented only in case of parameters of cellular energy systems versus development of mucosal damage and its prevention (in both HCl and ETOH models);

**4.** The changes in the parameters in oxygen free radicals and in cellular energy systems do not indicate close correlations in the gastric fundic mucosa of rats, during the development of mucosal damage produced by the intragastric administration of 1 mL of 0.6 M HCl or 1 mL of 96% ethanol and of mucosal-protective effects of $PGI_2$ and β-carotene in these models, depending on time after the administration of necrotizing agents and on different doses of mucosal-protective agents;

**5.** $PGI_2$ is a physiological regulatory component but not a scavenger in living bodies, while β-carotene is one of the typical scavengers; however, both of them are able to produce practically the same changes in the parameters of oxygen free radicals and in cellular energy systems in association with the gastric mucosal-protective effects on gastric

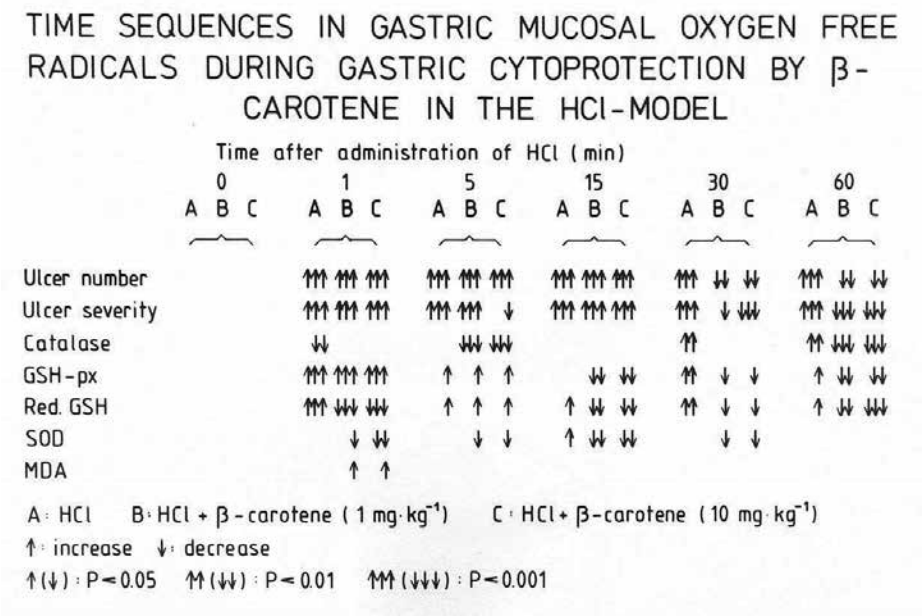


**Figure 255.** Summary of the changes in the parameters of oxygen free radicals in the gastric fundic mucosa during the development of gastric mucosal protection of β-carotene in rats, in which the gastric mucosal damage was produced by intragastric application of 1 mL of 0.6 M HCl (HCl model), depending on the extents of mucosal protection, doses of β-carotene dependent on the time after administration of necrotizing and protecting agents. (Note that the $PGI_2$ was given 30 minutes before the HCl.) [Mózsik, Garamszegi, Jávor, Sütő, Vincze, Tóth, Zsoldos: Mechanisms of gastric mucosal protection. In: Tsuchiya et al., 1988, Free Radical in Digestive Diseases. Elsevier Science Publishers, pp. 111–116 (with kind permission).]

mucosal damage produced by HCl and ETOH, suggesting that the changes run besides each other both in the development of gastric mucosal damage and its prevention.

### 8.12. Comparative cellular molecular pharmacology of development of gastric acid secretion in ethanol-induced gastric mucosal damage and in $PGI_2$-induced gastric mucosal prevention

Various drug actions and cellular mechanisms were demonstrated in the gastric fundic mucosa (especially in animal experiments). The changes of the cellular energy systems in the gastric fundic mucosa – in different animal models – suggested that "active metabolic adaptation" exists in the gastric tissues against the different ulcerogenic agents during the development of gastric mucosal damage.

There was no doubt that the prostacyclin also enhances mucosal biochemistry (including the cellular energy systems), when it was given to protect the gastric mucosal damage in rats. It is also clear that the gastric acid secretion is a consequence of an active metabolic process (involving the cellular energy systems).

In this chapter, the effects of different drugs having different subcellular mechanisms were studied in the following experimental models:

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1. In gastric acid secretion of 4-hour pylorus-ligated rats;
2. In the development of gastric mucosal damage (number and severity) produced by 1 mL of 96% ethanol intragastrically applied in 1 hour; and
3. In the gastric mucosal prevention (number and severity) of prostacyclin ($PGI_2$; 5 μg/kg s.c. given at 30 minutes before the administration of 1 mL of 96% ethanol) as in point 2;

The inhibitory effects of different drugs having different subcellular mechanisms were observed in different experiments, and thereafter their affinity and intrinsic activity curves were calculated according to the methods of Csáky (1969). The value of atropine was taken to be equal to 1.0 in calculation of intrinsic activity ($\alpha_{atropine} = 1.0$).

The following drugs were used in these observations: atropine, actinomycin D, cimetidine, epinephrine, histamine, mannomustine, pentagastrin, $PGI_2$, ouabain and tetracycline (Table 43).

| Drugs | Subcellular mechanisms |
|---|---|
| Atropine | Decrease of ATP transformation into ADP by membrane ATPase, Small increase of ATP transformation by adenylate Cyclase |
| Actinomycin D | Inhibition of RNA synthesis depending on DNA |
| Cimetidine | Inhibition of ATP-cAMP transformation by adenylate cyclase |
| Dinitrophenol | Inhibition of oxidative phosphorylation |
| Epinephrine | Decrease of ATPtransformation into ADP by membrane ATPase Increase of ATP-cAMP transformation by adenylate cyclase |
| Histamine | Increase of ATP-cAMP transformation by adenylate cyclase Decrease of ATP-ADP transformation by membrane ATPase |
| Mannomustine | Inhibition of *de novo* synthesis of DNA |
| Pentagastrin | Increase of ATP-cAMp transformation by adenylate cyclase Decrease of ATP transformation into ADP by membrane ATPase |
| $PGI_2$ | Increase of ATP-cAMP transformation by adenylate cyclase Decrease of ATP-ADP transformation by membrane ATPase |
| Ouabain | Inhibition of ATP transformation by membrane ATPase |
| Tetacycline | Inhibition of protein translation |

**Table 43.** Subcellular mechanisms of drugs used in the experiments. For detailed references for drug actions, see Mózsik and Jávor: Dig. Dis. Sci. 33:92–105, 1988 (with kind permission).

*8.12.1. Affinity and intrinsic activity curves for drugs inhibition the gastric acid secretion in 4 hours pylorus ligated rats*

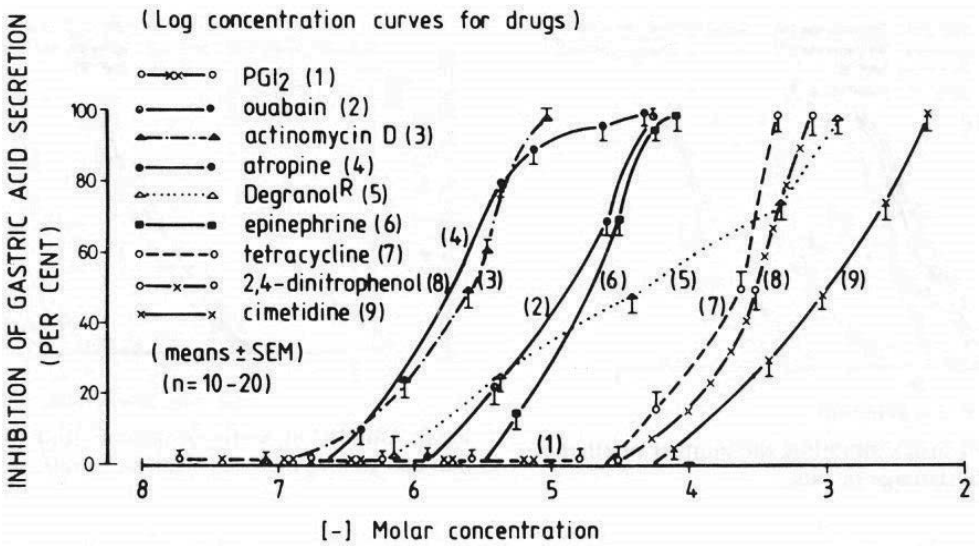


**Figure 256.** Affinity curves for the drugs inhibiting the gastric acid secretion in 4-hour pylorus-ligated rats. [Mózsik and Jávor: Dig. Dis. Sci. 33:92–105, 1988 (with kind permission).]

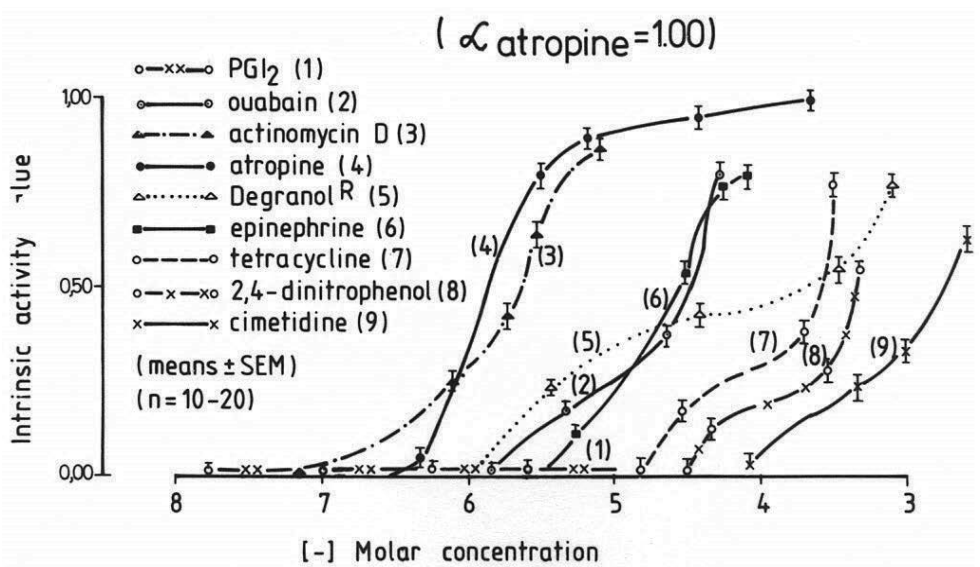


**Figure 257.** Intrinsic activity curves for the drugs inhibiting gastric acid secretion in 4-hour pylorus-ligated rats. [Mózsik and Jávor: Dig. Dis. Sci. 33:92–105, 1988 (with kind permission).] The values of $pD_2$ (dose necessary to produce 50% inhibition in the affinity curves) and of $pA_2$ (dose necessary to produce 50% inhibition in the intrinsic activity curves) were calculated from the affinity and intrinsic activity curves (Table 44).

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| Drugs | $pD_2$ | $\alpha$ | $pA_2$ |
|---|---|---|---|
| Atropine | 5.75 | 1.00 | 5.80 |
| Actinomycin D | 5.63 | 0.87 | 5.86 |
| Cimetidine | 3.00 | 0.64 | 3.20 |
| Mannomustine(Degranol) | 4.25 | 0.78 | 4.90 |
| Dinitrophenol | 3.50 | 0.56 | 3.75 |
| Epinephrine | 4.95 | 0.80 | 4.75 |
| $PGI_2$ | 0.00 | 0.00 | 0.00 |
| Oaubain | 4.75 | 0.80 | 4.50 |
| Tetracycline | 3.63 | 0.78 | 3.75 |

**Table 44.** Values of affinities ($pD_2$) and intrinsic acivities ($\alpha_{atropine} = 1.00$ and $pA_2$) for drugs inhibiting the gastric acid secretion in 4-hour pylorus-ligated rats. Values are in [−] molar. [Mózsik and Jávor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

### *8.12.2. Affinity curves for the drugs inhibiting the development of ethanol-induced gastric mucosal damage in rats*

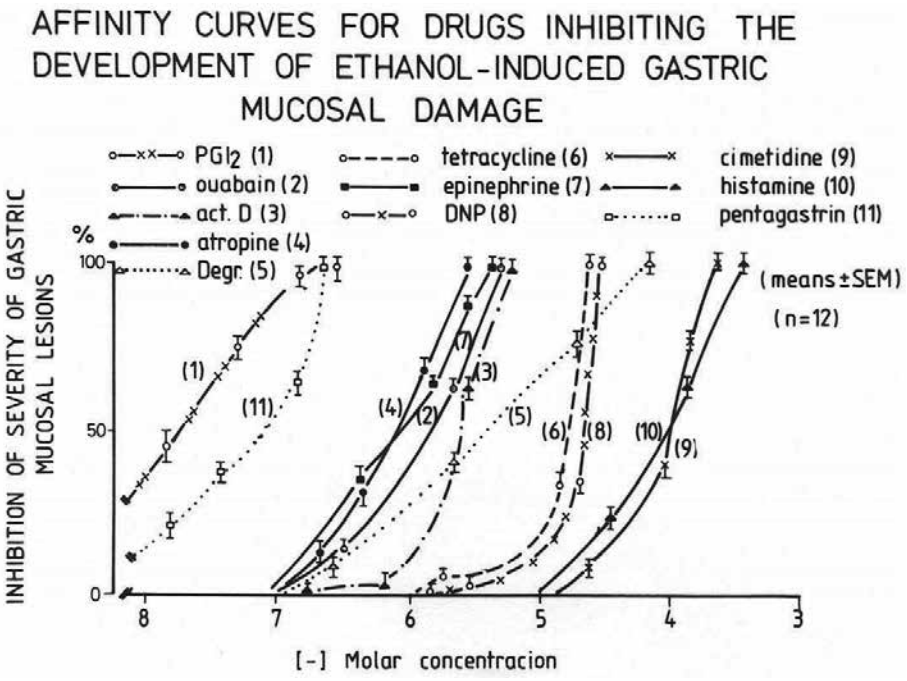


**Figure 258.** Affinity curves for the drugs inhibiting the development of gastric mucosal damage produced by ethanol administration in rat. [Mózsik and Jávor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

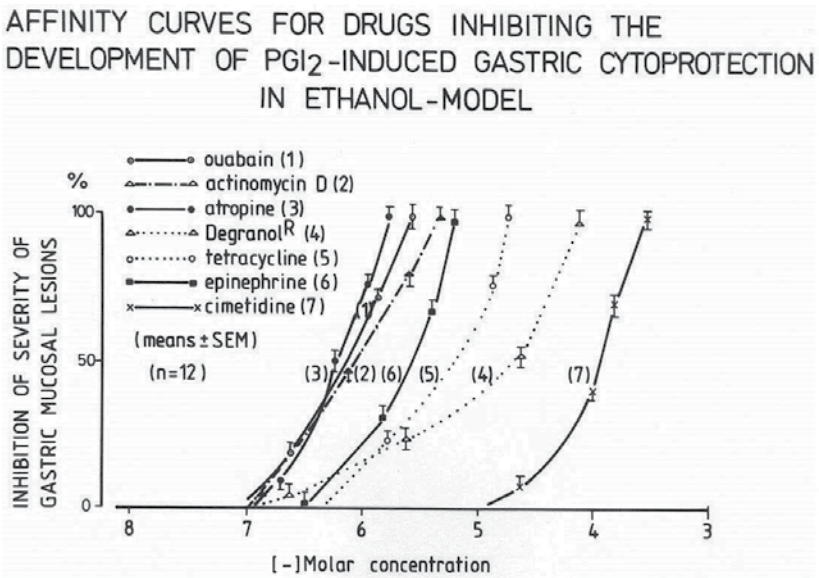


**Figure 259.** Intrinsic activity curves for the drugs inhibiting the development of gastric mucosal damage produced by ethanol administration in rats. [Mózsik and Jávor: Dig. Dis. Sci. 33: 92–105, 1988 (with kind permission).]

*8.12.3. Affinity and intrinsic activity curves for the drugs inhibiting the $PGI_2$-induced gastric mucosal prevention on the gastric mucosal damage produced by ethanol in rats*

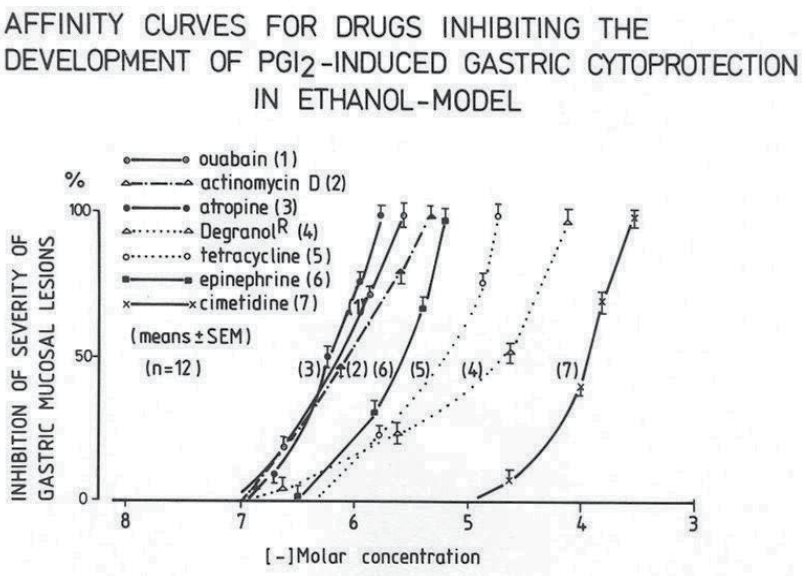


**Figure 260.** Affinity curves for the drugs inhibiting the $PGI_2$-induced gastric mucosal protection of the ethanol-produced gastric mucosal damage in rats.

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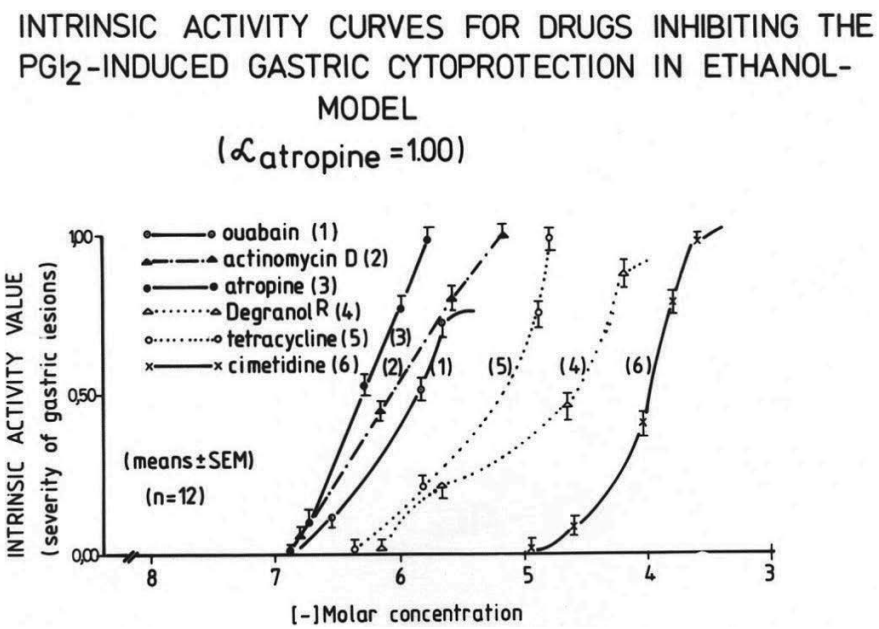


**Figure 261.** Intrinsic activity curves for the drugs inhibiting the $PGI_2$-induced gastric mucosal protection of the ethanol-produced gastric mucosal damage in rats.

*8.11.4. Short discussion of results obtained from the observations presented in Sections 8.14.1–8.14.3*

There were different unexpected results, when we practically noted that the examined drugs (with different subcellular mechanisms) were able to produce very similar actions on the inhibition of gastric acid secretion, ethanol-induced mucosal damage and $PGI_2$-induced gastric mucosal protection in the ethanol-produced gastric mucosal damage in rats (Figure 262).

The histamine, pentagastrin and dinitrophenol enhanced the $PGI_2$-induced gastric mucosal protection, and histamine and pentagastrin stimulated the gastric acid secretion in 4-hour pylorus-ligated rats. The contrary action of dinitrophenol (namely its inhibition of gastric acid secretion and its stimulation of the $PGI_2$-induced gastric mucosal protection) can be explained by the significant time difference of the experiments.

When the values of $pD_2$ and $pA_2$ were calculated, these values were practically in all of three different experimental models.

The same drugs were used to study the subcellular mechanisms of damage to the gastric mucosa by ethanol. Pentagastrin and histamine, while stimulating acid secretion, were able to reduce gastric mucosal damage due to ethanol. The effects of all drugs on both acid secretion and mucosal protection were dose-dependent. Furthermore, the molarities were similar in both types of experiments.

The following can be concluded:

1. The same subcellular mechanisms are involved in acid secretion and mucosal protection;
2. Active metabolic processes are involved in both;
3. Significant changes in membrane-bound ATP-dependent energy systems, oxidative phosphorylation, DNA and RNA synthesis, and *de novo* protein synthesis in both;
4. Ethanol-induced gastric mucosal injury can be prevented by drugs which inhibit active metabolism or those which increase active metabolic responses (histamine, pentagastrin);
5. No significant difference in effective doses on gastric acid secretion or protection against ethanol-induced mucosal injury was seen between drugs having different mechanisms of subcellular action;
6. The same subcellular mechanisms are involved in the $PGI_2$-induced gastric mucosal protection in ethanol-model.

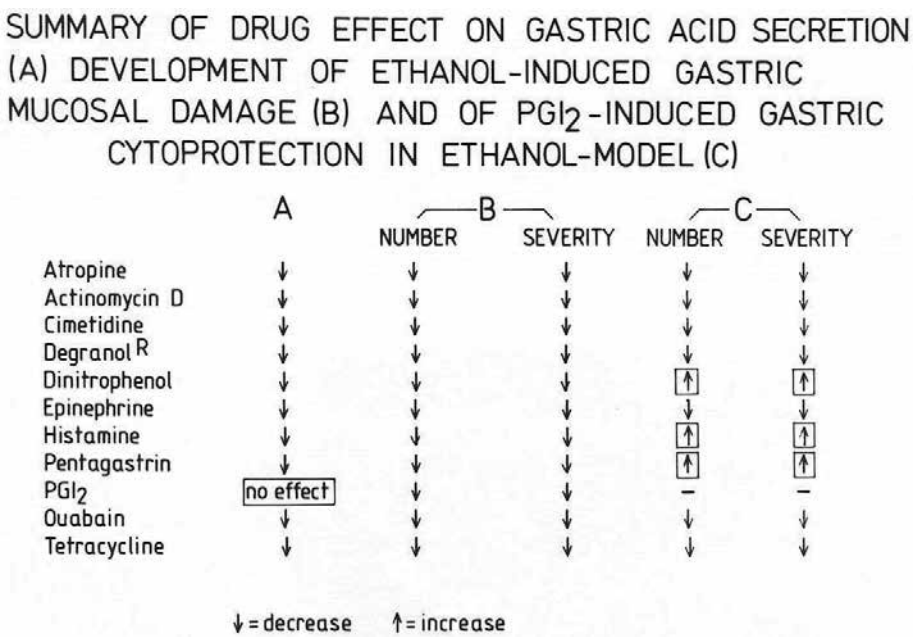


**Figure 262.** Schematic summary of the effects of different drugs having different subcellular mechanisms on the gastric acid secretion, development of ethanol-induced gastric mucosal damage and $PGI_2$-induced gastric mucosal protection in the ethanol-produced gastric mucosal damage in rats.

### 8.13. Epinephrine-model

As demonstrated earlier, epinephrine, given immediately after pylorus ligation, inhibited the gastric acid secretion and ulcer development (Nagy et al., 1976; Sethbakdi et al., 1970 a, b; Pfeiffer, 1971; Pfeiffer and Sethbakdi, 1971; Pfeiffer and Mózsik, 1990) (Figure 263).

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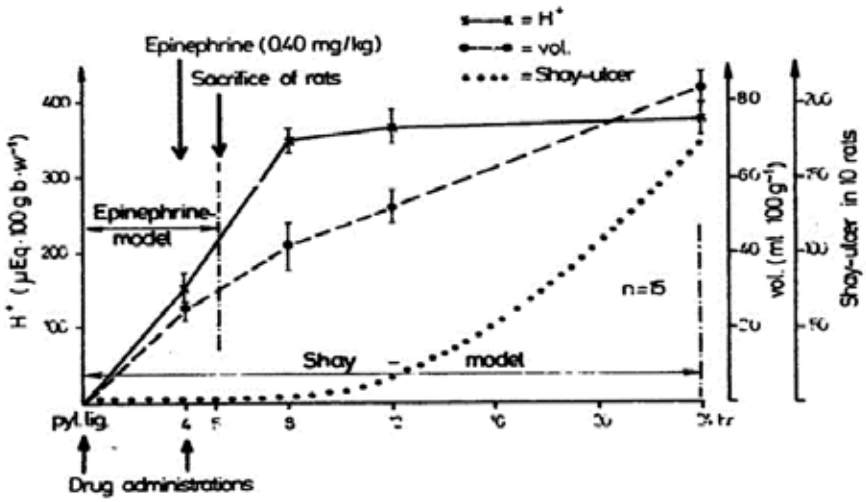


**Figure 263.** The schematic representation of the different ulcer provocations in pylorus-ligated rats and in epinephrine model. [Nagy, Mózsik, Tárnok, Jávor (1979) Drugs Exp. Clin. Res. 5, 87–96 (with kind permission).]

Sethbakdi et al. (1970 a, b) elaborated a new experimental model, "epinephrine model," in which the gastric ulceration appeared in the glandular stomach. Sethbakdi et al. (1970 a, b) applied epinephrine at 4 hours after pylorus ligation and the animals were sacrificed at 5 hours after the pylorus ligation. The biochemical analysis of this epinephrine model led to a new possibility to understand the mechanisms of this model (especially membrane-bound energy systems) (Mózsik and Pfeiffer, 1992).

Figure 263 indicates the time correlation between the 24-hour pylorus-ligated rats versus epinephrine model. Sethbakdi et al. found that the optimal dose of epinephrine is 0.4 mg/kg given at 4 hours after pylorus ligation, whereas the animals were sacrificed at 5 hours after pylorus ligation (that is 1 hour after the epinephrine application) (Nagy et al., 1976; 1981a; Mózsik, 1971) (Figure 264).

When we applied different doses (0.1, 0.4 and 1.0 mg/kg), the $H^+$ concentration, $H^+$ output and volume of gastric secretion dose-dependently decreased (Figure 263).

The peak of gastric mucosal injury was found after the application of 0.4 mg/kg, when different doses of epinephrine were given at 4 hours; however, the ulceration was smaller when epinephrine was given at a dose of 0.1 or 1.0 mg/kg (Figures 264, 265).

When the effect of epinephrine (given at the time of pylorus ligation) was studied (applied in a dose of 0.4 mg/kg), the tissue level of cAMP was significantly higher only in the pylorus-ligated rats; however, when epinephrine (in doses of 0.1, 0.4 and 1.0 mg/kg) was given at 4 hours after pylorus ligation, we could not notice any increase in the tissue level of cAMP (Mózsik and Pfeiffer, 1992) (Figures 267, 268). In other words, the different doses of epinephrine were not able to produce an elevation in the tissue cAMP. Therefore, cAMP-induced gastric mucosal-protective effects completely disappeared in 4-hour pylorus-ligated rats.

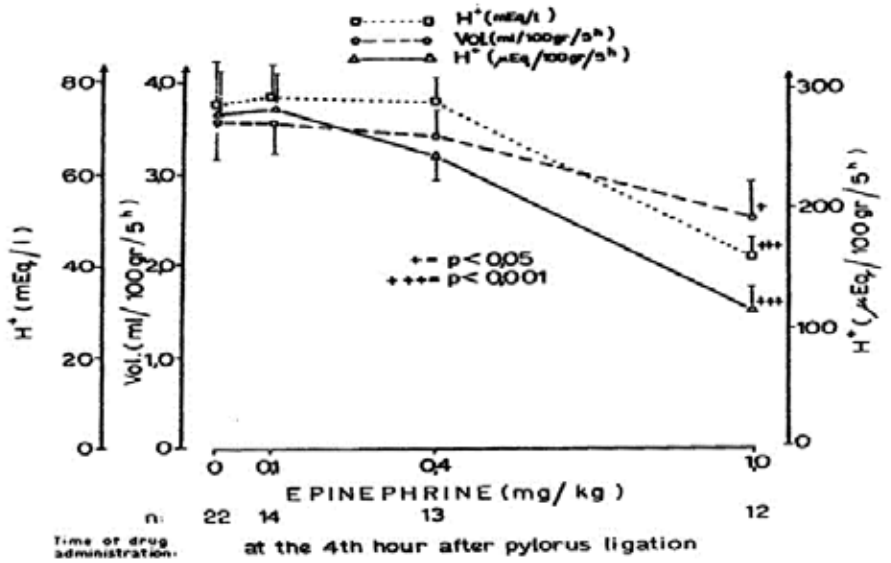


**Figure 264.** Dose–response curves for epinephrine on the gastric secretory responses (volumes $H^+$ output and $H^+$ concentration) in 5-hour pylorus-ligated rats, when the different doses of epinephrine were given at 4 hours after pylorus ligation. The animals were sacrificed one hour later (means ± SEM). [Nagy, Mózsik, Tárnok, Jávor (1979), Drugs Exp. Clin. Res. 5, 87–96 (with kind permission).]

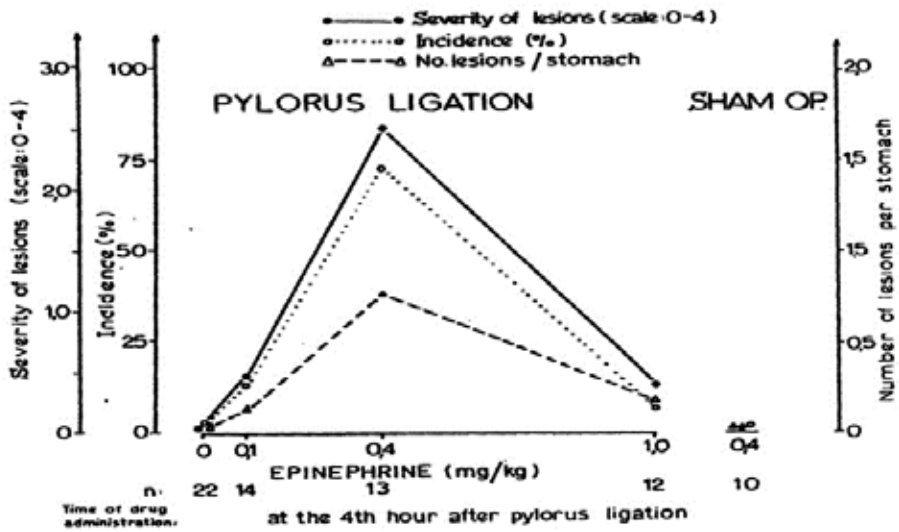


**Figure 265.** The extent of gastric ulcer provocation by epinephrine in 5-hour pylorus-ligated rats when the different doses of epinephrine were i.p. at 4 hours after pylorus ligation. The right side of the figure indicates epinephrine effect on the same parameters given at a dose of 0.4 mg/kg immediately after pylorus ligation (means ± SEM). [Nagy, Mózsik, Tárnok, Jávor (1979). Drugs Exp. Clin. Res. 5, 87–96 (with kind permission).]

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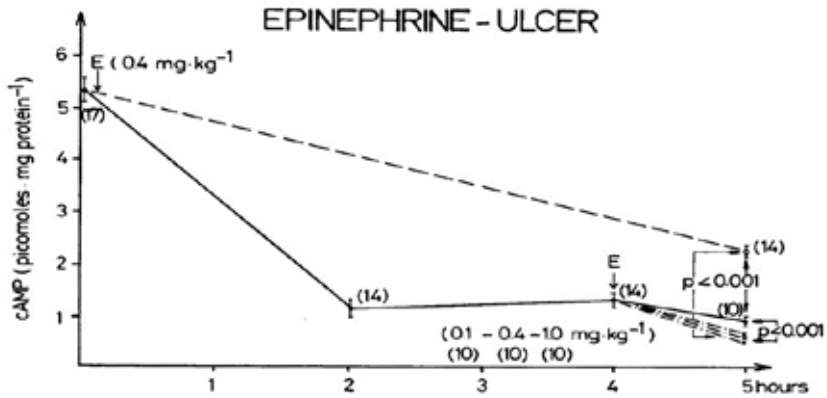


**Figure 266.** Changes in the tissue level of cAMP in pylorus-ligated rats when epinephrine (given at a dose of 0.4 mg/kg) was given immediately after pylorus ligation (E) and in different doses at 4 hours after pylorus ligation. The animals were sacrificed at 5 hours after pylorus ligation (means ± SEM). [Mózsik et al. (1981g). Acta Medica Acad. Sci. Hung. 36: 1–29 (with kind permission).]

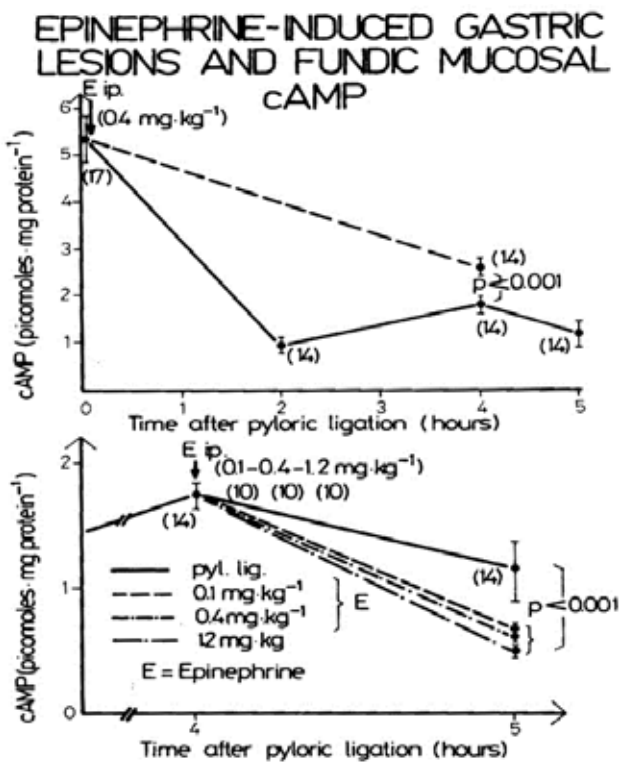


**Figure 267.** The epinephrine (E) induced changes in the rat gastric fundic mucosal levels of cAMP depending upon the time and doses (means ± SEM). [Mózsik et al. (1981g). Acta Medica Acad. Sci. Hung. 36: 1–29 (with kind permission).] For further explanation, see Figure 263.

### 8.14. Drug actions depend on the stomach cellular energy systems in dependence of different functional states of target organ

The Δ changes in the gastric fundic mucosal ATP, ADP, AMP and cAMP were detected by the application of different doses of epinephrine (0.1, 0.4 and 1.0 mg/kg) given in intact, 1 hour and 4 hours pylorus-ligated rats (Figures 268–271). These values differed significantly from each other. The largest action was obtained in intact animals, smaller in 1 hour and less values in 4-hour pylorus-ligated rats. These results together clearly indicate that the drug actions depend on the actual functional state of the target organ.

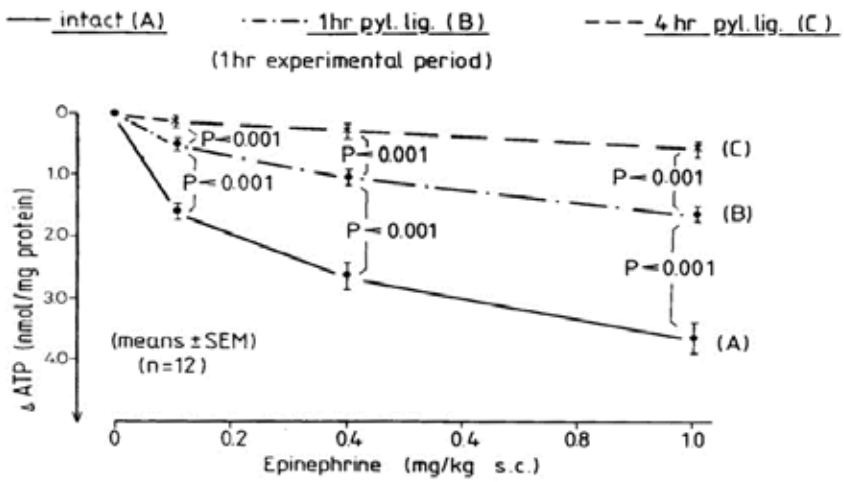


**Figure 268.** Δ changes in the epinephrine-induced gastric mucosal levels of ATP depending upon different functional activities of the rat gastric fundic mucosa (means ± SEM). [Mózsik and Pfeiffer, Exp. Clin. Gastroenterol 2, 190–194, 1992 (with kind permission).]

The results of observations presented in Sections 8.15 and 8.16 clearly indicate that the drug (epinephrine) effects significantly differ depending on different functional states of the target organ (presently, of stomach). Furthermore, the differences in the drug actions on the stomach – depending on the differences of its functional state – show dose-dependent actions.

In everyday medical practice, the clinicians never know exactly the etiology of peptic ulcer, when patients are diagnosed with the existence of the disease. These different conditions of the functional states of GI tract surely involved in the final efficiency of the medical treatments in patients with GI disorders as well as pathogenic roles of different noxious agents.

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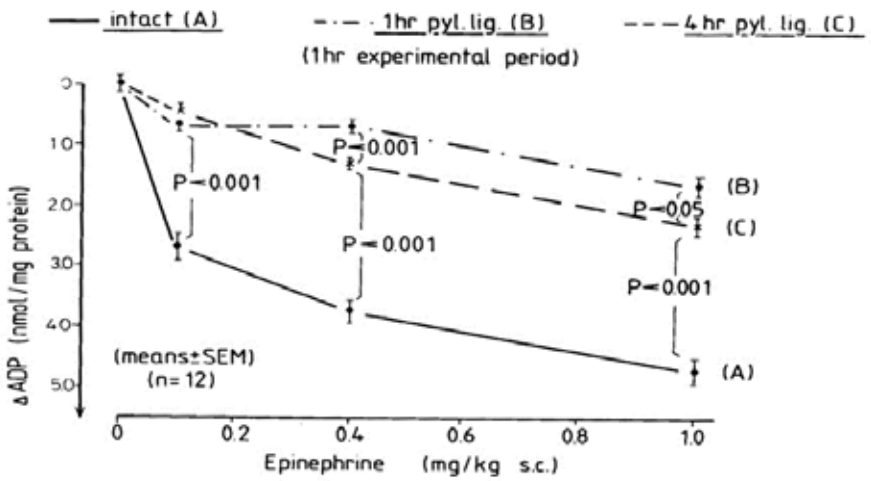


**Figure 269.** Δ changes in epinephrine-induced gastric mucosal level of ADP depending on different functional activities of the rat gastric fundic mucosa (means ± SEM). [Mózsik and Pfeiffer, Exp. Clin. Gastroenterol 2, 190–194, 1992 (with kind permission).] For further explanation, see Figure 263.

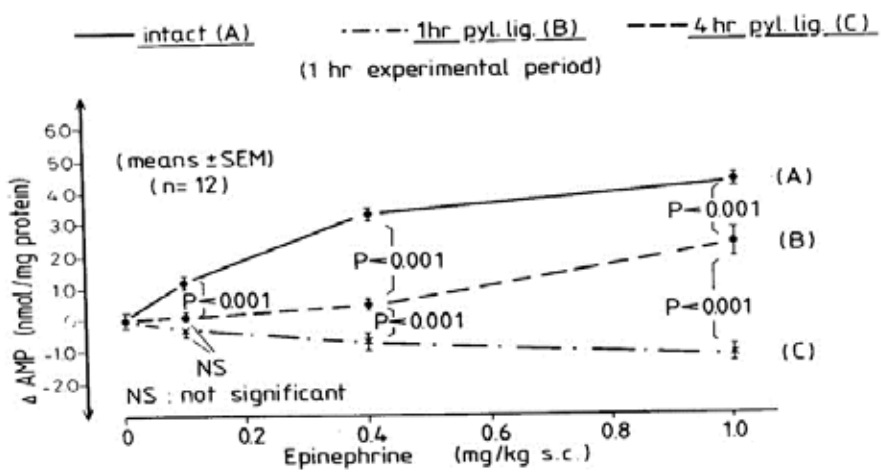


**Figure 270.** Δ changes in epinephrine-induced gastric mucosal levels of AMP depending upon different functional activities of the rat gastric fundic mucosa (means ± SEM). (Mózsik and Pfeiffer, Exp. Clin. Gastroenterol 2, 190–194, 1992). For further explanation, see Figure 263.

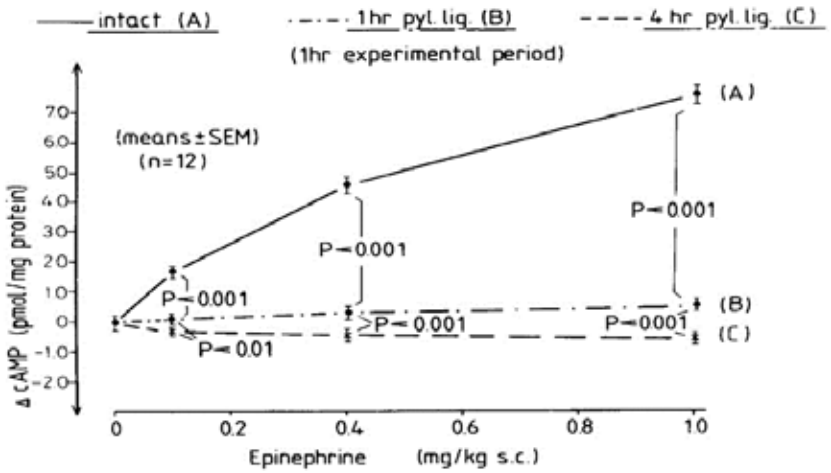


**Figure 271.** Δ changes in the epinephrine-induced gastric mucosal levels of cAMP depending upon different functional activities of the rat gastric fundic mucosa (means ± SEM). [Mózsik and Pfeiffer, Exp. Clin. Gastroenterol 2, 190–194, 1992 (with kind permission).] For further explanation, see Figure 263.

### 8.14. Surgical vagotomy and atropine-, cimetidine-, $PGI_2$- and ß-carotene-induced gastric mucosal damage in ETOH-model

There was no doubt after the works of Robert et al. (1979) that the small dose of $PGI_2$ (5 μg/kg) is able to prevent the chemically induced gastric mucosal damage.

Figure 272 clearly demonstrates that the ETOH-induced gastric mucosal damage was enhanced after bilateral surgical vagotomy. (Jávor et al., 1981; Mózsik et al., 1981a).

This observation was the first evidence to suggest that the intact vagal nerve is necessary not only in the aggression but also in the defense in the stomach (Jávor et al., 1981a; Mózsik et al., 1992 ; ütő et al.,;1989, 1992; Vincze et al.; 1992; 1993 a 1997; Király et al.,; 992 a b).

After bilateral surgical vagotomy, the gastric mucosal-protective effects of atropine (Figure 273), cimetidine (Figure 274) β-carotene (Figure 275) and $PGI_2$ (Figure 276) were not present.

Miller et al. (1983) published an excellent review paper on the PGs, which indicated that the gastric mucosal effect of PGs disappear after surgical vagotomy. In other papers written by his team (1983), our original observations were mentioned; however, those were completely forgotten later on in the literature (Henagan et al., 1983; Forte and Lee, 1977).

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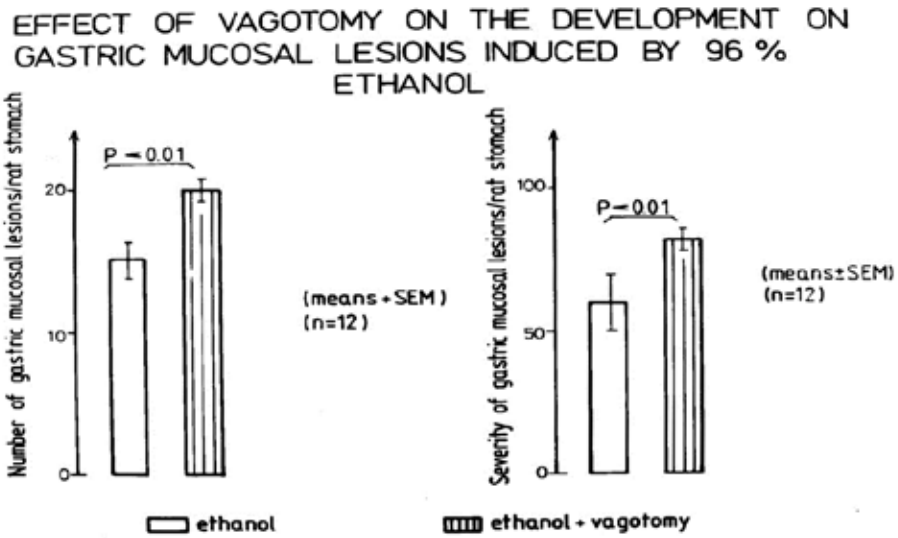


**Figure 272.** The ethanol-induced gastric fundic mucosal lesions (number and severity) with and without bilateral surgical vagotomy (means ± SEM). [Mózsik et al., Life Sciences 49, 1383–1388, 1991 (with kind permission).]

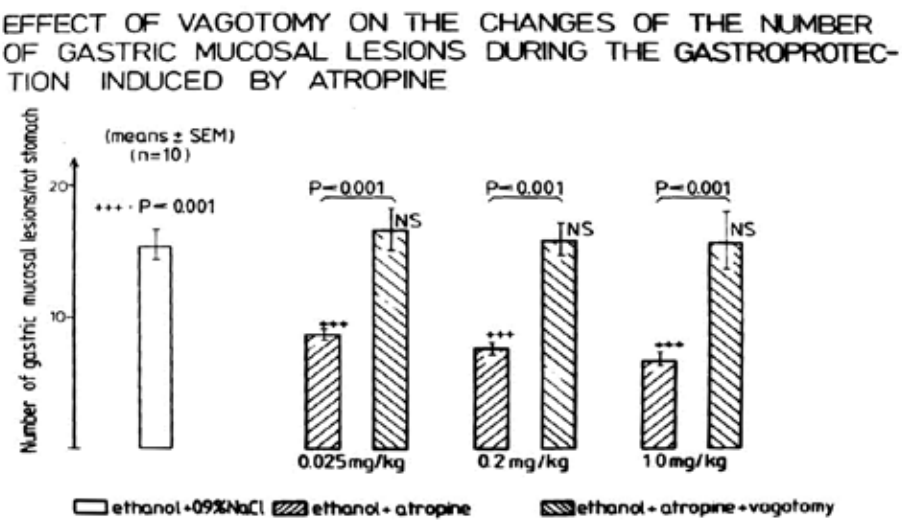


**Figure 273.** The atropine (given in cytoprotective and antisecretory doses)-induced gastric mucosal-preventive effects with intact vagal nerve, which disappeared completely after bilateral surgical vagotomy in ETOH model (means ± SEM). [Mózsik et al., Life Sciences 49, 1383–1388, 1991 (with kind permission).]

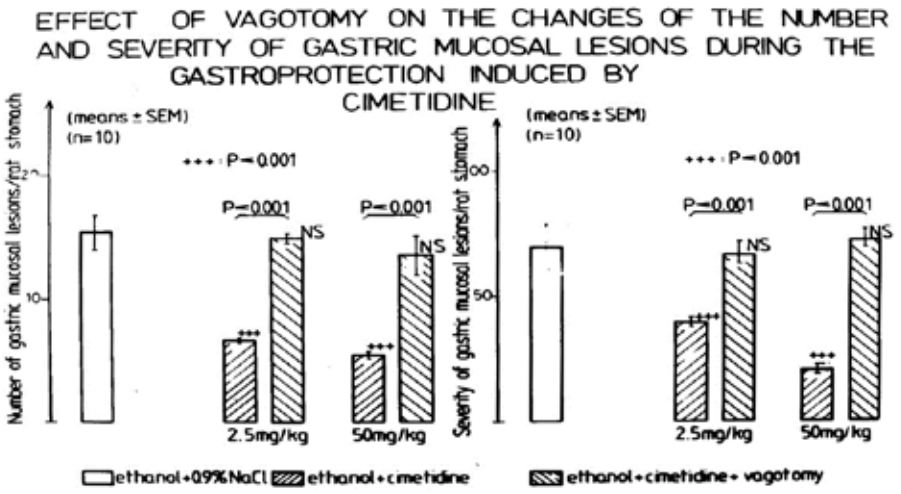


**Figure 274.** The cimetidine (given in cytoprotective and antisecretory doses)-induced gastric mucosal-preventive effects on the number and severity in the rat gastric fundic mucosa with intact vagal nerve and after bilateral surgical vagotomy (means ± SEM). [Mózsik et al., Life Sciences 49, 1383–1388, 1991 (with kind permission).]

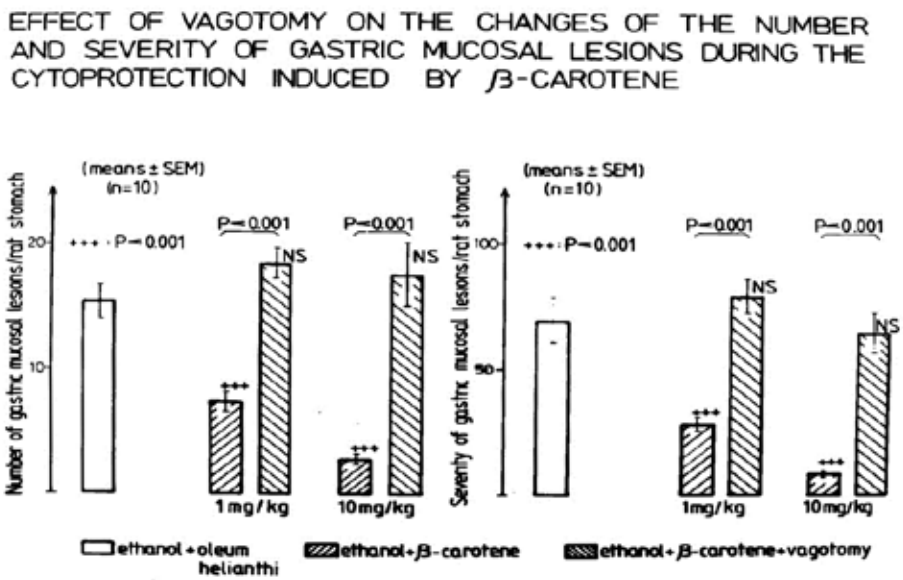


**Figure 275.** The β-carotene (given at doses of 1 mg and 10 mg/kg doses)-induced gastric mucosal-preventive effects on the number and severity in rats with intact vagal nerve and after bilateral surgical vagotomy in ETOH model (means ± SEM). [Mózsik et al., Life Sciences 49, 1383–1388, 1991 (with kind permission).]

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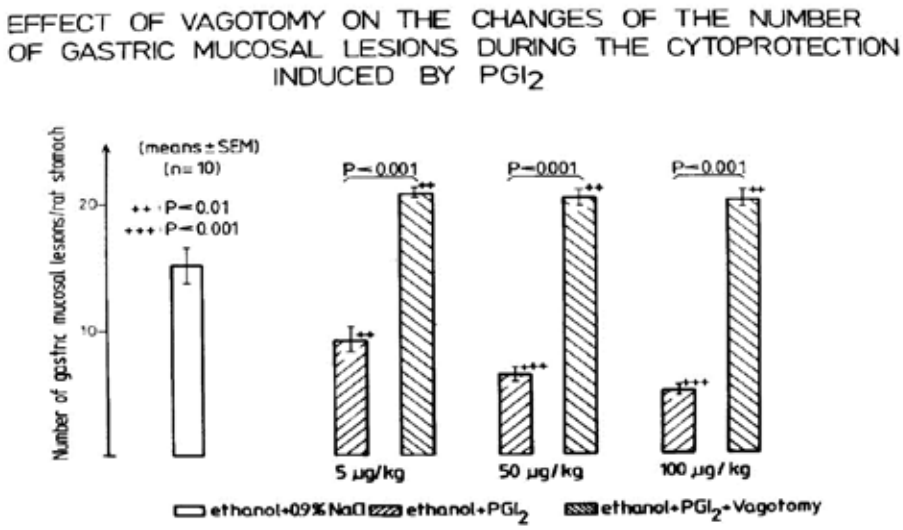


**Figure 276.** The $PGI_2$ (given at doses of 5, 50 and 100 μg/kg i.g.)-induced gastric mucosal-protective effect on the number in the rat gastric fundic mucosa treated with ETOH – in intact vagal nerve and after bilateral surgical vagotomy (means ± SEM). [Mózsik et al., Life Sciences 49, 1383–1388, 1991 (with kind permission).]

### 8.16. Gastric mucosal protective effects of ß-carotene and adrenals

Previously, β-carotene was used as a scavenger material. When the surgical vagotomy was able to neglegee β-carotene-induced gastric mucosal-preventive effect, we suggested that the intact adrenals are also important key factors for the development of its gastric mucosal-protective effects (Szabo et al., 1983; Sütő et al., 1989; Vincze et al., 1997).

No gastric mucosal-preventive effects of β-carotene were observed after the surgical removal of adrenals. When supplementation with glucocorticoid was performed, the gastric mucosal effects of β-carotene resumed. When supplementation with mineralocorticoid was performed, the gastric mucosal-protective effects of β-carotene did not appear.

This was another interesting observation, because the scavenger agent cannot act in rats without adrenals (Mózsik et al., 2001a).

### 8.16. Surgical vagotomy and tissue levels of $PGE_2$ and $PGI_2$

After surgical vagotomy, both $PGE_2$ and $PGI_2$ (6-keto-$PGF_{-1}\alpha$) significantly decreased (Sütő et al., 1992) (Figure 277).

The gastric mucosal-protective effect of β-carotene was dose-dependent; however, a significant difference was obtained between the effects (number) registered in animals with intact vagal nerve versus post bilateral surgical vagotomy (Figure 278).

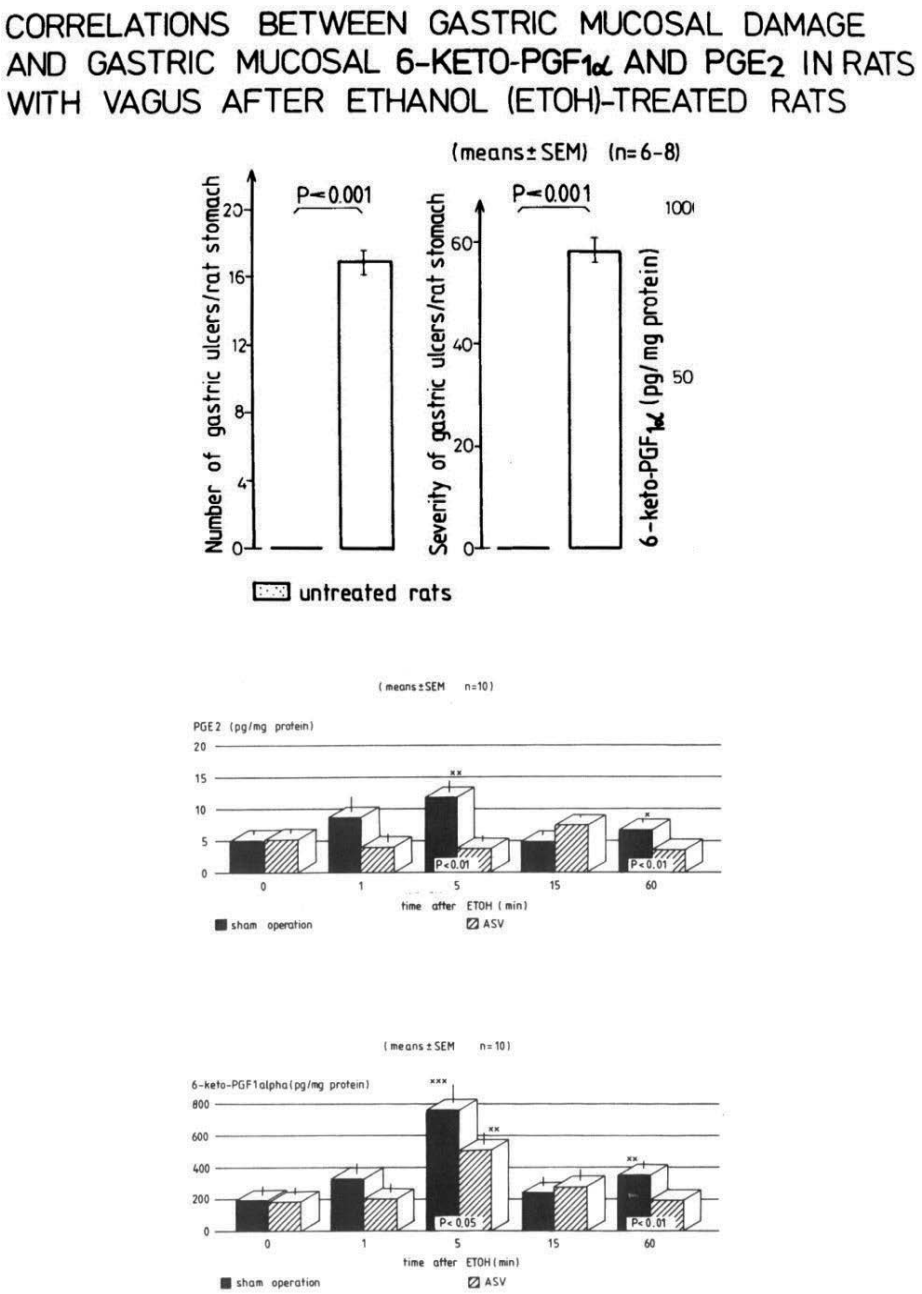


**Figure 277.** Surgical vagotomy-induced changes in ethanol-induced gastric mucosal damage without (untreated, sham-operated rats) and with surgical vagotomy (a) changes in levels of $PGI_2$, (b) 6-keto-$PGF_{1\alpha}$ (as the final product of) and (c) in the gastric mucosa (means ± SEM). [Sütő et al., Acta Physiol Hung 80, 205–211, 1992 (with kind permission).]

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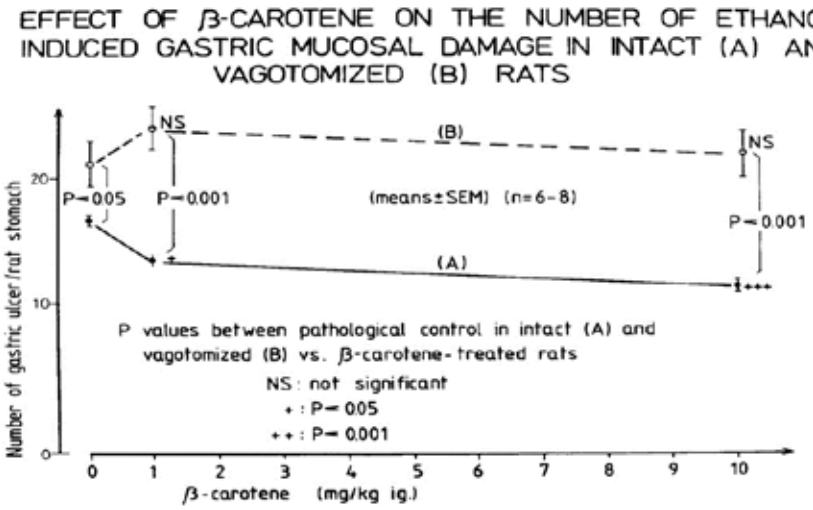


**Figure 278.** Dose–response curves for β-carotene in gastric mucosal damage (number) in rats treated with ETOH in animals with intact vagal nerve and after bilateral surgical vagotomy (means ± SEM). [Sütő et al., Acta Physiol Hung 85, 207–213, 1993 (with kind permission).]

### 8.17. Biochemical changes in the gastrointestinal tract and oxygen free radicals, scavengers in of intact vagal nerve, "surgical" and "chemical" vagotomy, intact adrenals, after adrenelectomy and supplemention of glucocorticoid and mineralocorticoid

There is no doubt that significant changes can be obtained in the oxygen free radicals during the development of the gastrointestinal mucosal damage (Mózsik et al., 1984 a, b, c). However, we expected the gastric mucosal-protective effects of retinoids in rats after surgical vagotomy (Jávor et al., 1983; Mózsik et al., 1988; Mózsik et al, 1991a; Mózsik and Jávor, 1991). Surprisingly, the gastric mucosal-protective effects of retinoids completely disappeared after bilateral surgical vagotomy (Mózsik et al., 1991a; Sütő et al., 1992). It was suggested that the presence of the intact vagal nerve and adrenals is necessary for the development of gastric mucosal-preventive effects of scavengers in animal observations.

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 9

# Direct Action of Helicobacter pylori on the Freshly Isolated Rat Gastric Mucosa Cells (GMCs)

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60103

## 9.1. General introduction

The etiological role of *H. pylori* has been suggested in the development of gastric and duodenal ulcer and it later advances to chronic atrophic gastritis and gastric cancer. Consequently, everyone tried to find suitable treatment for *H. pylori*-induced disorders. Finally, "evidence-based" therapeutic recommendations were established consisting of antibiotic combination, metronidazole and proton pump inhibitors (PPI). The efficacy of various therapeutic schemes differing by small modification has been widely studied by randomized, multiclinical, multinational studies and their meta-analysis (www.cancer.gov/cancertopics/factsheet/Risk/p-pylori-cancer; Wong et al., 1998; Molloy et al., 1998; Molloy and Sonneberg, 1997; Mabe et al., 2009).

The researchers all over the world can be divided into two groups based on their view of *H. pylori*'s importance. A larger group of researchers totally accepts the role of *H. pylori* in all abovementioned pathological conditions, including the etiological role and eradication treatment in these H. *pylori*-induced diseases. A smaller group of researchers shows a little skepticism toward the H. *pylori* as being a factor in gastric and duodenal ulcer, gastric atrophic gastritis and gastric cancer development (Mózsik et al., 2014). We did a critical analysis of gastric secretory responses (BAO, MAO) on patients with duodenal ulcer with respect to their age and duration of complaints ($n$ = l20) (Mózsik et al., 1981f). Surprisingly, their MAO values remained the same in old age as those in the younger age, meanwhile the BAO values increased with respect to their age and duration of complaints. We had no information about the presence of Hel*icobacter pylori* infection at that time (for details, see Mózsik et al., 2014 a, b, c, d).

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The aim of this chapter is to give a summary of the observations that are not in agreement with the main line of *H. pylori* infection and the necessity of its eradication treatment (Mózsik et al., 2014 a, b, c, d).

## 9.2. Testing of *Helicobacter pylori* (*H. pylori*) cultures on the isolated gastric mucosal cell

As the letters of Warren and Marshall in *Lancet* (1983) were published, a very active research chain was started throughout the world. Researchers tried to find the explanation on how *H. pylori* can lead to inflammation in the stomach and peptic ulcer.

The existence of *H. pylori* as the cause for direct damaging effect in the stomach was one of the possibilities. To answer the question, we analyzed the direct cellular effect of sonicated *H. pylori* cultured (at the level of membrane, mitochondrion and DNA) alone and with different combinations on the freshly isolated gastric mucosal cells of rat. The aggravation effect of this sonicated *H. pylori* was also examined in ethanol-induced cell injury model to evaluate the changes in cell resistance.

### *9.2.1. H. pyloric culture*

Bacteria were obtained from human gastric and duodenal biopsy samples. Urease test and histological positivity were both required to be definite in the tissue samples. Bacterial suspensions ($10^6$ bacteria/mL in 20 Tris/HCl buffer, pH 7.0) were sonic on ice (30 W) in six consecutive treatments lasting 30 seconds.

### *9.2.2. Preparation of mixed Gastric Mucosal Cells (GMCs)*

Gastric mucosal cells from 1 or 2 unfasted Sprague-Dawley strain rats were isolated by the method of Nagy et al. (1994). Briefly, the segments of glandular stomach without blood vessels surrounding the connective tissue were sequentially incubated in a physiological solution containing 0.5 mg/mL Pronase E (Type XXV Sigma Chemical Co.) and $10^{-3}$ EGTA. After washing for several times, the cells were resuspended and kept in shaking water bath at a temperature of 37°C in a solution (0.157 M, pH 7.4) freshly produced with the following ingredients: 98.0 mM NaCl, 5.8 mM KCl, 2.5 mM $NaH_2PO_4$, 5.1 mM Na pyruvate, 6.9 mM Na fumarate, 2 mM glutamine, 24.5 mM HEPES Na, 1.0 mM Trizma base, 11.1 mM D-glucose, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 2 mg/mL (w/v) bovine serum albumin. Mixed population of isolated rat GMCs contained at least three types of cells: parietal (20–25%), chief (40%) and epithelial cells (40–45%). An initial viability of 85–95% of the isolated cells was maintained for 6–7 hours. The examinations were carried out through the same media of solution.

### *9.2.3. Toxicological studies*

#### *9.2.3.1. H. pylori culture*

Cells were incubated with sonicated *H. pylori* ($10^6$–$10^8$ bacteria /mL) for 30 minutes in the shaking water bath at 37°C. Equivalent volume of both diluted toxic agent and cell suspension were added. At the end of 30 minutes of incubation, the cells were separated from the

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supernatant by centrifugation. Cell pellet was carefully resuspended and incubated for 10 minutes in the shaking water bath, before it was centrifuged again (500 g, 10 minutes) to obtain the toxic-agent-free supernatant. Cells were resuspended for further biochemical examinations.

*9.2.3.2. Ethanol*

Pretreated and washed cells of sonicated *H. pylori* were incubated with 15% ethanol (EtOH) for 5 minutes in shaking water bath at 37°C after the cells were treated as described above.

*9.2.3.3. Indomethacin*

Pretreated and washed cells were incubated with $10^{-8}$–$10^{-4}$ M indomethacin (IND) (Chinoin, Hungary) for 5 minutes in shaking water bath at 37°C. Then the cells were treated as described above.

*9.2.3.4. Determination of cell viability by Trypan Blue exclusion test*

Trypan Blue (TB) is excluded by viable cells, while being taken up by damaged cells, staining the cytoplasm blue (Bauer et al., 1972). A solution of 0.4% TB (Sigma Chemical Co., St Louis, USA) was mixed with the same volume of cell suspension, and 5 minutes later the rate of stained (dead) and unsustained (viable) cells were calculated as percentage of counting 100 cells in a hemocytometer.

*9.2.3.5. Biochemical assays*

*9.2.3.5.1. Lactate dehydrogenase assay*

The enzyme, lactate dehydrogenase (LDH) can be found in the cytoplasm of the cells. If it is present in the supernatant, it indicates membrane damage of cells. Its activity was determined in samples of both toxic-agent-free supernatant and the cell pellet destroyed by freezing. The calorimetric assay was based on the reduction of $NAD^+$ to NADH, catalyzed by LDH in the presence of lactate as a substrate (Bergmayer and Bern, 1972). The color produced by the reduction of phenazine methosulfate (Sigma Chemical Co., St. Louis, USA) and tetrazonium salt isinicotinic acid hydrazide (Sigma Chemical Co., St. Louis, USA) was measured at 520 nm by a Hitachi 124 spectrophotometer. The results were expressed as mU/min/$10^6$ cells.

*9.2.3.5.2. Succinic dehydrogenase assay*

The enzyme succinate dehydrogenase (SDH) can be found in the mitochondria. The mitochondrial integrity was tested in $2\times10^6$ previously treated and redispersed cells (Mosmann, 1983). The callus formazon product was quantified using a Hitachi 124 spectrophotometer at 500 nm and calculated as nmol/min/$2\times10^6$ cells.

*9.2.3.5.3. Ethidium bromide-DNA flourescence assay*

The nuclear damage of cells due to ethidium bromide–DNA-binding was assessed by nuclear rluorescence duey and Majumder, 1988) .To the ethinium bromide (EB) solution, $10^7$ cells were mided withma Chemical Co., St. Louis, USA), and the fluorescence intensity was measured bny aHitachi F-3000 spectrophotometer at 325–-385 m (excitation–-emision). The results were expressed as arbitrary flouorencenceunits / $10^7$clls.

*9.2.3.6. Direct cellular effect of H. pylori, EtOH and IND*

*9.2.3.6.1. H. pylori culture alone*

Sonicated $10^6$–$10^8$ bacteria/mL had no direct cellular toxicity on freshly isolated rat GMCs by Trypan blue exclusion test (Figure 279), LDH activity (Figure 280) or ethinium bromide–DNA fluorescence (Figure 281) (Bódis et al., 1995 a, b; 1996, 2000; Mózsik et al., 1996c).

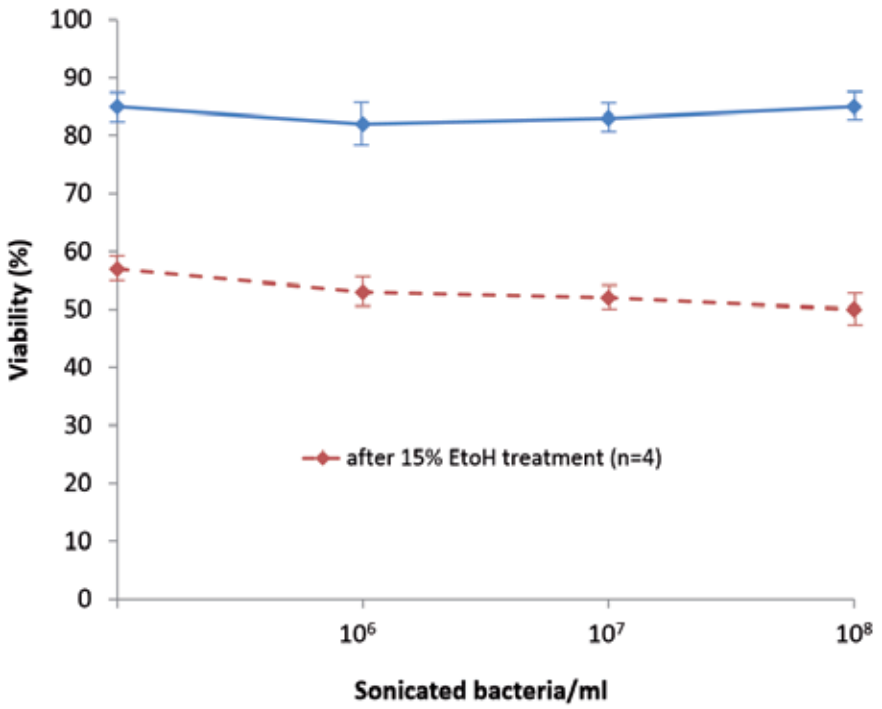


**Figure 279.** Effect of 30 minutes incubation with sonicated $10^6$–$10^8$ bacteria/mL *H. pylori* on the viability of freshly isolated gastric mucosal cells of rat detected by Trypan blue exclusion test with or without 5 minutes of 15% ethanol treatment. [Bódis, Karadi, Voros, Mozsik (1995b): Has sonicated Helicobacter pylori any direct cellular effect? In: Bajtai A., Papp J., Rácz I., Simon L. (Eds.). Helicobacter pylori. Hungarian Society of Gastroenterology. Medicom, Budapest. pp. 137–142 (with kind permission).]

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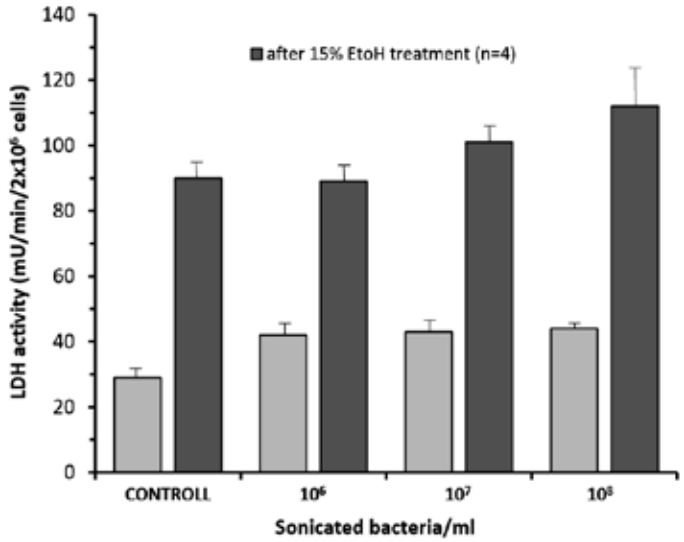


**Figure 280.** Changes in lactate dehydrogenase activity of freshly isolated gastric mucosal cells of rat after 30 minutes incubation with sonicated $10^6$–$10^8$ bacteria/mLL *H. pylori* withoor without 5 minutes of 15% ethanol treatment. [(ódisBoKaradi, Voros, Mozsik (1995b): Has sonicated Helicobacter pylori any direct cellular effect? In: Bajtai A., Papp J., Rácz I., Simon L. (Eds.). Helicobacter pylori. Hungarian Society of Gastroenterology. Medicom, Budapest. pp. 137—142) with kind permission).]

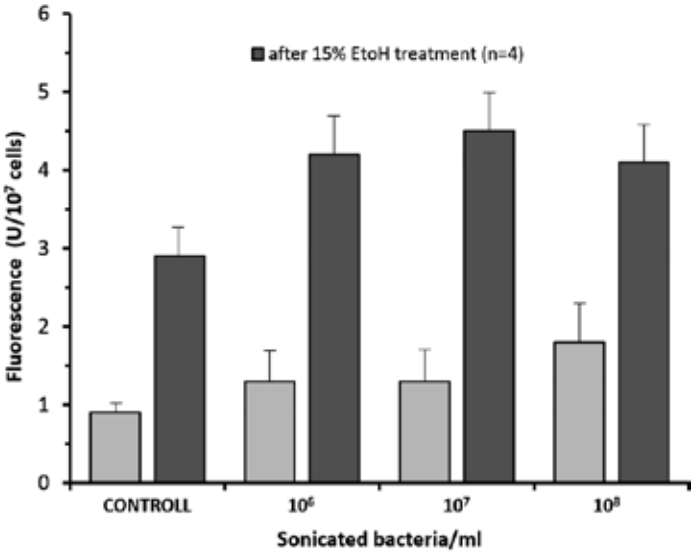


**Figure 281.** Changes in ethidium bromide–DNA fluorescence of freshly isolated gastric mucosal cells of rat after 30 minutes of incubation by sonicated $10^6$–$10^8$ bacteria/mL *H. pylori* with or without 5 minutes of 15% ethanol treatment. [Bódis, Karadi, Voros, Mozsik (1995b): Has sonicated Helicobacter pylori any direct cellular effect? In: Bajtai A., Papp J., Rácz I., Simon L. (Eds.). Helicobacter pylori. Hungarian Society of Gastroenterology. Medicom, Budapest. pp .137–142 (with kind permission).]

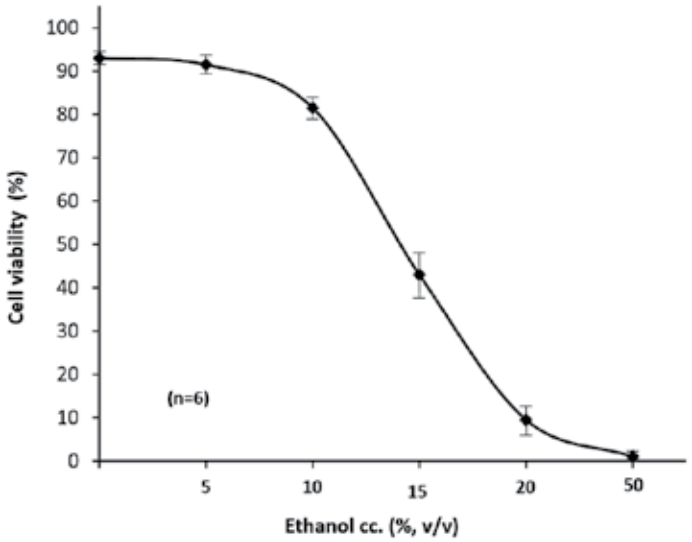


**Figure 282.** Changes in the viability of acutely (freshly) isolated rat gastric mucosal cells after 5 minutes incubation of 5–50% (v/v) ethanol detected by Trypan blue test. The $EC_{50}$=13.5%. [Bódis et.al., (1996): Dig.Dis.Sci.41:430; Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

*9.2.3.6.2. Combined effect of H. pyloriand EtOH*

Pre-incubation of cells with sonicated *H. pylori* ($10^6$–$10^8$ bacteria/mL) did not aggravate the 15% EtOH-induced cell injury detected by TB (Figure 282) and biochemical assays (Bódis et al., 1995b, 1996, 1997 a, b, 1998).

*9.2.3.6.3. Combined effects of H. pylori and Indomethacin (IND)*

After 5 minutes incubation of $10^{-8}$–$10^{-3}$ M indomethacin, only high doses ($10^{-4}$–$10^{-3}$ M) of IND significantly decreased ($P< 0.01$) the number of viable cells (Figure 283).

When the IND and *H. pylori* ($10^7$ sonicated bacteria/mL) were applied in combination, the number of viable cells was not changed compared with IND treatments alone (Figure 283), and no changes were obtained by biochemical assays (Figures 284, 285).

*9.2.3.6.4. Combined effect of EtOH and IND*

Using these toxic agents, IND and all doses significantly aggravated the 15% EtOH-induced cell injury determined by Trypan blue exclusion (Bódis et al., 1995 a, b, 1996, 1998).

In these studies, the direct effects of sonicated *H. pylori* were examined under different experimental conditions (when it was given alone or in combination with EtOH and IND).

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This model was suitable to eliminate the additional effect of immune systems, which is usually involved in a bacterial infection.

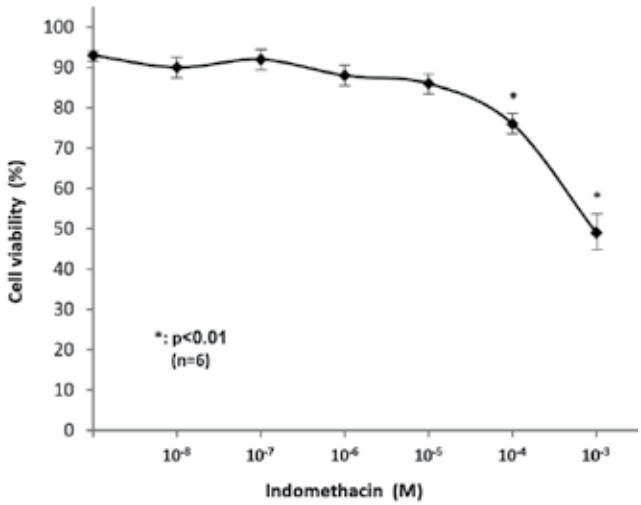


**Figure 283.** Changes in the viability of acutely (freshly) isolated rat gastric mucosal cells after 5 minutes incubation of $10^{-8}$–$10^{-3}$ M indomethacin (IND) detected by Trypan Blue exclusion test.*$P$<0.01 compared with untreated cells. [Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

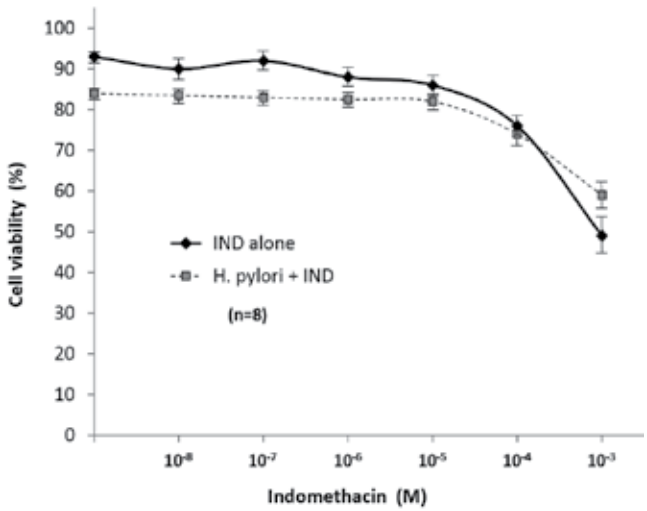


**Figure 284.** Effects of indomethacin (given in doses of $10^{-8}$–to $10^{-3}$ M concentrations) with and without co-administration of sonicated *H. pylori* (given in a dose of $10^{7}$ bacteria/mL together with all doses of indomethacin) on the viability of acutely (freshly) isolated gastric rat mucosal cells by Trypan Blue test. The incubation time was 30 minutes in all cases. [Bódis, Németh, Mózsik 1998. Akadémiai Kiadó, Budapest (with kind permission).]

We found that sonicated *H. pylori* had no direct cellular toxicity.

These types of observations with freshly isolated gastric mucosal cells from rat stomach were widely used to study the direct actions of various aggressive and protective compounds under experimental conditions.

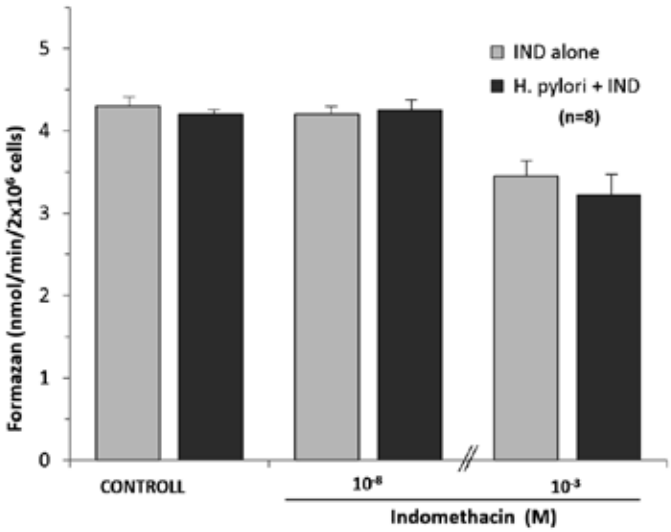


**Figure 285.** Effects of indomethacin (given in doses of $10^{-8}$–$10^{-3}$ M concentrations) alone and in co-administration with sonicated *H. pylori* (given in $10^7$ bacteria /mL together with all doses of indomethacin) on the succinate dehydrogenase activity of acutely (freshly) isolated rat gastric mucosal cells. The incubation time was 30 minutes in all cases. [Bódis, Németh, Mózsik (1998). Akadémiai Kiadó, Budapest (with kind permission).]

For the correct understanding, the results obtained with H. pylori (given alone or in combinations with EtOH or IND) have to be emphasized:

1. Rat (as animal strain) might not be the best model to study the possible gastric mucosal damaging mechanisms involved in *H. pylori* infection. Mongolian gerbil might be a better animal model to evaluate the mechanisms of *H. pylori*-induced pathology (Takahashi et al., 1998);

2. This *in vitro* model is an excellent model to study the direct cellular reactions of different chemical and hormonal, natural compounds (EtOH, IND, pentagastrin, histamine, body protective compound, BPC, etc.);

3. This experimental model is widely used in the research for preclinical selection of various drug candidates (Nagy et al., 1994);

4. No absolute correlation exists between the direct cellular damaging and defending effects of different biological agents and chemical compounds obtained in freshly isolated gastric mucosal cells and *in vivo* observations (Bódis et al., 1998).

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## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 10

# Studies on the Stable Cell Lines (Gastric Cancer, Hepatoma, Colorectal Cancer, Mouse Myeloma)

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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## 10.1. Introduction

The original term "gastric cytoprotection" described by Robert in 1979 (Robert, 1979; Robert et al., 1979) has new meaning nowadays. The "cytoprotection" expression has been limited to the level of cells, and new concepts have been defined, such as histoprotection and organoprotection (gastroprotection), which are widely accepted and recently studied in experiments searching for other fields of gastroenterology (e.g., hepatology). In these *in vivo* investigations, many environmental factors, such as central nervous system (Grijalva and Novin, 1990), vagal nerve (Mózsik et al., 1993 a, b, 1992 a, b, c, d), blood flow (Szabó et al., 1985; Guth et al., 1984), vascular permeability (Szabo et al., 1985) and rapid epithelial restitution of neck cell (Lacy et al., 1984), were studied as a part of defense mechanisms of the gastric mucosa.

The *in vivo* and *in vitro* experiments essentially differ from each other. *In vitro* investigations on isolated cells have many advantages. Ali of the environmental effects originated from other organs, tissues can be eliminated, so the behavior of a single cell can purely be examined. Unfortunately, these kinds of studies might have harmful consequences. The acutely isolated cells are less suitable for pharmacological studies than the gastric mucosa *in vivo* experiments because of their isolation procedure (Nagy et al., 1994).

The stress may reduce the cell responsiveness; surface receptors are damaged during the digestive stages of the isolation procedure. These effects are eliminated by using stable cultured cells in the experiment, though they are few in number and some properties of the tumor origin can be seen in their behavior.

Ethanol (EtOH) is widely used as a toxic agent in the gastric cytoprotection investigations *in vivo* (Nagy et al., 1994; Djahanguiri, 1969; Brodie et al., 1970; Karádi et al., 1994; Lacy et al., 1982). Indomethacin (IND) is also generally used for experiments (Djahanguiri, 1969; Brodie et al., 1970; Nagy et al., 1994). It inhibits prostaglandin synthesis, which is responsible for the maintenance of gastric mucosal integrity due to the stimulation of mucosal blood flow,

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preservation of cellular-ion transport and protection of the mucosal proliferative zone (Lacy and Ito, 1982). These chemical agents have been widely studied *in vivo* experiments, but only a few experiments have been carried out *in vitro* on isolated cells or cell lines with these toxic agents.

Various chemicals have been known and used as cytoprotective agents in the investigation of gastric, liver, pancreatic and large bowel protection; quite a few of them are scavengers (β-carotene, DMSO, DMPO, GSH and mannitol).

### 10.2. The aims of this present study were the followings

- To analyze the toxic effect of 5-minute EtOH treatment *in vitro* on acutely isolated mixed gastric mucosal cells (GMC) and on stable cultured cell lines;
- To compare the differences between the GMC and stable cultured cells – myeloma (Sp2/0-Ag14) and hepatoma (Hep G2) cell lines;
- To evaluate the differences between the myeloma (Sp2/0-Ag14) and hepatoma (Hep G2) cell lines;
- To study the effect of IND on GMC and Sp2/0-Ag14 cells;
- To examine the combined effect of EtOH and IND on these two types of cells;
- To analyze the toxic effect of EtOH after an alcohol-dehydrogenase enzyme inhibitor treatment (we used pyrazole for inhibition);
- To examine the scavenger effect of dimethyl sulfoxide (DMSO), glutathione (GSH) and β-carotene on these two types of stable cultured cells.

### 10.3. Materials and methods

#### *10.3.1. Preparation of mixed gastric mucosal cells*

Gastric mucosal cells from Sprague-Dawley rats were isolated by the method of Nagy et al. (1994). The segments of the glandular stomach were separated from the blood vessels and the surrounding connective tissue and were incubated in a physiological solution containing 0.5 mg/mL pronase E (type XXV, Sigma Chemical Co.) and $10^{-3}$ mol/L EGTA. After several washings, the cells were resuspended in a solution (0.157 mol/L, pH 7.4) produced freshly with the following ingredients: 98.0 mmol/L NaCl, 5.8 mmol/L KCl, 2.5 mmol/L $Na_2P0_4$, 5.1 mmol/L sodium pyruvate, 6.9 mmolJL sodium fumarate, 2.0 mmol/L glutamine, 24.5 mmol/L HEPES-Na, 1.0 mmol/L Trizma base, 11.1 mmol/L D-glucose, 1.0 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$ and 2.0 mg/mL (w/v) bovine serum albumin. AH examinations were carried out in this solution.

#### *10.3.2. Stable cultured cells*

Sp2/0-Ag14 (CRL 1581) is a non-secreting mouse myeloma; Hep G2 is a human hepatocellular carcinoma cell line obtained from the American type culture collection (ATCC). Cells were

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cultured and the examinations were carried out in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in a humidified incubator that held 95% air and 5% $CO_2$ at 37°C.

*10.3.3. Toxicological studies*

The cells were incubated with different concentrations of EtOH (1, 5, 10, 15, 20 and 50%) (v/v), IND ($10^{-8}$–$10^{-3}$ mol/L dissolved in 5% $NaHC0_3$, pH 7.4 with 5 N HCI) and their combination (15% EtOH and $10^{-3}$ mol/L IND) for 5 minutes in a shaking water bath at 37°C. Each study used $10^5$ cells. Alcohol-dehydrogenase inhibitor pyrazol was used during and after the EtOH treatment in the concentration of $10^{-5}$ mol/L. After 5 minutes of incubation, the cells were separated from the supernatant by centrifugation (500 g, 10 minutes), washed out (10 minutes water bath and centrifugation again) and resuspended in a toxic-free medium.

*10.3.4. Cytoprotective studies*

In the cytoprotective studies, the DMSO was used in the concentration of $10^{-3}$ mol/L. The GSH was applied to our medium in two concentrations: $10^{-4}$ mol/L and $10^{-3}$ mol/L. The 10% water-soluble β-carotene from Sigma was used in three concentrations: $10^{-8}$, $10^{-7}$ and $10^{-6}$ mol/L.

*1.3.5. Trypan blue exclusion test*

Trypan blue is taken up by damaged cells, staining the cytoplasm blue; viable cells can resist this staining. Trypan blue (0.2%) was mixed with the same volume of cell suspension and, after 5-minute latency, the numbers of stained (dead) and unstained (viable) cells were calculated as percentages in a hemocytometer. In comparison with stable cultured cells, we examined the viability for longer periods (5 minutes, 60 minutes, 4 hours and 24 hours) after the 5-minute EtOH incubation.

**Statistics**

Values in figures and text are expressed as means ± SEM. Comparisons were performed using the unpaired Student's *t*-test, and *P*-values were considered significant at $P < 0.05$.

## 10.4. Results

*10.4.1. Result of the toxicological studies*

*10.4.1.1. Effect of EtOH on GMC*

EtOH (1, 5, 10, 15, 20 and 50%) concentration-dependently decreased the viability of GMC. The $EC_{50}$ was 13.5% (Figure 286).

*10.4.1.2. Effect of EtOH on the Sp2/0-Ag14 cell line*

The EtoH decreased the viability of the stable cultured cells in concentration- dependently. In the case of Sp2/0-Ag14 cells, there is no significant difference between the viability values

obtained at 5 and 60 minutes, but 4 hours after the incubation with 10 and 15% of EtOH, a significant cell loss (secondary cell destruction) could be detected (Figure 287).

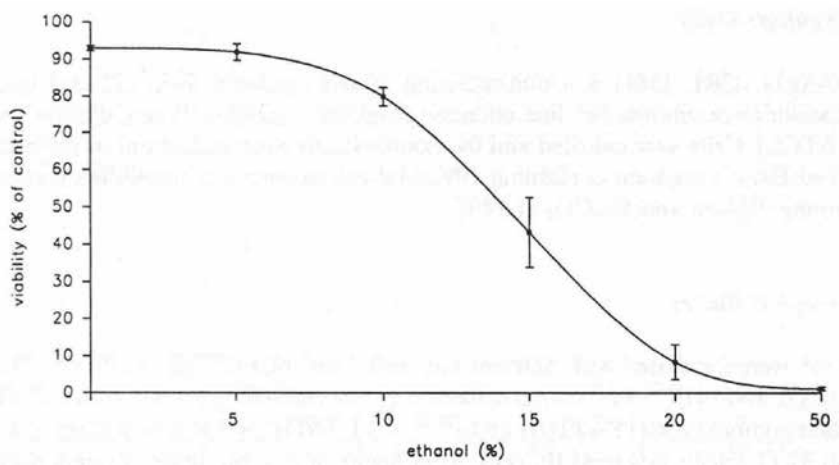


**Figure 286.** Changes in the viability of acutely isolated rat gastric mucosal cells after 5 minutes of incubation with 1–50% ethanol, detected by Trypan blue exclusion test (percentage of control). The results are expressed as means ± SEM ($n = 5$).

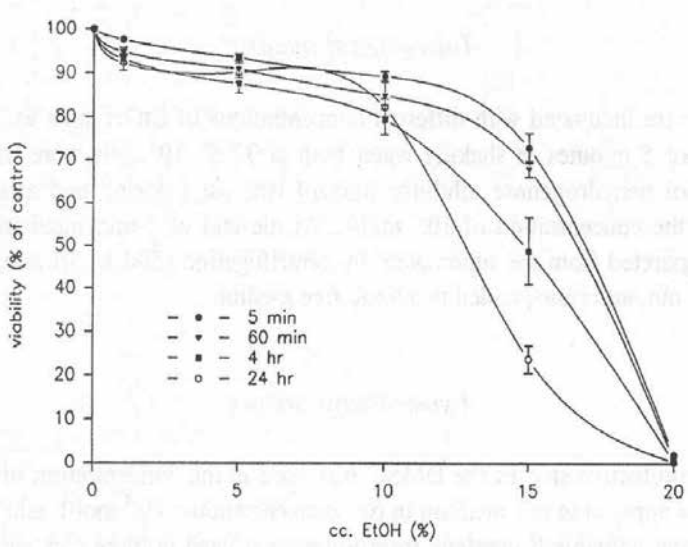


**Figure 287.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 1–20% ethanol (EtOH) counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$=5). * : $P < 0.01$ compared to 4-hour value. [Szabo et al. (1997a) Inflammopharmacology 5:20–28 (with kind permission).]

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*10.4.1.3. Effect of EtOH on the Hep G2 cell line*

In the case of Hep G2 cell line, a higher resistance was found up to 15% of EtOH concentration than in the case of Sp2/0-Ag14 cells. Above that concentration, a similar level of cell destruction occurred. During the 5-minute incubation of Hep G2 cells with various concentration of EtOH (1–20%), secondary cell destruction could not be detected (Figure 288).

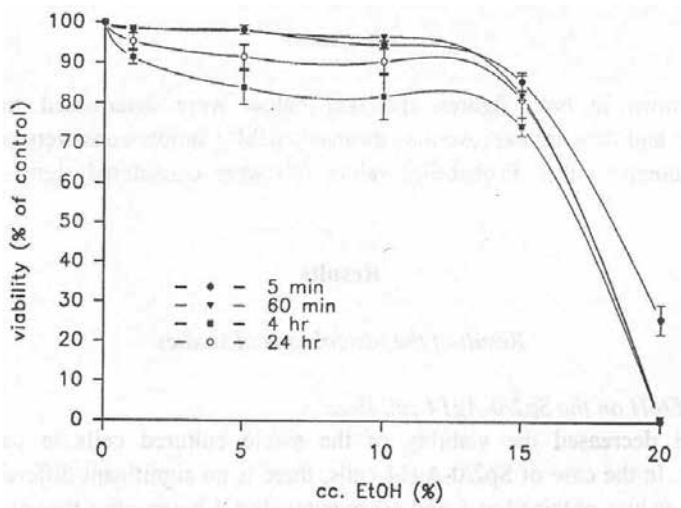


**Figure 288.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 1–20% ethanol (EtOH) counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$=5). * : $P < 0.01$ compared to 4-hour value. [Szabo et al. (1997a) Inflammopharmacology 5:20–28 (with kind permission).]

*10.4.1.4. Comparing gastric mucosal cells(GMCs) and stable cultured cells*

At all concentrations, the EtOH decreased the viability of GMC much more potently than the viability of the stable cultured cells. The $EC_{50}$ for GMC was 13.5%; the $EC_{50}$ for Sp2/0-Ag14 was 16% (Figures 287 and 288).

*10.4.1.5. Effect of IND*

Five minutes of incubation with $10^{-8}$–$10^{-3}$ mol/L IND had no effect on the viability of Sp2/0-Ag14 cells. In the case of GMC, only the highest dose ($10^{-3}$ mol/L) of IND decreased the number of viable cells significantly ($P < 0.02$) (Figure 289).

*10.4.1.6. Combined effect of EtOH and IND*

After the combined treatment, greater cell destruction could be detected. While using different concentrations, the $10^{-3}$ mol/L dose was the most aggressive; the amount of necrotic cell loss

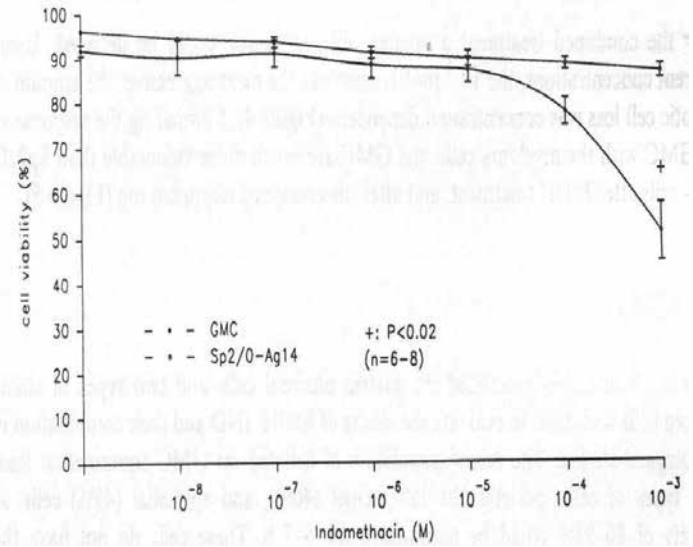


**Figure 289.** Changes in the viability of GMC and Sp2/0-Ag14 cells after 5 minutes of incubation with $10^{-8}$–$10^{-3}$ mol/L indomethacin, detected by Trypan blue exclusion test (percentage of control). The results are expressed as means ± SEM ($n$ = 6–8). * :$P$ < 0.02 compared with GMC. [Szabo et al. (1997a) Inflammopharmacology 5:20–28 (with kind permission).]

was concentration-dependent (Figure 290). Comparing the response of the GMC with the myeloma cells, the GMC are much more vulnerable than Sp2/0-Ag14 cells after the EtOH treatment and after the combined treatment too (Figure 291).

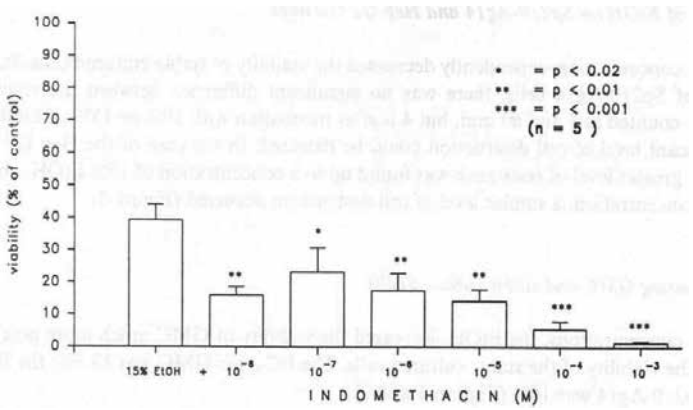


**Figure 290.** Changes in the viability of gastric mucosal cells (GMCs) after combined incubation with 15% ethanol and $10^{-8}$–$10^{-3}$ mol/L indomethacin, detected by Trypan blue exclusion test (percentage of control). The results are expressed as means ± SEM ($n$ = 5). * : $P$ < 0.02, ** : $P$ < 0.01, *** : $P$ < 0.001 compared with 15% EtOH alone. [Szabo et al. (1997a) Inflammopharmacology 5:20–28 (with kind permission).]

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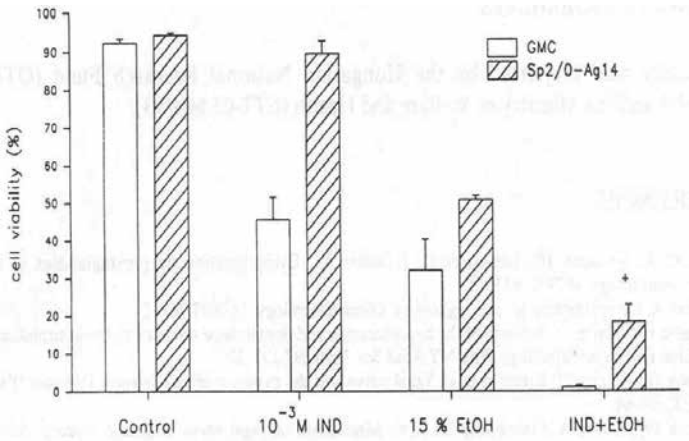


**Figure 291.** Changes in the viability of GMC and Sp2/0-Ag14 cells after 5 minutes of incubation with 15% ethanol (EtOH), $10^{-8}$–$10^{-3}$ mol/L indomethacin (IND) and 15% EtOH and $10^{-3}$ mol/L IND combined, detected by Trypan blue exclusion test (percentage of control). The results are expressed as means ± SEM ($n$ = 6–8). + $P < 0.01$ compared with GMC. [Szabo et al. (1997a) Inflammopharmacology 5:20-28] with kind permission).]

### *10.4.1.7. Comparison of myeloma (Sp2/0-Ag14) cells and hepatoma (Hep G2) cells after pyrazole treatment*

The viability values of the Sp2/0-Ag14 cells, compared to the values of EtOH treatment, did not change during the 5-minute co-incubation of the alcohol-dehydrogenase inhibitor pyrazole and EtOH (Figure 292).

In the case of Hep G2 cells, adding pyrazole to the incubation medium decreased the viability values, subduing the high resistance observed during the EtOH incubation (Figure 293).

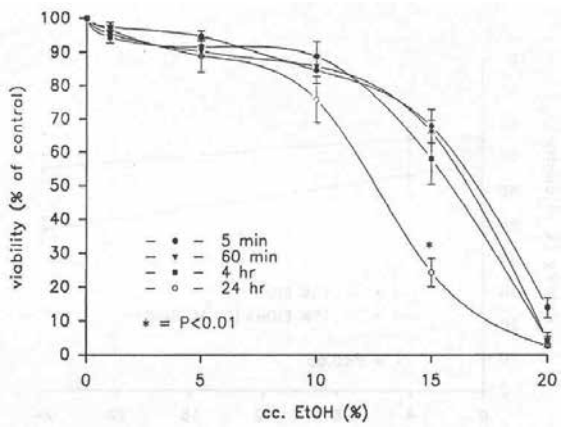


**Figure 292.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 1–20 % ethanol (EtOH) and continuous $10^{-5}$ mol/L pyrazole treatment counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$ = 5).

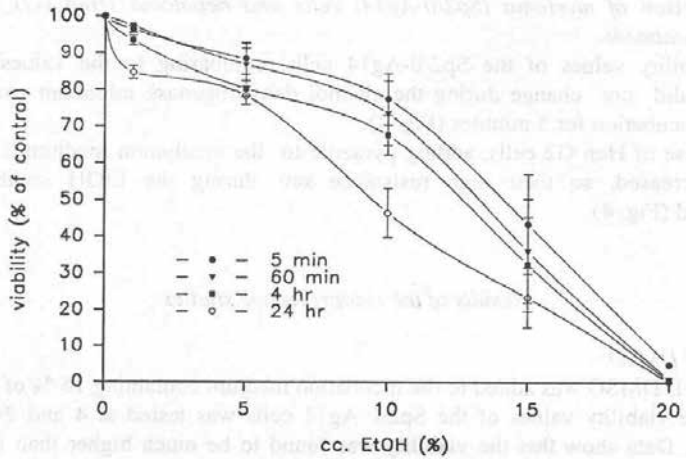


**Figure 293.** Changes in the viability of Hep G2 cells after 5 minutes of incubation with 1–20% ethanol (EtOH) and continuous $10^{-4}$ mol/L pyrazole treatment counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$ = 5). [Szabo et al. (1997a) Inflammopharmacology 5:20-28] with kind permission).]

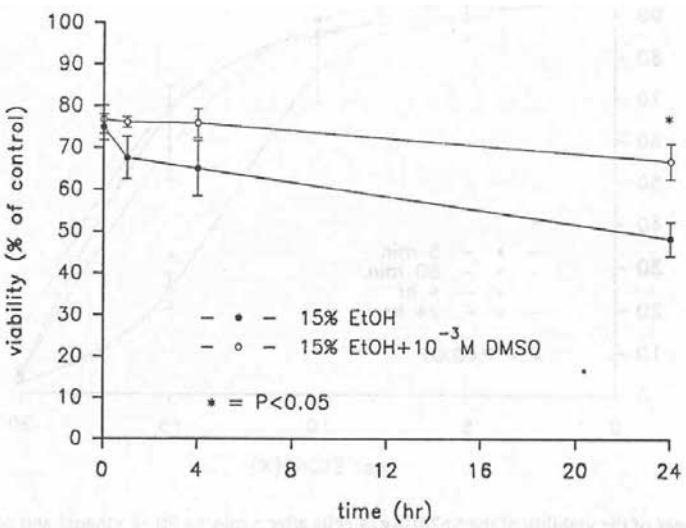


**Figure 294.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 15% ethanol (EtOH) and continuous $10^{-3}$ mol/L dimethyl sulfoxide (DMSO) counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$ = 5). *$P$ < 0.05 compared to 24-hour value of 15% EtOH incubation. [Szabo et al. (1997b) Usage of scavengers on stable cultured (Sp2/0-Ag14 and Hep G2) cell lines as an approach to reveal the mechanisms of cytoprotection. In: Mózsik Gy., Nagy L., Király Á. (Eds). *Twenty Five Years of Peptic Ulcer Research in Hungary (1971–1995)*. Akadémiai Kiadó, Budapest. pp. 275–283 (with kind permission).]

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*10.4.2. Results of the cytoprotective studies*

*10.4.2.1. Effect of DMSO*

A $10^{-3}$ mol/L DMSO was added to the incubation medium containing 15% of EtOH (for 5 minutes); the viability values of the Sp2/0-Ag14 cells were tested at 4 and 24 hours of incubation. The $10^{-3}$ mol/L DMSO significantly decreased the secondary cell destruction of the Sp2/0-Ag14 cell line (Figure 294).

*10.4.2.2. Effect of GSH*

Both concentrations ($10^{-4}$ and $10^{-3}$ mol) of GSH used during and after the EtOH incubation significantly increased the viability of the Sp2/0-Ag14 cells at the time period of 4-hour toxic-free incubation (Figure 295). Four hours after the incubation of EtOH, the GSH effect to protect was lost, probably due to its short-life span.

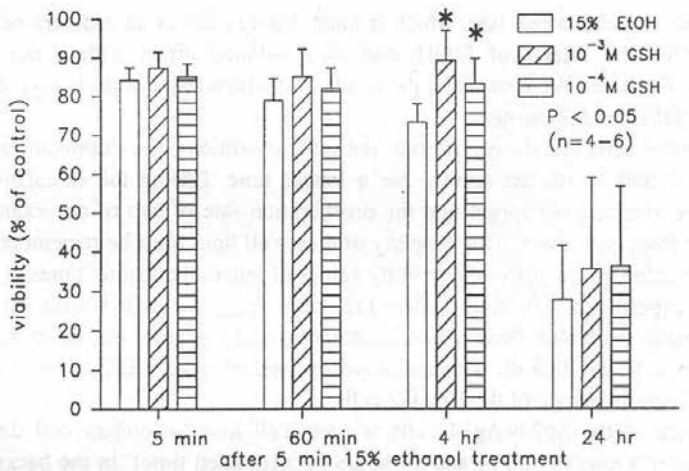


**Figure 295.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 15% ethanol (EtOH) and continuous $10^{-4}$–$10^{-3}$ mol/L glutathione counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$ = 4–6). *$P$ < 0.05 compared to 4-hour value of 15% EtOH incubation. [Szabo et al. (1997b) Usage of scavengers on stable cultured (SP2/0-Ag14 and Hep G2) cell lines as an approach to reveal the mechanisms of cytoprotection. In: Mózsik Gy., Nagy L., Király Á. (Eds). *Twenty Five Years of Peptic Ulcer Research in Hungary (1971–1995).* Akadémiai Kiadó, Budapest. pp. 275–283 (with kind permission).]

*10.4.2.3. Effect of β-carotene*

During the co-incubation with 15% of EtOH and $10^{-8}$–$10^{-6}$ mol/L – β-carotene, we could not find any protective effect. The viability values of the Sp2/0-Ag14 cells were much lower than they were obtained in the 15% EtOH incubation. The $10^{-7}$ mol/L dose of β-carotene significantly aggravated the 15% EtOH-induced cell destruction of the myeloma cells ($P$ < 0.01) (Figure 296).

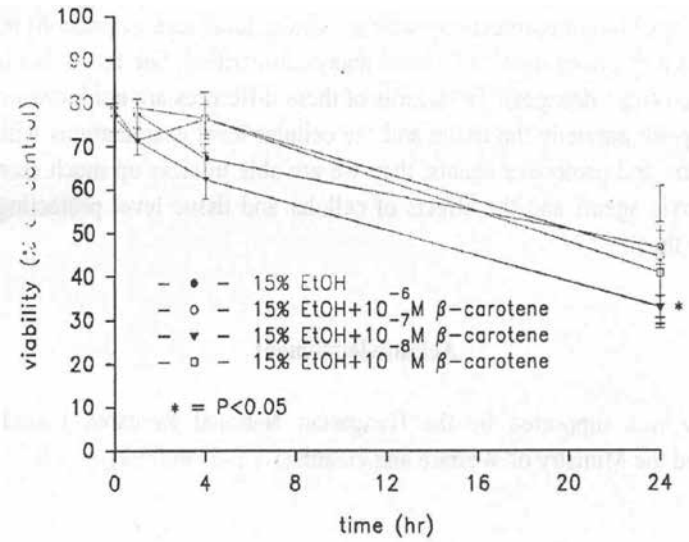


**Figure 296.** Changes in the viability of Sp2/0-Ag14 cells after 5 minutes of incubation with 15% ethanol (EtOH) and continuous $10^{-8}$–$10^{-6}$ mol/L β-carotene counted at 5 minutes, 60 minutes, 4 hours and 24 hours by Trypan blue exclusion test. The results are expressed as means ± SEM ($n$ = 6). *$P$ < 0.05 compared to 24-hour value of 15% EtOH incubation. [Szabo et al. (1997b) Usage of scavengers on stable cultured (SP2/0-Ag14 and Hep G2) cell lines as an approach to reveal the mechanisms of cytoprotection. In: Mózsik Gy., Nagy L., Király Á. (Eds). *Twenty Five Years of Peptic Ulcer Research in Hungary (1971–1995).* Akadémiai Kiadó, Budapest. pp. 275–283 (with kind permission).]

## 10.5. Discussion

In these studies freshly isolated rat gastric mucosal cells and two types of stable cultured cells were used to evaluate the effects of EtOH, IND and their combination in toxicological studies. The mixed population of isolated rat GMC contained at least three types of cells: parietal (20–25%), chief (40%) and epithelial (45%). A viability of 80–95% could be maintained for 6–7 hours. These cells do not have the potential for proliferation. Cultured cells were always kept in the same conditions, tests are reproducible and cells can survive for a longer time. During the calculation of viability, it must be remembered that the two cell lines have different proliferation rates. In particular, this should be borne in mind during the interpretation of the different viability values of longer incubation times. Our results indicate that the acutely isolated cells are more vulnerable than cultured cells. After EtOH treatment, in the case of Sp2/0-Ag14 cells, the $EC_{50}$ (16%) was higher than that of GMC (13.5%). IND had no damaging effect on stable cultured cells. The combined treatment reduced the viability in both types of cells, but this effect was much smaller in Sp2/0-Ag14; almost all the GMC were destroyed. These results show that cultured cells are more resistant to toxic agents than acutely isolated cells. Though different types of cells were used in this study, the differences in behavior may derive from the differences in isolation procedure rather than their differences in type. *In vivo* experiments have shown that gastrointestinal ulceration can be produced by IND administration (Nagy et al., 1994; Djahanguire et al., 1969). It is known that IND inhibits the activity of cyclooxygenase, producing less prostaglandins and excessive vasoconstrictor leukotrienes (Rainsford, 1992),

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and decreases the mucosal level of adenosine triphosphate (Rainsford, 1987). These factors reduce the gastric mucosal resistance to acid. In the *in vitro* study described here, IND was applied without any other aggressive factor, such as EtOH, and was not toxic for these cells. However, after the combination treatment, cell viability was considerably decreased compared with the effect of EtOH alone. It is likely that the decreased levels of endogenous prostaglandins might play a role in the enhanced toxic effect of EtOH. These results are in good agreement with those of Tarnawski et al. (1988) who observed a protective effect of exogenous prostaglandins on human-isolated gastric glands against IND and EtOH injury.

In our experiment, two types of stable cultured cells were used for cytoprotective studies with DMSO, GSH and β-carotene. In our experiment, when the Hep G2 cells were incubated with an alcohol-dehydrogenase inhibitor, their higher resistance against ethanol disappeared. This observation indicates that the alcohol- dehydrogenase enzyme might have an important role in the high resistance of the Hep G2 cells.

In the case of the Sp2/0-Ag14 cells, a great cell loss (secondary cell destruction) was derived after a long period (4 and 24 hours of incubation time). In the background of this phenomenon, the role of oxygen-free radicals had been suspected and proved (Bódis et al., 2000). Therefore, we tested the effect of the applications of some commonly known scavengers. Dimethyl sulfoxide and glutathione were effective, but the 10% water-soluble β-carotene had no cytoprotective effect. It was possible that the β-carotene with high molecular weight did not have enough time to be taken up by the cells in our experiments. In order to support this assumption, we have studied the effect of a 24-hour preincubation of $10^{-7}$ mol/L β-carotene, but in this case, we also found similar toxic behavior.

The tissue level (organoprotection) and cellular level (cytoprotection) mechanisms of the stomach "cytoprotection" have many similarities, but they also have many differences (e.g., dosages). The details of these differences are not clearly known yet. If we accomplish the tissue and cellular level examinations by choosing different toxic and protective agents, then we will easily be able to understand the effects of toxic agents and the cellular and tissue level protecting reactions generated by them.

## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

Chapter 11

# Discussion

Gyula Mozsik and Imre Szabó

Additional information is available at the end of the chapter

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In the discussion, we will focus our attention on different main points of our present work:

11.1. Etiological and terminological problems of gastrointestinal mucosa (ulcers in humans);

11.2. Clinical pharmacology;

11.3. Biochemical results of the human gastrointestinal tract;

11.4. Results of biochemical–pharmacological studies in animal experiments;

11.5. Direct cellular damaging effects of ethanol, indomethacin and *Helicobacter pylori* on freshly isolated rat gastric mucosal cells;

11.6. Observations with stable cell lines;

11.7. General conclusions;

11.8. Present and future observations.

## 11.1. Etiological and terminological problems of gastrointestinal mucosa damage (ulcers in humans)

The developmental mechanisms of the acute (dominantly in experimental models) and chronic antral, duodenal and jejunal ulcers are unknown. Before the use of the term "gastroduodenal ulcer" – without any recognition of the causative factor(s) – we used the term "genuine ulcer" "(in about 80% of total number of patients with GI ulcer).

After the publications of Waren and Marshall (1983) and Marshall and Waren (1983), most gastroenterologists accepted the presence of *Helicobacter pylori* as the real cause of PUD – an infectious disease; however, some experts are of the opinion that the presence of *Helicobacter pylori* is only one of the different causative factors (hyperacidity, physical and chemical stress, drugs, psychical status, trauma, different diseases of central nervous system, etc.). These

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problems of the etiological role of *Helicobacter pylori* in peptic ulcer disease have been reviewed very recently [Buzas Gy. (Ed.) *Helicobacter pylori*: a Worldwide Perspective 2013. Bentham Science Publishers. Sharjah, UAE – Oak Park, IL, USA]. The overall role of *Helicobacter pylori* infection in peptic ulcer disease has been emphasized after Barry James Marshall and John Robin Warren (Royal Perth Hospital, Australia) received the Nobel Prize. The experimental approach to ulcer development and prevention was based on different physiological, pathological and pharmacological fields (with the exception of experimental observations with Mogolian gerbils which is sensitive to *Helicobacter pylori* infection) (Takahashi et al., 1998). Hence, there is a contradiction between the etiologies of human gastric and duodenal ulcers and experimental ulcers in animals.

Changes in the suggested etiological factors of ulcer diseases appeared in the actual levels of applied drugs in the medical treatment of peptic ulcer patients (parasympatholytics, antacid compounds, histamine$_2$ receptor blockings, antigastrin compounds, proton pump inhibitors and the eradication treatment since 1980s).

It is important to clarify that the "classical histological definition of peptic ulcer diseases" is given by the presence of gastrointestinal mucosal damage which can be histologically characterized as a mucosal defect reaching the muscularis mucosae.

The term "secondary ulcer" was introduced as peptic ulcer disease associated with stress, burn, chronic liver, kidney and pulmonary diseases (in about 20% of patients with GI ulcer).

The connotation and use of the term PUD changed significantly in the past decade, when NSAID-induced gastrointestinal mucosal injury was found to be the cause of GI bleedings (ulcers) in patients in everyday medical practice. A couple of years ago, the term "non-ulcer disease" (NUD) was published by Scandinavian researchers, which indicated clinically the same symptoms as active peptic ulcer, however, without any endoscopic and histologic features.

Earlier it was thought that the primary cause for peptic ulcer disease (PUD) was unknown in patients. Furthermore, the inhibition of gastric acid secretion was taken as the primary tool for the treatment of PUD.

The classically used experimental ulcer models practically do not accept the histological definition of gastrointestinal ulcers (these models are widely used in the research on ulcer).

The peptic ulcer disease is similar to the infectious disease caused by *Helicobacter pylori* – in terms of classical (and old) terminology of this disease – which can be accepted partially, and now classical eradication treatment seems to be successfully used in the medical treatment (for details, see Mózsik et al., 2014 a, b, c, d). No clear histological evidence has been given by international experts that the infections caused by *Helicobacter pylori* produce histologically "classical" ulcer.

However, there is no doubt that many results of classical human observations were given earlier, and these results remained with us after Garrick and Warren received the Nobel Prize in 2005 [for the etiological role of *Helicobacter pylori* infection as a causative (or main) factor to human peptic ulcer disease]. During that time, the attention of physicians was only focused

on reality to prove the role of *Helicobacter pylori* infection. This approach suggested two different important points, namely, a). the eradication treatment was widely accepted in every medical practice and b). the results of classical gastroenterological research were forgotten after 2005 (when Warren and Garrick received the Nobel Prize for their research with *Helicobacter pylori*) in our everyday medical practice.

Clinicians have very frequently met patients with gastrointestinal mucosal damage, who obtained different drugs (dominantly non-steroidal anti-inflammatory compounds). The histological features of these drug-induced gastric mucosal damages do not indicate the classical ulcer structures.

The term "peptic ulcer" is not used in everyday medical practice. The characterization of ulcer by the name of "peptic" potentially suggested the overproduction and function of pepsin in the development of ulcer diseases.

### 11.2. Clinical pharmacology

Our interest in peptic ulcer disease started in the 1960s. We studied different clinical symptoms, functional parameters of the stomach and small intestine, "actual traditional medical treatment", and efficiency of different drugs used in the medical treatment of peptic ulcer (gastric ulcer, duodenal ulcer, typical complaints of detectable symptoms resembling hyperacidity; however, no ulcer was detectable).

In the 1960s, there was no methodology to approach these complicated fields of gastroenterology; however, there was hope that a new tool would be invented.

The basic problems of human clinical pharmacology are the following: measurements of absorption, metabolism, excretion of different drugs, serum levels of drugs and to find correlations between the obtained clinical pharmacological parameters and drug actions (inhibition of gastric acid secretion, gastric emptying, healing rate, side effects). Our studies started with the establishment of methodology for parasympatholytics in the 1960s. These observations enabled in excluding different drugs from the human "classical medical treatment," because these compounds were not absorbed from the gastrointestinal tract.

The classical pharmacological research tried to produce new quaternary ammonium compounds (instead of tertiary ammonium compounds). The theoretical background of these research works was as follows:

1. To produce drugs having long-lasting drug actions (the therapeutic time interval of atropine is 4–5 hours) in the medical therapy;
2. The tertiary ammonium compounds can pass across the hemato-encephalic barrier, while the quaternary ammonium compounds cannot pass over across this barrier;
3. The quaternary ammonium compounds produced have stronger blocking effects on peripheral neural ganglions than those produced by tertiary ammonium compounds.

Clinical pharmacological studies have proved clearly that the absorption of quaternary ammonium compounds from the human gastrointestinal tract is bad (or not absorbed). We

received a clear explanation for why some drugs are without any objective effects in the human medical therapy (so we received another explanation from our observations why different indications resulted in gastric surgery for peptic ulcer). We had a quaternary ammonium compound (Gastropin®) in our medical practice (at that time) which is absolutely not absorbed from human GI tract (e.g., 1000 pills given orally); however, when this drug was given as injection, we were able to detect its serum level and excretion of the drug in the same person.

On the contrary, the results of our previous clinical pharmacological examinations demonstrated clearly that the results of experimental pharmacological research cannot be applied directly to the human medical therapy.

One of the most important results was (obtained by clinical pharmacology) that the duodenal ulcer completely healed during classical atropine treatment in patients with duodenal ulcer; however, the gastric acid secretory responses of these patients were unchanged (1965). It was also important to emphasize that the clinical pharmacological parameters of atropine treatment were also unchanged.

Another important question was in medical therapy (especially after establishing the clinical pharmacological methodology). "What kind of therapy" can be taken as "basic therapeutic state"? In other words, what happens to patients who are not given an effectively acting drug (or compounds)? Halter and coworkers published a paper in gastroenterology (Scheurer et al., 1977), which described that gastric and duodenal ulcers were able to heal without the administration of any drug (however, the description of this placebo compound – without any pharmacological actions – was not clearly defined chemically in the aforementioned paper).

In our clinical pharmacological practice, we used an antacid tablet (without the neutralization capacity of gastric acid secretion) as placebo drug (aluminum hydroxide).

In randomized and prospective studies, the efficiencies of atropine (3 × 1–2 tablets/day orally given), cimetidine (1.0 g/day orally given), carbenoxolone (3 × 100 mg/day orally given for 2 weeks and 3 × 50 mg/day orally given for 3rd and 4th weeks) and placebo (3 × 1 placebo tablet/day orally given) for one month was analyzed. Different laboratory examinations, such as gastrofiberoscopic examinations (the presence of ulcer was detected, and the measurement of ulcer size was carried out planimetrically), were carried out at the beginning, 2nd and 4th weeks of the study, while dairy card observations (complaints, appetite, body weight,, etc.) were recorded day to day from patients with duodenal ulcer during the whole time period of the study (Tárnok et al., 1989).

The results of this study clearly indicated the following:

1. The beneficial effects of atropine, cimetidine and carbenoxolone were superior to that of placebo in a multicenter, randomized, prospective and comparative study in duodenal ulcer (DU) patients;

2. No significant difference was obtained in the beneficial effects of atropine versus cimetidine versus carbenoxolone in DU patients;

3. Because the carbenoxolone has no inhibitory action of gastric acid secretion in DU patients, the ulcer healing effects (due to stimulation of mucus) could be considered independent of any effect on gastric acid production (Tárnok et al., 1979; Mózsik et al., 2011).

Thus, these and earlier demonstrated studies of chronic atropine treatment in DU patients (during the 1960s–1970s) were performed before the classical concept of "gastric cytoprotection" was formulated by André Robert, yet it is clear now in retrospect that we had been observing acid-independent gastroduodenal protection with atropine and other drugs before Robert et al. had defined gastric cytoprotection (1979).

On the contrary, these observations clearly indicated us that the beneficial effects of different drugs (or drug candidates) were compared to that of placebo effect. This was the first step in the gastrointestinal clinical pharmacology, when the effects of different drugs (compounds) were compared to that of placebo. In our days, the basic requirement for new drug production is that the suggested beneficial effect should be better than that of the most effective drug in different medical fields.

Clinical pharmacology offers a new possibility to suggest a proof for the development of tolerance (Mózsik et al., 1965 a, b, 1966 a, b, c, 1969 a, b, c, d, e, f) and cross-tolerance (Mózsik and Jávor, 1968 a, b, 1969 a, b, c, d, e, f) without any inhibition in gastric basal and supraliminal (but submaximal) stimulated gastric secretory responses in patients with chronic GI ulcers (Mózsik et al., 1966, a, b, c, 1970 a, b, c). In other words, the PUD healed without any inhibition of gastric acid outputs (so clearly the PUD healed just on the dependence of gastric mucosal protective mechanisms).

The facts for the existence of "gastric cytoprotection" were generally and clearly indicated by the works of Robert et al. (1979) in experimental observations in rats. This phenomenon was proved specifically to prostaglandins in rat observations (Grossmann, 1979).

The gastric mucosal healing without any inhibition of gastric acid secretion was proved by our team with anticholinergic cimetidine and retinoids in animal observations (Jávor et al., 1981; Mózsik, 2005; Mózsik et al., 1967c, 1969a; Morón et al., 1984c) and in patients (Rumi et al., 1997, 2001 a, b).

We also demonstrated – in randomized multiclinical, multicentric and prospective study in gastric ulcer patients – that vitamin A (3 × 50.000 IU/day orally given) has an ulcer healing effect in GU patient (without any gastric acid inhibitory action) (Patty et al., 1982, 1983; Mózsik et al., 1986). Vitamin A is an important nutritional component having scavenger properties (Mózsik et al., 2005, 2007).

To the best of our knowledge, this study was first carried out in multiclinical, randomized and prospective study with nutritional components (as scavenger) in patients worldwide.

Clinical pharmacology studies helped us in comparing the beneficial effects of different drugs in gastric and duodenal ulcers in patients. The key roles in these clinical pharmacological studies were the follow-up of changes in the incidence of ulcer sizes at different times of randomized, multiclinical, prospective and multiclinical studies. To understand and evaluate

the dynamism of ulcer healing rates of different drugs, clinical pharmacological studies of the changes of ulcer sizes in incompletely healed patients are necessary.

The general hypothesis (the increased side of aggression, that is, the overproduction of HCl secretion) responsible for the development of GI ulcers in patients was applied for many years. As we can see from the literature and our observations, this standpoint has been invalid both in animal experiments and in patients (Mózsik et al., 1985).

### 11.3. Biochemical results of the human gastrointestinal tract

After obtaining the new clinical pharmacological results in patients with gastric and duodenal ulcer, it was another important standpoint that the classical gastric and duodenal ulcers develop as a result of tissue hypoxia (like myocardial infarction). However, it was also interesting to note that many drugs (used in the medical treatment of peptic ulcer) inhibit the biochemical metabolism of gastrointestinal mucosa (parasympatholytic drugs, histamine$_2$ receptor blockers, later the proton pump inhibitions). As it is well known, these compounds inhibit the breakdown of energy storage molecule (adenosine triphosphate, ATP). In other words, these drugs inhibit the liberation of energy (by splitting up of ATP into ADP). However, it was (and is) suggested that the resynthesis of ATP can be inhibited under hypoxemic conditions in GI mucosal tissues. Therefore, we medically applied different drugs in the medical treatment of peptic ulcer, which was a priori able to inhibit the metabolic adaptation. Our primary aims were to stimulate positive metabolic adaptation. The aims of medical treatment and suggestions in the development and healing of gastric and duodenal ulcers are absolutely in contradiction. These (and other) arguments and counter-arguments (or suggestions) led us to start with the biochemical examinations of gastrointestinal mucosal tissues.

It is important to learn the basic methodologies applied in the biochemical examinations in GI mucosal tissues. We tried to follow up the updated, so-called general, trends in the biochemical observations. We have to emphasize that the progression of biochemical research (in detail) is practically an impossible challenge for clinicians. In consequence of many circumstances, we started with simple biochemical examinations of gastrointestinal mucosa from 1966. We spent more time in learning these biochemical methodologies in animal experiments (see Chapters 3, 4 and 5).

Our basic standpoints were, during the "learning" period, to learn and demonstrate the involved cellular mechanism of gastrointestinal mucosal damage and prevention. The results of many of our biochemical observations proved that the cell membrane, mitochondrion, proteins, RNA and DNA are involved in the development of gastric mucosal damage and prevention in animal experiments.

Probably the best representative key moments in these tissue reactions were to understand the details of the changes in cellular energy systems under different pathological and experimental conditions. The acid-soluble inorganic and organic phosphates were measured in GI mucosa. The components of acid-soluble organic phosphates were not known in the earlier times (in the 1960s–1970s). Later, we could separate adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenine–adenosine from the acid-soluble

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organic phosphates. Consequently, we were able to not only follow up with the changes to the extent of ATP transformation into ADP but also approach the possible extent of rephosphorylation. Similar observations were made by Atkinson (1968) to approach the general metabolism of living cells, including some calculation index from the results of measurements [such as ATP/ADP, adenylate pool (ATP+ADP+AMP), "energy charge" (ATP + 0.5 ADP)/(ATP+ADP +AMP)]. The calculated values of "energy charge" give information on the extent of phosphorylation (and dephosphorylation). When the value of "energy charge" is equal to 1, then all adenosine compounds are phosphorylated (e.g., in the form of ATP); however, if its value is 0, then all adenosine compounds are in dephosphorylated form (e.g., practically not in the form of ATP). The results of these observations gave us a possibility to prove (or to exclude) the presence of tissue hypoxemia (which was a key point in the development of mucosal damage during ulceration).

Biochemical observations from the resecates of human gastrointestinal tract after the surgical intervention in patients with peptic ulcer were carried out in the 1970s (see Chapter 7 for details). These biochemical examinations were performed under the nationally accepted medical practice, our scientific knowledge and ethical laws; however, our methodological abilities were limited by the current knowledge in medicine (e.g., no possibility for cAMP).

From the evaluation of these biochemical examinations, we emphasize the following:

1. Simultaneously 6–10 biochemical measurements were performed (from the same resecate obtained from a patient);
2. All biochemical examinations (measurements of ATP, ADP, AMP, lipid phosphates, RNA and DNA preparation of membrane ATPase) – from the tissue of one patient – were simultaneously carried out;
3. Tissue samples obtained by biopsy were not suitable enough for simultaneously carried measurements (the technical problems of the measurements were excluded by these simultaneously carried out biochemical examinations).

All biochemical examinations from the gastric tissues (obtained from one patient) were also simultaneously carried out.

There are different important points applicable from the biochemical methodology from 1969–1970, when we learned the existence of the details of the specific sodium pump (substrate enzyme with its classic biochemical behaviors) and "second messenger" systems in other tissues (as GI tract). Because we succeeded in preparing the classical $Na^+$–$K^+$-dependent ATPase and adenylate cyclase from rat heart muscle, rat and human gastric mucosa, we received an absolutely new possibility to understand some parts of regulations of cellular energy systems (Mózsik and Øye, 1969; Mózsik, 1969 a, b, 1970; Mózsik et al., 1970 a, b).

We were the first authors to prove the presence of $Na^+$–$K^+$-dependent ATPase (Mózsik and Øye, 1969; Mózsik, 1969 a, b) and adenylate cyclase (Mózsik et al., 1970) in rat and human gastric fundic mucosa (Gheorghui, 1976; Mózsik et al., 1975 a, b, 1976 a, b, 1978 a, 1982 a, b).

These observations were carried out in earlier time period under different *in vitro* conditions to describe the existence of feedback system between two membrane-bound ATP-dependent

energy systems (Mozsik, 1969 a, b, 1970 a, b). We also measured the changes in these energy supply systems by the direct *in vivo* measurements of different adenosine compounds by different enzymatic kits and RIA method (such as ATP, ADP, cAMP, AMP) in the gastrointestinal tract. (We did not have these conditions at the time of our human biochemical examinations.)

Because no systematic biochemical examinations from the human gastrointestinal tract were carried out and published, out attention is focused on the results obtained from GI resecates of human GI tract obtained at the time of surgical intervention (treatment) in peptic ulcer patients.

There are important notes to the evaluation of human biochemical results obtained from the GI resecates of patients with peptic ulcer:

1. We had no information (at the time of gastric resection) on the suggested etiological factors for this disease;

2. The gastric secretory responses (basal acid output, BAO; maximal acid output, MAO) were – as the gastric secretory responses and the suggested main aggressive factor for peptic ulcer disease – permanently measured in these patients. The extents of these secretory responses were used to create different groups of biochemically evaluated patients;

3. We had no information on the presence of *Helicobacter pylori* infection in these patients. Now we know well that practically all DU patients were infected with *Helicobacter pylori*, and its infection rate is less in GU patients. In our days, we had no knowledge on *Helicobacter pylori* infection in jejunal ulcer patients (after Billroth II-type surgical intervention);

4. We had no information on the details of medical treatment (applied drugs, time periods of medical treatments) on GI mucosal cells in the ulcerated and control animal and patients' tissues;

5. The necessary indication for surgical intervention was done based on a special consultation between the internist and the surgeons; however, we did not participate personally in this process;

6. In the critical evaluation of ATP, membrane ATPase, and ADP, the possible role(s) was used as an energy supply system (namely energy liberation) for different regulations of enzymes and a critical biochemical parameter of GI tissues to prove (or to exclude) the presence of tissue hypoxemia (by the measurements of dephosphorylation and oxidative phosphorylation). This enzyme system exists in all animals tissues;

7. We wanted to know more on the biochemical structure of cells in the human GI tract in peptic ulcer patients.

8. An important fact is that the $Na^+$–$K^+$-dependent ATPase and $H^+$–$K^+$–ATPase differ from each other; however, the ATP is a common substrate for the function of both enzymes (see Chapter 5);

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9. The activity of membrane ATPase (ATP transformation into ADP by $Na^+$–$K^+$–ATPase) and its extent can be used as an important energy biochemical marker function related to the functional state and to the general buildup of target organs;

10. The $H^+$–$K^+$–ATPase is located only in the parietal cells of the stomach (see Chapter 5) and not in other cells of the GI mucosal tissue.

The results of human biochemical examinations (from the resecates of GI tract) indicated clearly that:

1. A very close and positive correlation exists between the $Na^+$–$K^+$-dependent ATPase activity (liberated quantities of inorganic phosphate from ATP by $Na^+$–$K^+$-dependent ATPase, measured in *in vitro* conditions) versus gastric basal acid output (BAO)($r = 0.88$; regression line: $Y = 0.49 + 0.39.X$; $n = 45$) (Mózsik et al., 1974d).;

2. The $Na^+$–$K^+$–ATPase system (from the human gastric fundic mucosa) works without any trouble under cholinergic influences (Mózsik et al., 1974c) and its activity can be inhibited by parasympatholytics, histamine, pentagastrin, $PGE_1$ and $PGE_2$ (Mózsik et al., 1974b; Mózsik et al., 1974a), epinephrine (Mózsik, 1969 a, b).;

3. There are positive and significant correlations between the BAO, MAO values and members of ATPase systems ($Na^+$–$K^+$-dependent ATPase activity, tissue levels of ATP and ADP in the human gastric fundic mucosa – including the positive correlations between these biochemical parameters) (Mózsik et al. 1981).;

4. The ATP transformation into ADP by $Na^+$–$K^+$–ATPase system can be regulated by drugs, hormones and mediators in smaller molar concentrations than the ATP transformation into cAMP by adenylate cyclase, and the drug actions are contraregulatory in these energy systems (Mozsik et al. 1997c).

5. The gastric acid secretion depends on the activation of $Na^+$–$K^+$-dependent ATPase and stimulation of adenylate cyclase, besides the activation of function of $H^+$–$K^+$–ATPase in human beings.

6. The active transport functions across the gastric mucosal cells depend on different cellular (however, ATP-dependent membrane-bound) energy systems (such as $Na^+$–$K^+$-dependent ATPase-, adenylate cyclase- and $H^+$–$K^+$-dependent ATPase systems) in human beings.

7. Biochemical and energetic gradients exist in the gastric fundic, antral, duodenal (jejunal) mucosal tissues dependent on the gastric basal acid secretory responses in patients with hyperacidity and normacidity; however, these gradients disappear in patients with hypoacidity (Mózsik— et al., 1976).;

8. The extents of ATP transformation into ADP by membrane ATPase are significantly higher in the mucosal tissues around the gastric (antral), duodenal and jejunal ulcer patients than those in their identical normal (non-ulcerated) mucosal tissues (in the same patients) (the values of "energy charge" remained unchanged in these tissue specimens). These results together proved clearly that the oxidative phosphorylation is intact in the ulcerated mucosa, which cannot be obtained in the presence of hypoxemic damage in the tissues.

For understanding these results, we have to mention different points:

1. We had no information on the existence of classical ulceration in the antrum, duodenum and jejunum in patients;

2. We had no information on the kinds (and their time periods) of medical treatments;

3. We did not receive any information on the reasons for the indication of surgical intervention in these patients;

4. We also had no suggestion on the possible healing processes of ulceration in these patients without any surgical intervention;

5. During surgical interventions, we did not hear anything on the presence of *Helicobacter pylori* infection; however, now it is clear that the incidence of *Helicobacter pylori* infection differs in the case of duodenal ulcer and gastric ulcer (no data are available on *Helicobacter pylori* infection in jejunal ulcer after surgical intervention in patients). Based on our opinion on these, we suggested that the presence of *Helicobacter pylori* infection is independent of these biochemical observations;

9. Our human biochemical results (obtained in the ulcerated and non-ulcerated mucosa) suggest the positive metabolic adaptation against ulcer development in the antral, duodenal and jejunal mucosa. The extent (capacity) of the metabolic adaptation depends on the general biochemical buildup of the tissues. There is no doubt that the highest metabolic activity can be obtained in the gastric fundic mucosa in patients with gastric hyperacidity and normacidity (because of the existence of biochemical and energetic gradients in these mucosal tissues). Consequently, the exhaustion of antral, duodenal and jejunal mucosa will appear in a shorter time than that in corpus mucosa. Probably that suggestion gives a real explanation for why we applied drugs (having metabolic blocking effect) for the medical treatment of peptic ulcer disease in patients.

We established a uniform biochemical explanation for the development and location of "genuine" gastric, duodenal and jejunal ulcers in patients (Mózsik, et al., 1997b).

9. These results, demonstrated earlier (Figures 67–71), clearly indicated that the extent of ATP–ADP breakdown is significantly higher in the ulcerated antral, duodenal and jejunal mucosa specimens than in the control (non-ulcerated) mucosa specimens. This fact can be proven by the increased membrane ATPase activity and increased level of ADP in association with significant increase in the tissue level of ATP. The following facts are found in the background:

    a. No impaired oxidative phosphorylation can be found in ulcerated mucosa specimens, which can be proven by increased tissue levels of ATP in time when the ATP–ADP breakdown was significantly increased (significantly higher membrane ATPase activity and increased level of ADP);

    b. The tissue levels of ATP and ADP are significantly higher in the ulcerated mucosa than those in the non-ulcerated mucosa (in the same patients);

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**c.** The membrane ATPase activity is also significantly higher in the ulcerated mucosa than that in the non-ulcerated GI mucosa (Mózsik et al., 1979);

**d.** The higher ATP tissue levels (in time when the ATP breakdown was increased in both directions) can only be obtained by the intact oxidative phosphorylation pathway;

**e.** The biochemical components of gastric mucosal tissue were expressed in accordance to 1.0 mg DNA, which represents the same number of cells (Figure 157). The values of adenine–adenosine, ATP, ADP and AMP were increased in the gastric fundic mucosa in patients with increased gastric secretory responses (BAO, MAO) and in the mucosa around chronic antral, duodenal and jejunal ulcer (Mózsik et al., 1979 a, b, d, f, h, 1981 a, b, 1987 a, b; Nagy et al., 1978, 1981b);

**f.** No physiological data exist in the literature to prove the presence of decreased GMBF in the gastric fundic mucosa in patients with gastric hyperacidity; nobody found an increased tissue level of lactate. Experts accept the increased energy turnover (increased capacities of ATP–ADP and ATP–cAMP transformation) in these gastric fundic mucosa specimens.

The chemical comparison of the biochemical results obtained in the human gastric fundic mucosa with different gastric acid secretory responses and non-ulcerated and ulcerated antral, duodenal and jejunal mucosa in patients is shown in Figure 297. The results are expressed as amounts of biochemical parameters/1 mg DNA.

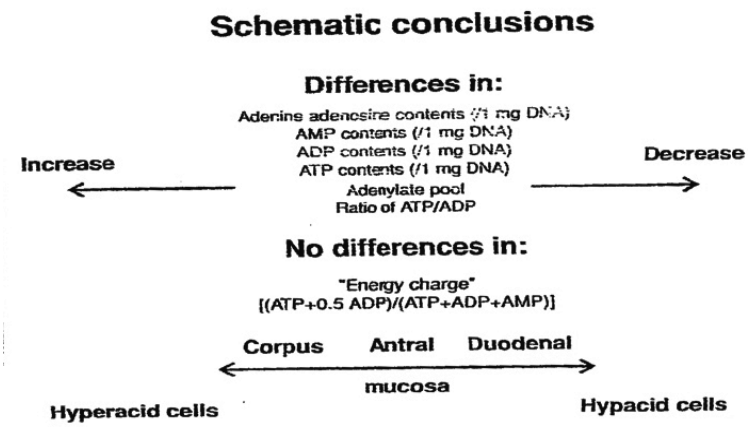


**Figure 297.** The schematic presentation of biochemical buildup of human gastrointestinal mucosa in patients with different gastric acid secretory responses. All of the adenine and adenosine compounds increased significantly (meanwhile the stream of ATP breakdown enhanced in both directions) in the gastric corpus mucosa in comparison with those results obtained in corpus mucosa patients with hyperacidity. The same biochemical parameters were obtained in the ulcerated antral, duodenal and jejunal mucosa in patients with chronic antral, duodenal and jejunal mucosa. So the biochemical structure of human chronic antral, duodenal and jejunal mucosa is the same as that obtained in the corpus fundic mucosa in patients with gastric hyperacidity. [Mózsik, Abdel-Salam, Király, Morón, Nagy, Sütő, Tárnok, Jávor (1997) in Mózsik Gy., Nagy L., Király Á. (eds) Twenty Five Years of Peptic Ulcer Research in Hungary: From Basic Sciences to Clinical Practice (1971–1995) pp. 159–170., Akadémiai Kiadó, Budapest; Mózsik, 2006. In: Discoveries in Gastroenterology (1960–2005). Akadémiai Kiadó. Pp. 139–224 (with kind permission).]

The biochemical buildup (chemical composition) of cells in the human gastric fundic mucosa with hyperacidity and in the ulcerated antral, duodenal and jejunal mucosa in patients are significantly different from that in gastric fundic mucosa with hypoacidity and in non-ulcerated antral, duodenal and jejunal mucosa. In consequence of these facts, the physiological, neural, hormonal and pharmacological regulations of these cells in the human gastric fundic mucosa in patients with hyperacidity and in ulcerated antral, duodenal and jejunal mucosa also differ (quantitatively) as those in the cells in gastric fundic mucosa with hypoacidity and in non-ulcerated antral, duodenal and jejunal mucosa in peptic ulcer. The physiological, pharmacological and pathological regulations of membrane-bound ATP-dependent energy systems had essential roles in these processes.

How can we explain the contradictions of the presence and absence of ulcerated gastrointestinal mucosa in animals and patients with peptic ulcer?

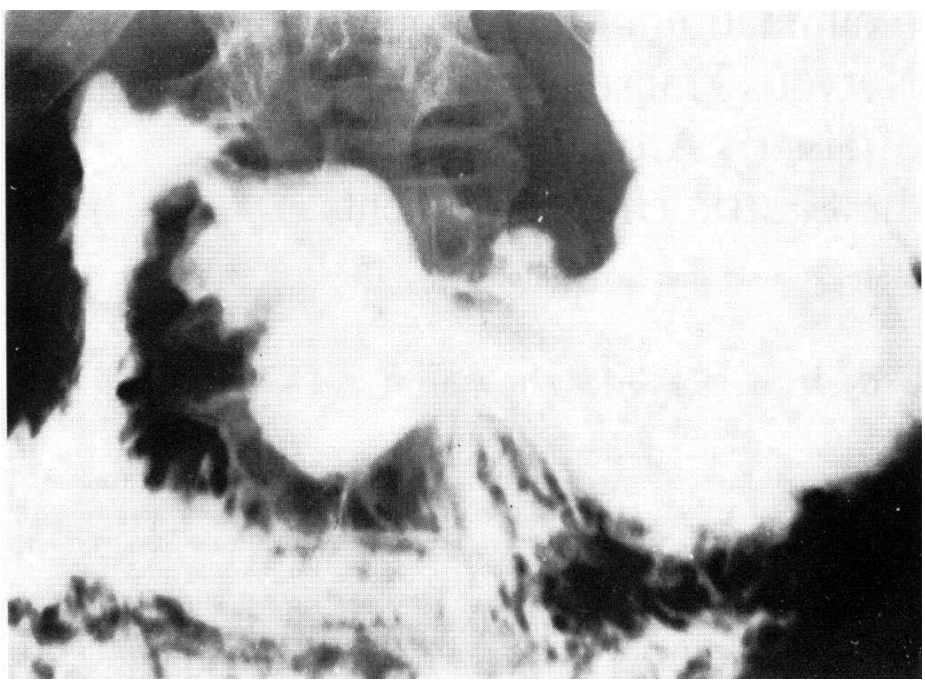

**Figure 298.** Roentgenogram of gastric ulcer localized on the lesser curvature of the stomach of a 56-year-old patient. The X-ray examination was performed by Z.Molnár M.D. at the Radiological Department, University of Medical School, Pécs, Hungary, in 1970. [Mozsik, Nagy, Tárnok , Jávor (1971): In: Pfeiffer C.J. (Ed). Peptic Ulcer. Munksgaard, Copenhagen – Lippincott, Philadelphia. pp. 323–328 (with kind permission).]

**1.** The results of experimental measurements in the animal gastrointestinal mucosa (used different – dominantly physiological – different experimental conditions, but not under the development of mucosal damage) and there bewer were proved the causative correlation between the physiologically measured decrease of GMBF versus insufficiency of oxidative phosphorylation in the same tissue samples in the same time (Kitajima, 1989);

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**2.** The experimental evidence of decrease in tissue ATP has been based on the observations of Menguy et al. (1974 a, b, c, d); however, the examined animals had a severe blood loss in the experiments. No similar changes of blood circulation existed in other experimental models (used by different experts all over the world);

**3.** The measurement of the tissue level of gastrointestinal mucosal tissues alone cannot give a scientific evidence for the existence of impaired oxidative phosphorylation (see Mózsik and Vizi, 1976 a, b; Mózsik et al., 1983). We have to measure the tissue levels of ATP, ADP, AMP and cAMP together (with the simultaneous measurement of lactate) in the same tissue samples in the same time;

**4.** The biochemical structures of the human gastric fundic mucosa with increased gastric acid basal (BAO) and maximal (MAO) secretory responses are the same as those obtained in the ulcerated antral, duodenal and jejunal mucosa in patients with peptic ulcer;

**5.** The tissue levels of ATP in the antral, duodenal and jejunal ulcerated mucosa are much higher than those in the non-ulcerated (control) mucosa. On the contrary, the tissue levels of ADP – in these tissue samples – are the same as ATP. However, the membrane (transport) ATPase ($Mg^{2+}$-dependent, $Mg^{2+}$–$Na^{+}$–$K^{+}$-dependent and $Na^{+}$–$K^{+}$-dependent) activities are also significantly higher in the ulcerated mucosal tissues than those in the control (non-ulcerated) ones.

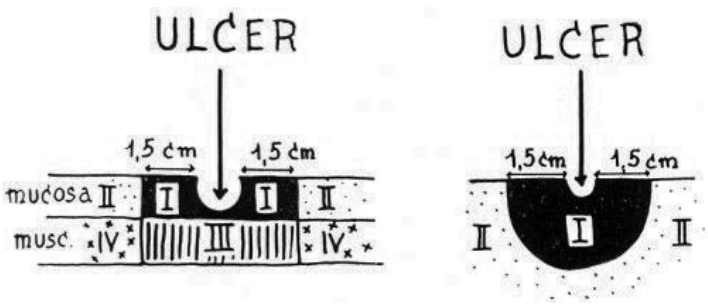


**Figure 299.** Schematic presentation of separation of gastric tissue surrounding a gastric ulcer localized on the lesser curvature of the stomach (same patients as in Figure 298). The left-hand side figure shows the transaction of the gastric wall; the right-hand side figure indicates the surface of the gastric mucosa. The gastric mucosa was separated from the muscular layer, and both layers were separated into two parts: gastric mucosa up to 1.5 cm around the gastric ulcer (I); gastric mucosa without ulcer (II); "callus" (III) i.e., the muscular layer under the gastric ulcer; and muscular layer (IV), i.e., the muscular layer under the gastric tissue without ulcer. These specimens of gastric tissues were used for the preparation of $Na^{+}$–$K^{+}$-dependent ATPase. [Mózsik, Nagy, Tárnok, Jávor (1971): In: Pfeiffer C.J. (Ed). Peptic Ulcer. Munksgaard, Copenhagen – Lippincott, Philadelphia. pp. 323–328 (with kind permission).]

The following main biochemical events are incorporated by these observations:

**a.** The increased membrane (transport) ATPase in the ulcerated antral, duodenal and jejunal mucosa produces an increased tissue level of ADP in these tissue samples;

**b.** The increased tissue levels of ATP in the ulcerated antral, duodenal and jejunal mucosa were compared with those in the non-ulcerated (control) mucosa tissue specimens;

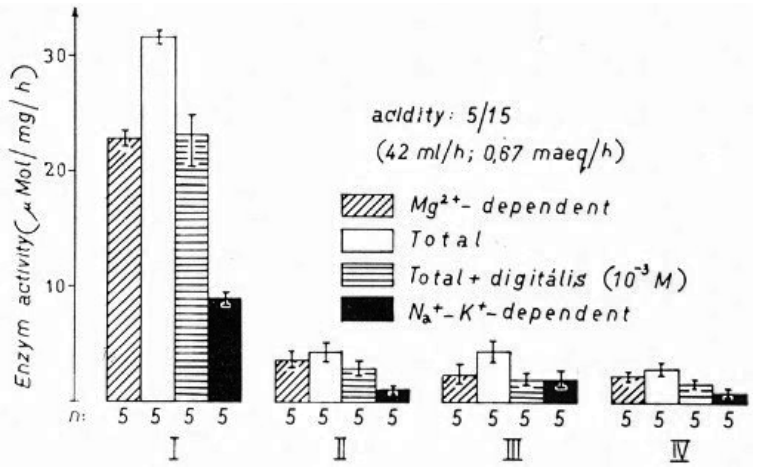


**Figure 300.** Alterations of the $Na^+$–$K^+$-dependent ATPase (transport) ATPase in the gastric tissues around the gastric ulcer in a patient. The ATPase activity was measured by the liberation of inorganic phosphorus and expressed in μMol/mg "dried membrane material"/hour. Secretory characteristics of the patient: volume of gastric secretion: 42 mL/hour; acidity, 0.67 mEq/hour or 5/15 clinical units, unstimulated; n, number of examinations (results presented as means ± SEM of five experiments). [Mozsik, Nagy, Tárnok, Jávor (1971): In: Pfeiffer C.J. (Ed). Peptic Ulcer. Munksgaard, Copenhagen – Lippincott, Philadelphia. pp. 323–328 (with kind permission).]

**c.** The existence of increased tissue levels of ATP with ADP and increased membrane (transport) ATPase activity gave a clear evidence for the increased (not impaired) oxidative phosphorylation in the ulcerated antral, duodenal and jejunal mucosa tissue specimens (excluding the presence of tissue hypoxia in the mucosal tissues around the antral, duodenal and jejunal ulcers in patients).

**6.** The GMBF increases with the increase in gastric acid secretory responses in human beings (Jávor, 1968).

The first molecular-pharmacological–biochemical examinations in gastric ulcer of patients with gastric ulcer was internationally demonstrated by us in 1970 at the 4th World Congress of Gastroenterology which was located in Copenhagen, Denmark (Mózsik et al.., 1971b). The forthcoming figures show these memory presentations (Figures 298–301). (Note: we did not mention – at that time – that the transformation of ATP into cAMP is probably also present in cases of higher molecular doses of atropine for the real explanation in the form of atropine-induced-inhibitory action curve on the $Na^+$–$K^+$–ATPase activity.)

### 11.4. Results of biochemical-pharmacological studies in animal experiments

The animal observations were carried out simultaneously with human observations. The rats were used for these observations (under the national law regulatory conditions). A majority of animal experiments were used as acute models, while the characteristics of peptic ulcer were used for chronic experimental models (in dogs dominantly) (see Table 45).

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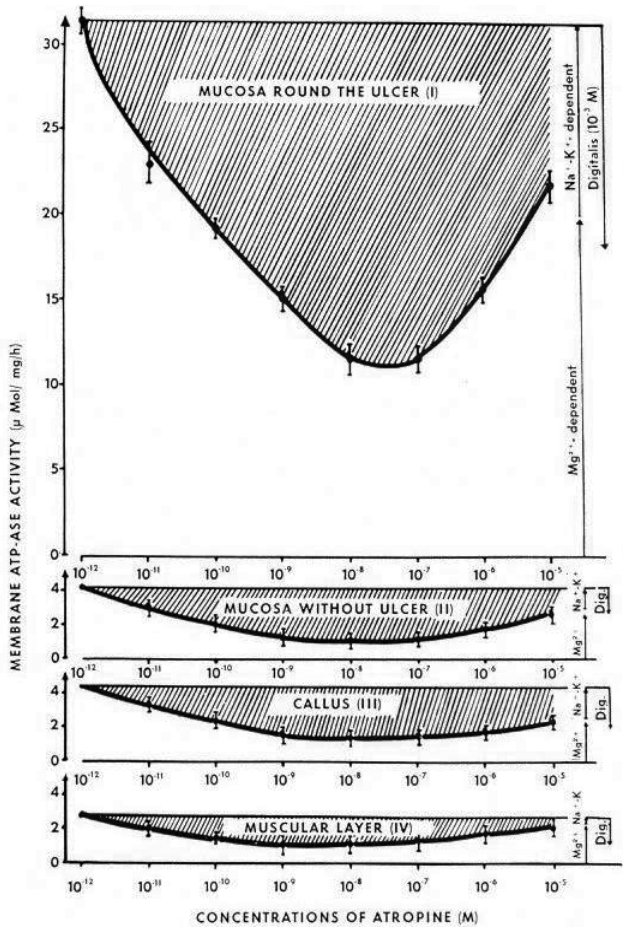


**Figure 301.** Inhibitory effect of atropine on $Na^+$–$K^+$-dependent ATPase activity prepared from different regions of gastric tissue around the gastric ulcer in the patient. The ordinate shows the ATPase activity by liberated, inorganic P, in μMol /mg dried "membrane material"/hour, atropine. Each point on the curves represents the means ± SEM of 5 experiments. The arrows on the right-hand side of the figure indicate the increased membrane ATPase by $Na^+$ (80 mM) and $K^+$ (33 mM) and the decreased activity of membrane ATPase by ouabain ($10^{-3}$ M). [Mózsik, Nagy, Tárnok, Jávor (1971): In: Pfeiffer C.J. (Ed). Peptic Ulcer. Munksgaard, Copenhagen – Lippincott, Philadelphia. pp. 323–328] (with kind permission).]

To elucidate the pathomechanism of human peptic ulcer, and to find effective drugs to treat patients, a series of animal models have been devised to mimic the natural history of ulcer disease. Unfortunately, the results contained – if not all – in these techniques do not process the main characteristics of human ulcer disease.

In spite of important differences, we do not have better direct methods for tackling the problems of ulcerogenesis than to use animal models. Generally, we use the expression of "experimental ulcer" for all sorts of experimentally induced gastric mucosal lesion irrespective of the true nature of the damage (erosion, acute and chronic ulcer). However, it is highly

| Main characteristics of Peptic ulcer disease | Experimental chronic gastric ulcers |
|---|---|
| - Appears unexpectedly | - No spontaneous gastric ulcer in animals |
| - May heal spontaneously | - Rapid healing after withdrawal of The ulcerogenic agent |
| - Reappear unexpectedly | - Do not reappear |
| - Gastric ulcre are solitary | - Mostly multiple |
| -Antral localization (mainly) | - Mainly in the fundus |
| - Role of Helicobacter pylori duodenal ulcers | - No proofs of Helicobacter pylori influence |

**Table 45.** Natural history of human ulcer disease and the experimental techniques, which do not process the main characteristics of the human disease.

important to differentiate between these three types of gastric lesions in both animal and man as their pathomechanisms are different. Erosions and acute ulcers differ from the chronic ones due to the underlying cause, which is mostly known. If properly treated, they disappear rapidly and do never become chronic. This does not mean that they represent harmless conditions; sometimes it might be difficult to treat them, and they may cause massive bleeding, perforation or even death.

The rat stomach is, however, quite different from that of the man, and the same is true for guinea pigs and other small rodents. The animals best suited for ulcer research are monkey, pigs and dogs. Even in these animals, there are some peculiarities that hamper the evaluation of the results, but with some precautions, the data obtained with some methods might be extrapolated to man. We had possibilities to do experimental observations only in rats, and consequently we were able to carry out acute observations. Biochemical pharmacological observations were carried out in rats under different experimental conditions.

Our attention was focused on evaluating the following:

1. The changes in the biochemical parameters of gastric tissues before the development of gastric mucosal lesions (produced by surgical intervention or administration of different drugs and chemicals) without and with administration of any protective drugs;

2. The study of interrelationships of the $Na^+$–$K^+$-dependent (transport) ATPase system (ATP transformation into ADP by membrane ATPase) and "second messenger system" (ATP transformation into cyclic 3′,5′-adenosine monophosphate by adenylate cyclase) in gastric tissues during the development of mucosal damage and its protection;

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3. The study of the role of vagus nerve of "surgical" and "chemical vagotomy" in mucosal protection against different drugs and chemicals;

4. The time-sequence study for the evaluation of oxygen free radicals and scavengers in the gastric tissues in acid-dependent and non-acid-dependent gastric ulcer models;

5. The presence (or absence) of tissue hypoxia in gastric tissues under different experimental conditions;

6. The comparative study for the actions of different drugs with different well-known subcellular mechanisms in gastric acid secretion, in the ethanol-induced model and prostacyclin-induced gastric mucosal protection in the ethanol-induced model.

The methodological problems (designs) and biochemical assays were detailed in some chapters.

We indentified very simple questions to clear these different scientific problems in the chapters, and we tried to give correct(s) answers to our previous questions in acute experiments. We are sure that we cannot give correct answers to the questions asked after chronic biological and pathological processes. We had to learn these methodological and scientific problems from human clinical pharmacology and human biochemical examinations of the resecated tissues of gastrointestinal tract (in patients who underwent the gastric surgery because of peptic ulcer disease). We tried to collect some important information from the ulcer research (including the results in animal experiments and human observations) in animal experiments.

Main discussion points of animal experiments are as follows:

1. The significant changes of membrane-bound ATP-dependent energy systems appear earlier in time in the rat gastric mucosa than those in the development of peak value of gastric acid hypersecretion of an ulcer development in 24-hour pylorus-ligated rats. The extent of ATP transformation into ADP by membrane ATPase increased, in association with decreased ATP transformation into cyclic AMP by adenylate cyclase in the gastric (fundic mucosa and forestomach) tissues in above-mentioned times of experiments (for further information, see Section 8.1.1).

When ouabain (as a specific drug to inhibit the $Na^+$–$K^+$–ATPase) was used in this experimental model, we found significant inhibition on both gastric acid secretion and ulcer development. Furthermore, these actions of ouabain also appeared in the same time as those in the untreated animals.

The results of these observations clearly proved the following:

a. The $Na^+$–$K^+$-dependent ATPase system (e.g., ATP transformation into ADP by membrane ATPase) plays a key role in the development of gastric acid hypersecretion and ulcer and in the inhibition (prevention) of gastric mucosal hypersecretion and ulcer production in 24-hour pylorus-ligated rats;

b. The gastric acid hypersecretion response by gastric fundic mucosa appears before the ulcer development of forestomach (rumen), and the inhibition of gastric acid secretion is

also associated with ulcer development in the forestomach (rumen) in 24-hour pylorus-ligated rats;

**c.** The biochemical changes in the gastric fundic mucosa and rumen – during the development of gastric hypersecretion together with ulcer development and prevention of gastric hyperacidity and ulcer development – indicated similar directions (different in their quantitative values) in both parts of the rat stomach.

**2.** The surgical vagotomy-induced decrease of gastric acid hypersecretion and the prevention of ulcer development are associated with decreased ATP transformation into ADP by $Na^+$–$K^+$-dependent ATPase in 24-hour pylorus-ligated rats.

These results suggest the following:

**a.** The breakdown of gastric mucosal ATP (both in directions of ATP transformation into ADP by membrane ATPase and of ATP transformation into cAMP by adenylate cyclase) is a basic biochemical background to the development of gastric acid hypersecretion and gastric ulceration and to their inhibitions by surgical vagotomy (for further information, see Section 8.1.2);

**b.** The measurement of tissue level of ATP (without measurements of tissue ADP, cAMP, AMP) does not give correct information on the approach of the actual levels of cellular energy systems (extents of dephosphorylation and oxidative phosphorylation, presence of tissue hypoxia);

**c.** The development of ulcer in the forestomach is only a hyperacid secretion process, independently from that of the ulcer location (in this experimental model), and is not related to human pathology of peptic ulcer disease in humans.

The gastric ulcer is located in the antrum of patients with peptic ulcer (which also has no acid secretory ability).

**d.** The results of the biochemical results (especially to energy supply systems of rat gastric fundic mucosa and forestomach) of 24-hour pylorus-ligated rats indicate a similarity to the biochemical background of the development of human antral ulcer, namely, the biochemical constituents (in terms of energy supply system, which significantly differs in the human gastric fundic mucosa vs. antrum in patients with gastric hyperacidity and normacidity). No antral ulcer can be found in the literature and in our practice (from 1960 to 2014) for the existence of antral location in patients with gastric hypoacidity. The results of biochemical examinations in rats and human gastric (fundic and antral mucosa tissues) give a very closed and acceptable biochemical explanation for antral ulcer development in patients as well as the forestomach of pylorus-ligated rats. These results explain that the gastric ulceration appears as a consequence of gastric hypersecretion (and not after *Helicobacter pylori* infection), which clinically appears after a significantly increased metabolic (energetic) adaptation in gastric tissues (antrum in humans and forestomach in rat). The level of metabolic adaptation in the energy supply systems is limited by the different biochemical structures (and their energetic background) of different parts of the stomach (in both humans and rats). Probably a similar (or the same) explanation is true

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for the biochemical explanation of the location of duodenal ulcer in patients (dominantly with hyperacidity) (see results presented in Sections 7.9.2 and 7.10) and jejunal ulcer after Billroth II gastric resection in patients.

3. Molecular pharmacological background of different drug actions in the gastric mucosa of intact gastric tissues under different pathological conditions.

The applications and uses of different drugs were widely used in the physiological, biochemical as well as in the pharmacological research. These methods have been applied to evaluate the characteristics of the effects of new compounds, or physiological events, by the modification in their effects. The atropine application is used to indicate the cholinergic influence and its participation in different physiological phenomena and development of new drugs.

The key points of these studies with "standard drugs" were to measure their actions at the level of final physiological (pathological) effects; however, we have no knowledge on the background of these drug actions. We studied the biochemical background of these "basic drugs" as well as the commonly used experimental standard pathological events (such as pylorus-ligated rats without the administration of any drugs or ethanol-induced gastric mucosa in rats). Our attention was focused on the changes in the membrane-bound ATP-dependent energy systems of the cells.

It was interesting to note that the *in vivo* observations of the interrelationships (extents) between the ATP transformation into ADP and the ATP transformation into cyclic AMP were changed from time to time (in hours in pylorus-ligated rats, in minutes in ethanol-induced model), and there were also absolutely contradictory changes (increased and decreased) between them. The results of these *in vivo* measurements were the same as those obtained by *in vitro* measurements due to the interrelationship of the cellular energy systems.

We had no knowledge on the cellular effects (on membrane-bound ATP-dependent energy systems) of atropine and epinephrine in the gastric mucosa. It was interesting to note that the biochemical background of atropine and epinephrine is very similar to each other; however, their molecular weights differ from each other.

The results of these biochemical pharmacological examinations offered a further explanation for the understanding of significantly different actions of the same drug after its administration during the actual functional (biochemical) state of the target organ (see Sections 8.12. and 8.13.).

4. The biochemical background of aspirin-induced gastric mucosal damage was studied in 4-hour pylorus-ligated rats. Aspirin (200 mg) was dissolved in 2 mL of 150 mmol HCl and intragastrically administered immediately after pylorus ligation. The ATP transformation into ADP significantly increased after pylorus ligation (without the administration of aspirin), while the extent of ATP-cAMP transformation decreased under this experimental condition. After aspirin administration, no metabolic adaptation both in ATP-ADP and in ATP-cAMP system, which can be explained by the direct damaging effect of non-ionized acetylsalicylic acid (as consequence of the presence of weak acid in presence of strong – gastric acid - , and it can to precipitate the membrane of cells), and by partly no

presence of any drug, which would be able to modify the membrane enzyme functions (Figure 90).

The administration of atropine (in doses of 0.1, 0.5 and 1.0 mg/kg) can stimulate ATP transformation into cAMP, which further inhibits the $Na^+$–$K^+$-dependent ATPase (besides the direct inhibitory effect of atropine on the $Na^+$–$K^+$-dependent ATPase). This active metabolic adaptation of the gastric mucosal tissue (increase in tissue level of cAMP) can produce a protective effect against aspirin (Figures 91–98). It is interesting to note that this metabolic adaptation is only dependent on the ATP–cAMP transformation (without the active participation of ATP–ADP transformation).

Vitamin A and β-carotene are important nutritional components in animals and humans; however, they have no antisecretory properties on the gastric acid secretion (Jávor et al., 1983; Mózsik et al., 1986). These compounds are also present in the aspirin-induced gastric mucosa damage (without any gastric acid inhibitory effects) (Figures 99–110 and Table 40). The biochemical background of these compounds is also similar to that of atropine, namely the ATP–cAMP transformation increased significantly by the application of vitamin A and β-carotene, which produce direct inhibitory action of $Na^+$–$K^+$-dependent ATPase (by the increased level of cAMP and AMP). Probably the actual level of ATP can reach to a critical level in the tissues, which can determine the ATP splitting processes by membrane-bound ATP-dependent enzymes.

The contradictory evaluation pathways of these results (obtained with aspirin administration in 4-hour pylorus-ligated rats alone and in combination with atropine, vitamin A and β-carotene) are given in the following:

**a.** The aspirin-induced gastric mucosal damage is a consequence of any positive metabolic adaptation in the cellular energy systems. The primary explanation given by Davenport (1965), namely the primary role of increased gastric $H^+$ back diffusion in the aspirin-induced mucosal damage, can be modified by the consequence of the absence of any active metabolic adaptation in the cellular energy systems (which is produced by non-ionized weak acetylsalicylic acid on the cell membranes);

**b.** If we can produce some active metabolic adaptation by membrane-bound ATPase-dependent energy systems, we can prevent the damaging effect of aspirin (independently on the presence or absence of gastric acid secretion).

**5.** The observation with indomethacin in different combinations offered further interesting results in the field of experimental ulcer research.

The indomethacin (20 mg/kg s.c. given) causes gastric mucosal damage in fundic mucosa in 4 hours time period. During this time period, the extent of ATP transformation into ADP increased significantly (together with the decreased extent of ATP transformation into cAMP) in the gastric fundic mucosa of rats (without the application of any protective agents).

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The atropine and cimetidine doses together decreased the ulcer development caused by indomethacin in 4 hours time period; however, the biochemical background of the preventive effects of atropine and cimetidine significantly differs in the rat gastric fundic mucosa.

Vitamin A also increases the IND-induced gastric mucosal damage, which is associated with the increased ATP–cAMP transformation. Similar types of observations were made with β-carotene in IND-induced model. With the biochemical background of these observations, we analyzed in detail the correlations between the changes in tissue levels of $PGE_2$ (its tissue level is inhibited by the application of indomethacin), cAMP, development of IND-induced mucosal damage and prevention of β-carotene. We have observed the following:

**a.** There was no direct correlation between the tissue levels of $PGE_2$ versus IND-induced mucosal damage and its prevention by β-carotene;

**b.** There was a very close correlation between the decreased tissue level of cAMP versus gastric mucosal damage produced by indomethacin (at 1 and 4 hours after indomethacin administration);

**c.** There was no significant difference between the decreased tissue levels of cAMP at 1 and 4 hours;

**d.** The indomethacin-induced decrease of tissue level of $PGE_2$ indicated a tendency to return to the normal level (in untreated animals) during the 4-hour experimental period;

**e.** The β-carotene-induced gastric mucosal protective effects are dose dependent and closely associated with the increase in tissue levels of cAMP (but not with changes in tissue levels of $PGE_2$);

**f.** The gastric mucosal protective effect of β-carotene differs from the prostaglandin system; however, it depends on the positive metabolic biochemical membrane-bound ATP-dependent energy systems.

**6.** The increased ATP transformation into cAMP (in association with the decreased ATP transformation into ADP) was observed during the development of stress-induced and reserpine-induced gastric mucosal damage in rats. It is also important to note that these changes in the membrane-bound ATP-dependent energy systems are detectable before the macroscopic appearance of gastric mucosal damage;

**7.** The observations with chemical (96% EtOH, 0.2 M NaOH, 0.6 M HCl and 25% NaCl solution)-induced models allowed us to conclude the following:

   **a.** The gastric mucosal damage by different chemicals in rats produced the same macroscopic features in the fundic part of the rat stomach, which appears at the same time after the application of necrotizing agents;

   **b.** There was no difference between the gastric mucosal features (and their characteristics in time and macroscopic pictures) produced by acid-dependent (HCl model) and non-acid-dependent (EtOH model) gastric ulcer models in rats;

**c.** The $ED_{50}$ doses of $PGI_2$ (5 μg/kg) and β-carotene (1 mg/kg) (which produce 50% prevention of gastric mucosal damage produced by intragastric administration of 0.6 M HCl and 96% ethanol) were identified in animal experiments. These doses and higher doses were used for studies to evaluate the correlations between the gastric mucosal damage, gastric mucosal biochemistry (ATP, ADP, AMP, cAMP, adenylate pool, "energy charge," ATP/ADP, lactate) and oxygen free radicals (catalase activity, glutathione peroxidase, superoxide dismutase, reduced glutathione and malondialdehyde) in the rat gastric fundic mucosa (see Figures 251–255). These observations were carried out at the same time and the same tissue samples were used for biochemical examinations. The results of these observations showed us the following facts:

- The gastric mucosal preventive effects of $PGI_2$ appear earlier (from 0 to 15 minutes), and β-carotene-induced gastric mucosal effects appear later (from 15 to 60 minutes) after the administration of necrotizing agents in both acid-dependent and non acid-dependent experimental models;
- The increased ATP–ADP transformations by membrane ATPase were obtained in the gastric fundic mucosal tissue (in association with the decreased extents of ATP–cAMP transformation) by both $PGI_2$ and β-carotene. These changes in the biochemical parameters of gastric fundic tissues were dose dependent and these appeared at the time of detectable characteristics of gastric mucosal preventions;
- The changes in the parameters of oxygen free radicals also were well detectable; however, the changes were not found to be dose-dependent actions.

We concluded the following from these observations:

**a.** There is a close and mathematically significant correlation between the protection of gastric mucosal damage by both $PGI_2$ and β-carotene and the changes in the biochemical parameters of membrane-bound ATP-dependent energy systems (with respect to extents in their changes and appearance in time). These correlations between the above-mentioned parameters were not dose-dependent and mathematically significant;

**b.** No differences were obtained in the appearance of gastric mucosal damage caused by acid-dependent (0.6 M HCl) and non acid-dependent (96% ethanol) mucosal damage, and their mucosal protective actions caused by a non scavenger ($PGI_2$) and a scavenger (β-carotene) have the same characteristics in these animal models;

**c.** The preventive actions of scavengers against tissue injuries involve significant changes in experimentally measurable parameters of oxygen free radicals; however, we suggest that the changes in the membrane-bound ATP-dependent energy systems have key roles in the development of tissue protective effects;

**8.** Selye (1936) emphasized the non-specificities of different stress reaching the living organs. The reactions of target organs give specific replies.

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We noted the results of the observations with 0.2 M NaOH, 25% NaCl, 96% ethanol and 0.6 M HCl (given orally), in the study of the development of mucosal damage, its biochemical background (Figures 181–183) and the development of prostacyclin-induced gastric mucosal damage (Figures 193–199). These results also clearly indicate its principal role and its action of stress theory established by Hans Selye (Szabo et al., 2012);

**9.** The epinephrine model offered a very special experimental opportunity to understand the possible action of one mediator (epinephrine) under different functional states of the target organ (target organ with different functional states). The epinephrine inhibited the development of gastric acid secretion and ulcer development in 24-hour pylorus-ligated rats; however, the epinephrine (given is in a suitable dose) at 4 hours later after pylorus ligation produces ulcer development. The biochemical background of the significant actions of epinephrine depend on the actual levels of membrane-bound energy systems, that is, the tissue level of ATP is normal immediately after pyloric ligation (therefore epinephrine produces a significant increase in the tissue level of cAMP); however, it was impossible at 4 hours after pylorus ligation when the tissue level of ATP was extremely low, and consequently the epinephrine application was not able to produce an increase in the tissue level of gastric fundic mucosal level of cAMP.

These results concluded the following:

**a.** The functional and regulatory steps of the feedback systems between the $Na^+$–$K^+$-dependent ATPase and adenylate cyclase systems work in a regulatory manner in the gastric tissues;

**b.** The regulatory mechanism, between the $Na^+$–$K^+$-dependent ATP and adenylate cyclase systems can be separated in some way under pathological conditions.

**10.** The results of animal experiments clearly indicated that the biochemical components differ significantly in the glandular stomach in comparison with the values measured in the forestomach (Mózsik et al., 1967 a, b, 1969 a, b, c, d; Mózsik et al., 1970). When we analyzed the time sequence and biochemical changes of the development of gastric hyperacidity and ulcer, in 24-hour pylorus-ligated rats, it was found that the gastric hyperacidity developed before ulcer, and the changes in the gastric mucosal biochemistry in both parts of the stomach appeared before the development of gastric hyperacidity(Mózsik and Vizi, 1976 a, b).

A significant biochemical gradient was biochemically proved in the gastric fundic, antral, duodenal and jejunal mucosa dependent on the gastric secretory responses (BAO, MAO) (Mózsik et al., 1976 a, b, 1979 a, b, c, e, f, g, h, 1981e).

The presence of tissue hypoxia was proved by Menguy et al. (1974 a, b, c) and Menguy and Master (1974) by hemorrhagic shock (Kitajima, 1989; Pihan et al., 1989). Pihan et al. (1989) explained the stasis of blood flow after the administration of chemicals. Under our experimental conditions, we could not prove the presence of tissue hypoxia (no elevation was obtained in the tissue levels of lactate, and the extent of phosphorylation was intact) (Mózsik

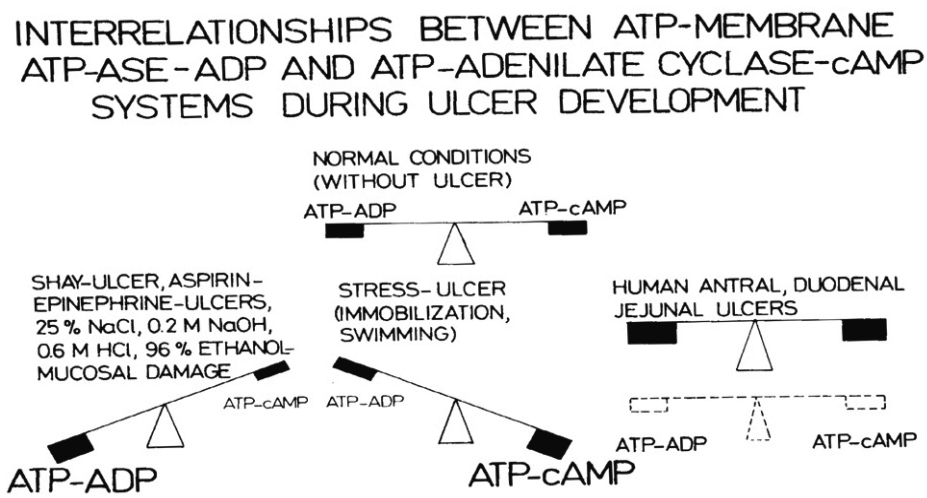


**Figure 302.** Changes in equilibrium between the ATP–ADP and ATP–cAMP transformations during the development of different experimental models (under significantly different experimental conditions). (For further details, see Chapters 7 and 8).

et al., 1971 a, b; Mózsik et al., 1979 b, f, h, 1981 e; Mózsik and Jávor, 1988e; Mózsik et al., 1990a, 1992c; Mózsik and Pfeiffer, 1992).

The application of biochemical–pharmacological methods in the peptic ulcer research offered a possibility to approach the developmental mechanisms (oxygen supply, biochemical changes, oxygen free radicals, vagal nerve, drug actions, scavenger actions, gastric mucosal secretory responses, ulcer development, drug-and chemical-induced GI mucosal changes) of aggressive and defensive factors in the GI mucosa. Our attention was dominantly focused on the actions of drugs acting at the mucosal level or efferent nerves. These studies analyzed the tissue reactions and not the programmed death of cells (apoptosis). The definition of tissue reactions differs from that of apoptosis.

Modern observations deal with the cellular mechanisms of apoptosis (Bódis et al., 1998; Pai et al., 2000, 2002; Szabó et al., 1996, 1997 a, b, 2000; Szabo, 2004; Tarnawski and Szabó, 2001).

In the future, we need to keep the integrity of living cells, tissues, animals and humans. We lost this integrity by receiving a great deal of detailed information. Our studies dealing with the membrane-bound ATP-dependent energy systems offered an excellent possibility to understand the integrity of these energy supply systems from the point of physiology, biochemistry, pharmacology and pathology.

**11.** Probably one of the most interesting results was obtained in surgical vagotomy when we observed that the $PGI_2$-induced gastric cytoprotective effect disappeared after bilateral surgical vagotomy (Jávor et al., 1981; Mózsik et al., 1982). The results of these observations first proved that the intact vagal nerve is necessary for gastric mucosal defense (not only for the gastric acid secretion). Similar results were published by Miller (1983). Unfortu-

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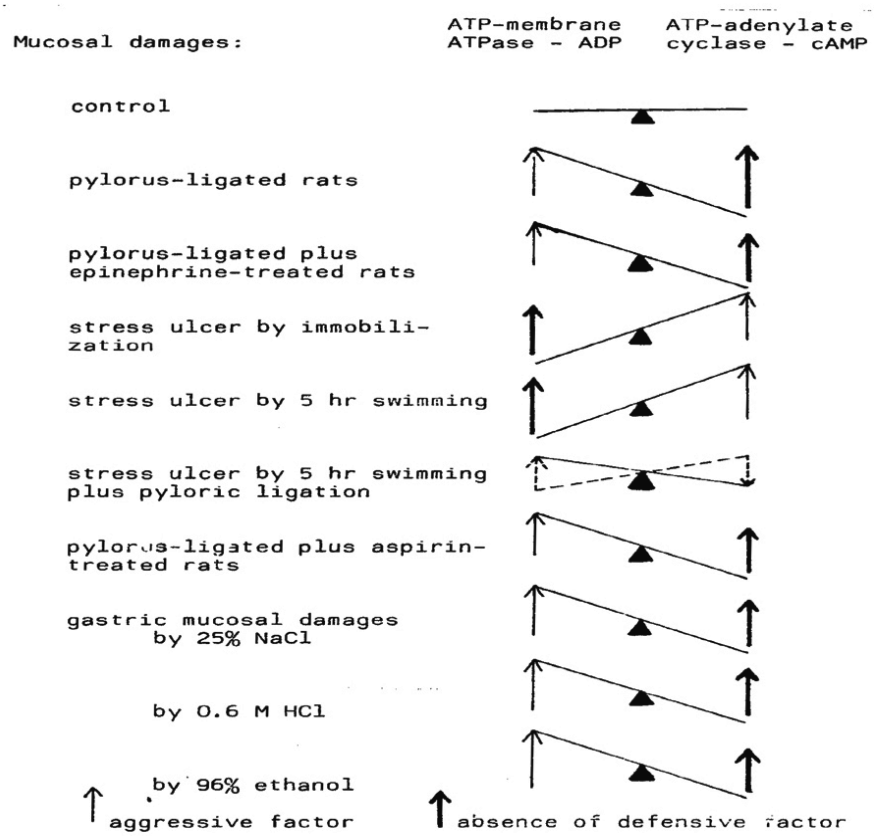


**Figure 303.** Correlations between the aggressive and defensive mechanisms during the development of gastric mucosal damage in different experimental models (a schematic summary of animal experiments). [Mózsik, Figler, Nagy, Patty, Jávor (1981). In: Mózsik Gy., Hänninen O., Jávor T. (Eds.) Advances in Physiological Sciences. Vol. 29. Gastrointestinal Defence Mechanisms. Pergamon Press, Oxford- Akadémiai Kiadó, Budapest. pp. 213–276 (with kind permission).]

nately, the necessary role of intact vagal nerve for the mucosal protection is known worldwide from the publication of Miller (we published this new observation earlier).

Later, Karádi and Mózsik (2000) studied surgical and chemical vagotomy (atropine treatment) in rats treated with indomethacin in detail, under different acute and chronic experimental conditions in the stomach, small intestine and large bowel. The indomethacin-induced mucosal damage (in the stomach and small intestine) enhanced after surgical vagotomy but not after "chemical vagotomy" (atropine treatment), and the small dose of atropine (cytoprotective effect) disappeared after surgical vagotomy.

Similar results were demonstrated with atropine, cimetidine, $PGI_2$ and β-carotene due to their failures in gastric mucosal protection after surgical vagotomy (see Section 8.17). The tissue levels of $PGE_2$ and $PGI_2$ (or its metabolite 6-keto-$PGF_{1\alpha}$) significantly decreased in the rat gastric mucosa after surgical vagotomy (see Section 8.17); however, we were of the opinion that these

facts (namely, decrease of $PGE_2$ and $PGI_2$) can only partially explain the disappearance of gastric mucosal protective effects of atropine, cimetidine, $PGE_2$, $PGI_2$ and β-carotene by surgical vagotomy (we emphasize better the roles of neural regulation of intact vagal nerve on cellular energy systems).

**12.** The actions of atropine, actinomycin D (inhibitor of RNA synthesis dependent on DNA), dinitrophenol (inhibitor of oxidative phosphorylation), epinephrine, histamine, mannomustine (Degranole$^R$) (inhibitor of *de novo* synthesis of DNA), pentagastrin, $PGI_2$, ouabain (specific inhibitor of $Na^+$–$K^+$-dependent ATPase enzyme) and tetracycline (inhibitor of protein translation) were studied and their dose–response curves were identified under three different experimental models, namely, in 4-hour pylorus-ligated rats (HCl secretion), ethanol-induced gastric mucosal damage and prostacyclin-induced gastric mucosal protection (see Section 8.14).

The affinity and intrinsic activity ($\alpha$) curves were calculated from the obtained results (the doses of these drugs are given in [−] molarities), and the values of $ED_{50}$, intrinsic activity and $pA_2$ were calculated. These molecular pharmacological results are able to approach the importance of different subcellular mechanisms involved in the development of gastric acid secretion, ethanol- and prostacyclin-induced gastric mucosal damage in rats.

The results (including the values of $ED_{50}$ and intrinsic activities and their $pA_2$ values) indicated practically the same results. It is tough to evaluate these results in understanding the cellular mechanisms of gastric mucosa under these experimental conditions.

**13.** The biochemical background of epinephrine ulcer (Section 8.15) clearly proved that the membrane-bound ATP-dependent energy systems (namely $Na^+$–$K^+$-dependent ATPase and adenylate cyclase) can be separated from each other depending on different functional states of target organs (Figures 263–267), which can give a schematic explanation for the gastric mucosal damage and prevention (Figures 302, 303). Similar conclusions can be drawn from the results of Figures 268–271.

**14.** The results of biochemical examinations in gastric mucosal tissues (in animals and humans) offered a general biochemical approach to the main biochemical cellular events (during the development and protection of mucosal damage). Changes in many mechanisms (at the levels of functions, cell membrane, mitochondrion, nucleic acids) are involved in these pathological and therapeutic processes of the mucosa of gastrointestinal tract.

There were and are many researchers who worked (or still working) on the study of gastrointestinal tract (pathologists, biochemists, physiologists, pharmacologists, internists, gastroenterologists, etc.) and they emphasized the importance of different fields from their research. An increased biochemical research has provided information on many cellular events in these target reactions, and we have to learn to evaluate the importance of these observations (dominantly done in animal experiments and isolated cells, cell lines).

The observation of the background of gastrointestinal mucosal damage and protection mechanisms shows that intact cellular energy systems are present in these processes.

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The results of our observations (done in humans and animals) show the key roles of membrane-bound ATP-dependent energy systems in different cell functions (under intact and different pathological conditions, without and with any drug treatments). These membrane-bound ATP-dependent energy systems also differ in the target organ under these investigational conditions.

We have described many observations in this book to understand different cellular reactions based on the key roles of membrane-bound ATP-dependent energy systems in both gastrointestinal mucosal damage and protection of patients and animals.

The chemicals can enhance or cause injury to the gastric mucosa (Figure 304). So a well-defined, regulated and dynamic system exists between the positive and negative metabolic adaptations to different (physical, chemical) stress. Furthermore, the GI mucosal injury can be healed (or some to extent prevented) by the positive and negative influences of the mediators, hormones and drugs (see Sections 5.7.1. and 5.7.2).

The mediator-, hormone- and drug-induced regulatory functions depend on the affinity and intrinsic activity curves of the membrane-bound ATP-dependent energy systems. The most important scientific information can be obtained by the comparison of values of $pD_2$ and $pA_2$ (when the doses are expressed in molarities).

Figures 303 and 304 demonstrate the presence of an active metabolic adaptation of gastric mucosa in rats and patients to increased gastric secretory responses and to chemical loadings. This metabolic adaptation is directed to ATP–ADP and to ATP–cAMP transformation in the cell membrane (with mitochondrial ATP). These facts suggest the existence of equilibrium between the two enzyme systems. This equilibrium is regulated by mediators, hormones, drugs (as first messengers) and different intracellular events. The different steps of these regulatory pathways were analyzed by the studies conducted on different acute animal models.

The PUD development is an acid- and biochemical (energetic)-dependent active metabolic adaptation; however, both membrane-bound ATP-dependent energy systems are involved in this active metabolic adaptation. These results suggest that the acid-dependent "genuine" ulcers appear as consequence of exhaustion of the metabolic adaptation (Mózsik et al., 1979 a, b, d, f, h, 1981a, 1982 a, b, 1987 a, b; Nagy et al., 1976, 1978, 1981 a, b) (Figure 73). The existence of the extremely high metabolic adaptation excludes the presence of tissue hypoxia.

### 11.5. Direct cellular damaging effects of ethanol, indomethacin and *Helicobacter pylori*

In this chapter, freshly isolated gastric mucosal cells were analyzed and the results were obtained. The effects of different mucosal damaging agents (such as *Helicobacter pylori* cultures, ethanol, indomethacin) were studied at the levels of cell membrane, mitochondrion and DNA, when these agents were administered individually and in combination).

This chapter concludes that the *Helicobacter pylori* alone does not produce any damage at the levels of cell membrane, mitochondrion and DNA, and it will not change by the administration of indomethacin.

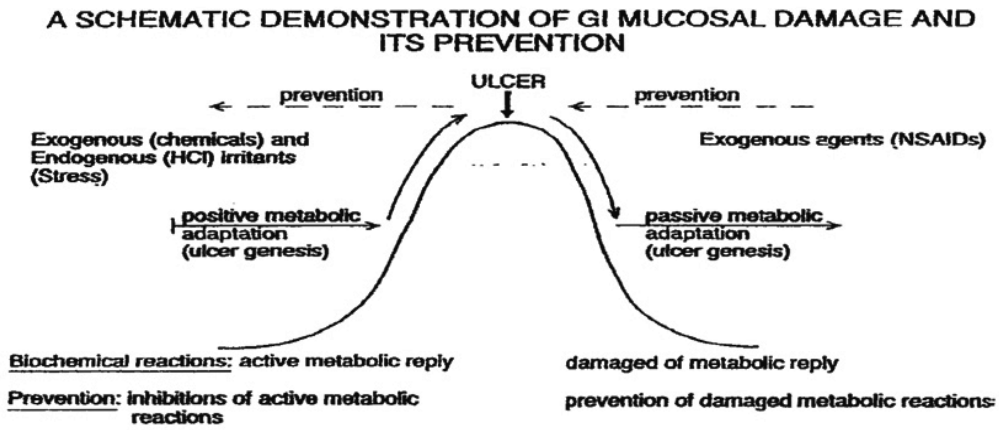


**Figure 304.** A schematic demonstration of gastrointestinal mucosal damage and prevention including human and experimental observations, summed from about 40000–50000 experiments. For further explanation, see Figure 303.

These results were surprising, because the presence of *Helicobacter pylori* in the gastric mucosa is the real cause for the development of peptic ulcer (gastric and duodenal ulcers) and chronic atrophic gastritis in patients.

The freshly isolated gastric mucosal cells are widely used to test the harmful and beneficial effects of different drugs (or drug candidates) in the experimental research of ulcer. It is well known that the rat (as experimental animal) is not the best experimental model to study and test the actions of *Helicobacter pylori* (see Chapter 9).

### 11.6. Observations with stable cell cultures

In earlier days, cell line cultures were widely available for research, because most of these cultures originated from human tissues. This fact suggests that the results obtained from these cell cultures are nearest to humans than those with rats (see Chapter 10).

The results of these observations were used to compare the results obtained from freshly isolated gastric mucosal cells and stable cell cultures. The mouse myeloma cell line is used as a "general cell line" in different fields of research, because this cell line has no secretory property.

The results obtained from these stable lines indicated different important notes to us:

1. The mechanisms of "cytoprotection" and "organoprotection" differ to some extent;
2. The freshly isolated gastric mucosal cells are more vulnerable than the stable cell culture lines against different chemicals (drugs, drug candidates);
3. The results obtained in the experiments with stable cell cultures can be accepted with a good criticism.

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### 11.7. General conclusions

It is very difficult to summarize the above-mentioned results (presented in the book). Furthermore, this study involves more serious work for clinicians than for people working just on experimental research. However, we have to mention a few points to explain this:

1. Patients with the same clinical diagnosis do not represent the same characteristics (such as their ages, body weights, life conditions, nutritional habits and states, genetics, etc.), whereas researchers have used the same animal strains (clearly identified their body weight, nutritional state, standard living conditions, genetic background, etc.);
2. The levels of clinical observations and animal experiments significantly differ from each other;
3. Clinical observations (exception of laboratory measurements from the blood samples) just can be done (owing many medical and ethical conditions and laws) in the before noon;
4. The conditions for carrying out clinical observations in human beings (patients) and animals cannot be compared to each other;
5. The clinicians know the borders of their knowledge in the everyday medical practice, but they practically are not able to carry out different research steps to solve these problems;
6. The clinicians do not have enough knowledge (from the modern biological research methods) and suitable conditions to carry out modern medical observations (exceptions are the clinical methods which depend on the modern diagnostic equipment, and these can be used for modern human research);
7. The fields of clinicians and researchers vary, and they have different conditions to do a well-planned research;
8. The clinicians have no correct information on the onset of diseases;
9. The fields of experimental research (in some meaning) are different from the problems of clinicians, and furthermore there are no collaborations between them;
10. Clinicians have consider important results of multiclinical, randomized, prospective, studies in their everyday medical practice (which somewhat depends on business), and there is no possibility to evaluate the background of clinical problems in patients' service;
11. General information (TV, radio, newspapers, Internet) on the so-called new observations are given by researchers (who are as far from the reality of clinicians and patients), and they are not participants in clinical realizations of experimentally discovered results (including the required time, economical support, etc.).

Earlier, it was an excellent practice for young physicians to learn more on the possibilities of methodologies (including new methods, general research laws, laws of presentations, etc.) when they stayed for years in research institutes before and after entering clinical practice. They learned much from the research institutes (e.g., the ways to approach different scientific problems). The postgraduate education offered an excellent possibility for them to recognize

the main problems of clinical practice (especially the details of medical treatments) and to plan the possibility pathways to scientifically approach them.

The authors worked as internists; however, they worked for 2–3 years in research institutes (in Europe and USA).

The following topics in the human and experimental research were:

**1.** We participated (as pioneers) in establishing the human clinical pharmacology in patients with peptic ulcer. This methodology offered us to do comparative clinical pharmacological studies of different drugs, to demonstrate the existence of drug tolerance (to the drug used chronically in the treatment of patients and to other chemically similar drugs that were not used in the treatment) together with the development of "pharmacological denervation phenomenon" in peptic ulcer patients – during a chronic drug therapy – however, these noted (proved) pharmacological observations disappeared in 6–10 days after cessation of drug therapy. These studies were carried out in the 1960–1970s, when we had the opportunity to use parasympatholytic drugs in the treatment of peptic ulcer.

Many observations were made later by us, which indicated that the surgical vagotomy was a harmful medical intervention in the treatment of patients with peptic ulcer. Many of our results clearly proved that the long-lasting effects of surgical and chemical vagotomy differ significantly in the gastric mucosa. It is important to note that "chemical vagotomy" can produce reversible pharmacological actions (development of tolerance to the medically used drug with "pharmacological denervation phenomenon") during the medical treatment of patients with peptic ulcer. The results of these observations proved clearly that the efficiency of the therapeutically used drugs decreased, and the complaints of patients increased during that time; however, these pharmacological effects can be reversed by the interruption of drug treatment (for 6–10 days). In other words, the sensitivity (efficiency) of the drug to the target organ can be tested clinically and pharmacologically by the administration of drugs intermittently in a long-lasting medical treatment of peptic ulcer patients. In contrast to the effects of chemical vagotomy, surgical vagotomy produces the final effects (including the compositions of gastric mucosal damage, the significant actions of their extra- and intracellular regulatory mechanisms, disappearance of gastric mucosal defensive actions by drugs and scavengers, changes in the tissue preventive effects of scavengers, etc.) on the target organs.

We applied the results of our observations in the chronic intermittent atropine treatment of patients with peptic ulcers, and the number of surgical innervations decreased significantly (from 160–170 patients to 2–5 patients in 1-year period in our surgical department). We followed up these patients for 10 years. Surgical intervention was not necessary in these patients as there was no complication.

It is true that we were away from the scientific problems of chronic atropine treatment in peptic ulcer patients; however, we learned the general problems of medical treatment with different modern drugs.

**2.** We noted the theoretical contradiction between the effects of therapeutically applied drugs (all of them inhibited the active metabolic adaptation of gastric tissues, while the

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ulcer disease appeared as a result of hypoxemic mucosal damage) and their desired actions, but we were not able to solve this significant contradiction in peptic ulcer treatment.

These problems were not studied in detail by the actually and traditionally known pathways in the 1970s. We had no concrete knowledge on the biochemical background of the development of gastric mucosal damage and prevention in both animal experiments and patients. This was the reason why we tried to start with the biochemical examination of gastric mucosal tissues.

The preliminary results of our biochemical observations in the gastric mucosa clearly indicated that significant changes can be found in the biochemical components of cell membrane, mitochondrion, proteins and nucleic acids.

It was observed that the changes in the biochemical composition of peripheral parts of cells (cell membrane, mitochondrion) are much more higher than those in proteins and nucleic acids.

In the early period of our biochemical observation, there was no internationally accepted methodology to approach the changes in the mitochondrial functions in detail (energy-dependent processes).

3. The preparations of $Na^+$–$K^+$-dependent (transport) ATPase and adenylate cyclase were carried out in the rat and human gastric mucosa. We noted that the functions of these enzymes can be regulated by mediators, hormones and drugs. Furthermore, the ATP is a common substrate for both the enzymes in the presence of $Mg^{2+}$. A very complex intra- and extracellular feedback mechanism system was proved to exist between the $Na^+$–$K^+$-dependent ATPase adenylate cyclase system under normal (physiological) and different pathological conditions. The significant changes in the equilibrium of regulation of the feedback system ($Na^+$–$K^+$-dependent ATPase and adenylate cyclase) are deeply involved in both development and prevention of gastrointestinal mucosal damage in animals and patients.

4. The results of measurements of tissue levels of lactate and the calculation of "energy charge" [(ATP+05 ADP)/(ATP+ADP+AMP)], together with simultaneous measurements of ATP, ADP, cAMP and AMP, are able to exclude the presence of impaired oxidative phosphorylation in the gastrointestinal mucosal tissues in both animal experiments and patients with peptic ulcer.

5. The cellular mechanisms of surgical and "chemical" vagotomy significantly differ in the rat gastrointestinal tract. The intact vagal nerve is basically necessary for gastric mucosal protection produced by $PGE_2$, prostacyclin, scavengers (vitamin A, β-carotene) and drugs (atropine, cimetidine).

6. *Helicobacter pylori* alone and in combination with indomethacin cannot cause any damage at the levels of cell membrane, mitochondrion and DNA on freshly isolated gastric mucosal cells.

7. A cellular biochemical explanation was given to the development and prevention of gastrointestinal mucosal damage in different animal models and humans.

### 11.8. Present and future observations

In the last decade, we summarized and reviewed our results obtained from our animal experiments and human observations for some actual scientific problems in different fields of biochemical pharmacological results (Mózsik, 2006), oxygen free radicals, antioxidants (Szabo et al., 2014) and role of *Helicobacter pylori* in the development of peptic ulcer disease in patients (Mózsik et al., 2014).

The actions (effects) of capsaicin on the gastrointestinal tract of animals since 1980 (Mózsik et al., 1997) and healthy human subjects and patients with different gastrointestinal disorders since 1997 were studied, with permission from the Hungarian Pharmaceutical Institute and Regional Ethical Committee of Pécs University (Hungary) since 1997 (Mózsik et al., 2009). These results clearly proved that the capsaicin (given in small doses) protects the development of gastrointestinal mucosal damage via the capsaicin-sensitive afferent nerves. These studies were conducted in collaboration with different institutes (Department of Pharmacology and Pharmacotherapy, Institute of Pharmaceutical Chemistry, Pécs University, Hungary) and therapeutic departments (Department of Gastroenterology, Petz Aladár Teaching Hospital, Győr, Department of Gastroenterology, Markusovszky Teaching Hospital, Szombathely, Hungary).

We observed the animal, preclinical and human examinations with capsaicin for innovative pharmacological and pharmaceutical research (since 2005).

We analyzed in detail the actions of different doses of capsaicin in animal experiments and stimulatory (small) doses on capsaicin-sensitive afferent nerves in healthy human beings and patients with different gastrointestinal disorders, principal problems of capsaicin chronic toxicology, preclinical dossier, basic problems of clinical pharmacology of capsaicin (tolerability, presence or absence of desensitization of afferent nerve to capsaicin during a chronic treatment), international (and national) permission of therapeutically (orally) applicable natural capsaicin preparation, classical clinical pharmacological phases I and II in order to produce new orally applicable drugs or combination of drugs in patients. These results were summarized and published recently (Szabo et al., 2012; Mózsik, 2014, Mózsik et al., 2014 a, b, c). Capsaicin-containing drugs are used alone or in different combinations in healthy human beings and patients with different gastrointestinal disorders.

## 12. Epilogue

The authors of this monograph are internists, who have been involved in various researches (physiological, pharmacological, molecular biological), from Hungary and other foreign countries (Norway, USA) either before or after becoming internists, gastroenterologists or clinical pharmacologists.

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The authors have focused on various aspects (including symptomatology, suggested etiologies, pharmacological and dietetical treatments, endoscopy and follow-up of patients for a long time period) of peptic ulcer disease, since the 1960s. Our knowledge, of course, has changed significantly from time to time in the above-mentioned fields. We joined the clinicians with some research questions (problems) in the field of peptic ulcer disease.

The clinical research study was conducted dominantly by surgeons in the first part of the last century. The possibility of surgical treatment of patients with peptic ulcer was emphasized dominantly by Germanian surgeons in the last part of the 19th century.

The use of different experimental (animal) models has internationally appeared to understand and approach the various problems (such as etiology, development mechanisms at different levels, prevention or treatment) of human peptic ulcer disease in the second half of the 20th century.

Professor Carl. C. Pfeiffer (Philadelphia, USA) was the pioneer researcher who organized an international workshop on peptic ulcer (in connection with the 4th World Congress of Gastroenterology) in 1970 at Copenhagen, Denmark. This workshop offered the "last opportunity" for the personal meeting of world famous (though older) researchers with the representative members of young generation. This workshop was named later as the "First International Conference on Experimental Ulcer" (Pfeiffer, 1971).

Following this conference, an international series of conferences on experimental ulcers has been established [Cologne (Germany), 1972; Parádfürdő (Hungary), 1976; Tokyo (Japan), 1980; Boston (USA), 1985; Jerusalem (Israel), 1988; Berlin (Germany), 1991; Kyoto (Japan), 1994; Hong Kong (China), 1997; Budapest- Pécs (Hungary), 2000; Dubrovnik (Croatia), 2003; Osaka (Japan), 2006; Split (Croatia), 2009; Tokyo (Japan), 2012 and the forthcoming conference will be located at Ottawa, Canada (2015)].

The Standing Committee of International Conference on Experimental Ulcer was officially established in 1976 [(C.J. Pfeiffer (Blackburg (USA) general secretary; members are T. Gheoghiu (Cologne, Germany), Gy. Mozsik (Pécs, Hungary), A. Robert (Kalamazoo, USA) and S. Umehara (Tokyo, Japan)]. One of the authors (Gy. Mozsik) was a member of the Standing Committee up to 2000, and he was general secretary of the Standing Committee from 2000 to 2009) (for details, see Mózsik 2006)].

Other series of international conferences (symposia) were established in connection with International Union of Pharmacology (currently with the name "International Union of Pharmacology, Basic and Clinical Pharmacology"). The Gastrointestinal Section of International Union of Pharmacology (GI Section of IUPHAR) was officially established in 1994 at Montreal, Canada. Before that, different satellite symposia were organized in connection with World Congress of Pharmacology (International Union of Pharmacology) in 1984 in London (UK), in 1990, in Amsterdam (the Netherlands) and in Pécs (Hungary) (1984, 1990). After the official establishment of GI Section of IUPHAR, special symposia dealing with GI pharmacology were incorporated in the program of main congresses [Munich (Germany) 1998, San Francisco (USA) 2002, Beijing (China) 2006, Copenhagen (Denmark) 2010, Cape Town (South African Republic) 2014; different satellite symposia were also organized in these main world

congresses of pharmacology [Pécs (Hungary) 1998; Honolulu, Hawaii (USA); Osaka (Japan) 2006]. Some other symposia of GI Section of IUPHAR were also organized in different parts of the world [Pécs (Hungary), 1995; Sperlonga (Italy), 1996; Kyoto (Japan) 2004; Honolulu, Hawaii (USA) 2012; Zagreb (Croatia), 2013; Budapest (Hungary), 2014].

One of the authors (Gy. Mozsik) was one of the establisher experts, member of different committees, the President (2002–2026) of the GI Section of IUPHAR and, of course, he organized several symposia in Hungary and other countries (see for details, Mozsik, Szabo, Takeuchi, 2006).

During the last four decades, we participated in establishing the processes of different international congresses:

1. Cell/Tissue Injury and Cytoprotection/Organoprotection in the Gastrointestinal Tract;
2. World Congress on Inflammation, Antirheumatics, Analgetics, Immunomodulators [Venice (Italy) 1984; Monte Carlo (Prinicipality of Monaco) 1986 and 1989; Geneva (Switzerland) 1995];
3. International Symposia of International Brain-Gut Society (Lake Arrowhead (Los Angeles) (USA) 1988; Pécs (Hungary) 1990; Florence (Italy) 1993; Pécs (Hungary) 1996)] (two symposia of this series were organized by Hungarian experts);
4. The First International Symposium on Gastrointestinal Cytoprotection was organized (established a new international series in this field) by Gy. Mozsik at Pécs (Hungary) in 1983, thereafter in 1987, 1991, 1995 and 2000;
5. World Congress of International Union of Physiological Sciences (Budapest, Hungary, 1980).

Summarizing our participation in the international flow of sciences, it can be interpreted that we tried to build up a functional bridge between the members working in Western countries and in Eastern (Central) European countries in the time period 1970–1995. This work was very difficult, but we believed that our work would result in a successful interaction between Western countries and Eastern (central) European countries in the field of scientific organizations and hopefully in their scientific activities. During that time, one of the authors (Gy. Mozsik) was the head of the First Department of Medicine, University of Pécs, Hungary (from 1993 to 2003), and his pupils spent approximately 51 years studying in foreign laboratories. We are proud that all colleagues who worked in Western countries returned to Hungary after finishing their research/study period.

Finally, we would like to summarize our scientific activities in the field of gastrointestinal research (focused mostly on peptic ulcer disease). We present this retrograde review of five decades starting from the 1960s; however, different observations and research were done in different clinical, instrumental conditions and research conditions. Consequently, we had to consider the actual time, when we made those observations and to give these critical (but, in some meaning, subjective) evaluations on our scientific activities.

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### 12.1. Clinical pharmacology of parasympatholytic drugs in patients with peptic ulcer

In the 1960s, the efficiency of medical treatment of patients with peptic ulcer was analyzed. The physicians had no objective methodology for the critical evaluation of efficiency of medical treatment of patients with peptic ulcer hade success of medical treatments ofpatients with peptic ulcer wereapproached only by the "subjective" signs of patients (to follow the changes in patients' complaints, body weight, appetite, etc.). These observations were given by the retrospective notes of the above- mentiond parameters.

The treatment of patients with peptic ulcer was done partly by internists (the so-called conservative treatment) and by surgeons. The indications for gastric surgery were dominantly based on the failure of medical treatments, which was one of the most important standpoints to us, the reason why we wanted to know more (in detail) about medical (pharmaceutical) treatments.

One of the most important questions was how to obtain objective data on the gastrointestinal absorptions of various drugs. Only the parasympatholytic drugs (atropine, scopolamine and some other tertiary and quaternary ammonium compounds) were used in clinical practice to decrease the suggested gastric acid hypersecretion.

No chemical methodology was found in clinical practice for the determination of parasympatholytics from different biological samples (serum, urine, bile) of treated patients. The biological methodology was used in our studies for the determination and measurements of different parasympatholytic drugs (in different biological samples) in patients treated with orally and parenterally applied doses of various drugs (see Chapter 2.).

The parasympatholytics (as water-soluble compounds) are not linked to albumin in the serum of patients; consequently, these compounds are excreted in the urine (after some metabolic processes) at the time of administration during the medical treatment. The liver (as the main metabolic organ) was suggested for the metabolization of drugs; so the results of this metabolic process could be followed by the detection of these drugs in the bile.

When the same dose of different parasympatholytics was applied orally and parenterally, we were able to identify the ratio of oral/parenteral dose of the drugs in the same patients. These results offered to control the suitable laws on the applicability of the "experimental pharmacology" in human medical treatment (no similar observations were carried out before in patients).

After careful systematic results from parasympatholytics in patients with peptic ulcer offered us to establish a "complete methodology for clinical pharmacology of parasympatholytics" in peptic ulcer patients (in 1960–1970), we received the possibilities for the identification of the following clinical pharmacological parameters:

**a.** Absorption from the gastrointestinal tract;

**b.** Ratio of oral/parenteral rate of different drugs;

**c.** Linkage of drugs to albumin;

**d.** Time course of drug actions by their urinary excretion;

**e.** Changes in the serum levels of different drugs during administration of different drugs.

These observations clearly indicated the following:

**1.** Different parasympatholytic drugs (dominantly quaternary ammonium compounds) are not absorbed from the gastrointestinal tract in patients with peptic ulcer;

**2.** The challenge of pharmacologists (namely to discover more new quaternary ammonium compounds to obtain longer blocking effects on the peripheral nervous system under experimental conditions) would help in clinical medicine (from the viewpoint of clinical pharmacology);

**3.** The failure of medical treatment (by quaternary ammonium compounds) does not give a real indication of gastric surgery (since these compounds are not absorbed from the human gastrointestinal tract).

### 12.2. Problems of chronic parasympatholytic drug in patients with peptic ulcer: Development of tolerance to drugs and together with appearance of "pharmacological denervation phenomenon" in patients with peptic ulcer

The decrease of gastric acid secretion was the main goal of medical treatment in parasympatholytics (in the 1960s) during a chronic (in about 4 weeks) treatment.

Systematic clinical observations were made in patients with chronic duodenal ulcer during chronic atropine treatment (4 weeks), when the gastric acid secretory responses (basal and secretory answer to superluminal but submaximal dose of histamine) and the ulcer healing (controlled by X-ray examination) were studied before and after the medical treatment.

It was surprising that the ulcers healed in patients with chronic duodenal ulcer, while no changes (no decreases) were found in the gastric acid secretion before and after a chronic atropine treatment (of course, without acute administration of atropine) (Mózsik et al., 1965). We could not interpret these results at the time of completion of these observations, since the "key role of gastric hypersecretion" was not present in these patients in order to heal duodenal ulcer in patients. These results are absolutely contradictory to the internationally suggested etiological standpoint of ulcer development (and healing) in patients with duodenal ulcer.

The theory of "cytoprotection" (e.g., existence of the gastric mucosal protection without any inhibitory action on the gastric acid secretion) was discovered and named by André Robert (1979), based on animal observations. Consequently, the existence of cytoprotection ("ulcer healing action without any decrease in gastric secretion") was proved in patients with chronic duodenal ulcer in 1965.

The inhibitory actions of the same doses of atropine were measured (from the viewpoint of clinical pharmacology) in these patients with duodenal ulcer before and after a chronic atropine treatment. Atropine was intramuscularly given (so the changes in the absorption were excluded). The magnitudes of the inhibitory effect of parenterally applied atropine significantly decreased the gastric basal and stimulated (given superluminal, but submaximal dose – 0.5 mg – histamine s.c.) dose after chronic atropine treatment.

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Thereafter, as atropine was orally given (before and after a chronic atropine), its levels were measured in the sera, bile and urine in patients who had no changes in these parameters. We concluded from these observations that no changes are present in the absorption, metabolism and excretion of atropine (in case of oral application) in the treated patients, during a chronic atropine treatment.

The vagal nerve is partially involved in the innervation of human parotid gland. The extents of salivary secretion of parotid gland were studies, using a coaxial capsule, during an acute administration of atropine (given orally or parenterally), and the inhibitory time indicated the same time as the excretion of drug from patients (when the titration of atropine in biological samples was parallel with the measurement of salivary excretion). When we injected patients with different agents (such as acetylcholine, epinephrine and histamine) before and after a chronic atropine treatment, the excretion of the basic salivary secretion significantly increased to acetylcholine (but not to epinephrine and histamine). These results focused our attention on the development of "pharmacological denervation supersensitivity" during a chronic atropine treatment.

In other words, "tolerance to atropine" and "pharmacological denervation supersensitivity" to acetylcholine appeared in patients with duodenal ulcer during the chronic atropine treatment. These phenomena (namely the development of tolerance to atropine and "pharmacological denervation supersensitivity" to acetylcholine) existed only together (for details, see Chapter 2). It was important from a clinical point of view that both the tolerance to atropine and "pharmacological denervation supersensitivity" and the tolerance to acetylcholine disappeared in 6–10 days after cessation of atropine treatment. We were the first researchers to demonstrate the existence of the development of tolerance to atropine and the "pharmacological denervation supersensitivity" to acetylcholine during a chronic parasympatholytic treatment in patients with peptic ulcer. In consequence of our observation, we applied only the intermittent atropine treatment in the medical treatment in patients with peptic ulcer.

### 12.3. Comparative clinical pharmacology in patients with peptic ulcer

The methodology of clinical pharmacology offered us to carry out randomized, prospective, multicentric and comparative clinical pharmacological studies with different therapeutically applied drugs (as an actual clinical examination) in patients with chronic gastric and duodenal ulcer (for details see Chapter 2). These studies were carried out with the permission of Regional Ethical Committee of Pécs University, Hungary. Different objectives, laboratories and subjective parameters were noted during the treatment of patients with peptic ulcer. The changes in the ulcer sizes were also calculated in the examined patients (before 2 and 4 weeks of treatments).

The most important note was from these observations that vitamin A produced gastric ulcer healing effect that was not less than those produced by other antisecretory drugs.

Earlier we suggested these observations that the antisecretory effect of drugs played the key role in medical treatment; however, vitamin A is a classical scavenger biological molecule

(without any antisecretory properties). Consequently, the phenomenon of gastric cytoprotection exists in patients with chronic gastric ulcer.

### 12.4. General biochemical examinations in the rat stomach under different experimental conditions

General biochemical examinations of the gastric mucosa (and other parts) were done in animals with different experimental conditions (effects of surgical and chemical vagotomy in acute experiments and in chronic observations) (for details, see Sections 4.2 and 4.3). These results clearly indicated that the "surgical" and chemical" vagotomy produced significantly different biochemical changes in the tissues of rat stomach. The key roles of cellular energy systems have also been suggested from these examinations.

### 12.5. Membrane-bound ATP-dependent energy systems in the gastrointestinal mucosa in animals and humans

These studies opened an absolutely new avenue for us to study the biochemical mechanisms and their regulations by mediators, hormones and drugs (*in vitro* and *in vivo* observations) (for details, see Chapter 6).

The classical $Na^+$–$K^+$-dependent ATPase and adenylate cyclase enzymes were first prepared from rat and human gastric mucosa by us. The examination of these enzymes gave an excellent possibility to study critically the changes of the membrane-bound ATP-dependent energy systems in the gastric mucosal tissues.

These types of observations are especially interesting (and important), because the ATP is a common substrate molecule for both $Na^+$–$K^+$-dependent ATPase and adenylate cyclase in the presence of $Mg^{2+}$. The actual level of ATP is also a limiting factor for the functions of $Na^+$–$K^+$-dependent ATPase. The ATP level decreases by the functions of membrane-bound enzyme systems; however, ATP resynthesis can be obtained only under intact oxidative phosphorylation. The measurements of tissue ATP (*in vivo* observations) performed alone do not give a correct argument to prove or to exclude the presence of tissue hypoxia. This question is a basic problem in the ulcer development (under experimental conditions of patients).

The results of these observations proved the existence of a very complex regulatory (feedback) mechanism system between the $Na^+$–$K^+$-dependent ATPase and adenylate cyclase in *in vitro* and *in vivo* conditions by mediators, hormones, drugs and by the actual level of tissue ATP in the gastric mucosa. Furthermore, the function of $Na^+$–$K^+$-dependent ATPase can be modified by smaller concentrations of these compounds, in comparison with those of adenylate cyclase. It is also interesting and important to note that the regulatory actions of these regulatory compounds produce contradictory directions (stimulation vs. inhibition or inhibition vs. stimulation) on the $Na^+$–$K^+$-dependent ATPase and adenylate cyclase from gastric mucosal tissues. We have to emphasize the extents of energy liberation by the transformations of ATP into ADP, and ATP and cAMP also differ from each other in terms of energy liberation.

The ATP–ADP transformation by $Na^+$–$K^+$-dependent ATPase represents the first-line metabolic adaptation of the gastric mucosal cells to different influences (drugs, hormones, media-

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tors), while the ATP–cAMP transformation by adenylate cyclase indicates the secondary metabolic adaptation of the cells in the gastric mucosa. The term "second messenger system" has been defined by Sutherland, who received the Nobel Prize in Physiology (Medicine) in 1971 for the discovery of ATP–cAMP transformation by adenylate cyclase in liver slides.

### 12.6. Biochemical measurements in the gastrointestinal mucosa of patients with peptic ulcer who underwent gastric surgery for ulcer disease

The gastric partial resection (dominantly according to Billroth II intervention) was frequently applied in the treatment of patients with peptic ulcer, because of the failure in "medical" (pharmacological, dietetic) treatment carried out by internists.

We never emphasized the importance of surgical treatment of peptic ulcer because we observed many early (dumping syndrome, diarrhea, etc.) and late (malabsorption syndrome, osteoporosis, stump cancer) complications of gastric surgery. These medical problems led us to evaluate the reason(s) why the "medical treatment" failed in these (1960s) years. These facts stimulated us to start with the critical analysis of the problems of pharmacological treatment in patients with peptic ulcer, and the methodology of clinical pharmacology appeared in consequence of these facts. The number of patients with peptic ulcer was 160–170/year who underwent surgical intervention at the First Department of Surgery, Pécs University, Hungary; however, after our clinical pharmacological results were introduced into everyday medical treatment, the number of surgically treated patients with peptic ulcer decreased to 2–3 patients per year in a very short time.

The necessity of gastric surgery was not indicated by internists, but by surgeons. Our biochemical observations were carried out on the resecates of human gastrointestinal tract obtained during surgical intervention.

The gastrointestinal resecates (obtained after surgical intervention) were biochemically studied (for details, see Chapter 7). Our biochemical examinations (using the internationally accepted methodology during the first half of 1970–1975) were expressed in relation to 1.0 mg DNA (representing the same number of cells).

The $Na^+$–$K^+$-dependent ATPase differs from the $H^+$–$K^+$–ATPase (for details, see Chapter 5); however, about 50–60% of $Na^+$–$K^+$–ATPase is incorporated into $H^+$–$K^+$–ATPase. The $H^+$–$K^+$–ATPase is located only in the parietal cells of stomach, while the $Na^+$–$K^+$-dependent ATPase can be found in all types of cells. Interestingly, the ATP is a common substrate molecule for both enzymes. (Note: We have to emphasize that we had no possibility to measure the cAMP directly from the surgically obtained resecates of the GI tract of patients with peptic ulcer at the time of gastric surgery.)

The gastric basal (BAO) and maximal (MAO) secretory responses were used as objective parameters for the stomach (these observations were carried out before the surgical intervention).

The biochemical results obtained from the gastric fundic mucosa and ulcerated antral, duodenal and jejunal mucosa must be observed.

These results were very surprising, since they were the same (note that the results were calculated as 1.0 mg DNA). The levels of ATP and ADP were higher in the gastric fundic mucosa with increased gastric acid secretory responses (together with the increased activity of $Na^+$–$K^+$-dependent ATPase). The same results were obtained in the ulcerated antral, duodenal and jejunal mucosa (1.5–2.0 cm around to ulcer), while these parameters were significantly lower (in the same patients) in the non-ulcerated (control) antral, duodenal and jejunal mucosa. The increased level of tissue ATP can be obtained by the increased oxidative phosphorylation, while the increased splitting (turnover) of ATP into ADP can be found by the increased $Na^+$–$K^+$-dependent ATPase in these tissue specimens. These results gave clear evidence for the exclusion of the presence of tissue hypoxia (impaired oxidative phosphorylation) in the ulcerated antral, duodenal and jejunal mucosa in patients with antral, duodenal and jejunal ulcers.

If we carefully observe the biochemical structures of corpus (fundus), antrum, duodenum in patients with peptic ulcer, we can notice that different energetic and biochemical gradients are present between fundic, antral and duodenal mucosa in these patients.

After a very careful analysis of these results, we can suggest that the clinically detectable appearance of antral, duodenal and antral ulcer is a consequence of a very active (increased) metabolic process (and not of decreased tissue metabolism). The possibility of adaptation to increased metabolic processes is determined by the original biochemical and energetic structures of fundus, antrum, duodenum and jejunum in patients with peptic ulcer. These structures, of course, depend on the general activity (such as gastric secretory parameters) of the stomach in humans (patients with peptic ulcer).

There are some problems with the critical evaluation of the presence of tissue hypoxia:

**a.** The decrease in gastric mucosal blood flow measured by different physiological methods does not prove the presence of tissue hypoxia in the ulcerated tissues in patients with peptic ulcer;

**b.** No clear and easily used methods were available in the case of humans (especially to measure the gastric mucosal blood flow (GMBF) in patients with peptic ulcer in time just before the development of ulcer);

**c.** The increased level of tissue level of lactate is one of the two main arguments to prove the existence of tissue hypoxia; the second one is the decreased tissue level of ATP due to impaired oxidative phosphorylation.

### 12.7. Messages of biochemical examinations in different ulcer models

Many animal observations were carried out to understand the changes in the gastric mucosa with and without mucosal damage and ulcer. A wide scale of biochemical methods were used in these animal studies (for details, see Sections 4.2 and 4.3) and different necrotizing agents (aspirin, indomethacin, ethanol, NaOH, HCL, concentrated NaCL solution and other drugs such as epinephrine, reserpine and stress situation) (or surgical intervention) were administered to cause gastric mucosal damage. The biochemical examination was done in a time-

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dependent manner after the administration of different necrotizing agents; consequently, we were able to study the biochemical mechanisms before the macroscopic appearance of gastric mucosal damage, and, of course, during the time of macroscopic appearance of gastric mucosal damage. The critical analyses of these results (obtained in different experimental models) helped in understanding the suggested key biochemical events responsible for the development of gastric mucosal damage (for details, see Chapter 8).

Our attention was focused on the changes in the members of membrane-bound ATP-dependent energy systems (e.g., ATP, ADP, AMP, cAMP) in connection with the measurements of tissue lactate levels in gastric mucosa. All biochemical measurements were carried out in the same tissue samples at the same time. The key goal was to understand the problems of tissue hypoxia in the development of gastric mucosal damage (in very different experimental conditions).

In other observations, we administered various drugs (atropine, cimetidine, drugs interfering with different subcellular mechanisms and vitamin A and β-carotene – as typical nutritional scavengers – prostacyclin) to prevent the development of mucosal damage produced by different chemicals or stress. The biochemical examinations were conducted under the same experimental conditions as those in the previous models.

We have concluded the following from the analyses of the results obtained from these biochemical examinations in different experimental models:

**a.** The ATP breakdown (by membrane-bound ATPase and adenylate cyclase) increased practically in all models, with the exception of aspirin-induced mucosal damage and pylorus ligation by after surgical vagotomy).

**b.** The changes in energy systems in cells appear before the macroscopic appearance of gastric mucosal damage, while these changes in the cellular energy systems can be prevented by the administration of different mucosal protective agents in the same time period as in the case of the development of mucosal damage.

**c.** The existence of feedback mechanism can be proven in different experimental models, in terms of development of mucosal damage and prevention.

**d.** The presence of tissue hypoxia can be excluded by biochemical observations in the gastric mucosa with the development of gastric mucosal damage.

**e.** The mucosal protection by drugs and scavengers, or biological agents (such as prostaglandins), exists in animals only in conditions of intact vagal nerve, while the gastric mucosal protection ("cytoprotection") disappears after surgical vagotomy, but not in the case of "chemical vagotomy." Consequently, the "surgical" vagotomy and "chemical" vagotomy differ significantly from each other. We were pioneers, who demonstrated first that the intact vagal nerve is involved in the increase of aggressive factors in the stomach, because no gastric mucosal protection exists after surgical vagotomy.

**f.** The gastric mucosal protection produced by scavengers appears in pathways of the changes in parameters of oxygen free radical system (peroxidase, glutathione peroxidase,

superoxide dismutase, reduced glutathione, MDA) and in the parameters of membrane-bound ATP-dependent energy system (ATP, ADP, AMP, cAMP). Interestingly, the mucosal protecting effects of scavengers (vitamin A, β-carotene) produced dose-dependent changes in the membrane-bound ATP-dependent energy system but not in the parameters of oxygen free radicals. It is important to note that these actions are associated with the changes of membrane-bound ATP-dependent energy system and oxygen free radical system; however, without the biochemical presence of tissue hypoxia in the gastric mucosa (in both the time of development of gastric mucosal damage and prevention by scavengers).

**g.** The mucosal aggressive and protective effects of different drugs (or other compounds) depend on the functional states of effector organ (including their biochemical background). The observation of these facts is an important factor in the medical treatment by different drugs (compounds). The clinicians have enough information on the onset of the disease and functional states of effector organ. Observing these facts, probably, we can better understand the efficiencies of various therapeutic drugs, but these suggestions are also true in the case of mucosal producing drugs.

It is well known that these experimental models cannot be interpreted in human pathology without criticism; however, we received more information in this field from experimental ulcers.

### 12.8. Observations on freshly isolated gastric cells from the rat gastric mucosa

The special cellular biochemical examinations were carried out on gastric mucosal cells freshly isolated from gastric mucosa of rat (for details, see Chapter 9). These methodologies were able to demonstrate the cellular damaging effects of different agents at the levels of cell membrane, mitochondrion and DNA.

The effects of ethanol, indomethacin and sonicated *Helicobacter pylori* cultures (obtained from the cultured strains of *Helicobacter pylori* isolated from the gastric mucosa in patients with duodenal ulcer) were studied.

It was very surprising to note that sonicated *Helicobacter pylori* were given alone in doses of $10^8$ to $10^6$ germs/mL, indomethacin and its combination had no actions at any level of cell membrane, mitochondrion or DNA of freshly isolated cells (for details, see Chapter 9). This experimental methodology is internationally used to screen the cellular mucosal damaging and protecting effects of compounds.

One of the authors (Gy. Mozsik) observed that gastric cancer did not develop in patients, who originally suffered from the classical duodenal ulcer (from 1960 to 2014), without gastric surgical intervention (Mózsik Szabó and Czimmer, 2014). It is well known that most patients with duodenal ulcer are infected with *Helicobacter pylori*. We are of the opinion that the presence of *Helicobacter pylori* infection is only one of many causes, which are able to produce gastrointestinal mucosal damage. It seems that researchers have forgotten the results of classic GI research when the role of *Helicobacter pylori* became emphasized. However, we have to

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remember the possibility that the single isolated cells from rat gastric mucosa are not able to produce prostaglandins.

### 12.9. Experimetal studies with different compounds on the stable cell lines

The tendency of drug (and some meaning physiological) research with stable cell cultures significantly increased during the last decades. We also used different stable cell lines to study the effect of various chemicals on cells and to do some toxicological examinations (for details, see Chapter 10).

The results obtained with different compounds in stable cell lines are important; however, these effects are not the same as those in living organs.

### 12.10. The "Take Home Messages" from the authors.

Several different mechanisms (existing at different levels of living organisms) run parallelly beside each other during the development of damage and protection at the levels of organisms, organs, tissues, cells or different subcellular particles (besides the genetic control in the whole living organisms). The researchers have many possibilities to modify a separated mechanism from this extremely large number of mechanisms. We can prove some correlation(s) between the examined parameters versus different phenomena. We believe that we are right and we discovered some new mechanism(s) in our field. Other researchers are in the same position to discover the actually studied phenomenon versus main problems of results in the field, and they also believed that they discovered some other new mechanism(s). Unfortunately, these new results together are not able to help to understand the human medical problems.

We are not able to understand the deep mechanisms, when we observe the whole organism; however, as we observe the very small pieces of cellular and subcellular mechanisms, we might lose the whole organism. This contradiction is also permanently present between clinicians and basic researchers.

Our attention was focused on the existing cellular regulatory mechanisms in the biochemical changes of gastric (gastrointestinal in patients) mucosa in patients with peptic ulcer and gastric mucosa in different experimental models (including the different subcellular particles) under basic (normal and different pathological conditions, without and with the application of different biologically active compounds, drugs).

We have faced the actual challenges of peptic ulcer disease in patients, namely, critical evaluation of the efficiency of medical treatment, principal role of gastric hypersecretion in ulcer development in patients, establishing the human clinical pharmacology, biochemical constitutes of gastric fundic mucosa with different gastric acid secretory responses, the presence of hypoxia in the ulcerated antral, duodenal, jejunal tissues around the ulcer, other detailed biochemical mechanisms of gastric ulcer in the human gastrointestinal mucosa under different conditions (fundic, antral, duodenal and jejunal) and in tissues (with and without ulcer).

A wide scale of experimental ulcers has been applied (together with different experimental conditions). The main attention was focused on the changes of cellular energy (membrane-bound ATP-dependent energy) systems versus the presence of tissue hypoxia (the lactate levels were measured together with other biochemical examinations to prove/to exclude the presence of impaired oxidative phosphorylation in the gastric mucosal tissues). The observations were done at the time of development of damage and its protection by different chemical compounds (drugs).

Answers to our (main) scientific problems are the following in the last half of the century:

**a.** Without clinical pharmacology, we were not able to critically evaluate the efficiencies (and their background) of the drugs used in the treatment of patients with peptic ulcer. We obtained an excellent reconfirmation by the international clinical pharmacological research to pioneer our work in establishing methodology of clinical pharmacology in patients with peptic ulcers.

The established clinical pharmacology gave scientifically based observations to understand the different medical questions and compare the efficiencies of drugs (with different actions of mechanisms).

**b.** The key role of decrease of gastric acid secretion (by therapeutically applied drugs) is not necessary for the healing of gastric and duodenal ulcer in patients. In other words, the phenomenon of "cytoprotection" exists in patients with both chronic duodenal and jejunal ulcers.

**c.** The development of "development of tolerance" to parasympatholytic drugs together with the "development of pharmacological denervation phenomenon" is one of the clinical pharmacological explanations for the existence of "cytoprotection" in patients with chronic atropine treatment. This explanation was never given for the existence of "gastric (duodenal) cytoprotection" in patients with chronic gastric and duodenal ulcer. However, it is also clear that the "gastric cytoprotection" is an existing phenomenon in patients with chronic gastric ulcer (for details, see Chapter 2) (without this clinical pharmacological explanation, vitamin A has really gastric mucosal damage, but without its inhibitory action on gastric acid secretion).

**d.** The presence of gastric hypoxia in the ulcerated antral, duodenal and jejunal mucosa (e.g., of impaired oxidative phosphorylation) biochemically can be excluded in the presence of gastric antral, duodenal and jejunal mucosa around chronic ulcer in patients with chronic peptic ulcer (because the biochemical compositions of these ulcerated mucosal tissues are the same as those in the gastric fundic mucosa with gastric hyperacidity).

**e.** The successful preparation of $Na^+$–$K^+$-dependent ATPase and adenylate cyclase by us opened an absolutely new gate to study and understand the classic biochemical mechanism of the equilibrium between different parameters of cellular energy system (ATP, ADP, AMP, cAMP) under normal (intact) and different pathological conditions).

The axis of cellular ATP–ADP–cAMP–AMP–ATP breakdown by the membrane-bound ATPase splitting enzymes (membrane ATPase, adenylate cyclase),– ATP resynthesis (under

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intact oxidative phosphorylation), tissue levels of lactate, gastric acid hypersecretion, the development of gastric (gastrointestinal) mucosal damage (ulcer), intact vagal nerve, damaging and protective effects of different drugs, decrease of gastric acid secretion and the protection of GI mucosal damage cover the main topics of gastrointestinal physiology, pathophysiology and pathology in animals and humans. The obtained results in this axis can be critically evaluated in respect with the results of the above-mentioned cellular parameters.

The drugs (and other compounds) represent the "first messengers," while the intracellular mechanisms together indicate the details of "second (intracellular) mechanisms" in the cellular energy systems in both normal (intact) and different pathological conditions, without and with the application of any drug (compounds). We have to emphasize that the changes in the cellular energy systems are in very close correlation with other intracellular mechanisms (for details, see Chapter 5). The details of these different mechanisms presented by international experts (over the world) are related closely to this axis. Our molecular pharmacological examination with the drugs interfering with different cellular mechanisms clearly indicated that the functions of the levels of cell membrane, mitochondrion, protein synthesis, RNA and DNA are involved in the development of gastric acid hypersecretion, mucosal damage and mucosal protection produced by PGI2 and scavengers (vitamin A, β-carotene) (for details, see Section 8.12).

The actions of first messengers and their intracellular changes in the energy systems depend on the actual functional levels of target organ (including other intracellular mechanisms) at the time after the onset of different pathological (drugs) processes.

**f.** The existence of "gastrointestinal cytoprotection" and drugs produced by different compounds (scavengers, drugs, other compounds) disappears after surgical vagotomy; however, it remains intact in the case of "chemical vagotomy." This is a great criticism on the clinical application (practice) of surgical vagotomy in the treatment of patients with chronic peptic ulcer.

**g.** The results of the observations obtained from freshly isolated gastric mucosal cells and stable cells lines (gastric cancer, hepatoma, colorectal cancer, mouse myeloma) gave important scientific information for researchers (clinicians); however, it is also important to note that these results differ – in some meaning – from those obtained in living organs;

**h.** The observations with capsaicin (in respect to all of the very strict international laws of clinical pharmacology) offer a new pathway for the medical treatment of patients with peptic ulcer and for the prevention of drug-induced gastrointestinal mucosal damage by the stimulation of capsaicin-sensitive afferent nerves via the stimulation of capsaicin receptor (TRPV1) and liberation of different neuropeptides (calcitonin gene-related peptide, CGRP; substance P, SP; somatostatin; glycagon).

Newer research fields in gastroenterology, such as the research with capsaicin or newer neural mediators, opened a new window to further approach the mechanism of gastrointestinal mucosal damage and prevention.

The clinicians have followed the theoretical and practical advantages of clinical medicine with the simultaneous development of basic research. It has been very difficult for clinicians to maintain this position (we have tried to live two lives in the last half century). We were basically clinicians; however, apart from that we foray into the experimental research, thus accepting the actual scientific questions of basic research in gastroenterology.

There are general problems that no critical and scientific communications exist between the clinicians and basic researchers in gastroenterology.

*The following list of references includes (incorporates) the most selective list of references, the detailed list of references is incorporated in the original publications.*

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## Author details

Gyula Mozsik[1*] and Imre Szabó[2]

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

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# Abbreviations

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http://dx.doi.org/10.5772/60496

ADP, adenosine diphosphate;

Adenylate pool, ATP+ADP+AMP;

ATP, adenosine triphosphate

AMP, adenosine monophosphate

cAMP, cyclic 3',5'-adenosine monophsophate;

CAT, catalase enzyme;

BAO, basal acid output;

COX, cyclo-oxygenase enzyme;

DNA, deoxyribonucleic acid;

DU, duodenal ulcer;

"energy charge", [(ATP+0.5 ADP)/(ATP+ADP+AMP)];

ETOH (EtOH), ethanol;

GMCs, gastric mucosal cells;

GMBF, gastric mucosal blood flow;

GSH-px, glutathione peroxidase;

GSH-red., reduced glutathione;

GU, gastric ulcer;

HCl, hydrochlorid acid;

Hep G2 cell line, human hepatoma cell line;

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H. pylori, Helicobacter pylori;

IND, indomethacin;

IP3, inositol triphosphate;

JU, jejunal ulcer;

6-keto-$PGF_{1\alpha}$, metabolite of prosptacyclin;

LDH, lactate dehydrogenase;

MAO, maximal acid ouput;

MARK, mitogen-avtivated proptein kinase;

NaOH, sodim hydroxyde;

MDA, malondialdehyde;

NOSAIDs, non-steroidal anti-inflammatory compounds;

NUD, non ulcer dyspepsia;

$PGE_2$, prostagladin $E_2$,

$PGI_2$, prostacyclin;

PGs, prostaglandins;

PUD, peptic ulcer disease;

RNA, nuleic acids;

ROS, reactive oxydative species;

SOD, superoxide dismutase;

Sp2/0-Ag14 cell line, mouse myeloma cell line.

## Author details

Gyula Mozsik[1] and Imre Szabó[2]

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

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Gyula Mozsik and Imre Szabó

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## Author details

Gyula Mozsik[1] and Imre Szabó[2]

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary

*Authored by Gyula Mozsik and Imre Szabo*

The book on Membrane-bound Atp-dependent Energy Systems and the Gastrointestinal Mucosal Damage and Protection deals with various aspects of peptic ulcer disease, like clinical pharmacology, nutrition, molecular biochemical pharmacology as well as clinical aspects, and especially with the evaluation of certain biochemical mechanisms in human gastric mucosa and in animal gastric tissues obtained from different ulcer models. This book can be useful to physiologists; biochemists; pharmacologists, particularly molecular and biochemical pharmacologists; internists; gastroenterologists; biologists; surgeons and pharmacists.

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