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

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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?

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 resectates 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 H₂ 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 resectates 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:

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 \pm 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 Dickinson, 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 NaI 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 \pm 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

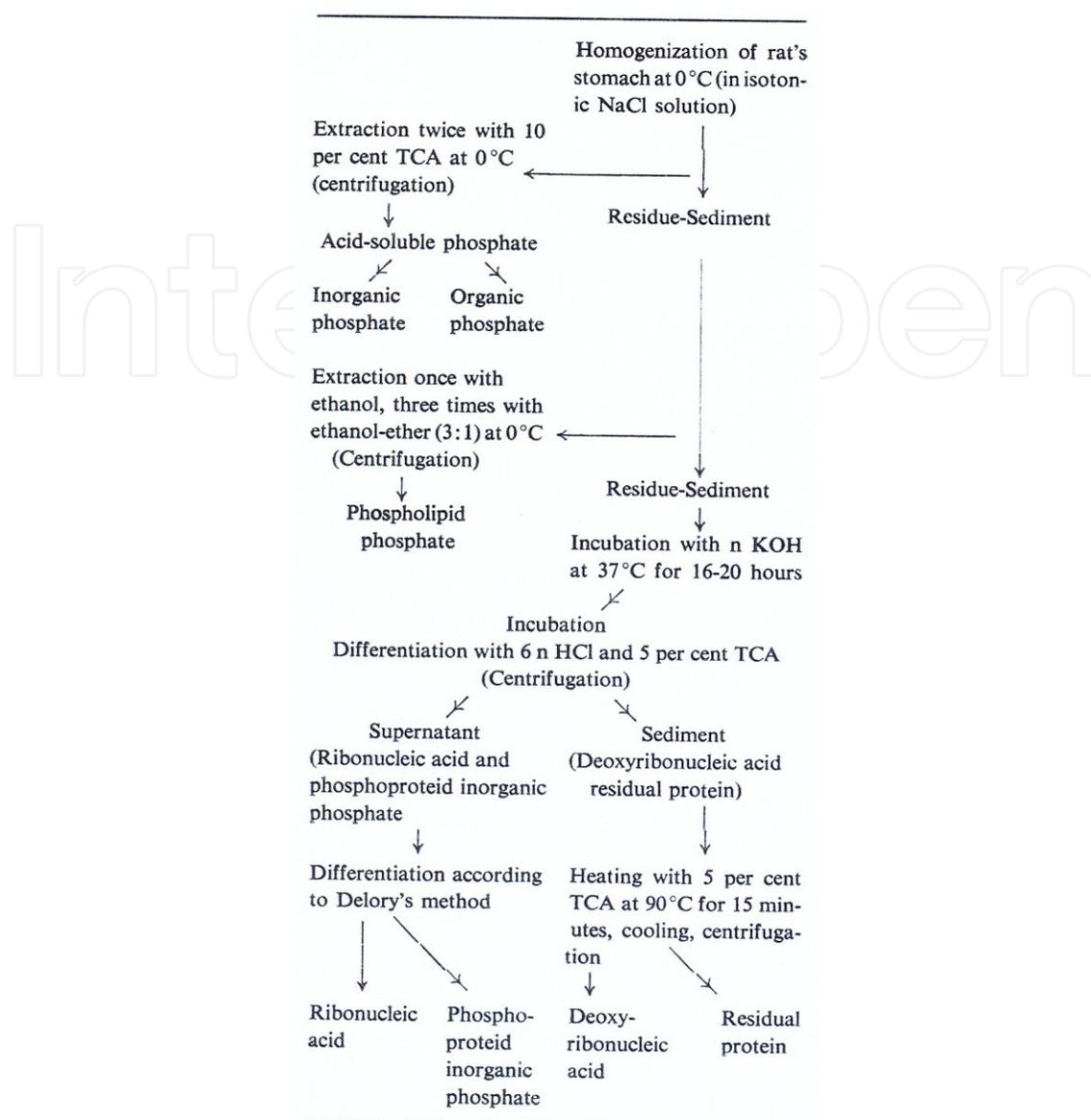


Table 6. Summarizing steps of the biochemical procedures for separation of phosphate fractions and nucleic acids of the rat's stomach and of resectates of human gastrointestinal tracts of patients with chronic gastric, duodenal and jejunal ulcer, who underwent surgical intervention because of peptic ulcer diseases. [Mózsik, Szabó, Krausz, Jávör: 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).

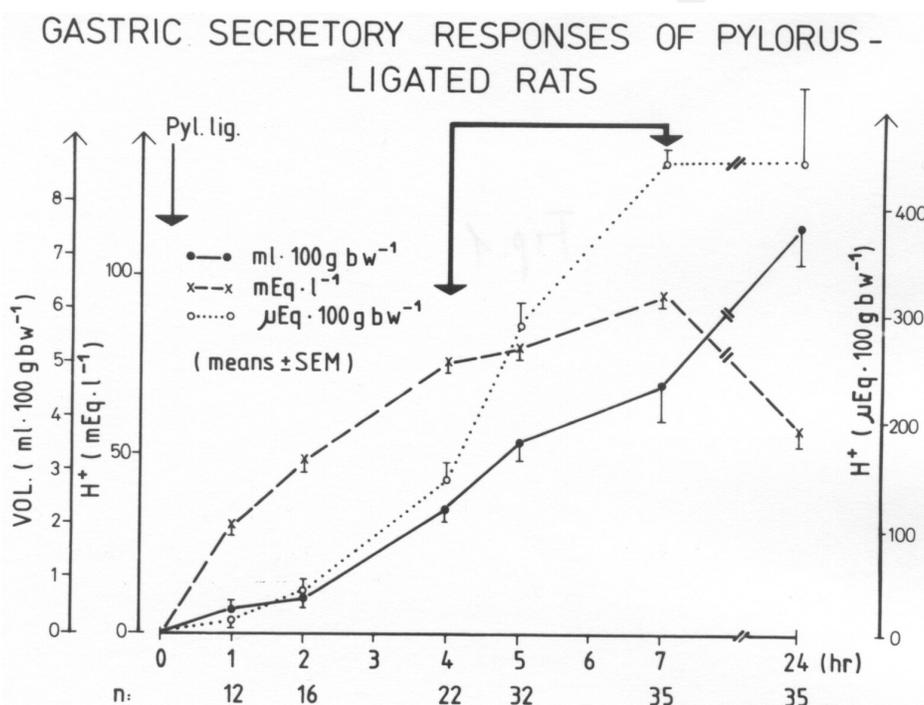


Figure 13. The pattern of gastric secretory responses in 24-hour pylorus-ligated rats. The volume of gastric secretory responses (mL/100 body weight), H⁺ concentration (mEq/L) and H⁺ output (μEq/100 b.w.) were measured and their results were expressed as means ± SEM, indicating the number of animals. 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.

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	7 HR	24 HR
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_o) 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;

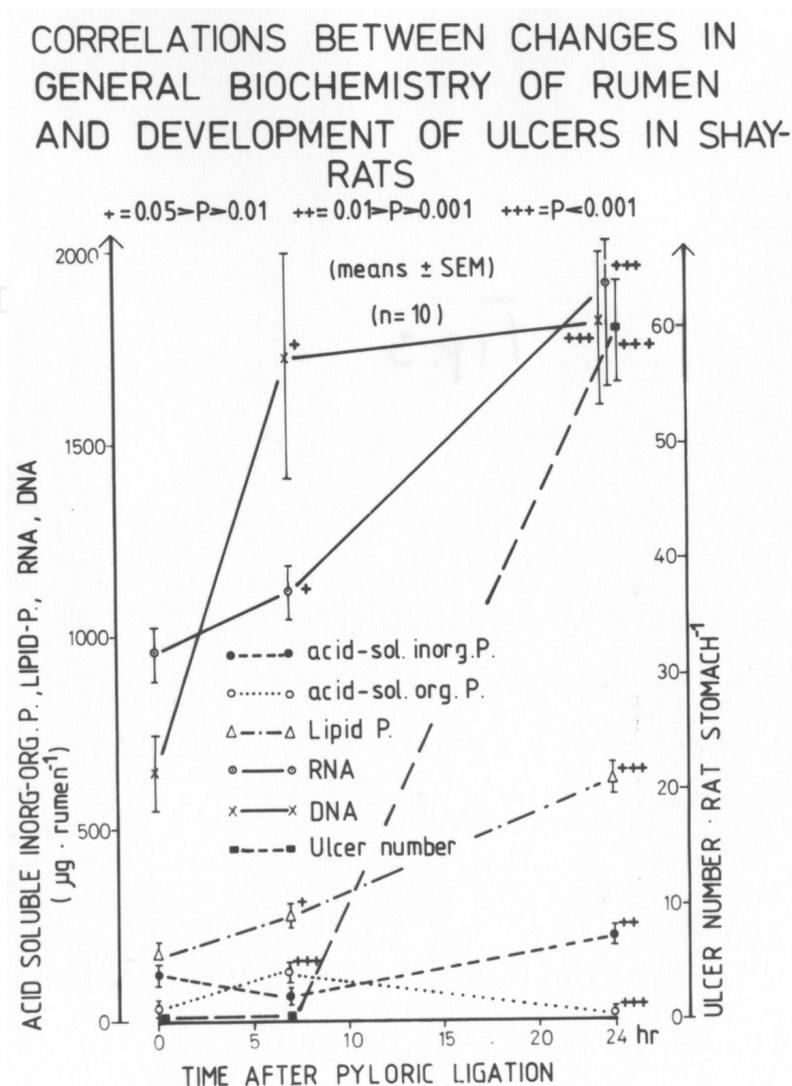


Figure 15. Correlation between the changes in the general biochemistry of ulcer development in forestomach (rumen) of the 24-hour pylorus-ligated rats depending on time after pyloric ligation. [Mózsik et al., *Scand. J. Gastroenterol.* 4: 633–640; 1969 (with kind permission).]

- 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).

BIOCHEMISMS OF GASTRIC FUNDIC MUCOSA FORE-STOMACH AND DEVELOPMENT OF GASTRIC HYPER-SECRETION AND ULCER IN PYLORUS-LIGATED RATS

	GLANDULAR STOMACH			FORESTOMACH (RUMEN)		
	0	7	24 HR	0	7	24 HR
H output	-	↑↑↑	↑↑↑	-	-	-
ULCER	-	-	-	-	-	↑↑↑
ACID-SOL. INORG. P		↓↓↓	↑↑↑	NS		↑↑↑
ACID-SOL. ORG. P		↑↑↑	↓↓↓	↑↑↑		↓↓↓
LIPID P		↑↑↑	↑↑↑	↑		↑↑↑
RNA		NS	NS	↑		↑↑
DNA		NS	↑↑	↑		↑↑↑

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

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;

CHANGES IN GENERAL BIOCHEMISTRY OF GASTRIC WALL OF PYLORUS-LIGATED (SHAY)-RATS DURING DEVELOPMENT OF GASTRIC HYPERSECRETION AND ULCER DEVELOPMENT

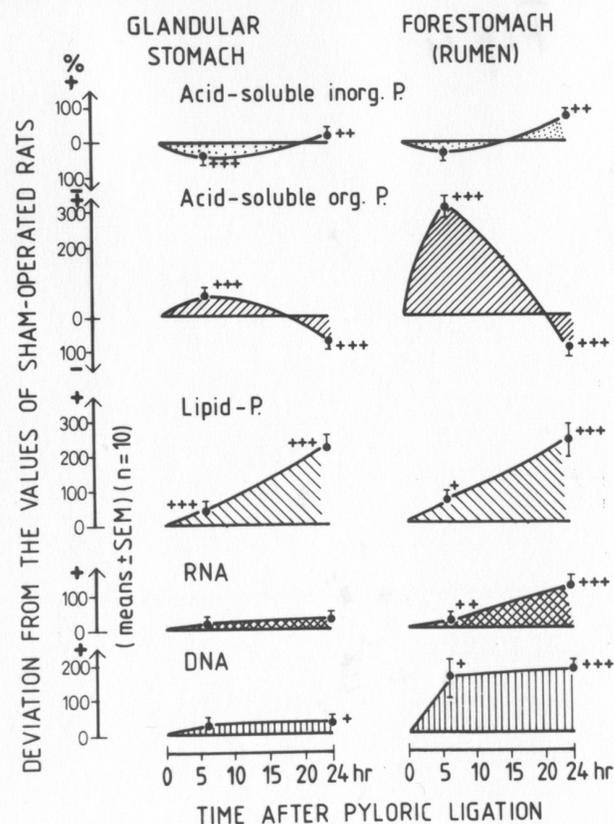


Figure 17. Schematic summary of the changes in gastric hypersecretion, ulcer development (forestomach) and biochemical compositions (acid-soluble inorganic and organic phosphates, lipid phosphates, RNA and DNA) observed in 24-hour pylorus-ligated rats. [Mózsik et al., *Scand. J. Gastroenterol.* 4:633-640;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

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., 1955; Proosdij-Harzeme, et al., 1955). 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.

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 + Iso-propamide (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 + pyroliclig. + 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 + Iso-propamide (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).]

Experimental groups	n	Acid-soluble inorganic phosphates		Acid-soluble organic phosphates	
		n	quantities	n	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		Acid-soluble organic phosphates	
		n	quantities	n	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).]

Experimental groups	Deoxyribonucleic acid			
	Glandular stomach wall		Membranaceous stomach wall	
	n	quantities	n	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				Membranaceous stomach wall			
	Experimental groups				Experimental groups			
	Control	After Atropine	After Isopropamide	After Gastrixone	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*.

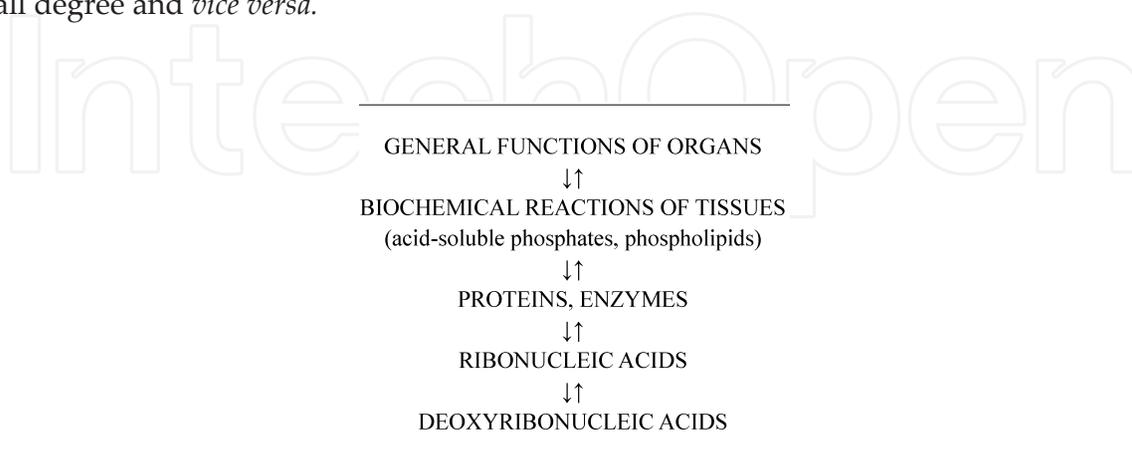


Table 17. Main steps of regulatory levels of cells in the living organs. The most stable regulatory steps are located at the level of DNA and the most instable regulatory steps are at the level of functions of organs.

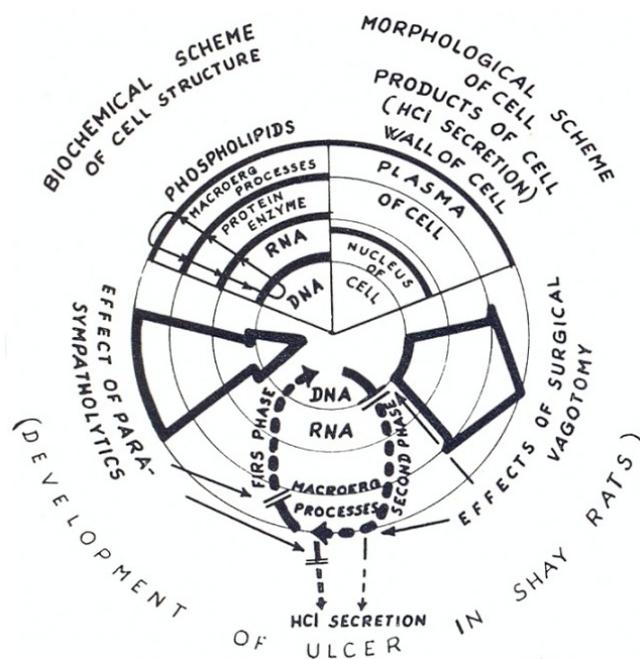


Figure 18. A schematic and possible biochemical–cellular–morphological explanation of parasympatholytics and surgical vagotomy. [Mózsik, Kiss, Krausz, Jávör: *Scand. J. Gastroenterol.* 4: 641–651, 1969c (with kind permission).]

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	
	<i>n</i>	quantities	<i>n</i>	quantities	<i>n</i>	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	
	<i>n</i>	quantities	<i>n</i>	quantities	<i>n</i>	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).]

	Controls (animals treated with physiological saline for 25 days)		Animals treated with atropine for 25 days		10 days after cessation of atropine treatment	
	<i>n</i>	quantities	<i>n</i>	quantities	<i>n</i>	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 \pm 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	
	<i>n</i>	quantities	<i>n</i>	quantities	<i>n</i>	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 \pm SEM in grams (*n* indicates the number of animals). [Mózsik, Kiss, Jávör, 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, Jávör, Krausz, Tóth, Pharmacology 2:45-59, 1969d (with kind permission).]

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 <i>Brown</i>)	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 <i>Seibert</i>)	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, Jávör, 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 <i>Brown</i>)	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 <i>Seibert</i>)	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, Jávör, Krausz, Tóth, Pharmacology 2: 45-59, 1969d (with kind permission).]

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 <i>Brown</i>)	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 <i>Seibert</i>)	10	1073 ± 113 (22)	10	1139 ± 217 (23)	7	2880 ± 492 (24)

Significance:

1-2: P > 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, Jávör, 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 be sensitive to mediators than that innervated organ. Emmelin and Rosenblueth (1951), Emmelin and Muren (1951 a, b; 1952), and Elin (1952, 1961) observed that the efficiencies of drugs and mediators changes after a prolonged treatment (including the atropine). In these observations, no surgical manipulation was done with the nerves of different organs, however, they chronically treated was done to inhibit the neural functions at the levels of synapses or at the levels of target 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 development of tolerance to drugs used in the treatment and cross-tolerance to the pharmacologically pharmacological drugs, but that are not used in the treatment, 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

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

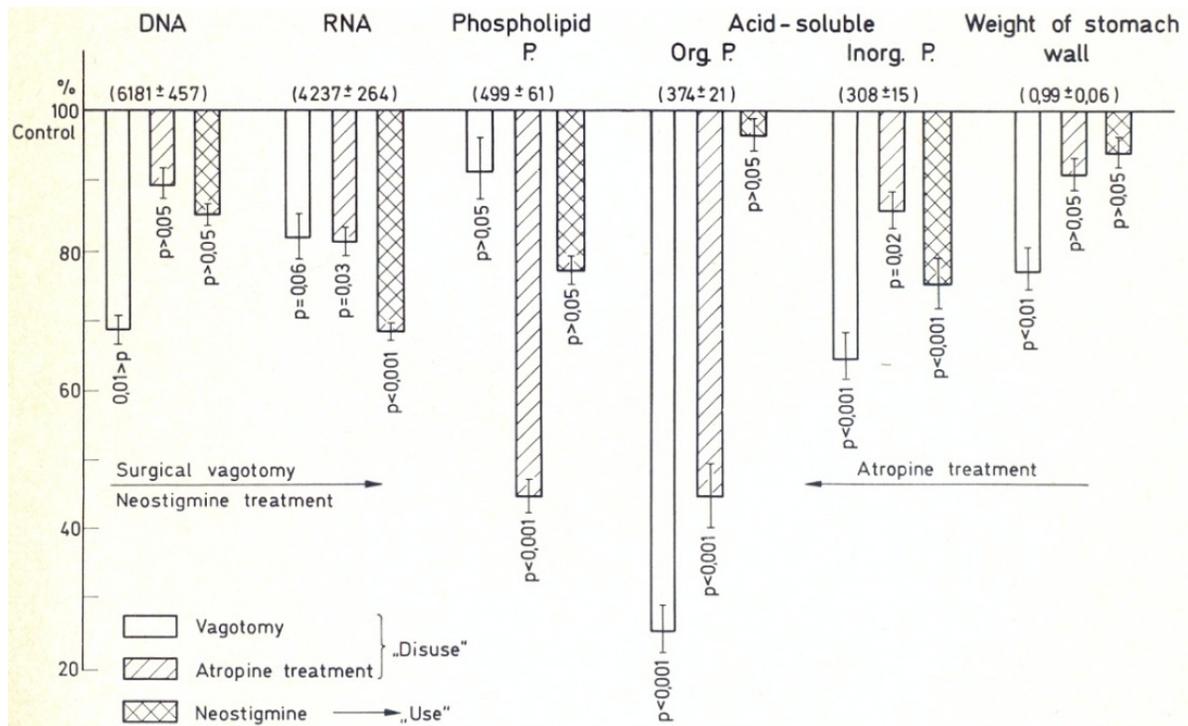


Figure 19. Biochemical backgrounds of “use” and “disuse” of cholinergic innervations on gastric tissue in the glandular stomach wall (in one month’s treatment or surgical vagotomy). The results are expressed as means ± SEM in percentage values of control (sham-treated) rats. [Mózsik, Kiss, Jávör, Krausz, In: Gregor O., Riedl O. (eds) Modern Gastroenterology, Schattauer Verlag, Stuttgart, 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.

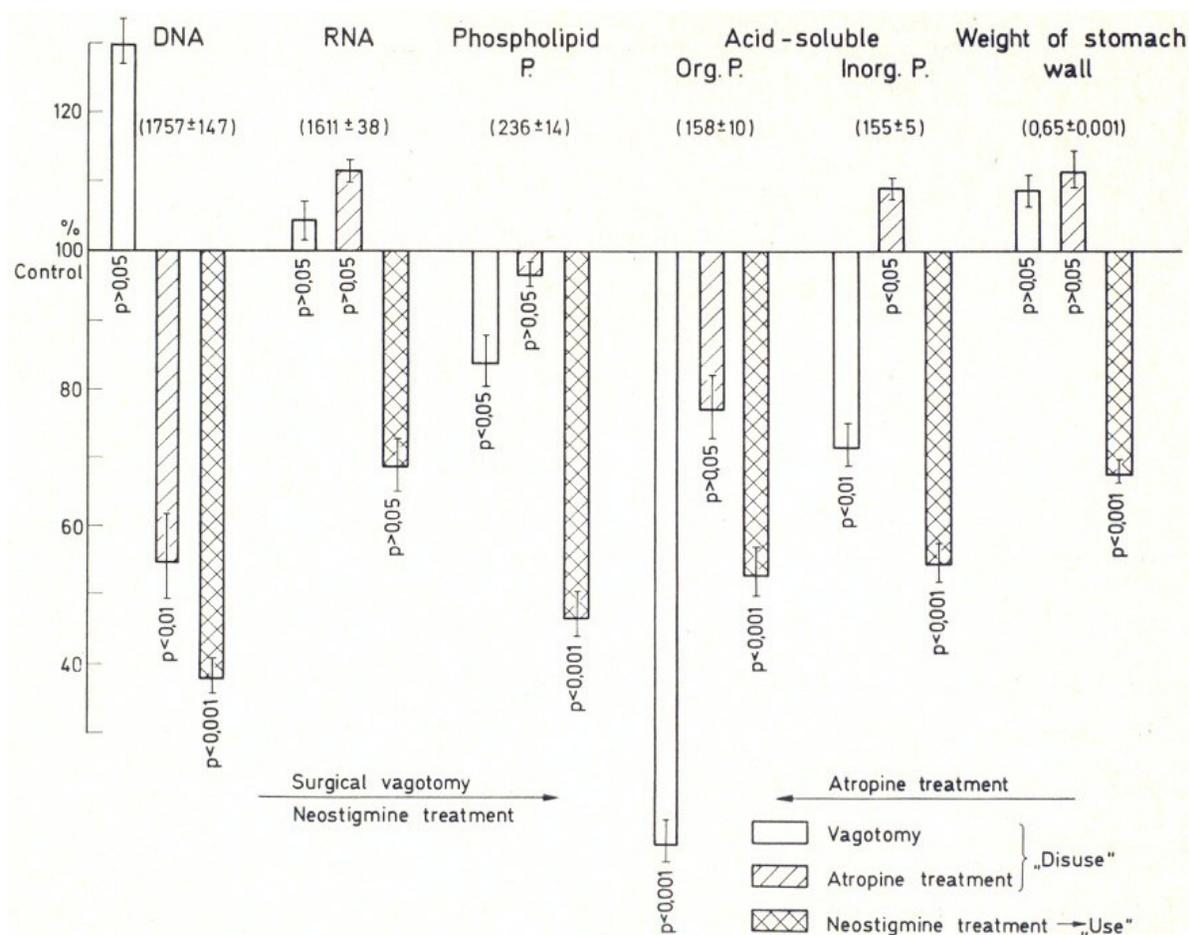


Figure 20. Biochemical backgrounds of “use” and “disuse” of cholinergic innervations at the biochemical level on gastric tissue in the membranaceous stomach wall (forestomach) (in one month’s treatment or surgical vagotomy). The results are expressed as means \pm SEM in percentage values of control (sham-treated)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

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