Animal Experiments in Medicine

Experimental techniques in practice I.
Gastrointestinal research

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The main physiological functions of gastrointestinal (GI) tract to provide the body with a continual supply of water, electrolytes, and nutrients. These multiple gastrointestinal (GI) function, including the propulsion of nutrients (motility), the transport of fluids, electrolytes and immune cells (microcirculation), the discharge of ions and bioactive compounds (secretion) and the regulation of epithelial barrier function are regulated and coordinated by enteric nervous system (ENS).

The experimental examining methods of GI can be grouped according to the main physiological functions of GI tract:
I. Food movement in the GI
II. Food digestion
   - Secretion of digestive juices
   - Absorption of digestive products, water and various electrolytes
III. Nervous and hormonal control of these functions
IV. Blood circulation to carry absorbed materials

I. GASTROINTESTINAL MOTILITY
The gut motoric functions are performed by different layers of muscle. The intestinal wall is composed of,
   a) Mucosa
   b) Muscularis mucosa
   c) Submucosa
   d) Tunica muscularis
      - inner
      - outer
   e) Tunica serosa or Tunica adventia

![Figure 1. Simplified representation of the layers of intestinal wall.](image)

I.1. Electrical activity of the GI smooth muscle
The gastrointestinal smooth muscles function as a syncytium. The smooth muscle fibers in the tunica muscularis are electrically connected through gap junctions that allow
ions to move from one cell to the next. Action potential elicited within the muscle mass travels in all directions in the muscle. GI muscles are excited by intrinsic electrical activity along their membrane. Two basic types of electrical activity (Figure 2):

a/ Slow wave
b/ Spikes

Figure 2. Two basic electrical activities of GI tract are the slow wave and spikes.

Features of GI slow waves
1. They are slow undulating changes of resting membrane potential, which are not regarded as action potentials.
2. The slow wave is a result from undulation of the activity of the sodium-potassium pump.
3. The rhythm of GI contractions is determined by the frequency of slow waves in the smooth muscle membrane potential.

Factors that depolarize the membrane and lead to smooth muscle contractions (Figure 3):
1. Stretching the muscle,
2. Stimulation by parasympathetic nerves that secrete acetylcholine at their endings,
3. Stimulation by gastrointestinal hormones.

Factors that hyperpolarize the membrane and lead to the inhibition of the smooth muscle contractions (Figure 3):
1. Norepinephrine or epinephrine on the muscle membrane
2. Stimulation of the sympathetic nerves that secrete norepinephrine at their endings.
Figure 3. Factors, which are depolarize or hyperpolarize the membrane.


The electrical activity of the intestinal smooth muscle could monitor with implantable bipolar Ag/AgCl extracellular sensor in the \textit{in vivo} experiments. These Ag/AgCl wire electrodes can sutured into the external surface of the intestine (or stomach or oesophagus). Leads are attached to connectors fixed to the skin on the nape of the animals. A forceps or Pean is guided between the skin and the muscle toward the nape opening; the electrodes are caught and pulled over to the abdominal area with the head-plug remaining about to its position on the nape. Pairs of electrodes (not less than 2 and not more than 4 pairs) are pushed through the serosal surface not reaching the deeper layers; the end of each electrode is carefully coiled, and the coil is placed on the surface (Figures 4-5).

Figures 4-5: Ag/AgCl bipolar sensors are for measurement of the electrical activity of intestinal smooth muscle. Leads are attached to connectors fixed to the skin on the nape of the animals.
1.3. Monitoring of intestinal smooth muscle contractions with strain gauge sensor

Strain gauge sensor is an implantable force/displacement transducer for *in vivo* application. The strain-gauge transducer can suture onto the different parts of small and large intestine and it is connected to an SG-M bridge amplifier and the signals were recorded continuously with a computerized data-acquisition system (SPEL Advanced Haemosys 1.72, Experimetria Ltd., Budapest, Hungary). The duration of sampling is minimum 10 min, with a sampling frequency of 500 Hz; the signal analysis can be performed off-line.

Figure 6: Strain gauge sensors are force/displacement transducer for *in vivo* application, which is implantable, manufactured in three different sizes and arched shape with suture-hole.

The motility pattern of the intestinal contractions consists of several components, including the amplitude, the rate and the tone. These components together are characterized by the motility index, which is determined by calculating the area under the motility curve as a function of time. The amplitude and frequency of intestinal contractions can be calculated, and the tone of the colon was given by the mean value of the minima in the motility curve (Figure 7).

Figure 7: The motility pattern of the intestinal contractions: the amplitude, the rate, the tone and the motility index.

**III. NEURAL CONTROL OF GASTROINTESTINAL FUNCTION**
III.1. The enteric nervous system

The autonomic neuronal regulation of GI motility and secretion is achieved by more than 10 billion neurons, which constitute the enteric nervous system (ENS); ENS is named as „brain in the bowel”.

All aspects of GI function, including the propulsion of nutrients (motility), the transport of fluids, electrolytes and immune cells (microcirculation) and the discharge of ions and bioactive compounds (secretion), are coordinated with the enteric nervous system (ENS). The network of neurones, which entwines the GI tract from the oesophagus to the anal sphincter, is capable of integrative functions independently of the central nervous system (CNS). This classical finding has been substantiated by numerous observations; on the one hand, the reflexes persist after all extrinsic connections are rendered ineffective; on the other hand, the organized propulsive and mixing movements cease if local neurone activity is inhibited by tetrodotoxin, or neurotransmission is blocked by nicotinic or muscarinic blocking agents. However, neurogenic control and coordination of the ENS is influenced by a reciprocal connection between the GI tract and the CNS: through the cross-connections of parasympathetic and sympathetic fibres, the intestines provide sensory information to the CNS, and likewise, signals from outside of the GI tract can be relayed to the digestive system.

The major functional components of the ENS are the subserous, the myenteric (Auerbach's, located between the two layers of the muscularis propria) and the submucosal (Meissner's, located in the submucosa) plexuses. These networks contain a variety of functionally distinct neurones, including primary afferent neurones, interneurones, excitatory and inhibitory motor neurones, synaptically linked to each other in microcircuits. In addition, enteric neurones are supported by glial cells, the ENS counterparts of astrocytes in the CNS, which can also modulate the enteric neurone function (Figure 8).

Figure 8. Simplified representation of the enteric neuronal system and its connections with the central nervous system. 1. An intrinsic primary afferent neuron (IPAN) with cell body in the submucosal plexus; 2. an IPAN with cell body in the myenteric plexus (1 and 2 are AH-type...
neurons); 3-5. muscle motor neurons; 6. an interneuron (3-6 are S-type neurons); 7. an extrinsic primary afferent neuron; S: serosa; LM: longitudinal muscle; MP: myenteric plexus; CM: circular muscle; SMP: submucosal plexus; MM: muscularis mucosae; E: intestinal epithelium, SY: sympathetic fibers; PSY: parasympathetic fibers.

**Myenteric plexus or Aurebach’s plexus** is located in tunica muscularis. Its stimulation increases the:

- tone of the gut wall,
- intensity of rhythmical contractions,
- rate of contraction and
- velocity of conduction
- inhibits the pyloric sphincter, which controls emptying the small intestine into the cecum.

**Submucosal plexus, or Meissner’s plexus** lies in the submucosa. It controls the function of the inner wall of the intestine and controls sensory signals originate from the GI epithelium and reach the submucosal plexus to control local intestinal secretion, absorption and contraction of the submucosal muscle.

### III.2. Functional types of movements in the gastrointestinal tract

The patterns of motility of the gastrointestinal tract include mixing and propulsive movements, which are more or less confined to regions, and organized patterns of movement such as swallowing and esophageal peristalsis, migrating complexes, vomiting, and defecation, which involve large sections of the digestive tract.

Two patterns of activity are recognized in the mammalian small intestine, the activity of the interdigestive state and the fed pattern of activity. The interdigestive pattern is characterized by the migrating myoelectric complex (MMC), which passes along the intestine every 80–110 min in humans. The complex takes about 6–10 min to pass any point in the intestine, and as it passes, that region undergoes intense rhythmic contractions of the circular muscle. These contractions are propagated through the region occupied by the migrating complex at a greater speed than the complex itself propagates. The migrating complex is also referred to as phase III activity. It is followed by a period of less intense activity (phase IV), then by relative quiescence (phase I) and by irregular contractions (phase II) that are interrupted by the reoccurrence of phase III activity. In continuously feeding animals, such as sheep and guinea pigs, rats the MMC passes down the intestine at regular intervals even when the animal is digesting. However, in intermittent feeders, such as humans and dogs, the MMC and the other cycles of the interdigestive state disappear soon after a meal is taken, to be replaced by the fed pattern of activity, which consists of ongoing phasic contractile activity.

**Peristalsis** is the basic propulsive movement of the gastrointestinal tract. Intestinal distension causes a contractile ring to appear, moves forward before ending. The gut relaxes down toward the **anus**. This process is the receptive relaxation, allowing the food to be propelled towards the anus (Figure 9). These are called myenteric reflex, or peristaltic reflex. Peristaltic reflex plus movement toward the anus is called “Law of The Gut”.

**Measurement of propulsion and peristalsis** could performer in rodents with using a non-absorbable marker dye (phenol red) as a function of time and position within the GI tract. Phenol red meal is administered with the aid of the oral feeding syringe. Following the treatment with marker dye, feeding the bowel content or faces are collected with time dependent manner. Phenol red can extracted from the collected samples and the concentration of dye is determined by colorimetric method at 560 nm.
Figure 9. Scheme demonstrates the peristaltic propulsion of chyme. This accomplished by the circular and the longitudinal muscle layers to move the chyme caudally.

IV. GASTROINTESTINAL CIRCULATION

The blood vessels of the GI tract are part of the splanchnic circulation, which consists of blood flow from stomach and gut, spleen, pancreas and liver. Splanchnic circulation flows into the liver by way of the portal vein and the blood passes through liver sinusoids and leaves the liver by way of hepatic veins.

IV. 1. Monitoring of the gastrointestinal circulation with gastric tonometry

The gastrointestinal mucosa is the target of blood flow redistribution during shock, trauma and major surgical interventions: vasoconstriction evolves in the mucosa in low cardiac output states. The small intestine is one of the first organs to suffer from hypoperfusion and one of the last to be restored upon resuscitation. Hence, a GI mucosa impairment caused by a splanchnic circulatory failure can play a crucial role in the etiology of sepsis and multiple organ failure. Gastric tonometry is an appropriate tool for the diagnosis of early gastric hypoperfusion (before the development of systemic symptoms). The idea of gastric tonometry was developed by Dr. Domokos Boda (professor of pediatrics at the University of Szeged)\(^5\).

The circulation of GI mucosa could be estimated with indirect measurement of the mucosal pH of the stomach/small intestine/sigmoid bowel. The counter-current exchanger system of the intestinal microcirculation results in the tissue \(pO_2\) content decreasing from the base of the villi toward the villus tip. During flow reduction, adequate tissue oxygenation can not be ensured at the apical part of the villus. The mismatch between regional perfusion and metabolism, however, leads to an imbalance between \(CO_2\) removal and production: as a result, \(CO_2\) accumulates in the mucosa. This phenomenon can be detected in the cavernous organs (the stomach and intestines) through the luminal measurement of \(CO_2\) (\(PgCO_2\)). In the stomach, the mucosal \(pCO_2\) (\(PgCO_2\)) is indicative of the balance between the \(CO_2\) production (metabolism) and removal (perfusion) (Figure 10). An increase in \(PCO_2\) (regional hypercapnia) can be a good indicator of an inadequate tissue blood flow and/or an exaggerated metabolism. Under physiological conditions, \(PgCO_2\) (normal value) is equal to the arterial \(pCO_2\) \((PgCO_2 = 45\ \text{mm Hg} (6\ \text{kPa})\).

The advantage of \(PgCO_2\) over \(pHi\) (the intramucosal pH calculated on the basis of the Henderson-Hasselbalch equation) is the better diagnostic value. The reason for this is that anaerobic \(CO_2\) production contributes to the increased \(PgCO_2\) when oxygen delivery reaches a critical level. Meanwhile, the arterial pH also decreases and contributes to the decrease in \(pHi\).
If we wait for the changes in pH in these cases (when pH is already low), therapeutic intervention can be late and ineffective.

Determination of $\text{PgCO}_2$ with different types of minimal invasive tonometry:
1. A special tonometry catheter and monitor are used to analyze $\text{PCO}_2$ with infrared sensor technology.
2. Saline tonometry: a multiple-lumen catheter includes a semipermeable silicone balloon at the distal end of the catheter, which is positioned in the stomach. CO$_2$ freely equilibrates between the gastric mucosa, the gastric lumen and the balloon. The partial CO$_2$ pressure of the saline sample can be determined with blood gas analyser.
3. Air-tonometry: a gas sample is drawn from the balloon and the partial CO$_2$ pressure of the air sample is analyzed following a 10-min equilibration time with automated capnograph. This technique can improve the precision of $\text{PgCO}_2$ determinations significantly.

IV. 2. Measurement of intraabdominal pressure

The abdominal compartment syndrome (ACS) is one of the major indications of intraabdominal pressure measurements. This is a severe, often life-threatening syndrome which usually develops as a consequence of pancreatitis, pneumoperitoneum (surgery), trauma, bleeding, ascites, malignancies, outer compression (burns), peritonitis or sepsis. Its pathophysiological basis is a sudden increase in intraabdominal pressure (IAP). As the abdominal pressure is transmitted to the urinary bladder, the IAP can be determined reliably and noninvasively by measuring the intravesical pressure. A Foley catheter is inserted into the bladder and is filled with a standard volume (e.g. 100 ml) of physiological saline. A pressure transducer is attached to the catheter through a 3-way stopcock. The fluid pressure in the bladder can be detected with a system used for the measurement of CVP. Attention should be paid to the setting of the zero point of the system at the level of the bladder. The values are expressed in cmH$_2$O (1 mm Hg = 1.36 cmH$_2$O = 0.13 kPa) during expiration after waiting for 1 min. The normal abdominal pressure is 5-7 mm Hg. The abdominal perfusion pressure is calculated as (mean arterial pressure - abdominal pressure). The ACS is thus defined as permanent, severe intraabdominal hypertension (>25 mm Hg), accompanied by a potential new organ failure.

IV.3. Methods in intestinal permeability measurements
1. Gravimetric analysis: determination of the rate of wet and dry weight informs us from the level of intestinal edema.
   1. Tissue sample taken and measurement of wet weight;
   2. Tissue sample is drying min 48 hrs (until the measured dry weight is not changed);
3. If the weight of dried sample is not changed, it is used as dry weight;

2. Vascular/capillary permeability

Changes in the vascular permeability of the gastric mucosa could be determined by using Evans blue, which binds rapidly to albumin and migrates into the extravascular space with it. Evans blue (a 10 mg/kg bolus in saline) is injected iv. Thirty minutes later, a blood sample taken occurs from the right ventricle (in rat). The examined part of GI is rapidly excised, the mucosal layer was scraped off with a microscope slide, and the scrapings were put in 1 ml of formamide and homogenized for 1 min. The homogenate was incubated at room temperature for 18 h and then centrifuged at 5000 g for 60 min. The absorbances of the supernatant and serum were determined at 650 nm against a formamide blank with a UV-1601 spectrophotometer (Shimadzu, Japan). The protein contents of the samples are determined. Gastric microvascular permeability was expressed as the permeability index (PI), defined as the ratio of the concentration of Evans blue in the mucosa to the concentration in the serum: PI = (Evans blue concentration in tissue)/(Evans blue concentration in plasma).

3. Epithelial permeability changes

Lumen-to-blood directional epithelial permeability changes is detectable by the Na-fluorescein (NaFl) clearance method. NaFl (5 mg/ml) is added to the intestinal lumen. Blood samples were taken from the vein system in 10-min periods, and the NaFl concentration of the plasma is determined with a spectrofluorometer. The blood samples (2 ml) were collected in prechilled tubes containing 250 IU heparin, and immediately centrifuged at 1000 g for 5 min. The samples were stored at 0 °C in the dark for a maximum of 120 min. NaFl concentrations were measured with an fluorescence spectrophotometer (ex: 455 nm, em: 515 nm). The lumen-to-plasma clearance of NaFl is calculated according to the following equation:

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\text{Inward NaFl clearance} = \frac{[\text{NaFl concentration}]_{\text{serum}} \times 100}{[\text{NaFl concentration}]_{\text{test solution}} \times \text{volume}}.
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V. EXPERIMENTAL MODELS IN GASTROINTESTINAL RESEARCH

V.1. Acute model of colon inflammation

Occlusion of the mid-transverse colon was maintained for 420 min in anesthetized dogs. The systemic hemodynamics, mesenteric circulatory changes and inflammatory mediators (leukocyte activation, iNOS) were monitored. Strain-gauge transducers were used to analyze motility changes on the hepatic and lienal flexures, respectively.

V.2. Chronic model of colon inflammation

In animal experiments, 2,4,6-trinitrobenzene sulfonic acid (TNBS) is an appropriate substance for the modelling numerous symptoms of human inflammatory bowel diseases (IBD) or colitis. After a single intracolonal administration it provokes colon ulceration lasting for more than 8 weeks accompanied by weight loss, visceral hyperalgesia, a significant elevation of tumor necrosis alfa (TNFα), inducible nitric oxide synthase (iNOS) and tissue leukocyte accumulation (shown by myeloperoxidase activity) after instillation of TNBS as compared with the control.
REFERENCES