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Research Article

Effect of intragastric administration on the morphology of laboratory rats' gastrointestinal tract

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BACKGROUND: In preclinical studies on laboratory animals, oral administration with special probe for test substances is very in demand. Most studies are devoted to the psychophysiological and clinical-biochemical reaction of the animal's body to this effect, but extremely few studies consider the effect of the manipulation itself on the development of pathology in the tissues of the gastrointestinal tract associated with the inevitable mechanical impact of the probe on the mucous membranes.

AIM: To identify pathological changes in the organs of the gastrointestinal tract of laboratory rats that are associated with the effects of intragastric administration of the tested substances, and compare the data obtained with the frequency of spontaneous diseases.

MATERIALS AND METHODS: The data of pathomorphological observations obtained from Wistar rats involved in the course of scientific work carried out at NPO Dom Pharmacy in the period from 2018 to 2021 were used. 1400 sentinel animals were analyzed, and the same number of intact rats. Intact animals were gavaged with a control substance (distilled water) for 14 days.

RESULTS: In less than 5% of clinically healthy animals, inflammatory diseases of insignificant intensity and prevalence in all parts of the intestine can be detected. As a result of the anipulation of intragastric administration, an almost twofold increase in the number of cases of catarrhal esophagitis and gastritis, erosive and ulcerative lesions of the mucous membrane of the esophagus and stomach in its glandular part and hyperkeratosis in its non-glandular part was noted.

CONCLUSIONS: The type and frequency of occurrence of the background pathology of the gastrointestinal tract of laboratory rats were determined. It has been proven that repeated traumatization of the mucous membrane associated with mechanical contact of a solid metal probe with the epithelium provokes the development of inflammatory diseases of the esophagus and stomach, without affecting the underlying parts of the intestine.

Keywords: biomedical research; rats; drug administration routes; pharmaceutical vehicles; digestive system; historical control data.

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Научная статья

Влияние внутрижелудочного введения на морфологию желудочно-кишечного тракта лабораторных крыс

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Актуальность. В доклинических исследованиях с использованием лабораторных животных широко востребовано пероральное введение тестируемых веществ при помощи специального зонда. Большинство исследований посвящено психофизиологической и клинико-биохимической реакции организма животного на это воздействие, но чрезвычайно мало исследований, рассматривающих влияние самой манипуляции на развитие патологии в тканях желудочно-кишечного тракта, связанных с неизбежным механическим воздействием зонда на слизистые оболочки.

Цель — выявить патологические изменения в органах желудочно-кишечного тракта лабораторных крыс, связанные с воздействием процедуры внутрижелудочного введения тестируемых веществ, и сравнить полученные данные с частотой возникновения спонтанных заболеваний.

Материалы и методы. Были использованы данные патоморфологических наблюдений, полученные от крыс линии Wistar, задействованных в ходе научных работ, выполненных в НПО «Дом фармации» в период с 2018 по 2021 г. Проанализированы 1400 сентинельных животных и такое же количество крыс интактных групп, которым многократно на протяжении 14 дней внутрижелудочно вводили контрольное вещество (дистиллированную воду).

Результаты. У менее 5 % клинически здоровых животных можно выявить незначительные по своей интенсивности и распространенности воспалительные заболевания во всех отделах кишечника. В результате проведения манипуляции внутрижелудочного введения было отмечено почти двукратное увеличение количества случаев катарального эзофагита и гастрита, эрозивно-язвенного поражения слизистой оболочки пищевода и желудка в его железистом отделе и гиперкератоза в безжелезистой части.

Выводы. Определены вид и частота развития фоновой патологии желудочно-кишечного тракта лабораторных крыс. Доказано, что многократная травматизация слизистой оболочки, связанная с механическим контактом твердого металлического зонда с эпителием, провоцирует развитие воспалительных заболеваний пищевода и желудка, без влияния на нижележащие отделы кишечника.

Ключевые слова: биомедицинские исследования; крысы; пути введения лекарств; фармацевтические носители; пищеварительная система; исторический контроль данных.

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BACKGROUND

Oral administration is the most common method of drug delivery in both medicine and veterinary medicine. In preclinical studies using laboratory animals, especially for substances that are tested for safety, oral administration is also widely employed because it simulates the most popular method of drug administration in clinical practice. Oral administration is convenient, relatively simple, safer for animals, and does not require long staff training and expensive equipment, and some animals can be trained to voluntarily take the substance. Although self-administration of the administered compound is ideal, this method of dosing in most cases is unreliable and unsuitable for all animals because of the dosage forms, individual taste preferences of animals in long-term studies, behavioral reactions, and organoleptic qualities of the drug. The dosing accuracy is also affected. A good alternative is the use of an intragastric tube. This is a widely used method for precise administration of oral solutions [1, 2], when the substance to be delivered cannot be added to the feed or is unpalatable [3]. An experienced staff member performs the procedure quickly, without damage to the animal, allowing the exact volume (or dose) of the substance to be delivered directly to the stomach and then to the intestine, from where it can be absorbed [4]. Intragastric probes vary, differing in materials (rubber, plastic, silicone, and metal), rigidity (flexible and rigid), and shape (straight and curved) [5], and lubricants can be also used. Despite this wide range, problems associated with the use of probes may occur, especially when used for long periods [1] or when multiple daily injections are required [2]. These complications can be divided into physiological, involving the response of the animal body to the stressful effects of manipulation, and iatrogenic, related to the procedure itself and staff qualification, such as aspiration pneumonia, unintentional insertion into the airways, severe esophageal trauma, and gastric rupture [2, 6–8]. Although the majority of studies are devoted to psychophysiological, clinical, and biochemical reactions of the animal to, undoubtedly, unpleasant actions, extremely few studies consider the influence of manipulation on the development of pathologies in the tissues of the gastrointestinal tract (oral cavity, esophagus, and stomach), which are linked to the inevitable mechanical influence of the probe on mucous membranes.

The main *purpose* of the study was to determine whether, and most importantly how, the use of intragastric probes affects pathomorphological changes in organs of the gastrointestinal tract of laboratory animals. However, there was one more question: is the detected pathology a result of the influence of manipulation or a spontaneous (background) change? This refers to the abnormalities already present in animals at the time of the study, which may be congenital or acquired, physiological or pathological, or a unique characteristic of the species. Thus, these changes in tissue morphology are outside the range of normal variation in the population [9, 10]. Often, they can interfere with the analysis of tissue morphology; thus, the researcher must be aware of them and be able to interpret the data obtained, differentiating the possible negative effect of the studied substances from spontaneously occurring diseases of laboratory animals. Unfortunately, although this problem is discussed in the available literature, these include very few works and have a scattered, rather than a descriptive character [11, 12], stating the possible presence of diseases, but not giving information about their share in the population. We tried to correct this situation to determine the background pathology of the gastrointestinal tract organs of laboratory rats and identify the frequency of its occurrence in clinically healthy rats. The obtained information will help researchers in preclinical studies to interpret the data they receive; however, we should not forget that a proportion of possible pathology depends not so much on the species and line of animals but on the living conditions.

MATERIALS AND METHODS

Before the study, to identify the incidence of background gastrointestinal pathology in laboratory rats and the effect of intragastric manipulation on this indicator, the literature sources available in PubMed, Medline, and eLibrary were analyzed. Searches were performed without regard to the year of publication, using the keywords “spontaneous,” “background,” “lesions,” “gastrointestinal,” and “control animals.” Further, the most relevant studies, which were less than one hundred, were selected by title and abstract. Among these few works, it was extremely difficult to find a quantitative, rather than descriptive, estimate of disease incidence. Only two sources indicated that approximately 5%–7% of rats have degenerative and inflammatory changes in esophageal epithelium, and inflammation of the gastric mucosa occurs in <5% of animals [13, 14]. The situation was even worse in determining the degree of the effect of intragastric administration on tissue morphology. Further searches for the keywords “gavage” and “intragastric” identified four articles reporting that catarrhal gastritis and esophagitis could be found in 1%–11% of animals receiving the control substance intragastrically for 2–52 weeks [8, 12, 13, 15].

Based on these data, background pathology could be detected in a maximum of 7% of sentinel animals and a maximum of 11% of rats against the background of manipulation. In accordance with these values, the required sample size was calculated using Statistica 10 software, which was determined based on the two-sided

independent *Z*-criterion used to compare proportions and the need to ensure at least 80% power at a significance level of α = 5%. The required number of animals in the study would be at least 1,356 in each group.

Sexually mature Wistar rats, without sex, weighing 200–300 g, were used as biological test systems. Animals subjected to routine euthanasia in the current study performed at the NPO House of Pharmacy (Leningrad Region, Russia) between 2018 and 2021 were selected for the study.

The animals were kept in standard conditions according to Sanitary and Epidemiological Regulations (SanPiN) 2.2.1.3218–14 and Directive 2010/63/EU dated September 22, 2010, concerning the protection of animals used for scientific purposes. For the whole period mentioned above animal diet was not changed, the composition of the used feed corresponded to GOST R50258–92, and water quality corresponded to the requirements of SanPiN2.1.4.1074–01.

Thus, in this study, we used pathomorphological observation data obtained from 1400 sentinel animals, which were not subjected to intragastric injection or other manipulations affecting their health. The same number of intact rats was repeatedly injected intragastrically for 14 days with a control substance, in which distilled water was used. The injection was performed by experienced laboratory assistants, trained accordingly, once a day using a special rigid metal probe equipped with "olive" on the end. The diameter of the "olive," probe length, and injection volumes did not exceed those recommended for this type of animal [16, 17].

In all cases, euthanasia was performed according to the schedule (day 14) of the study using carbon dioxide (CO_2) in a special chamber, followed by external examination of the animals, autopsy, and evisceration of internal organs. The obtained material was fixed in 10% formalin and transferred to the laboratory of histology and pathomorphology, where histological processing of samples according to the conventional methods was performed, and micro preparations stained with hematoxylin and eosin were made.

Statistical analysis

Descriptive statistics were applied to all data. Data were checked for their compliance with the normal distribution using the Shapiro–Wilk criterion. Nominal data were compared using Fisher's exact test. Statistical significance was evaluated at the level of $p < 0.05$. Statistical analysis was performed using GraphPad Prism 9.0 software (GraphPad, USA).

RESEARCH RESULTS

In the majority of animals in both groups, the structure of the studied gastrointestinal organs corresponded to the norm, both macroscopically and microscopically. However, separate pathologies were detected in some animals (Table).

The results of the data analysis revealed statistically significant differences in the incidence of pathology between the groups, as expected, exclusively in the upper gastrointestinal tract (esophagus and stomach), which were subjected to the mechanical impact of the probe. Besides, we should note the comparability of macro- and microscopic manifestations of diseases, which once again proves the necessity of a thorough visual examination of the objects under study (macroscopic examination) and not only "tissue collection" for subsequent histological analysis.

Esophagus

In both groups, the animals macroscopically showed slight hyperemia and small pinpoint erosions. Moreover, hyperemia was observed twice as often during the injection procedure. The result of the histological analysis of esophageal tissue revealed that diseases occurred about twice as often in animals with intragastric injection. Esophagitis presenting small clusters of lymphocytes and plasma cells with a small admixture of neutrophil granulocytes was located predominantly in the lamina of the mucosa (Fig. 1, *a*). Quite often, areas of hyperkeratosis, in which the thickness of the horny layer increased, with slight thickening of the thorny layer, could be detected.

Erosions of the esophageal mucosa in the presence of weakly to moderately expressed mixed lymphoplasma inflammatory infiltration, with moderate amounts of neutrophilic granulocytes, affected small areas of the epithelium and spread deep into the wall up to the mucosa plate, without penetrating deeper than the submucous layer.

A deeper lesion is ulcerous, mostly without signs of healing, deep down to the muscular layer, with moderate and pronounced inflammatory infiltration and numerous neutrophil granulocytes (Fig. 1, *c*). However, in certain cases, signs of mucosal defect healing were observed, such as marginal hyperplasia and "overhanging" of the epithelium along the edge of the ulcer, slightly pronounced fibrosis in the fundus area. The number of neutrophils was significantly reduced, and inflammatory infiltration was mostly represented by lymphocytes, plasmocytes, and macrophages.

Exclusively in animals subjected to the intragastric injection procedure, we visually observed the consequences of iatrogenic pathology, i.e., perforation of the esophageal wall with the probe resulting in a purulent process (phlegmon) confined to the neck fascia, and in two cases spreading into the mediastinum (Fig. 1, *d*). Subsequently, histological examination revealed the pathological process as split inflammation

Table. Incidence of gastrointestinal pathology (%) in Wistar rats

Таблица. Встречаемость патологии желудочно-кишечного тракта (%) у крыс линии Wistar

Microscopically			Macroscopically		
Identified pathology	Group		Identified pathology	Group	
	WG (n = 1400)	Sentinel. (n = 1400)		WG (n = 1400)	Sentinel. (n = 1400)
Esophagus					
Esophagitis	4.5*	2.4	Erosive and ulcerative lesions	1.5	0.9
Erosion	2.1*	1.0	Hyperemia	2.2*	1.1
Plagues	0.8*	0.1	Perforation	0.6*	0.0
Hyperkeratosis	2.9*	1.7			
Perforation	0.6*	0.0			
Epithelial hyperplasia	2.6	2.0			
Stomach					
Glandless erosions	1.1	0.6	Erosive and ulcerative lesions of the gland-free part	1.5	0.9
Glandular erosions	2.2*	1.1	Erosive and ulcerative lesions of the glandular part	3.1*	1.9
Gastritis	6.9*	5.0	Hyperemia	10.6*	8.4
Glandless ulcers	1.0	0.4	Hemorrhages	5.3	4.4
Glandular ulcers	1.9*	0.8			
Hyperkeratosis	5.0*	3.3			
Glandular expansion	5.2	3.9			
Epithelial hyperplasia	0.8	0.5			
Duodenum					
Duodenitis	2.6	2.2	Hyperemia	3.9	4.2
Erosion	0.5	0.6	Hemorrhages	2.2	1.7
Plagues	0.4	0.2			
Small intestine					
Enteritis	4.0	2.9	Hyperemia	3.8	3.4
Erosion	0.7	0.6	Erosive and ulcerative lesions	0.6	0.8
Plagues	0.6	0.5	Hemorrhages	2.8	3.1
Large intestine					
Colitis	5.4	4.4	Hyperemia	3.3	3.8
Erosion	1.2	1.0	Erosive and ulcerative lesions	0.6	0.4
Plagues	0.6	0.7	Hemorrhages	2.4	1.9

Note. WG, animals subjected to intragastric injection procedure; Sentin., sentinel animals. *Differences are statistically significant compared with the sentinel group, Fisher's exact test, $p < 0.05$.

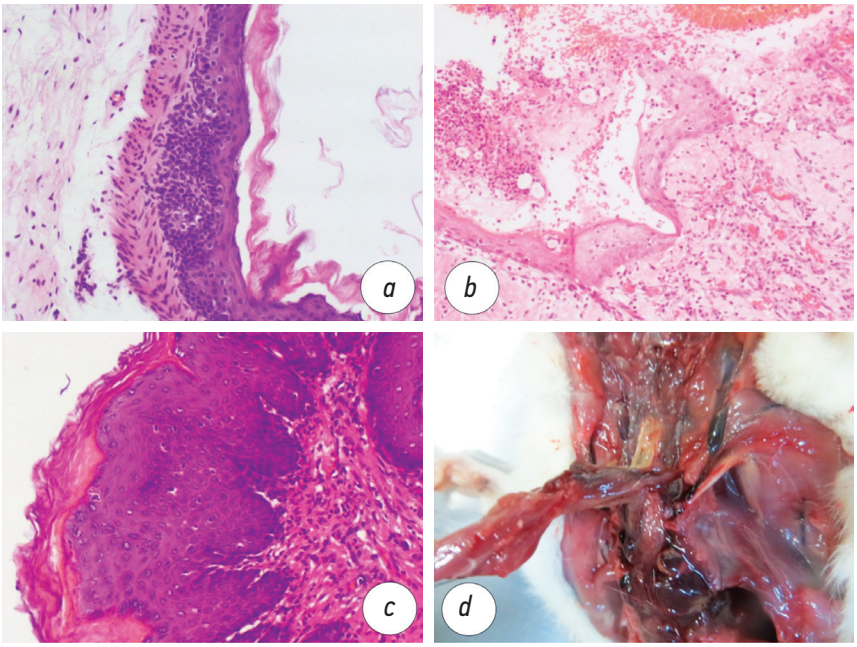

Fig. 1. Pathomorphological changes in the rat esophagus: (*a*) catarrhal esophagitis, hematoxylin and eosin staining, magnification ×100; (*b*) acute ulcer, hematoxylin and eosin staining, magnification ×100; (*c*) epithelial hyperplasia, hematoxylin and eosin staining, magnification ×100; (*d*) soft tissue phlegmon of the neck resulting from the perforation of the esophageal wall by a probe

Рис. 1. Патоморфологические изменения пищевода крыс: *a* — катаральный эзофагит, окраска гематоксилином и эозином, увел. ×100; *b* — острая язва, окраска гематоксилином и эозином, увел. ×100; *c* — гиперплазия эпителия, окраска гематоксилином и эозином, увел. ×100; *d* — флегмона мягких тканей шеи в результате перфорации стенки пищевода зондом

affecting all layers of the esophageal wall and surrounding soft tissues. The cellular composition of the inflammatory infiltrate was mixed with numerous neutrophilic granulocytes. Large areas of tissue necrosis were present in the center and periphery of the focus. In several animals, the inflammatory process changed from an alterative to a proliferative stage, and necrotized tissue replacement by connective tissue with abscess formation could be observed at the periphery of the focus.

Focal hyperplasia of multi-row squamous epithelium of the esophageal mucosa predominantly due to basal layers was observed in both groups in approximately equal numbers (Fig. 1, *c*).

Stomach

In the majority of animals in both groups, the structure of the stomach both macroscopically and microscopically was normal. The stomach was represented by two sections, i.e., glandless and glandular, with distinct borders between them. The mucous membrane was shining, and folding was strongly expressed.

In animals subjected to intragastric injection procedure, we could observe hyperemia of the gastric mucosa more often, which predominantly affected its richly vascularized glandular part, and erosive–ulcerative lesions. Small pinpoint erosions and ulcers could be also seen mostly in the glandular part of the rat stomach and they differed from hemorrhages by a visible change in mucous membrane luster in the defect area, presence of "crater-like depression," and rarely black bottom (Fig. 2, *a*). However, estimating the depth of spread is not possible macroscopically, and a more detailed differentiation was performed during the histological examination.

Dot hemorrhages, from single to multiple, and erosive–ulcerous lesions of the glandless part of the stomach were found in both groups with statistically equal frequency.

Histological examination

Mild manifestations of catarrhal gastritis were detected in both groups (Fig. 2, *b*), namely, focal lymphocytic accumulations with a small or moderate number of neutrophils accompanied by slight edema and full bloodiness of the vascular bed. Inflammatory infiltration was limited to the intrinsic lamina of the mucous membrane; however, spread to the submucosa and glandular epithelium, which was often accompanied by gland enlargement, were noted, and in single cases, microabscesses were formed in the lumen. Several animals showed signs of transition to a chronic disease course (Fig. 2, *c*). Inflammation in the non-glandular part of the stomach occurred in isolated cases. When intragastric manipulation was performed, the number of detected cases of gastritis was statistically higher (Fisher's exact test, $p < 0.05$) than in sentinel animals. Erosions and ulcers in the glandular region of the stomach were detected nearly twice as often in animals from the experimental group. In most cases, the lesion was acute and accompanied by a pronounced perifocal inflammatory reaction of the tissue (Fig. 2, *d*).

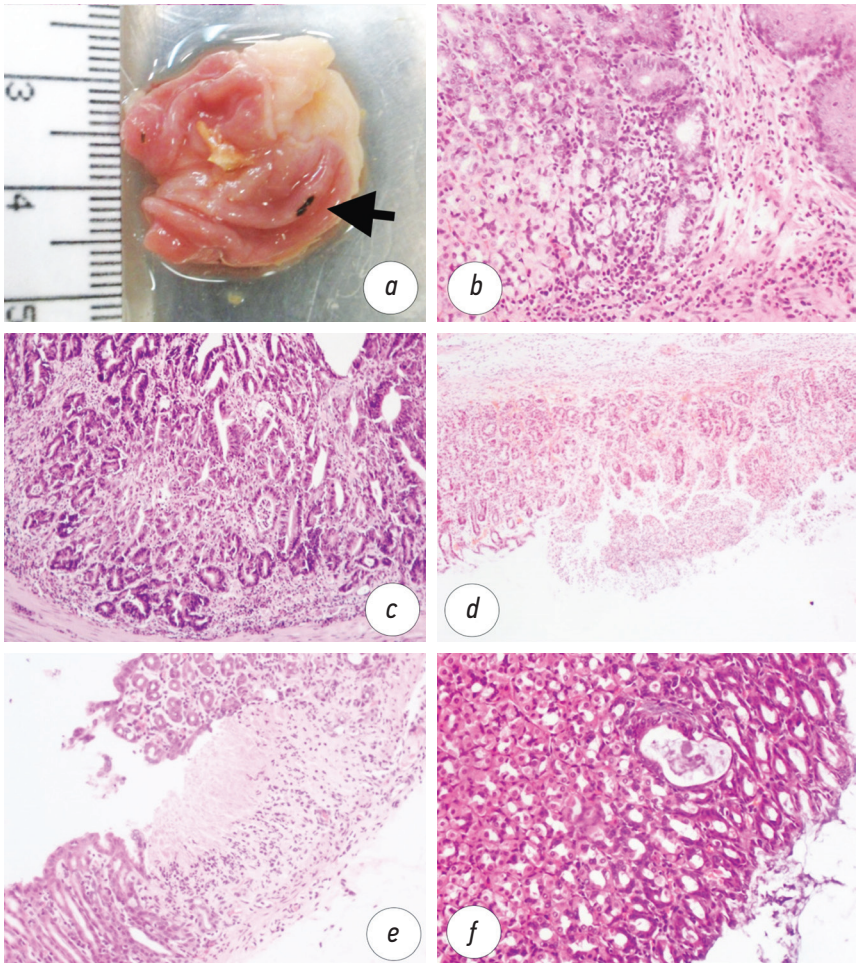

Fig. 2. Pathomorphological changes in the rat stomach: (*a*) erosive and ulcerative defect in the glandular region (arrow); (*b*) acute catarrhal gastritis, magnification ×100; (*c*) gastritis with signs of chronicity, magnification ×100; (*d*) acute glandular erosion, hematoxylin and eosin staining, magnification ×40; (*e*) glandular ulcer in healing stage, magnification ×40; (*f*) dilated glands, magnification ×200, hematoxylin and eosin staining

Рис. 2. Патоморфологические изменения желудка крыс: *a* — эрозивно-язвенный дефект в железистом отделе (стрелка); *b* — острый катаральный гастрит, увел. ×100; *c* — гастрит с признаками хронизации, увел. ×100; *d* — острая эрозия железистого отдела, окраска гематоксилином и эозином, увел. ×40; *e* — язва железистого отдела в стадии заживления, увел. ×40; *f* — расширение желез, увел. ×200, окраска гематоксилином и эозином

Less often, we could observe old ulcers that passed through the acute phase and were in the healing phase (Fig. 2, *e*). Moreover, in the gland-free part of the gastric mucosa, the injection procedure did not significantly affect the development of erosions and ulcers, although an increase in their frequency in this area was observed (0.5%–1%). In both groups, small areas of hyperkeratosis of the multilayer squamous epithelium, which was often combined with epithelial hyperplasia, were detected in the gland-free area. However, the number of pathologies detected in sentinel animals was significantly lower than that in experimental animals (3.3% and 5%, respectively, at $p < 0.05$).

Equally ($p > 0.05$), in both groups, small areas of weak hyperplasia of the glandular epithelium could be observed; however, it occurred in <1% of the animals.

A not uncommon phenomenon found in 3.9% of sentinel animals and 5.2% of experimental rats was the presence of local sites of gland enlargement: in the lumen of closely located enlarged glands, weakly basophilic mucus accumulated, and epithelial cells acquired a cubic shape or were flattened, but showed no signs of atrophy. In addition, this condition was not accompanied by any inflammatory phenomena around the affected area (Fig. 2, *f*).

Intestines

All detected pathological changes were present in both groups, in both sentinel and animals with intragastric manipulation, and no statistically significant differences were found in the frequency of occurrence (Fisher's exact test, $p > 0.05$).

In the macroscopic analysis of all sections of the small and large intestines in 3.2%–4.7% in both groups, hyperemia of separate sections of the intestine could be found, ranging from 0.5 to 4.5 cm in length. In most cases, further histological examination of these sections

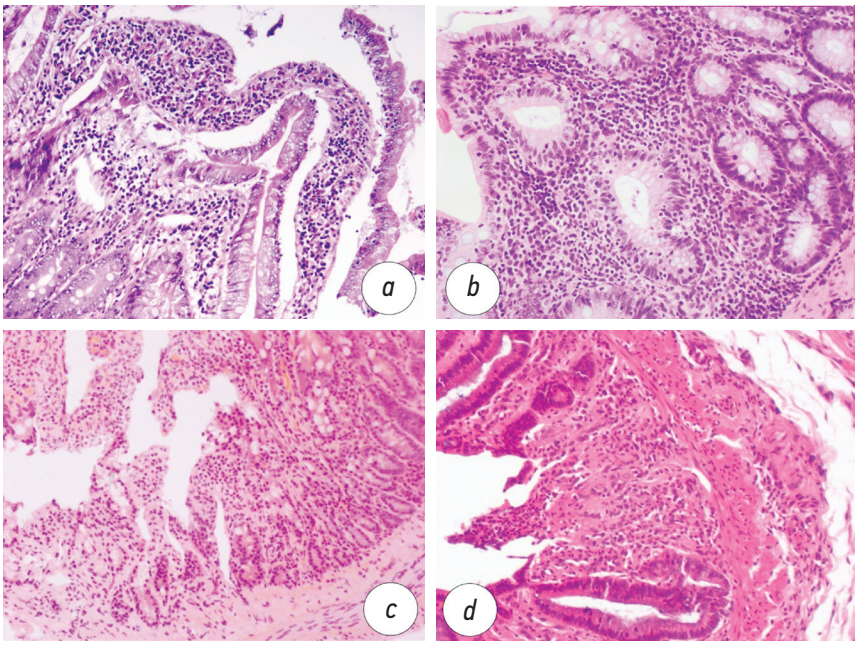

Fig. 3. Pathomorphological changes in the rat intestine: (*a*) catarrhal enteritis, ×100 magnification; (*b*) catarrhal colitis, ×100; (*c*) acute small intestinal ulcer, ×40; (*d*) acute colorectal ulcer, ×100. Hematoxylin and eosin staining
Рис. 3. Патоморфологические изменения в кишечнике крыс: *a* — катаральный энтерит, увел. ×100; *b* — катаральный колит, ×100; *c* — острая язва тонкой кишки, ×40; *d* — острая язва толстой кишки, ×100. Окраска гематоксилином и эозином

revealed that the microscopic structure of the intestinal wall was not disturbed, and only dilation of blood vessels without any accompanying inflammatory reaction was noted.

In 1.9%–2.2% of animals in both groups, small pinpoint hemorrhages from single to scattered in long areas were diagnosed. Microscopically, they represented limited areas imbibed with blood, predominantly in the submucosal layer, partially affecting the mucosal lamina. In the absence of any secondary inflammatory reaction, their appearance was assumed to be the result of the euthanasia of the animals.

The frequency of erosive and ulcerative lesions of the intestinal mucosa also did not depend on the intragastric injection manipulation ($p > 0.05$). Small single erosions and ulcers measuring up to 0.2 cm were detected in both groups in <1% of the rats.

Histological examination

The use of an intragastric probe for substance administration also did not affect the incidence of inflammatory intestinal diseases, which can be characterized by catarrhal duodenitis, enteritis, and colitis of minimal activity (Fig. 3, *a*, *b*). Inflammatory infiltration, represented by lymphocytes, plasmocytes, histiocytes, and neutrophils of a weak or moderate degree of severity, could be found in small areas of the intestinal mucosa in both sentinel and experimental animals. More often, it was limited to the superficial epithelium and its lamina. It had a pronounced character in only a few animals, spreading to the submucosa and, in a few cases, to the muscular layer. Moreover, the height of duodenal and ileum villi was slightly decreased, and reactive hyperplasia of lymphoid follicles was noted. In the colon, crypts were slightly shortened, bocal cells were slightly hypertrophied, and the number and size of lymphoid follicles increased. Chronic inflammatory processes such as mucosal atrophy with damaged structural elements and scleroses occurred in single animals.

No statistical differences in the frequency of erosions and ulcers of the intestinal mucosa were found between the animal groups (Fisher's exact test, $p > 0.05$). Erosions and ulcers were found in <1% of animals, and the number of erosions increased to 1.3% only in the colon. Predominantly small in size, single ones were in the acute stage (Fig. 3, *c*, *d*); however, one could observe in different stages of healing marginal epithelialization, inflammatory infiltration with lymphocytes, plasma cells, and neutrophils in the periphery of the mucosal defect, and at the bottom, numerous fibroblasts are present. In some cases, signs of scarring were observed.

Some findings were not included in the statistical analysis because they were exceptionally rare and found in isolated cases. A congenital malformation of the soft tissues of the gastric mucosa such as a squamous cell cyst located in the ridge area extending to the submucosa and muscularis was detected in one animal (Fig. 4, *a*). The cyst, which was clearly separated from the surrounding tissues, contained keratin plates in the center, and very weakly expressed lymphocytic infiltration was present on the periphery.

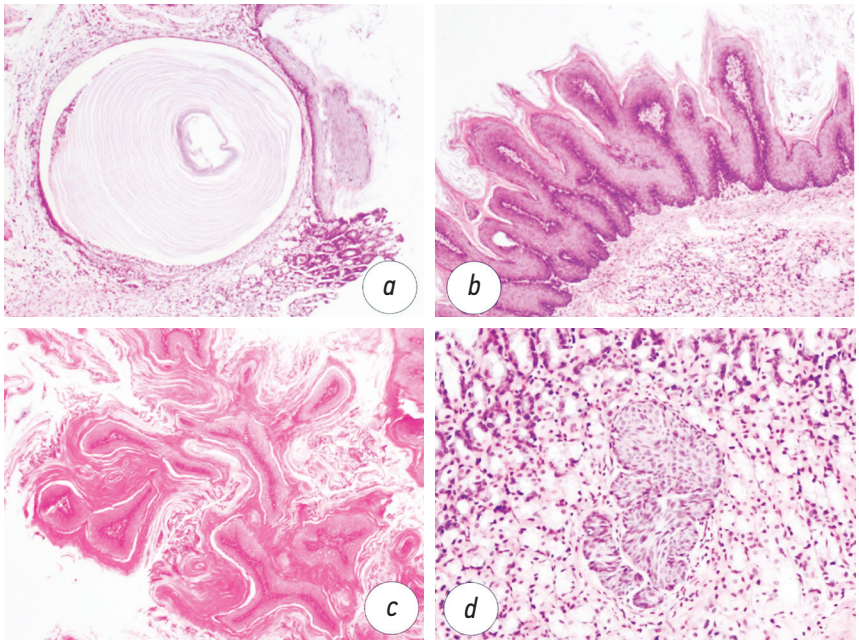

Fig. 4. Pathomorphological changes in the rat intestine: (*a*) gastric squamous cyst, ×40; (*b*) glandless gastric squamous hyperplasia, ×100; (*c*) glandless gastric squamous papilloma, ×40; (*d*) basal cell hyperplasia of glandular epithelium of the stomach, ×100. Hematoxylin and eosin staining

Рис. 4. Патоморфологические изменения в кишечнике крыс: *a* — плоскоклеточная киста желудка, ×40; *b* — плоскоклеточная гиперплазия в безжелезистом отделе желудка, ×100; *c* — плоскоклеточная папиллома безжелезистого отдела желудка, ×40; *d* — базальноклеточная гиперплазия железистого эпителия желудка, ×100. Окраска гематоксилином и эозином

One sentinel animal and two animals that were injected showed areas of squamous cell hyperplasia in the non-glandular part of the stomach (Fig. 4, *b*). In these areas, the squamous epithelium was sharply thickened at the level of the thorny layer, and there was a pronounced proliferation of epithelial cells with increased mitotic activity and increased keratinization. The formation of unbranched papillary outgrowths without a pronounced fibrovascular layer was noted. In one case, a squamous cell papilloma that had a branched well-defined stroma was detected, and the epithelium had no signs of dysplasia but had pronounced hyperkeratosis (Fig. 4, *c*).

In one rat, basal cell hyperplasia was detected. An area of basophilic stained cells was clearly separated from the glandular epithelium of the stomach by an intact basal membrane (Fig. 4, *d*).

CONCLUSIONS

This study revealed that spontaneous pathologies of the gastrointestinal tract in laboratory Wistar rats are not uncommon. In clinically healthy animals, inflammatory diseases of minor intensity and prevalence can be detected in all parts of the intestine. However, these pathological conditions are sporadic and can be detected in not more than 5% of animals for individual diseases. Thus, these indicators are not absolute for the whole species of animals but reflect the state of health in a particular vivarium. The frequency of their development can be affected not only by the sex and age of animals but also by the conditions of the vivariums, i.e., although standardized, they are kept in vivariums with relatively different conditions, such as the composition of the feed or bedding and water quality. Moreover, the psychological state of animals is not an unimportant factor [11, 19, 20].

Determining the frequency of background pathologies in sentinel and intact animals proves that direct manipulation during intragastric insertion influences the morphological state of organs and tissues of the digestive system, but exclusively in its upper part. Imminent repeated traumatization of the mucous membrane associated with mechanical contact of the epithelium with the solid metal probe induces the development of inflammatory diseases of the esophagus and stomach. In this study, in cases of catarrhal esophagitis and gastritis, the incidence of erosive–ulcerative lesions of the mucous coat of the esophagus and stomach in its glandular part and hyperkeratosis in its non-glandular part increased by nearly twofold. However, the injection procedure did not affect the lower intestinal parts, and no differences in the frequency of pathology development were revealed.

The exceptionally damaging effect of manipulation can explain the few cases of iatrogenic pathologies, i.e., esophageal perforation, followed by severe purulent processes in soft tissues. However, the probability of such severe damage directly depends on the qualification of the personnel, duration of insertion, and psychological state of the animals, i.e., their readiness to tolerate this manipulation [1–3].

In the course of the morphology study of the gastrointestinal tract, several single pathologies were reported, as described in the literature [10, 11, 20], but are extremely rare. These include squamous cell cysts, basal cell and squamous cell hyperplasia of the gastric epithelium, and papilloma of its gland-free part. If the pathomorphologist is "lucky" to find these structures in the study of animal organs and tissues, they should not immediately attribute their appearance to the influence of the studied substances. The object being tested, its supposed mechanism of action, and its study duration must be evaluated. However, this also applies to the more frequently observed pathologies of the digestive system. The researcher must not only record the presence of diseases but also, when forming a conclusion, differentiate the background pathology from the pathology provoked by the administered substance. For example, one should not expect a picture of chronic atrophic gastritis after a single injection and an acute ulcer at the end of a delayed 2-week follow-up. Nevertheless, this is a topic for a separate discussion, and the results of this study will be useful to both pathomorphologists who directly study organs and tissues of laboratory animals and specialists who conduct preclinical studies to analyze the information obtained and more fully compare the results.

ADDITIONAL INFORMATION

Authors' contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. The contribution of each author: Ya.A. Gushchin, M.N. Makarova — manuscript drafting, writing and pilot data analyses; M.N. Makarova, P.D. Shabanov — paper reconceptualization and general concept discussion.

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