# NEUROHISTOCHEMICAL STUDY OF INTESTINAL INNERVATION IN EXPERIMENTAL SPREADING PERITONITIS

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UDC 616.381-002.3-092.9-07:616.34-018.8-008.9-074

KEY WORDS: spreading peritonitis, intestine, innervation.

Paralytic ileus usually accompanies spreading peritonitis and is frequently the cause of death [3, 9]. Investigations have yielded indirect data on the role of disturbance of the autonomic innervation of the intestinal wall in the development of intestinal paresis [1, 4, 7]. However, this problem has been inadequately studied at the present-day level. Accordingly, the object of this investigation was a histochemical study of the cholinergic and adrenergic innervation of the muscular layer of the intestinal wall in experimental peritonitis.

## EXPERIMENTAL METHOD

Spreading peritonitis was induced in 25 noninbred male albino rats weighing 180-220 g injecting a 10% rat fetal suspension in physiological saline intraperitoneally in a dose of 0.8 ml/100 g body weight. The rats were decapitated under superficial ether anesthesia 2, 4, 24, 48, and 72 h after injection of the suspension, five animals at each time; seven rats constituted the control group. Pieces of the wall of the terminal ileum and the rectosigmoidal portion of the large intestine were obtained from the rats, and frozen sections 20 μ thick were cut from them. Cholinergic nerve fibers were stained by the method of Karnovsky and Roots [2, 8]. The intensity of the reaction for acetylcholinesterase (AChE) was assessed in the sections on a 4-point scale, and the density of cholinergic fibers, namely their number in a standard area of 1 $mm^2$, was determined by means of an ocular grid under magnification of the microscope objective of 40 times. The mean length of the nerve fiber and the mean number of varicose thickenings per fiber were measured on photographs and the number of varicose thickenings per 100 μ length of fiber was calculated. Adrenergic structures were revealed by condensation with glyoxylic acid [6]. The sections were examined in the LYUMAM-IZ luminescence microscope. The length of individual fibers and the diameter of the varicose thickenings were measured on the photographs, the number of thickenings per fiber was counted, and the number of varicose thickenings per 100 μ length of fiber was calculated.

## EXPERIMENTAL RESULTS

At autopsy on the rats 2 h after injection of the suspension, a very small quantity of serous exudate was observed in the peritoneal cavity, with hyperemia of the peritoneum. After 4 h about 5 ml of seropurulent exudates was found in the peritoneal cavity, and the peritoneum was dull. After 24 h, 4 ml of seropurulent exudate was present in the peritoneal cavity, the parietal and visceral layers of the peritoneum were adherent to the omentum, dull, and hyperemic, and covered with fibrinous deposits. After 48 h, the peritoneal cavity contained about 4 ml of purulent exudate, there were masses of fibrinous deposits on the peritoneum, and loose adhesions between the peritoneum and loops of intestine, the latter being swollen and filled with liquid contents. After 3 h the greatly distended intestinal loops with fibrinous deposits were fused into a single conglomerate, adherent to the peritoneum. Thus macroscopically paresis of the intestine was observed at all times of the experiment, but was most severe on the 2nd-3rd day of spreading peritonitis. Histochemical examination of the control rats revealed a rich network of cholinergic fibers in the smooth-muscle membrane of the small and large intestine, and the intensity of the reaction for AChE was estimated at 3 points. Marked AChE activity was determined in ganglionic structures, and weaker activity (2 points) in the intermuscular bands (Fig. 1a). The cholinergic

Department of Pathological Anatomy, Second Faculty of Internal Medicine, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 240-243, February, 1988. Original article submitted May 26, 1987.

  

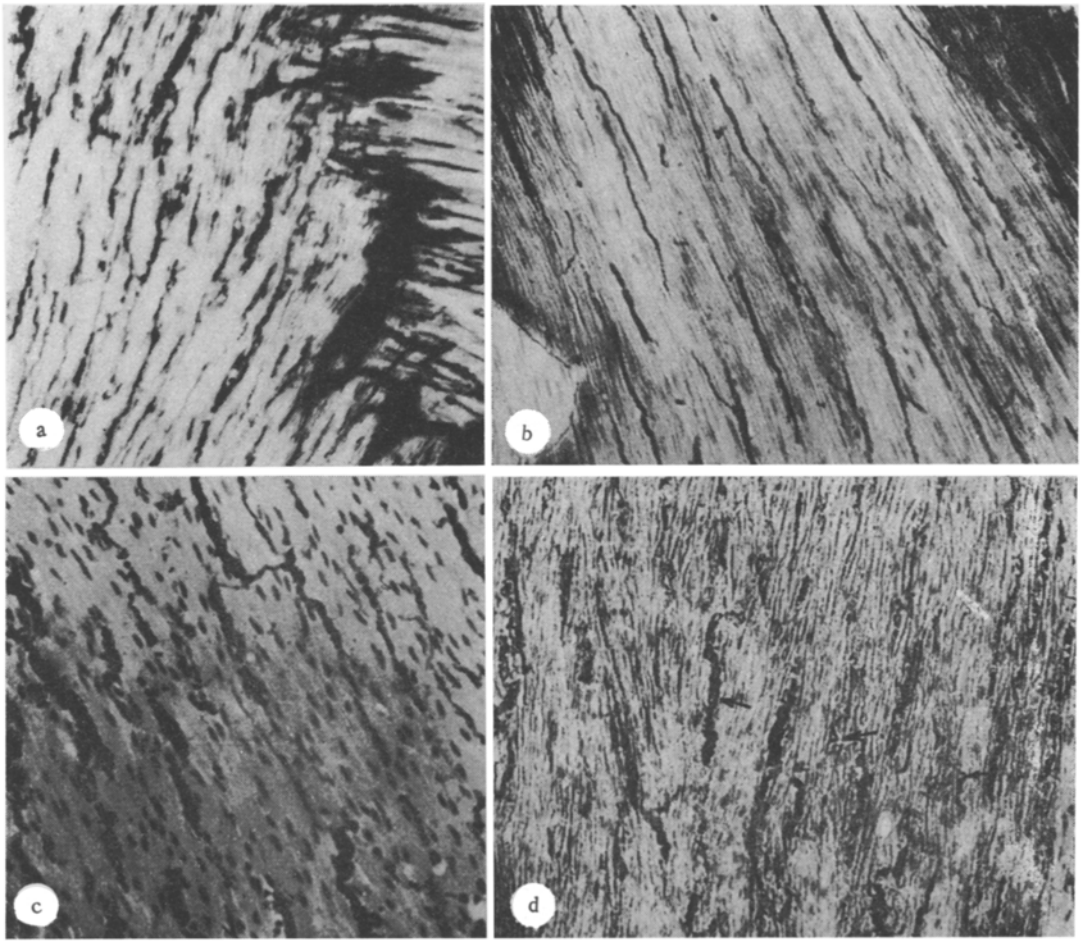


Fig. 1. Cholinergic innervation of muscular layer of small and large intestine of rats under normal conditions and at various times of spreading peritonitis. Histochemical reaction for AChE, longitudinal sections. a) Control, large intestine. Marked (3 points) AChE activity in ganglionic structures and nerve fibers forming a rich network; weaker reaction (2 points) in intermuscular bundles. 240 ×. b) Spreading peritonitis, 4 h, large intestine. Swelling, fragmentation of nerve fibers. AChE activity absent in intermuscular bands. 375 ×. c) Spreading peritonitis, 24 h, large intestine. Unification of network of nerve fibers with AChE activity reduced to 2 points; d) diffuse peritonitis, 48 h, small intestine. Decrease in AChE activity of nerve fibers to 2 points and disappearance of reaction in some varicose thickenings. 240 ×.

fibers were uninterrupted, uniform in thickness, contained separate varicose thickenings, and were located between the muscle cells or groups of them, and in some places they formed bundles. Nerve fibers in the large intestine appeared thicker. In spreading peritonitis, swelling, fragmentation, and tortuosity of the fibers were observed in both the small and the large intestine (Fig. 1b). AChE activity in the intermuscular bands had disappeared. Changes in the structure of the nerve fibers 24, 48, and 72 h after reproduction of peritonitis were uniform in type, but were more severe, and their network appeared to be joined together (Fig. 1c). The intensity of the reaction for AChE was appreciably modified after 2 days of peritonitis. For instance, after 48 h AChE activity of the fibers still remained at 2 points, but in some varicose thickenings the reaction had disappeared, and they were replaced by round empty spaces (Fig. 1d). After 72 h the intensity of the AChE reaction in the fibers and their varicose thickenings fell sharply to 1 point. Quantitative analysis (Table 1) showed that the length of the cholinergic fibers at different times of peritonitis was virtually unchanged. The time course of the number of varicose thickenings was similar in the small and large intestines, and followed a wave like course. For instance, in the small intestine their number was reduced by 33-50% after 2 h of peritonitis ($p < 0.01$), it returned to its original level at the 4th hour of the process, increased somewhat up to 24 h, and then decreased again by 33-50% after 72 h of peritonitis.

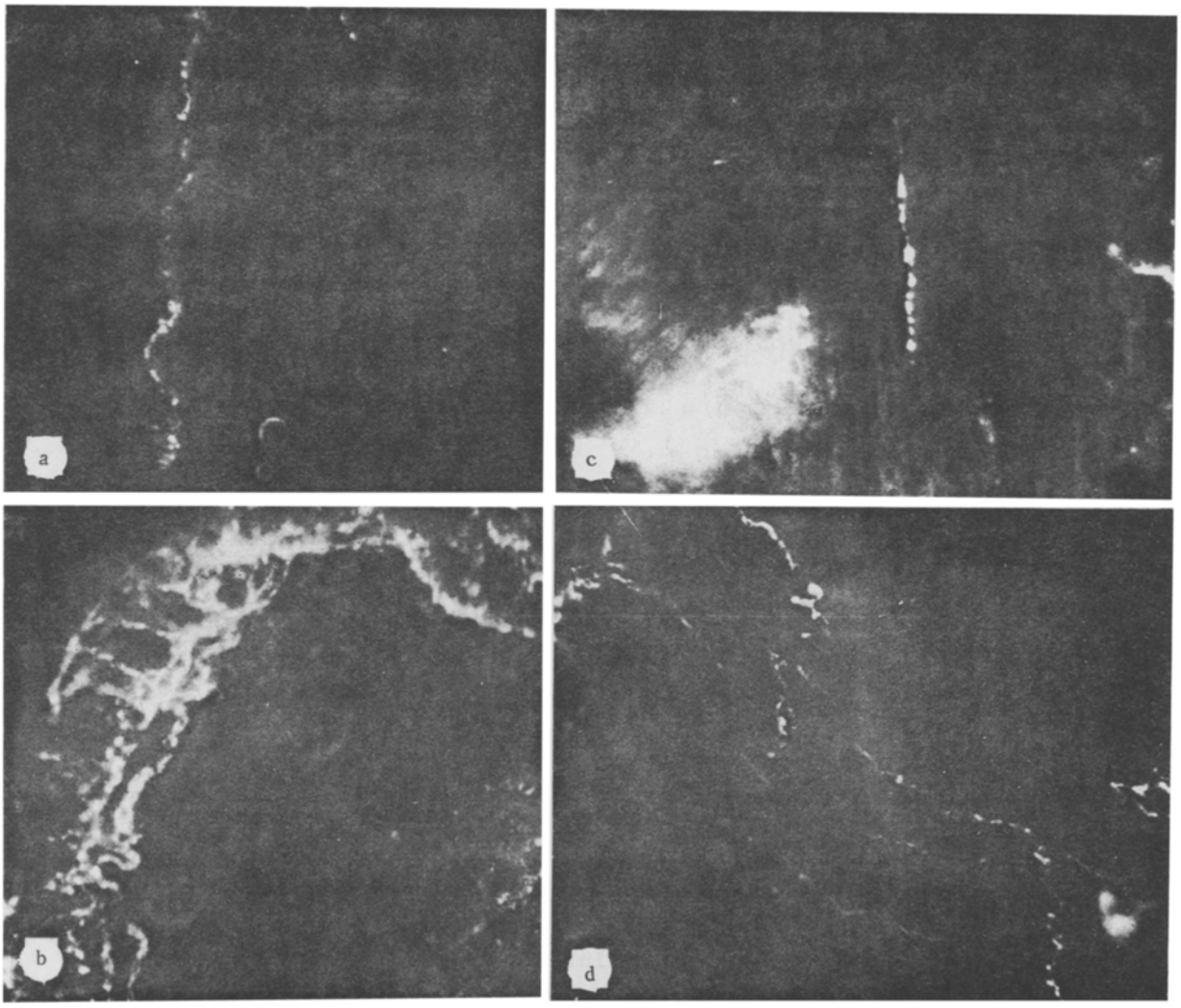


Fig. 2. Adrenergic innervation of muscular layer of small and large intestine under normal conditions and at different times of diffuse peritonitis. Reaction with glyoxylic acid, longitudinal sections. a) Control, large intestine. Single nerve fiber in circular muscular layer. 750 ×. b) Control, large intestine. Dense plexus on nerve fibers in adventita of intramural artery. 120 ×; c) Diffuse peritonitis, 24 h, large intestine. Separate nerve fiber with numerous intensely fluorescent varicose thickenings. 450 ×; d) Diffuse peritonitis, 72 h, small intestine. Reduction of intensity of fluorescence of nerve fibers. 450 ×.

These changes developed somewhat later in the large intestine. By 48 h the number of varicose thickenings was reduced by 67-75% compared with the control, and by 89-90% compared with 24 h of peritonitis. After 72 h the number of varicose thickenings was beginning to recover (Table 1). The density of the fibers was reduced after 72 h of peritonitis by 67-75% ($p < 0.01$).

The adrenergic network of nerve fibers in the muscle coat of the small and large intestines of the rats was thinly distributed, and the nerve fibers were found mainly in the circular muscle layer between groups of smooth muscles and they contained many varicose thickenings, giving off fluorescence several times more intense than that in other parts of the fiber (Fig. 2a). Nerve fibers penetrated into the intestinal wall along the course of blood vessels and formed dense plexuses in the adventita of the intramural arteries and arterioles of the intestine, surrounding the vessel on all sides, and resembling cuffs in shape (Fig. 2b). In spreading peritonitis, the structure of the adrenergic fibers and the density of their network remained visually unchanged. However, after 2, 4, 24, and 48 h of the pathological process the intensity of fluorescence of the fibers was increased somewhat (Fig. 2c), but after 72 h it was reduced (Fig. 2d). The morphometric parameters of the adrenergic plexus were more significantly changed than the parameters of the cholinergic

TABLE 1. Quantitative Characteristics of Cholinergic Nerve Fibers at Different Times of Spreading Peritonitis (M ± m)

| Time of sacrifice of animals | Small intestine | | | Large intestine | | |
|---|---|---|---|---|---|---|
| | number of varicose thickenings in 100 μ length of fiber (n = 30) | density of fibers per mm² (n = 16) | length of fibers, μ (n = 30) | number of varicose thickenings in 100 μ length of fiber (n = 30) | density of fibers per mm² (n = 16) | length of fibers, μ (n = 30) |
| Control | 6,06±0,39 | 191,46±5,00 | 86,32±8,51 | 5,46±0,46 | 214,79±6,67 | 102,59±8,08 |
| Peritonitis | | | | | | |
| 2 h | 3,78±0,45* | 160,21±6,67 | 66,13±7,6* | 4,47±0,45* | 191,25±11,88* | 104,53±10,45 |
| 4 h | 6,39±0,45 | 145,83±6,46 | 69,95±6,45* | 7,95±0,87 | 161,46±10,83 | 73,87±9,39 |
| 24 h | 6,6±0,51 | 109,38±5,42* | 79,81±6,91 | 12,76±0,17* | 118,54±7,29* | 70,32±6,27 |
| 48 h | 5,65±0,38 | 58,54±6,88* | 87,92±6,03 | 1,89±0,23* | 33,96±4,79* | 86,32±7,23 |
| 72h | 3,04±0,22* | 54,79±7,29* | 86,40±6,12 | 3,11±0,31 | 58,54±3,96* | 115,62±8,64 |

Legend. Here and in Table 2: n) number of experiments, *p < 0.05 compared with control.

TABLE 2. Quantitative Characteristics of Adrenergic Nerve Fibers at Different Times of Spreading Peritonitis (M ± m)

| Times of sacrifice of animales | Small intestine | | | large intestine | | |
|---|---|---|---|---|---|---|
| | number of varicose thickenings in 100 μ length of fiber (n = 30) | diameter of varicose thickenings, μ (n = 30) | length of fibers, μ (n = 16) | number of varicose thickening in 100 μ length of fiber (n = 30) | diameter of varicose thickenings, μ (n = 30) | length of fibers, μ (n = 16) |
| Control | 10,65±0,80 | 3,03±0,19 | 49,84±5,06 | 18,28±0,83 | 3,03±0,55 | 38,67±4,49 |
| Peritonitis | | | | | | |
| 2 h | 12,33±0,59 | 4,68±0,05 | 64,37±5,15 | 16,23±2,53 | 2,93±0,12 | 67,09±12,76 |
| 4 h | 17,64±1,80 | 5,73±0,36* | 41,11±3,58 | 13,43±1,95 | 5,04±0,29 | 56,80±6,02 |
| 24 h | 15,64±1,53 | 5,00±0,24* | 55,56±5,27 | 20,70±2,06 | 7,11±0,42* | 60,69±5,87 |
| 48 h | 19,06±2,07* | 6,04±0,33* | 80,69±7,24 | 23,97±2,28 | 4,84±0,31 | 91,53±5,30* |
| 72 h | 20,65±2,46* | 3,16±0,18 | 75,98±7,78 | 29,27±3,45 | 4,22±0,20 | 65,56±5,96 |

fibers (Table 2). A tendency was noted for the fibers to lengthen toward 48 h of the experiment. The length of the fibers in the large intestine was increased by 50-100%, but in the small intestine the change in this parameter was not significant. A tendency was observed for the number of varicose thickenings per 100 μ length of fiber to increase as the spreading peritonitis progressed. However, whereas in the small intestine this parameter increased by 50-100% after 72 h ($p < 0.01$), its change in the large intestine was not significant. An increase in diameter of the varicose thickenings was observed after 4, 24, and 48 h of peritonitis ($p < 0.01$), with a peak after 48 h in the small intestine and after 24 h in the large intestine. After 72 h the diameter of the varicose thickening decreased and approached the control values.

The investigation thus showed that intestinal paresis associated with spreading fecal peritonitis in rats was accompanied by a marked disturbance of the cholinergic innervation of the small and large intestine, and the time course of the number and AChE activity of the varicose thickenings, which are sites of synthesis and storage of neurotransmitter, assumes particular importance under these circumstances [5]. The adrenergic innervation, which under normal conditions also is represented in the intestinal wall in the form of infrequent fibers, was less severely affected. It can be concluded from these results that intestinal paresis associated with peritonitis is due primarily to a disturbance of the cholinergic branch of the autonomic innervation of the muscular coat of the intestine.

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# MICROCIRCULATION AND ANGIOGENESIS DURING WOUND HEALING BY FIRST AND SECOND INTENTION

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UDC 617-001.4-003.9-07:[616.16-005+616.16-003.9]-031.84-07

KEY WORDS: microvessels, wound inflammation, microcirculation, angiogenesis.

A favorable course of wound healing largely depends on the state of the microcirculatory bed, the depth of spread, intensity, and duration of the inflammatory reaction, and also the promptness and intensity of blood vessel formation in the wound region [2, 6, 8, 9, 11-14]. Impairment of tissue nutrition through direct injury to microvessels as a result of trauma, and the disturbance of their function during inflammation lead to the appearance of areas of primary and secondary necrosis [5, 6, 12]. Newly formed blood vessels account for the main mass of granulation tissue [2], and the accumulation of fibroblasts in the wound and collagen production, which processes are possible only if the blood supply is adequate [6, 11, 13] and, finally, an adequate blood flow in the course of healing, all exert a favorable influence on the mechanical strength of the resulting scar [9]. Changes in the microcirculation during wound inflammation [6, 8, 11, 12, 14] and the times, sequence, and possible changes in stimulation of angiogenesis [4, 11, 12] have now been well studied. However, virtually no research has been undertaken with the aim of a direct analysis and quantitative characterization of these processes under conditions close to those found during the natural course of reparative regeneration of soft tissue wounds.

The most labile parameters of the microcirculatory bed are the diameter of the microvessels and the number of capillaries simultaneously involved in the blood flow (the functioning capillary density — FCD) [7, 14]. The study of their dynamics and a description of the changes in the microcirculation during healing of experimental wounds of the subcutaneous areolar tissue were the aims of the investigation described below.

## EXPERIMENTAL METHOD

Experiments were carried out on 72 Wistar rats weighing 150-200 g, divided into three groups. Under sterile conditions a linear wound of the skin and subcutaneous areolar tissue 3 cm long, in the interscapular region, was inflicted with a sharp scalpel on the 32 animals of group 1 under sterile conditions. The incision in the subcutaneous areolar tissue was closed by interrupted gauge 5-0 silk sutures on an atraumatic needle, and the skin wound was closed by a continuous intradermal silk suture. In the 32 animals of group 2 the wound was left open and infected with a suspension of a 24-h culture of *Staphylococcus aureus* ($10^9$ microbial cells/ml) and covered with a gauze dressing. The eight animals of group 3 were used to study the microvessels in the intact subcutaneous areolar tissue.

The microvessels were selectively stained by a modified method [10] based on the study of the spread of a peroxidase preparation of plant origin, injected into the blood stream. Workers who have used a similar method to reveal microvessels in the omentum and skeletal muscles also have noted its advantages over traditional methods, namely the possibility of detecting functioning microvessels, and the minimal deformation of the vessels compared with intravital conditions [3], so that the evaluation can be based on the criteria of biomicroscopy.

Research Institute of Laser Surgery, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 243-246, February, 1988. Original article submitted January 5, 1987.

 