

*Materials of Conferences***BLOOD CIRCULATION LEVEL IN GASTRIC WALL IN EXPERIMENT**

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The state of gastric wall and vagus nerve blood circulation was investigated rheographically and by the method of hydrogen clearance according to Smith D.R. (1977) in 17 animals (dogs). The main group consisted of 12 dogs, the control one – of 5 dogs. The animals' average age was 3,4 years, their weight –  $6,8 \pm 0,5$  kg. The methodology is based on the polarographic registration of the tissue hydrogen clearance. The registration was carried out through platinum and silver-chloride electrodes introduced into the gastric wall in the course of operation, then – once a day. The main group consisted of experimental animals, which submaximal doses of substrate antihypoxant "reamberin" in combination with prostaglandin were applied to. The given preparations were not used in the control group. The investigation purpose was to define the role of the substrate antihypoxant and prostaglandin in the gastric blood circulation disorders prophylaxis. It was found out that the gastric wall blood flow value (B) in both groups for the moment of the operation performance were high enough and were registered as  $185 \pm 15,3$  and  $175 \pm 16,3$  ml/min /100. Thereat, these values were a bit higher in the main group. In the postoperative period a significant blood flow index decrease was registered in both groups in the space of an hour after suturing. The highest decrease was registered in the control group – up to  $59,5$  ml/min /100 (2,9-fold), in the main group -  $79,8$  ml/min/100 (2,3-fold). The analysis of gastric wall blood circulation establishment dynamics in the following seven days testified that the process intensity in the groups differed in the degree of approximation to the norm. In the main animal group the values changed with a greater intensity – on the first day after the operation already  $B = 126,2 \pm 42,1$  ml/min /100 (or 46,5% of the original one). Later a relatively uniform blood flow level increase was found out in both groups and with the same value increase. However, these values' increase rate downtrend in both groups was registered since the 4<sup>th</sup> day. It was found out that a higher blood circulation in the gastric wall in the early postoperative period was one of the conditions for the gastro-intestinal tract motor function recovery. The application of substrate antihypoxant and prostaglandin increasing the level of tissues' oxygenation and microcirculation improves the gastric blood flow indexes both during the operation and in the postoperative period.

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**BLOOD CIRCULATIONS LEVEL IN VAGUS NERVE AFTER GASTRIC OPERATION**

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The state of blood flow in the vagus nerve gastric branches was studied in 17 animals (dogs). The main group consisted of 12 dogs, the control one – of 5 dogs. The animals' age was from 2 to 5 years, they weighing  $6,8 \pm 0,5$  kg. The blood flow was defined in the nerve stem of the nervus Vagus by the hydrogen clearance method according to Smith (1977). Active electrodes were introduced through an epineurium puncture hole subepineurially. The introduction and fixation were performed in the enlargement of a surgery microscope. The blood flow was calculated according to Aukland (1964). The main group consisted of the animals, which submaximal doses of substrate antihypoxant "reamberin" in combination with prostaglandin were applied to. The specified preparations were not used in the control group. The n. Vagus blood flow level comparison in the experimental animals testified that an analogous picture was traced in the gastric wall. In the main group the blood flow values were higher during the operation, than in the control one ( $66,2$  and  $46,2$  ml/min /100 accordingly). This value fall was significant enough after the operation. Especially in the control group – up to  $22 \pm 3,1$  ml/min /100 (that is 2,1 times less, than the original one). Such a decrease of the blood flow level was registered in the main group as well, but the given parameter value didn't fall lower, than the average mark of  $32,1 \pm 3,1$  ml/min/100. The dynamics of blood flow establishment in the n.Vagus testified that the given process took course most intensively in the nerve tissue on the first day after the operation. Then a relative retardation of the establishment rate was registered. On the average, during a day the value increases by  $1,5$  ml/min /100. By the end of the investigation the average values of the blood flow volume were at the level of  $41,1 \pm 6,1$  in the main group, and  $34,1 \pm 6,0$  ml/min /100 – in the control one. The findings' analysis testifies that the blood flow values are not constant in the course of the experiment; they are subjected to significant fluctuations. These fluctuations are unidirectional both in the animals with the prophylactic application of the substrate antihypoxant and prostaglandin and without it. The blood flow decreases considerably in nerve stems after the operation irrespective of the original level (twice). The most low blood flow values are registered in the group of the animals, which didn't receive the substrate antihypoxant in combination with prostaglandin.

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### STRESS-RELATED ADAPTATION CHANGES IN THE SPLEEN DURING EARLY POSTNATAL DEVELOPMENT

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Interconnections between the integrative systems, such as nervous, endocrine, and immune ones, are clearly seen during a stress response. The sources of such a cooperation should be sought at the earliest stages of development. Early negative life events, especially during the neonatal period, resulted in long lasting, irreversible effects on well being. Neonatal stress has implications for host resistance to infection throughout life. Thus, long lasting effects of negative life events on health and disease may be the basis for the individual differences in host susceptibility to infection, malignancy and autoimmune disorders. Age-related aspects of the reduced immunity following stress exposure in terms of possible mechanisms of their development remain not fully understood (**I.G.Akmaev et al., 2002; S.K.Butcher et al., 2005; R.Avitsur et al., 2006**).

The objective of this study was to compare immunomodulatory changes in the spleen as a peripheral organ of immune defense in different age groups of the growing experimental animals under the chronic effect of a severe stressor.

Thirty two Sprague-Dawley rats of the two age groups, each of which included 16 animals, were either exposed to the severe chronic (restraint) stress (**R.Kvetnansky et al., 1970**) with 7 daily 5-hour sessions (eight animals per subgroup) or used as an age-matched control (eight animals per subgroup). The 1<sup>st</sup> age group contained weaning animals aged 21 days and the 2<sup>nd</sup> age group included early postweaning animals age 30 days.

After the last session of stress the animals were weighed and euthanized by cervical dislocation. The lymphoid organs (thymus, spleen and mesenteric lymph nodes) were collected, weighed and processed for histological examination. Formalin-fixed paraffin sections were stained with haematoxylin-eosin and immunohistochemically stained by monoclonal antibodies (Serotek, UK) against rat CD8 (T-suppressor/cytotoxic lymphocytes), CD20 (B-lymphocytes) and CD68 (macrophages) surface markers using ABC-method (**J.Polack, 2000**) with subsequent image analysis of the profiles of the immunoreactive cells on the NIKON camera-captured digital pictures using Image Pro Plus 4.5 software.

At the end of the last stress sessions the body mass of the experimental animals was significantly

reduced in both age groups ( $p < 0,05$ ). Relative splenic mass was also decreased in the experimental animals compared to the age-matched control groups in the weaning and early postweaning pups ( $p < 0,01$ ).

The results of the microscopic investigation presented dramatic immunomodulatory changes in different compartments of the spleen which were mainly localized in the splenic white pulp, with red pulp and marginal zones being also involved. The lymphatic follicles of the experimental rats of both age groups were reduced in number and size, lacked germinal centers and were filled with tingable-body macrophages containing numerous apoptotic bodies. Periarterial lymphoid sheaths also decreased in size mainly at the expense of their inner zone. Tingable body macrophages filled with apoptotic bodies were less common for the periarterial lymphoid sheaths compared to the lymphoid nodules. The marginal zone of the lymphoid nodules and periarterial lymphoid sheaths was reduced in width while this reduction was more prominent in the animals of the weaning experimental group.

The immunohistochemical staining for the CD8 of the control animals spleen exhibited immunoreactive cells localized mainly in the periarterial lymphoid sheaths with fewer cells in the red pulp, marginal zone and mantle zone of the lymphoid follicles. The accumulation of the CD8+ cells increased with age. After chronic exposure to the severe stressor the number of immunoreactive cells in the periarterial lymphoid sheaths was notably reduced with single immunopositive cells still present in the red pulp and marginal zone. Staining for CD20 revealed concentration of the immunopositive cells in the lymphoid nodules with less dense distribution of the immunoreactive cells in the marginal zone and red pulp. After the last stress session the number of the immunoreactive cells appeared to be reduced in the splenic B-zones of the experimental animals of both age groups. Staining for CD68 demonstrated crowding of the immunopositive cells in the red pulp of the experimental and control animals of both age groups. In the lymphoid nodules and the marginal zones of the control rats they were very rare and in the splenic T-zones they were absent. In the experimental animals single immunopositive cells were also visible in the splenic T-zones.

Quantitative immunohistochemical analysis showed that the volume and numeric density of the CD8+ lymphocytes was significantly reduced in the weaning and early postweaning ( $p < 0.001$ ) experimental rats against the control groups of animals. The volume and numeric density of the CD20+ lymphocytes was meaningfully decreased in the weaning ( $p < 0.001$ ) and postweaning ( $p < 0.05$ ) experimental animals accordingly. The changes in the volume density of the CD68+ cells in the experimental animals did not reach the level of significance in both age groups compared to the control rats.