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FUNCTIONAL AND MORPHOLOGICAL STATE OF THE AMPUTATION STUMP MUSCLES AFTER VARIOUS METHODS OF THE STUMP PLASTY

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An assessment of the nature of histochemical and morphological processes in limb stump muscles after various methods of the amputation plasty was given. There were five series of experiments carried out on 55 dogs with the amputation of the hind limb in the middle third of the thigh. There was fascioplasty, myoplasty with suture of the antagonist muscles performed, as well as myodesis with the muscle tension of 916–962 μN, 980–1100 μN, and 650–800 μN. The observation periods consisted of 3 days, 4 weeks, 8 months. The histochemical reactions for determining of succinate dehydrogenase (SDG), NAD·N-dehydrogenase (NAD·H-DH), beta-hydroxybutyrate dehydrogenase (β-HBDG) and lactate dehydrogenase (LDG) were performed on the fresh muscle pieces. The histological examination of the muscles was also carried out. By the end of the observation period, only single unchanged muscular fibers were detected by fascio- and myoplasty. Most of them were replaced by the coarse fibrous connective tissue with fat cells. There was a significant increase in LDG activity (glycolysis) and growth of SDG (Krebs cycle). The activity of NAD·H-DH was high, and the level of β-HBDG significantly reduced. By myodesis with the muscle tension of 916–962 μN the degenerative-dystrophic processes were less significant. There was the decrease in the intensity of the terminal oxidation and oxidation of the ketone bodies observed. The activity of the Krebs cycle increased, and glycolysis grew up. By muscles tension of 980–1100 μN and 650–800 μN there were changes similar to those by fascio- and myoplasty observed.

Key words: amputation, fascioplasty, myoplasty, myodesis, muscles, histochemistry, histology.

Introduction. A lot of works have been devoted to the limb amputations. Recently, the problems of wound healing prognosis [1, 4], pain syndrome [9, 11, 12], amputation techniques in various modifications have been intensively studied [5, 7, 8, 10]. Unfortunately, in these works such important issues as bone stump healing, factors affecting the process of reparative regeneration and stump muscles state were not reflected. We took into account the fact that unsatisfactory outcomes of bone stump healing were observed by 97,1 % of the examined patients [2], and the formation of the functional bone stump occurred only in 10 % of cases [6] during 1–1,5 months after the amputation. Taking into account such disappointing results, it seemed reasonable to study the state of the stump muscles after various methods of its plasty in the experiment.

Objective. To assess the nature of the histochemical and morphological processes in limb stump muscles after various methods of the amputation plasty.

Material and Methods. There were five series of experiments performed on 55 dogs. Under the intravenous thiopental anesthesia (25 mg per 1 kg of weight) the amputation of the right hind limb in the middle third was performed.

During the first series there was the fascioplastic closure of the saw-line performed. During the second series – the antagonist muscles were sewn under the saw-line. During the third, fourth and fifth series – the muscles were fixed to the saw-line through the drilled holes at its edge, respectively, with the tension of 916–962 μN, 980–1100 μN, and 650–800 μN. The muscle tone was monitored by the tonomiometer. The observation periods consisted of 3 days, 4 weeks, 8 months for the I–III series and 8 months for the IV and V series.

For the histochemical study the pieces of the fresh muscle were used which were quickly frozen in the petroleum ether, cooled to –75 °C, and placed in the cryostat where at –18 °C the sections were made with a thickness of 7 μm. To determine the

peculiarities (active and directional) of the metabolic processes in the muscle fibers on different stages of the experiment, the histochemical reactions for determining succinate dehydrogenase (SDG) were established with the help of Nachlas and others method (1957), NAD·H-dehydrogenase (NAD·H-DG), its total activity was determined by the same method, beta-hydroxybutyrate dehydrogenase (β-HBDG) and lactate dehydrogenase (LDG) – by Hess and others method (E. Pierce, 1962) [3]. To determine the activity of the enzymes, the serial preparations of the skeletal muscles were examined on the automatic cytospectrophotometer MPV-2 of "Leitz" company (Germany). The enzyme activity was expressed in absorbance units. The statistical analysis of the material was carried out using the standard methods with application package "MS Excel XP" and "Statistica SPSS 10.0 for Windows" (license number 305147890).

To perform the histological examination, the fragments of the rectus muscle of the femur stump, of 1 × 1 cm in size, taken 2 cm from the end of the stump, were placed in the Schaffer fixation mixture (2 parts of ethanol, 1 part of formalin), then dehydrated and poured into paraffin. Subsequently, the sections of 7 μm thickness were dewaxed and colored with hematoxylin and eosin.

The experiment was carried out in accordance with the principles of humane treatment of animals set out in the directives of the European Community (86 (609) EEC) and the Helsinki Declaration on Humane Treatment of Animals.

The Results and their Discussion. The histochemical examination allowed revealing that all metabolic pathways studied by us were observed in the muscles of the intact dogs (table 1). At the same time, the most significant were the processes of the terminal oxidation, which were judged from the activity of NAD·H-DG and oxidation of the ketone bodies (according to β-HBDG), which are the main substrates of oxidation supplying energy. The second most important supplier of substrates for the terminal oxidation was glycolysis. Attention is drawn to the fact that significantly less attention was paid to the Krebs cycle in these processes. The data obtained correspond to the literary ones.

Table 1. **Activity of the enzymes in the skeletal muscles fibers of the intact animals ($M \pm m$)**

Enzyme	Activity, absorbance units
SDG	5,09 ± 0,15
NAD·H-DG	10,86 ± 0,21
β-HBDG	12,06 ± 0,18
LDG	8,25 ± 0,26

In 3 days after fascioplasty the edema and proliferation of the endomysial cells, as well as activation of connective tissue cells were microscopically determined in the muscles. A large number of muscle fibers had significant degenerative-dystrophic changes. In the sarcoplasm of many of them, there were large vacuoles found, located between myofibrils and sarcolemma. There was the substitution of the dead muscle fibers by the connective tissue. At the same time, in the muscle fibers (table 2) there was a slight increase in the activity of SDG and β-HBDG and, to a greater extent, of NAD·H-DG and LDG, which indicated the activation of the terminal oxidation processes, glycolysis and oxidation of the ketone bodies.

After 4 weeks all the indicated changes progressed. Especially intensive there was the formation of the loose connective tissue.

In comparison with the results of the previous observation period, the activity of NAD·H-DG and LDG decreased in the muscle fibers, as the levels of LDG and β-HBDG increased. Thus, by the end of the 4th week the intensive development of the young connective tissue was accompanied by the decrease in the activity of the glycolytic processes in the muscle tissue. Along with this, the SDG activity was higher than during the first days after the fascioplasty (see Table 2). After 8 months only single unchanged muscle fibers were found, and their main mass had signs of deep degenerative and necrobiotic disorders. A considerable part of them was already replaced

by the formed coarse fibrous connective tissue, in which there were quite a great number of fat cells detected. In some places these cells formed an assembly. There was a sharp increase in LDG activity and the further increase in the level of SDG. The activity of NAD·H-DG was still high. At the same time, the β-HBDG level significantly reduced.

Table 2. **Activity of the enzymes (in absorbance units) in the various types of the skeletal muscle fibers after fascioplasty (*M* ± *m*)**

Enzyme	Postoperative period		
	3 days	4 weeks	8 months
SDG	5,90 ± 0,20	7,64 ± 0,24 *	8,14 ± 0,74 *
NAD·H-DG	17,07 ± 0,31	13,30 ± 0,34 *	12,47 ± 1,06 *
β-HBDG	13,78 ± 0,33	17,05 ± 0,43 *	6,74 ± 0,54 * #
LDG	11,98 ± 0,18	10,99 ± 0,19 *	19,90 ± 0,77 #

*P < 0,05 relative to the indicator on the 3rd day after the operation.
#P < 0,05 relative to the indicator on the 4th week after the operation.

By the morphological study of the muscles of the amputation stump in 3 days, 4 weeks and 8 months after myoplasty, there were the structural changes discovered generally similar to those after fascioplasty.

Some differences were as follows: in the formation of the stump by means of myoplasty, the bands of muscle fiber contraction were more clearly defined and in more quantity, their destruction in the scar tissue was less significant, and there were less number of fat cells found in the muscle fibers. In three days there was the activation of NAD·H-DG observed in the muscle fibers in comparison with the conditional standard. Its level was higher than after carrying out the fascioplasty. Attention was drawn to the decrease in SDG activity in comparison with both the conventional standard and the level after the fascioplasty. The activity of β-HBDG did not change. The level of LDG increased not significantly as after the fascioplasty (table 3).

Table 3. **Activity of the enzymes** (in absorbance units) **in the skeletal muscles fibers after myoplasty (*M* ± *m*)**

Enzyme	Postoperative period		
	3 days	4 weeks	8 months
SDG	3,09 ± 0,39	4,44 ± 0,31 *	13,59 ± 0,34 * #
NAD·H-DG	18,42 ± 0,45	11,74 ± 0,24 *	11,16 ± 1,02 *
β-HBDG	12,21 ± 0,33	15,51 ± 0,47 *	8,79 ± 0,77 * #
LDG	9,16 ± 0,32	8,49 ± 0,41 *	22,98 ± 0,51 * #

*P < 0,05 relative to the indicator on the 3rd day after the operation.
#P < 0,05 relative to the indicator on the 4th week after the operation.

In 4 weeks after the myoplasty, the activity of NAD·H-DG significantly reduced in the muscle fibers in comparison with the previous observation period, and the level of SDG and β-HBDG increased. After 8 months in the skeletal muscle fibers SDG (Krebs cycle) and LDG (glycolysis) were sharply activated. The level of NAD·H-DG decreased somewhat, and β-HBDG activity also tended downward (see table 3).

In 3 days after myodesis with the muscle tension of 916–962 μN there was the interstitial edema and metachromasy of sarcoplasm of the muscle fibers found. In most of them, both longitudinal and transverse striations were well identified. In the course of the individual fibers there were areas of re-contraction, in which the transverse striation was not visible. In a number of fibers there was the granular degeneration. In sarcoplasm of a small number of fibers, there were large vacuoles found, located under the sarcolemma; they were detected also between the myofibrils. The muscle fibers nuclei had an oval shape; they contained a delicate network of chromatin and large nucleoli. The activity of the detected enzymes reduced (table 4).

However, despite of some general decrease in the activity of oxidation-reduction enzymes by myodesis with muscle tension of 916–962 μN, the leading value in the metabolism of muscle fibers appeared to belong to the terminal oxidation (the marker of which was NAD·H-DG), as usual.

Table 4. **Activity of the enzymes** (in absorbance units) **in the skeletal muscle fibers after myodesis with the muscle tension of 916–962 μN (*M* ± *m*)**

Enzyme	Postoperative period		
	3 days	4 weeks	8 months
SDG	3,98 ± 0,24	5,15 ± 0,22 *	7,94 ± 0,46 *#
NAD·H-DG	8,53 ± 0,33	10,43 ± 0,35 *	7,67 ± 0,41 #
β-HBDG	7,55 ± 0,39	11,56 ± 0,66 *	5,90 ± 0,53 *#
LDG	6,73 ± 0,30	9,10 ± 1,25 *	15,32 ± 0,70 *#

*P < 0,05 relative to the indicator on the 3rd day after the operation.
#P < 0,05 relative to the indicator on the 4th week after the operation.

The main suppliers of substrates for it became oxidation of the ketone bodies and glycolysis during the decrease of the Krebs cycle role. In 4 weeks after the operation there were already the muscle fibers with degenerative changes of different degrees of intensity present. As a rule, in the fibers with the significant degenerative-dystrophic changes, the nuclei occupied an unusually central position. The inflammatory cellular infiltration was observed in such areas, the signs of activation of the loose connective tissue became more distinct. Metachromasy of the myoplasm of the individual fibers was still detected. In many of them, the longitudinal and transverse striations were not detected; there were areas of re-contraction of the fibers. In comparison with the previous observation period, there was the increase in the activity of the studied enzymes in all muscle fibers. Despite of the significant dystrophic changes in myocytes, the activity of the terminal oxidation enzymes, the Krebs cycle, and oxidation of the ketone bodies approached the initial values. At the same time, there were signs of increased glycolysis, which gradually became the main source of the substrates for the terminal oxidation (see table 4).

In 8 months the degenerative changes were no longer detected in most muscle fibers. There were both longitudinal and transverse striations well seen in them, in the course of only separate fibers there were still areas of re-contraction. The substitutions of the muscle fibers by the connective tissue were almost not observed. Nevertheless, there was the decrease in the intensity of the terminal oxidation and oxidation of the ketone bodies, but the activity of the Krebs cycle and, in particular, glycolysis increased (see table 4). In 8 months after myodesis with the tension of 980–1100 μN, there were the signs of albuminous degeneration in many fibers, as well as areas of destruction with their replacement by the connective tissue. There was the significant edema and proliferation of endomysium. The activity of SDG and LDG was lower and the level of NAD·H-DG and β-HBDG was significantly higher than after myodesis with the tension of 916–962 μN in the same period (table 5).

Table 5. **Activity of the enzymes** (in absorbance units) **in the skeletal muscle fibers in 8 Months after myodesis with the different muscle tension (*M* ± *m*)**

Enzyme	Muscle tension, μN	
	980–1100	650–800
SDG	6,70 ± 0,16	5,15 ± 0,17 *
NAD·H-DG	15,03 ± 0,45	21,21 ± 0,45 *
β-HBDG	16,16 ± 0,23	21,68 ± 0,31 *
LDG	9,75 ± 0,24	13,10 ± 0,24 *

*P < 0.05 relative to the indicator by muscle tension of 980–1100 μN.

In 8 months after myodesis with the tension of 650–800 μN, in addition to the described changes, there were a large number of fat cells observed, which in some

places formed the extensive assemblies. Many muscle fibers had the signs of degeneration, but there were less of them than in the experiments with the muscle tension of 980–1100 μN. The activity of NAD·H-DG, β-HBDG and LDG was significantly less, and the level of SDG was lower than in the previous experiments with the use of myodesis (see table 5).

Conclusions. 1. The study of the morphofunctional processes in the stump muscles revealed their direct dependence on the essentials and nature of the plastic measures during amputation. The most significant degenerative-dystrophic changes occurred after fascioplastic amputation, when the muscle was losing one of the attachment points and there was the dysfunction occurred. 2. After the myoplastic amputation with suture of the antagonistic muscles, the degree of intensity of the degenerative-dystrophic processes was less and was explained by the chronic super-tension due to the influence of the antagonistic muscles on each other. 3. The least significant changes occurred by the myodesis with the muscle tension of 916–962 μN. 4. The excessive muscle tension caused changes in them, similar to those by the myoplasty. The insufficient tension led to the processes similar to fascioplasty.

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ФУНКЦІОНАЛЬНИЙ ТА МОРФОЛОГІЧНИЙ СТАН М'ЯЗІВ АМПУТАЦІЙНОЇ КУКСИ ПІСЛЯ РІЗНИХ СПОСОБІВ ЇЇ ПЛАСТИКИ

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Наведено оцінку характеру гістохімічних та морфологічних процесів у м'язах кукси кінцівки після різних способів ампутаційної пластики. Проведено п'ять серій дослідів на 55 собаках з ампутацією задньої кінцівки на рівні середньої третини стегна. Виконано фасціо- і міопластику із зшиванням м'язів антагоністів, міодез з натягом м'язів 916–962 мкН, 980–

1100 мкН і 650–800 мкН. Терміни спостереження – 3 дні, 4 тиж, 8 міс. На шматочках свіжих м'язів виконували гістохімічні реакції з визначенням сукцинатдегідрогенази (СДГ), НАД·Н–дегідрогенази (НАД·Н-ДГ), бета-оксибутиратдегідрогенази (β-ОБДГ) та лактатдегідрогенази (ЛДГ). Виконували гістологічне дослідження м'язів. Встановлено, що наприкінці терміну спостереження при фасціо- і міопластиці виявляли лише поодинокі незмінені м'язові волокна. Більшість їх була заміщена грубою волокнистою сполучною тканиною з жировими клітинами. Спостерігали різке збільшення активності ЛДГ (гліколіз) та підвищення СДГ (цикл Кребса). Також високою була активність НАД·Н-ДГ на фоні істотного зниження рівня β-ОБДГ. При міодезі з натягом м'язів 916–962 мкН дегенеративно-дистрофічні процеси були менш вираженими. Відмічали зниження інтенсивності термінального окислення та окислення кетонових тіл. Збільшувалась активність ферментів циклу Кребса, підвищувався гліколіз. При натягу м'язів 980–1100 мкН і 650–800 мкН спостерігали зміни, подібні таким, як і при фасціо- та міопластиці.

Ключові слова: ампутація, фасціопластика, міопластика, міодез, м'язи, гістохімія, гістологія.

ФУНКЦИОНАЛЬНОЕ И МОРФОЛОГИЧЕСКОЕ СОСТОЯНИЕ МЫШЦ АМПУТАЦИОННОЙ КУЛЬТИ ПОСЛЕ РАЗЛИЧНЫХ СПОСОБОВ ЕЁ ПЛАСТИКИ

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Приведена оценка характера гистохимических и морфологических процессов в мышцах культи конечности после различных способов ампутационной пластики. Выполнены пять серий опытов на 55 собаках с ампутацией задней конечности в средней трети бедра. Проводили фасцио- и миопластику со сшиванием антагонистов, миодез с натяжением мышц 916–962 мкН, 980–1100 мкН и 650–800 мкН. Сроки наблюдения – 3 дня, 4 нед, 8 мес. На кусочках свежей мышцы ставили гистохимические реакции на сукцинатдегидрогеназу (СДГ), НАД·Н–дегидрогеназу (НАД·Н-ДГ), бета-оксибутиратдегидрогеназу (β-ОБДГ) и лактатдегидрогеназу (ЛДГ). Проводили гистологическое исследование мышц. Выявлено, что к концу срока наблюдения при фасцио- и миопластике обнаружены лишь единичные неизменённые мышечные волокна. Большинство их было заменено грубой волокнистой соединительной тканью с жировыми клетками. Отмечалось резкое увеличение активности ЛДГ (гликолиз) и повышение СДГ (цикл Кребса). Высокой была активность НАД·Н-ДГ, а уровень β-ОБДГ существенно снижался. При миодезе с натяжением мышц 916–962 мкН дегенеративно-дистрофические процессы были значительно менее выражены. Отмечалось снижение интенсивности терминального окисления и окисления кетоновых тел. Увеличивалась активность цикла Кребса, повышался гликолиз. При натяжении мышц 980–1100 мкН и 650–800 мкН наблюдали изменения, аналогичные таковым при фасцио- и миопластике.

Ключевые слова: амптутация, фасциопластика, миопластика, миодез, мышцы, гистохимия, гистология.

МЕДИЦИНА И ПРАВО

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ПЛАНУВАННЯ ОРГАНІЗАЦІЇ ГРОМАДСЬКИХ РОБІТ У СФЕРІ ОХОРОНИ ЗДОРОВ'Я ЯК ГАРАНТІЯ ПРАЦЕВЛАШТУВАННЯ МЕДИЧНИХ ПРАЦІВНИКІВ

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Проаналізовано основні етапи планової діяльності з організації оплатних громадських робіт в Україні, що відповідають специфіці трудової функції медичних працівників. На