Joint contractures are loss of passive range of motion of the diarthrosis joint. Immobilization contractures are one of the common types of joint contractures. The study aimed to determine the effect of immobilization on the formation of knee joint contracture in laboratory animals (rats) and assess the possibility of restoring mobility using low-frequency vibration during and after immobilization. Three groups of laboratory rats, ten animals each, were studied: I — 4 weeks of immobilization, 4 weeks of observation without rehabilitation measures; II — 4 weeks of immobilization, 4 weeks of low-frequency therapy; III — 4 weeks of immobilization, and 4 weeks in a row rats received a course of vibration therapy. The knee joint range of motion was measured weekly. Vibration development was carried out in the mode of 20 Hz with an amplitude of 1.5 mm. The rapid growth of contracture occurs from the 2nd week of immobilization. In the animals of the I and II groups, the process happened in the same way. In the III group, contractures were formed by the 4th week, but significantly less than in the previous groups. After the removal of immobilization, a slow increase in movements was observed in the first group, which did not reach average values by the 4th week after the removal of immobilization. Group II rats had a rapid reduction in contracture, and recovery occurred by week 4. In the III group, recovery was complete in 2 weeks. Low-frequency vibration reduces the impact of immobilization and significantly accelerates the recovery of the joint after its completion.

Key words: laboratory animals, experiment, contracture, knee joint, vibration.

Connection of the publication with planned research works.

The research was carried out with the scientific topic “To study the mechanisms of formation of immobilization contractures and to investigate the effect of low-frequency vibration on the restoration of joint function”, state registration number 0121U111555.

Introduction.

The term “joint contractures” describes the loss of the passive range of motion of diarthrosis joints, the most common and mobile type of joint. Measuring the passive or active range of motion of a contracture joint is critical to assessing the importance of joint contractures [1]. Joint contractures caused by immobilization have both myogenic and arthrogenic components. In a rat model, less than two weeks of immobilisation periods produced contractures associated with muscle restriction, and the contractures were reversible after remobilisation. In the case of immobilisation for four or more weeks, the joint structures contributed more to limiting the range of motion, and the resulting contractures were irreversible [2, 3].

The aim of the study.

To determine the impact of immobilization on the formation of contracture of the knee joint in laboratory animals (rats) and to evaluate the possibility of restoring mobility in the case of using low-frequency vibration during and after immobilization.

Object and research methods.
The experimental study was conducted on 30 non-linear white male rats aged six months.

Experimental studies were carried out in compliance with the requirements of humane treatment of experimental animals, regulated by the Law of Ukraine “On the Protection of Animals from Cruelty” (№ 3447-IV dated 21.02.2006) and the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other scientific goals (Strasbourg, March 18, 1986).

Before immobilization, the angle of extension and flexion of the knee joint was measured for all animals. Hindlimb immobilization at an angle of 140° was performed by holding it under a plaster cast and an elastic bandage in unanaesthetized rats.

Rats were divided into three groups of 10 animals each: Group I – 4 weeks of immobilization, 4 weeks of observation without rehabilitation measures; II group – 4 weeks of immobilization, 4 weeks of low-frequency therapy; Group III – 4 weeks of immobilization and 4 weeks in a row rats received a course of vibration therapy.

Vibration development of the immobilized knee joint was performed using a vibration stand in the mode of 20 Hz with an amplitude of 1.5 mm.

The immobilization bandage was removed, and the animal’s limb was shaved from the fur to determine the amount of movement in the animal. Markers were placed on the hip, knee and hock joints. The animal was placed on a special stand, and the limb was hanging down. To determine the angle of flexion, the hind limb was maximally flexed. A 50-g weight was tied to the hock joint to determine the extension. The range of motion was measured from photographs.

A similar procedure was performed for the opposite limb. Measurements were carried out weekly, four times during immobilization and four times after the end of immobilization.

We determined the angle of extension, the angle of flexion, and based on this data, the amount of movement. Contracture was defined as the difference between the measured volume of movements and the volume of movements before the start of the experiment for each animal separately.

The experimental data were processed statistically. The mean (M) and its standard error (SD), minimum and maximum values per group were determined. Comparisons between opposite limbs were performed using the T-test for repeated measurements, comparisons between experimental data and primary range of motion measurements were made using a one-sample T-test, and comparisons between groups were made using a one-way analysis of variance (ANOVA). Calculations were performed in the IBM SPSS Statistics 20.0 application program package.

**Research results.**

Before the start of the experiment, the rats were measured for the range of motion in the knee joint (Table 1). Movement volume was measured weekly. The dynamics of knee joint contracture formation in laboratory rats is shown in Table 2.

Contracture on the 7th day of the experiment averaged 5° (from 0° to 13°). No noticeable difference between groups of animals and immobilized and opposite joints was found (p>0.05).

On the 14th day of the experiment, an increase in the progression of mobility limitation in the knee joint of rats of groups I and II was observed. In the III group of rats, the formation of a contracture on the immobilized joint was not detected; in the animals of the I and II groups, a rather noticeable contracture was formed in the range of 20-25°, which was significantly more prominent than in the animals of the III group.

On the 21st day of the experiment, a significant increase in contracture was noted in animals of the I and II groups. The first signs of limitation of movements began to be observed in animals of the III group at an average of (12±4)°; in animals of I and II groups, the contracture reached 50°. In some animals of all groups, the phenomenon of mobility restriction on the opposite joint began to be noted — about 10° in animals of the I and II groups and about 7° in animals of the III group.

On the 28th day of the experiment, the animals of the I and II groups developed a contracture of the immobilized joint of more than 60°. On the opposite joint, the development of movement limitation in the range from 2° to 26° continued to be observed. In the III group of rats, the contracture of the immobilized joint was smaller and averaged (24±6)°. On the opposite joint, the restriction of movement reached (13±8)°.

After 28 days, immobilization was removed from the animals. And first group of animals was kept free in the future. The second group of animals underwent daily vibration procedures for 10 minutes. Group III continued the daily course of vibration development in the same mode.

The dynamics of the restoration of mobility of the knee joint are shown in Table 3.

A week later, on the 35th day of the experiment, the angles in the knee joint were measured in the animals.

A decrease in knee joint contracture was observed in all animals. The most significant changes were observed in the III group of animals. The limitation of movements was, on average, (14±4)°. The worst results were in the I group – (59±6)°; in the II group – (40±7)°. The limitation of movements on the opposite limb decreased sharply to 5-6°.

---

**Table 1 – Control measurements of the volume of movements in rats before the start of the experiment**

<table>
<thead>
<tr>
<th>Control measurement</th>
<th>Volume of movements, degree</th>
<th>Extension</th>
<th>Flexion</th>
<th>Volume of movements</th>
</tr>
</thead>
<tbody>
<tr>
<td>M±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min−max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M±SD</td>
<td></td>
<td>32±2</td>
<td>151±1</td>
<td>119±2</td>
</tr>
<tr>
<td>min−max</td>
<td></td>
<td>29±35</td>
<td>149±153</td>
<td>117±122</td>
</tr>
</tbody>
</table>

**Table 2 – Dynamics of contracture formation of the knee joint of rats during immobilization**

<table>
<thead>
<tr>
<th>Group</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
<th>28th day</th>
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<tr>
<td></td>
<td>lm</td>
<td>C</td>
<td>t/p</td>
<td>lm</td>
</tr>
<tr>
<td>I</td>
<td>5±3</td>
<td>0±13</td>
<td>0,478</td>
<td>27±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12±38</td>
</tr>
<tr>
<td>II</td>
<td>5±3</td>
<td>0±11</td>
<td>0,497</td>
<td>20±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3±35</td>
</tr>
<tr>
<td></td>
<td>4±4</td>
<td>0±11</td>
<td>0,497</td>
<td>21±4</td>
</tr>
<tr>
<td>III</td>
<td>3±3</td>
<td>0±07</td>
<td>1,186</td>
<td>5±4</td>
</tr>
<tr>
<td></td>
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<td>0,790</td>
<td>24,518</td>
</tr>
<tr>
<td>p</td>
<td>0,464</td>
<td></td>
<td></td>
<td>0,001</td>
</tr>
</tbody>
</table>
On the 42nd day of the experiment, slow recovery of movements was observed in the animals of the I group – (52 ± 7)°; in the II group, the contracture decreased to (30 ± 8)°; in the III group, the restrictions were minimal and averaged (12 ± 5)°. The limitation of mobility in the opposite joint remained without noticeable changes.

On the 3rd week after the removal of immobilization (49 day), in most of the animals of the III group, full recovery of mobility was noted, with some restrictions remaining up to 15°, and joint mobility was restored on the opposite limb. Good recovery dynamics up to (14 ± 6)° were observed in the II group. In the I group, the changes were slow – (37 ± 4)°.

At the end of the experiment (56 day), all animals of the III group had a full recovery of movements. In the II group, only some rats had a slight mobility restriction. In the I group, a rather pronounced contracture was noted at (34 ± 4)° with no limitation of movements on the opposite joint.

**Discussion of research results.**

This study aimed to determine the formation of immobilisation contracture of the knee joint and identify the effects of limiting the joint’s range of motion after immobilisation in laboratory rats.

Four weeks after the removal of immobilisation under the condition of vibration development both during the immobilisation process and after the removal of immobilisation (III group), a complete restoration of the range of motion in the joint was observed. In animals that underwent vibrostimulation only after immobilisation removal (II group), the average residual contracture was (72 ± 4)°. In animals that were kept after immobilisation removal without rehabilitation (I group), the contracture remained within (34 ± 4)°.

The obtained data coincide with the results of other authors. Chimoto et al. [4] immobilized rat knee joints with an internal fixator while the knee joint was flexed at 150° to develop an animal model of flexion contracture and investigated the progression of arthrogenic limitation of motion after immobilization. The study showed that joint contracture progressed rapidly up to 8 weeks. Although our experiment lasted only four weeks, we observed an increase in contracture each week. Similar results were reported by other authors [5].

According to research, full recovery of joint movements after 30 days of immobilization does not occur for eight weeks [6] according to Sato Y. et al. [7], only on the 32nd week, when the animals are kept freely, it is possible to talk about the restoration of movements.

During the experiment, it was found that a rapid increase in movement limitation occurs starting from the 2nd week of immobilisation. In the animals of groups I and II, which were kept during the immobilisation period, the formation of movement limitation occurred in the same way. In group III, movement limitation occurred slowly during the first three weeks of immobilisation, and noticeable changes were observed only during the 4th week, but significantly less than in groups I and II.

After removal of immobilisation in animals, normalisation of extension occurs. In the I group, in which the recovery took place naturally, that is, without external intervention, a slow increase in movements was observed. On the 4th week after the removal of immobilisation, following the dynamics of recovery, a full return to normal extension can be expected sometime after the 10th week. In the rats of the II group, after immobilisation removal, vibration development was carried out, a rapid decrease in contracture was observed, and a return almost to the original values, judging by the dynamics of recovery for 5-6 weeks.

In the animals of the III group, a noticeable contracture began to form only after the 3rd week of immobilisation. It did not reach the level of the I and II groups. Therefore, recovery after removing immobilization was complete and relatively fast – in 2 weeks.

Analysis of contracture, or limitation of movements in the joint, provides more valuable information for studying joint changes. Therefore, immobilization of the knee joint (fig.) in the I and II groups of animals gave a contracture of 60°, while in the III group, the restrictions did not exceed 25°. And accordingly, the recovery in the groups with vibration development was rapid. In the III group, full recovery was achieved; in the II group – recovery of up to 5° residual contracture; in the I group, we observed a residual contracture of almost 35°, which is more than the formed immobilisation contracture in the III group.
During the experiment, it was found that during the progression of the contracture on the immobilized joint, there is a limitation of movements on the opposite side. We associate the decrease in the volume of movements, mainly extension on the non-immobilized limb, with the fact that to maintain balance during movement without full support, rats must reduce their body height, thereby bending the supporting limb. Prolonged locomotion in this way leads to a functional reduction in the range of motion (i.e. disuse contracture), which quickly disappears when normal locomotion is restored. We did not specifically study this aspect, and it may be the subject of further research.

The reason why the animals did not fully restore the range of motion when vibration was used can be explained by the fact that, after all, the limitation of mobility also affects the condition of the muscles, and the short-term effect of vibration cannot fully replace physiological joint due to improved blood circulation. It is not enough to maintain physical exertion, but only restore hydrodynamics joint due to the fact that to maintain balance during movement without full support, rats must reduce their body height, thereby bending the supporting limb. Prolonged locomotion in this way leads to a functional reduction in the range of motion (i.e. disuse contracture), which quickly disappears when normal locomotion is restored. We did not specifically study this aspect, and it may be the subject of further research.

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Мета дослідження.

Визначити вплив іммобілізації на формування контрактури колінного суглоба у лабораторних тварин (щурів) та оцінити можливість відновлення рухливості у разі використанні низькочастотної вібрації в процесі іммобілізації та після її завершення.

Об’єкт і методи дослідження.

Експериментальні дослідження було проведено на 30 неідентичних білих щурах-самцах віком біля 6 місяців.

Перед проведенням іммобілізації всім тваринам вимірювали кут розгинання та згинання колінного суглоба. Іммобілізація задньої кінцівки під кутом 140° була виконана шляхом її утримання під гіпсовою пов’язкою та еластичним бинтом у ненаркотизованих тваринах у режимі 20 Гц з амплітудою 1,5 мм.

Тварини були поділені на 3 групи по 10 тварин: I група – 4 тижні іммобілізації, 4 тижні спостереження без реабілітаційних заходів; ІІ група – 4 тижні іммобілізації, 4 тижні проводилась низькочастотна терапія; ІІІ група – 4 тижні іммобілізації та 4 тижні посіль щури отримували курс вібраційної терапії.

Вібраційну розробку іммобілізованого колінного суглоба виконували з використанням вібраційного стенду в режимі 20 Гц з амплітудою 1,5 мм.

Визначали кут розгинання, кут згинання, і за цими показниками вимірювали обсяг рухів у тварин колінного суглоба. Об’єкт визначали середнє (М) його стандартну похибку (SD), які використовувалися для дослідних та інших наукових цілей (Страсбург, 18.03.1986 р.).

Динаміка формування контрактури колінних суглобів була незворотною [2, 3]. Основне значення мали морфо-функціональні зміни суглобових структур, які вплинули на обмеження об'єктивно виявлені при огляді суглобів.

Для визначення обсягу рухів проводили щотижня. Динаміка формування контрактури колінних суглобів у лабораторних щурів наведена в табл. 2. Контрактура на 7 добу експерименту становила в середньому 5° (від 0° до 13°). Помітної різниці між групами тварин іммобілізованим і протилежним суглобом не виявлено (р>0,05).

На 14 добу експерименту спостерігали збільшення прогресування обмеження рухливості в колінному суглобі щурів I та ІІ груп. В ІІІ групі щурів формування контрактури на іммобілізованому суглобі не виявлено, у тварин I та ІІ груп сформувалась дозволена помітна контрактура у межах 20-25°, яка значною була більшою, ніж у тварин ІІІ групи.

На 21 добу експерименту у тварин I та ІІ груп відмічали суттєве збільшення контрактури. Перші ознаки обмеження рухів стали спостерігатися у тварин ІІІ групи в середньому (12±4°), у тварин I та ІІ груп контрактура сигнала за 50°. У деяких тварин всіх груп стали відмічатись явища обмеження рухливості на протилежному суглобі – біли 10° у тварин I та ІІ груп, біли 7° у тварин ІІІ групи.

На 28 добу експерименту у тварин I та ІІ груп сформувалась контрактура іммобілізованого суглоба більше 60°, на протилежному суглобі продовжувалося спостерігатися розвиток обмеження рухів у межах від 2° до 26°. В ІІІ групі щурів контрактура іммобілізованого суглоба була меншою і становила в середньому (24±6°), на протилежному суглобі обмеження рухів сягнуло (13±8°).

Після 28 доби у тварин було знято іммобілізацію. І I III група тварин у подальшому були обстежені. ІІІ група тварин щоденно проходила вібраційні процедури по 10 хв. ІІІ група продовжувала щоденний курс відносно нього.

У всіх тварин іммобілізація не вплинула на розтягову рухливість щурів. У тварин І та ІІ груп обмеження рухливості на протилежному суглобі були незначними, при цьому у тварин І групи обмеження були значно меншими.

На 42 добу експерименту у тварин I групи спостерігали повне відновлення рухливості щурів, у тварин ІІ групи обостре рухливість щурів зменшилося на 20-25% в порівнянні з контролем.

Для оцінки можливості відновлення робіт у тварин використали методи вимірювання обсяга рухів в колінному суглобі (табл. 1).
Це текст вихідної документа на російській мові. Він стосується досліджень, що виконані в лабораторіях на тварин, що підлягають іммобілізації. Описаний процес включає в себе порівняння динаміки відновлення рухів суглобів після іммобілізації.

На кінець експерименту (56 доб) у тварин III групи відмічали повне відновлення рухів у всіх тварин, у тварин I группі відмічали повне відновлення рухів у всіх тварин, в ІІІ групі відмічали повне відновлення рухів у всіх тварин.

В процесі проведення експерименту було виявлено, що стрімке нарощування обмеження рухів відбувається в тиждень 2 та 3 тижні.

Таблиця 3 – Динаміка зміни контрактури колінного суглоба у лабораторних шурів після зняття іммобілізації

<table>
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</tr>
<tr>
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ФОРМУВАННЯ ІММОБІЛІЗАЦІЙНОЇ КОНТРАКТУРИ КОЛІННОГО СУГЛОБУ У ЛАБОРАТОРНИХ ТВАРИН ТА ВІДНОВЛЕННЯ ЇГО ФУНКЦІОНАЛЬНОСТІ ПІД ВПЛИВОМ НІЗЬКОЧАСТОТОНОЇ ВІБРАЦІЇ (експериментальна дослідження)

Хасавнех А. А. М., Карпінська О. Д.

Резюме. Термін «суглобові контрактури» використовується для опису втрати пасивного діапазону руху ділянок суглобів, найбільш поширеного та рухомого типу суглобів. При іммобілізації протягом чотирьох і більше тижнів суглобові структури більше сприяли обмеженню обсягу рухів, і контрактури, що виникають в результаті є незворотними.

Мета дослідження. Визначити вплив іммобілізації на формування контрактури колінного суглоба у лабораторних тварин.

Об’єкт і методи дослідження. Експериментальне дослідження було проведено на 30 нелінійних білих щурів. Спроби проведено на колінних суглобах, прикладні та підключені на щурів під гіпсовою пов’язкою. Результати дослідження. Було виявлено, що стрімке наростання обмеження обсягу руху відбувається починаючи з 2 тижня іммобілізації. У тварин І та ІІ груп обмеження руху відбувалося одночасно, в ІІІ групі вже на 4-й тиждень спостерігалася помітна зміна, але значно менше, ніж в І і ІІ групах. Після зняття іммобілізації у тварин відбувається розтягування пасивних з'єднань, як на 4-й тиждень після зняття іммобілізації не досяглись відповідних значень. У щурів ІІІ групи спостерігалися стрімке зменшення контрактур, і
FORMATION OF IMMOBILIZATION CONTRACTURE OF THE KNEE JOINT IN LABORATORY ANIMALS AND RESTORATION OF ITS FUNCTIONALITY UNDER THE INFLUENCE OF LOW-FREQUENCY VIBRATION (experimental study)

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Abstract. The term «joint contractures» is used to describe the loss of passive range of motion of diarthrosis joints, the most common and mobile type of joints. When immobilized for four or more weeks, the joint structures contribute more to limiting the range of motion, and the resulting contractures are irreversible.

Objective of the study. To determine the effect of immobilization on the formation of knee joint contracture in laboratory animals (rats) and to evaluate the possibility of restoring mobility in the case of low-frequency vibration during immobilization and after its completion.

Object and research methods. The experimental study was conducted on 30 nonlinear white male rats aged 6 months. We studied 3 groups of 10 animals each: Group I – 4 weeks of immobilization, 4 weeks of observation without rehabilitation measures; Group II – 4 weeks of immobilization, 4 weeks of low-frequency therapy; Group III – 4 weeks of immobilization and 4 consecutive weeks of vibration therapy.

Before immobilization, the angle of extension and flexion of the knee joint was measured in all animals. Immobilization of the hind limb at an angle of 140° was performed by holding it under a plaster cast and elastic bandage in unanesthetized rats. Vibratory development of the immobilized knee joint was performed using a vibration stand in the 20 Hz mode with an amplitude of 1.5 mm. Determination of contracture was performed weekly.

Research results. It was found that a rapid increase in movement restriction occurs from the 2nd week of immobilization. In animals of groups I and II, the limitation of movements occurred in the same way, in group III only at week 4 there were noticeable changes, but significantly less than in groups I and II. After removal of immobilization, the animals showed normalization of extension. In group I, a slow increase in movements was observed, which did not reach normal values by week 4 after immobilization was removed. In rats of group II, a rapid decrease in contracture was observed, and a return to almost the original values. In group III, the recovery was complete and relatively fast – in 2 weeks.

Immobilization of the knee joint in groups I and II resulted in a contracture of 60°, while in group III the restrictions did not exceed 25°. Recovery in the groups with vibration development was rapid, in group III a complete recovery was achieved, in group II – recovery of up to 5° of residual contracture, in group I the residual contracture was about 35°, which is more than the formed immobilization contracture in group III.

Conclusions. Low-frequency vibration can reduce the effect of immobilization and significantly accelerate joint recovery after its completion. If vibration therapy is not possible during the period of immobilization, it should be started as early as possible.

It should be noted that today there are few studies on the effect of low-frequency vibration on the development of immobilization contractures and their subsequent treatment. The data obtained require further research with longer immobilization periods, immobilization options, and modes of vibration exposure to joints.

Key words: laboratory animals, experiment, contracture, knee joint, vibration

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