

Morphological examination with prolonged introduction of acupuncture needles MM.  
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#### SUMMARY

Changes in the skin after prolonged administration (up to 3 months) of acupuncture needles made of an alloy of gold and stainless steel were studied in experimental animals - rats. In the early stages, aseptic (or septic) inflammation develops at the injection site, reaching a peak after 2 weeks. As the inflammatory reaction subsides, activation of epidermal cells is noted, which overgrow the acupuncture needles from the periphery, isolating them from the surrounding tissues. By 2–2.5 months from the beginning of the experiment, as the needles are inserted under the skin, an implantation canal (acurate), lined with epidermis, is formed.

Key words: prolonged reflexology, micro-acupuncture, cellular reaction of the skin, accurate.

Various technologies of acupuncture are presented in scientific, methodological and reference medical publications [4, 9, 10]. Among them is the method of micro-acupuncture, which is one of the varieties of classical corporal and auricular acupuncture. It is assumed that prolonged irritation of acupuncture points by microneedles creates an artificial, long-lasting dominant in the central nervous system, contributing to the "inhibition" of the components of pathological reactions [1].

However, with all the positive aspects, this method also has certain disadvantages. Often, the duration of exposure to microneedles is limited due to the natural regeneration of the skin and exfoliation of the upper layer of the epidermis, which contributes to the rejection of the fixing adhesive plaster, and with it the loss of needles. The imperfection of the method can be attributed to the fact that under the patch occurs maceration of the skin, inflammatory complications, there are aesthetic inconveniences due to the presence of an adhesive plaster on the skin. Therefore, constant medical supervision, frequent replacement of the plaster and periodic pinning of the dropped microneedles are necessary. In addition, apparently, an intermittent and relatively short exposure (from 1 to 5 days with steel needles,

Taking into account the existing shortcomings, a new method of prolonged exposure to acupuncture points was proposed for implementation [5]. The essence of the new method is to determine the points of entry and exit of the needle. The needle is then passed through the selected points, leaving both ends of the needle outside the patient's skin. Next, the needle is fixed by means of clamps installed at the ends of the needle, and

leave the needle in the patient's body for a predetermined time, forming an implantation channel or acus tract (acurate). In this case, points located in the area of the acupuncture point and / or reflexogenic zone, and / or beyond the border of the acupuncture point and / or reflexogenic zone, are selected as points of entry and / or exit of the needle [7]. In this way, acupuncture points can be influenced, both on the auricle and on the body. To perform this procedure, specially designed and duly registered acupuncture needles are used [6]. If the needles are inserted correctly, then patients do not experience any inconvenience, they can work and take water and other hygiene procedures without restrictions.

The advantages of this method of acupuncture v possibilities simultaneously to puncture a group of points, in the absence of inflammatory complications and maceration of the skin under the plaster that fixes the needles. And, most importantly, in the reality of long-term - prolonged reflexotherapy, when, after placing the needles, the patient may not visit the doctor until a certain period of time.

In previous publications, the results of clinical studies were presented, showing the effectiveness of this method in complex reflexotherapy of excess body weight [2, 8]. The aim of this work was to study the reactions and the sequence of morphological changes in the skin during prolonged exposure to acupuncture needles.

#### Materials and methods

The experiments were carried out on 30 male Wistar rats. Rats under ether anesthesia were injected with one sterile acupuncture needle made of 585 gold alloy, GOST 30649-99 grade or stainless steel under the skin of the auricle and under the skin of the withers. The needles were used from the "Anti-adaptation kit for acupuncture according to the method of Mukhina M.M." according to TU 9398-001-770066072007, registration certificate No. FSR 2008/02508 dated July 14, 2009. The experimental modeling was fully consistent with the clinical use of the method of influencing acupuncture points.

The control was the contralateral auricle of the same rat and the skin of the withers away from the injection site. Three rats were operated on for each term. In the early stages (including 2 weeks), only gold needles were inserted, while for later periods (from 1 month to 3 months), both gold and steel needles were used. In this case, it was supposed to reveal differences in the late terms of the formation of the implantation canal. The animals were kept in a vivarium on a standard diet and housed one at a time in a cage. Before and after the introduction of acupuncture needles, the skin areas of the entrance and exit of the needles (on the auricle and withers) in all experimental animals were not subjected to antiseptic treatment.

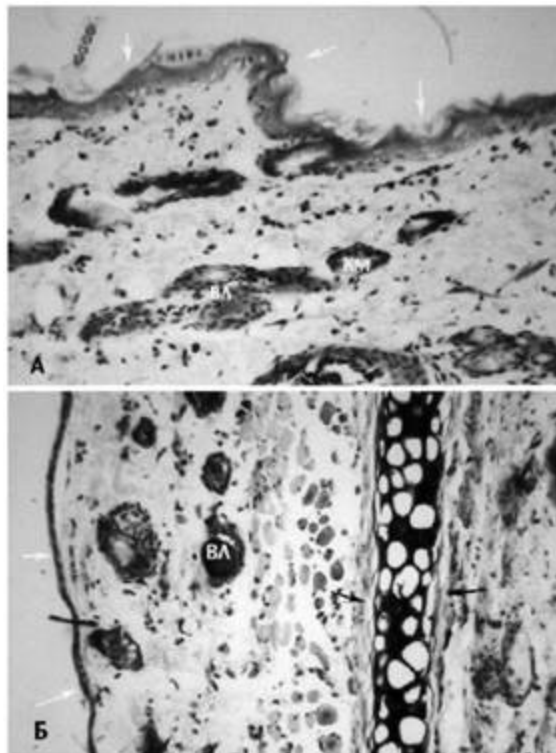
Material - pieces of skin and adjacent tissues of the withers (1.0 x 0.5 cm) and ears of rats - were taken on the 1st, 3rd, 7th and 14th days, as well as after 1, 2 and 3 months after setting acupuncture needles. Rats were sacrificed with ether overdose.

4 pieces of tissue were taken from each rat: auricle and skin of the withers with an acupuncture needle, contralateral auricle and a piece of skin from the site

withers away from the needle. The material was fixed in 10% neutral formalin, dehydrated in a battery of ascending alcohols, chloroform, and embedded in paraffin. Material from each rat was poured into 1 or 2 blocks, which were decomposed into sections 5–7  $\mu\text{m}$  thick. First, the tissue was cut along the implantation canal, and then the same blocks were turned over and cut across the canal. Cellular elements were detected with azure2-eosin according to Romanovsky, fibrous elements were stained by Van Gieson's method. The preparations were examined and photographed under a light microscope.

### Results and discussion

The skin of rodents and, in particular, rats, has a number of features in comparison with human skin. At the same time, in the scientific literature it is noted that rats should be considered sufficiently adequate animals for experimental morphological studies of the skin, in particular when exposed to acupuncture needles [11]. In fig. 1A shows the normal rat withers skin and a section through the rat auricle (Fig. 1B), in which the cartilaginous tissue is determined.

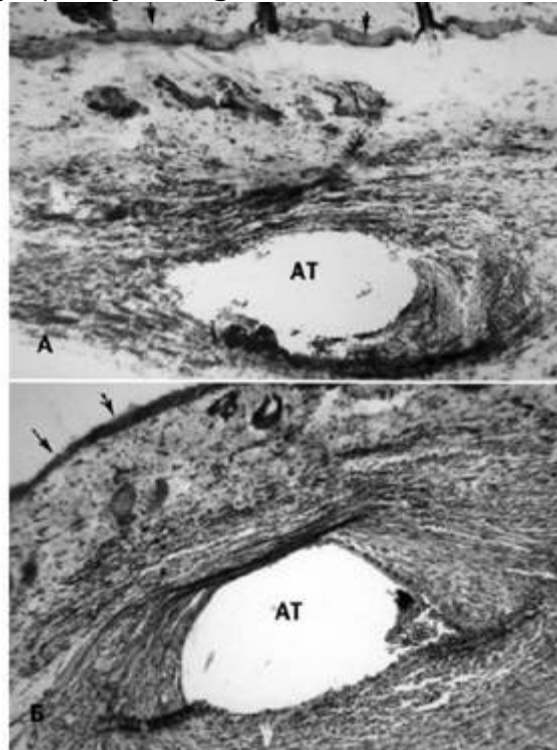


Rice. 1. The skin of the withers and auricle of the rats is normal.

A - The skin of the withers of the rat. Above, the epidermis is covered with keratin (indicated by white arrows on A and B), in the underlying layers - hair follicles (vl) and blood microvessels (km). Romanovsky staining, magnification: vol. 20x, approx. 10x.

B - Tissues of the rat auricle, norm. A feature of the auricle is the presence of cartilaginous tissue (indicated by black arrows). Romanovsky staining, magnification: vol. 10x, approx. 10x.

In the early stages (1-3 days) after the start of the experiment, all rats developed an inflammatory reaction in the area of needle insertion in the auricle and withers. At the same time, the process proceeded in parallel in the auricle and the skin of the withers. Edema formed around the implantation canal (acustruct), constriction of arterioles and dilatation of plethoric venules were observed. A large number of mast cells appeared, some of which were in the stage of degranulation, neutrophils and small lymphocytes (Fig. 2A).



Rice. 2. The skin of the rat withers in the early stages after the introduction of the acupuncture needle.

A - 3 days after the introduction of the gold alloy needle. Around the acustruct (AT), a cellular reaction, edema, is observed. The epidermis is indicated by arrows. Coloring by Romanovsky, increase: about. 10x, approx. 10x.

B - 2 weeks after insertion of the gold alloy needle. Around the acustruct (AT), there is a pronounced inflammatory infiltrate, edema. Formation observed collagen fibers and numerous blood capillaries.

Romanovsky staining, magnification: vol. 10x, approx. 10x.

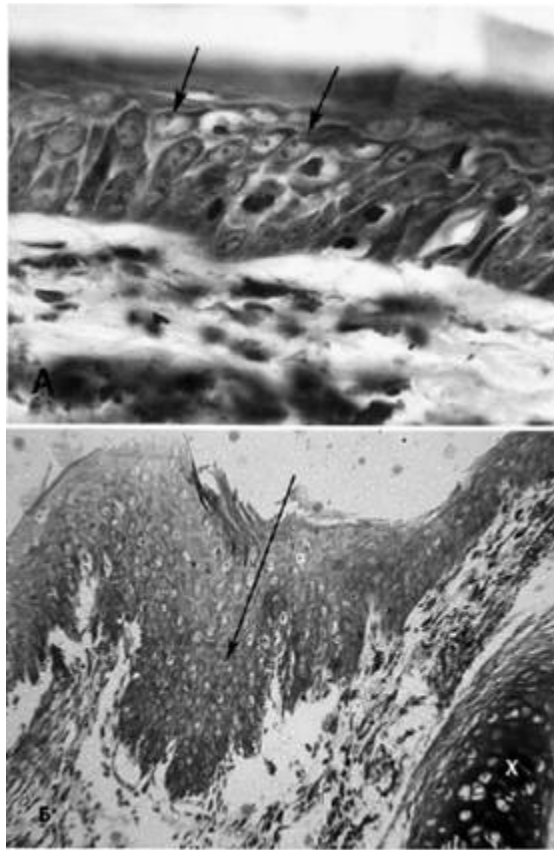
After one week, the further development of the inflammation process continued. However, an important fact was the appearance of young fibroblasts, which began to form collagen fibers to organize the walls of the implantation canal. Along with this, there was also activation of the epidermis at the entrance and exit of the acupuncture needle, which is the initial stage of its delimitation from the surrounding tissue. Sometimes constrictions were observed between the cells of the epidermis, which, apparently, is evidence of their amitotic division (Fig. 3A).

During this period, attention is drawn to the appearance in the cellular

infiltration near the implantation channel of a significant number of eosinophils and individual plasma cells, which indicates the development of an allergic reaction to the acupuncture needle. After 2 weeks, the inflammatory changes continued, but to a lesser extent. In the auricle, activation of the epidermis and mitotic activity were observed. At the same time, there were also pictures indicating amitotic division of epidermal cells. In individual cells, the nuclei had two or more nucleoli, which indicates the activation of protein synthesis in the epidermis. An epidermal layer is formed to isolate the needle from the surrounding structures. At the withers, one could also observe a hole from an acupuncture needle, around which connective tissue with accumulations of mast cells, lymphocytes, fibroblasts and collagen fibers was determined (Fig. 2B).

After 1 month, the process of delimiting the acupuncture needle from the surrounding tissues continued, both in the auricle and in the skin of the withers. It was possible to observe the ingrowth of the epidermal layer from the skin surface into the implantation canal. At this time, some animals continued to reveal an eosinophilic reaction near the needle. 2 months after the introduction of the needles, the high activity of the integumentary epidermis remains. Both in the auricle and in the skin of the withers, it overgrows an acupuncture needle, isolating it from the surrounding tissue (Fig. 3 B). In a number of cases, regeneration of cartilaginous tissue was observed in the auricle of rats, which was the result of passing a needle through this histological layer.

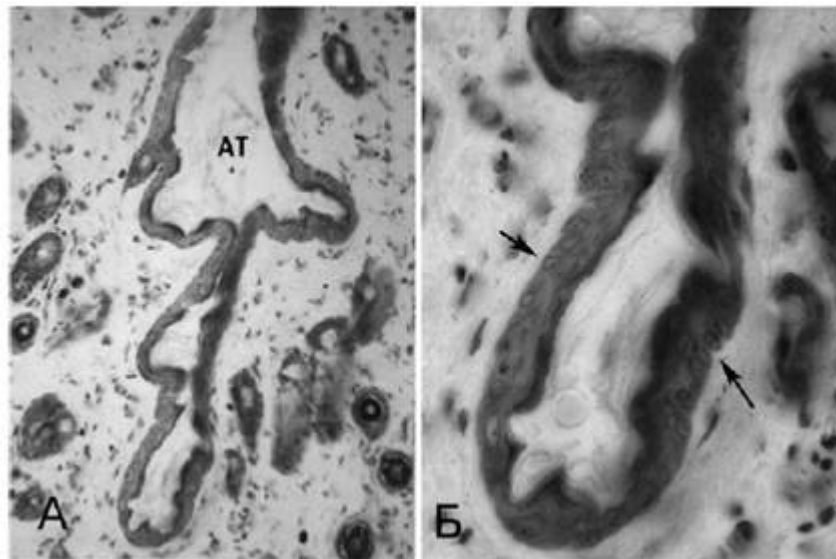
After 3 months from the beginning of the experiment, the epithelialization process inside the implantation canal both in the ear and in the skin of the withers ends and as a result the needle is completely isolated from the surrounding tissue (Fig. 4A and B). It should be noted that the young epidermis inside the canal was more multilayered than on the surface of the skin of the ear and withers, and a layer of keratin from exfoliated cells was also formed on its surface, which can be released from the canal in the form of plugs.



Rice. 3. Activation of the epidermis after the introduction of an acupuncture needle  
A - Skin of the withers of a rat, 1 week after insertion of a gold alloy needle. Initial activation of the epidermis. The appearance of cells in the nuclei of which there are 2 nucleoli.

Sometimes a constriction is observed between the cells, which, apparently, is evidence of amitosis (indicated by arrows). Coloring according to Romanovsky, increase in volume. imm. 60x, approx. 10x.

B - Auricle of a rat, 2 months after insertion of a stainless steel needle. Ingrowth of the epidermis from the skin surface into the implantation canal was noted (according to direction of the arrow). Bottom right - cartilage (X). Coloring according to Romanovsky, increase: about. 20x, approx. 10x.



Rice. 4. Auricle of a rat, 3 months after insertion of the needle made of gold alloy  
A - A formed accurate (AT), lined with the epidermis, is noted. There is keratin inside the canal. Around - blood microvessels, hair follicles.

Romanovsky staining, magnification: vol. 20x, approx. 10x.

B - Detail of the previous preparation. Young cells of the epidermis are clearly visible (indicated by arrows). There is keratin inside the canal.

Romanovsky staining, magnification: vol. imm. 60x, approx. 10x.

In experiments with stainless steel needles, the formation of an implantation channel occurs similarly to experiments with needles made of gold alloy, but skin irritation, as well as the degree of scratching of the area where the steel acupuncture needle is inserted, were more pronounced than with the introduction of a gold alloy. Since the changes were studied only at later stages, it can be assumed that the inflammatory reaction in these rats was more pronounced, and as a result of this, the scar connective tissue around the formed canal was coarser.

Thus, in response to the introduction of needles made of an alloy of gold under the skin of the auricle and withers of rats, starting from the 1st day, aseptic (in some cases - septic) inflammation occurs, the peak of which falls on the 2nd week after insertion of the needles. At the same time, regardless of the nature of the inflammation, the changes around the implantation channel (acustract) are unidirectional. As a rule, aseptic inflammation develops, which, as noted in the specialized literature, is not a contraindication for micro-acupuncture [4]. Very early (after 1 week) in the area of the developing acustract, eosinophilia is determined, which persists for at least 2 months. Eosinophilia is an indicator of an allergic reaction that may occur to some component of the gold alloy. It is not possible to assess the significance of this local allergic reaction to the body as a whole. It can also be assumed that this is a feature of experimental animals. However, eosinophilia and the appearance of plasma cells are undoubtedly an indicator of involvement in the process

the immune system. Starting from 2 weeks after the insertion of the needles, activation of the epidermis begins on the inner surface of the acustract in the auricle and the skin of the withers. It is important to note that keratinocytes are not formed *de novo* in the channel, and grow into the implantation canal from the skin surface, multiplying mitotically and amitotically, and, as the inflammatory process subsides, gradually isolate the acupuncture needle from the surrounding tissue. By 2–2.5 months from the beginning of the experiment, along the insertion of a needle made of an alloy of gold and stainless steel, an implantation canal (acurate) lined with the epidermis and containing free nerve endings is formed under the skin of the ear and withers of the rats.

In general, the subcutaneous injection of acupuncture microneedles is possible for a long time without harm to the skin and underlying tissues.

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