A stress situation thus leads to considerable changes in the structural and functional apparatus of chromaffin cells, which are expressed as marked emptying of the CA-storage granules, swelling of the mitochondria and endoplasmic reticulum, and partial destruction of the reticulum. The depth of these structural changes depends on the type of stress and is more marked in a situation dominated by physical stress.

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EFFECT OF MONOCHROMATIC RED LIGHT OF A HELIUM-NEON LASER ON THE MORPHOLOGY OF ZYMOSAN ARTHRITIS IN RATS

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Considerable experience has been gained in recent years in the treatment of rheumatoid arthritis by low-intensity laser radiation. The pathological basis for such treatment has not yet advanced beyond the stage of a working hypothesis. Judging from preliminary changes in the morphology of synovitis toward the end of treatment, laser radiation reduces tissue edema and the amount of fibrin on the synovial surface and between the synovial lining cells, reduces infiltration of the subjacent layer with lymphocytes and macrophages, and activates fibroplasia [2]. However, since laser therapy is usually given after preliminary anti-inflammatory therapy, and also because of the very varied picture of synovitis at the beginning of treatment and the incomparability of biopsy specimens obtained before and after treatment, it is difficult to assess the action of the laser beam on the inflamed synovial membrane.

The object of this investigation was to study the effect of laser radiation on the dynamics of the inflammatory process in experimental arthritis, in which the negative factors mentioned above were reduced to a minimum.

MATERIAL AND METHOD

Zymosan arthritis was chosen as the experimental model. Keystone et al. [3] induced arthritis in mice aged 6-8 weeks by injecting zymosan (a mixture of insoluble glycans from the yeast cell wall) into the knee joints in a dose of 0.02 ml of a sterile 1.5% suspension in physiological NaCl solution. In the present experiments, noninbred rats weighing 100-175 g were used as experimental animals. A 1.5% suspension of zymosan was injected into both

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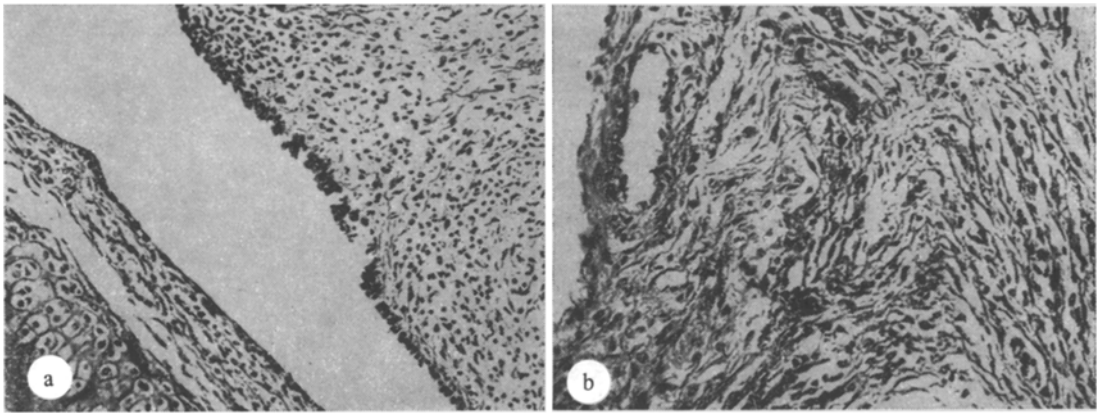

Fig. 1. Synovial membrane of a rat. a) Zymosan synovitis. Proliferation of synovial cells, infiltration of macrophages and lymphocytes. Pannus present on surface of articular cartilage. Hematoxylin—eosin, 100 ×; b) postinflammatory fibrosis of synovial membrane of right knee of rat after 21 sessions of laser radiation. Marked proliferation of fibroblasts. Hematoxylin—eosin, 150 ×.

knee joints in a dose of 0.04 ml, which is a dose 6-10 times smaller, relative to body weight, than that injected into the mice. Sixteen rats were used in the experiments and divided into two groups. The rats of group 1 (n = 10) were treated by 21 sessions of laser irradiation with a frequency of five times a week. The rats of group 2 (n = 6) were irradiated by the same scheme as those of group 1, but with nonmonochromatic red light (an ordinary incandescent lamp with a simple red filter). The rats were killed 28 days after the beginning of the experiment and 48 h after the last session of irradiation. Sessions of laser irradiation were given by means of an LG-75 helium-neon laser, and the power density on the surface of the irradiated joint was 1-1.5 mW/cm^2. The session lasted 2 min. The right knees were irradiated in all rats and the left knees served as the control. The duration of the irradiation session and the power density on the surface of the joint during irradiation with ordinary red light were the same as during laser irradiation.

After excision of small pieces of synovial membrane and articular cartilage for electron microscopy, the two knee joints as a whole were fixed with formalin, decalcified with Trilon B, and embedded in paraffin wax. Material for electron microscopy was fixed with glutaraldehyde and osmic acid, dehydrated in alcohols, and embedded in Araldite. Paraffin sections were stained with hematoxylin and eosin and with picrofuchsin. Ultrathin sections were stained with uranyl acetate and lead citrate, and then studied in the electron microscope.

The severity of synovitis was assessed from the results of histological investigation in points (from 0 to 3): 0) synovitis absent, 1 point) inflammation of one-third of the synovial membrane, 2 points) inflammation of more than one-third but less than two-thirds of the synovial membrane, 3 points) inflammation of more than two-thirds of the synovial membrane.

EXPERIMENTAL RESULTS

After injection of zymosan into the knee joints arthritis developed toward the end of the 1st day and reached maximal intensity by the 2nd-3rd day. Macroscopically the joints were puffy, the skin over them hyperemic, and passive movements were limited and painful. Under the influence of laser irradiation the signs of arthritis in the right knee joints regressed whereas in the left, control joints and in joints irradiated with ordinary red light, the signs of inflammation persisted.

Histologically, after a complete course of laser therapy synovitis was absent in the control joints of the animals of group 1 in three cases, its severity was rated at 1 point in two cases, at 2 points in two cases, and at 3 points in three cases (Fig. 1a). In the right knee joints, which were irradiated with the laser beam, synovitis was absent in most cases (six), it was rated at 1 point in two cases, at 2 points in one case, and at 3 points in one case.

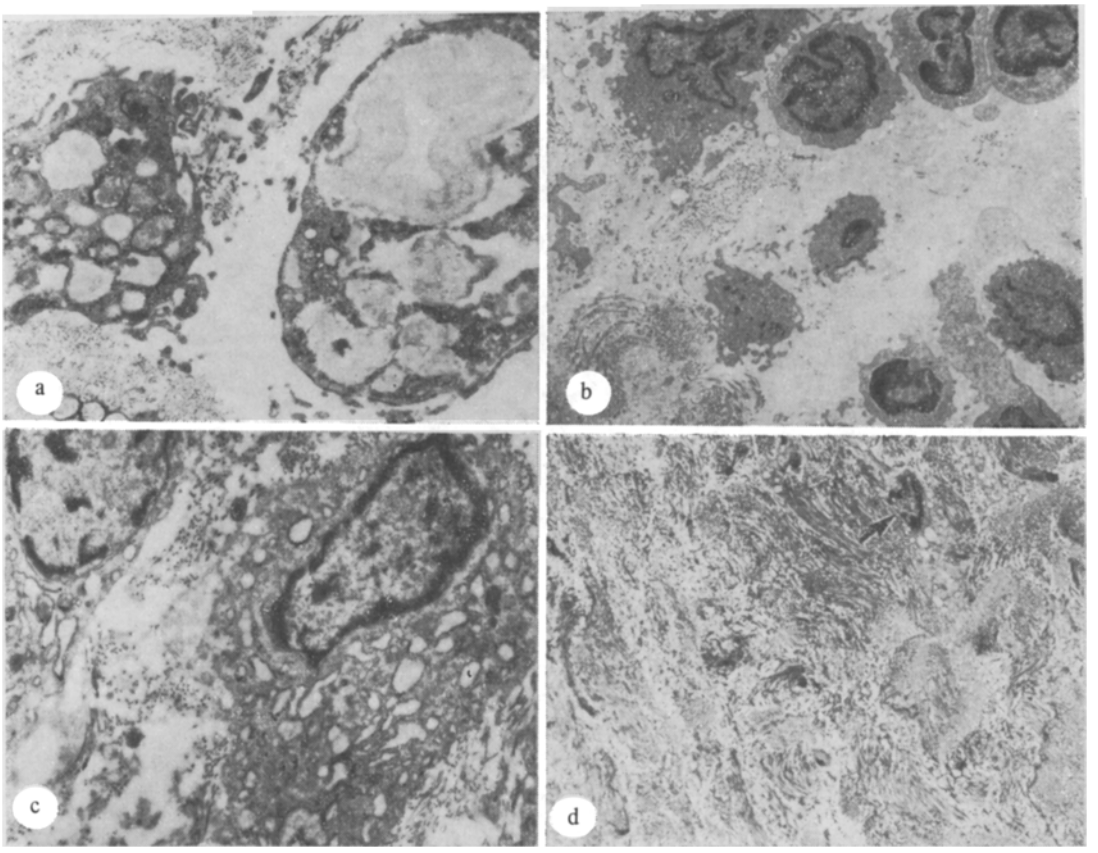

Fig. 2. Ultrastructure of synovial membrane in zymosan synovitis. a) Synovitis of macrophagal type with phagocytic zymosan particles in control joint, 10,000 ×; b) edema, infiltration of subendothelial layer with macrophages and lymphocytes in control joint, 2000 ×; c) type B synoviocyte with dilated cisternae of rough endoplasmic reticulum in an irradiated joint, 7500 ×; d) area of fibrosis of synovial membrane in an irradiated joint. Arrow indicates fibrocyte along collagen fibrils, 3500 ×.

Analysis of the histological sections and of the electron-microscopic data revealed organized fibrin on the synovial surface and in the lining layer in the control joints in most cases (seven), with edema of the stroma, plasmarrhagia, dilatation of the blood vessels and accumulation of erythrocytes in them, and widening of the spaces between the endothelial cells. In the right knee joints, irradiated with monochromatic red light, the features described above were found in only three cases. Neutrophilic infiltration was discovered in the control joints in four cases and in the irradiated joints in two cases; lymphocytic infiltration was found in six and three cases respectively (Fig. 2b). Proliferative changes in the synovial membrane in the control joints were discovered in seven cases: type A synoviocytes with a well-marked lysosomal apparatus, an extensive Golgi complex, and numerous filopodia were predominant in these cases. Synoviocytes and macrophages contained zymosan particles in their cytoplasm (Fig. 2a). Type B synoviocytes predominated in the synovial membrane from irradiated joints in eight cases; in their ultrastructure they were close to normal or showed signs of increased metabolic activity (Fig. 2c). Only in a few synoviocytes were degenerative changes and reduction of organelles noted. Zymosan particles in the cytoplasm of the lining cells and macrophages of the subjacent layer were present in single instances. Proliferation of fibroblasts in unirradiated joints was observed in two cases, whereas in the irradiated joint it was found in all 10; fibroblasts rich in rough endoplasmic reticulum and with a large Golgi complex, and continuous areas of collagen studded with fibrocytes occupied a larger area of the subendothelial layer (Fig. 2d).

A comparative study was made of the articular cartilage in the irradiated and control joints. Spreading of the pannus over the articular cartilage in the control joints was observed in five cases (Fig. 1a), but in joints treated with laser irradiation in only one case. Death of chondrocytes was observed in both control and irradiated joints (seven of each), but the scale of this process was greater in the unirradiated joints.

In the animals of group 2 histological analysis revealed absence of synovitis in the control joints in one case and the presence of synovitis rated at 1, 2, and 3 points in one, two, and two cases respectively. In the right knee joints, which were irradiated with ordinary red light, synovitis rated at 1 point was found in one case, at 2 points in two cases, and at 3 points in three cases. Analysis of the histological and electron-microscopic data in this group revealed no significant difference between the control joints and those irradiated with red light.

The results demonstrate the therapeutic action of external irradiation of joints in most rats with zymosan arthritis by monochromatic coherent polarized red light of a helium-neon laser, as reflected in diminution or disappearance of the swelling and hyperemia of the joints, and an increase in their range of movement. The time course of improvement of the arthritis corresponded to a decrease in the severity of the synvitis, i.e., inflammatory infiltration of the synovial membrane, and acceleration of the processes of replacement of the infiltrating macrophages and lymphocytes of the inflammatory reaction of cells of the fibroblastic series. During this improvement, at the end of the course of laser irradiation practically no zymosan particles remained in the irradiated joints, whereas in the un-irradiated joints type A lining synoviocytes and macrophages, containing zymosan particles, were still found. Zymosan particles lying extracellularly, among collagen fibers, also were found. These cases are interpreted as a result of stimulation of phagocytosis and digestion of the zymosan particles by macrophages, and they support the hypothesis that laser radiation activates macrophages [1].

Laser radiation, by stimulating cells of the fibroblast series, evidently promotes the more rapid development of postinflammatory tissue fibrosis. If predominance of type B synoviocytes in the lining layer at the end of the course of laser irradiation can be regarded as a process of normalization of the structure of that layer, proliferation of fibroblasts in the subjacent layer, intensification of collagen fibril production by them, and the formation of areas of fibrosis in which branching fibroblasts can be seen between indistinctly outlined collagen fibers can be regarded as the result of activation of collagen production by fibroblasts and of neofibrillogenesis. Ultimately the phases of inflammation of the synovial membrane follow one another in more rapid succession.

As a rule single cells (lining synoviocytes, macrophages, cells of the fibroblast series) in a state of degeneration, necrosis, and disintegration appeared in the irradiated joints at the end of the course of treatment, but most cells, to judge from their ultrastructure, did not undergo any considerable changes. With the power of laser radiation, its duration, and the number of sessions of irradiation used in these experiments it is evident that the helium-neon laser radiation has minimal or no destructive action.

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