THE EFFECT OF RADIOACTIVE RADIATION OF VARIOUS STRENGTHS FROM Co^{60} ON THE REGENERATING POWER OF SKELETAL MUSCLE

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In the present research we studied the effect of total, prolonged exposure to γ-radiation from Co^{60} on the processes of regeneration in striped muscle, using different doses of irradiation, and different distributions of the same dose in time, and compared this with the effect of a single total exposure of short duration, as described by us in an earlier paper [4]. So far as we know, no research has been done on this subject.

EXPERIMENTAL METHOD

Experiments were carried out on male white mice of the same age and weight. More than 350 animals were used, and were kept on the same diet. The test object was the tibialis anterior muscle, regenerating after mechanical trauma. For this purpose an incision was made through the skin and halfway through the middle of the muscle in its depth. The course of the process of regeneration was examined at the following times: 12, 24 and 48 hours, and 3, 5, 8, 10, 12, 15, 17, 20, 25 and 30 days after infliction of the trauma. The material was fixed in Zenker-formol; the whole of the tibialis anterior muscle was embedded in celloidin-paraffin wax. Serial sections were stained with Carazzi's hematoxylin and with Mallory's eosin, with Heidenhain's iron-hematoxylin, with azocarmine and with azure II-eosin.

In all, 8 series of experiments were carried out in which animals were exposed to total, prolonged, continuous irradiation. Two groups of mice acted as controls and were not irradiated. In addition, for comparison, we used animals irradiated for short periods of time, exposed to a single total irradiation with γ-radiation from Co^{60} in a GUT-400 telecobalt apparatus, in doses of 250, 500, 800, 1000 and 1500 r, and at a dose rate of 21 r/min.

The total, prolonged irradiation of the animals was carried out in a special room, in which was a stable telecobalt apparatus which was in operation 24 hours a day. The range of dose rates used was from 0.003 to 0.07 r/min. In order to obtain the desired dose rate, the animals were placed at different distances from the source of radiation.

In these series of experiments, the posttraumatic regeneration was studied after a total dose of 800 r, given over a period of 8 days, $1\frac{1}{2}$ months and 6 months of continuous irradiation, and after a dose of 1500 r, given over periods of 15 days, $1\frac{1}{2}$ months and 6 months. Two series of experiments comprised animals which were given a dose of 2300 r over periods of 5 months and $10\frac{1}{2}$ months of continuous, prolonged and total irradiation. In all cases the trauma was inflicted on the day following cessation of the irradiation. The control animals underwent operation at the same time.

EXPERIMENTAL RESULTS

All the irradiated animals developed radiation sickness, the severity of which depended on the dose and also on its intensity. The character of the radiation sickness was assessed by the peripheral blood picture, the weight of the spleen and the general condition of the animals.

Microscopic analysis of the material showed that in the control animals, after mechanical trauma, destructive changes developed in the striped muscle fibers immediately exposed to injury, which were expressed, primarily, as disappearance of the transverse and longitudinal striation, lumpy and granular destruction of the sarcoplasm and death of the muscle nuclei. The degenerative changes spread from the focus of direct injury for a certain distance in either direction. The areas of muscle fibers suffering destruction were cleared by lysis and phagocytosis by the blood connective cells. On the 3rd-4th day, resorption of the necrotic masses was mainly completed.

Side by side with these processes of active degeneration and resorption, on the 2nd day changes of a regenerative character were found in the injured muscle. Fibroblasts, undergoing mitotic division, were seen in considerable numbers, which were especially large on the 4th-5th day. At the ends of the areas of viable muscle fibers there was an accumulation of basophilic sarcoplasm, which stained a light or dark blue color with azure II-eosin. These areas of basophilic sarcoplasm later were the sources of muscle buds and myosymplasts. The latter were formed not only from growth of muscle buds but also by cleavage of the basophilic sarcoplasm along the length of the fiber in the form of myosymplastic bands with chains of nuclei.

On the 5th day the defect was filled with granulation tissue and myosymplasts, in which later appeared a myofibrillary system, so that they became converted into muscle tubes. On the 8th day cartain muscle tubes were already differentiated into young muscle fibers (see Figure 1, a).

The process of regeneration of the skeletal muscle from the 8th-10th day to the 30th day after mechanical trauma was simply the further differentiation of muscle tissue. On the 30th day a muscle and connective tissue area of regeneration was formed, consisting of adult muscle fibers with a well-marked cross striation and sparsely arranged nuclei beneath the sarcolemma; between the fibers were seen small connective tissue septa. This area of regeneration differed from normal muscle tissue only by the irregular arrangement of its muscle fibers (see Figure 3, a).

We have previously reported [4] that in uninjured muscle, after single, total irradiation, insignificant morphological changes were from time to time observed. The muscle tissue of animals exposed to prolonged irradiation showed absolutely no morphological changes. Under these circumstances, however, it was found that the muscle had suffered considerable radiation injury, which became apparent only during posttraumatic regeneration, the course of which was significantly depressed by comparison with the controls.

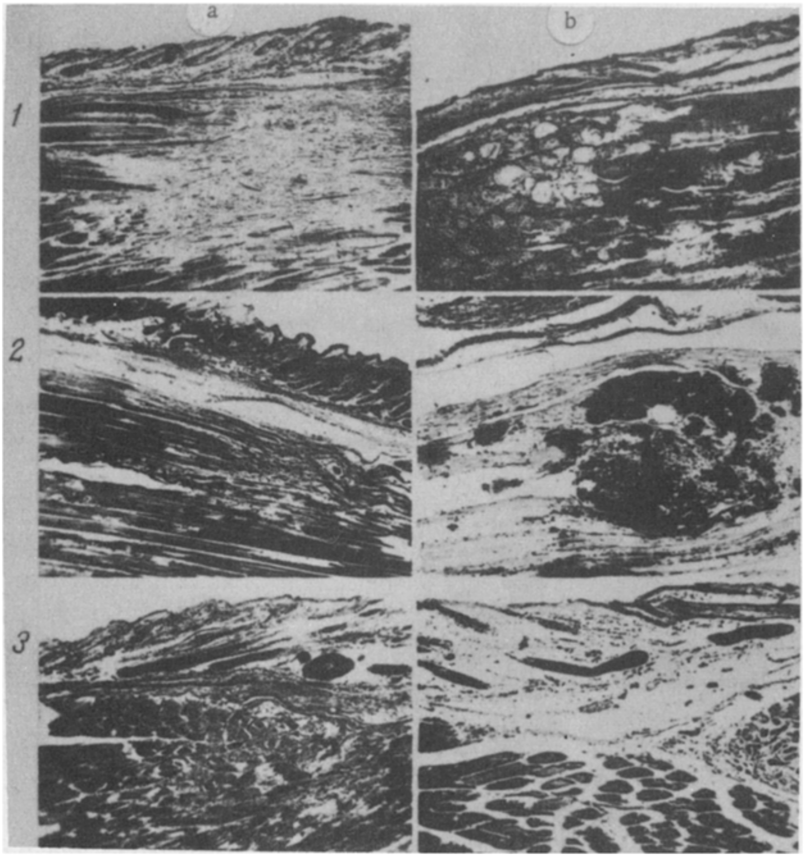
As a result of chronic irradiation of animals with a dose of 800 r, the exposure to radiation continuing uninterruptedly for 8 days and $1\frac{1}{2}$ months, an appreciable depression of the process of regeneration of muscle tissue was observed; it was especially noteworthy in the experiments in which mice were irradiated with a dose of 1500 r, extending over periods of 15 days and $1\frac{1}{2}$ months.

Detailed histological analysis showed that with a total dose of 1500 r, given over a period of 15 days, in the first stages of regeneration the process was indistinguishable from that observed after a single, total irradiation with the same dose. In either case it was shown as a sharp depression of the leucocytic reaction and of the reaction of the connective tissue cells and as very extensive destructive changes throughout almost the whole extent of the divided part of the muscle, especially at its distal end. Destructive processes were predominant in the region of the wound for a long time, and the regenerative processes were sharply depressed. Whereas in the control animals, at the end of the second day the involvement of new areas of muscle fibers in the destructive process was already restricted, in the case of animals irradiated for 15 days, destruction at a distance from the immediate area of trauma continued until the 8th or even the 10th day.

On account of the depression of the leucocytic reaction, of the diminished lysing function of the microphages, and also the lowered phagocytic activity of the macrophages, even on the 8th day resorption of the necrotic masses from the wound was completely absent, as after a single, total exposure of short duration to irradiation (see Figure 1, b). At the site of the defect, in contrast to the controls, lay untouched necrotic masses, occasionally a large quantity of effused blood and fibrin threads. Excessive and prolonged edema of the tissues was also observed.

Even on the 8th-10th day after total chronic irradiation, there were still hardly any signs of the appearance of regeneration. The solitary buds developing at this time were often defective and underwent regeneration; in the region of the defect, as a rule, no muscle tubes were found, and often even no muscle buds.

The proliferative power of the fibroblasts, and also their collagen-forming function, were depressed to an equal degree. Whereas in the control animals, on staining by Mallory's method, slender collagen fibrils could be seen on the 4th-5th day, in animals irradiated for 15 days, just as in those given a single irradiation of the same dose (1500 r), hardly any collagen fibrils were to be seen on the 8th day. In the controls, on the 2nd-3rd day after trauma, a large number of fibroblasts undergoing mitotic division could be seen, but in the experiments with irradiation for 15 days, and also after a single irradiation, no mitotically dividing cells could be observed in the films on the 5th nor on the 8th-10th days; under these circumstances large numbers of giant fibroblasts and binuclear cells were encountered, which had previously been observed by other authors after local irradiation with x-rays [1, 3, 6].



The state of the regenerating muscle tissue in mice exposed to irradiation and in control animals. 1,a) Control, 8th day after trauma; 1,b) total, chronic irradiation for 15 days; dose – 1500r, 8th day after trauma; 2,a) control, 15th day after trauma; 2,b) total chronic irradiation for 15 days, dose 1500 r, 15th day after trauma; 3,a) control, 30th day after trauma; 3,b) total chronic irradiation for 5 months, dose 2300r, 30th day after trauma. Microphotographs. Magnification: ocular 5 x, objetive 8 x.

Comparison of the findings shows that the depression of the process of regeneration in skeletal muscle after single irradiation of short exposure and irradiation of mice for 15 days with radiation from Co^{60} in a dose of 1500 r was very similar, despite the fact that the general effects on the body differed sharply in the two cases. Whereas with single, total irradiation of short exposure, in a dose of 1500 r, all the mice had died by the 9th-10th day, after total irradiation for 15 days the animals survived for 30 days. This permitted the histological changes to be observed at later periods. It could be seen that even on the 15th day the necrotic masses were still unabsorbed, and remained in large quantities in the region of the defect (see Figure 2,b). Naturally these acted as an obstable to the completion of regeneration of the skeletal muscle.

It is interesting that at this time a considerable number of myosymplasts was observed in the distal end of the injured area of the muscle, at the periphery of the defect, whereas at the proximal end necrotic masses of muscle tissue remained, as it were, static, like the appearance on the 15th day after a single total exposure to a dose of 1000 r. No signs of regeneration of muscle fibers could yet be seen at the proximal end. In the controls, on the 15th day, as a rule the region of the defect was filled with young muscle fibers, with connective tissue septa between them (see Figure 2,a). On the 30th day, no necrotic masses could be seen in the irradiated animals of this series, and the region of the defect was filled mainly with fatty tissue, with undifferentiated muscle cells scattered here and there.

In the experiments in which a dose of 1500 r was attained in $1\frac{1}{2}$ months, the process of regeneration of muscle tissue was also appreciably depressed. The young muscle cells which appeared were retarded in their growth by comparison with the controls, and did not always reach the center of the wound. For example, on the 8th day, in contrast to the controls, only muscle buds and short myosymplasts were seen at the periphery of the defect, and in the center there remained remnants of necrotic masses and loose connective tissue (see Figure 1,b). The connective tissue and leucocytic reations were, however, depressed to a much less degree than in the previous series of experiments (1500 r over a period of 15 days). On the 30th day the differences in the course of the process of regeneration in these two series of experiments (1500 r over periods of 15 days and $1\frac{1}{2}$ months) were not very pronounced.

In the chronic irradiation experiments, in which the same total dose (1500 r) was given over a period of 6 months, some signs of depression of regeneration of the muscle tissue were also observed, but to a much less degree.

In the next series of experiments with chronic, continuous γ-ray irradiation we used a total dose of 2300 r. One group of animals received this dose over a period of 5 months, another over $10\frac{1}{2}$ months. The dose rate in the first case was almost double, and in the second case equal to that used in the preceding series of experiments (1500 r over a period of 6 months).

The depression of regeneration of muscle tissue by a dose of 2300 r was expressed to a far greater degree than that by a dose of 1500 r, given over a period of 6 months. This is all the more interesting because in the course of the inflammatory process, no essential differences were observed. In all these experiments (1500 r over a period of 6 months, 2300 r over 5 months and 2300 r over $10\frac{1}{2}$ months) the leucocytic reaction and the reaction of the connective tissue cells were depressed to a considerably less extent than in the series in which the irradiation was given as a single exposure or spread over 8 and 15 days. The intensity of spread of the regeneration of muscle fibers away from the zone of direct injury was hardly indistinguishable from that of the controls; there was no delay in the liquidation of necrotic masses from the wound. The proliferative power of the fibroblasts, and also their collagen-forming function, were depressed to a less extent.

Despite these favorable circumstances, which distinguished the course of the process of regeneration essentially from that observed after a single exposure or after irradiation lasting 8 or 15 days, a total dose of 2300 r, whether given over a period of 5 months or $10\frac{1}{2}$ months, perceptibly depressed the regenerative power of the muscle tissue. Muscle cells, appearing in the course of regeneration, sometimes had not reached the center of the defect even on the 30th day (see Figure 3,b).

We have previously mentioned that after a single irradiation of short exposure, when the same dose rate was used, the degree of depression of the process of posttraumatic regeneration of skeletal muscle depended on the dose and on the severity of the radiation sickness [4]. The most pronounced depression of regeneration was observed with γ-ray irradiation from Co^{60}, given as a single dose of 1500 r; with doses of 1000 r and 800r the degree of depression was proportionately reduced. Doses of 500 and 250 r caused no essential differences in the course of the process of regeneration by comparison with the controls; merely certain changes in the process of inflammation were observed, as has been pointed out also by other authors [2, 4, 6].

Our conclusion from an analysis of our findings is as follows. In the case of total, chronic, continuous irradiation of mice with γ-rays from Co^{60}, depression of the regenerative power of striped muscle tissue takes place. The degree of this depression is less expressed at early stages when the irradiation is spread over a long period of time than when the same total dose of irradiation is given in a single exposure; this is due, it must be assumed, to the difference in the severity of the radiation sickness. The influence of the general condition of the animal on the course of the process of regeneration in the traumatized muscle is all the more likely because degeneration of the muscle fibers, distortion of the inflammatory process and delay in the resorption of the necrotic masses from the wound were much more pronounced after a single total exposure to irradiation than in the case of chronic exposure.

Not all these factor, naturally, could affect the process of regeneration of the muscle tissue, but they are not, however, the only cause of delay in and depression of regeneration.

After total irradiation of an animal, direct injury of muscle tissue also takes place, which is capable of lasting for a long period of time, as in the case of local irradiation os muscle [6]. In case of chronic irradiation, this is a latent radiation injury, appearing only during posttraumatic regeneration, and also capable of summation. The property of striped muscle to retain and to summate radiation damage is presumably explained, as G. S. Strelin believes, by the almost complete absense of power of physiological regeneration of this tissue, during which process recovery could take place [5].

SUMMARY

Experiments were performed on mice with total continuous (Co^{60}) irradiation of short and long duration. After discontinuance of the irradiation mechanical trauma was inflicted to the anterior tibial muscle. Regeneration in irradiated and control animals was studied in histological preparations. It was demonstrated that regeneration is completely inhibited by a single irradiation with 1500 r (the dose rate being 21 r/min); the regenerative function, although considerably depressed, is retained with the doses of 1000 r and 800 r, and hardly differs from the control, with the dose of 500 and 250 r.

In chronic irradiation (with the dose rate of from 0.003 to 0.07 r/min), only the dose of 800 r, administered within 6 months, did not alter the course of regeneration in comparison with controls. In all the remaining experiments (800 r for the period of 1-5 months and 6 months, 1500 r for the period of 15 days, $1\frac{1}{2}$ months and 6 months; 2300 r for the period of 5 and $10\frac{1}{2}$ months) a clear-cut depression of the regenerative power of the muscle tissue could be observed, which rose along with an increase in the dose administered.

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