EXPERIMENTAL BIOLOGY

ZENKER'S DEGENERATION OF SURVIVING MUSCULAR FIBERS AS A REACTION TO LOCAL IRREVERSIBLE INJURY

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D. N. Nasonov and D. L. Rozental [1] established that the basic impetus which produces Zenker's degeneration in vitro and which also propagates the wave of excitation is the bioelectric voltage, in this case the injurious voltage.

On the basis of the work of these authors, it would be expected that Zenker's degeneration should occur after any injury to the surviving muscle fiber, not only after mechanical injury. In connection with the theories of D. N. Nasonov and D. L. Rozental it was interesting to find out what methods of applying an injurious agent to a muscle would produce Zenker's degeneration.

The results obtained during the study of these problems are presented here.

EXPERIMENTAL METHOD

In the first series of experiments we determined the possibility of reproducing Zenker's degeneration by the application of various injurious substances to an area of muscle fiber: hypotonic salt solutions, ethyl alcohol, chloral hydrate. The experiment was carried out on the sartorius muscle of the frog in the fall and winter. After the muscle was prepared, it was kept in Ringer's solution for an hour; it was used in the experiments only if microscopic examination did not reveal any signs of injury. All the experiments were carried out in a moist chamber at 18-20°. Most of the muscle lay on the surface of a slide with a polished edge. Near this edge, the muscle was folded and approximately half of the hanging portion (the proximal section) was immersed for an hour in 20 ml of the injurious liquid.

Control experiments proved that folding the muscle at the edge of the slide for as long as two hours did not cause any visible changes in the outer surface of the muscles.

Later, all observations were made only on fibers located on this surface.

If a muscle which had been cut across was placed in the moist chamber as above, so that the surface came in contact with the Ringer's solution, Zenker's degeneration developed and progressed above the surface of the solution. Since Zenker's degeneration does not develop to any extent under the ordinary conditions of a moist chamber, as is known [1], in this case the destructive process apparently occurs as a result of the rise of the Ringer's solution by capillary action along the intercellular spaces.

The hypotonic solutions were prepared from a basic isotonic solution and were expressed in fractions of it (distilled water, 0.1; 0.2; 0.3; 0.4 and 0.5 R). The alcohol and chloral hydrate solutions were prepared with Ringer's solution.

We studied the action of the following concentrations of the narcotics: alcohol – 6, 5, 4, 3, 2, and 1 M; chloral hydrate – 0.08; 0.065; 0.04; 0.025 and 0.125 M.

EXPERIMENTAL RESULTS

In spite of the variety of active agents, the results obtained were identical, so we will present their general characteristics.

After the end of the immersion, the muscle fibers were changed not only in the submerged area, but also to some height above the surface of the liquid. The amount of injury to the fiber of the entire submerged portion was the same: cloudiness, swelling and shortening of the muscular striae, destruction of their structure, and the appearance of structural nuclei. The extent to which these changes were evidenced was proportional to the strength of the stimulus employed on the muscle.

Above the level of the solution, the signs described above diminished gradually as the distance increased.

Alteration of the muscle fibers above the level of the solution was connected with the infiltration (as a result of diffusion and by means of capillary action) of the injurious agent (alcohol, chloral hydrate) into the area. As the concentration of the agent decreased away from the surface, the injury observed decreased in the same direction. As regards the hypotonic solutions, in this case also the movement of the electrolytes in the direction of lower salt concentration determined the amount of injury above the surface of the solution and the gradual decrease of changes as the distance increased.

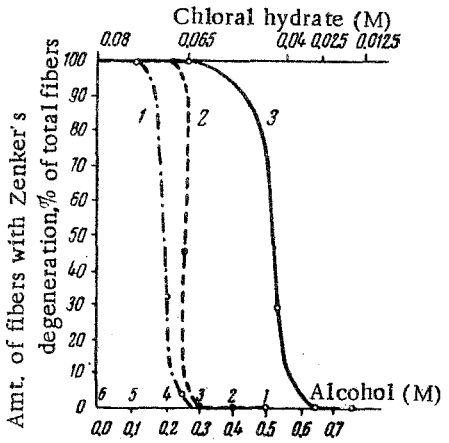
Thus, the border between the changed and intact parts of the muscle fibers was practically non-existent for purely physical reasons – a gradual transition from one part to the other was observed.

Zenker's degeneration was never observed immediately after the end of the immersion (not after hour-long, nor after more extended, immersion). The development of this process was only observed after the injured fibers were transferred to Ringer's solution. The relationship of the amount of fibers with Zenker's degeneration to the concentrations of the injurious agent (ether, chloral hydrate) and the decreases in the salt concentrations in the hypotonic solutions are shown in the illustration. The figures used for each point on the curves are averages which were obtained on examination of all fibers located on the surfaces of 3-10 muscles (250-850 fibers); the tabulations were carried out an hour after transfer of the muscles to Ringer's solution; as many experiments indicated, there was practically no increase in the number of fibers with Zenker's degeneration after the muscles had been kept in Ringer's solution for a longer time. As the illustration shows, the number of fibers with Zenker's degeneration decreases as the strength of the stimulation decreases.

By means of microscopicalexamination combined with vital staining (0,025% solution of neutral red in Ringer's solution) and determination of the threshold for excitation of the submerged portions of the muscle, we found that Zenker's necrosis developed only after irreversible injury to the submerged portions of the muscular fibers. The weaker the stimulus, i.e., the lower the concentration of alcohol, chloral hydrate, or the less the dilution of the Ringer's solution, the fewer was the number of fibers in the submerged piece which suffered irreversible injury, and so the smaller the number of those in which Zenker's degeneration was to be observed.

This condition was fulfilled in some of the experiments at the time of submersion: for example,6 M alcohol, 0.08 M chloral hydrate or distilled water injured the submerged portion of the muscle irreversibly only a few minutes after immersion; under these conditions, there was enough time left for the development of Zenker's degeneration before the end of the experiment, but, nevertheless, it developed only after transfer of the muscle to Ringer's solution. Systematic observation showed that this process was only apparent after the reparably damaged areas of muscle fibers repaired the damage. As a result, a sharp boundary was formed with the injured area, which was absent at the time of injury. The first tears of the muscle fibers appeared at this boundary, heralding the beginning of Zenker's degeneration, which later spread only within the living portion of muscle cells. These observations formed the basis for the suggestion that, in addition to irreversible injury, the presence of a sharp boundary to the injury, i.e., a steep gradient of trauma, is a necessary condition for the appearance of Zenker's degeneration.

In order to test this assumption, two variations of the experiments were carried out using thermal stimulation to produce irreversible changes in the fibers. In both variations, one part of the muscle was submerged for an hour in Ringer's solution of such a temperature as to produce irreversible changes in the muscle fibers (from 42° to 39°). In the first variation, the areas of the muscle which were located above the surface of the liquid were also heated, and the injury above the boundary extended even further than in the experiments described above, gradually decreasing away from the surface of the liquid; thus, the boundary of the injury was practically absent, as in the



Number of muscle fibers with Zenker's degeneration compared with the concentration of the injurious agents. 1) Ringer's solution; 2) Ethyl alcohol; 3) chloral hydrate.

earlier experiments. As a result, Zenker's degeneration only arose after transfer of the muscle to Ringer's solution at room temperature. In the other variation, arrangements were made to limit the heating of the areas which were located above the level of immersion, lowering their temperature to a harmless point. Because of this, the gradient of injury in these experiments was very steep. Under these conditions, Zenker's degeneration developed at the time of stimulation.

The results of these experiments confirmed the assumption that a sharp boundary to the injury is necessary as a second condition for the development of Zenker's degeneration.

Another proof of this assumption was the absence of Zenker's necrosis in muscles of fall-winter frogs which had been completely immersed in the injurious solutions (chloral hydrate and alcohol of the concentrations indicated above, hypotonic solutions, Ringer's solution heated to 42°, 40°, 38° and 36°), regardless of the speed with which the muscles were killed under these conditions. Zenker's degeneration did not develop during stimulation by the injurious agent, nor later in the Ringer's solution under these conditions. This could be considered the result of the uniformity of the intensity of the injury throughout the length of the muscle fibers, i.e., as the consequence of the maximal horizontality of the gradient of injury. However, this result was observed only if the injury to the muscle fibers was uniform for their entire length (muscles of newly-caught frogs in the fall and winter).

In experiments on summer frogs which had been kept in the laboratory (surrounding temperature 23-28°) on a starvation diet for 5-10 days, Zenker's degeneration invariably developed after total immersion, as well. In addition, the degeneration was evident only on the use of threshold, almost necrotizing doses of the injurious agents (hour-long submersion in hypotonic solutions of 0.25-0.3 R, 3-2 M alcohol, 0.25 M chloral hydrate, Ringer's solution heated to 34-36°). In contradistinction to the usual picture of Zenker's degeneration, the disintegration of the muscle fibers in these experiments often began simultaneously at various points on a single fiber; it could already be observed in some fibers at the time of immersion in the injurious agents, but it developed and progressed, primarily, in Ringer's solution.

With the assistance of vital staining (neutral red), it was possible to establish the fact that, even before immersion of these muscles, a number of signs of injury (increased diffusive staining, absence of granulations) became apparent. The most characteristic trait was the irregularity of the staining of these fibers, noticeable not only as a difference in the staining of separate fibers, but also as differences in the intensity of the staining of sections of the same fiber (mosaic-like staining). In addition, about one third of these fibers showed contracted nodes which occurred as a result of the action of the stain itself (0.25% solution of the stain during 15 minutes).

All this pointed to markedly different basic physiological conditions of the various areas of muscle fibers, as a result of which the susceptibility to injury varied.

Thus, Zenker's degeneration does not arise during total stimulation if the susceptibility of the muscle fibers to injury is uniform throughout their length. If the susceptibility to injury is not uniform, the same stimulus produces changes which are incompatible with life in some areas of a fiber, while in other areas the injury is reversible.

Zenker's degeneration developed in these experiments only when threshold doses of the agent were used. This is quite understandable, since it is these doses which are conducive to unequal injury along the length of the fiber. Stronger doses soon kill all of the areas, in spite of differences in their sensitivity; weaker ones do not destroy the sensitive areas.

We observed that, as a rule, Zenker's degeneration appeared in these cases after the muscles had been transferred to Ringer's solution, not while they were immersed in the injurious liquid. This fact correlates with the role which we are assigning to the steepness of the gradient of injury in the production of Zenker's degeneration. Truly, it is in Ringer's solution that the contrast between the necrotic areas and the adjacent living ones becomes sharpest as a result of the regenerative processes; it is here that the gradient of injury becomes steepest.

Thus, the results of these experiments confirm the assumption that irreversible injury to an area of a fiber and a sharp boundary with this change is necessary for the development of Zenker's degeneration.

All of this correlates very well with the electrical theory of transmittal of injuries in muscle fiber which was proposed by D. N. Nasonov and D. L. Rozental. In accord with this theory, the reason for the development of and the moving force behind Zenker's degeneration is the difference in the bioelectric potentials arising at the boundary between injured and intact protoplasm. The electric current which arises here, is of a high enough voltage to affect the adjacent area and destroy it; in this way, the injury is transmitted along the fiber. In view of this, the development of Zenker's degeneration on the boundary of the injured area with the living part of the fiber, and not within the injured portion, is understandable. Further, this theory explains the requirement of a steep gradient of injury for the development of Zenker's degeneration. Indeed, if areas with gradually decreasing changes follow the irreversibly injured area, there is practically no difference in potential—the current is insignificant.

LITERATURE CITED

[1]. N. D. Nasanov and D. L. Rozental. General Biology (USSR), v. VIII, No. 4, 1947.