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INFLUENCE OF PLANT PROTEASES UPON THE STRUCTURE AND ENZYMATIC ACTIVITY OF SCELETAL MUSCLES

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For the acceleration of the tenderness in beef, nowadays in the meat industry are used plant and microbial proteolytic enzymes. The application of the proteases as artificial tenderizers, gave us the possibility to study and elucidate more comlitely the role of the proteolytic enzymes in the muscles during the natural ripening. Wang et al (1), Aduckevich (2) establish, that the changes in protein substrates and the morfological structures of tissues under the influence of the endogenic and exogenic proteolytic enzymes,nevertheless quite similar are not indentical. While the catepsins act electoraly on chosen parts of the muscle fibre, the applied tenderizers act more nonspecifically, influencing the tital muscle structure.

In the last years, there are many announcements and data about the changes in the activity of enzymes in the muscles during the process of natural rupening of meat (3,4,5). In some of the previous works (6,7,8,9,10,11,12) we have used liver tissue as a model for studying the action of plant proteases on the structure of enzymes of the cell.

In our present study we had as object to follow the influence of plant proteolytic enzymes on the structure and activity of some oxydoreductasae and hydrolasae of sceletal muscles in rat.

Material and Methods.

The studies were made on the seceletal muscles (m.m.gastrocnemius, soleus) of rats from the Wistar breed, with a livewaight of 150-200 grams. From the plant proteases we used Papain (Miles Chem.Co.) and Ficin (Calbiochem), introduced in the organism intra peritonealy 25 mg and in veins 15 mg in single doses to each animal ,in solution in 1 ml saline solution. One group of the animals were killed by decapitation 90 minutes after the injection. M.m.

gastrocnemius and soleus, we separated and kept at 0-3°C temperature till the 44th hour. The investigations were made on the 2-nd, 20th and 44th hour of the injection and storage of the tissues, in vitro. In a second group of animals the action of the proteases was followed in vivo, as the animals were killed and investigated on the 3-rd, 20th, and 44th hour of the venous and interperitoneal injection of the proteases. To each investigated group there was a control group, which was injected with 1 ml saline solution. The materials for the investigation were refrigerated in dry snow and were fixed in 10% neutral formol.

On crysostatic cuts, thick 10 m we carried out the following enzymohystochemical reactions : succindehydrogenasae (SDH) and NAD-diaphorasae after Nachlas et al (13), lactatdehydrogenasae (LDH) and glucoso-6-phosphatdehydrogenasae (G_6PDH) after Hess et al (14), As-esterasae after Burston, the alcalic (AP) and acid (KP) phosphatasae after Grogg and Pearse (Barka et al 15). The paraffin cuts were stained with Chemalaun-eosin and after VanGison.

Results and Discussion.

With the animals killed on the 90th minute after the venous and intraperitoneal injection of ficin and papain, investigated on the 3-rd hour after the injection (group I), and with those investigated on the 2-nd hour after the injection, immediately after being killed , in the hystological cut predominated normal muscle tissue. On certain spots, however, were observed unevenly stained fields-pale sections stained poorly by eosin. In these sections and around them, the myofibriles are swollen, the transversal furrows and the nucleus are vaguely seen. Basic differences in the changes between the two groups are not observed, while in degree they are stronger in the venous application of the proteases.

In the given time terms for action of papain and ficin, in the muscle tissue is established a light increase of the activity of the investigated oxydoreductases (SDH, NAD-diaphorasae, LDH, and G_6PDH). On certain sections the reaction has its normal character: the sedimented formasan is coarse grained and a diffusion is observed. As with the venous, thus with the intraperitoneal injection of papain and ficin, the activity of the acid phsphatasae is strongly increased. Higher, in comparison with the control is the activity of the non specific esterasae.

With the increase in the period of action of the proteases, in the experiments in vivo as well as in those in vitro, the changes in the structure of the muscle tissue and the activity

of the studied enzymes increase. On the 20th hour after injection, the quantity of sections with swelling of myofibriles is increased. Envolved are whole muscle fibres, in which on places the sarcolem, the nuclei and the transversal furrows are observed very hardly. Prevalent are the poorly stained, almost unstained sections, in which are seen only comparatively pale nuclei. Strongly expressed quantitavely and qualitatively, are the changes in the investiga tions in vitro, and with the venous introduction of the proteases.

On the 44th hour the changes in the structure of the muscle elements, in the in vivo investigations, with both methods of injection of the papain and ficin, are similar with those obser ved on the 20th hour. In the in vitro investigations, in the described changes is observed a progresive evolution, which is demonstrated strongly with the venous injection. The changes are observed most often near the blood vessels and are acompanied by lymphoyd cell accumulation. In these sections the greater part of the muscle fibres are affected. Demonstrated is very strong uneven staining of the muscle tissue, while because of the dec reased staining ability very often are seen almost unstained sections. The cell nuclei of the muscle tissue in these sections are stained poorly, while a part of them are in a partial state of cariolyses. The transversal furrows is poorly observed or are totally lacking. Established is a strong swelling, desintegration, fragmentation and desorganisation of the muscle fibres. In a milder form are affected the connective bonds in the structure of the muscles.

Specific are the changes in the activity of the oxydoreducta ses in the process of the action of the proteases on the muscle tissue. On the 20th hour after injection, in the experiments in vivo, the sections with comparatively unaffected structure, exibit an activity similar to that of the control. On places where changes are observed, the activity of the enzymes is decreased : the sedimented formasan is presented in coarse granules while a strong diffusion is also observed. The decrease in the activity of the oxydoreductases in the envolved sections, with the intravenous injection in the experiments in vitro is stronger. The transver sal furrows of the myofibriles under the action of the enzymes are almost unseen while a strong diffusion is observed. In the influenced activity for the separate oxydoreductases only quanti tative changes are observed.

Inthe experiments in vivo, the changes in the activity of

of SDH, NAD-diaphorasae, LDH and G_6PDH on the 44th hour, are similar to those observed on the 20th hour after the injection. With the venous and intraperitoneal injection and storage of the muscle tissue in vitro 44 hours, the decrease in the activity of the oxydoreductases is strongest. Along with the general decrease in the activity in the investigated tissue, on places are observed sections with very weak reaction, and with singular depositions of formasan. Alsmost over the whole cut is observed a strong diffusion. On the 20th hour the activity of the acid phosphatasae and the non specific esterasae in all experimented muscles is higher than that of the control, while on the 44th hour is decreased and is almost equal with that of the cpntrols. A strong diffusion is observed.

The derived results show a specific action of papain and ficin on muscle tissue, expressed in an uneven staining to almost total lack of stained material, swelling of the muscle fibres, almost lack or very poorly expressed transversal furrows of the myofibriles, desorganisation of the muscle fibres, pale staining of the nuclei with signs of cariolyses, decrease in the activity of the oxydoreductases and an increase in the activity of the acid phosphatasae with strongly expressed changes in the character of the enzymatic reactions.

With the venous and intraperitoneal introduction of the proteases, the hystological and hystochemical changes have the same character, while the changes are stronger and more evenly distributed in the muscle tissue with the venous application. These results confirm the opinion of other authors for the expedience of introduction of proteolytic enzymes for tenderizing of meat, thru the blood vessel system, which ensures their even distribution and action (16,17). Further to that in killing the animals 90 minutes after injection and storage of the muscle tissue under 0-3°C the processes have a progressive with the time character, expressed with the envolvement of the greater part of muscle tissue and an increase in the changes. With the venous and intraperitoneal application and action in vivo after the 20th hour, is observed an inhibition of the hystological and hystochemical changes to one level, most probably because of the inclusion of the natural paths for the elimination of the introduced proteases and the general protective and repairing forces of the organism.

Papain and ficin act not only on the muscle but as well as on the connective tissue elements of the muscles, while the influence on these is weaker.

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