Histological changes of bovine M.longissimus dorsi frozen at various temperatures (-10°, -22°, -33° and -78°C)

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Samples of M.longissimus dorsi of bovine weighted ca.600 g. were frozen at -10°, -22°, -33° and -78°C. Samples of the last group were frozen by dry ice.

Investigations of histological preparations by light microscope have proved the following features for the samples frozen at :

-10°C - large groups of fibers are separated by large spaces indicating interfibrillar formation of large crystal agglomerations. In center of some fibres it is found the hole. The fibres are damaged at the surface and sometimes in the depth ;

-22°C - fiber groups are smaller and separated with smaller spaces. The holes are formed in center of much more fibers. These findings indicate that ice crystals are formed inter and intrafibrillarly. Fibers are more damaged by sarcoleme tearing as well as fragmentation ;

-33°C - fiber groups are still smaller being sporadically separated by greater spaces, i.e. there appeared interfibrillar formations of smaller ice crystals agglomerations. Fibers are less damaged than in the previously described samples ;

-78°C - the fibers are lying close to each others. In every fiber there is a hole what indicates that ice crystals were formed intrafibrillarly . In some fibers there are more smaller holes which are confluent sometimes. Even the holes of some adjacent fibers are confluent with the result of larger round holes formations. Fibers are less damaged but there are found frequently fibril tearing in them.

Fibres of muscles frozen at -22°C are mostly damaged.

Histologische Veränderungen von M.longissimus dorsi des Rindes bei verschiedenen Temperaturen (-10°, -22°, -33° und -78°C)

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Die Proben von M.longissimus dorsi des Rindes, von etwa 600 g, wurden auf -10°, -22°, -33° und -78°C gefroren. Die Proben der letzten Gruppe wurden mit trockenem Eis gefroren.
Durch die Untersuchung der histologischen Präparate mit einem Lichtmikroskop wurde folgendes festgestellt:

-10°C - grössere Fasergruppen sind durch grosse Flächen getrennt, was darauf hinweist, das sich grosse Kristalaglomerationen interfibrilär bilden. In manchen Fasern ist in der Mitte ein Loch zu finden. Die Fasern sind auf der Oberfläche beschädigt und manchmal auch in der Tiefe ;

-22°C - die Fasergruppen sind kleiner und durch kleinere Flächen getrennt und in einer grö - sseren Fasernzahl befinden sich Löcher in der Mitte. Dieser Befund weist darauf hin, dass sich die Eiskristale inter- und intrafibrilär bilden. Die Fasern sind durch Reissen der Sarkoleme und durch Fragmentation stärker beschädigt ;

-33°C - die Fasergruppen sind noch kleiner als bei den vorher beschrieben Proben und sind nur stellenweise durch grössere Flächen getrennt, bzw. haben sich interfibrilär kleinere Aglomerationen der Eiskristale gebildet, die Fasern sind weniger beschädigt als bei den bisher beschriebenen Proben ;

-78°C - die Fasern liegen aufeinander und Löcher sind vorhanden, was darauf hinweist, dass sich die Eiskristale interfibrilär bilden. In einigen Fasern befinden sich mehrere kleinere Löcher, die manchmal konfluieren. Die Löcher von benachbarten Fasern konfluieren auch manchmal. Die Fasern sind weniger beschädigt, es kommt aber oft zum Reissen von Fibri - llen.

Die Schädinung von Muskelfasern ist am grössten bei -22°C.

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Les changements histologiques de M.longissimus dorsi des bovins,gelé aux differentes températures (-10°,-22°,-33° et -78°C)

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Les échantillons de M.longissimus dorsi des bovins,poids environ 600 g,gelés à -10°,-22°, -33° et -78°C.Les échantillons du dernier groupe étaient gelés par une glace sèche.Par l'examen des préparations histologiques au moyen de microscope à lumière,on a constaté les caractéristiques suivantes sur les échantillons gelés à:

-10°C - plus grands groupes de fibres sont séparés par grands espaces,ce qui montre gue de grandes agloméràtions de cristaux de glace se produisent interfibreusement.Au milieu de quelque fibre on trouve un trou.Les fibres sont endommagées à la surface mais il-y-en a avec des endommagements en profondeur;

-22°C - les groupes de fibres sont moins grands et ils sont séparés par des éspaces plus petits et dans un nombre de fibres il-y-a des trous au milieu.Cette constatation montre que les cristaux de glace se forment inter-et intrafibreusement.Les fibres sont encore plus endommagées par l'arrachement de la sarcolème et par fragmentation;

-33°C - les groupes de fibres sont encore plus petits qu'aux échantillons précédemment décrits et ils ne sont séparés que par endroits avec des espaces plus grands, c'est-à dire,interfibreusement sont formées de moines agloméràtions des cristaux de glace,les fibres sont moines endommagées qu'aux échantillons précédement décrits;

-78°C - les fibres sont posées les unes sur les autres et elles ont des trous ce qui indique que les cristaux de glace se forment intrafibreusement.Dans quelques fibres il-y-a de petits trous qui confluent parfois.Les trous des fibres voisines confluent aussi et forment de plus grands trous ronds.

Гистологические изменения M.longissimus dorsi рогатого скота замороженого на разных температурах (-10°, -22°, -33°,-78°C)

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Образцы M.longissimus dorsi рогатого скота весом 600 г заморожены на -10°, -22°, -33° и -78°C.Образцы последней группы заморожены сухим льдом.Исследованием гистологических преппаратов световым микроскопом утверждены следующие характеристики образцов замороженых на:

-10°C - большие группы волокон отделены одни от других,что показывает агломерации кристаллов льда формирующихся интерфибрилярно. У некоторых волокон имеются отверстия в середине.Волокна повреждены на поверхности а некоторые в глубине;

-22°C - группы волокон небольшие и отделены одни от других а в большинстве волокон есть отверстия в середине.Это показывает,что кристаллы льда формируются интер-интрафибрилярно.Волокна больше повреждены вытаскиванием сарколем и фрагментацией;

-33°C - группы волокон еще меньше,чем у вышеуказанных образцов и только на несколько мест отделены между собой,т.е. интерфибрилярно сформированы небольшие агломерации кристаллов льда,волокна меньше повреждены,чем у предварительно описываемых образцах;

-78°C - волокна налегают одно к другому а в их имеются отверстия,что показывает формирование кристаллов интрафибрилярно.У некоторых волокон есть большое количество небольших отверстий,которые в некоторых случаях соединяются.Соединяются и отверстия соседних волокон образующие большие круглые отверстия.Волокна немного повреждены,но в них часто отделяются небольшие волокна.

Больше всех повреждены волокна мускул замороженых на -22°C.

Histological changes of muscle longissimus dorsi of beef frozen at various temperatures (-10°, -22°, -33° and -78°C)

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There are in literature a lot of published data on histological changes of the muscles frozen at various temperatures. All of those papers quote the fact that in slowly frozen muscles ice crystals are formed interfibrillarly while at those fastly frozen intrafibrillarly (Tuchschneid-Emblik, 1959; Love, 1966; Lawrie, 1966; Cassens, 1971; Rahelić and Mihalković, 1972). Cassens (1971) have indicated that in the muscles frozen even at -40°C ice crystals are formed in the fibers, while at those frozen at -48°C they are formed also in fibers but at the periphery. Goma and Biro (1970) have proved that ice crystals are formed interfibrillarly in the muscles frozen at -40°C. In the case of muscles frozen at -150°C (Love, 1966; Lawrie, 1966; Cassens, 1971) and at -193°C (Tuchschneid-Emblik) small ice crystals are formed equally distributed inter- and intrafibrillarly.

Such differences in the course of freezing involve different muscles damages, whereas in the muscles frozen slowly these damages are greater, but smaller in those fastly frozen. Contrary to the mentioned findings, Crigler and Dawson (1968) have observed that the rate of freezing and the degree of damages proved not to be always in agreement, as in some ranges of higher freezing temperatures damages are relatively smaller and at slower temperatures greater.

Most of the cited papers give the results of investigations on the effect of a few freezing temperatures, i.e. freezing temperatures in the smaller ranges on the structure of muscles. This initiated investigations of histological changes in the muscles frozen at higher temperatures in the range from -10° to -193°C in order to give the picture of changes in the continuity of lower freezing temperatures. This paper contains the review only of a part of these results.

Material and methods

For the described investigations we have used M. longissimus dorsi of steers of red spotted breed, weighing about 450 kg. Samples were cut from the left halfs of carcasses, weighed of about 600 g, frozen at -10°, -22°, -33° and -78°C. Samples were frozen in refrigerator at -10°C, in freezing room at -22° and -33°C and those frozen at -78°C with dry ice. At each of these temperatures have been frozen in group of six samples. The course of freezing has been registered for the surface layer and the middle of the sample by thermocouple, Ellab-Copenhagen.

Immediately after freezing, samples were cut by Cry-Cut, American Optical Comp., at -20°C, into slices thick 10, 15 and 20 nm. Slices were stained by hematoxylin - iosine and fixed by canada balsam. After the analysis of samples, typical places were photographed on the muscle preparations frozen at all investigated temperatures.

Results and discussion

Muscles frozen at -10°C were during freezing in the range of critical temperature from 0° to -5°C, in the middle of sample, for 798 min.

Histological changes of muscles on the cross-section (Fig. 1) were characterized by the groups of several tens of fibers. These groups were set apart with great interfibrillar spaces created as the result of crystals formations. Fibers in these groups leans one on another being here and there separated. Individual groups are connected with one-or

twofold rows of fibers.
On the longitudinal section of these samples (Fig. 2) there are rare groups of connected fibers, branched into groups of few fibers or even individually. These spaces between fibers are in some places large. Fibres are mainly curved here and there broken, sarcolemma is often damaged and in some places even the deeper layers are destroyed.
Muscles frozen at -22°C were in the range of critical temperature in the middle of sample for 336 min.
On the cross-section of the preparations (Fig. 3 and 4) it is seen that fiber groups are smaller than in those frozen at -10°C, separated one from another with smaller interspaces. This proves that ice crystals are formed interfibrillarly. However, there are in some places holes in the middle of fiber as the result of ice crystals formed in the fiber. This finding proves that in these samples ice crystals are formed inter- and intrafibrillarly. Such arrangment of ice crystals in muscles frozen at this temperature was established for three groups of samples.
On longitudinal section of the frozen muscle (Fig. 5) the previous finding is proved that the fibers are agglomerated in smaller groups as well as that the spaces between them are markedly smaller. However, fiber damages are significantly greater. They are expressed by sarcolemma tearing, or even by tearing of deeper layers or ofter fibers tearings into fragments. It is obvious that the breakages are appeared in various degrees.
Samples frozen at -33°C were in the range of critical temperature in the middle of samples for 54 min.
Characteristic of the muscle preparations frozen at this temperature proved to be of the net structure of fibers (Fig. 6 and 7). Namely, fibers are separated one from another with more or less equal spaces, arranged in rows connected in such a way that they resemble to the net. Another characteristic of the histological picture of these samples proved to be that there are hardly any hole in the fibers, what indicates intrafibrillar ice crystals formations.
On the longitudinal section in the preparation (Fig. 8) the picture described under cross--section of fibres is repeated - fibers are separated with smaller spaces creating the net structure of tissue. Fibers are surfacely damaged and even teared in deeper layers, but these damages are smaller than on muscles frozen at -10° and -22°C.
Samples frozen at -78°C were in the range of critical temperature in the middle of samples for 22 min.
Histological structure of these muscles on the cross-section proved that the fibres are mainly close to each other (Fig. 9), i.e. that they lean one on another, being only here and there separated with small interspaces obtained due to ice crystals formations. However, holes are there in all fibers. In the greatest number of fibers these holes are in the middle. But, in many fibers there are more smaller holes remained after ice crystals formations at those places. These holes become confluent in some fibers or even between a few other fibers, creating greater spaces of irregular shape, but mainly of rounded surfaces (Fig. 10).
The described picture repeats even on the longitudinal section (Fig. 11) but in another projection. Namely, it is seen that the fibers lean one on another being relatively hardly separated. However, the longitudinally extended spaces within the fibers are dominant. These finding indicate that ice crystals in fibers frozen by dry ice are formed mainly intrafibrillarly.
Due to this location of ice crystals there is established the damage of fibrils (Fig. 12). This phenomenon of fibrils damaging in fibers is observed only in the preparations of these muscles. This proved to be logical as by freezing at this temperature (-78°C) ice crystals are formed intrafibrillarly and are so great that at the formation tear the elements

of intrafibrillar structure.
Fibers damages in these muscles are the smallest in comparison with the damages on previously described frozen muscles.

If the degree of damages is compared for the samples of all four groups of muscles, it is possible to conclude that the damages are mostly expressed on the muscles frozen at -22°C. This state could be explained by the fact that in the muscles frozen at this temperature great ice crystals are created interfibrillarly and even often intrafibrillarly. Due to this fact fibers are extended by crystals formed in them and pressed by those formed between them, whereas these pressures directed conversely damage the fibers even more. In the muscles with the created ice crystals mainly interfibrillarly (frozen at -10°C and -33°C) or even intrafibrillarly (frozen at -78°C) fiber damages are smaller.
There still remains the question why the crystals of ice are formed in fibres of muscles frozen at -22°C, but nearly don't appear in those frozen at -33°C ? However, Cassens (1971) quotes that ice crystals are formed in fibers of muscles frozen at -40°C while in those frozen at -48°C they are peripherly located. On the contrary, Goma and Biro (1970) have established that in the muscles frozen at -40°C ice crystals are formed interfibrillarly. It is necessary to mention the finding of Crigler and Dawson (1968) that the damages of fibers are not always in agreement with freezing temperatures. If we take into account the finding obtained in this paper as well as the data cited by Cassens (1971), or Crigler and Dawson (1968) it is possible to ask whether the arrangment of ice crystals in muscles changes regularly with the decrease of freezing temperatures?

Conclusion

According to the results of these investigations it is possible to conclude:

- freezing of the muscles at -10°C involves the formation of ice crystals, mainly, only interfibrillarly, at -22°C inter- and intrafibrillarly, at -33°C mainly, only interfibrillarly, and at -78°C mainly intrafibrillarly;
- mostly damaged fibers of the muscles are of those frozen at -22°C, because ice crystals are formed inter- and intrafibrillarly;
- in the muscles frozen at -78°C there are very often tearing of myofibrils.

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Fig. 1. Cross-section of muscle frozen at -10°C (x 30)

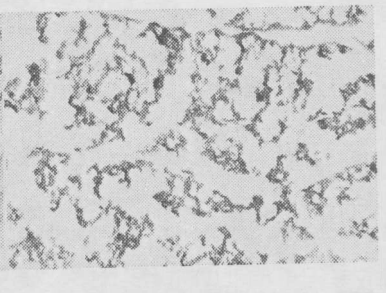

Fig. 2. Longit. section of muscle frozen at -10°C (x 40)

Fig. 3. Cross-section of muscle frozen at -22°C (x 25)

Fig. 4. Cross-section of muscle frozen at -22°C (x 100)

Fig. 5. Longit. section of muscle frozen at -22°C (x 40)

Fig. 6. Cross-section of muscle frozen at -33°C (x 25)

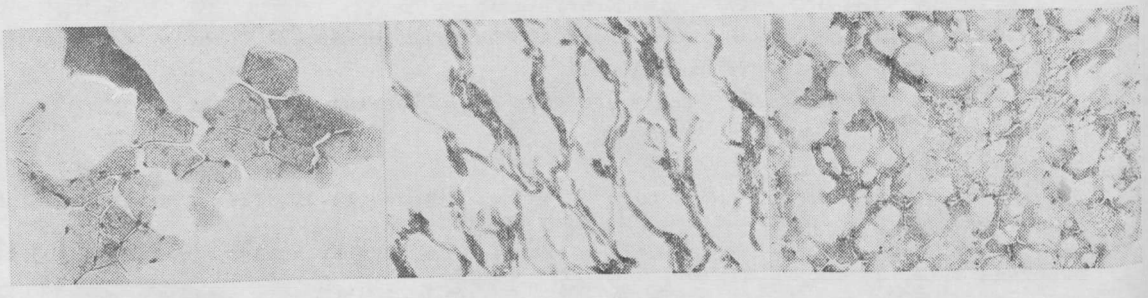

Fig. 7. Cross-section of muscle frozen at -33°C (x 150)

Fig. 8. Longit. section of muscle frozen at -33°C (x 40)

Fig. 9. Cross-section of muscle frozen at -78°C (x 100)

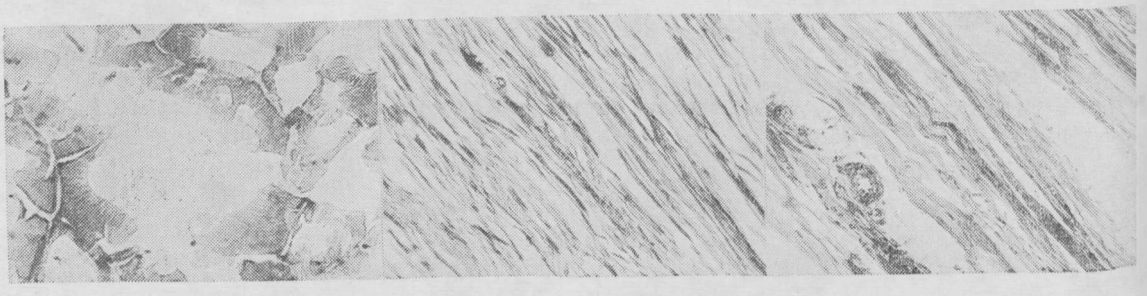

Fig. 10. Cross-section of muscle frozen at -78°C (x 250)

Fig. 11. Longit. section of muscle frozen at -78°C (x 25)

Fig. 12. Longit. section of muscle frozen at -78°C (x 100)