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HISTOLOGICAL STUDIES ON MUSCULAR TISSUES OF SHEEP INJECTED WITH ADRENALIN

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INTRODUCTION

Meat tenderization is closely related to biochemical changes of muscaes which take place after slaughtering.

These changes have a great effect on the structure of muscular tissues. The relationship between meat tenderness, the changes in the shape of muscular fibers and their contraction have been investigated by many authors (Paul, 1944 ; Zender, 1958 ; Sakalov *et al.*, 1963 ; Sakalov and El Dashlouty, 1963).

Sakalov and El Dashlouty (1963) studied the changes in the muscular tissues of sheep during tenderization using the normal and the electronic Microscope. Their studies revealed that the contracted snd uncontracted fibers were observed in the mean time of autolysis. They also added that at the time of full rigor the majority of fibers were observed to be contracted.

Tinikov and Makaiev (1966) studied the changes in cow muscles after slaughtering based on the observed changes in sarcomere lengths. They detected 4 stages of autolysis. They also (1966) agreed with Sakalov and El Deshlouty (1963) that during and after rigor mortis, both contracted and relaxed fibers exist. El Ashri and El Dashlouty (1967) found that animal injection with adrenalin before slaughtering gave meat of a better quality. The pH value and the solubility of the actomyosin complex were found to undergo only slight changes while the water holding capacity and the tenderness of the meat of injected animals were of better qualities. They also concluded that injection with adrenalin minimized autolysis and rigor motis. However, no studies concerning the changes in the fine stucture of the muscular tissues injected with adrenalin were yet carried out.

MATERIALS AND METHODS

Meat samples were taken from injected and noninjected sheep (El Ashri and El Dashlouty, 1967). Sampling took place immediately after slaughtering, after 24, 48, 120, and 192 hours of storage at 0-1°C. Fixation was held in 10% neutral formalin solution.

The samples were first dehydrated, then blocked in collodoin. Two methods of staining were used, namely Hematoxlin-iosin and Vangeison's method. Sections from *Longissimus Dorsi* (L.D.), *Biceps Femoris* (B.F.), and *Triceps Brachii* (T.B.) were examined.

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RESULTS AND DISCUSSIONS

Table 1 shows the observed changes of the sarcomere lengths during autolysis. The data presented revealed that contraction in muscular tissues during autolysis was irregular. During the same period of autolysis sarcomeres were observed to be of different lengths, not only in different fibers, but also within the same fiber. Therefore the lengths were presented in the forms of minima and maxima and not as averages.

On the basis of the data shown in table I, three stages of autolysis could be detected :-

1. *The stage of relaxation.*—which is characterized by sarcomeres ranging between 3.59 and 2.89 microns.

2. *The stage of full rigor.*—where the majority of the sarcomeres ranged from 2.06 to 1.66 microns in length, no measurements are obtained in the rest of sarcomeres due to the absence of cross-striation.

3. *The stage of tenderization.*—where the majority of the sarcomeres were of longer lengths (2.34-2.39 microns).

Immediately after slaughtering sarcomeres of injected and non-injected animals were observed to exhibit the same range of sarcomere length variation. However, it is worth mentioning that in injected animals sarcomeres of maximum lengths predominated. In the case of non-injected animals small spaces between fibers (vacules) were found. Such spaces were not observed between the fibers of injected animals. Existence of vacuoles may be explained on the basis that the fibers of injected animals were swollen with water besides that the average diameter of fibers was found to be smaller in the case of injected animals than non-injected ones. The variation of fiber diameters is presented in Table II.

In the tissues of both injected and non-injected animals, immediately after slaughtering, it was also noticed that the cross-striation of muscular fibers was clear. Collagen and elastin fibers were straight and filled the spaces between muscular bundles. Muscular fibers are observed to be straight or slightly undulated (wavy).

After 24 hours of slaughtering (at full rigor), sarcomeres were found to be shorter. Heterogenity of sarcomere lengths was also noted. The minimum sarcomere lengths were found to be predominant in the case of non-injected animals. Fibers with no cross-striations were encountered in the case of non-injected animals. Hence it could be suggested that this was a more developed stage of contraction.

TABLE 1.—SARCOMERE LENGTHS OF MUSCULAR TISSUES ADURING UTOLYSIS.

(in Microns)

Periods of autolysis in hours	Non injected animals			Injected animals		
	L.D	B.F.	T.B.	L.D.	B.F.	T.B.
0	2.15–2.86	2.34–.198	3.59–2.39	2.14–2.86	3.98–2.14	3.59–2.39
24	2.06–1.66	1.71–1.61	1.54–1.79	2.57–1.98	3.67–2.14	2.15–1.79
48	2.34–1.71	2.86–2.27	1.67–2.27	2.14–1.98	3.67–3.21	3.33–2.27
120	1.98–1.71	—	2.15–3.50	2.24–2.14	—	3.95–3.08
192	2.34–1.084	—	2.29–3.50	—	—	3.59–2.69

TABLE 2.—THE DIAMETERS OF MUSCULAR FIBERS DURING AUTOLYSIS

Periods of autolysis in hours	Non injected animals			Injected animals		
	L.D.	B.F.	T.B.	L.D.	B.F.	T.B.
0	20.56–30.15	25.70–33.41	20.00–30.15	20.56–30.84	20.56–28.27	12.90–30.12
24	25.70–41.12	28.27–38.55	30.15–38.76	25.70–35.98	23.13–28.27	15.08–30.12
48	25.70–33.41	20.56–28.27	21.15–32.30	17.99–28.27	15.24–25.70	25.84–27.99
120	17.99–30.84	—	15.79–32.30	15.42–25.70	—	15.08–25.84
192	15.42–28.27	—	17.23–25.48	—	—	15.08–23.63

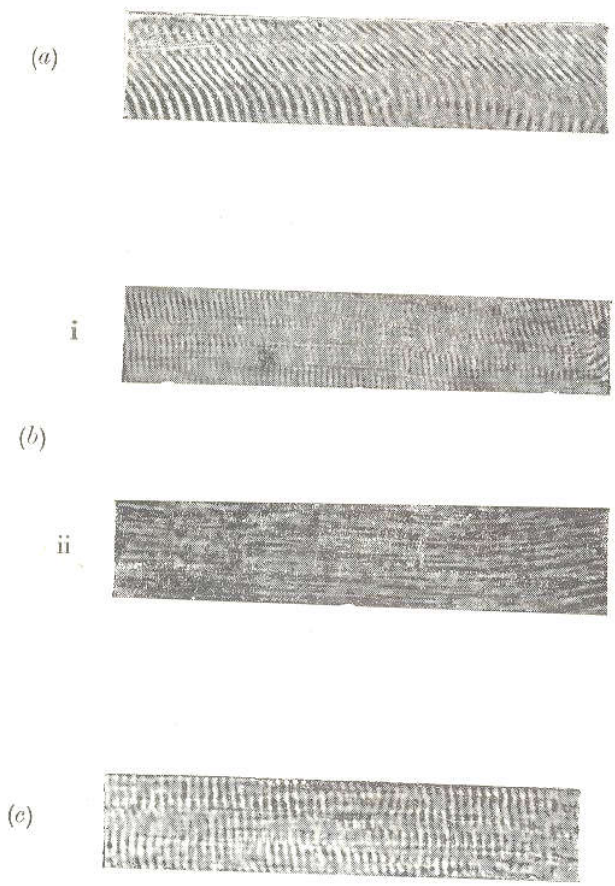


FIG. 1.—The Stages of Autolytic Changes

(a) The Stage of relaxation sarcomere lengths range from 3.59 to 2.86 microns).

(b) The stage of full rigor:

(i) Sarcomere lengths range from 2.06 to 1.66 microns.

(ii) no cross-striations are observed.

(c) The stage of tenderization (sarcomere lengths range from 2.34 to 2.39 microns.).

The maximum vacuolations between fibers and their undulations were observed after 24 hours of slaughtering (Fig. 2). These maxima were of less values in the case of injected animals than those encountered in the case of non-injected ones.

The authors suggest the following explanations for the above mentioned phenomena :

1. The high ability of water swelling observed in the fibers of injected animals, which may be due to the high pH value.

2. Undulated fibers were less frequent in the case of injected animals, which may reflect a more regular fiber contraction.

After 48 hours of slaughtering the fibers of injected animals were observed to be more undulated compared with those of non-injected ones. It was also noticed that the lengths of sarcomeres were longer in the injected animals than those of the non-injected ones. However, the sarcomere lenghts in both cases were longer and the diameters of fibers were smaller after 48 hours of storage compared with the lenghts measured after 24 hours of slaughtering.

The degree of rigor mortis and its development were less marked in the case of injected animals. This may explain the higher solubility of extracted myosin, which was observed with injected animals by El Ashri and El Dashlouty (1967).

The effect of injection with adrenalin before slaughtering on the degree of fiber contraction during autolysis requires an explanation. This explanation has to take into consideration the chemical changes which are responsible for the fiber contraction.

Many authors proved that the contraction of tissues was due to the break-down of ATP (Bendall, 1951; Bate Smith, 1956 ; Perry, 1956 ; and Hamm, 1968). Whenever, the factor of Marsh-Bendall is markedly active, no break-down of ATP takes place, due to that the ATP-ase of myosin looses its activity. The activity of Marsh-Bendall factor does not take place unless low concentration of free cations exists. It is observed that the lower the pH value of a solution (extracted from a muscle) the higher the amount of free Ca^{++} ions in this solution. El Ashri and El Dashlouty (1967) observed that the injected tissues show a higher pH value compared with non-injected tissues. Whence the residual amount of ATP are not expected to be broken down in the case of injected animals which could be taken as an explanation for the observed low degree of contraction.

Another explanation could be given in the following : The injection of animals with adrenalin before slaughtering results in conversion of ATP into inorganic phosphorus which is secreted with urine (El Ashri and El Dashlouty, 1967). The residual ATP is sufficient to produce only a small amount of energy which can produce only a low degree of contraction. However, experimental studies are suggested to confirm any of the above mentioned explanations.

During the period from the second to the eighth day of storage a gradual increase in the sarcomere lengths and a decrease in the diameters of muscular fibers were observed. These changes were noticed to be of a higher order in the case of injected animals. The small amounts of vaculations observed in the case of injected animals may be interpreted as due to the high degree of protein swelling (Fig. 3). In the case of injected animals, it was also observed that the fibers were highly undulated. This high degree of undulation may explain the high amount of fiber breaks observed after 8 days of storage.

CONCLUSIONS

From the previously mentioned discussions the following could be concluded :

1.—The autolytic changes that take place during the storage of sheep meat are subdivided into three stages :

(*a*) The stage of relaxation which is characterized by sarcomeres ranging from 3.59 to 1.86 microns in length.

(*b*) The stage of full rigor in which the majority of sarcomeres ranges from 2.06 to 1.66 microns in length, in the rest of the sarcomeres no measurements are obtained due to the lack of cross-striations.

(*c*) The stage of tenderization in which the majority of sarcomeres are of longer lengths (2.34–2.39 microns).

2.—Injection of animals with adrenalin, besides its chemical effect, is accompanied by remarkable changes in the histological structure of muscular tissues.

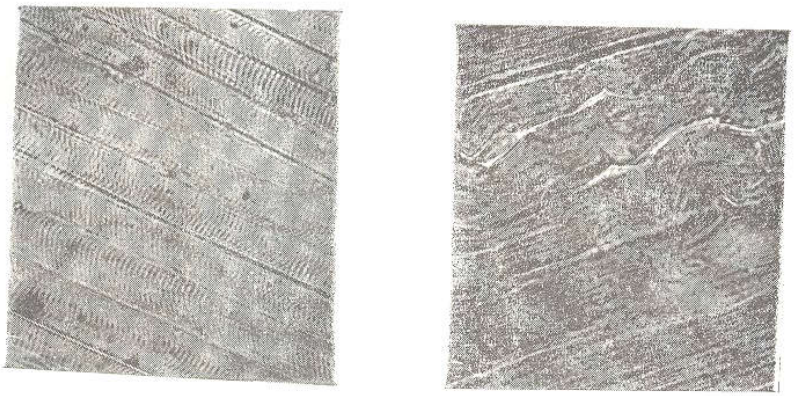
At full rigor, in the case of injected animals the sarcomere lengths were found to be longer, and the fiber diameters smaller, when compared with the results obtained in the case of non-injected animals. This may indicate that the animals injected with adrenalin before slaughtering offer more tender meat.

3.—The results of the examination of the fibers and the spacing between them revealed that in the case of injected animals muscular tissues had higher water holding capacity.

4.—The high solubility of actomyosin complex in the injected animals was interpreted to be due to a less contraction in the actomyosin fibers.

5.—Mechanism of contraction was discussed through glycogen and ATP amounts, pH values, the relation between Marsh-Bendall factor and free calcium ion content. This discussion was used to explain the effect of adrenalin on the degree of fiber contraction of the injected tissues after slaughtering.

FIG. 2.—Muscular fibers of *triceps brachii* at full rigor
(After 24 hours of slaughtering)



(*a*) (*b*)

FIG. 3.—Muscular Fibers of *Triceps brachii* after 24 hours of Slaughtering

(*a*) Injected with adrenalin animal. (*b*) Non-injected animal.

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دراسات هستولوجية على الأنسجة العضلية للأغنام المحقونة بهرمون الآدرينالين

الملخص

اجريت الدراسة على لحوم مجموعتين من الأغنام الأوسيمى الأولى حقنت بالادرينالين قبل الذبح وأخرى غير محقونة وأخذت منها عينات من العضلات التالية :

بعد الذبح مباشرة ثم بعد ٢٤ ، ٤٨ ، ١٢٠ ، ١٩٢ ساعة من الذبح والتخزين على درجة حرارة صفر — ١ مئوية .

وظهر من الدراسة الهستولوجية ما يأتى :

١ — يمكن تقسيم التحلل الذاتى الى ثلاثة مراحل أساسية تبعا لاتساع السار كومير وهى :

(أ) مرحلة الارتخاء ويتميز بسار كومير متسع (من ١ر٨٦ — ٣ر٥٩ — ميكرون) .

(ب) مرحلة التيبس الرمى ويتميز بالسار كومير الضيق (١ر٦٦ — ٢ر٠٦ ميكرون) وفى بعض الأحيان يختفى التخطيط نهائيا .

(جـ) مرحلة التطرية وفيها يكون اتساع السار كومير أكبر (٢ر٣٤ — ٢ر٣٩ ميكرون) .