The Electrical Stimulation of Lamb Carcasses.

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Introduction

The biochemical changes which are caused by the electrical stimulation of the carcass in the muscle fiber were investigated during the last years. Nevertheless, there are still many other problems, which are related to ES and these are mentioned at various levels of meat technology.
Along with the study of the biochemical changes of the muscle fiber, many investigators studied the structural changes which the electrical stimulation causes to muscle fiber. However, the search of the literature in this area did not provide a complete view about these which it happened in the muscle fiber during the action of the electric current.
In case this is taken into consideration, the fact that the tenderness of meat is significantly induced by the structural changes of the muscle fiber, at that time, the interest of the search is obvious for the alterations in the morphology of the muscle fiber which occur during the electrical stimulation of the meat.

Materials and Methods

Carcasses of sheep weighing about 20 Kg were used in these experiments. 30 min after stunning the Longissimus dorsi and Semitendinosus muscles were removed from the right sheep carcass half and then were electrically stimulated (ES) of 250 Volts for 50 sec (I=23 mA). The same muscles from the left sheep carcass half served as a control sample. The electrically stimulated samples and the control samples were stored in the cold storage chamber at ≃ 2°C.
On all samples (electrically stimulated samples and the control samples) the following determinations were performed.
Measurement of pH, 10 min, 1st, 2nd and 5th day after the ES, GÖFO value, Water Holding Capacity (WHC), the lactic acid and the total creatinine determination were performed, 1st 2nd and 5th day after the electrical stimulation.
Furthermore, the samples which were taken at 1st, 5th and 24th hour after slaughter were studied at the light and the electron microscopic levels.

The samples which could be examined under the light microscope were fixed in a 10% neutral formalin solution and then they were enclosed in paraffin.
Each specimen which could be examined under the electron microscope was fixed in a 2,5% glutaraldehyde solution and a 1% Osmium Tetroxide (OsO_4) solution.
Dehydration was followed by acetone and 1,2-Propylene oxide and they were enclosed in EPON 812.

Results and Discussion.

The results of the biochemical determinations are shown on the table 1. It is obvious from the results that:
There is a significant difference in the pH value (table 1) between the control and the electrically stimulated samples. This difference which remained stable until 5th day after slaughter ranged about 0,5 of unit (from 0,1 to 0,6 of unit).
The observations of other workers (Carse 1973, Dutson et al. 1981, Eikelenboom et al. 1981, Georgakis et al. 1981) have also indicated the same differences to pH value. They generally accept that this significant decrease of the pH value is due to a more rapid increase of lactic acid levels which is caused by the acceleration in the rate of post mortem glycolysis inside of the muscle. The electrical stimulation is the cause of this alteration.
This point of view is confirmed by the data of talbe 1, which clearly demostrates that the concentration of lactic acid in the electrical stimulated samples is greater than the control samples. This difference begins to become clear on the 1st day and it continuously increases up to the 5th day after slaughter (and the ES).
The decrease of the pH value it seems to have an indirect favorable influence in tenderness of meat. Furthermore, Dutson et al. (1981), formulate the thought that this low pH creates suitable conditions for other probable alterations for the muscle tissue proteins. This low pH in conjunction with the high carcass temperature, before the development of rigor mortis, enhances the activity of acid proteases (Dutson et al., 1977). This fact, with relation to the development of rigor mortis at high temperatures, it seems to be the cause of significant increase of tenderness of meat.
McKeith et al., (1981) reported that the time in which the ES takes place it seems to have no influence on the colouring, the taste and the tenderness of the muscle.
There were differences in the total creatinine concentration (table 1) and the GÖFO value (table 1) among the samples which were examined.
There is a universal acceptance of the fact that muscles for electrically stimulated carcasses are more bright than those from unstimulated carcasses. This phenomenon is probably caused by some alteration of myoglobin. Furthermore, it is obvious from the table 1 that the Water Holding Capacity is induced by the electrical stimulation (ES).
Light microscopic examination revealed a lack of uniformity contraction of the myofibrils of

many muscle fibers. Savell et al., (1978) first, observed the morphological changes of the muscle fibers with the light and the electron microscope and they called them "contraction zone". These workers, just like George et al., (1980) observed that the contraction bands desappeared and Z line was disintegrated in the experimental samples. The rupture of sarcoplasmic reticulum in some cases was observed. The "contraction zone" which is created by the influence of ES seems to be due to a very intense contraction of the sarcomere.
The hypothesis of George et al., (1980) that this morphological alteration is caused by a sediment of proteins seems not to have many supporters at least at present.
The structural changes of the M. Semitendinosus as well as of the M. Longissimus dorsi were alike and for that reason they will be described together. No difference was observed between the muscles of the two test animals.
Light microscope:
On the control samples of one hour after the slaughter a fluctuating route of minor degree of many muscle fibers was observed. This fact is considered as a typical change in the muscles during the storage of meat because of the nonuniform contraction of all of the muscle fibers.
In the electrically stimulated muscles at the same hour (the 1st hour after slaughter), fibers with alternating areas of thickness and thinness were observed in addition to fluctuating route of the muscle fibers mentioned above.
At the 5th hour after slaughter, on the control samples at certain areas the cross-striation of the fibers were not distinct but an intense fluctuating route of many muscle fibers was observed (fig. 1).
The electrically stimulated muscles at the same hour (5th hour after slaughter) presented, at certain positions, muscle fibers with more intense fluctuating route as well as upright muscle fibers bearing intracellular areas of thickness and thinness (fig. 2). In these thick areas the cross-striation (of the muscle fibers) were not present. Several nuclei appear to be hyperchromatic and several of them were pyknotic.
At the 24th hour on the control samples, intense intracellular oedema was observed which resulted in the removal of the sarcolemma from the rest of the structures of the cell. The fluctuating route of many muscle fibers was intense and the distance between myofibrils was increasing. Furthermore, pyknosis of the nucleus was often observed (fig. 3)
The electrically stimulated muscles (24th hour after slaughter) had the above mentioned changes of the control samples but at a more intense degree. Furthermore, areas of thickness and thinness were observed on the upright muscle fibers. In the areas of thinness, the cross-striation of the muscle fibers were retained (fig. 4).

The mean value of the sarcomere length of the muscle fibers in the electrically stimulated samples (H) and the control samples (M).

M. Semitendinosus

	S.M.285	S.H.285	S.M.286	S.H.286
1 h	1,5 μ	2 μ	1 μ	1,7 μ
5	1,8 μ	1 μ	1,5 μ	1,5 μ
24	1,7 μ	1,5 μ	1,5 μ	1,3 μ

M. Longissimus dorsi

	LDM 285	LDH 285	LDM 286	LDH 286
1 h	1,9 μ	1,8 μ	1,7 μ	2 μ
5	2 μ	1,7 μ	1,8 μ	1,8 μ
24	1,5 μ	1,5 μ	1,5 μ	1,6 μ

Electron microscope:
The control samples of the 1st hour contained myofibrils at a different degree of contraction. The "I band" was not dinstinct in most cases. A starting intracellular oedema removed the sarcolemma from the myofibrils as well as myofibrils from each other. The swelling spaces were occupied by a lot of granules of glucogen and dilated cisternae of sarcoplasmic reticulum. Several mitochondria were swelling and presented a disintegration of their cristae (fig. 5).
The electrically stimulated muscles of the same hour (1st hour) did not present the above mentioned contraction in the control samples, of the myofibrils and the "I band" in most cases was distinctly appeared. The intracellular oedema could be characterized as more extensive and slightly more intense than that of the control samples and thus more dilated cisternae of the sarcoplasmic reticulum were observed (fig. 6) as well as more swelling mitochondria. At certain positions there was also observed a breakdown of the myofibrils on "Z line" as well as in other positions of the sarcomere (fig. 6a).
The typical finding, however, in the electrically stimulated muscles was the strong contraction at certain areas of the myofibrils which were corresponding to the areas of the thickness of the fibers as was observed at the light microscope. The contraction in these areas reached the point where the identification of the structure of the sarcomeres was impossible and their limits (Z line) were hardly distinct or were not even present (fig. 7).
At the 5th hour after slaughter the sarcomeres of the myofibrils on the control samples were less contracted in comparison with those of the 1st hour. The intracellular oedema was more intense and was characterized by disintegration of cristae and vacuolation of the mitochondria and dilations of the sarcoplasmic reticulum (fig. 8). Many myofibrils, especially those which are located near the sarcolemma, where the more intense oedema is found, are destructived (fig. 8a). During this time (5th hour after slaughter) a starting extracellular oedema mainly in the spaces around the vessels was also observed.
At the 5th hour after slaughter, the electrically stimulated muscles presented a more contraction in relation to the 1st hour which was more intense in the M. Semitendinosus (short muscle) and it was slight in the M.Longissimus dorsi (long muscle). From the histopathological aspect the autolytic changes presented a clear difference to those on the control samples. The intracellular oedema was most intense resulting in the removal of the myofibrils from each other or even their disruption (fig. 9). The extracellular oedema appeared also to be intense mainly in the spaces around the changed vessels.
The typical non-uniform contraction of a great number of myofibrils was also evident. This

non-uniform contraction often resulted in their disruption (fig. 9a,b). Finally the nuclear changes were frequent and appeared with the presence of hyperchromatic or pyknotic nuclei which accompanied great changed fibers (fig. 10).
At the 24th hour after slaughter the autolytic changes on the control muscles were great and were characterized by intense up to complete vacuolation of the mitochondria (fig. 11), dilations of sarcoplasmic reticulum, in presence of mylin figures and phagosomes. The myofibrils are disrupted and great swelling areas mainly in the periphery of the cell are flooded with granules of glucogen. The nuclear changes were frequent and the extracellular oedema was intense.
The length of the sarcomere presented a further decrease which differed in the two muscles (see table).
The electrically stimulated muscles of the same hour (24th hour) present the above autolytic changes but at a more intense degree. Here, the spaces leaved by the disrupt myofibrils and the vacuolation of all the membranaceous structures were large (fig. 12). Most of the remaining myofibrils were opaque and their structure was indistinct. Some of them lost their structure completely and appeared as being fibrillating masses having no form (fig. 12a).
The contraction of the sarcomeres does not uniform in the two muscles as it is indicated in the table.
The above findings lead to the following conclusions.

a. The electrically stimulated muscles are contracted much later than the control muscles (at about the 5th hour).
b. One parameter of the contraction of the muscle is its length.
c. In the electrically stimulated muscles, the autolytic changes from the 5th hour onwards occur at a greater speed than those of the control muscles.
d. The empty spaces which exist between the myofibrils of the electrically stimulated muscles are greater and more numerous.
e. The disruption in the electrically stimulated muscles is more intense.
f. The extracellular fluid in the ES muscle is greater in quantity.
g. The disruption of the myofibrils does not refer only to Z line but more so to "I band".

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		Times after Electrical Stimulation																			
		10'			24 h					48 h					5 days						
		pH	W.H.C.	GÖFO values	pH	W.H.C.	GÖFO values	L.A.	Cr.C.	pH	W.H.C.	GÖFO values	L.A.	Cr.C.	pH	W.H.C.	GÖFO values	L.A.	Cr.C.		
M. longissimus dorsi w. Somatotropin	Control sample	6,85	44,00	90	6,05	36,33	86,5	0,62	0,904	5,95	35,31	89	0,73	1,219	-	20,30	-	0,81	-		
	Treated sample with ES	6,25	35,68	92,5	5,85	37,79	84,5	0,66	1,209	5,80	28,19	86	0,79	0,924	-	12,17	-	1,02	-		
M. longissimus dorsi	Control sample	7,00	47,43	91	6,00	47,04	90,5	0,54	1,119	5,95	26,86	90	0,56	0,900	6,00	24,14	91	0,74	0,774		
	Treated sample with ES	6,55	30,54	93,5	5,90	45,31	87,5	0,57	1,374	5,85	33,34	89	0,66	1,093	5,90	21,17	91,5	0,88	1,024		

pH = pH values, W.H.C. = Water Holding Capacity of the meat, GÖFO = GÖFO values, L.A. = Lactic Acid Concentration in mg/g, Cr.C. = Total Creatinine Concentration in mg/g.

Figures

Fig. 1. Light micrograph of Longissimus dorsi (5th hour after slaughter) where the fluctuating route of the muscle fibers is indicated. X 336.

Fig. 2. Light micrograph of Longissimus dorsi (ES), 5th hour after slaughter, where upright muscle fibers with intracellular areas of thickness and thinness are indicated. X 336.

Fig. 3. Light micrograph of Longissimus dorsi (24th hour after slaughter) where we observed the intracellular oedema, the fluctuating route of the muscle fibers, the pyknosis of certain nuclei and the loss of the cross-striation of muscle fibers. X 336.

Fig. 4. Light micrograph of Longissimus dorsi (ES), 24th hour after slaughter, in which we observed the intense alternation of areas of thickness and thinness. Many of the fibers disrupt in the areas of the thinness. X 224.

Fig. 5. Electron micrograph of Semitendinosus, 1st hour after slaughter, in which the intracellular oedema is indicated. M. swelling mitochondria. X 14.400.

Fig. 6. Electron micrograph of Semitendinosus (ES), 1st hour after slaughter, where an intracellular oedema was observed (Oed) dilated cisternae of sarcoplasmic reticulum (arrows) and phagosomes (heads of the arrows). X 14.400.

Fig. 6a. Electron micrograph of Longissimus dorsi (ES), 1st hour after slaughter, where destruction of the myofibrils is observed on Z line (arrows) as well as in other positions of the sarcomere. X 5.400.

Fig. 7. Electron micrograph of Semitendinosus (ES), 1st hour after slaughter, in which the non-uniform contraction of the myofibrils is indicated. X 9.000.

Fig. 8. Electron micrograph of Longissimus dorsi (5th hour after slaughter) where the disintegration of cristae in the mitochondria is observed. X 14.400.

Fig. 8a. Electron micrograph of Semitendinosus (5th hour after slaughter) in which we observe a disruption of myofibrils and concentration of a great number of granules of glucogen in the area of the intracellular oedema (Cly). Oed., intracellular oedema. X 14.400.

Fig. 9. Electron micrograph of Longissimus dorsi (ES), 5th hour after slaughter, in which we see the intense disintegration of cristae up to complete vacualation of mitochondria. X 14.400.

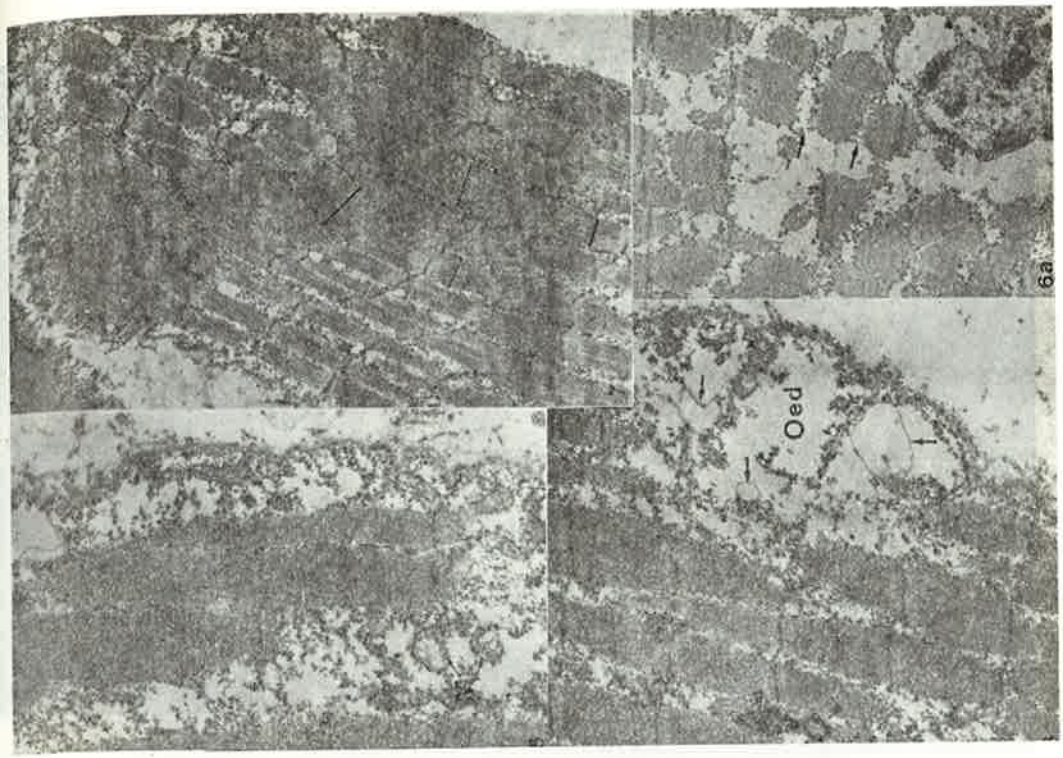
Fig. 9a. Electron micrograph of Semitendinosus (ES), 5th hour after slaughter. The typical non-uniform contraction of the myofibrils is observed. X 5.400.

Fig. 9b. Electron micrograph of Semitendinosus (ES), 5th hour after slaughter. Non-uniform contraction of the myofibrils and their destruction in the areas of looseness (arrows), intense intracellular and extracellular oedema, E, erythrocytes inside a changed capillary vessel are observed. X 5.400.

Fig. 10. Electron micrograph of Longissimus dorsi (ES), 5th hour after slaughter. We observe the difference of the change between the two muscle fibers. The myofibrils of the bottom left muscle fiber have been homogenized, the mitochondria are completely changed and the nuclei are pyknotic. X 14.400.

Fig. 11. Electron micrograph of Semitendinosus (24th hour after slaughter). The disruption of myofibrils and complete vacuolation of all the membranaceous structures of the muscle fibers are observed. N, heterochromatic nucleus. X 14.400.

Fig.12,12a. Electron micrographs of Longissimus dorsi (ES), 24th hour after slaughter. Intense itracellular and extracellular oedema, opaque, disruption and homogenation of myofibrils are observed. X 14.400.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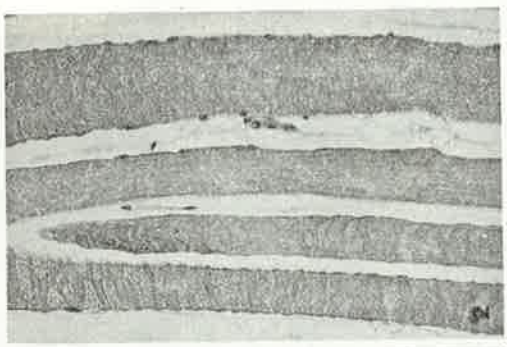
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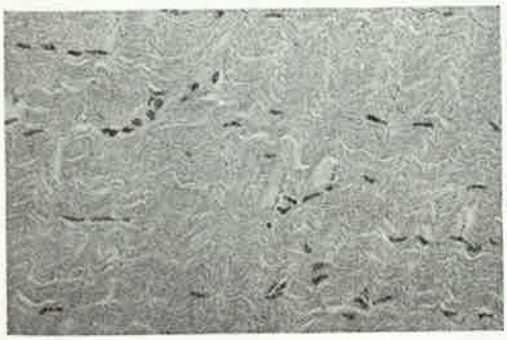

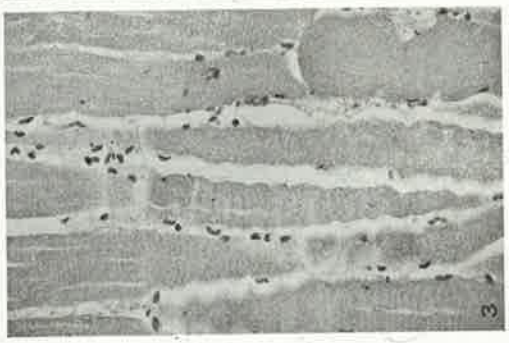
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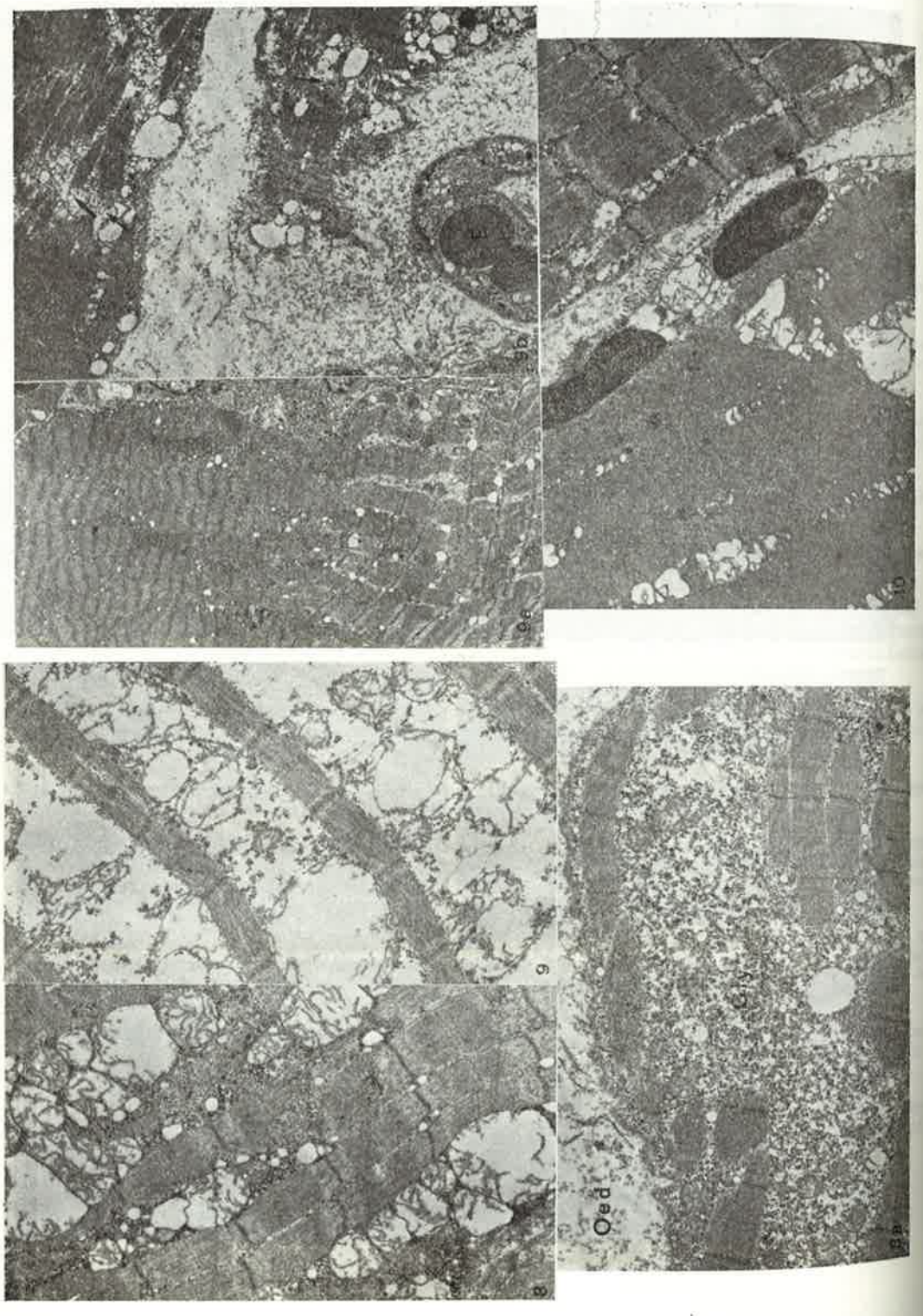
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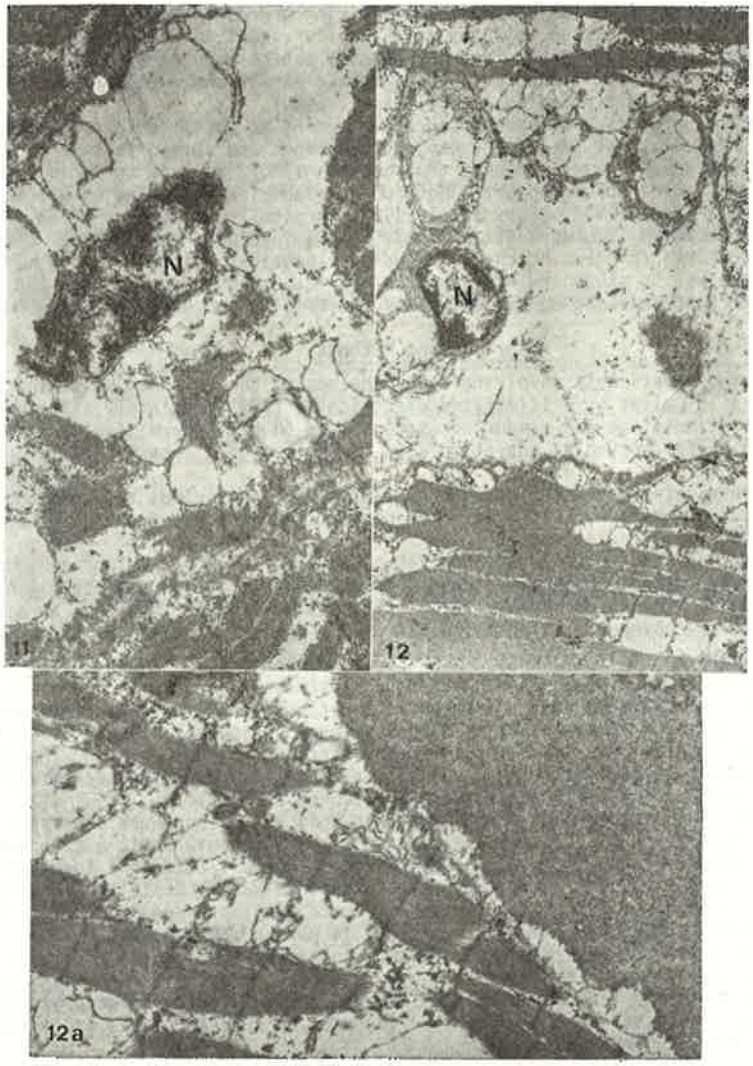
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