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SOME OBSERVATIONS ON THE HISTOLOGICAL APPEARANCE OF POST MORTEM MUSCLE.

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SUMMARY

The microscopical appearance of muscle fibres at stages from immediate post-slaughter to completion of rigor, has been followed. Marked differences are apparent but this does not necessarily mean that these are applicable to the actual tissue when within the carcase.

Fibres still contracting are shown to possess a constant volume, the visible shortening being accompanied by an increasing diameter.

The findings of Bendall & Pedersen on pale watery pork have been confirmed, and a limiting rate of diffusion of locally produced lactic acid is suggested as a possible contributory factor.

SOME OBSERVATIONS ON THE HISTOLOGICAL APPEARANCE OF POST-MORTEM MUSCLE.

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For some years this Laboratory has been developing histological methods which would make a fairly accurate assessment of the meat tissues used in comminuted meat pies. As we were also interested in determining some of the changes during rigor, it was hoped that some of the techniques employed might provide a means of studying the rapid changes which occur between death, the onset of rigor, and the end of rigor.

We feel, however, there is little conclusive evidence to show that this microscopical approach has provided any significant advance in elucidating some of the differences in quality of meat post-rigor, although our results tend to confirm the work of Bendall and Pedersen from their studies of watery pork. We nevertheless feel, however, that the following account of some of our findings may be of general interest and by discussion stimulate further investigation.

1.

Structural Changes during rigor

Muscle was obtained from an electrically stunned pig, within 5 minutes of the commencement of bleeding. This was homogenised immediately with normal saline, and examined microscopically. Similar samples were taken from the carcase at various intervals afterwards.

0 Hours

At 0 hours after slaughter, as shown in Fig. 1, all the fibres were fully contracted, and show the typical cross corrugations of the sarcolemma giving a rough appearance to the fibres. The myofibrils are just distinguishable in parts of some fibres.

1 Hour

(Fig. 2) The general appearance after 1 hour was much the same as for 0 hours, with perhaps a slightly less rough appearance of the fibres. The myofibrils also tended to show through a little clearer.

3 Hours

(Fig. 3) As for 1 hour, but roughness not quite so marked.

9 Hours

(Fig. 4) Much less roughness of the fibres, and the myofibrils easily evident. There was however some degree of distortion appearing in about one-third of the fibres visible in any one field. This distortion of symmetrical surface peaks and valleys, alternating on each side of the fibre, at first sight resembles a helical twist. Closer inspection however shows that this is not so, the valleys appear to run at right angles to the length of the fibres, and are not helical. In addition, the myofibrils are still aligned longitudinally, without any twist, which should be evident if the fibre as a whole had twisted spirally.

As far as we are able to judge, this must represent an uneven relaxing of some of the individual myofibrils within some fibres. This may well be due to a fibre position within a fibre bundle.

An extremely marked example of this was found in a 20 hours old sample, (Fig. 5) although this was not at all typical of that stage.

24 Hours

(Fig. 6) After 24 hours the majority of the fibres had the usual post-rigor, smooth relaxed appearance, with the myofibrils and their characteristic bands very well evident.

Discussion

Although these fibres at different stages are so markedly different in appearance, this need not necessarily mean that the fibres have this appearance in the actual tissue. We are of the opinion that on severing from the carcase and homogenising in saline, the fibres will be free to fully contract, probably depending on the amount of available ATP at the time. Thus the early samples soon after death, give immediate and full contraction on homogenisation; the intermediate samples show partial contraction, even to the extent of varying amounts within a single fibre; whereas post-rigor, the fibres are incapable of contracting. No evidence was found in the early (pre-rigor) samples, of the transient ring structures found by Lorincz(1). Search was also made for these in pre-rigor beef, but even in very fresh material (2 minutes after death) we were unable to demonstrate the presence of these structures.

When the degree of contraction was very marked, however, the roughened appearance of the sarcolemma, although usually recognisable as being only a surface corrugation, did sometimes appear to be ring-like, as shown in the accompanying Figs. 7, 8, 9, 10, 11, 12.

It is therefore apparent that sometimes during homogenisation, individual fibres will undergo unequal contraction or relaxation, either on different parts along the length of the fibre, or even in the same part the internal myofibrils may contract, leaving the outermost layers of myofibrils still relaxed thereby causing a distorted shape of the whole fibre. Although these phenomena sometimes give rise to ring-like appearances, we cannot confirm the general presence of the 'rings' or 'tyres' as found by Lorincz in immediate pre-rigor muscle.

2. Fibre - Contraction

In some of the homogenates from immediate post-slaughter muscle, it was noticed that sometimes a few fibres were still undergoing some delayed contractions. Photomicrographs of one of these fibres taken at short intervals are shown in Figs. 13, 14, 15 and 16. Measurement of the lengths of this fibre and the diameters calculated from the measured areas, gave the following results :-

TABLE I

CHANGE OF LENGTH & DIAMETER OF A FIBRE IN A SALINE HOMOGENATE.

FIBRE FROM BEEF MUSCLE IMMEDIATELY POST-SLAUGHTER (10 MINUTES).

	0 mins.	1½ mins.	3 mins.	6 mins.
Area (A)	34.5	34.2	32.2	31.1
Length (L)	25.3	24.8	22.0	20.5
Diameter $(D)\frac{A}{L}$	1.36	1.38	1.46	1.52
Volume (D^2L)	46.8	47.1	46.9	47.3

It is of interest to note that the visible shortening that occurred, was accompanied by an increased diameter, such that the volume of the whole fibre appeared to be unchanged.

Pale Watery Pork

We have noticed that the microscopic appearance of homogenised tissue after completion of rigor, is very different for acid than for alkaline muscles.

Figs. 17 and 18 show the different extent to which the fibres are broken down. The muscle from low ultimate pH meat tends to break across the fibres during maceration, whereas the high ultimate pH muscle fibres tend to break longitudinally into masses of myofibrils.

We have obtained some histological photographs which support the findings of Bendall and Pedersen (2) that with pale watery pork, the post-rigor fibres show the presence of coagulated protein.

Our findings on this whole phenomena of watery pork are in agreement with the conclusion of Bendall and Pedersen, that a rapid rate of glycolysis (particularly at warmer temperatures) gives rise to this coagulation of sarcoplasmic protein, and the watery tissue typical of this kind of pork.

We would further like to suggest that this is probably brought about when the rate of glycolysis in the muscle reaches a rate which exceeds the rate of diffusion of the resulting lactic acid.

If one accepts the hypothesis that glycolysis proceeds for example, inside the fibres, then all the findings become logical developments. Thus, a slow rate of glycolysis gives a gradual build-up of lactic acid which diffuses out into the sarcoplasm. With increasing rates of lactic acid production, however, the local concentration, being limited by the rate of diffusion, may be so high, that local pH values may drop below the normal equilibrium pH, giving rise to local coagulation of the sarcoplasmic protein, where it first meets the concentration of lactic acid, i.e. just outside or just inside the sarcolemma where the coagulations are in fact mostly found.

The coagulation of protein associated with watery pork can therefore be explained as primarily due to the diffusion rate of lactic acid becoming limiting under conditions of fast acid production.

This hypothesis, however, presupposes that the lactic acid production is local, either inside the fibres, the myofibrils, or perhaps the sarcolemma itself. This again must be because either the glycogen, or the amylases concerned, are local to these sites, and of these two alternatives the localisation of the enzymes would appear to be the most likely.

References

(1) Lorincz. F. VII Meeting European Meat Research Workers. Warsaw. 1961.

(2) Bendall. J.R. & Wismer-Pedersen.J. J. Food Sc. (1962) 27. p.144.

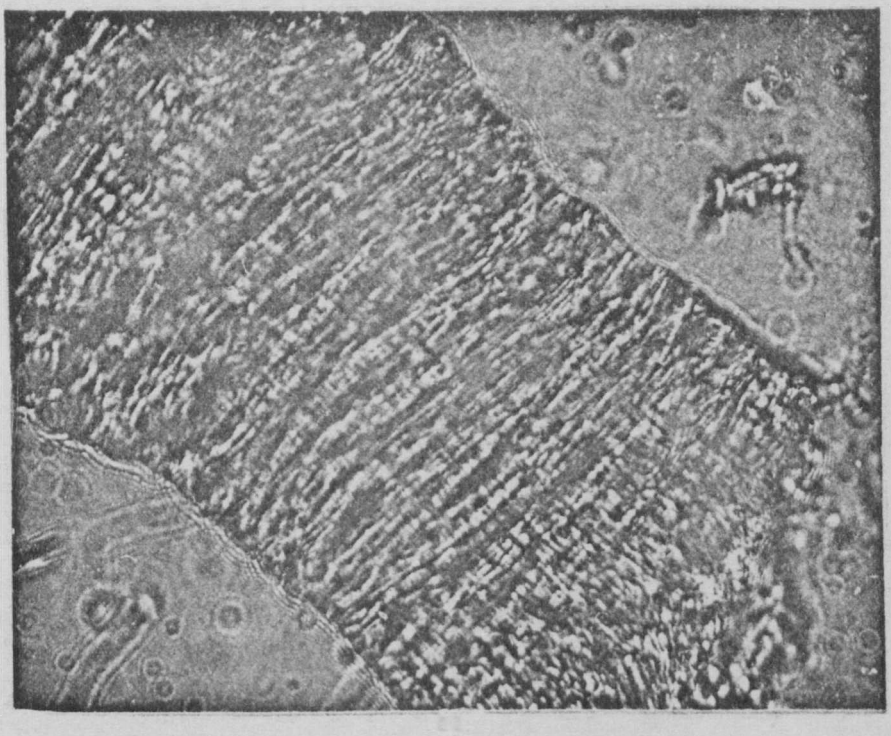

Fig. 1.

Pork Muscle Homogenate. 0 Hr.

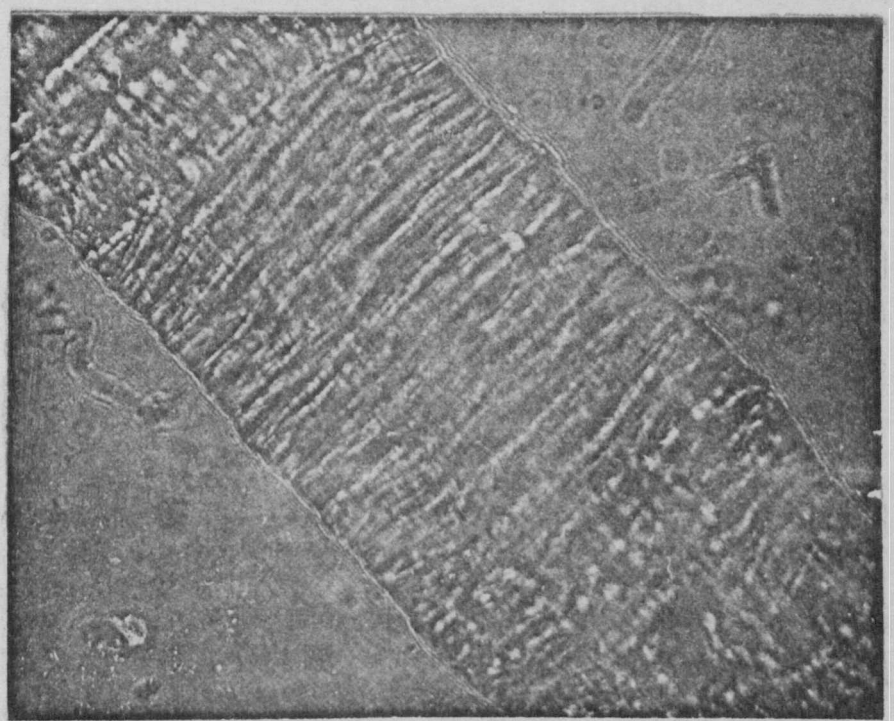

Fig. 2.

Pork Muscle Homogenate. 1 Hr.

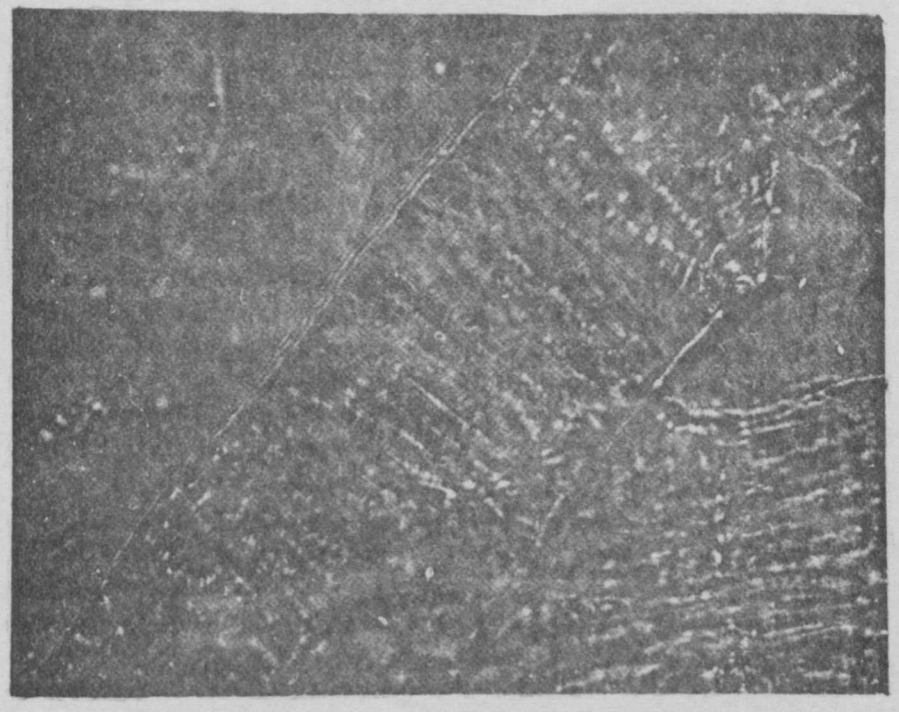

Fig. 3.

Pork Muscle Homogenate. 3 Hrs.

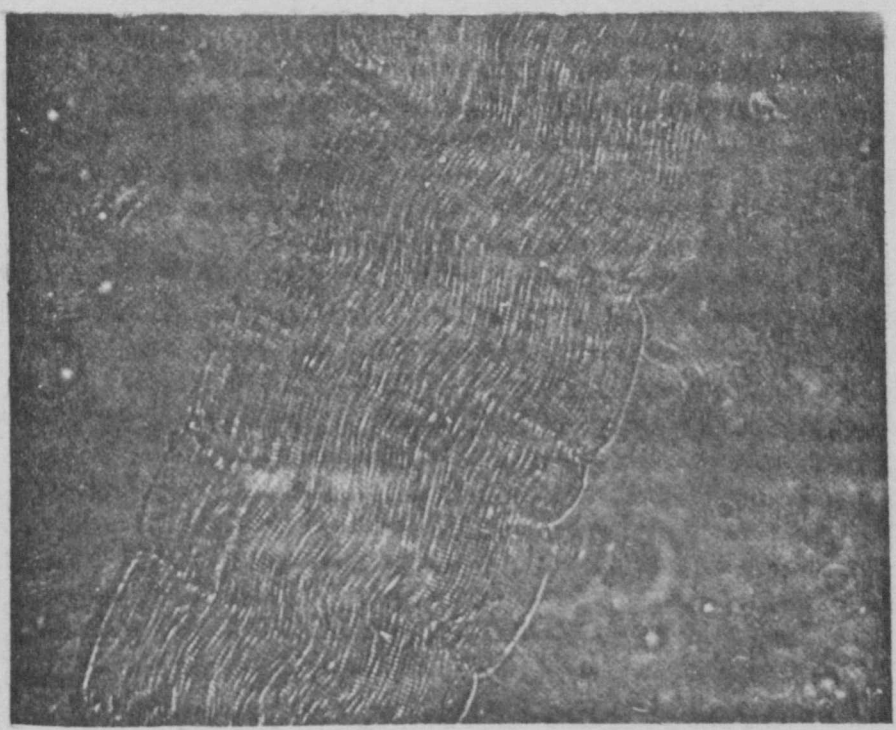

Fig. 4.

Pork Muscle Homogenate. 9 Hrs.

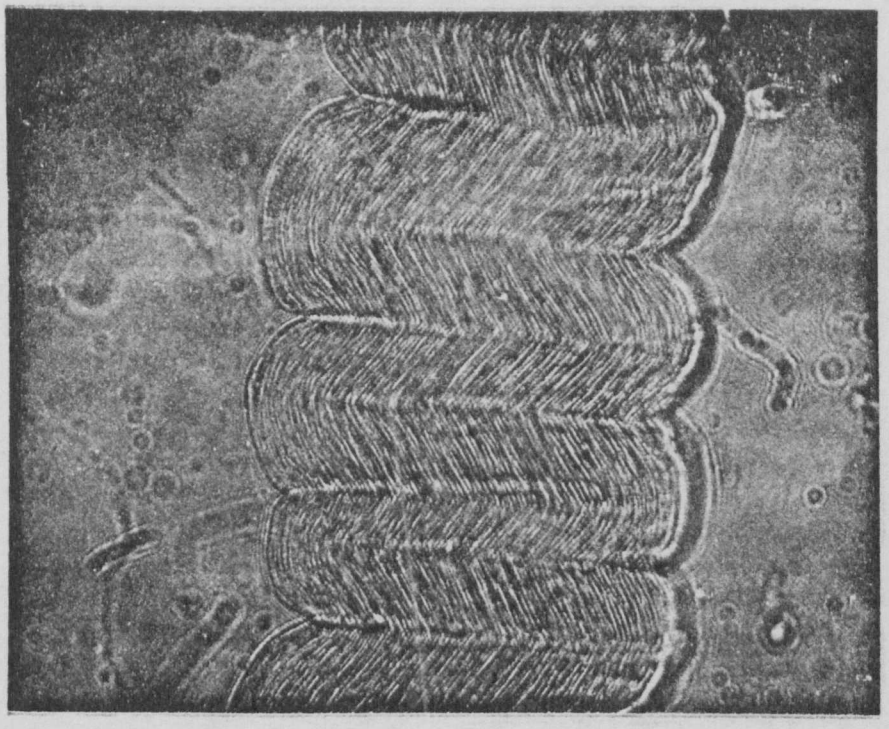

Fig. 5.

Pork Muscle
Homogenate.
20 Hrs.

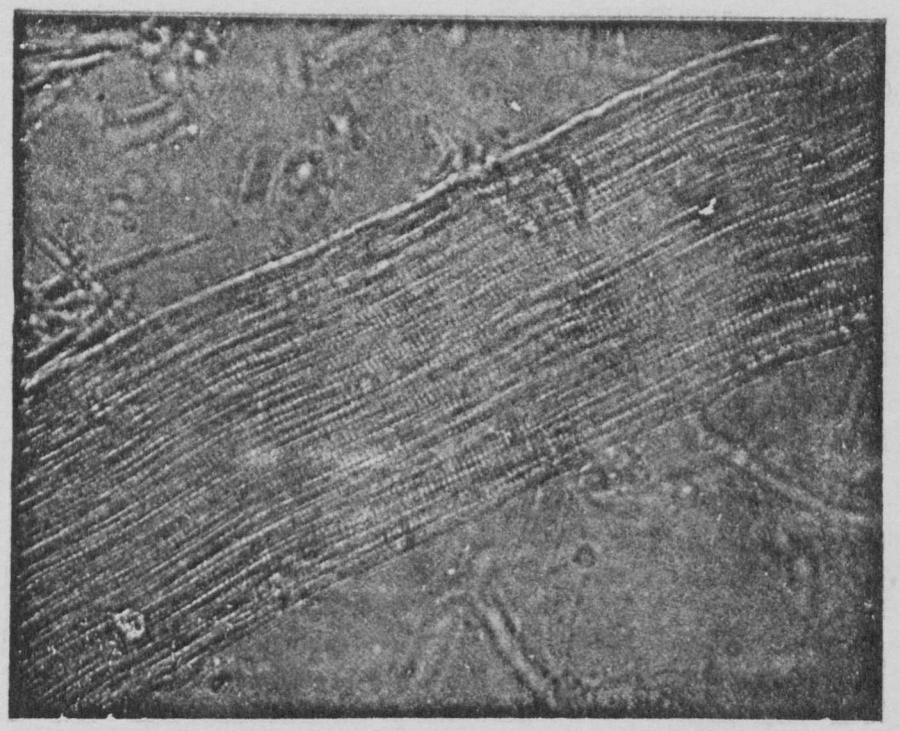

Fig. 6.

Pork Muscle
Homogenate.
24 Hrs.

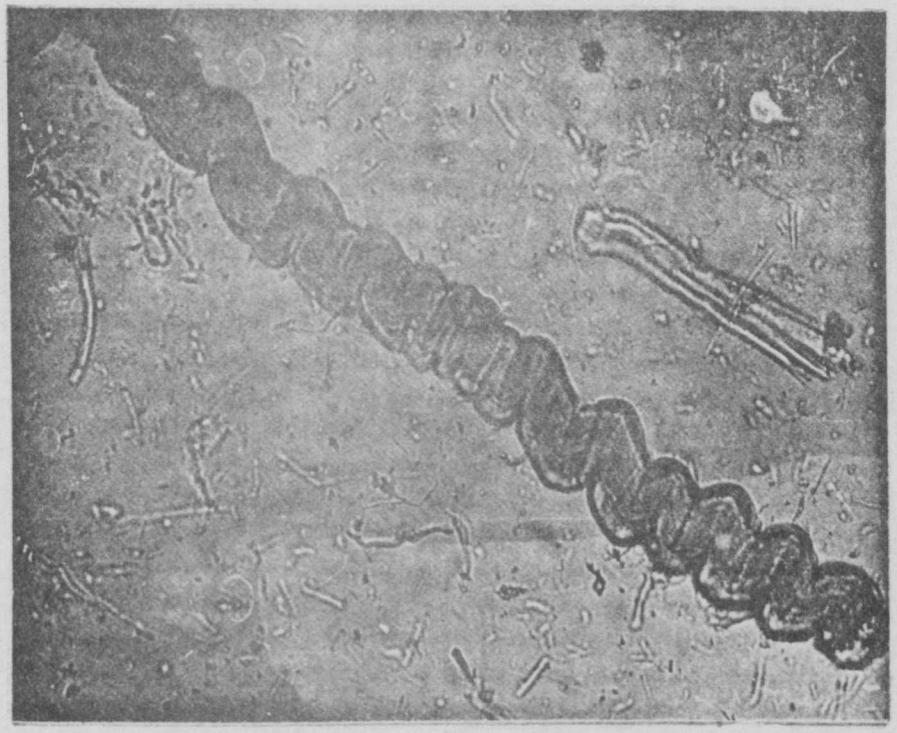

Fig. 7.

Muscle Fibre.
Pork.
Pre-Rigor.

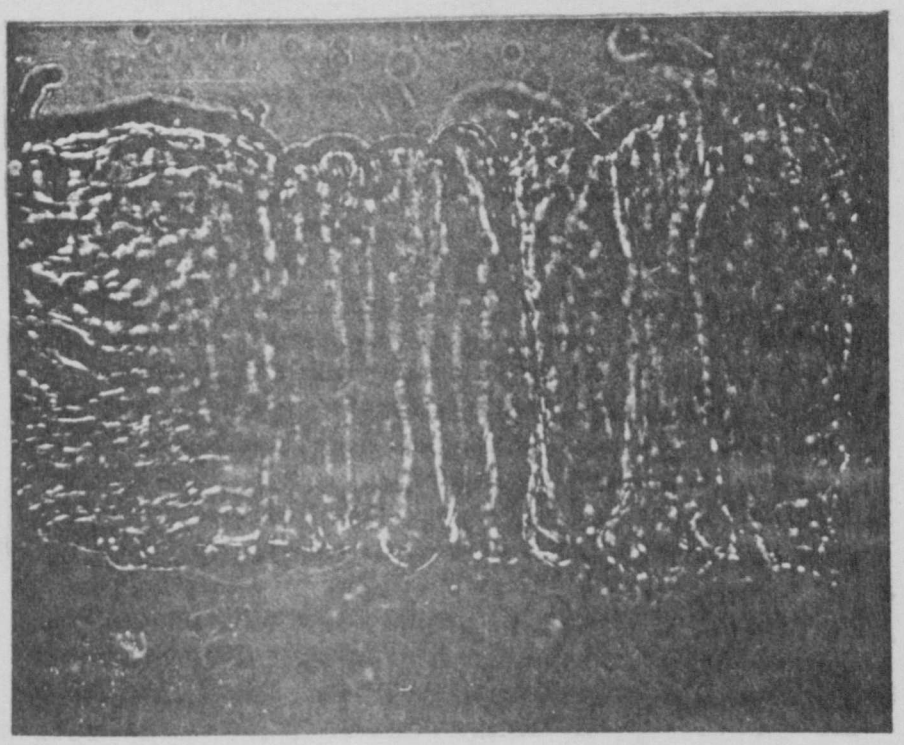

Fig. 8.

Muscle Fibre.
Rat.
Pre-Rigor.

Fig. 9.

Muscle Fibre.
Pork.
Pre-Rigor.

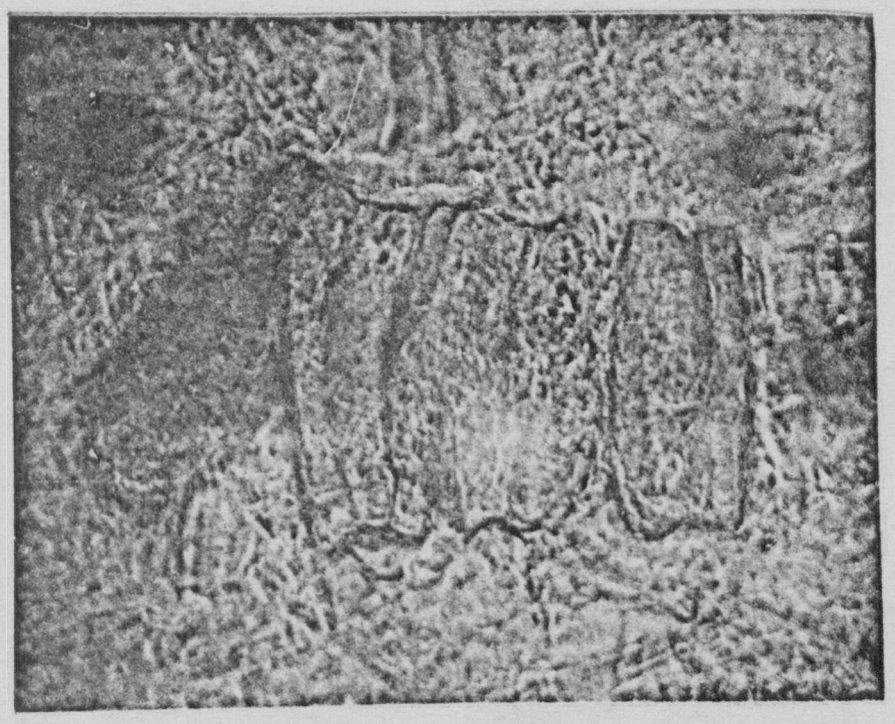

Fig. 10.

Muscle Fibre.
Pork.
Pre-Rigor, after
prolonged maceration.

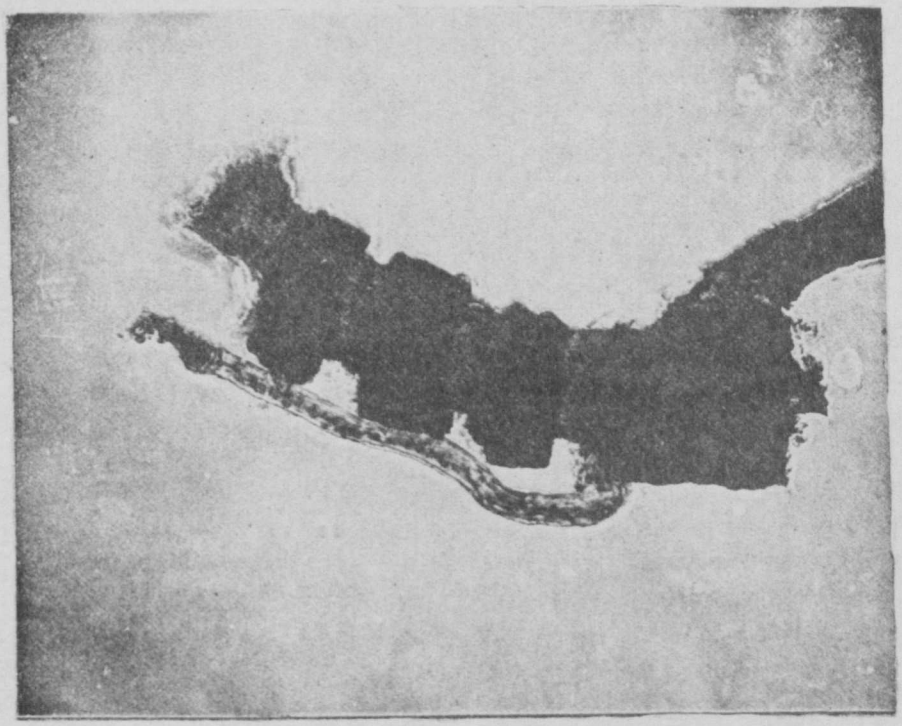

Fig. 11.

Muscle Fibre.
Pork.
Pre-Rigor.

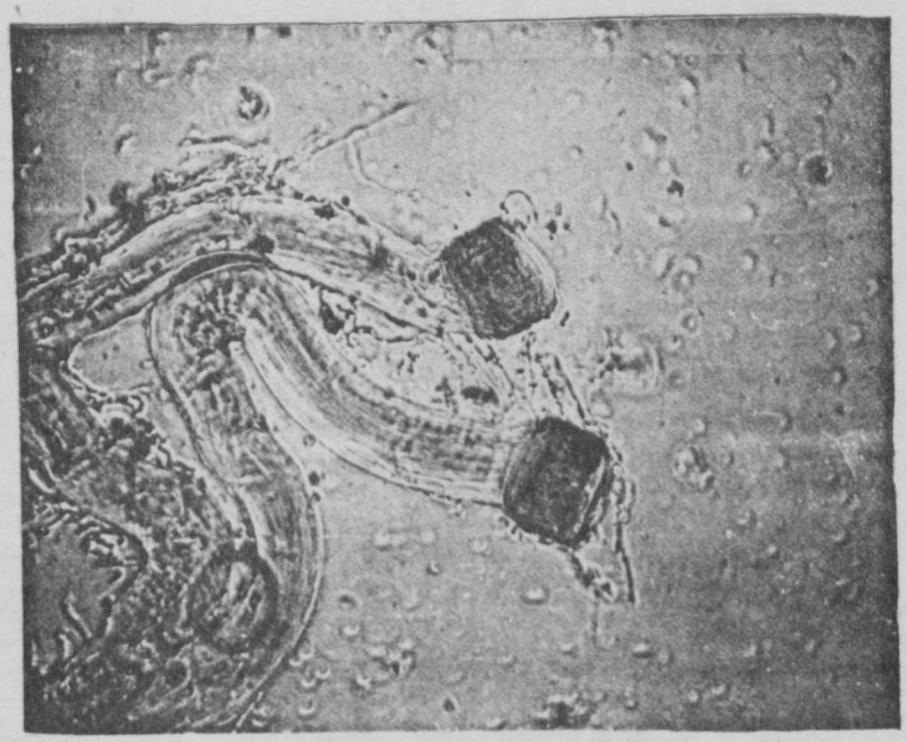

Fig. 12.

Muscle Fibre.
Pork.
Pre-Rigor.

Fig. 13. 0 Mins.

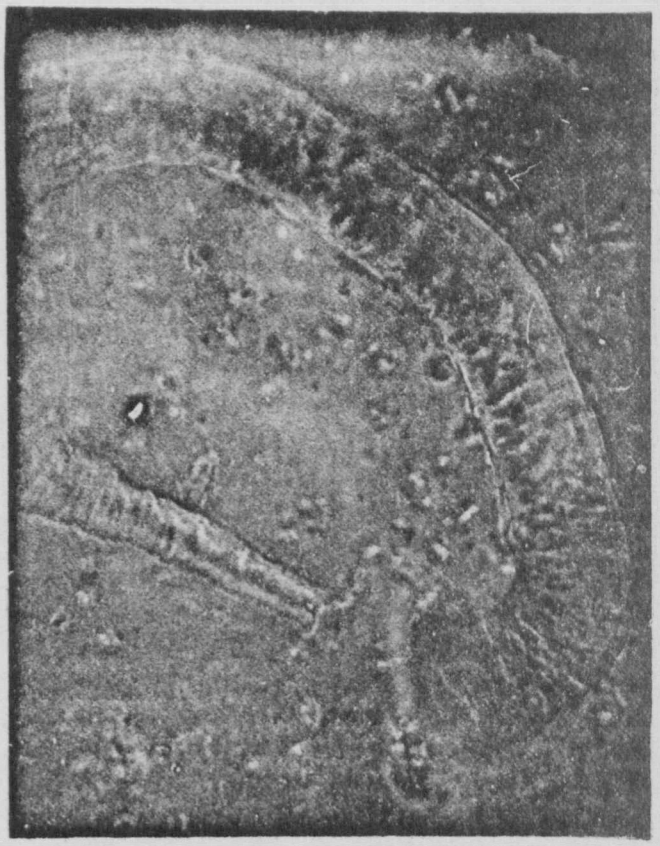

Fig. 14. 1½ Mins.

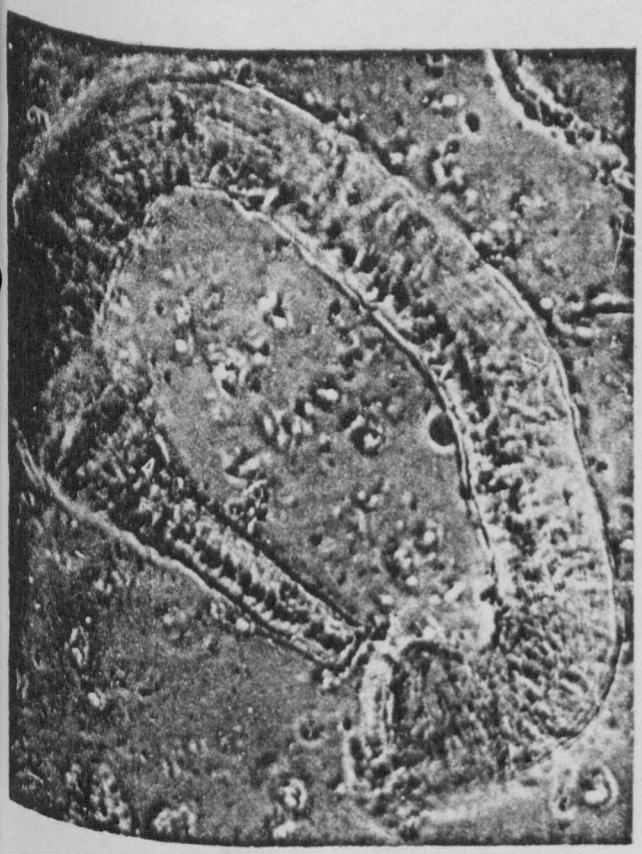

Fig. 15. 3 Mins.

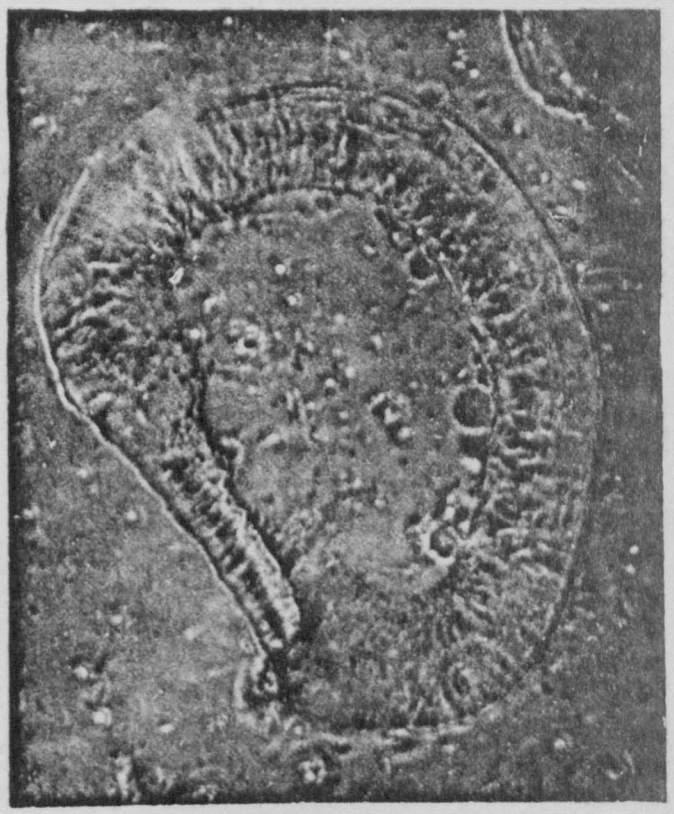

Fig. 16. 6 Mins.

PORK MUSCLE FIBRE HOMOGENISED PRE-RIGOR.

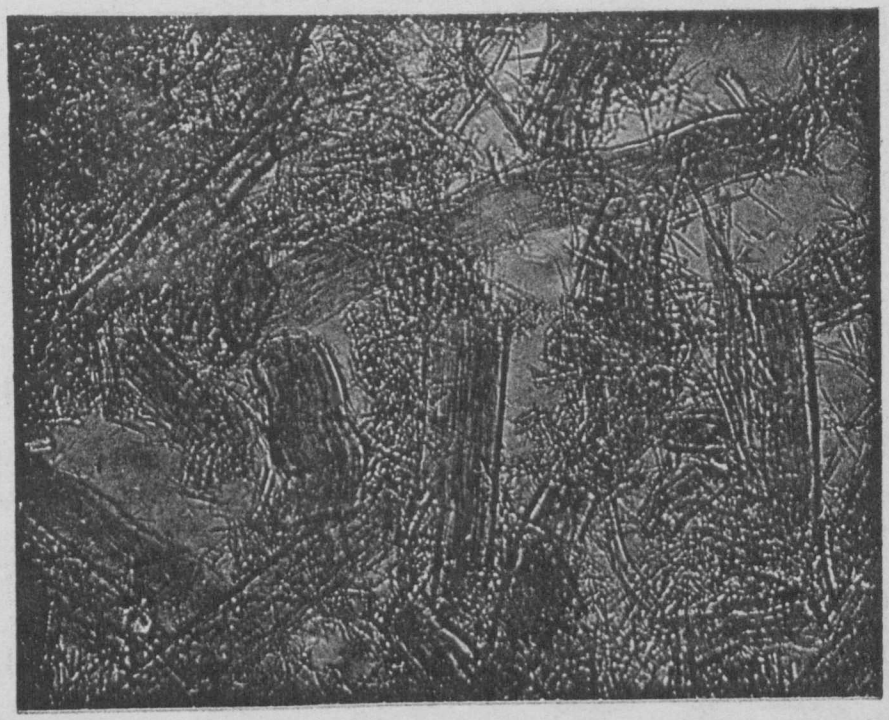

Fig. 17.

Homogenate.
Pork Muscle.
Post-Rigor.
Low ultimate
pH. 5.2

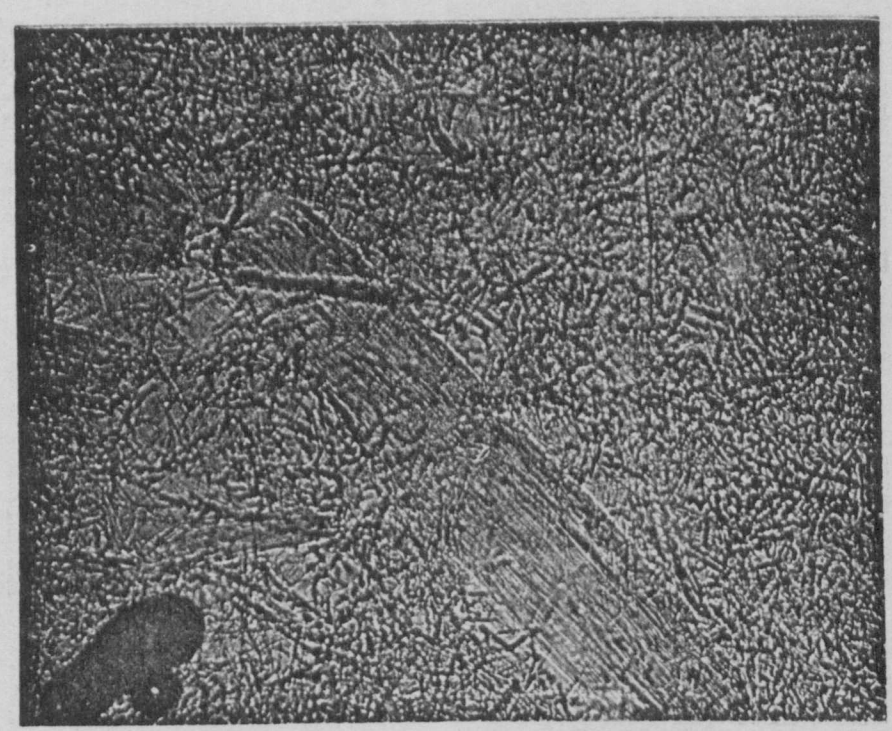

Fig. 18.

Homogenate.
Pork Muscle.
Post-Rigor.
High ultimate
pH. 5.8

EINIGE BEOBACHTUNGEN ÜBER DIE HISTOLOGISCHE ERSCHEINUNG VON MUSKELFLEISCH POST MORTEM.

ZUSAMMENFASSUNG

Die mikroskopische Erscheinung von Muskelfasern ist in Stufen von unmittelbar nach dem Schlachten bis zur vollendeten Totenstarre nachgeforscht worden. Deutliche Unterschiede sind erkennbar, aber das bedeutet nicht unbedingt, dass diese auch im Gewebe innerhalb des Tierkörpers vorhanden sind.

Es wird gezeigt, dass Fasern, die sich noch zusammenziehen, ein konstantes Volum haben, so dass der Durchmesser gleichzeitig mit dem sichtbaren Zusammenziehen wächst.

Die Befunde von Bendall & Pedersen über Muskeldegeneration werden bestätigt, und eine begrenzende Diffusionsgeschwindigkeit von örtlich produzierter Milchsäure wird als ein vielleicht beitragender Faktor unterbreitet.