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7TH MEETING OF EUROPEAN MEAT RESEARCH WORKERS

WARSAW, 13th to 23rd September, 1961

OBSERVATIONS ON ASEPTIC AUTOLYSIS IN MUSCLE

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Observations have been made on the changes taking place in certain protein fractions of the longissimus dorsi muscle of rabbits and oxen during storage under aseptic conditions at 5^{o} and 37^{o}.

Two procedures were followed to obtain samples of muscle in an aseptic condition. (a) In most of the preliminary rabbit experiments the dissected muscles were dipped in 70% ethyl alcohol and then flamed to sterilise the surface. The muscle sections had an outer denatured pellicle about 2 mm thick, but the tissue in the interior was apparently unaltered. Tissue from the interior free from the outer pellicle was taken for analysis.

(b) In later experiments on both rabbit and beef muscle the flaming procedure was cut out. After dissection to expose the longissimus dorsi, the intact muscle was painted with an alcoholic solution of the dyes, Crystal Violet and Brilliant Green. The dyed surface layer was removed aseptically and test samples were taken from the interior for storage in nitrogen or air in sealed containers at different temperatures. By this procedure over 70% of the dissected samples were obtained in a sterile condition.

The stored samples were homogenized and extracted firstly with 0.1 M KCl, secondly with 0.2 M disodium hydrogen phosphate at pH 8.9, and finally with 0.1 M Na Citrate at pH 3.3. The dialysed extracts and insoluble residue after hydrolysis in HCl were analysed for TN, for hydroxy-proline by Neuman & Logan's method (1) and for tyrosine by absorption at λ 293.

This procedure when applied to muscle immediately post rigor gives a rough separation into sarcoplasmic proteins (0.1 M KCl), fibrillar proteins (0.2 M Na_2HPO_4), and a residue of denatured proteins and connective tissue proteins. Any soluble collagen present appears in the phosphate and citrate extracts (2).

The total soluble non-protein N (TCA sol N) was estimated in an aliquot of the KCl homogenate after extraction with 10% trichloroacetic acid.

Adrenaline treatment. Two rabbits were treated subcutaneously 4 hours ante-mortem with 1.5 mg adrenaline to give final pH's of 6.6 and 6.5 in the longissimus dorsi.

Papain treatment. In connection with other studies on texture, a sheep was injected intravenously ante mortem with papain equivalent to 30 mg papain/lb live weight (3). The animal was killed 5 minutes after injection and the dressed carcase held for 24 hours at 5^{o} before storage at -20^{o} pending analysis.

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Texture observations. In this series of experiments, few organoleptic observations on texture were made, but in the later work certain observations were made on homogenates of test samples prepared in .02 M phosphate : 0.1 M KCl buffer at pH 7 in a Marsh-Snow machine (4) under standard conditions. The homogenisation was carried out at two speeds, low - approximately 600 r.p.m. and high - approximately 1800 r.p.m.

The proportion and dimensions of the fibres and fibrils present in a representative field were observed microscopically and photomicrographs were taken to illustrate the state of the fibres and fibrils.

Samples were cooked mildly by holding in a closed container in a water bath at 80° for 15 minutes followed by 15 minutes with bath temperature of 70°. The interior of the sample was therefore at 65° to 70° for 20 to 25 minutes. Certain samples were fully cooked by holding for 15 minutes at a bath temperature of 80° followed by 60 minutes at a bath temperature of 95-100°.

Results

It is clear from the values plotted in Fig. 1 that the rate of production of TCA sol N is greater in rabbit than in beef muscle. In both cases after a very rapid increase in TCA sol N over the first 8 - 12 days, the rate decreases greatly. Over the first 10 days, the rate of production of TCA sol N is roughly 10 μ mols and 21 μ mols/day for beef and rabbit respectively at the normal ultimate pH in the range 5.6 to 5.8. In rabbit treated with adrenaline to give a high, ultimate pH (6.5 - 6.6) the rate of production of TCA sol N is reduced to roughly 0.5 μ mols/day over the same period. In untreated rabbit and beef muscle at pH 5.6 - 5.8, over a period of 5 - 6 months at 37°, the TCA sol N increases from original values of 10 - 12% to values of 37% and 31% respectively of the TN.

In flamed rabbit samples, appreciable amounts of hydroxy-proline (OHP) amounting after 2 months to about 23% of the total OHP present were found in the hydrolysed 0.1 M KCl extracts. No OHP was found in any other extract of the flamed samples nor in any extract of the non-flamed samples. The values for OHP soluble in 0.1 M KCl are given in Fig. 1.

In the absence at present of values for control samples analysed immediately after flaming, the possibility remains that the act of flaming may cause a change in the collagen leading to the immediate production of a fraction soluble in 0.1 M KCl. This effect continues with storage as shown by the values for three individual rabbits D, Q and Z.

It is interesting to note that in the adrenalised animals (flamed) no soluble OHP was found in samples of either rabbit (1) or rabbit (2) after 15 and 22 days respectively at 37° but in rabbit (2) muscle an appreciable proportion was present after 64 days. Therefore, although at pH 6.5 - 6.6 the rate of production of TCA sol N was very low, the rate of formation of soluble OHP was not reduced to the same degree.

The difference in texture between homogenates of normal samples and adrenalised samples after storage at 37° for 60 - 80 days was very striking. During homogenisation the control sample became broken down to short sections of fibres and fibrils whereas the adrenalised samples showed almost entirely long smooth fibres with little sign of deterioration.

Texture. The dimensions of the fibres and fibrils present in homogenates are represented in Fig. 2. They demonstrate clearly the different degrees of disintegration brought about in the samples by standard conditions of homogenisation. Photomicrographs of the fields represented by these figures are not reproduced here but will be shown at the Conference.

It must be noted that the control -20° samples during homogenisation at low speed did not disintegrate like the stored samples into individual fibres. In low speed homogenates of the control sample several clumps of fibre bundles were present indicating greater cohesion of the tissue structure.

The photomicrographs show several additional points of interest:-

1) Irrespective of the state of the tissue, whether raw or cooked, and the temperature of storage, the basic structure of the myofibrils as shown by the presence of cross striations remained apparently unaffected even after 6 months at 37°.

2) In homogenates of the -20° control samples and samples stored at 37° the fibres showed almost entirely longitudinal cleavage and very little transverse cleavage. On the other hand, samples stored at 5° showed mainly transverse cleavage.

3) Differences in texture between homogenates of raw samples were also apparent between the homogenates of similar samples after cooking.

4) The degree of disintegration of the tissue structure was greater in samples stored at 5° than in samples stored at 37°. This is presumably due to the strengthening of the fibres by heat denaturation at 37°. After storage at 5° a large proportion of the proteins was still undenatured.

Comparison of the organoleptic assessment of texture and the state of the fibres and fibrils in corresponding homogenates was made only in the case of beef held for 30 days at 5° and muscle from a sheep treated with papain and held at -20°.

The cooked beef samples were very tender but still had a reasonably cohesive structure, whereas the papain treated mutton samples were over tender and fell to pieces with little remaining strength of structure. On the other hand, in homogenates of similar test samples (Fig.2) the mutton fibres were much longer and showed less deterioration than the fibres of the beef homogenates.

Further observations of this kind will require to be made on directly comparable samples before any relationship between organoleptic texture and the texture of homogenates by microscopic analysis can become established.

References:

1. Neuman R.E. & Logan, M.A., J.Biol Chem. (1950) 184, 299.

2. Nature and Structure of Collagen, J.T. Randall, (1953) p.214.

3. Beuk, J.F., Hinsdale, A.L. et al, U.S.Patent No.2,903,362, Sept. 1959.

4. Marsh, B.B. & Sno , A., J.Sci.Fd Agric. (1950) 1, 190.

Summary

The rate of aseptic autolysis at pH 5.6 - 5.8 in muscle at 37° as measured by the production of trichloroacetic acid soluble nitrogen was 10 μ and 21 μ mol N/g/day respectively in beef and rabbit muscle. The corresponding rate in rabbit muscle after treatment with adrenaline to give an ultimate pH of 6.6 was only 0.5 μ mol N/g/day.

No change in the collagen fraction as evidenced by the formation of soluble fractions containing hydroxyproline was observed in untreated, sterile, raw muscle samples during storage for 6 months at 37°.

The effect of different storage treatments on the structure of sterile muscle tissue has been investigated by comparing the dimensions and condition of the fibres and fibrils in homogenates prepared under standard conditions.

Résumé

La vitesse d'autolyse aseptique à pH 5.6 - 5.3 dans le muscle à 37°, qu'on a mesurée par la production du nitrogène soluble dans l'acid trichloroacétique, était 10 μ et 21 μ mol N/g/jour respectivement, pour le muscle du boeuf et du lapin. La vitesse d'autolyse aseptique dans le muscle d'un lapin, injecté avec adrénaline à produire un pH final de 6.6 était 0.5 μ mol N/g/jour.

On n'a pas observé d'alteration de collagène démontré par la production de hydroxyproline soluble, dans les échantillons contrôles de muscle cru et stérile pendant conservation à six mois à 37°.

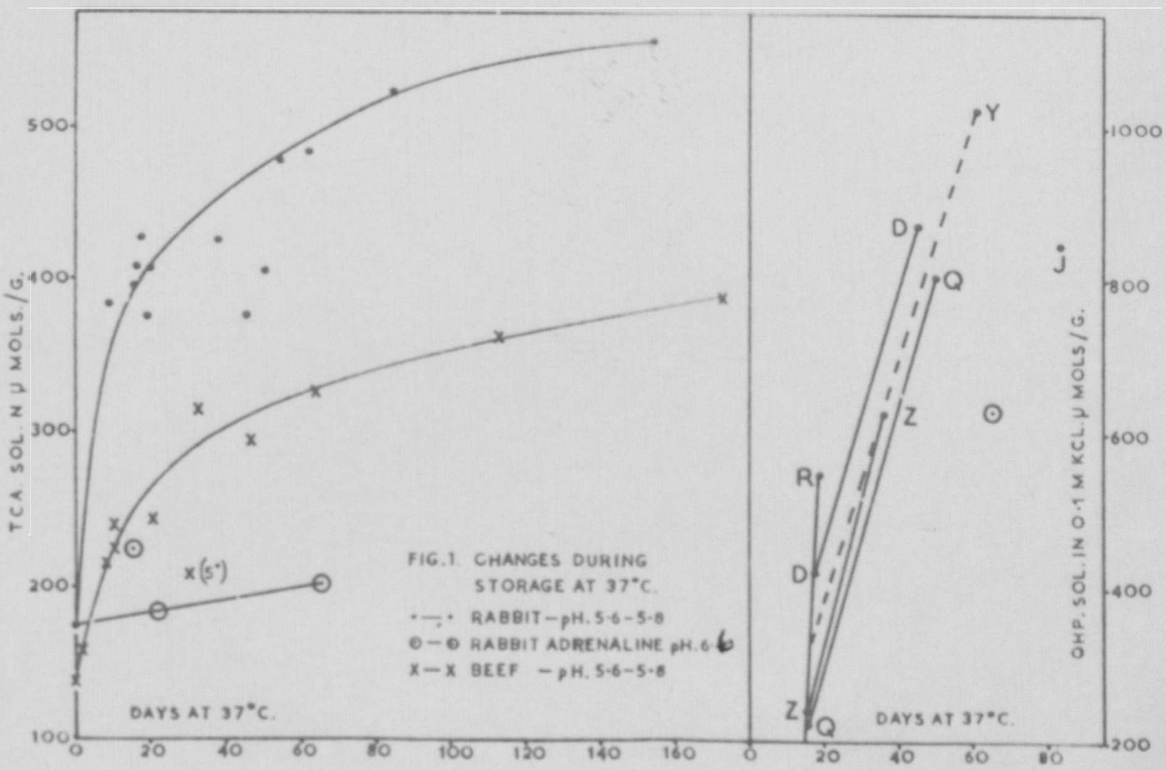
On a examiné l'effet des conditions différents de conservation sur la structure de muscle stérile par la comparison des dimensions et de l'état des fibres et fibrilles en les homogenates qu'on a preparé dans les conditions standardisés.

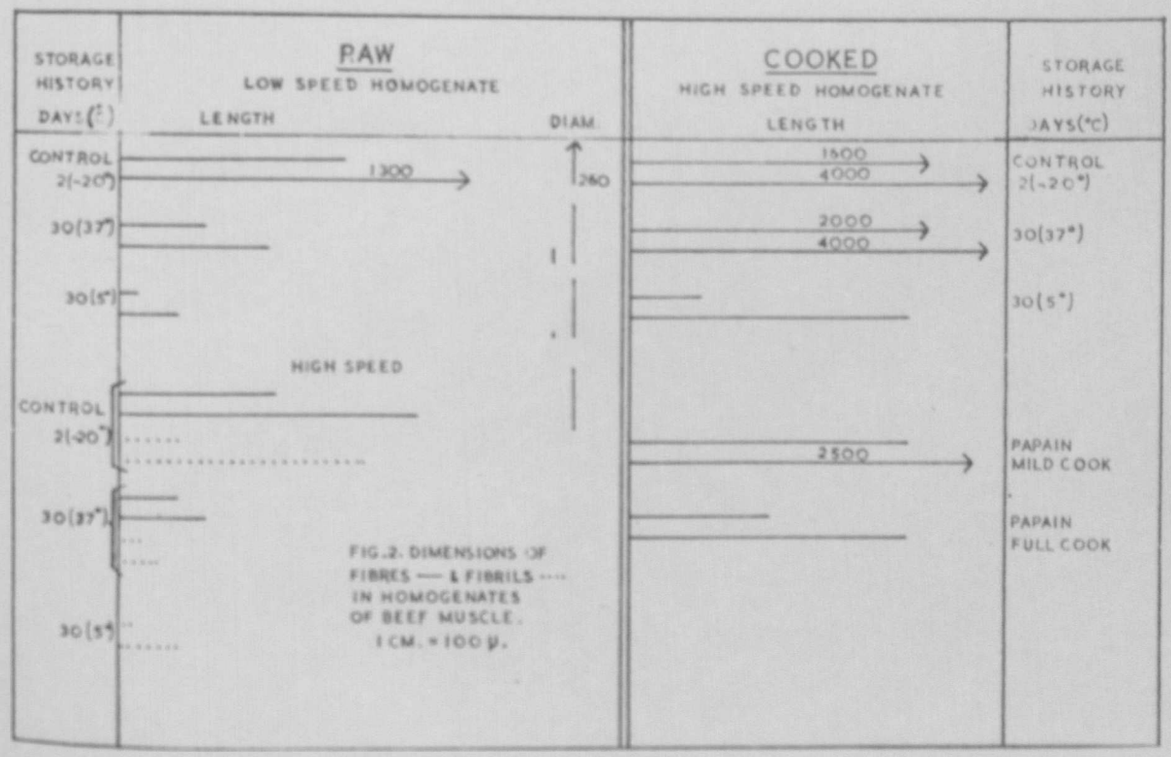
Zusammenfassung

Die Rate der aseptischen Muskelautolyse bei 37° und einem pH von 5,6 - 5,3 wurde bestimmt wobei die Bildung von Trichloressigsäure löslichem N als Mass galt. Dieses war 10 μ beziehungsweise 21 μ Mole N/g/Tag in Rinder- oder Kaninchenmuskel. Die entsprechende Rate im Kaninchenmuskel nach vorheriger Behandlung mit Adrenalin, wodurch das pH auf 6,6 stieg, war nur 0,5 μ Mole N/g/Tag.

In Proben von unbehandelten, sterilen, rohen Muskel während 6-monatiger Aufbewahrung bei 37°, kein Hydroxy-proline in die löslichen Anteilen war gefunden. Dies ergab sich daraus dass all der Collagen unauflöslich blieb.

Der Einfluss von Unterschieden in den Lagerungsbedingungen auf die Struktur von sterilem Muskelgewebe wurde fernerhin untersucht durch einen Vergleich der Dimensionen und des Zustandes der Fasern und Fibrillen in Homogenaten, die unter Standard-Bedingungen hergestellt waren.


TCA. SOL. N μ MOLS/G.
DAYS AT 37°C.
FIG. 1. CHANGES DURING STORAGE AT 37°C.
•—• RABBIT—pH. 5·6–5·8
⊙—⊙ RABBIT ADRENALINE pH. 6·6
X—X BEEF —pH. 5·6–5·8
X(5°)
QHP. SOL. IN 0·1 M KCL. μ MOLS/G.
DAYS AT 37°C.
Y
D
Q
Z
R
D
Z
Q


STORAGE HISTORY DAYS(°C)
RAW
LOW SPEED HOMOGENATE
LENGTH
DIAM.
CONTROL
2(-20°)
1300
260
30(37°)
30(5°)
HIGH SPEED
CONTROL
2(-20°)
30(37°)
30(5°)
FIG. 2. DIMENSIONS OF FIBRES — & FIBRILS ···· IN HOMOGENATES OF BEEF MUSCLE. 1 CM. = 100 μ.
COOKED
HIGH SPEED HOMOGENATE
LENGTH
1500
4000
2000
4000
2500
STORAGE HISTORY DAYS(°C)
CONTROL 2(-20°)
30(37°)
30(5°)
PAPAIN MILD COOK
PAPAIN FULL COOK