LIV. THE AUTOLYSIS OF BEEF AND MUTTON.

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I. THE AUTOLYSIS OF BEEF AND MUTTON COMPARED.

The study of the course of autolysis in beef and mutton described in the following experiments was undertaken in the hope that it would reveal some explanation of the inherent differences observed in the effect of cold storage on these two kinds of muscle.

It is a fact of common knowledge that beef cannot be frozen and thawed again without marked changes taking place in the appearance, palatability, and general physical state of the meat, while nothing of the sort happens when mutton is similarly treated. Frozen beef on thawing becomes bluish in colour, flabby to the touch, and, most serious of all, there is an exudation of juice which contains valuable nutrient material. "Frozen" beef must be carefully distinguished from "chilled" beef. The latter is beef which has been cooled to a temperature low enough to prevent bacterial action, but not so low as to cause the tissue fluids to become solid. Meat so treated is not immune from attack from moulds, and can only be preserved for a limited length of time.

The fundamental differences in the two kinds of flesh underlying the differences in their behaviour on freezing and subsequent thawing may be chemical or physical. Autolysis is a term used to describe a series of post-mortem chemical changes in tissues, and it was hoped that any radical difference between the chemical constitution of beef and that of mutton would reveal itself in the course of the autolysis of the two kinds of meat.

Autodigestion was the term used by early workers on this subject, and it implies that, under certain conditions, tissues digest themselves. The digestion of an organ by its own self-contained enzymes must clearly be distinguished from that produced by external agencies, as, for instance, the bacteria of putrefaction. If an excised organ is kept aseptically *in vitro*, it undergoes characteristic autolytic changes. Since certain antiseptics inhibit bacterial action, while they are without effect on enzymes, autolysis pursues a normal, or nearly normal, course *in vitro* in the presence of antiseptics such as toluene, chloroform, or acriflavine. In some pathological conditions autolysis can occur in the living animal.

As in the case of digestion by the ordinary enzymes of the alimentary canal, autodigestion is a series of processes by which the complex compounds of the tissues are broken down into simpler ones. It is certain that there are changes in the carbohydrates and fats, but the course of the protein autolysis is that which has received most study. Proteolysis implies a degradation of coagulable

protein material into simpler substances, which are not coagulated by heat, nor precipitated by certain protein reagents, and hence autolysis is accompanied by a relative increase in the non-coagulable, or soluble, nitrogen. As this is capable of easy estimation, the course of autolysis is usually followed by making successive determinations of the soluble nitrogen present.

All animal tissues are subject to post-mortem autolysis, but the process proceeds at very different rates in different organs, being most rapid in glandular tissue, such as the liver, and slowest in striated muscle. In order to develop the details of technique, a preliminary examination and comparison was made of the course of autolysis in sheep and ox liver, and essentially the same methods were later applied to the study of autolysis in muscular tissue.

Experimental details.

About one and a half pounds of the liver were transferred immediately after the death of the animal, and with as little handling as possible, to a sterile, covered dish. On arrival at the laboratory it was cut into small slices and scraped to a fine pulp with sharp scalpels on a glass plate. In this way the glandular material could be almost entirely freed from connective tissue. The pulp was well mixed, weighed in a glass dish, and made up into a 20 % suspension in toluene water. For example, in one case (Exp. 12), to exactly 400 g. of well-mixed liver pulp, 50 cc. of toluene were added and sufficient distilled water to make a total volume of 2 litres. The suspension was poured into a sterile flask with a well-fitting cork which had previously been soaked in toluene. The mixture was incubated, generally at 37°, the flasks being shaken thoroughly twice a day. The course of the autolysis was followed by successive estimations of the soluble nitrogen in an aliquot part of the whole suspension. Fractions were obtained by withdrawing a known volume by means of a blunt-ended pipette. The tip was cut off an ordinary, rather wide, 20 cc. pipette, which was then found to deliver 19·4 cc., and this was used throughout all the experiments. Provided that the suspension had been carefully made up, no difficulty was found in obtaining representative samples by this means. Samples were withdrawn immediately after making up the suspension for determination of the initial total and soluble nitrogen, and then at regular intervals, generally once a day for the first four days, and later every two or three days.

The total and soluble nitrogen were estimated by Kjeldahl's method, and the amino-nitrogen by Sörensen's formaldehyde method. The value for the total nitrogen was obtained by incinerating 20 cc. of the suspension, corresponding to a known weight of muscle, and that of the soluble nitrogen by incinerating an aliquot part of the filtrate after precipitating the coagulable protein. In all cases the ammonia was steam-distilled into the standard acid, which was N for the total, and $N/10$ for the soluble nitrogen. The caustic soda was used in equivalent strengths, and was CO_2 free. The indicator used was methyl red.

The coagulable protein was precipitated either by 25 % metaphosphoric acid, or 2·5 % trichloroacetic acid, according as the filtrate was to be used for the determination of amino-nitrogen or not. It was found impossible to do a formaldehyde titration in the presence of so large an excess of phosphate, while trichloroacetic acid showed no such "buffer" action.

The following is a detailed account of the procedure. 20 cc. of the suspension were run into a stoppered graduated cylinder, and 10 cc. of a 25 % solution of glacial phosphoric acid (freshly made) added, and the volume made up to 100 cc. After thorough shaking, the cylinder was allowed to stand over night. The precipitate was then removed by filtration through a dry, fluted paper, when a crystal clear filtrate was obtained. The nitrogen was estimated in 10 cc. of this filtrate. Duplicate estimations were always made. When trichloroacetic acid was used, 50 cc. of a 2·5 % solution were added instead of the phosphoric acid, but the procedure was otherwise the same.

Expression of results.

In reviewing the literature on this subject, a great diversity in the method of expressing results has been found. The most general practice has been to give the number of cc. of acid neutralised in the Kjeldahl estimation of the nitrogen; sometimes the amount of alkali equivalent to the excess of acid. As each observer chooses his own standards, this method is far from satisfactory, and it is suggested that a uniform method of expressing results should be adopted. We have found that the clearest picture of the course of autolysis is presented by expressing the amount of non-coagulable nitrogen present as a percentage of the original total nitrogen. In this way the progressive degradation of the nitrogenous complexes is most easily followed and graphically represented.

THE AUTOLYSIS OF OX AND SHEEP LIVER.

It was found that the autolysis of the livers of both these animals follows a normal course, and they are strictly comparable. There is nothing in the breakdown of the chemical complexes after death to suggest any fundamental difference during life. The curves representing the increase in soluble nitrogen follow the same course and are similar to those obtained by other workers. Estimations were not made sufficiently soon after death to show the latent period, but both show a rapid initial increase followed after above five days by equilibrium. The curves obtained from amino-acid titrations show the same characteristics. Curves from a typical experiment are appended (Figs. 1 and 2).

THE AUTOLYSIS OF SHEEP AND OX MUSCLE.

In the main the methods used for the examination of the autolysis of liver were applicable to muscle tissue, but slight modifications were necessary in preparing the suspension. The meat was separated from fat and connective tissue, and then shredded on a glass plate. The resulting pulp was passed

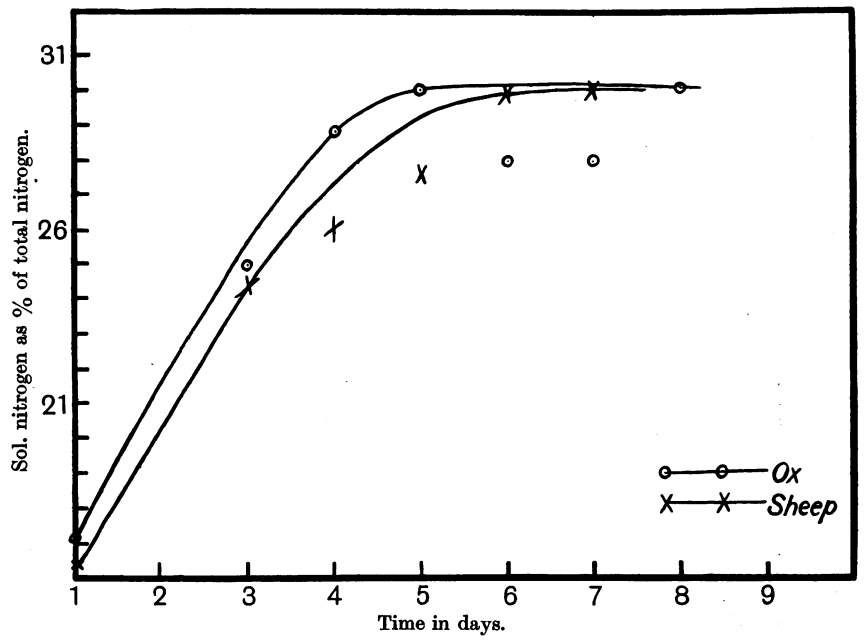

Fig. 1. The autolysis of sheep and ox liver.

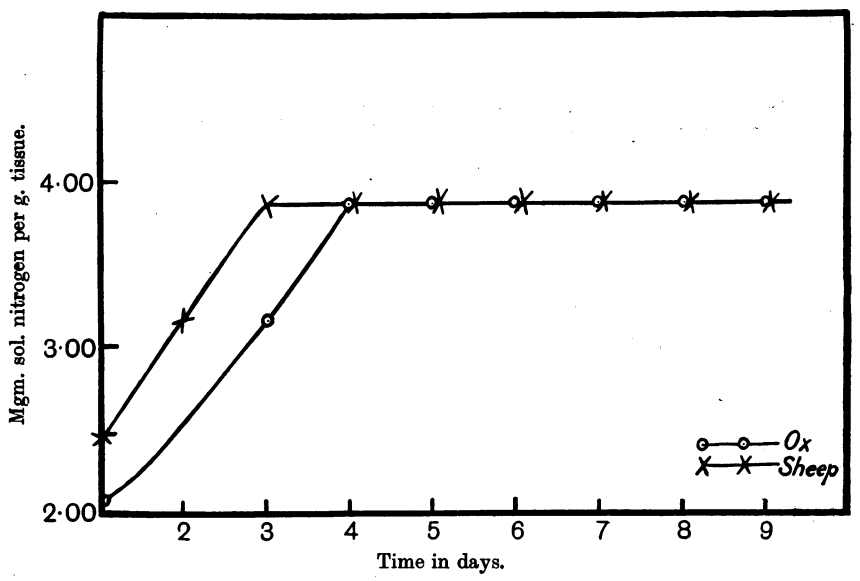

Fig. 2. The amino-nitrogen curve of liver autolysis.

twice through a fine mincer, and then rubbed to a paste in a mortar. A definite amount (*e.g.* 100 g.) was then weighed on a tared clock glass and transferred quantitatively to the mortar, and well mixed with water and toluene. The suspension was washed into a cylinder, and made up to a known volume; a fixed volume of water was then used to wash this into a sterile flask, so that the total volume of suspension was accurately known. The flask was closed by a cork soaked in toluene. In general, 20 % solutions in 5 % toluene were used, and 20 cc. fractions withdrawn as already described. The technique of precipitation etc. was exactly the same as for liver autolysis.

As in the case of the liver, the study of post-mortem autolysis in these two species of muscle failed to reveal any intrinsic differences. It was found,

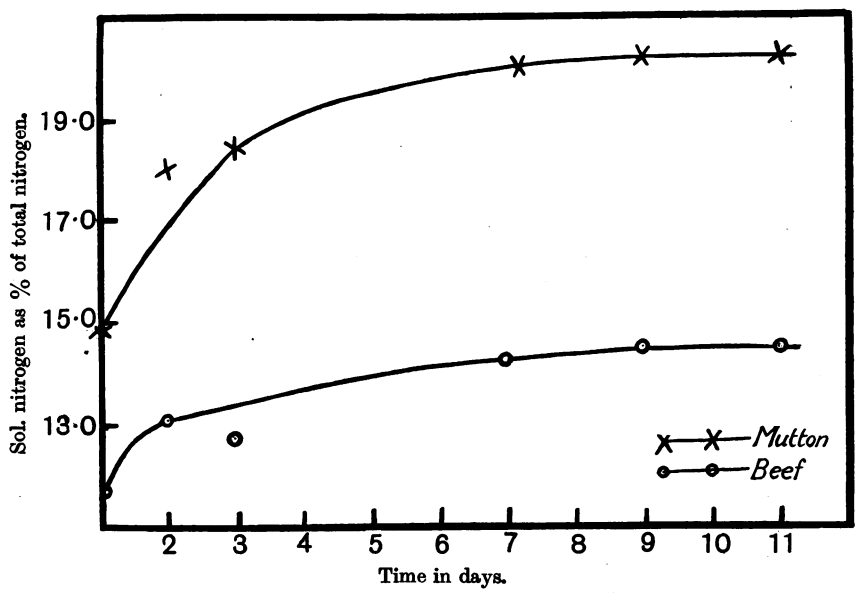

Fig. 3. The autolysis of beef and mutton at 37°. (Exp. 13.)

however, that in mutton the soluble nitrogen forms a larger percentage of the total nitrogen, so that the curves are parallel, but not superposable. The course of both, however, is perfectly regular and typical, and there is nothing to point to any important differences in the chemical constitution of these two kinds of muscle sufficient to account for their different behaviour in the cold store.

The progress of the autolysis is markedly slower in muscle than in glandular tissue, and equilibrium is not reached till about the eighth day. Moreover, the degradation stops short at a smaller percentage of soluble nitrogen.

Curves are given of a typical autolysis at 37°, and also of one at a much lower temperature. It is to be noted that though autolysis is retarded at the lower temperature, the two curves remain parallel (Figs. 3, 4 and 5).

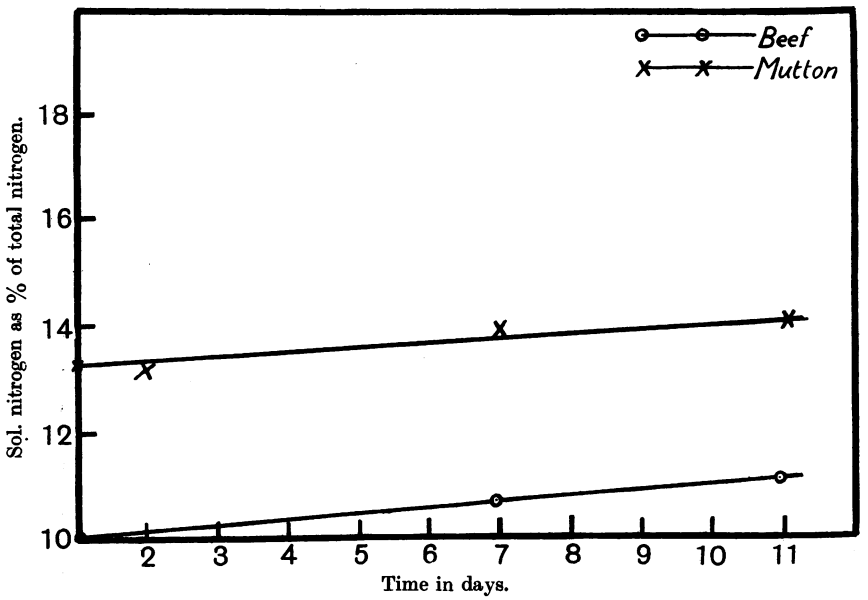

Fig. 4. Autolysis of beef and mutton at 6°. (Exp. 13.)

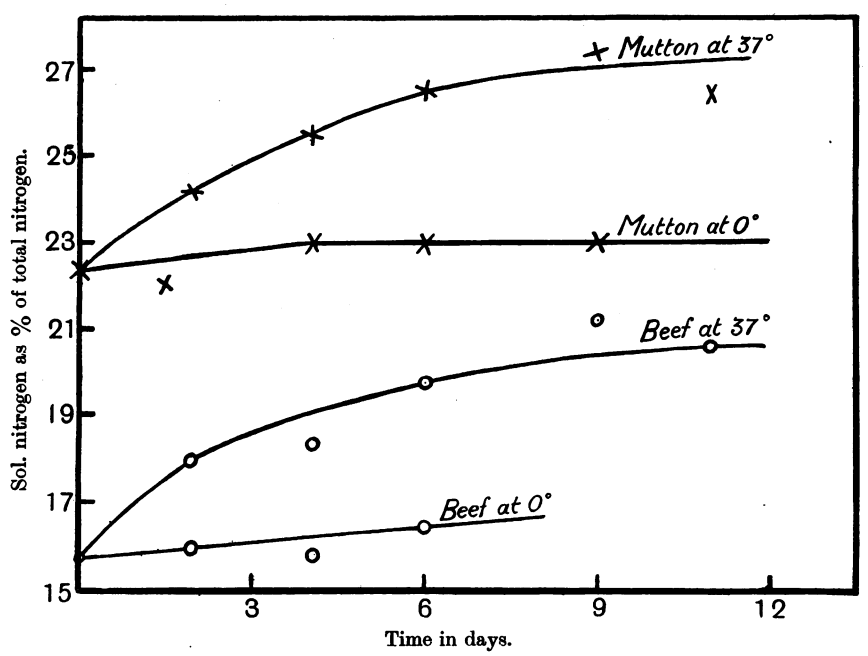

Fig. 5. The autolysis of beef and mutton at 37° and at 0°. (Exp. 14.)

GENERAL CONCLUSIONS AND SUMMARY.

An examination of the post-mortem autolysis of beef and mutton has thrown no light on the cause of their different behaviour after being frozen. The processes in both are exactly parallel, both at incubator temperature and at low temperatures. In the case of mutton, equilibrium is reached at a higher percentage of soluble nitrogen than in beef; but the initial non-coagulable nitrogen is higher, so that the curves are comparable. It is probable, therefore, that the differences in the two kinds of muscle in this respect lie not in their chemical constitution, but in the structure and physical properties of the fibres.

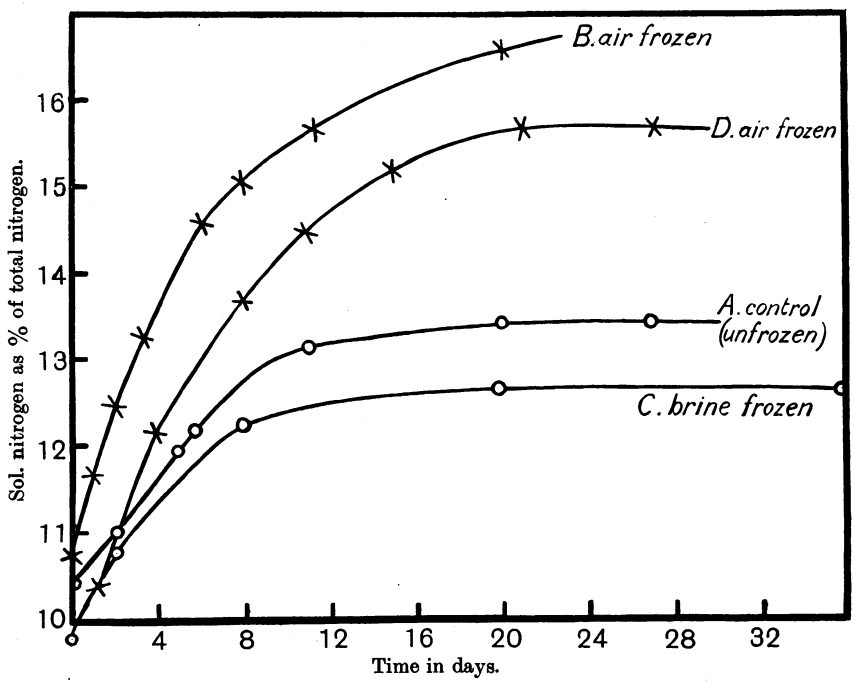

Fig. 6. The autolysis of frozen and unfrozen beef.

II. THE AUTOLYSIS OF FROZEN BEEF.

While working on the autolysis of beef it seemed of interest to determine whether previous freezing had any influence on the course of autolysis after thawing. It was found that not only was the course and degree of autolysis profoundly altered by such treatment, but that the method of freezing, that is the rate of freezing, is of great significance. This is made clear by the curves given in Fig. 6. It will be seen that previous freezing of the muscle results in a much greater degradation of the nitrogenous constituents during autolysis, and also that equilibrium is greatly delayed. The rate of freezing of carcasses is known commercially to be most important, and recent work has shown that, if frozen sufficiently rapidly, the meat on thawing approaches

more nearly in appearance and palatability to fresh beef. On the large scale, rapid cooling is achieved by immersing the carcass in brine at about – 20° C. instead of freezing in air chambers. The beneficial effect of rapid freezing is borne out by the autolysis curve of beef frozen in brine. This curve approaches much more closely to that of the control from fresh beef, than that of air-frozen meat.

The following are the details of one such experiment. The animal was killed at 2.30 p.m. on June 29th, and immediately, before skinning, about 2½ lbs. were cut off the shin, and placed in a sterile pan in ice. On arrival at the laboratory, the skin and fat were removed, and the muscle divided into four portions.

"A" was used as control, and a 20 % suspension was made by the method described above, and placed in the incubator at once.

"B" was suspended in a glass jar, and placed in the cold store at 18° F. at 3.20 p.m. It was removed the next day, and allowed to thaw for six hours at room temperature, and then the autolysis suspension made up as "A."

"C" was treated in the same way as "B" except that, in the cold store, it was sunk in saturated brine at 18° F.

"D" was kept at 0° for 20 hours, and then placed in cold store on June 30th, after which it received the same treatment as "B."

In each case the autolytic mixtures consisted of 100 g. muscle in 5 % toluene. Samples were removed at the times indicated on the curve, and the subsequent procedure was exactly as previously described.

Curves are given for one experiment only, but similar relationships were found to hold in other experiments.

It is very difficult to explain this effect on autolysis produced by freezing. Obviously the equilibria of the cell have been disturbed by the rupture of the membranes which occurs during slow freezing. As there is an absence of "drip" after brine freezing it would seem that by this method any extensive damage to the cell is avoided, and this is borne out by the autolytic changes in the brine-frozen beef.

Our sincere thanks are due to Prof. Hopkins for his interest and help throughout this work.