I. THE AUTOLYSIS OF THE MUSCLE OF THE COD FISH.

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FEARON and FOSTER [1922] showed that ox muscle, after being frozen rapidly in brine at -8° and then thawed, autolysed at about the same rate (in the presence of toluene) as normal muscle. On the other hand, muscle which had been frozen slowly in air at -8° and then thawed showed a greater amount of autolytic change and a more rapid rate of autolysis. The present investigation is an extension of Fearon and Foster's work to the muscle of cod. No reference to the autolysis of cod muscle could be found in the literature. The material proved to be very difficult to manipulate and it was only after much preliminary work that satisfactory methods were worked out.

METHODS.

The following procedure was finally adopted. The muscle, with connective tissue, was minced by passing twice through a very fine mincer, and then mixed thoroughly by hand; 400 g. were quickly weighed out for the digest. At the same time about 15 g. were transferred to a stoppered weighing bottle and used for determinations of total nitrogen; in each case six estimations of total nitrogen were carried out, the average being adopted. The 400 g. of mince were transferred in small quantities to a mortar and triturated with toluene until each portion was saturated, 150 cc. of toluene being used in all. The mixture was then quantitatively transferred to a sterile 2-litre flask, the total volume of suspension being made up to 2 litres with distilled water. The flask was shaken at intervals for two hours. After this, a sample was withdrawn and the flask placed in the incubator or cold room. Every 12 hours the flask was shaken vigorously, and every 24 hours two samples were withdrawn with a blunt-ended pipette delivering 24.7 cc. Each sample was placed in a stoppered 100 cc. measuring flask containing 50 cc. of a 4 % solution of trichloroacetic acid. The contents of the flask were made up to 100 cc. by addition of distilled water. The mixture was left for 24 hours and then filtered. Estimations of the nitrogen content of the filtrate (so called "soluble nitrogen") were made by Cole's alcohol distillation method [Cole, 1920]. Nitrogen determinations of the reagents were carried out and due allowance made.

In order to compare fresh and frozen muscle, a fish was bisected longitudinally. Muscle was taken from one half and minced for autolysis; this served for a control. The other half was frozen, stored, thawed and then minced for autolysis. For the processes of freezing and storing, halves of two different fish were placed together in an oiled-silk bag, so that the thickness would be the same as that of a whole fish. Thus the fish was affected neither by penetration of salt during brine freezing, nor by desiccation during air freezing and storage.

EXPERIMENTAL RESULTS.

Exp. 1. The Autolysis of Fresh Cod at + 37°. In order to test the accuracy of the method, preliminary autolyses were carried out. The following experiment shows that the experimental error involved is quite small.

Fresh cod was minced in the usual way, and duplicate autolyses were carried out. Fig. 1 shows that such duplicates gave practically identical results, thus illustrating the accuracy of the method.

Exp. 2. The Autolysis of Fresh Cod at + 1°. Fresh cod was minced and put up in four similar flasks, two of which were incubated at + 37° and sampled daily. The other two flasks were kept in a room at + 1° and samples were removed at intervals of several weeks. The experiment commenced on October 5th, 1923.

Table I.

Date examined	Soluble N	
	Flask 1	Flask 2
	%	%
October 9, 1923	10.9	10.9
January 7, 1924	10.8	10.9
May 23, "	11.7	11.7
October 5, "	12.4	12.2

The results of the experiments at + 37° are shown in Fig. 1 whilst Table I shows the results at + 1°. It will be seen that the rate of autolysis at + 1° is very much reduced. From these data it was calculated that the rate of autolysis at + 37° was 223 times more rapid than at + 1°. (This gives a temperature coefficient of 4.5 for each 10°.)

Exp. 3. The Effect of Brine-freezing at - 19° on the Rate of Autolysis.

Halves of two different fishes were placed together in an oiled silk bag and frozen in brine at - 19° for 2½ hours, and then stored at - 11° for 10 days. They were thawed by hanging in air at + 1° for 96 hours, then minced and autolysed at + 37° in the usual way. The other halves of the two fishes were minced without having been frozen, and incubated to give a measure of normal autolysis.

Fig. 2 shows that the brine-frozen fish gave results practically identical with those of the unfrozen control. This agrees with the results of Fearon and Foster [1922] who found that brine-freezing does not cause any appreciable change in the tissues of ox muscle.

Exp. 4. The Effect of Air-freezing at -11° on the Rate of Autolysis. Two cod were bisected longitudinally. Half of each fish was immediately minced for a control experiment, suspensions being made in the usual way and incubated at $+37^{\circ}$. The other halves were placed together in an oiled-silk bag and slowly frozen by hanging in a cold chamber at -11° for 10 days. Portions of muscle were then cut from each half-fish and placed in two separate covered

Fig. 1. Duplicate autolyses

Fig. 2. The autolysis of brine-frozen cod muscle

beakers. The beakers were placed in a room at $+1^{\circ}$ for 66 hours to ensure slow thawing. During this process liquid ("drip") separated out. The muscle was then minced and the mince and "drip" thoroughly mixed together. Suspensions were made up and autolyses were carried out at $+37^{\circ}$ in the usual way.

The results of a typical experiment (see Fig. 3) show that in the case of air-frozen cod there is slightly more autolytic change than occurs in the unfrozen control. This result is also in agreement with Fearon and Foster's [1922] conclusions on the air-freezing of ox muscle.

Fig. 3. The autolysis of air-frozen cod muscle

Exp. 5. The Autolysis of "Drip." "Drip," that is the fluid exuded by slowly frozen tissues on thawing, was collected from a cod frozen in air at -19° . 100 cc. of this fluid were mixed with 37.5 cc. of toluene and the volume made up to 500 cc. with distilled water. The mixture was incubated at $+37^{\circ}$ and samples were withdrawn daily and analysed. It was found that "drip" contained a higher initial percentage of soluble N (27.9 %) than cod muscle, and that the increase of soluble N (to 36.4 %) during the course of autolysis was also greater than in the case of muscle.

DISCUSSION.

Previous work [Stiles, 1922; Richardson and Scherubel, 1908; Kallert, 1923] on the freezing and thawing of animal tissues has shown that the minimum of alteration is obtained when the tissues are frozen rapidly and thawed slowly. The reason for this is that during freezing ice crystals separate out—the slower the freezing the larger the ice crystals formed and *vice versa*.

Obviously the ideal method is to freeze so rapidly that the size of the ice crystals is a minimum. Brine freezing at low temperatures appears to give a rate rapid enough for most practical purposes.

It has been observed that, on thawing frozen tissues, the water formed from the ice is slowly reabsorbed by the tissues [Kallert, 1923; Richardson and Scherubel, 1908.]

Even after complete thawing has been accomplished, this process of absorption still goes on. In the present investigation this fact was borne in mind,

the muscle tissues being given ample opportunity to reach equilibrium before the autolyses were carried out.

The results of this investigation show that air-frozen cod suffered a much greater destruction of its tissues than brine-frozen cod. Cod muscle frozen rapidly in brine at -19° gave no "drip" and presented a perfectly normal appearance on thawing, whereas cod frozen slowly in air at -19° and -11° always "dripped" on thawing and felt noticeably flabbier. The volume of "drip" which could be collected in such cases was of the order of 10 % of the weight of the fish. Also, rapidly frozen cod muscle autolysed at the same rate as the unfrozen control whilst with slowly frozen cod muscle there was a slight but appreciable increase in the amount and rate of autolysis. Thus for cod muscle as for other animal tissues the best method of preservation is to freeze rapidly.

Experiments carried out at $+1^{\circ}$ showed only slight autolysis even after 12 months. This is of importance as it indicates that no appreciable changes are likely to occur in the proteins of *frozen* cod stored for prolonged periods at low temperatures such as -11° .

Note on a Protein of Cod Muscle.

When "drip" was incubated at $+37^{\circ}$ with toluene, a coagulum was observed after 24 hours. It consisted chiefly of protein. The following experiments with cod muscle offer further evidence for the existence of a protein which coagulates at $+37^{\circ}$.

1. Air-frozen cod muscle whilst still in the frozen condition was minced, suspended in a 5 % solution of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and left over night. The suspension was then filtered and the filtrate gently heated to $+37.5^{\circ}$ when a faint opalescence was observed. Another experiment on the same filtrate gave an opalescence at $+38^{\circ}$.

2. The following experiment proved that the presence of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ had no appreciable effect on these results. Shavings of air-frozen cod were ground up in a mortar with sand and distilled water. The filtrate from this extraction also contained a protein which commenced to coagulate at $+36^{\circ}$. This experiment was repeated with fresh cod with the same result.

SUMMARY.

1. At $+37^{\circ}$ autolysis of cod muscle was practically complete after seven to eight days but at $+1^{\circ}$ autolysis proceeded very slowly and even after 12 months the changes were quite slight.

2. Cod frozen rapidly (in brine at -19°) presented a normal appearance on thawing, and on autolysis at $+37^{\circ}$ gave results identical with those of the unfrozen control.

3. On thawing cod which had been frozen slowly (in air at -11°), a fluid ("drip") separated out. Autolysis of the thawed tissue with the appropriate amount of "drip" proceeded at a slightly greater rate than that of the unfrozen control.

4. "Drip" was found to contain a higher initial percentage of soluble N and to autolyse to a greater extent than normal cod muscle.
5. Cod muscle was found to contain a protein which coagulates at $+ 37^{\circ}$.

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