THE HISTOLOGICAL CHANGES IN FROZEN FISH AND THE ALTERATIONS IN THE TASTE AND PHYSIOLOGICAL PROPERTIES OF THEIR FLESH.

A RESUMÉ OF DR K. REUTER'S INVESTIGATIONS[1].

BY G. H. F. NUTTALL AND J. STANLEY GARDINER.

(With Plate I.)

[UP to recent years, fish were frozen in a dry chamber; this was not found entirely satisfactory, the fish tending to become dry and lose their flavour. We were ourselves intending to experiment on freezing fish in brine prepared in different ways and made a proposal to the Fish Food and Motor Loan Committee to this effect. Our attention was then drawn to Ottesen's process which we have since tested through the kindness of Mr J. V. Pryor of Cambridge[2], who has supplied us with ice and allowed us to use his cold storage chambers. The confirmatory results, which we have obtained practically, are well explained in the light of Dr Reuter's report on his careful investigations, an abstract of which follows.

The preservation of fish and other food is clearly a matter of great national importance, and it already exercises the attention of the Governments of all the Allies. We believe, therefore, that the results of the researches herein summarized should become widely known.]

The expansion of water on freezing formerly dominated our views as to its effects on the dead animal body. The idea arose that the body fluids on freezing rupture the tissues so as to burst such organs as the

[1] "Ueber die histologischen und geschmacksphysiologischen Veränderungen gefrorener Fische," von Dr med. Karl Reuter, Hamburg. *Abhandlungen zur Volksernährung*, Berlin, 1916, Heft 5, pp. 211–242, figs. 36–55.

A copy of the foregoing publication was obtained recently through the good offices of the Fisheries Branch of the Board of Agriculture and Fisheries. We reproduce nine of the author's illustrations.

[2] "Frozen Fish." *Fish Trades Gazette*, Jan. 19, 1918, p. 23.

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intestine, the urinary and gall bladders. The tissues on thawing were supposed to be broken up and putrefaction to result, since putrefactive changes became more apparent after freezing. In fish it was thought that the rupture of the gall bladder would especially lead to a deterioration in the flesh. However, no such bursting actually occurs, the walls of such hollow organs possessing sufficient elasticity to resist the expansion due to freezing.

The fine histological changes which tissues undergo through freezing are of greater significance. They chiefly affect the blood and muscle. The haemoglobin of the former becomes dissolved in the serum (haemolysis). It then diffuses into the muscles staining them faintly red; a similar change also occurs as a result of putrefaction. Such diffusion in fish is small, discolouration being seen only near the vertebral column in the neighbourhood of the large caudal artery and vein; elsewhere the muscles showing the grey-white colour characteristic of fish muscle.

Fish, whether frozen rapidly in brine or slowly in air, present at first a similar external appearance. The bright glossiness due to the slime is, however, quickly lost in the air-frozen fish, the skin of which soon commences to present a shrivelled appearance somewhat approaching that seen in dried fish. In such fish loss of weight may be considerable, complete drying being ultimately effected if the frozen fish is kept in air for any length of time.

Preliminary examinations were made of the muscles of various fish immediately on killing. Small pieces were cut out and frozen by liquid CO_2. They were then cut into sections of 0·01 mm. thickness by a freezing microtome. Such sections show the principal changes in the muscles due to freezing and are best understood by reference to schematic Figs. 1 and 2 (Pl. I) of unfrozen and frozen muscles. In Fig. 1 the muscle fibres appear as fine prismatic columns, consisting of the sarcolemma enclosing the sarcoplasm, or contractile substance. In rapidly frozen muscle (Fig. 2), the contents of the muscle columns have become separated into central more fluid and peripheral less fluid parts. The latter are pressed against the sarcolemma, and the fibres simulate hollow cylinders enclosing ice columns. While the whole muscle is frozen hard, histological changes only occur in the fibres, not in the connective tissue binding them together.

In fact, freezing separates the water from the albumin in each muscle fibre so that it comes to lie axially. Such a separation clearly can only take place during the freezing process, for with complete hardening there can be no further histological changes. Most fish begin to freeze

at about − 1° C., and at this temperature by far the greater amount of fluid contained in the fibres separates out. It may be assumed that the water which collects in the centre of the fibre contains salts. Furthermore, sections treated with alcohol exhibit a finely granular amorphous precipitate of albumin in this fluid, which it is suggested has about the same chemical composition as the juice which escapes on thawing.

Numerous histological examinations of fish muscle had necessarily to be made in the course of the investigations. Killing in 4 % formalin, hardening in graded alcohols and embedding in celloidin gave the best results. The shrinkage was slight and large sections were cut, at times extending through the whole body of the fish (Pl. I, Figs. 6 and 7). The study of these sections gives rise to three questions:

(1) Can the change in the tissues be altered by the method of freezing? Is rapid or slow freezing the better?

(2) Can the process of thawing be modified so as to induce the tissues to return to their original state?

(3) How far is the value of fish as food changed by freezing? Keeping qualities, nutritive value and flavour?

In the first experiments, made both with fish rapidly frozen at a low temperature in brine and with fish slowly frozen at a moderate temperature in the air of a refrigerating chamber, a difference was visible to the naked eye on cutting the muscles. In the slowly frozen fish relatively large crystals of ice formed between the muscular fibres giving rise to clear spaces after thawing, the flesh appearing translucent. In rapidly frozen fish no structural changes were thus visible, the flesh appearing uniformly opaque. After thawing these differences became less marked, because, as the tissues softened, the interspaces disappeared. Under otherwise similar conditions more of the juice drained off the slowly frozen than off the rapidly frozen fish.

The microscopic appearances differed considerably. Where the freezing was most rapid, viz. close under the skin, a number of small ice columns were formed within the muscle fibres (Pl. I, Fig. 3). In deeper muscular fibres, where freezing was less rapid, the number of such columns decreased so that finally each fibre contained a single column. In large fish, in the centre of the musculature the escape of fluid out of the fibres was observable, this being due to the rupture of the sarcolemma (Pl. I, Fig. 4).

In fish frozen slowly in the air vacant spaces corresponding to the ice columns no longer occur in the sarcoplasm. The muscle fibres form compressed bands or separate bunches of sharp-edged little columns,

while irregular spaces appear in the connecting tissue into which the fluid from the muscle fibres has exuded. In other words the fluid congeals outside the muscle fibres (Pl. I, Fig. 5).

The explanation of these differences is to be found in the colloidal state of the muscle. It has been seen that water is separated from the gelatinous content of the muscle fibre. In all colloids the time required for imbibition, and the reversal of this process, is much longer than in bodies having a crystalloid structure. The assumption is therefore justified that the separation of water from a colloid will be more complete the more slowly it is cooled down to the point at which it solidifies. Conversely the separation of fluid will be lessened in proportion to the rapidity with which the temperature of solidification is attained. By using liquid CO_2 small pieces of muscle could be so rapidly frozen that no changes were produced. Freezing took place within one or two seconds, and sections only showed the effect of freezing on their edges, the greater part of the tissue presenting the appearance of normal unfrozen muscle (Pl. I, Fig. 8). No exudation of fluid into the connective tissue, and no aggregation into columns within the separate fibres could be seen. The muscle illustrated in Pl. I, Fig. 9, the freezing of which lasted five to ten minutes, may be compared to that in Fig. 8, the latter being practically the section of a normal unfrozen muscle and comparable to schematic Fig. 1, and Fig. 9 corresponding to schematic Fig. 2.

The conclusion from the above observations is that in very rapid freezing the water of the muscle albumin freezes in an invisible molecular state. In less rapid freezing a number of small columns of fluid are formed in each muscle fibre, if time allows, these fusing to form a single column. In still slower freezing the fluid ruptures the sarcolemma and escapes into the connective tissue, forming large spaces filled with ice.

In brine-frozen fish the spaces between the muscle fibres are not visible to the naked eye, but in air-frozen fish ice crystals may be seen, although at times the fibres may remain intact. Indeed it is evident that in different methods of freezing all gradations may be found. The size and character of any fish, its possession of a thick skin, an isolated layer of fat, a swimming bladder, or a peculiar shape may influence the changes brought about by freezing and explain occasional small divergences. The essential difference observable in a fish frozen in the air of the refrigerating room at $-7°$ to $-12°$ C. and in one frozen in brine at $-15°$ C. is always attributable to the difference in sizes of the ice crystals formed. It explains why on thawing less juice escapes from

the flesh of brine-frozen fish, its small lacunae possessing greater capillary attraction. The juice, however, can by slight pressure be squeezed out, and this is a drawback to frozen fish, readily differentiating it from the unfrozen[1]. It is of practical importance that such thawed fish should not be exposed to pressure and moreover desirable that it should in preparation for table be cut as little as possible.

When slow thawing occurs in the presence of a great deal of juice, the cells being ruptured, the muscle fibres only act as passive bodies in the fluid. Experiments show that the muscle albumin does not reabsorb its fluid, unless the duration of the frozen condition has been very short. In the latter case there is a slight recovery after thawing, but muscle albumin does not give off and reabsorb water appreciably as do such colloids as glue and gelatin.

The best results in thawing were obtained by floating the fish in a large vessel of cold water. According to our investigations the speed of thawing has no influence on the recovery of the tissues from the effects of freezing. These observations are not in agreement with those of the Dutch Report wherein the authors considered that they had observed such a recovery. As the measure of the changes may be subject to the personal equation, a series of measurements are now being made. The author's observations convince him that recovery is very improbable and of no practical importance. The fact that the muscle albumin does not reabsorb the expelled fluid indicates that its colloidal condition has undergone changes closely similar to those of coagulation. In this respect Ostwald states of colloids that when they freeze "in der Regel gleichzeitig mit der Kristallisation des Eises das Kolloid koaguliert, obschon in vielen Fällen die Homogenität der räumlichen Verteilung annähernd gewahrt bleibt," a statement which does not always apply to muscle. The muscles of cod were compared frozen both in brine and in the air, allowing them to thaw at room temperature. Coagulation seemed to increase with the length of time that the fish remained frozen. If the freezing has only been maintained for a short time, the muscle retains some of its viscosity and elastic-gelatinous consistency; if frozen for a long time, it loses its elasticity and becomes dry and friable. The difference is one which can easily be determined if pieces of the muscle are pressed between the fingers.

[1] [Our experience with brine-frozen herring does not agree with Dr Reuter's statement which apparently applies to cod; the juice cannot be easily expressed in this manner, and differentiation between frozen and unfrozen herrings is exceedingly difficult. G. H. F. N. and J. S. G.]

The nutritive value of frozen fish may be reduced owing to the loss of meat juice, but on the other hand freezing is an advantage in that it prevents putrefaction. The digestibility of fish muscle may possibly be increased by the loosening of the fibres by freezing. Experiments were made by digesting with artificial gastric juice at body temperature. Fresh, slowly and quickly frozen fish were compared, but no differences were found. The flesh of slowly frozen fish is somewhat firmer than that of fresh fish, the muscle fibres being pressed together and of a straw-like consistency. The differences in quickly frozen fish are so small that they can neither in texture nor in taste be readily distinguished from fresh fish.

The natural smell of fish depends on volatile substances and may be of considerable value in rendering them appetising. These substances are absorbed by the brine or given off into the air, being doubtless present mainly near the surface of the fish. To such a degree is this the case that brine before being used for further freezing has to be filtered through charcoal. All haemoglobin is converted into oxy-haemoglobin so that the blood appears bright red in frozen fish instead of dark coloured as in ordinary dead fish.

The progressive drying of frozen fish during storage, accompanied as it is by gaseous interchange (oxidation) and loss of aroma, may make a considerable difference to the flavour, leading to a tastelessness, which is also a frequent source of complaint against frozen meat. It is probably owing to this oxidation that fish, which contains a small quantity of fat such as mackerel and herring, may acquire a marked rancid flavour. To prevent this care must be taken to exclude air either by covering the stored fish by an impermeable membrane or by other means.

Finally, Dr Reuter desires to make it clear that his conclusions only apply to fresh fish. If putrefaction has begun prior to freezing, other changes may take place in the fish during freezing and storage: his experiments do not cover such fish. He assumes that all bacterial and enzyme action ceases in frozen fish as otherwise meat could not be preserved by cold storage. The bacteria and enzymes, however, return to activity on thawing. Moulds, moreover, may grow on frozen fish or meat if the temperature in the storage chamber rises above a certain point, air being allowed free access.

EXPLANATION OF PLATE I.

Figs. 1–5 *are schematic. For description, see the text.*

Fig. 1. Unfrozen muscle (corresponds to Fig. 8).

Fig. 2. Frozen muscle (corresponds to Fig. 9).

Fig. 3. Rapidly frozen muscle (brine process, at −14° to −16° C.).

Fig. 4. Less rapidly frozen muscle (deep-seated muscle of fish treated as under Fig. 3).

Fig. 5. Slowly frozen muscle; the connective tissue not represented (fish exposed to air in chamber at −7° to −12° C.).

Figs. 6–7. *Photographs of cross sections of whole fish (reduced in size).*

Fig. 6. Brine-frozen cod. But few interspaces are visible between the muscles; the interspaces are partly attributable to displacement in cutting the flesh and partly to shrinkage in alcohol.

Fig. 7. Slowly air-frozen cod. Numerous large interspaces arranged symmetrically are visible between the muscle bundles, the grouping of the latter is due to the distribution of connective tissue amongst the bundles. There is considerable shrinkage.

Figs. 8–9. *Photomicrographs of cross sections of fish muscle.*

Fig. 8. Very thin section of fresh cod's muscle frozen by liquid CO_2 in a few seconds. Photographed whilst in salt solution and unstained, × 180 (corresponds to schematic Fig. 1 and shows the appearance of normal muscle).

Fig. 9. Somewhat thicker section than that shown in Fig. 8. Fresh plaice's muscle frozen by liquid CO_2 in 5–10 minutes. Photographed in salt solution and unstained, × 135 (corresponds to schematic Fig. 2).

LETTERING TO FIGURES.

f, muscle fibre; *i*, connective tissue; *k*, nuclei of muscle fibre; *l*, large lacunae due to expansion from ice; *p*, sarcoplasm; *R*, small intramuscular spaces (see description of Fig. 6); *s*, sarcolemma; *Sr*, shrinkage leading to folds in skin; *w*, ice columns; in Fig. 9 they represent spaces previously occupied by columns of ice as shown in schematic Fig. 2.

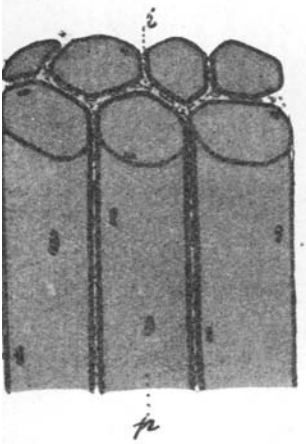

Fig. 1

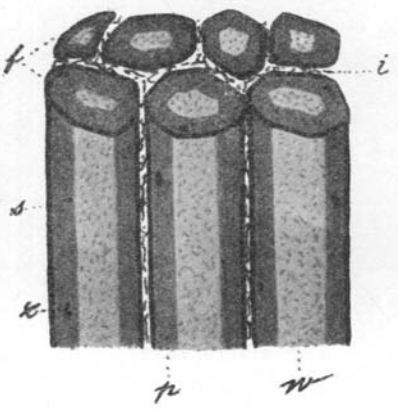

Fig. 2

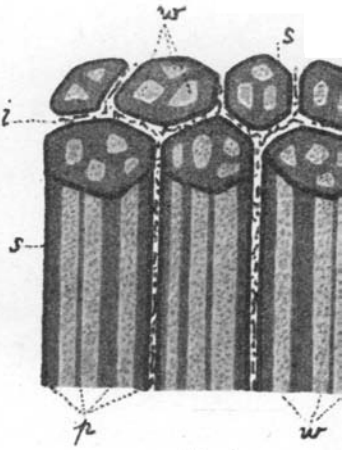

Fig. 3

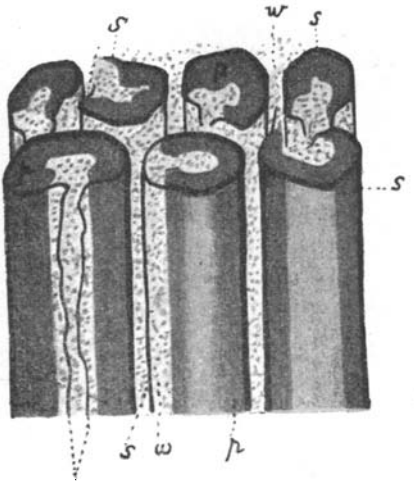

Fig. 4

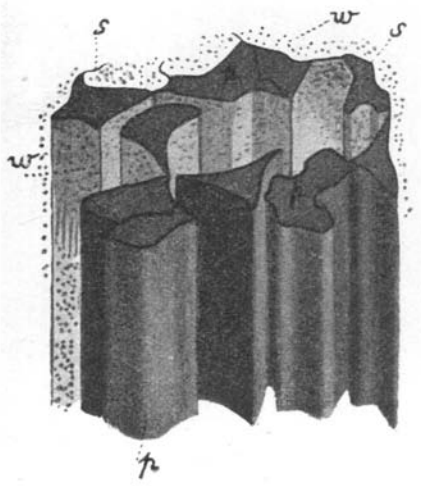

Fig. 5

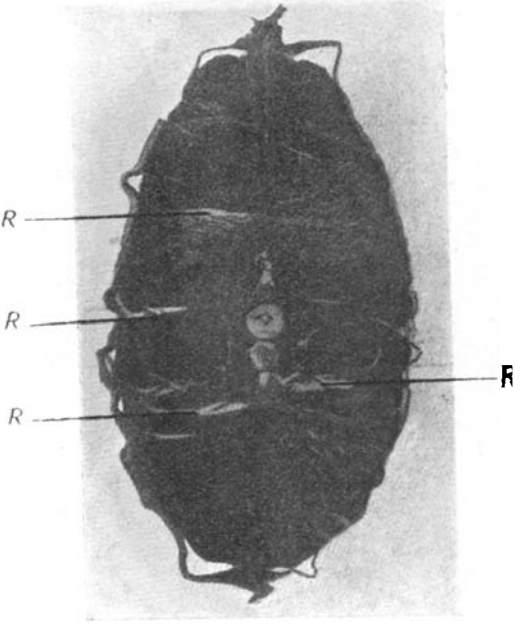

Fig. 6

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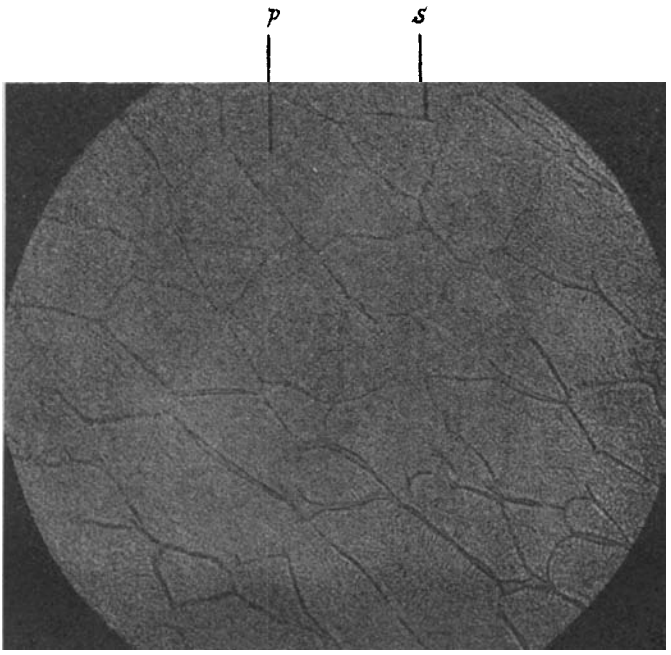

Fig. 8

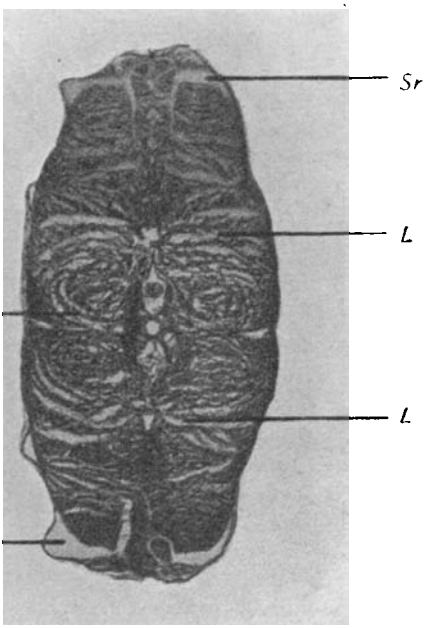

Fig. 7

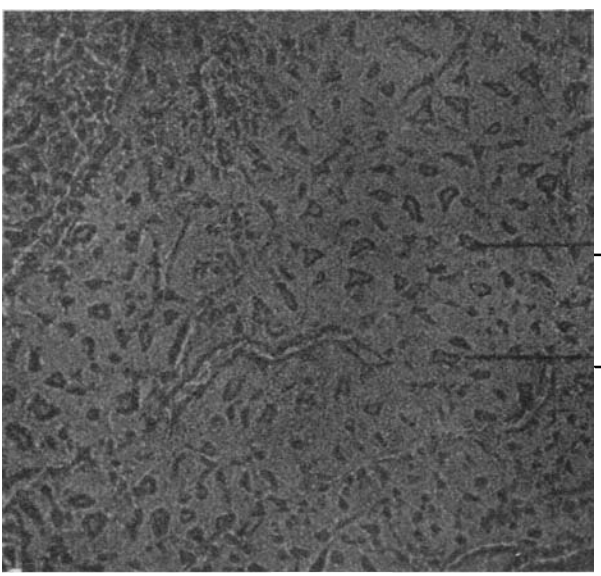

Fig. 9

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