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Structural Changes of Fish During Freezing

By D.D. MacKay and C.B. Weld

In the work on brine freezing which was done at the Station the macroscopic comparison of frozen with fresh fish was the main criterion of the success of the method.

Mr. Walter Boutilier of the National Fish Company told us that frozen fish could not be smoked successfully; and as we could see little real difference between fresh and brine frozen fish this statement seemed interesting and worth confirming.

One of us (C.B.W.) in the course of his investigation was examining microscopically the surface changes of smoked fish and he agreed to use some of the frozen material in his work. This made it possible for us to carry out the two following experiments.

Experiment I

Four fresh split haddocks from the National Fish Company were frozen by immersion in brine at 3°F. for one hour. They were rinsed with water at room temperature (60°F.) and stored in the freezing room at a temperature of about 5°F. for two days. They were then brought up to the laboratory, defrosted in air, brined in brines of different concentrations ranging from 98 to 45 per cent. saturated. After being dried in the drier for one hour they were smoked for eight hours.

Sections through the smoked surface showed a zone about 0.5 mm. wide and about 0.5 mm. under the surface where the muscle fibres seemed macerated (See plates I and II). These haddies were flabby throughout, even the backbone being unusually flexible. The surfaces were rougher and duller than those of fresh haddies smoked in the same way.

Experiment II

We decided to repeat the above experiment to confirm our observations of the microscopic structure. Six haddocks were frozen and left without washing in the freezing room for three days. When they were brought up to the laboratory three were thawed in air, and three in brine. The drying and smoking was

the same as in Experiment I. The sections were cut both after drying and after smoking; in each case the zone of macerated tissue was observed in every way similar to that noticed in Experiment I. The haddies, like the former, were duller than usual.

These observations encouraged us to consider the possibility of investigating the general changes in fish muscle during freezing. We had supposed from Dr. F.S. Jackson's work (6) that brine frozen fish did not exhibit the marked structural changes observed in the slowly frozen (sharp freezer) fish in which the cells were distorted and broken by ice crystals. However the above experiments do show changes and would indicate the necessity of comparing the appearance of air frozen with our own brine frozen fish. The changes shown in plates I and II are very interesting, in an abstract way, but we have no satisfactory explanation for the phenomena.

Experimental Technique

The method of obtaining the sections described in experiments I and II, and which is used in all the following experiments, is as follows:-
Small pieces of the flesh to be examined, measuring some 4 to 5 mm. square and 2 to 3 mm. thick were cut out with a sharp scalpel. These pieces were placed on a microtome block (wood) moistened with water, and frozen as rapidly as possible by directing a stream of carbon dioxide onto them from above. The pieces were frozen in 5-10 seconds. Several sections were then cut from these frozen blocks (which were carefully kept frozen hard) and the crumpled and now soft mass of sections then floated off the knife into water. Here they were carefully straightened out with dissecting needles and floated onto a slide where they were stained a few seconds with neutral red and examined while still wet. This method had been used for some months by Mr. Weld in examining the surface changes of haddies during smoking and had proven satisfactory and comparatively simple.

In the following experiments the expression "examined in the frozen state" will be frequently used. This refers to blocks of fish which have been frozen in various ways, small pieces of which have been put onto the microtome while still hard, kept hard by the carbon dioxide used as above, and sectioned before being allowed to soften at all. The sections after being cut softened almost immediately on the knife and were handled as above. When the expression "thawed" has been used, the blocks of fish were allowed to completely soften and then small pieces of the thawed fish were mounted on the microtome, frozen, and cut as above.

Thus in every case the section examined is soft, and in every case it has been frozen hard before cutting. The words "frozen" and "thawed" refer only to the handling before the sections were cut.

It will also be seen that the appearance of the fish cut "in the frozen state" differs materially from that cut "after thawing". It thus appears that while the cells of a block of frozen tissue, when thawed, show changes in their form and density, these same cells when cut in slices before thawing, as when they are sectioned" in the frozen state" do not change in appearance. Why this phenomenon should occur is rather inexplicable and we will not discuss the many possible explanations. Suffice it to say that by using the above technique the frozen fish do show a very different appearance than the thawed fish and this difference seems to be the true difference which does obtain between the frozen and thawed fish.

When prepared by the above technique, the microscopic appearance of sections of normal fresh cod and haddock is as shown in plate III. The muscle fibres are of even density throughout and they are placed close to one another. They show no perforations such as are seen in plates IV,V and VI and when cut in cross section their outline is smoothly rounded. If cut longitudinally they show nearly straight parallel sides. As shown by plate III the cells present a stippled appearance. When brined fish are examined by this same method this stippling is not shown (plates I and II) and they seem more firmly bound together.

Changes in Microscopic Structure

Experiment 3

Two fillets were frozen, kept for 3 days at 5-8°F., and then sectioned. One of these was frozen in brine at 5°F. and required one-half hour to freeze, while the other was frozen in air at 5°F. and required four hours to freeze. The microscopic appearance of the sections was as follows:

The brine frozen fillet, examined in the frozen state The sectioned cells were markedly distorted in outline and showed both large and small perforations. Thawed. The recovery of the cells to normal was marked but several are still distorted and show large perforations.

The air frozen fillet. In frozen state. The sectioned cells were markedly distorted in outline, the cell substance abnormally dense in consistency, and large but no small perforations present. Thawed. The recovery was marked but several cells are still distorted and show large perforations.

Obviously the structure of the fish muscle is changed by the freezing, even though the normal appearance is largely regained on thawing.

The results of experiments 1, 2 and 3 encouraged us to consider the advisibility of a systematic examination of frozen and defrosted fish muscle, as far as our time allowed. They suggested the following questions:

1. Will a microscopic examination be a means of determining
   (a) the changes in fish muscle due to freezing?
   (b) the changes due to storage?
   (c) the changes due to various methods of thawing or defrosting?

2. Is brine frozen fish on defrosting the same as fresh fish?

Experiment 4

A cake of fillets frozen by Mr. Finn on January 28, 1926 in brine at 0°F., wrapped, and kept in storage in the freezing room at the North Atlantic Fisheries, was cut in two in the centre and a section about two inches wide and running across the block from side to side sawed out. This was wrapped in paper, then in about one inch thickness of cotton wool, with a further wrapping of paper on the outside. This piece of frozen fish was brought up to the Station and sections made from the centre immediately on unwrapping. Pieces of the block were thawed in water, directly, and wrapped in oiled paper; in air; and a large piece wrapped in cotton wool and paper so that it would thaw very slowly. As each piece of material became defrosted sections were cut by the usual method.

An examination of the sections shows the following:

Treatment of pieces of fish prior to cutting sections	Appearance of sections
(1) "In the frozen state"	The cell tissue is markedly denser than normal. Holes are found in and between the cells which often appear very distorted
(2) Defrosted by immersion in water. Defrosting time was 5 minutes	The holes in the fibres are mostly closed up, but distorted cells and holes between the fibres still remain.

(3) Wrapped in oiled paper and immersed in water. Thawing time was 10 minutes	The same as (2).
(4) Defrosted directly in air Thawing time was 20 minutes	The same as (2) and (3) except that more holes in the fibres remain visible.
(5) Wrapped in a thick layer of oiled paper and absorbent cotton and left in air. Thawing time was about 10 hours.	The same as (4)
(6) Defrosted in 10 per cent. formalin.	Holes are seen between the fibres but not so many in the fibres themselves.

The two large pieces of the cake were each cut in two and the resulting large pieces thawed in air, in water by direct immersion, and by immersion in brine. The thawing times are of course proportionately longer than those of the small pieces used in the first part of this experiment. Sections were cut before and after thawing and showed the following:-

Treatment of fish	Appearance of sections
(7) In the frozen state.	See (1). The appearance is worse than of cod No.6, Experiment 5.
(8) Defrosted in air. Thawing time about 5 hours	The cell substance has swollen somewhat from (7) but the cells are still abnormally dense and distorted. They are now perforated by many small holes (swe below).
(9) Defrosted in water. Thawing time about 1 hour.	The same as (8).
(10) Defrosted in saturated brine. Thawing time about 1 hour.	The cells have swollen more than (8) but are still dense and distorted. The small perforations noted in (8) are now larger and more distinct.

As will be shown later (Experiment 6) fresh fish, if frozen unusually slowly by carbon dioxide, will show the presence of small holes such as seen in (8). We had no reason to believe that the small perforations of (8) were due to this cause, but the possibility had to be considered. However as this phenomenon does not occur when brined fish is frozen in the same way, the finding of these perforations in (10) constitutes strong evidence that their appearance in (8) is significant and not merely an experimental artefact.

From these results it would seem that the degree of recovery of the normal appearance on defrosting is the same whether the defrosting is in the air, or in water or fast or slow.

It was then thought of interest to examine more closely the different effects on the tissue in freezing in different ways. It was also thought necessary to see whether different types of fish showed the same changes on freezing. Consequently a cod and a hake were frozen in air, in brine at 0°F. and in a calcium chloride-ice mixture at -13°F. and sectioned in the frozen state. These fish were stored at 5 to 8°F. and again sectioned after 24, 48 and 72 hours, both in the frozen state and after thawing in air. A piece of beef was also run through at the same time in parallel with these fish in order to compare its picture with that of the fish. The results are given below in Experiment 5.

Experiment 5

A cod and a hake of about the same size were obtained from the National Fish Company. They were filleted and one fillet from each was air frozen at from 0 to 5°F. The other fillet from each was cut in pieces and packed in the small cans or boats used in all the small scale freezing experiments. A piece of beef was also packed into a can, and these three cans immersed in the brine for one hour. Pieces of each fish and of the beef were taken to the laboratory and microscopic sections cut in order to get the normal picture of these fish.

Data from Freezing Operation.

Time	Temperature of $CaCl_2$ brine	Temperature of NaCl brine	Temperature of fish (cod)
11.00 a.m.	1.5°F.	3°F.	40°F.
11.15	1.5	2.5	30.0
11.30	1.2	1.8	29.0
11.45	1.0	1.5	26.0
12.00 noon	0.8	1.0	8.0

Thus it would appear as though the fish had frozen completely in 45 minutes.

After the hour pieces of the flesh were dug out of the cans and taken to the laboratory for examination. We had found that if frozen fish was well wrapped in paper and cotton wool they showed no signs of softening at all when arriving at the laboratory. In fact one piece, weighing a couple of ounces, was left in cotton and found still to be hard after eight hours. These pieces were sectioned in the frozen state and examined. After being allowed to thaw in air they were again sectioned and examined. The remainder of the cans of flesh in the freezer were kept at an air temperature of 5 to 8°F. for 24 hours and 72 hours, an examination being made after each period. The fish frozen in air probably froze in about 6 hours but was not examined until next day and was labelled "twenty-four hours after freezing". It was examined both in the frozen and in the thawed state, as in the case of the brine frozen fish. The cod and hake were in addition frozen in a calcium chloride-ice mixture at -13°F., the fish were found to be hard throughout in less than half an hour. After one-half hour exposure to this temperature the pieces were examined in both the frozen and thawed condition.

Cod

Fresh (1) The cells are marked by minute dots and so present a granular or stippled appearance. They are invariably rounded in outline (in cross section) and lie well together, with their walls undistorted. Seeplate III.

Frozen in Sodium Chloride Brine

Immediately after freezing.

(2) Frozen State. The cell material seems denser than normal, spaces exist between cells and many large holes appear in the section. These holes are usually eccentrically placed and indeed may break through the cell wall. The general appearance is such as to indicate that some fluid has been withdrawn from the cell substance to these cavities, thus pushing aside the surrounding protoplasm and giving the tissues a denser, condensed or more concentrated appearance. See plate IV.

(3) Thawed. The section shows a great change from the last. Most of the gaps have closed up and the tissue presents a much less dense appearance than the last. However the section is not like that of the normal tissue, because quite a few gaps and distorted cells still remain.

Twenty-four hours after freezing.

(4) Frozen state. The appearance is very like that of the material examined immediately after freezing - (2). The impression is gained that the distortion and condensation of the cells is more marked than at first, however the difference is only slight.

(5) Thawed. The cells have swollen but many gaps still remain and the recovery is not so complete as it was in the case of the piece thawed immediately after freezing.

Seventy-two hours after freezing.

(6) Frozen State. The appearance again is not greatly dissimilar to that of (2) or (4). Now however the impression gained in (4) has become distinct. The cells are definitely more condensed and the cavities correspondingly larger.

(7) Thawed. The cells swell somewhat as in (5), but they remain much denser than normal and many cavities are still present. Recovery from (6) is not great.

Frozen in calcium chloride brine.

Immediately after freezing.

(2a) Frozen State. The appearance of this section is quite different from that of the sodium chloride brine frozen fish (2). The cavities or holes are smaller and more numerous than in (2), appearing as definite perforations through the section. The cell substance has been made considerably denser but not as much as (2) and the outlines of the cells are not as greatly distorted as in (2). In other words many more but smaller holes See plate V.

(3a) Thawed. The recovery here is almost complete. There are fewer abnormal cells than found in (3) even, though some are present. Some parts of the section appear quite normal.

Frozen in air.

Twenty-four hours after freezing.

(4a) Frozen State. (This is twenty-four hours after the fish were put in the cold air. The fish have probably been completely frozen for sixteen hours or more. They are grouped with the twenty-four hour frozen fish because they have been subjected to the freezing temperature for that length of time.) The appearance is quite like that of the brine frozen twenty-four hour fish (4). The distortion of the cells however is definitely more pronounced, the cavities larger and the cell substance more concentrated.

(5b) Thawed. The cells have swollen somewhat, being less concentrated than (4b), but they are still considerably distorted and many gaps remain. Recovery is not so great as that of (5).

Seventy-two hours after freezing.

(6b) Frozen state. The appearance is very much like that of (6) or of (4b). There does not seem to have been much change since (4b).

(7b). Thawed. Concentration of tissue, distortion and cavitation is greater than (5b) and even greater than (7). Recovery has been slight, less even than the case of (7) the seventy-two hour brine frozen fish.

Hake

Some difficulty was experienced in obtaining good sections consistently from the hake. The same method of sectioning was used as for the cod and beef, as it was not possible in the short time at our disposal to work out a more satisfactory one. A few seconds after floating the sections off the knife into water they "fluffed" up. The sections on being separated one from another in the water lay flat and smooth at first as did the cod sections, but suddenly they lost their even appearance, became hazy in outline, and crinkled up. "Fluffy" describes their appearance very well. It was difficult to work fast enough to be able to mount a section on a slide before this phenomenon took place. When once it did take place the cells of the section seemed to lie in no regular order, they were piled one on top of another and were therefore difficult to examine. However we think that the following descriptions are accurate, though in some cases many sections had to be cut in order to obtain one satisfactory one. No explanation of the phenomenon is suggested, though it is to be noted that all the sections, whether from fresh or from frozen hake, showed it. It must be remembered however that even the fresh fish was frozen on the microtome before cutting so that the possibility of its having been due to freezing cannot be ruled out. The freezing of the piece of fish on the microtome did not occupy more than 5 or 10 seconds.

Fresh

(1) The cells are larger than those of the cod and are similarly, though not so markedly granular or stippled. They lie close together both in cross and longitudinal section. When the sections have been "fluffed" the cells themselves may seem to take on a different appearance. In cross section the central parts may be lightly stained or thin, while surrounding this part is a broad darker band. This appearance was not always seen and it is not known whether it was an actual change of state or a technical artefact.

Frozen in sodium chloride brine.

Immediately after freezing.

(2) Frozen state. The appearance is like that of the cod (2) - treated in the same way. The cells may have shrunk more leaving wider spaces between them but they are not so distorted.

(3) Thawed. Recovery seems to be practically complete. Possibly the cells are not lying as close together as in the fresh (1), and they may be somewhat denser but this is uncertain.

Twenty-four hours after freezing.

(4) Frozen state. Appearance as in (2).

(5) Thawed. Recovery is marked but is not quite complete. Some cells are still distorted and are slightly denser and more separated than (3).

Seventy-two hours after freezing.

(6) Frozen state. Appearance as in (2) or (4).

(7) Thawed. Recovery is considerable. Some fluffing is present which makes examination difficult. Cells are distinctly not as shrunken and dense as (6) but distortion is still present.

Frozen in calcium chloride brine.

Immediately after freezing.

(2a) Frozen state. On close examination the central parts (in cross section) of the cells seemed paler and less dense than the periphery. Some cracks and crevasses were seen in the middle of the cells, although their outline and arrangement was normal.

(3a). Thawed. Recovery seemed complete. No difference could be seen between these cells and those of (1).

Frozen in air.

Twenty-four hours after freezing.

(4b). Frozen state. Appearance is not unlike that of the brine frozen hake (2) or (4). However there is more distortion of the cells.

(5b) Thawed. As (5).

Seventy-two hours after freezing

(6b). Frozen state. The cells are dense, distorted and widely separated from one another. The changes from (4b) are great and the density and distortion is much more marked than that of the brine frozen hake (2,4,6).

(7b). Thawed. The cells have swollen somewhat from (6b) but are still definitely denser than normal, and are considerably distorted and separated. There is some recovery from (6b) but it is not nearly as great as that shown by (7).

Beef

Fresh

(1). The muscle fibres are much smaller than in the fish. They are not so closely rounded in outline, being somewhat angular, but lie close together and show no perforations.

Frozen in sodium chloride brine.

Immediately after freezing.

(2). Frozen state. Many gaps have appeared between the cells, giving the section a somewhat honey-combed appearance. The cells themselves in the section do not seem to be perforated or distorted.

(3). Thawed. Appearance as in (1).

Twenty-four hours after freezing.

(4). Frozen state. As in (2).

(5). Thawed. Recovery complete as in (1) and (2).

Seventy-two hours after freezing.

(6). Frozen state. As in (2) or (4).

(7). Thawed. Recovery complete as in (1,3,5).

From the above data it is seen that the appearance of the hake and the cod is markedly changed by freezing in air at 0-5°F., in brine at 1°F. or in brine at -13°F. The difference between the effects of the air freezing and brine freezing at 1°F. is not great, not nearly as great as we had expected, but it is definitely in favour of the brine freezing. The sections of the fish frozen at -13°F. are quite different in appearance to the air frozen. The cells being perforated by many more but much smaller holes, their substance not so dense, and their outline not so distorted. On thawing these fish they regain, but not entirely, the appearance of fresh fish.

When these fish have been stored in air at 5-8°F. for three days and are then thawed, the air frozen fish do not regain the appearance of normal fish as well as do the brine frozen fish, and neither recover on thawing nearly as completely as they did at first. Thus it appears that the changes which take place during freezing tend to become more permanent as the fish are stored. This tendency is much more pronounced in the case of the hake than the cod. The appearance of the fish frozen by Finn (Expt. 4), eight months previous to our examination, frozen in brine at 0°F., also tends to confirm this statement. Their appearance is worse both in the frozen and thawed state than are our seventy-two hour cod. The fact that it is not much worse may indicate that this alteration in the permanence of the change takes place largely during the first few days storage, but of course as these are not the same fish used in experiment 5, not much weight can be put on small differences in appearance.

Experiment 6.

The results of the last experiment suggested to us the advisibility of obtaining if possible the picture of fish frozen at varying rates. To this end an attempt was made to vary the rate at which the small blocks of fish were frozen on the microtome. These blocks of fish- measuring perhaps 5 mm. long by 3 mm. broad by 2 mm. thick, or smaller - were placed on the microtome and the carbon dioxide freezing gas directed onto them at different rates. In the case of those taking some minutes to freeze it was not easy to tell when they were frozen but it may be said with considerable confidence that they were frozen somewhere within the limits given and for this preliminary experiment this was considered satisfactory. The fish used was a fresh haddock.

Time of freezing	Appearance of section
(1,2). 5 and 10 seconds. The fastest rate easily obtained. This was about the range obtained in the freezing of our routine specimens	Sections looked exactly like the pictures of normal cod shown in plate III, which of course was also obtained in this same way.
(3). 15 seconds	Similar to the above except that the stipple seems to be more marked. In a few of the cells the stipple presents a pin-hole appearance and in others some of the cell material seems to have concentrated towards the periphery.

(4). 20 seconds	The stipple definitely consists of pin-holes.
(5). 30 seconds	The pin-holes have increased in size, pushing the cell material aside, giving it a ragged and more dense appearance.
(6). 45-60 seconds	Most of the cells are as (5) but in some of them the holes have become larger and fewer.
(7). 60-90 seconds	As (6) but more of the cells show the larger, fewer holes.
(8). 1.5-2 minutes	Nearly all cells now show the condition of (7). There are still several holes to a cell and the cell material is markedly denser.
(9). 2-3 minutes.	The cell material is still denser and the holes more confluent, still larger and fewer.
(10). 3-5 minutes	Some of the cells are distorted in outline: the protoplasm is still denser: there are only a few quite large holes in each cell: The general appearance is very similar to that shown in Plate V.
(11). 5-8 minutes	More of the cells are distorted: more of the holes are confluent, there being now only one or two holes to each cell. The protoplasm is still more concentrated. The appearance approaches fairly closely to that of Plate IV.

From the above results it is seen that the more rapidly the fish is frozen, the fewer are the changes in the structural appearance. Distinct changes in the appearance were noted in the pieces frozen in 15-20 seconds, while those pieces frozen in 5-8 minutes took on an appearance very similar to that of a fillet frozen at 1°F. (in brine) and taking about three-quarters of an hour to freeze. It must be remembered that our normal sections are from pieces of fish which have been frozen in 5 or 10 seconds and that they too may have changed somewhat.

Possibly the stippled appearance of these cells may be due to changes which have taken place even at this rapid rate of freezing. We have had no opportunity to prepare sections of fresh fish by the paraffin imbedding method, but even so the fixing solutions used (such as formalin, alcohol, etc.) would alter the appearance sufficiently to make a close comparison unfair.

It would seem on first thought to be impossible to conceive of a commercial brine freezing process being developed which would freeze a fish in 5-10 seconds. Unless this is done then these results would indicate that some degree of change in the tissues will take place. This is not the case, however, as we shall endeavour to show.

In this experiment the piece of fish used was so small as to freeze practically as a whole. That is, all parts of it froze at the same instant or nearly so. If a large fish is being frozen it will not freeze all at once: the outside will harden before the inside. Let us suppose that a cubic millimetre portion of this fish on the surface is frozen five seconds after putting it in the freezer. A c.mm. portion one-half way in may not freeze until a half hour afterward but it may do all its solidifying within five seconds. Similarly a c.mm. portion in the centre of the fish may still be soft after one hour, and yet five seconds later it may be frozen. Thus while this hypothetical fish took over an hour to freeze, no one portion of it took more than five seconds to freeze, and it is much more conceivable that the refrigerating conditions be made good enough to achieve this result. (Incidentally this conception has also been developed by the Australian workers[4] who use a term "The latent period of freezing" to express it. This is discussed later in this report when their paper is under consideration).

Thus we conclude that the rapidity of freezing of a unit volume of fish is the important factor. This is entirely dependent on the rapidity of removal of heat from the fish which in turn is controlled chiefly by the:

(1). Thermal conductivity of the frozen fish flesh.
(2). Surface of fish exposed to cooling medium.
(3). Temperature of the cooling medium.
(4). Rate of circulation of the cooling medium.

This thermal conductivity is a constant, and until it has been determined it is impossible to know whether any practical combination of factors 3 and 4 can be devised which will remove heat fast enough to freeze fish without changing its structure.

The Difference Between Fresh Fish and Fish which Have Been Frozen

From the above work it is noted that during the freezing of fish changes occur in the microscopic appearance of the fish and on thawing the original appearance is to a large extent regained.

When the macroscopic appearance of the fresh fish is compared with that of thawed fish certain differences are noted. The dissimilarity is intangible but most fish dealers claim that it is a simple matter to differentiate between the two. From our observations we would say that the following are the most obvious differences:

1. The flesh of the fresh fish is soft and yielding and more plastic than is the thawed flesh; this by comparison is less yielding, less plastic and more resilient to slight pressure.
2. On being left in air the surface of the frozen sample seems to dry out and become hard more rapidly than that of the fresh.
3. When the frozen sample is pressed the liquid is extruded and the myomeres tend to separate, while with the fresh sample the flakes of the fish remain adherent and little if any fluid comes out.
4. On allowing the frozen specimen to stand fluid (often of an opaque milky character) exudes of its own accord.

These macroscopic differences enumerated above are not great, Are the microscopic differences sufficient to account for them? In a close comparison of the photomicrographs of fresh and thawed specimens, one sees that quite a considerable proportion of the cells of the latter are distorted and contain cavities. These cavities probably represent fluid which has not been reabsorbed and they give a somewhat spongy appearance to the flesh. Hence on pressing the material this non-absorbed fluid would readily be squeezed out. The shape of the cells would render the material resilient.

The fluid that exudes out of the thawed specimen is frequently of a milky character, obviously containing a considerable solid element. The following conception may help to explain this. During the freezing process crystals of ice form removing from the cell protoplasm a large proportion of its water content (see plate IV). These ice crystals are chiefly composed of water but they may contain small amounts of salts, protein material and such substances which have been forcibly squeezed from the cell by freezing. Now, however, on defrosting a large amount of the fluid is reabsorbed back into the cells.

Probably most of this reabsorbed fluid is pure water and that a lesser proportion of its solid contents are absorbed. This would result in a concentrating of the solid elements in the fluid remaining extra-cellular to such an extent that it would be of a milky character. Then too this infers that the recovery on defrosting as seen by comparing Plates IV and VI, is due to the reabsorption of water and not to the reabsorption of all the lost constituents. It would be reasonable to expect this water to be more readily lost now by evaporation than before.

That there is a definite pressure in the cells on freezing is pretty certain. We have repeatedly observed an increase in volume of a block of fish during freezing of about one-eighth its volume. This occurs in each cell, a core of crystals of ice forming within the cell, increasing its total volume, During normal contractions the muscle cell becomes shorter and fatter, its total volume remaining unchagged. In this case, however, its volume is forcibly increased and the squeezing out of part of its substance seems reasonable.

Hence though special histological technique has not been utilized to especially demonstrate the presence and shape of all the tissue components, such as connective tissue, blood vessels, etc., the changes which we have observed can account for the macroscopic differences between fresh and thawed fish.

The method is simple and it may be of value to be able to judge the degree of alteration in freezing by looking for such positive changes as deformed, cavitated cells rather than to try to figure out some grading scheme based on the intangible physical differences of macroscopic characters.

Discussion of the Literature

After completing the above description of our own experimental work we have had access to copies of papers dealing with a similar type of work. The following section consists of a discussion of these papers as applicable to our experiments.

A resume of the investigation of Reuter, Plank and Ehrenbaum (1) by Nuttall and Gardner (2) records results identical with our own and gives similar pictures of the changes. The following points are, however, of particular interest.
1. They state that the perforations ~~chrocytic~~ such as we have described are in reality columns or cores of ice or fluid which run longitudinally throughout the whole length of the fibre. As far as our pictures are concerned we would not be able to tell that this is the case. They might just as well be short crystals of irregular size and shape. Serial sections would have to be made in order to prove this point.

2. The following conclusion also agrees exactly with our findings and is worth quoting here. "The conclusion from the above operations is that in very rapid freezing the water of the muscle albumen freezes in an invisible molecular state. In less rapid freezing a number of small columns are formed of fluid in each muscle fibre, if time allows, these freezing 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." They also pictured muscle frozen at 20°F. (that is much more slowly than any we have examined). In this case the fluid congeals entirely outside the muscle fibres. The muscle fibres themselves form compressed bands or separate bunches of sharp edged little columns, while irregular spaces appear in the connecting tissue into which the fluid has exuded. There are no cavities within the fibres, yet the protoplasm has been even more greatly condensed than in a fish such as that shown in Plate III where large cavities are still present.
3. With regard to recovery of the tissues to their original state during thawing, they state "Experiments show that the muscle albumen does not reabsorb its fluid unless the duration of the frozen period has been very short. In the latter case there is a slight recovery after thawing, but muscle albumen does not give off and reabsorb water appreciably as do such colloids as glue and gelatine." We have observed a much greater degree of recovery than this quotation would seem to indicate. However we have noted that the longer the duration of the frozen state the less complete is the recovery on thawing. These authors state too." The speed of thawing has no influence on the recovery of the tissue from the effects of freezing." This observation is not in agreement with that of the Dutch report (3). Our findings support the view of Reuter.

Through the kindness of Dr. Wasteneys of the University of Toronto we were able to consult two papers which were published in the Australian Journal of Experimental Biology and Medical Science (4 and 5). These papers it is true deal with the refrigeration of meat but it seems to us that it would be wise to correlate the freezing process in fish muscle with that in meat so that use can be made of the researches of meat as an aid to solving the problem of freezing fish muscle.

The Australian workers endeavoured to develop "such methods of treatment that the beef underwent no changes during the freezing process which could not be reversed during thawing". In the course of this investigation they made use of the term "latent period" of freezing or thawing, which is a "measure of the rapidity with which freezing or thawing takes place, irrespective of the thickness of the meat or the conditions of freezing and thawing employed."

The "latent period" is defined as "the period of time corresponding to the distinct flattening of the freezing or thawing curves at the time when a very considerable portion of the water content of the meat solidified." As this water which solidifies plays an important part in the reversibility of the process, the term is a useful one. Another term which they use which needs explanation is "drip". This is the clear reddish coloured fluid which is exuded from all cut surfaces of beef which has been frozen slowly and thawed. The drip contains about 9 per cent. protein and shows by its presence that the cycle of changes of phases of the beef from liquid to solid and back to liquid produces permanent alterations in the tissue structure whereby water and protein are liberated.

These workers observed that "Increasing the latent period of freezing increased the changes in the beef as determined by the quantity of drip, the net nitrogen loss on thawing, and by the microscopic appearance in the frozen condition."

"The degree of reconstruction of the colloid material was increased by increasing the latent period of thawing, since the quantity of drip was reduced when the meat was thawed slowly. It could also be reduced by storage at 1°C. after thawing, showing that the imbibition of liquid by the colloid still went on after thawing was complete".

It is doubtful if results obtained in meat refrigeration can be directly applied to the freezing of fish.

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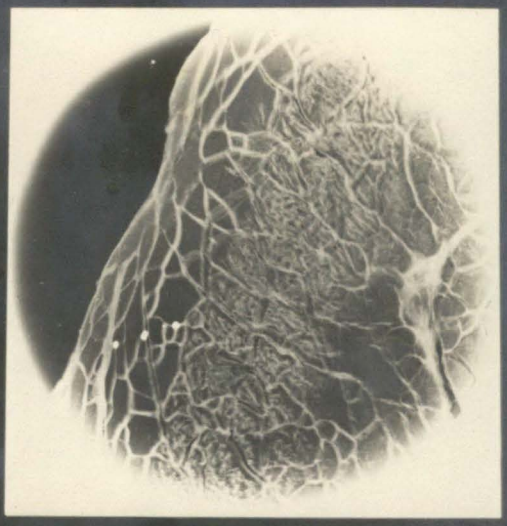
Explanation of Plate

1. Section through surface of smoked frozen haddock, showing the subsurface macerated zone.
2. Same, higher magnification.
3. Section of fresh cod.
4. Section of brine frozen cod, in the frozen state.
5. Section of cod frozen in calcium chloride brine, in frozen state.
6. Section of brine frozen cod of (4), after thawing.

1



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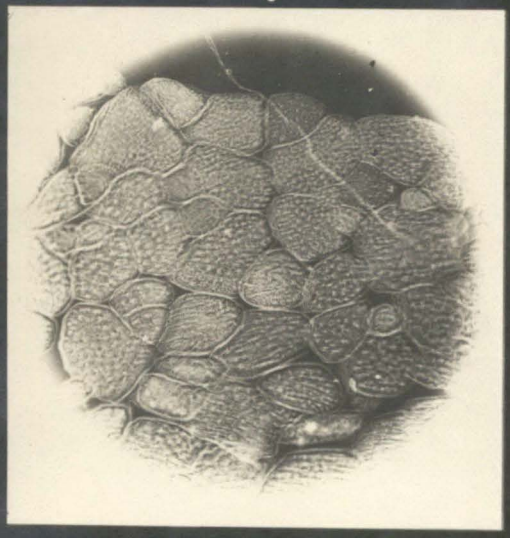

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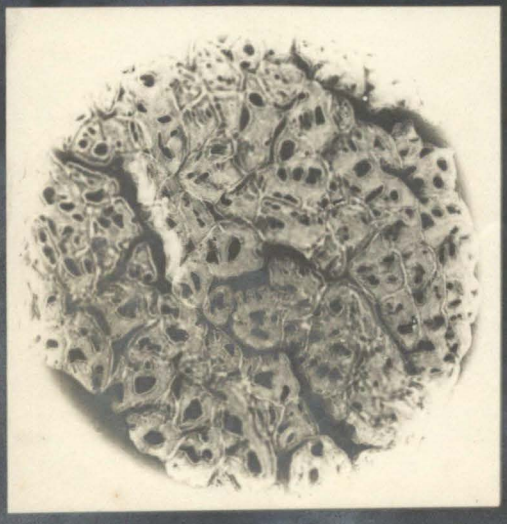

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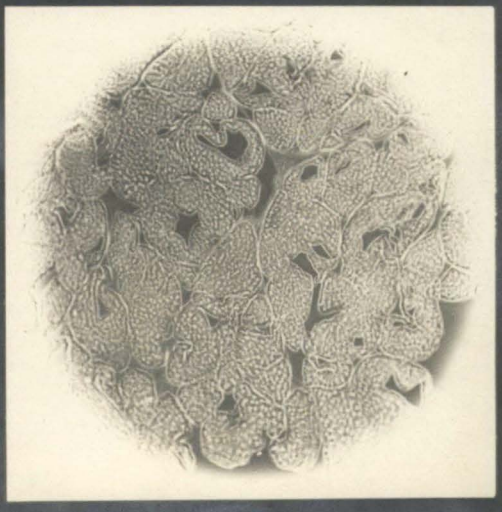

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