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Structural Changes in Fish During Freezing and Storage

By C.B. Weld, M.A.

It has been known for years that many of the undesirable changes which render frozen fish and meats unpopular on the market are due to the formation of ice crystals in the flesh.

Workers such as Reuter (1); Molisch (1); Plank and Ehrenbaum (1); Kuhne (1); Moran (2); Cook, Love, Vickery and Young (4); Ogura and Fugikawa (3); Callow (2); Jackson (5); and many other workers have proven this to be without doubt.

Most of the work done heretofore however has been rather qualitative in nature. This paper is to present an attempt to apply quantitative methods to the microscopic investigation of the frozen material and so to record precisely the changes which take place under known conditions of freezing and storage.

All the work reported in this paper has been done with Cod, except where definitely stated to the contrary.

The problem has been grouped under the following headings;

(1) Introduction and technique

(2) Thickness of the fish

(3) Freshness of the fish

(4) Rate of freezing

(5) Duration and conditions of storage

(6) Method and rate of defrosting

(7) Kind of fish

(8) Eating - the final test.

(9) General Summary and bibliography

(10) Appendix

(a) MacFadyen's work on "Drip" and expressible fluid, rate of freezing, storage, discussion

(b) Some theoretical aspects

The Histological Method

Material may be prepared for histological examination in one of three common methods.

(1) Embedding in paraffin
(2) " " celloidin
(3) Freezing

In the first two methods the material is fixed and hardened in some suitable solution, and then embedded and infiltrated with the substance named. The sections on thin slices are then cut with a microtome, and mounted on slides. They are rather tedious and elaborate processes and so the freezing method which is much faster even if less delicate was adopted.

In preparing slides of fresh fish, small pieces of fish are put on to the microtome block, a stream of CO_2 is directed onto it from above from a CO_2 tank, which freezes the portion of fish almost instantaneously. The thin sections are then cut from this frozen fish and floated from the knife into water which is slightly brackish. These sections are then floated onto a slide and allowed to dry, and then stained for a few minutes with dilute eosin (aequous). The slides (with the sections now attached to them) are now placed in 50% alcohol for 5 or 10 minutes, and then in two changes of 95% alcohol for 5 or 10 minutes each. A few drops of Oil of cloves is now placed on the section for 5 minutes or more, and after this is blotted off the section is covered with a solution of Canada Balsam in benzol, a coverslip applied, and the section is thus permanently mounted and ready for examination.

When fresh fish is prepared in this way the muscle fibres are evenly and smoothly stained and they lie close together - leaving no obvious gaps or holes between them. The cross striations are often visible when the fibres are cut longtitudinally and so too are longtitudinal striations, denoting the presence of the fibrils. In cross section the fibres present a stippled appearance, due to the cutting across of the bundle of fibrils. No nuclei are seen.

Between the fibres are thin strands of connective tissue which are rarely visible unless the fibres have been somewhat separated, either by freezing or during the manipulations.

A pinch of salt is added to the water into which the section is floated from the knife because, unless it is done, an occasional section will "fluff up." That is, it swells up and crinkles in all directions, looking quite fluffy, and is quite useless as a histological preparation.

Frozen fish prepared in the same way present an appearance varying with their previous treatment. Reuter (1), as recorded by Stiles (1), fixed frozen fish in 4% formalin, and embedded in celloidin, and noted the following points which agree exactly with those observed by us, using the freezing method. Reuter's work was with cod and Haddock while ours has been almost entirely with cod. He reports that, (1) Pieces of fish frozen almost instantaneously in liquid carbon dioxide show a structure identical to that of the fresh fish. (2) Fish frozen in brine at -14°C. show cavities within the muscle cells, which may be one or multiple, small or large, and the sarcolemma may be broken, especially by large irregular crystals. (3) In air frozen fish the cavities in the sarcolemma are not seen, but the muscle fibres are pressed together in large aggregates which are separated by irregular shaped spaces occupying the position of the connective tissue, and which contain large ice crystals.

One point which may be very confusing to the reader is a failure to separate properly (a) the freezing method of preparing sections and (b) frozen fish. All the fish has to be frozen before it is examined. Our standard however, which of course is fresh fish, is frozen only on the microtome block and this is done almost instantaneously by CO_2. As far as we know the appearance of this fish is exactly what it would be if the sections were prepared by another method. In any event it is our standard of comparison. We have three types of sections to deal with;- (1) Fresh: never frozen in the gross, but frozen on the microtome block, and sections thawed on the knife immediately after cutting. (2) Frozen: by which we mean frozen hard in the gross, and still hard when preparations are to be made. Care is taken, by working rapidly and in a cooled place, to fix the piece of fish on the microtome block before softening has occurred. The piece is now kept hard by CO_2 and sections are cut, and these sections of course soften at once

on the knife. (3) Thawed; by which we mean fish which has been frozen in the gross (in brine, or air, or some such method) and allowed to thaw in the gross. Then a piece of this thawed fish has been mounted on the microtome block and treated as though it were fresh fish. (That is, frozen by CO_2, and sections cut, which then again thaw on the knife).

It is important to understand clearly what actually is done when these fish are examined "fresh", "frozen", and "thawed", because the differences in appearance are striking and yet, in reality, they are defrosted in every case. For instance, suppose a mass of fish is frozen- by some commercial method- and examined "frozen". One would find it to be a mass of ice crystals. On the other hand, if it was examined "thawed" it might be almost identical in appearance to that of the fresh fish

It would seem logical to first investigate the effects of varying the "rates of freezing" on the microscopic structure of the fish. However, as preliminary studies of this variable had been done (Weld and McKay (6), Reuter (1), Tamman (2), Taylor (3) Stiles (1), Jackson(5), and as much more would of necessity have to be done in considering the effect of storage, it was decided first to examine the relationship of the thickness of the fish and the structural changes during freezing.

The Thickness of the Fish

It is obvious that the larger the piece of fish, the longer it will take to freeze. This does not necessarily mean that the rate of freezing differs in the two cases however. If one examines a fish while it is freezing, one sees quite a sharp division between the frozen and still soft material. It is at this zone where the freezing is taking place and where the ice crystals are forming. In a big fish it may take two hours say for this zone to reach the centre of the fish, and perhaps only one half an hour if the fish is small; and yet the zone may be moving inward at the same rate in each case. In other words, even though the fish took four times as long as the small one to freeze, each minute cell or portion of the fish may have taken the same time to become solidified.

This conception may be true when we consider that the specific heat of the outer layer of frozen fish is less and the conductivity greater than of the inner still unfrozen portion. On the other hand one is inclined to doubt its truth when one considers (Stiles (1)) that in air freezing the time taken for the mass to freeze is roughly proportional to its thickness, while in brine freezing it is roughly proportional to the square of its thickness. Thus, a mass of fish 1 cm. thick freezes 30 times as fast in brine

(-21°C.) as in air (-10°C.), while fish 5.5 cm. thick freeze 20 times as fast in brine as air, fish 10 cm. thick about 10 times as fast, and large masses like hindquarters of beef only 2 to 4 times as fast. This finding of the Australian workers (2 and 4), marks the end of the attempt to apply commercially the quick freezing theory to eliminate drip completely from such a large mass as a hindquarter of beef.

That the rate of freezing does become slower as the depths of the fish are reached is well shown by obtaining temperature cooling curves at different depths during the cooling process. Such curves show a much greater flattening over the freezing range when taken from the depths than they do when taken from the surface of the fish. Such curves are shown in Stiles (1), and have been confirmed in this work as will be shown later.

However such curves after all only show the changes of temperature in the fish and though one can deduce much about what is happening, it was thought wise to actually see whether or not the microscopic examination of the frozen tissues would show the expected changes.

To this end, several masses of cod were frozen and specimens taken from the outside and inside of the masses examined. It was found necessary of course to work out some way of giving some quantitative value to the changes in the tissue and it was finally decided to determine the average diameter of the crystals in the tissue. What actually was done was to measure - using a ocular scale on the microscope calibrated in microns by means of an object micrometer - the width of the spaces in the sections where the crystals had been. A dozen or twenty of what seemed to be the most typical crystal holes of each section were measured and averaged.

At first an attempt was made to calculate the proportion of crystal hole area to tissue area in the section but this was soon abandoned as there was often a shrinkage of tissue during the manipulations which interfered greatly with such determinations and which did not greatly affect the mere size of the crystal holes measured. In an experiment reported later (Exp. III A) there was an variation of 10% (5% each way) in five supposedly identical preparations, and it is probable that a possible error of 10% either way in any result given will have to be borne in mind.

Experiment I

Masses of cod fish muscle, roughly spherical in shape, from three to five inches in diameter, were wrapped in waxed paper and frozen in different ways, as noted below. After the freezing, specimens of the frozen material were obtained from the outside or surface portions of the masses, and from the inner or central portions. The masses of fish were then placed in storage in the "Cold Box" and examined from time to time later. The "Cold Box" is a galvanized iron rectangular box kept surrounded by a salt and ice mixture. The temperature of the air within it was usually at about -5 or -6°C. but ranged from about -2°C. to -10°C.

Table I

Means of Freezing	Size of Ice Crystals in Microns		Time of Storage
	Outside	Inside	
A. In brine, at -1°F for 2 hours	60	67	
B. In an Ice and Salt mixture for 6 hours	120	140	
C. Brine at 2°F for 2 hours	85	90	
D. In air, in the cold box, at about -8 to -10°C. for 2 days	285	430	
E. Brine at 15°F. for 3 hours	70 135	125 170	(Storage for 24 hrs)
F. In a $CaCl_2$-Ice mixture for 3 hours	90 110	115 150	(Storage for 24 hrs)
G. In a NaCl-Ice mixture for 3 hours	120 145	120 200	(Storage for 24 hrs)
H. Brine at -1°F for 3 hours	110 105	100 140	(Storage for 24 hrs)

From these figures it is seen that in practically every instance, the crystal growth has been greater in the centre of the mass (by an average of 20 percent. of all eight cases) than at the surface. In the two cases in which it is not so the measurements are so close together as to be within the limits of experimental error; and these two cases when again examined after 24 hours of storage at -6°C. give results which fall in line with the rest.

In none of these determinations was it possible to determine accurately just how deep in the tissue that the sections were obtained from; and the waxed paper bags were not always effective in keeping out the brine, besides being easily torn and hard to handle. In order to obtain more precise, and better controlled results, the following experiments were done.

Experiment 1 (I)

A roughly cylindrical mass of fish measuring 30 cm. X 12 cm. X 9 cm. was wrapped in heavily waxed parchment paper. This was surrounded by a one-half inch layer of dry absorbent cotton which was itself wrapped in waxed paper. This cotton surrounded the sides of this cylinder but covered neither end. Then the whole was wrapped in heavy wrapping paper and this was liberally painted with melted paraffin. Hence we have a cylinder of fish whose sides are covered with four thicknesses of waterproof paper and a half inch layer of cotton, but whose end is covered only by a double layer of waxed paper.

This was immersed to a depth of about 20 cm. in brine at 2.5°F. and left there for 20 hours, at which time the brine was 16°F. The parcel was then removed and unwrapped (the cotton was found to be quite dry) and the fish mass split longtitudinally. Specimens taken from different zones show the following crystal measurements.

Zone	Crystal Measurement
(a) 0-1 cm. from end	100 microns
(b) 5-6 " " "	215 "
(c) 12-13 " " "	360 "

When one considers that the size of crystals found in freshly air frozen fish (as will be shown in later experiments, eg. Exp. III and already shown in (D) of this experiment) is of the same order of magnitude as this last figure, this result becomes very significant. When this mass was examined in the gross it was readily apparent that the grain or texture of the material, was very much finer at the end than 5 or 6 cm. in, while at 12 or 13 cm. of depth the mass showed very large and coarse ice crystals.

Experiment 1 (J)

An old jam tin, cylindrical, measuring 5" in diameter and 4" deep was used in this experiment. Its bottom was insulated with a ½" layer of cork board and so all the cooling of the contained fish was through the walls of the tin. Three thermometers were embedded to a depth of 2" in the contained fish (which filled the tin) and the temperature cooling curves recorded. The thermometers were protected by being enclosed in loose fitting glass tubes containing a drop or two of mercury. The tin was frozen in rapidly circulating brine at -1°F., and after freezing was complete the mass was split (by sawing in two) and specimens taken for sectioning from areas adjacent to the thermometer bulbs.

Table II. Temperature record

Time elapsed	Temperatures at different positions		
	1" from tin wall	1¾" from wall	2½" from wall
0 minutes	68°F.	68°F.	68°F.
20 "	33	59	63
27. "	27.5	57.5	61
40 "	22.5	51.5	56.5
55 "	18	45	51
1 Hr. 10 min.	14.2	38.5	43.5
1 " 25 "	11.5	34.5	38.5
1 " 40 "	9.5	31.5	34
1 " 55 "	8	30	32.5
2 " 25 "	6	24.5	30.5
2 " 40 "	4.7	21.5	30.5
3 " 20 "	2	12.5	24.5
3 " 35 "	0.5	8.5	14.5

These results are incorporated in the graphs shown in Chart I.

The crystals in the sections, cut "frozen",

1" from tin are 85 microns in size
1½" " " " 115 " " "
2½" " " " 155 " " "

When sections are cut "thawed", no crystals at all are visible.

Experiment 1 (K)

This is a duplicate of (J), except that the tin was frozen in brine at 15°F.

Table III. Temperature record

Time elapsed	Temperatures at different positions		
	½" from tin	1½" from tin	2½" from tin
6 minutes	62°F.	61.5°F.	61.0°F.
2 "	61	61.5	61
3 "	60.5	61.5	61
4 "	60.0	61.5	61
5 "	58.5	61.5	61
6 "	57	61.5	61
7 "	56	61.5	61
8 "	55	61.3	61
10 "	52.7	61.2	61
15 "	48	60.2	61
30 "	40	55	60
45 "	35.5	50	57
1 hr. 0 min.	32	45	52.5
1 " 20 "	28	39.5	45
1 " 35 "	27	37.5	42
1 " 45 "	~~25~~ 26.5	~~36~~	40
2 " 0 "	25.5	34	37
2 " 15 "	24.7	32.7	35
2 " 30 "	23.7	31.7	33.5
2 " 45 "	23	31	32.2
3 " 0 "	22.7	30.7	32
3 " 15 "	22	30	31.5
3 " 30 "	22	29.7	31
3 " 45 "	21.5	29	31

Table III. continued

Time elapsed	Temperature at different positions		
	½" from tin	1½" from tin	2½" from tin
4 hrs. 0 min.	21.2	28.7	30.7
4 " 30 "	21	27.5	30.7
4 " 45 "	20.5	27.2	30.2
5 " 0 "	20	26.5	30.2
5 " 15 "	19.5	26	30.2
5 " 45 "	19	25	30
6 " 40 "	18	23	29.2
6 " 50 "	17.7	22.7	28.2
7 " 0 "	17.7	22.2	27
7 " 10 "	17.5	22	25.5
7 " 25 "	17.2	21.2	24
7 " 30 "	17.2	21	23.2

These results are incorporated in the graphs shown in Chart II.

The crystal holes in the sections show as follows:

Position	Size of crystal holes	
	"Frozen"	"Thawed"
½" from tin	175 microns	none
1½" " "	200 "	-
2½" " "	240 "	none

Summary of Experiment 1 Showing the Effect of Size of the Fish on the Results of Freezing

Masses of fish have been frozen in different cooling media and at different rates, and a histological examination made of specimens of the frozen material taken from different depths in the fish. In every case, no matter how efficient or inefficient the refrigerating medium, those specimens taken from the depths of the fish show larger crystals than those taken from near the surface. Indeed, in one instance,-

that of a very thick mass of fish frozen in a non-circulating brine- the crystals found five inches deep in a brine frozen mass of fish were as large as those which are found in air frozen fish.

These findings, in conjunction with the temperature cooling curves, show that the findings of the Australian workers (4) in respect to meats, may be applied to fish. They also lend credence to Taylor's (3) statement that the rapidity of freezing is the conductivity of the fish muscle itself. limiting factor of

Brine freezing methods are seen to be most efficacious and advantageous when applied to small or thin masses of fish, such as fillets; and the advantages of the brine freezing method are greatly minimised when applied to large masses of fish, such as would be presented by thick boxes or cakes of fish.

The Effect of Freshness of the Fish on the Changes Occurring in Freezing

It was thought wise to determine whether or not the freshness of the fish had any important bearing on the character of the freezing process.

Experiment II

To this end, experiment II was devised. Twenty cod were caught by C.K. Darrach early one morning (July 24,1927) and were at once killed by a blow on the head, bled, gutted, and iced. One fish was put into a tub of water and kept alive. On arrival at the laboratory (about 10 a.m.) the fish were heavily and carefully iced, and were carefully kept so during the duration of the experiment. Two were removed to room temperature and left there, protected from flies.

The live fish was frozen immediately after killing; one was found to be in rigor 6 hours after being caught, and was frozen then; and the remainder were frozen one by one at daily intervals.

All the fish were filleted, and the flesh packed in the same tin, for freezing, which was done in brine at 0°F. The tin measured 2¼" X 4" X 4½" and was well filled with fish for each freezing. It was submerged as deeply as possible consistent with its open top and brine was kept out. The temperature cooling curves were obtained in every case, the bulb of the thermometer being placed as nearly as possible

in the centre of the mass. The thermometer was protected by a surrounding glass tube, containing a drop or two of mercury about the bulb. The sections for examination were cut from specimens taken from the immediate vicinity of the thermometer bulb.

Both the cooling curves and the crystal sizes are somewhat irregular, and it is suggested that most of the inconsistencies are due to variations in positions of the thermometer bulb. The weight of the thermometer tended to tilt it and to push it down, while the expansion at the freezing zone in the tin tended to raise it. Hence there was undoubtedly a variation in the position of the bulb in the tin and for future work it was decided necessary to fix it more firmly in place. For this reason only the major trends of the results can be considered significant. All the figures obtained in the cooling curves will be given but the graphs obtained from them will not be recorded. After freezing the fish were stored in the $CaCl_2$ room at the North Atlantic Fish Company, and were kept glazed.

Table IV

Exp. II	Freshness	Storage Time after Freezing	Size of Crystals (Microns)	Notes
A.	Just killed	0	94	1 crystal per cell, cells unbroken
		49 days	120	1 crystal per cell, some cells broken
B.	6 hours iced (in rigor)	0	92	1 crystal per cell, cells unbroken
		50 days	140	1 crystal per cell few cells broken
C.	28 hours iced	0	106	1 crystal per cell, cells unbroken
		49 days	120	1 crystal per cell, some cells broken
D.	2 days iced	0	120	1 crystal per cell, most cells broken
		48 days	150	1 crystal per cell, most cells broken

Table IV continued

Exp. II	Freshness	Storage Time after freezing.	Size of Crystals (microns)	Notes
E.	3 days iced	0	115	crystals extracellular
		48 days	135	crystals extracellular
F.	4 days iced	0	125	crystals extracellular
		46 days	125	crystals extracellular
G.	5 days iced	0	105	crystals extracellular
		43 days	200	crystals extracellular
H.	7 days iced	0	120	crystals extracellular
		44 days	195	crystals extracellular
I.	10 days iced	0	150	crystals extracellular
		42 days	130	crystals extracellular
J.	12 days iced (fish smells stale)	0	125	crystals extracellular
		40 days	140	crystals extracellular
K.	16 days iced (fish smells stale)	0	125	crystals mostly extracellular
		36 days	95	crystals mostly extracellular
L.	1 day at room temp. (fish smells stale)	0	95	crystals mostly extracellular
		49 days	160	crystals extracellular
M.	2 days at room temp. (fish is putrid)	0	110	crystals extracellular
		49 days	160	crystals extracellular

During the first three days of preserving of the fresh fish in ice, the character of the frozen material was not constant, Fish frozen on the first day showed crystals 92 to 94 microns in diameter and these were intracellular. Fish frozen on the second day showed intracellular crystals which were 106 microns in diameter. Fish frozen on the third day showed intracellular crystals 120 microns in diameter but most of the sarcolemma was broken. Fish frozen on the fourth day showed crystals 115 microns in diameter but the sarcolemma were so widely gaped that the crystals must be described as extracellular. Fish frozen on all later days show the same character of material as does this last.

From these results one may conclude that unless fish are frozen almost as soon as caught, it makes no difference when they are frozen - insofar as changes in the fish specifically due to freezing are concerned. Even the advantage which is obtained by freezing fish as soon as caught seems not great enough to be commercially important.

The temperature cooling curves obtained in this experiment are recorded below.

Table V.-Temperature cooling curves of Experiment II.

Series	Time elapsed	Temperature °F.	
		Fish	Brine
A	0 minutes	63	0
	5 "	62	
	10 "	56	
	17 "	47	
	22 "	42	-1
	27 "	39	
	32 "	35	
	37 "	34	
	47 "	29	-1.5
	54 "	26	
	60 "	18	
B	0 "	66	0
	4 "	62	
	5 "	59	
	23 "	34	-1
	31 "	31	
	36 "	27	
	40 "	22	
	80 "	3	-1

Table V.-Temperature cooling curves of Experiment II. (continued)

Series	Time elapsed	Temperature °F.	
		Fish	Brine
C	0 minutes	64	0
	5 "	64	
	10 "	63.5	
	15 "	60.5	
	20 "	57	
	25 "	52	-1
	32 "	48	
	37 "	43	
	47 "	36	
	62 "	32	
	65 "	31	
	75 "	26	
	78 "	22	-2
	85 "	18	
	92 "	12	
	96 "	10	
	102 "	5	
D	0 "	62	0
	4 "	61	
	6 "	60	
	11 "	58.5	
	22 "	48	
	28 "	42	
	38 "	36	
	48 "	33	0
	53 "	31	
	58 "	30.7	
	63 "	30.2	
	68 "	30	
	73 "	29	
	78 "	28	0
	83 "	24	
	88 "	16	

Table V.-Temperature cooling curves of Experiment II. (continued)

Series	Time elapsed	Temperature °F.	
		Fish	Brine
E	0 minutes	62	0
	5 "	62	
	10 "	60	
	13 "	57	
	17 "	53	
	24 "	45	-1
	32.5 "	37.5	
	38 "	35.2	
	47 "	33	
	52 "	32	
	60 "	30.5	
	63 "	30.2	-1.5
	67 "	29.2	
	69 "	28.5	
	70 "	27.5	
	73 "	24	
	76 "	21	
	80 "	16	
F	0 "	60	0
	5 "	60	
	17 "	51	
	25 "	43	
	41 "	33	-1
	47 "	32	
	50 "	30.5	-1.5
	68 "	22	
	74 "	15	
	77 "	11.5	
G.	0 "	60	0
	5 "	60	
	10 "	60	
	15 "	56	
	20 "	51	
	25 "	46	
	30 "	41	-1
	35 "	37.5	
	40 "	34.5	
	45 "	33.5	
	50 "	31.5	
	55 "	31	
	60 "	30.2	-1
	63 "	29	

Table V.-Temperature cooling curves of Experiment II. (continued)

Series	Time elapsed	Temperature °F.	
		Fish	Brine
G	66. minutes	27.5	
	68 "	26	
	71 "	23	
	75 "	19	
	78 "	16	
H	0 "	54	0
	5 "	54	
	11 "	52	
	16 "	49	-1
	26 "	42	
	38 "	35	
	48 "	33.5	1
	53 "	32	
	55 "	31.5	2
	60 "	31	3
	67 "	30.5	
	73 "	30	
	78 "	27	2
	85 "	19	
	96 "	12.5	
	101 "	10	1.5
	106 "	9	
	110 "	7	1
I	0 "	41	1
	6 "	42	
	15 "	40	
	19 "	39	0
	21.5 "	37	
	24 "	36	
	27 "	35	
	30 "	34	
	33.5 "	33	
	36 "	32.2	
	37 "	32	
	38 "	32	
	39 "	32	-1
	40 "	31.5	
	41 "	31.2	
	42 "	31	
	43 "	31	
	47 "	31	

Table V.-Temperature cooling curves of Experiment II. (continued)

Series	Time elapsed	Temperature °F.	
		Fish	Brine
I	67 minutes	30	
	76 "	25	-1
	80 "	21	
	85 "	17	
	90 "	14	
J	0 "	46	0
	7 "	45.5	
	13 "	42	
	18 "	39.5	0
	22 "	37	
	30 "	33.5	
	38 "	31.5	-0.5
	43 "	31	
	50 "	31	
	56 "	30.7	-1
	60 "	29.7	
	64 "	27	
	69 "	17.5	
K	0 "	48.5	1
	5 "	48.5	
	13 "	46	0
	34 "	34	
	39 "	31.7	
	47 "	31	0
	59 "	29.5	
	65 "	25	
	71 "	16	0
L	0 "	74	0
	5 "	74	
	10 "	72	
	18 "	65	-1
	26 "	53	
	32 "	43	
	39 "	38	
	48 "	33	
	57 "	30.5	
	63 "	30	
	70 "	29.5	-2
	76 "	28	

Table V.-Temperature cooling curves of Experiment II. (continued)

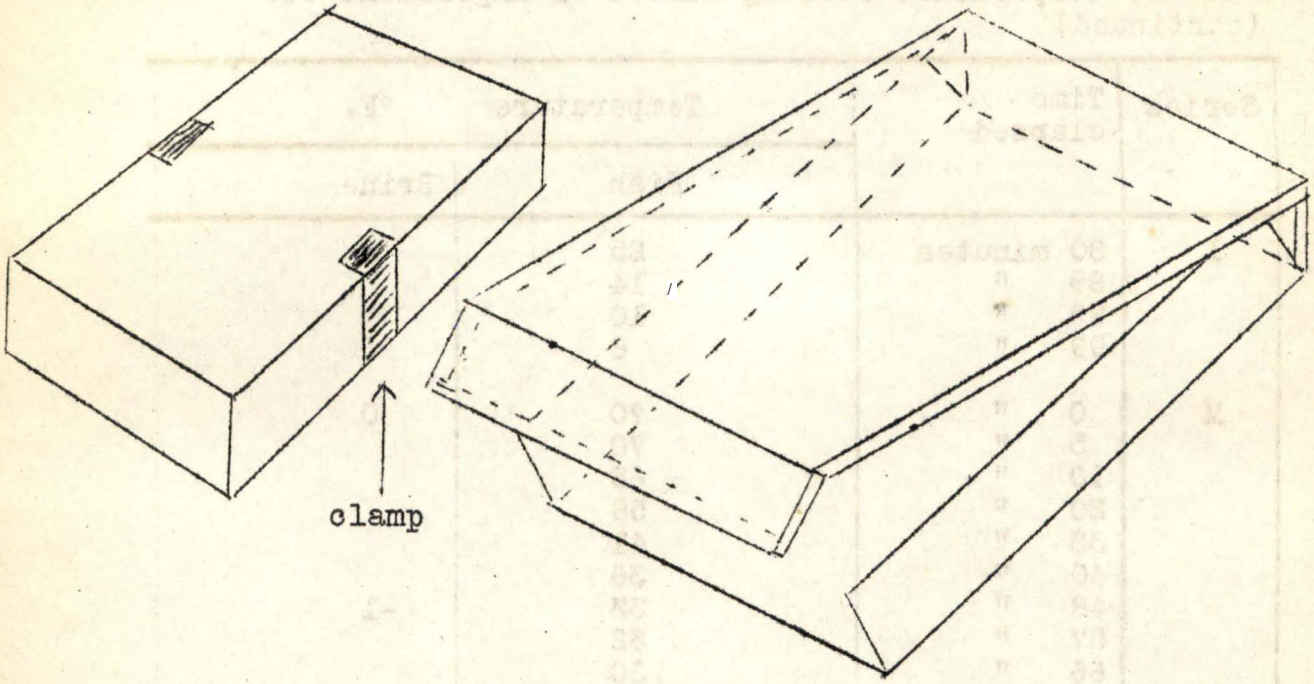
Series	Time elapsed	Temperature °F.	
		Fish	Brine
L	80 minutes	25	
	89 "	14	
	95 "	10	
	99 "	8	
M	0 "	70	0
	5 "	70	
	10 "	68	
	20 "	58	
	33 "	41	
	40 "	36	
	48 "	33	-1
	57 "	32	
	66 "	30	
	70 "	28	
	75 "	25	
	80 "	19.5	
	85 "	14	-1.5
	90 "	9	

The Rate of Freezing

In conjunction with D.A. MacFadyen who was working on the problem of "drip" from frozen fish, the following experiment was devised to explore the effect of varying the rate of freezing.

All the fish used in the experiment were caught by C.K. Darrach, bled, gutted, and kept for 24 hours in ice before being frozen. They were still in rigor mortis. Cod fish were used throughout.

The freezing was done in tins measuring 6" X 4" X 1", which gives a nice cake of fish approximating in thickness that of a thick fillet. These tins were cut out of a single sheet of tin, as is



indicated in the diagrams above. When filled with fish and closed, they were reinforced by heavy galvanized sheet iron clamps (see diagram), and the seams were sealed by being liberally painted with melted paraffin wax. This collapsible form often was adopted in order that the frozen blocks of fish might be removed without having to be softened or warmed on the surface. A thermometer was included in one tin of every batch and the temperature cooling curves recorded. A glass tube, just large enough to take a thermometer, was held rigidly - by being fixed through a wooden block and screwed on to the tin in such a position that the thermometer bulb was in the centre of the tin.

Experiment III

Three or four of the above described tins were filled with 24 hour caught cod fish, sealed, and frozen. One tin was then examined both histologically and from point of view of drip, and the others were at once well wrapped in paper and absorbent cotton and taken to the North Atlantic Fisheries Co. for storage. The tins were opened in the storage room and the cakes of fish glazed (by momentary immersion in cold water from time to time). Thus the fish, storage conditions, and handling methods were kept as constant as possible, and only the freezing method varied. The cooling curves and histological findings are given below.

Experiment III (A) Brine at 0°F.

Table VI - Cooling Temperatures.

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	53	0
7 "	34.5	
7.5 "	33	
8.5 "	32	0
9 "	31.5	
10 "	31	
10.5 "	30.5	
11 "	30	0
12 "	29	
13 "	28.5	
17.5 "	25.5	0
19.5 "	22.5	
22 "	18	
28 "	12	0.5
31 "	8	
33.5 "	5	
38 "	3.5	1.0
41 "	3	

Sections

"Frozen" - crystal holes = 100 microns, one to a cell, and many cells are broken.

"Thawed" - no crystal holes, Recovery to the original unfrozen appearance seems to be complete.

As a check on the accuracy of the measuring methods, two tins were packed with the flesh of a fresh caught cod. These were immersed in brine at 0°F. for 45 minutes.

Sections were then cut from three different areas in each tin and the crystals measured in the usual way.

Tin I - Slide a 95 microns
b 105 "
c slide broken

II - a 105 microns
b 95 "
c 100 "

These figures compare well with the 100 microns figure given for Exp. III (A) proper, and represent probably the maximum limits of accuracy obtained in this work.

They represent a variation of 5 per cent. either way, and would indicate that for a working average, a variation of 10 per cent. either way should be allowed for .

Experiment III (B) Brine at 9°F

Table VII - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	67	8.5
3 "	59	
4 "	56	9.5
6 "	48	
8 "	43	9.5
10 "	38	
12 "	35	
17 "	30	
19 "	29	
22 "	28.5	9.0
24 "	28.2	
25 "	28	
27 "	27.5	
29 "	27	9.0
32.5 "	26	
35 "	25	8.7
37.5 "	24	
41 "	22	9.0
47 "	17	
51 "	14	

Sections

"Frozen" - Crystal holes 130 microns. They are extracellular, i.e. the cells are crescent shaped, shrunken, and of such an appearance that the crystals would be described as outside the cell.

"Thawed" - No crystal holes, recovery apparently complete.

Experiment III (C) Brine at -4°F.

Table VIII - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	65	-5
1 "	65	
1.2 "	64	
2 "	62	-4.5
2.5 "	59	
3.5 "	54	
4.2 "	51	-4.0
5 "	47	
6 "	43	
7 "	40	-4.0
8 "	37	
9.2 "	34	
10.5 "	32	
11.5 "	31	
13 "	30	
15 "	29	
16.5 "	28	
18 "	26.5	-4.0
18.5 "	26	
19.5 "	25	
20.2 "	24	
21.2 "	23	
22 "	22.2	
23 "	21.2	-4
24 "	19.5	
25 "	18	
26.5 "	15	
30 "	8	

Sections

"Frozen" - Crystalsholes 31 microns - are 2 or 3 to a cell and the cells are unbroken.

Experiment III (D) Frozen in the "Sharp" freezer - air at -20°F.

Table IX - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 hours	66	-20
1 "	50.5	-20
1.5 "	29.2	-20.0
2 "	27.7	-20
2.2 "	27	-21
2.5 "	26	-21
3.5 "	19.5	-22
4 "	9	-23

Sections

"Frozen" - Crystal holes 350 microns. The holes are large enough as to show no relationship to any individual cells.

"Thawed" - No crystal holes. Recovery weems complete, except that the cells are not as closely bound together as is normal with fresh fish - some separation of cells.

Experiment III (E) Frozen in the "Cold Box" In air at 23°F. About

Table X - Cooling Temperatures

Time elapsed	Temperature °F	
	Fish	Air
0 minutes	70	23
5 "	67	
15 "	64	
48 "	52	
70 "	46.5	25
2 hrs. 10 min	36	
2.5 hrs.	34	23
18.5 "	30	
19.5 "	30	
24.7 "	30	21
43.2 "	26	23

Sections

"Frozen"- Crystal holes 350 microns. The holes are relatively few and are separated from one another by large aggregates of fairly normal appearing cells.

"Thawed" - Much swelling of tissue has taken place, and the crystal holes have disappeared, but the cells are somewhat separated from one another, being no longer closely held together.

Experiment III (F) Brine at -7°F.

Table XI - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
ϕ minutes	67	-8.5
2 "	63	
8.5 "	37	-7.0
10.2 "	32	
10.7 "	31	
11.2 "	30	
11.7 "	29	-7.0
12.2 "	28	
13 "	27	-7
14 "	26	-7
15 "	25	-7
16.7 "	24	
18	23	
19.5 "	22	-7
21 "	21	-7
22.5 "	21	
24.2 "	20	
25.2 "	19	-7
26 "	18	
28 "	15	
28.2 "	14	
30 "	10	-7

Section

"Frozen" - Crystal holes - several to a cell which is unbroken - 62 microns.

Experiment III (G) Carbon dioxide-Ether mixture at about -108°F.

Small pieces (weighing up to 70 gms) of fish were dropped into this mixture in a Dewar flask . Plenty of carbon dioxide snow was added and the fish was left in until active boiling off of carbon dioxide ceased. With the largest piece this took about 2 minutes.

Sections

"Frozen" - No crystal holes- tissue is as "fresh".

"Thawed" - " " " " " " "

Experiment III (H) Frozen in an Ice - Ca Cl_2 mixture at about -5.8° F.

Table XII - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	71	12°F
3 "	66	
6 "	59	
8 "	55	
11 "	49	
15 "	44	3
18 "	39	
22.5 "	36	
24.5 "	34	0
28 "	32	
31 "	31	-3
32 "	31	-4
34 "	30.5	-5.8
36.5 "	30	"
38 "	30	"
40 "	29.5	"
42 "	29	"
43 "	29	"
44 "	28.2	-5
46 "	27.7	-6.7
48 "	27	-5.8
50.5 "	26	
54 "	25	-0.4

Table XIII - Cooling Temperature

(continued)

Time elapsed	Temperature °F.	
	Fish	Brine
56 minutes	23	
58 "	22	-0.4
60 "	20	
61 "	19	
64 "	15	
66 "	12	-4.0
66.5 "	11	

Note:- The ice and $CaCl_2$ were mixed in a bucket. There was no forced circulation of brine as in the "small freezer."

Sections

"Frozen" - Crystal holes 125 microns, one to a cell and most cells broken.

"Thawed" - No crystal holes; recovery seems complete.

Experiment III (I) Brine at -3°F

No freezing curves were taken, but fish was left in brine for 30 minutes. The curve should correspond to that of the hake (experiment IX) fairly closely. The fish was thoroughly frozen.

Sections

"Frozen" - Crystal holes 40 microns, averaging about six holes per cell, and the cells are not broken.

Experiment III (J) Brine at 15°F.

Table XIII - Cooling Temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	57	15.5
1 "	56	
2.5 "	53	
3 "	51	
4 "	47	
5 "	45	15.5
7.5 "	39	
8.5 "	38	
10.5 "	35.5	16
11.5 "	34	
12.5 "	33	
13.5 "	32	
14.5 "	31.7	
15.5 "	31.5	
16.5 "	30.7	
17.5 "	30.5	30.5
20 "	30	
22.5 "	29.5	
29 "	28.7	
34.5 "	27.5	
50 "	23	
53.5 "	21.2	15.5
63.5 "	18	16

Section

"Frozen" - Crystal holes 175 microns. The crystals are extracellular, the cells being obliquely cut are shrunk and widely separated.

Experiment III (K) Frozen in the sharp Freezer - air at about 8°F.

Table XIV - Cooling temperatures

Time elapsed	Temperature °F.	
	Fish	Brine
0 hours	57	14
3½ "	30	10
6 "	24	7
7 "	17	2

Section

"Frozen" - crystal holes 300 microns. They are extracellular and show no relationship to any individual cell.

Summary of Results of Experiment III

The temperature cooling curves are shown in Charts III IV and V.

If the histological findings are grouped with the freezing rates as shown below in Table XV, their relationships will be more easily noted.

Table XV

Experiment III Section	How Frozen	Time interval for for temp. to drop from 32°F. to 20°F.	Diameter of crystal holes microns	Position of crystal holes
E	air at 23°F.	2 days	350	Surrounded by cell aggregates
K	air at 8°F.	3 hours	300	Surrounded by cell aggregates
D	air at -20°F.	2½ "	350	Surrounded by cell aggregates
J	Brine at 15°F	42 minutes	175	Extracellular
H	Ice-$CaCl_2$ mixture	32 "	125	cells broken
B	Brine at 9°F.	29 "	130	Extracellular
A	Brine at 0°F.	13 "	100	One per cell. Cells broken
I	Brine at -3°F.	12½ "	40	Several per cell. Cells unbroken.
C	Brine at -4°F.	13 "	31	Several per cell. Cells unbroken
F	Brine at -7°F.	14 "	62	Several per cell. Cells unbroken
G	CO_2-Ether	seconds	nil	

From these figures it is seen that there is undoubtedly some relationship between rate of freezing and size of crystal formed. Reuter's (1) findings to the effect that a few large between cell crystals are formed in slow freezing, many smaller intracellular crystals are formed in fast freezing, and no crystals are formed in very fast freezing, are quite confirmed. There are some slight irregularities in some of the figures in the table, but on the whole this tendency is plainly visible. There are not nearly enough figures, nor are they carefully enough ~~enough~~ checked, to make it possible for one to work out crystal size as a mathematical function of freezing rate, even though such a relationship probably exists.

It is interesting to note that even though the time taken for the temperature of the fish to drop from 32°F. to 20°F. (in which range the solidifying of the fish has taken place) is constant in the instances frozen in brine at 0°F. and below, the crystals formed are much smaller, the colder the brine. This would indicate a more rapid freezing at the lower temperatures despite the freezing curve figures, and suggests that the rather crude method of recording these temperature changes was not sufficiently delicate. Not only was the thermometer bulb and surrounding glass and mercury relatively so large as not to record temperature changes of a point, but this mass of material must have caused a considerable lag to have occurred in the apparent temperature changes.

With cod fish frozen in these tins, of this degree of freshness, frozen in a well stirred brine, a temperature just below 0°F. seems to be the critical one - above which the crystals which form on freezing are large enough to break through the sarcolemma of the cell; and below which several small crystals form within the cell but without breaking through the sarcolemma. Without the preparation of "serial sections" and their very careful examination however, this statement must be considered only as a suggestion, and its significance may be slight.

Experiment IV

As a check on the last experiment, and to enable MacFayden to obtain some more "drip" results, two fish equal in weight and length were caught together, kept in crushed ice for 24 hours, filleted, packed in the tins of Experiment III, and frozen as below. All the freezings were done during the one day.

A. Immersed in brine at 15°F. for 40 minutes
The crystal holes are 125 microns, and are mostly extracellular.

B. Brine at 9°F. for 35 minutes.
Crystal holes 100 microns, mostly extracellular.

C. Brine at 3°F. for 30 minutes

Crystal holes 90 microns, mostly extracellular but many intracellular.

D. Brine at 0°F. for 30 minutes

Crystal holes 95 microns, mostly extracellular, but many intracellular.

E. Brine at -2.5°F. for 30 minutes

Crystal holes 95 microns, many extracellular, but mostly intracellular.

F. Block of fish, not in tin, packed in carbon dioxide dry snow for 10 minutes.

Crystal holes 65 microns, mostly intracellular.

These results confirm in the main those of Experiment III.

Effect of Storage

It was noticed early in this investigation that the size of the crystals in the fish seemed to increase during storage, and this led to a discussion as to whether or not a weak brine solution frozen solid with extreme rapidity and then stored at a temperature much above the freezing temperature but below the freezing point of the solution, would change in any way during the storage. It was thought that there certainly would be a change but no one could figure how rapidly. Accordingly it was decided to try it and see, and a portion of the experiment will be reported on here before giving data obtained from fish.

Experiment V.

About 5 c.c. of 2.5%, 5%, and 10% solutions of NaCl in distilled water, in small Pyrex test tubes, were submerged in carbon dioxide ether mixture at -108°F. The solutions solidified within 10 seconds and the blocks of ice removed by warming the tube momentarily, and placed in air at a temperature varying between 21°F. and 4°F.
Immediately after freezing, all the tubes were solid, opaque, and white. Early in the freezing irregular crystal markings show in the tubes but the mass solidifies as a homogeneous whole, which then becomes permeated with a multitude of minute cracks, caused probably by uneven shrinkage of the solid mass in the CO_2-Ether.

After three hours storage (1) The 2½% NaCl ice is more translucent than at first, is not so hard, and is granular when crushed between the fingers. (2) The 5% NaCl ice is quite translucent and so soft as to be almost mushy. (3) The 10% NaCl ice is translucent and practically slushy, free fluid having formed.
After 24 hours storage (1) As after 3 hours, except that the mass is of coarser texture than before. (2) As after 3 hours. The mass still retains its shape but is very soft. Some free fluid has formed. (3) Soft and slushy. The crystals seem coarser and broader than before.
After one week's storage (1) The crystals are large and rock-like. The material is still hard but it crumbles readily. (2) Crystals are coarser than after 24 hours. (3) Crystals are very large and are floating freely in liquid.

Thus it is seen that almost immediately the unstable brine-ice mass is put into storage, it begins to change its character, i.e. to soften and attain the equilibrium it would attain if cooled gradually. The process however, insofar as actual growth in size of crystals is concerned, takes a week or more to finally come to equilibrium.

Experiment VI

The Growth of Crystals in Fish during Storage

The fish reported here are those of Experiments I, II, and III, which were put aside after freezing into storage under the conditions noted. It is unfortunate that more temperatures, and more alterations of constancy of temperatures could not have been explored, but facilities for doing so were not at hand.

Table XVI - Size of crystals in microns after storage of frozen fish for various periods in the "cold box" at from 14-28°F.

Expt.	Time of Storage							
I	0 days	1day	2 days	1 week	2 weeks	1 mos.	2 mos.	3 mos.
A	60			88		135		
	67			110		160		
B	120			145		216		
	140			160		250	230	200
C	85		105			180		
	90		150			300	150	215
D*			280	515	550			
			430	610	650		1250**	1015***
E	70	135						
	125	170		230	225			
F	90	110		135	150			
	115	150		175	240		190	205
G	120	145						
	120	200		230	200		175	190
H	110	105						
	120	140		170	170		170	230

* Air frozen ** 6 weeks *** 10 weeks

These results have been incorporated in table XVII in the following way. All the measurements shown in each column showing storage have been averaged, and the corresponding specimens in the "0 days" column averaged for comparison. The difference between the two has been worked out in terms of percentage of the latter. The air frozen fish (D) has been left out of the averages and its percentage increases are included in a separate column.

Table XVII

Average size of crystals after storage for		Percentincrease in size of crystal	The air frozen fish -D-
0 days 106μ	1 day 147μ	39%	
" " 88	2 " 128	45	0%
" " 104	1 week 160	54	53
" " 110	2 " 183	66	64
" " 94	1 month 207	111	250
" " 113	2 " 183	62	183
" " 116	3 " 208	71	-

Table XVIII

These frozen fish were stored in the calcium chloride room at the North Atlantic Fisheries, whose temperature varied from 0°F. to 10.5°F. They were glazed and wrapped in paper, but some drying-noticeable at the surface-took place.

Expt. II.	Size of crystals in microns after various storage periods		
	0 days	1 month	2 months
a	94	130	120
b	92	145	140
c	106	125	120
d	120	110	150
e	115		135
f	125		125
g	105		200
h	120		195
i	150		130
j	125		140
l	95		160
m	110		160
Average for a,b,c,d,	106	128(+21%)	133(+25%)
Average for all	113		148(+31%)

From these figures it is clear that the ice crystals in frozen fish will increase in size while the fish are kept in storage, and that in a given time this increase is less at a cold temperature than at a warmer. In one month at a variable temperature averaging about 22°F. the crystals doubled in size, and only increased by one-quarter in a month at a variable temperature averaging about 6°F.

Other, less obvious findings to be pointed out perhaps are:-

(1) The crystals do not seem to grow to a common maximum size. If they start large they remain large throughout the storage period. Indeed the largest crystals of all - namely, the air frozen crystals of Exp.I.(D) - showed a larger percentage increase than did any of the small crystalled specimens.

(2) There are a sufficient number of examples which show at least a temporary decrease in size that one is led to the belief that it is a true phenomenon and not purely an artefact. To develop this further one would say that once equilibrium is reached between water in the form of ice and water in the tissues - and this occurs relatively rapidly when the fish are stored at such a high temperature as 22°F. - that the actual size of the crystal then present will vary inversely with slow changes of temperature. This is because the amount of water available for ice crystals - presuming that the temperature changes are slow enough to allow equilibrium to be maintained- is a function of the absolute temperature and the salinity of the tissue fluids. The growth of the crystal (in storage, not during the freezing process is probably due to two factors. (A). The growth of the larger ones at the expense of the smaller, as reported before by Oyura and Fugikawa ((2). (B) That all the crystals varying in size inversely with the temperature -would become more prominent. However, this is pure surmise which the figures given neither confirm nor deny.

It was thought desirable to store some fish at a constant temperature and others in a variable temperature of about the same degree. Methods of definitely controlling this variation were discussed, but none seemed likely to be very precise, and all seemed certain to require a lot of attention. This was inconvenient at the time and so the following precedure was adopted. An incubator was taken to the calcium chloride room at the North Atlantic Fisheries Co. and set at 12°F. This temperature was adopted because the temperature of the room was known to vary between about 0°F. and 10.5°F. and the incubator to be kept a constant temperature would have to be set higher than the maximum attained by the containing room. Unfortunately during the experiment it was found that the temperature did not remain as constant as was anticipated. The temperature record for the period of the experiment is given below.

Table XIX - Temperature record of Storage Chambers

Date		$CaCl_2$ Room	Incubator
August	15	3.0°F.	12°F.
	16	3.0	13.5
	17	6	13.5
	18	0	14
	19	1	14
	22	7.5	12
	23	3.5	12.5
	24	3.5	12.5
	25	3.0	12

Table XIX (continued)

Date		$CaCl_2$ Room	Incubator
August	26	6.5	11.5
	27	6.5	12
	29	3.5	12.5
September	2	2.5	13
	7	6	11
	12	9	11
	13	8.5	10.5
	14	8.5	8.5 (Electricity off)
	15	8	11
	21	5	15
	22	8	12
	23	10.5	10.5

Some of the fish of Experiment III were used in this experiment. As reported before these fish were taken to the storage room before being allowed to warm up, and the tins were removed from the cakes without having to melt or soften the surface of the cake. The blocks of frozen material were glazed however (one coat) and kept glazed.

Table XX

Expt. III	Size of Crystals in Microns		
	Fresh frozen	Stored one month	
		in incubator at 12°F.	in $CaCl_2$ room at from 0-10°F.
A	100	135	175
B	130	130	130
C	31	110	50
D	350	300	215
F	72	62	61
G	0	12	18
Average	114	125 (+9.6%)	108 (-5.3%)
Average without D (the air frozen)	67	90 (+34%)	87 (+30%)

These figures show a great deal of variation, so much so indeed that no very positive conclusions should be drawn from them. However, if the results for D are discarded - and they may well be because the crystals are so large from the start as to exert a preponderant effect on the average including them - the remaining figures show an average increase of crystal size of 34 per cent. when stored in the incubator and 30 per cent. when stored in the $CaCl_2$ room. If there is a tendency for fluctuation in temperature to hasten the growth of crystals it is not shown by these figures. Such a showing though would be counterbalanced by:- (1) the lack of constancy of the incubator temperature and (2) the lower average temperature, even though slightly more variable, of the calcium chloride room.

Table XXI - The final size of crystals attained after long storage

(a) Fish frozen by Dr. Leim in 10 pound cakes, in brine at 0°F., about 9 months previously, and stored in the calcium chloride room at the North Atlantic Fisheries Co. at a temperature ranging from about 10°F. to about 0°F.
Crystals measure 135 microns.

(b) As a), but frozen by Mr. Finn 20 months previously.
Crystals measure 150 microns.

The size of the crystals formed originally during the freezing was not known, but on the basis of the figures reported in the "Rate of Freezing" section of this report, it is probable that it would at least be greater than 100 microns. The present size is of the same order of magnitude as those shown in Table XVIII which are fish frozen in brine at 0°F. and stored for 1 and 2 months in the same place. Thus it seems reasonable to conclude that under these conditions of storage growth is complete after perhaps one month.

Experiment VII

Qualitative Histological Changes Occurring During Storage

When a freshly frozen fish is cut "Thawed", i.e. allowed to thaw in the gross before sectioning, the appearance of the section is practically indistinguishable from that of "fresh" or unfrozen fish. The water elements of the tissue, which have been withdrawn into ice crystals during the freezing process, are reabsorbed back into the protein colloidal system of muscle tissue, with consequent swelling of the shrunken tissue substances to completely fill up the spaces previously occupied by the crystals. This phenomenon has been noted in every case examined, without exception.

Some permanent change must have occurred during the freezing, because the thawed material has a somewhat resilient or rubbery consistency not shown by the fresh; but this is not demonstrable histologically, by the somewhat crude method used in this investigation at least. It is only in the case of the very slowly frozen of the specimens that any difference histologically is seen between the fresh and thawed freshly frozen specimens, and even here it is not great. The individual cells have swollen to their normal size and shape, but are not quite so regularly and closely bound together as when fresh. In these cases the crystals formed are large and outside the cells, pushing them apart, thus suggesting that the phenomenon is due to the disruption of the delicate connective tissue fibres normally holding the cells close together.

When these frozen specimens are stored for some time however, and then cut "thawed", this recovery of the tissue to its original or "fresh" appearance is not noted. In fact there is no apparant difference in histological appearance between the "frozen" specimen and the "thawed" stored one. The tissue seems to have lost its ability to reabsorb the water elements withdrawn from them into the ice crystals during the freezing. Thus "Time" is another factor helping to determine whether or not the tissue changes occurring in frozen fish are reversible or irreversible. Some illustrative figures are given in Table XXII. The pieces of fish were left in air at room temperature until defrosted.

Table XXII

Expt. Number	Length of time in storage	Diam. in Microns of Crystals Holes in sections cut		Histological Notes
		"Frozen"	"Thawed"	
I (b)	fresh frozen	145	0	Recovery Complete
	1 month	250	200	No swelling of tissue
	2 "	230	150	" " " "
	3 "	200	250	" " " "
I (c)	fresh frozen	90	0	Recovery complete
	2 months	150	140	Considerable swelling of tissue
	3 "	215	155	No swelling of tissue
I (d)	fresh frozen	430	not done	
	2 months	1250	700	
Ross' fillets	fresh frozen	70	0	Recovery complete
	1 week	84	0	" "

Table XXII (continued)

Expt. Number	Length of time in storage	Diam. in Microns of Crystals Holes in section cut		Histological Notes
		"Frozen"	"Thawed"	
Ross' fillets	2 weeks	115	0	The tissue is uneven and patchy in appearance, unlike fresh fish.
	1 month	100	100	No recovery on thawing.
	1½ "	140	50	Much swelling of tissue
	2 "	160	95	Some swelling of tissue.
Leim TableXXI (a)	9 months	135	110	Some swelling of tissue
Finn TableXXI (b)	20 months	150	135	No apparent recovery
III (a)	fresh frozen	100	0	Complete recovery
	2 weeks	110	29	Uneven swelling of tissue
	1 month	135	80	Considerable swelling of tissue.
III (g)	fresh frozen	0	0	
	1 month	12	14	No recovery
III (h)	fresh frozen	125	0	Complete recovery
	1 month	120	100	Some swelling of tissue
III (d)	fresh frozen	350	0	Recovery practically complete
	1 month	350	100	Considerable swelling of tissue

Details of the freezing and handling of these fish may be obtained by referring to the experiments indicated by the reference numbers in the left hand column, with the exception of the series labelled,"Ross' Fillets". These were a batch of fillets (cod) frozen by Messrs. Ross and Mitchell on July 8,1927. Each fillet was individually contained in a heavily waxed paper envelope; they were frozen by immersion in brine at 3°F. for 25 minutes; then they were taken to the calcium chloride room at the North Atlantic Fisheries Co. for storage (being left in their envelopes). At the intervals shown in the table one fillet was brought to the laboratory for examination. Each pair of determinations therefore is on a separate fillet and hence some variation in results might readily be expected.

Summary of Effects of Storage

(1) The crystals which form in the fish during the freezing process, increase in size during storage.

(a) They increase more rapidly and to a greater degree at a high temperature (e.g. 22°F.) than at a low temperature (e.g. 5°F.).

(b) Crystals which are large to begin with, increase to the same proportional extent as those which start small.

(c) Growth seems to be practically complete within a month of storage at these temperatures.

(2) The changes which have occurred in the tissues during the freezing - namely, a concentration or dehydration of the tissue elements - while largely reversible at first, become practically irreversible after storage for some period less than a month at these temperatures.

This factor would seem to be of greater importance than the growth of crystals, which after all, at the lower temperature is only an increase of 25 or 30 per cent.

Defrosting Methods

It is quite generally reported in the literature that if defrosting is slow, there is some reabsorption of fluids by the tissues on thawing which does not take place if defrosting is rapid. (Taylor (3), Stiles (1)). Plank and Ehrenbaum (1)

however thawed various fish in crushed ice, water, and warm air, and concluded that the velocity of thawing influence neither the taste nor keeping properties of the fish. During this investigation we have thawed fish by putting them directly into the frying pan, and thawed others slowly in air, frying them subsequently, and could observe no difference in texture and flavour.

The following observations have been made on this phase of the subject from the point of view of histological appearance.

Experiment VIII

A few of the fish of Exp. III were defrosted in different ways, after storage, and the results are shown below in Table XXIII.

Table XXIII

Reference Number	Length of Storage	Size of Crystal Holes in Microns		Treatment of "Thawed" fish before sectioning
		"Frozen"	"Thawed"	
Exp.III(d)	1 month	300	150	Thawed in 3 hours in air at room temperature
			100	Thawed by immersion in water at 90°F. for 20 minutes.
III(k)	2 weeks	350	135	Thawed in 3 hours in air at room temperature
			145	Thawed in 15 minutes immersed in water at 90°F.
III(f)	1 month	62	50	Thawed in 3 hours in air at room temperature
			48	Thawed in 3 hours in air at room temperature and then immersed in water for 2 hours at room temperature.

Judging from these results there would seem to be no difference in the degree of reabsorption of water by the tissues whether the fish was thawed slowly in air or rapidly in water. Even slow thawing in air followed by immersion in water did not seem to cause any greater degree of recovery of the original unfrozen appearance.

However, as the defrosting methods were so widely different that it was thought wise to vary defrosting rates without subjecting the fish to such different conditions. To this end, Experiment VIII (A) was designed in which the fish was sealed in tins, all frozen together, stored for one week in the incubator (12°F.) at the North Atlantic Fisheries Co., thawed in water baths of different temperatures, and sectioned as soon as thawed.

Experiment VIII (A)

A 15 pound cod was obtained from the National Fish Co. (it was five or six days old), and several ordinary ½ lb. cans were filled with pieces of its flesh, and sealed. These cans were then all frozen together in brine at 0°F. (45 minutes). The temperature cooling curve was obtained by sealing a glass tube in the center of one of the tins with sealing wax, and putting mercury and a thermometer into the tube. The tins were then stored for one week at about 12°F., when they were defrosted by almost complete immersion in water at 100°F., 68°F., 50°F., and 32°F. The defrosting temperature curves were obtained by boring a hole through the top of the tin into the center of the mass of fish, pouring in some mercury and inserting a thermometer. After defrosting they were sectioned, and were examined by MacFadyen for "drip" and expressible fluid. The histological results are incorporated in Table XXIV, and the temperature curves in Table XXX and Chart VI.

Table XXIV

Expt. VIII-A	Defrosted at	Defrosted for	Histological Appearance
A			Section cut "Frozen" Crystal holes 105 microns mostly extracellular
B	100°F	½ hour	Two cells show crystal holes of 60 microns, but otherwise, except for a slight cell separation, recovery seems complete.
C	68°F	1 hour	One cell shows a crystal hole of 40 microns-slight cell separation-recovery otherwise complete.
D	50°F	2 1/3 hr.	No crystal holes-very slight cell separation, recovery otherwise complete.
E	32°F	8½ hours	Recovery seems complete. It is doubtful if there is even any more cell separation than might be considered normal in a fresh fish.

These results would indicate that in freshly or recently frozen fish, very slow defrosting does allow a greater recovery of tissue to its original state than rapid thawing. The differences noted however are so slight as to probably be of no commercial or practical importance.

Table XXV

The Temperature Cooling Curve

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	62.5	0
3	61	
4	60	1.0
6	57	
8	55	
11	51	1.0
13	46	
16	41.5	0.5
20	36	
23	33	0.5
24	32	
26	31	0
28	30	
31	29	
34	26	
37	21.5	
45	13	0

A - Defrosted in water at 100°F.

Time elapsed	Temperature °F.	
	Fish	Water
0 minutes	19	100
6	22	
8	23	
10.5	24	
13	25	102
16	25.5	
17	26	
19.5	27	100
21.5	28	
24	29	

Table XXV
A - continued

Elapsed Time	Temperature °F.	
	Fish	Water
25.5 minutes	30	
27 "	31	100
28 "	32	
29 "	33	
30 "	34	

B - defrosted in water at 68°F.

Elapsed Time	Temperature °F.	
	Fish	Water
minutes 0	15	
2.5	17	65.5
4	18	
5	19	64.5
7	20	
9.5	21	68
11.5	22	68
14.5	23	68
18	24	68
23	25	70
28.5	26	70
37	27	69
48	28	68
58	34	67

C - Defrosting in water at 50°F.

Elapsed Time	Temperature °F.	
	Fish	Water
minutes 0	19	50
2	20	46.5
4.5	21	48
7.2	22	50
10.5	23	
15	24	
19.5	25	50

Table XXV
C - continued

Elapsed Time		Temperature °F.	
		Fish	Water
minutes	26.5	26	
	36.5	27	
	52.5	28	50
1 hour	11	29	51
	33.5	30	52
	47.5	30.5	50
2 hours	00.5	31	
	06.5	31.5	50
	12.5	32.5	
	14.5	33	50
	16.5	34	
	18.5	35	50

D - Defrosting in water at 32°F.

Elapsed Time		Temperature °F.	
		Fish	Water
minutes	0	13	32
	8	17	
	10	18	
	12	19	
	14	20	
	16.7	21	32
	20	22	
	25	23	32
	31	24	
	40	25	
	53	26	32
1 hour	11	27	
	50	28	
2 hours	19	28.5	32
	50	29	
3 "	55	29.5	32
5 "	15	30	33
8 "	24	37	64.5

Summary of Experiment VIII

The problem of methods of defrosting has not been completely investigated, but the results shown tend to indicate:-

(1) That with fish freshly or very recently frozen, too rapid defrosting does not give the tissues quite sufficient time to completely reabsorb all the water withdrawn into the ice crystals. The difference however between very rapid and very slow defrosting seems to be too slight to be of practical importance.

(2) When fish have been frozen and stored for two weeks or a month, the changes have become so permanent that varying the rate of defrosting exerts no effect on degree of recovery. Five hours was the longest time given however, and it is possible that if twenty-four hours or some such time were given, some more swelling of the tissues would occur.

Kind of Fish

All the work reported in Experiments I to VIII-A have been done with Cod. A few isolated observations have been made with other fish, which tend to show that essentially the same processes occur as in the case of the Cod.

Experiment IX-Hake

A hake, twenty-four hours caught (kept in ice), was packed in the tins of Exp. III and frozen in brine at -3°F One tin was then examined and another stored in the incubator at 12°F. for one month. Its treatment in every way is comparable to that of the cod in Exp. III.

Table XXVI - Cooling Temperature

Time elapsed	Temperature °F.	
	Fish	Brine
0 minutes	68	-3
2	67	
2.2	64	-2.5
3	59	
4	54	
5.5	46	-2.7
7	41	
8	37	
9.5	33	
10	31	
10.5	30	-2.7

Table XXVI - continued

Time elapsed	Temperature °F.	
	Fish	Brine
11 minutes	29	
11.5	28	
13	27	-3
14.5	26	
16	25	
18.5	23.5	-3
20	22	
22	21	
23.5	19	-3
25	17	
26.5	13	
29	8	-3
40	5	

Sections Show

A - Fresh frozen - "Frozen" - 95 microns, 1 crystal per cell, most broken.
"Thawed" - no crystals - complete recovery (air).

B - Stored 1 month - "Frozen" - 105 microns, extracellular-tissue much shrunken.
"Thawed" - 3 hours in air 75 microns-much swelling.
½ hour in water 90 microns much swelling.

If these results are compared with those of the cod frozen under the same conditions, no marked differences are noted.

Cod - Exp. III (I) (Table XV) - 40 microns - several crystals per cell.
Exp. IV (E) - 95 microns - intracellular mostly.

To be sure, the crystals are mostly intracellular in the cod, and smaller in one determination, but on the basis of the one experiment no definite conclusions can be drawn. Cod frozen at this temperature seems to be below the "Critical" point - i.e. instead of crystals broken through the sarcolemma, most of them are definitely intracellular - and it is possible that this "Critical" temperature is lower in the case of the hake.

Haddock

A haddock was also packed in the tins of Exp. III and frozen with the hake tins described above. The fish was 24 hours caught - kept in ice - and should be exactly comparable with the hake, and with the cod examples given above. It was not stored.

Sections Show

"Frozen" - 74 microns - intracellular, 1 or 2 per cell, cells unbroken.
"Thawed" - in air for 3 hours - no crystals - recovery complete.

It would seem that under these conditions this haddock was frozen at a temperature below the "Critical" temperature - like the cod but unlike the hake.

Herring

Herring were frozen by Dr. Chipman (August 5 and 8) in the course of a "Rust" experiment and were examined histologically as shown below. They were fine large fat herring, and were frozen by direct immersion, in the round, in brine at 0°F. for 45 minutes. Sections were made before and after freezing, and two batches were frozen. All were caught together, and stored in crushed ice prior to freezing, one batch for 3 days and one batch for 6 days.

Table XXVII Herring

Storage before freezing	Size of crystals
3 days	"Fresh"- No crystals "Frozen"- Crystals 52 microns. A large number of oil globules were seen to be scattered freely through and about the section
6 days	"Fresh"- No crystals. A few oil globules are present. "Frozen"- Crystals 50 microns. More oil globules are seen than in the "fresh" specimen.

These "frozen" or "fresh" specimens did not differ qualitatively from corresponding specimens of cod, except for the presence of the oil globules. These were extracellular, no special fat stains being available for study of intracellular fat. The results are too few to be conclusive but would seem to indicate that fat is liberated from the tissue substances during the freezing. If so the process could hardly be reversible, and may be a factor to be considered in problems of refrigeration as applied to fatty fish.

Mackerel

A mackerel, large and fat, was frozen by the North Atlantic Fisheries Co. (Sharp freezer) probably a year previously, stored by them for 6 months, and then stored in the "Cold Box" at the Laboratory for a further 6 months. The temperature of the "Cold Box" ranged from about 29°F. to 4°F. Considerable "Rust" is seen on the fish, especially in the region of the pectoral fins and the gills, and the superficial layer of adipose tissue is distinctly yellow.

Macroscopic sections of the frozen material show the presence of large plates of crystals (measuring perhaps 3 or 4 mm. x $\frac{1}{2}$-$\frac{3}{4}$ mm. x $1/10$ mm.), making the flesh a tough fibrous spongy mass. After defrosting the tissue seems just as fibrous and spongy as if no reabsorption of fluid had taken place.

Microscopic sections of this mackerel show a much larger amount of connective tissue interconnecting the muscle fibres than do the sections of cod.

Salmon and Halibut

A Salmon fillet and a Halibut fillet, from a commercial brine freezing establishment were examined. Except that they were brine frozen nothing is known of their history. (The salmon though can hardly be more than 2 months old and may have been very recently caught). Both fillets were very attractive in appearance and in addition to being examined histologically they were cooked and eaten. They were placed directly in the frying pan and defrosted during the frying; both were delicious and could pass readily as fresh fish.

Table XXVIII

Salmon	"Frozen"	-Crystals 100 microns, extracellular, the cell tissue being very shrunken and dense.
	"Thawed"	-in air- Crystals 90 microns. Much swelling of tissue has taken place, but the tissue is still somewhat shrunken. Many of the crystal holes have disappeared.
Halibut	"Frozen"	-Crystals 150 microns, extracellular, the cell tissue being shrunken and dense.
	"Thawed"	-Crystals 90 microns, Very little swelling of tissue has taken place, and as many crystal holes seem present as before defrosting (in air).

Experiment X

Eating - The Final Test of Frozen Fish

A - Ross' Fillets (see Table XXII) Brine Frozen

One fillet of this batch was left unfrozen, and was fried. One of the frozen fillets was also cooked, being defrosted in the frying process. No difference in flavour or texture was noted between the two.

One of the frozen fillets which had been stored for two months in the calcium chloride room at the North Atlantic Fisheries was cooked, being defrosted during the frying process. The myomeres were firmer and the fibres somewhat tougher than normal, but to the uninitiate the fish would have passed as fresh.

B - Leim's fish (see Table XXIa) Brine Frozen

This cake of fish was brine frozen, and stored for nine months. It was defrosted during the frying process. The flavour was good enough but the texture was tough and fibrous. It was quite palatable however and no worse than many orders of restaurant fish.

C - Finn's fish (Table XXI b) Brine Frozen

This cake of fish had been stored for twenty months. It was defrosted during the frying process. It was tough and fibrous and somewhat tasteless, but was still edible enough that it was all eaten - served to six of the laboratory staff for lunch.

D - Air Frozen

Two fillets were obtained from the same fish. One was stored in cracked ice, and one was put into the cold box (air, Temperature 23°F.). After 24 hours one half of each fillet was removed for examination and cooking. The frozen fillet was hard but not rigid, was gritty to the knife and its cut surface mottled by the presence of large crystals (365 microns) It was defrosted in air and both were fried. By close comparison one could pick out the frozen fish by its slightly greater firmness, but either piece if alone would have been called fresh.

After 48 hours the remaining halves of the two fillets were treated as above. The frozen fillet was now more rigid than before, but it could readily be cut with a knife. It is probable that at this temperature it would never get any harder. (Crystals were 480 microns). On cooking it was practically impossible to tell which of the pieces of fish had been frozen.

E - Air Frozen

A commercially (sharp freezer) frozen cod was obtained from Boutilier's, which was said to have been stored for three weeks. It was a gutted fish, weighing 10 pounds. It was steaked, and was defrosted during the frying process, being fried with Finn's fish (c). Its crystals were - "Frozen" 530 microns and "Thawed" 500 microns.

The cooked fish was exceedingly tough and fibrous, and was absolutely tasteless. None of us(six)attempted to finish our portions, but ate the twenty months stored brine frozen fish instead. (see "c")

Summary of Experiment X

(1) Both brine and air frozen fish, when freshly frozen are when cooked, practically indistinguishable from fresh fish.

(2) Brine frozen fish slowly, over a period of months, become tough and fibrous and tasteless.

(3) Air frozen fish very rapidly, over a period of weeks, become tough and fibrous and tasteless.

(4) These results are quite in keeping with the histological findings heretofore reported in this paper.

General Summary

An attempt has been made to study the changes occurring in fish during freezing and subsequent cold storage, in an endeavour to determine how the present commercial product may be best improved.

This has included a careful microscopic examination of the fish treated in various ways, as well as a general macroscopic examination, and a final comparison of the products by the culinary method.

Working with cod it has been shown that :-

(1) The more rapidly the fish are frozen the smaller the ice crystals which form during the freezing.

(2) The character of the frozen material may be roughly divided into four classes:

(a) Where large crystals form between fibres, separated from one another of aggregates of muscle cells.

(b) Where small, but more numerous crystals form, which however are large enough to have broken through the sarcolemma and to appear to be extracellular (or intercellular)

(c) Where still smaller crystals have formed; - about one to each fibre- which are definitely within the fibre but which have broken the sarcolemma.

(d) Where many very small crystals have formed, several in each cell, which have not broken the sarcolemma.

(3) With cod, in tins one inch thick, the appearance of class C will be formed if the fish is frozen in brine at about 1°F. The same tins frozen at -3 or -4 °F. will give the appearance of group D.

(4) These ice crystals grow in size during storage, rapidly at a high temperature and slowly at a low temperature. The rate of growth is not dependent on the original size of the crystals. The extent of growth is complete within a month or so of storage and depends upon the temperature of storage.

(5) The changes occurring in the tissues - a dehydration or concentration of the tissue substances - is reversible (partially at least) when the fish have just been frozen by any method. After they have been stored for a month or less however the changes have become irreversible, and the fluids withdrawn from the tissues into the ice crystals are not reabsorbed by the tissues during defrosting.

(6) The rate of defrosting is of no practical importance in determining the amount of recovery (reabsorption of fluids by the tissues).

(7) It is impossible to freeze large masses of fish without causing a considerable degree of crystal growth in their central portions.

(8) The freshness of the fish frozen has very little bearing on the structural changes occurring during the process. This is chiefly from a commercial standpoint: very fresh fish do show a slightly smaller degree of crystal growth during the freezing than do fish caught two or three days before and kept well iced.

(9) While there is little difference from a culinary standpoint between brine frozen and air frozen cod, when freshly frozen, the brine frozen shows a very marked superiority after storage.

Even the brine frozen fish however is not as good after storage as when freshly frozen. This suggests the advisability of freezing fish only as a means of increasing the marketing or distribution time, and not for indefinite storage periods. A lower storage temperature would prolong the period of usability of the fish.

(10) Other varieties of fish have been examined, though less thoroughly, and the changes occurring in them are in the main similar to those shown by the cod. Differences in the salinity and fat content of the muscle, and the amount of connective tissue present will modify somewhat the changes occurring during the freezing. This may necessitate a more rapid freezing rate for one type of fish than for another in order to obtain a satisfactory product.

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Appendix

During the course of this work, D.A. MacFadyen also started to explore the realm of freezing, but from a different viewpoint.

It has been long known, that flesh, which has been commercially frozen, drips on defrosting. That is, a fluid exudes from the thawed meat spontaneously or on application of a light pressure, which will not come from fresh unfrozen material. This fluid contains valuable nutritive elements and what is probably more important, many of those complexes to which the typical flavour of the substance is due. Besides this, it is objectionable to the housewife, and is evidence that permanent sturctural changes have occurred in the meat.

MacFadyen's problem was to investigate this drip in relation to the problem of refrigeration of fish. His preliminary work has consisted of measuring the quantity of the fluid, after finding suitable methods, leaving till later any possible chemical examinations.

I will not attempt to describe his method or details of the work, as his report will cover this, but a broad statement of general findings is of interest because many of the specimens examined by him have also been reported on in this paper. For example most of the fish of Experiments II, III (Tables XV, XX, and XXII), IV, VI, VII (Ross' fillets) VIII and VIII-A, have been used by MacFadyen in his investigation.

He found it necessary to divide the liquid obtained from the frozen fish into two classes.

(a) That which exuded from the fish spontaneously during the defrosting process, which is called the "Drip", and
(b) That which could be pressed out, in his special press by a pressure of one or two atmospheres.- after defrosting.

Rate of Freezing

The more rapidly the fish are frozen, the greater the amount of fluid that can be expressed from them but the smaller amount of drip there is. The rates of freezing ranged from being almost instantaneous (frozen in carbon dioxide - snow and ether) to being very slow (frozen in air at 20°F. over a period of many hours).

Fresh or unfrozen fish gave no drip and practically no fluid could be squeezed out. The most rapidly frozen fish - that frozen in carbon dioxide ether - too gave no drip, but on pressure much fluid was expressed, and expressed rapidly. Brine frozen fish gave very little drip but fluid was readily expressed on pressure, though less in amount and more slowly than in the rapidly frozen fish. Air frozen fish gave still more drip, but very little fluid could be expressed by pressure.

Storage

After the fish had been stored for two weeks or so the amount of drip and the amount of expressible fluid both increased greatly. Air frozen, brine frozen, and carbon dioxide frozen specimens all gave approximately the same amount of total fluid, but the proportion of drip to expressed fluid varied greatly.

Brine frozen fish continued to show but little drip, and carbon dioxide frozen none, while air frozen gave considerable. In each case considerable fluid could be expressed, but less was obtained from the air frozen than the brine frozen, which was in turn less than the carbon dioxide frozen.

Discussion

These results and those of the histological examination are very complementary.

The microscopical findings of larger crystals and greater tissue damage occurring with slow freezing explain nicely the greater amount of drip in the slowly frozen fish.

The becoming permanent of the changes due to freezing, during storage as shown by the microscope also explains the large increase of drip and expressed fluid. The air frozen fish, containing a relatively few but large crystal holes might be likened a very coarse rubber sponge which would hold but little water. The brine frozen fish with its much larger number of smaller crystal holes (yet with the ratio of crystal hole to total volume of fish about the same as in the air frozen) might be likened to a very fine grained sponge, which by capillary attraction will hold much water, even though comparatively slight pressure will express it.

On the other hand freshly frozen fish, especially if brine or carbon dioxide frozen, when allowed to thaw show considerable expressible fluid and yet no histological changes seem to have occurred. Probably a finer histological technique would show some changes to account for this expressible fluid.

Thus the positive findings of the two methods confirm one another; and the fact that some positive findings by one method are associated with negative findings by the latter, points rather to a lack of delicacy of the latter.

Some Theoretical Aspects

Tammann (1) , working with pure supercooled liquids, reports that while the crystallizing velocity actually decreases with a decrease in temperature, there is a temperature zone in which this does not hold true. For example, at just below the freezing point, when the crystallizing velocity should be high, the heat of solidification is sufficient to raise the temperature slightly and so stop the process altogether. On the other hand, at a slightly lower temperature, the heat of solidification doesn't make such great differences and so crystallizing continues. Hence the apparent or real crystallizing velocity increases with decrease in temperature (from the freezing point to a few degrees below) to a maximum, and then decreases as the temperature is lowered still further.

The number of crystallizing centres however increases with the degree of supercooling to a maximum, and then decreases to nearly zero if the degree of supercooling is very great.

One would judge that in freezing the fish (a) the air freezing produced such a small degree of supercooling as to bring out but a few crystallizing points; (b) the brine freezing at 0° to 15°F. caused a greater degree of supercooling and allowed many more crystallizing centres to develop while the rate of crystal growth was still large; (c) the brine freezing at -3° to -7°F. slowed slightly the crystal growth rate; and (d) carbon dioxide freezing practically stopped crystal growth and possibly reduced the numbers of crystallizing centres. The expressed fluid findings however would indicate the probability of submicroscopie crystals forming, and in unknown numbers.

This consideration suggests the advisability in experimental work of endeavouring to separate "rate of freezing" from "temperature of freezing". Probably in any fast freezing the degree of supercooling would have to be great and many crystal centres would form; but the nearer the freezing point that the process was kept at, the slower would be the crystal growth, and so if the cooling conditions were sufficiently effieient (amount of exposed surface, circulation and specific heat of cooling agent, etc.) the resulting product would contain small crystals . This is precisely what brine freezing does: by increasing cooling efficiency, it freezes more rapidly and at a higher temperature.

Another aspect of the problem, equally hard to dissociate from rate of, and temperature of freezing, is the final temperature attained by the frozen material. If any colloidal solution is cooled slowly, water is withdrawn in the form of crystals of ice and the degree of dehydration of the colloid that occurs, depends entirely on the temperature to which the solution is cooled.(Working with gelatine - Moran (2)). Of course if the solution is very rapidly cooled this does not at once occur but does so later during storage. This question of phase law equilibrium and dehydration of colloid brings up the question of "Free and bound water" (Callow (2)), and in the case of fish is complicated by the importance of the size of the crystals present as well.

The term "Free water" is used merely to name that fraction of the colloidal solution which can be removed from the solute or disperse phase in the form of ice. It is nearly all removed as ice during the first few degrees of cooling below the freezing point, and but little more can be removed by lowering the

temperature many more degrees. However this small portion of water (dispersion medium) which is removable by lowering the temperature may be all important in determining whether or not the change is irreversible. For example we know (Moran (2)) that egg yolk frozen at a temperature of less than -6°C. is irreversibly changed, and the changes are reversible if the freezing is above this temperature. Our figures fail to show whether or not there is any such critical temperature for fish.

Thus, from the point of view of tissue or colloid dehydration at equilibrium, storage at high temperatures and freezing at high temperatures would seem advisable. However, the other factors predominate, and render this consideration of minor importance.

The fish are so rapidly frozen that the phase law equilibrium is not obtained, and some unstable or metastable ice complexes are formed. The more of these that are formed, the less dehydration there has been, the faster the freezing must have been, and the greater the degree of supercooling which has been obtained. No matter how great the rate of crystal growth which has been present, if the fish is frozen in a short enough time crystals could not have grown large. Then, during storage in order for phase rule equilibrium to be attained, these "brine-colloid-ice" complexes have to be broken up into brine, colloid, and water ice. This process is much more rapid at a high temperature than at a low. Hence while the degree of dehydration ultimately obtained at a low temperature may be greater than at a high, the difference is not great and the low temperature is desirable because the equilibrium will be so long delayed.

Storing at a low temperature is also indicated for another reason. The crystals which have formed during the freezing may all increase or decrease slightly in size, with fluctuations of temperature. On the other hand, without any change in the degree of dehydration of the colloid, some of the crystals may grow large at the expense of the smaller. This is important because these large crystals may be said to bruise the tissues (Taylor (3)), and autolysis is promoted by bruising. This growth of some at the expense of others through the medium of the free phases or degrees of freedom present is greatly slowed by a low storage temperature. The maintaining of a stationary storage temperature too should assist in preventing this phenomenon from occurring. (Ogura and Fugikawa (2))

Very small live entities have been frozen so rapidly that no internal crystallization can have occurred as is evidenced by the regaining of the living functions on defrosting. For examples see Stiles (1), reporting on (a) Staminal hair of the Tradescantia virginica, (b) Frog muscle (c) Rotifers and Nematodes. However it is impossible to freeze pieces of fish of commercial size rapidly enough to prevent any crystal formation, and being impossible, adherence to the methods suggested above, should give the best results-namely-fast freezing, and low constant storage temperatures.

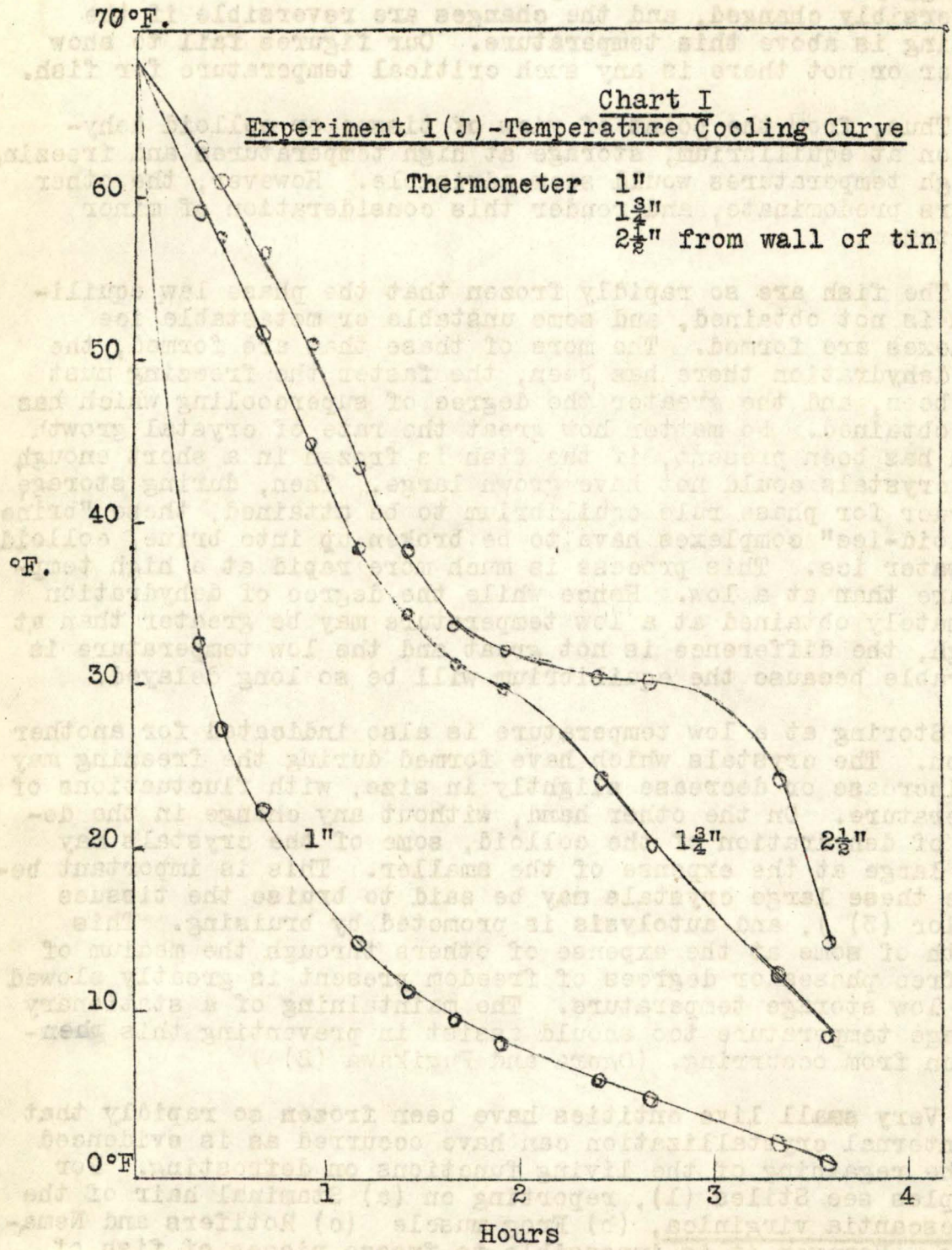


Chart I

Experiment I(J)-Temperature Cooling Curve

Thermometer 1"
1¾"
2½" from wall of tin

Chart II

Experiment I(K) - Temperature cooling curves

Thermometers 0.5"
1.5"
2.5" from tin

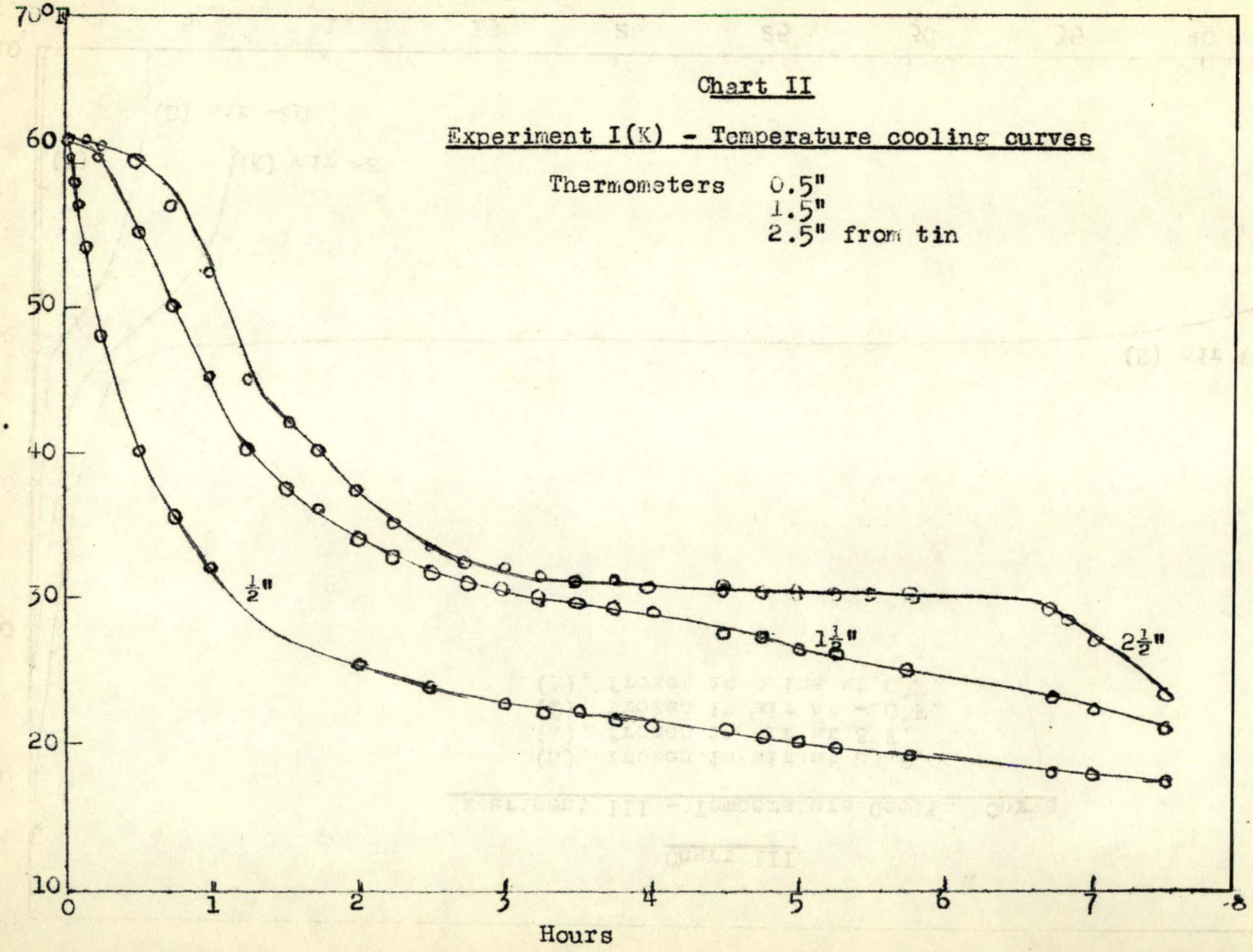
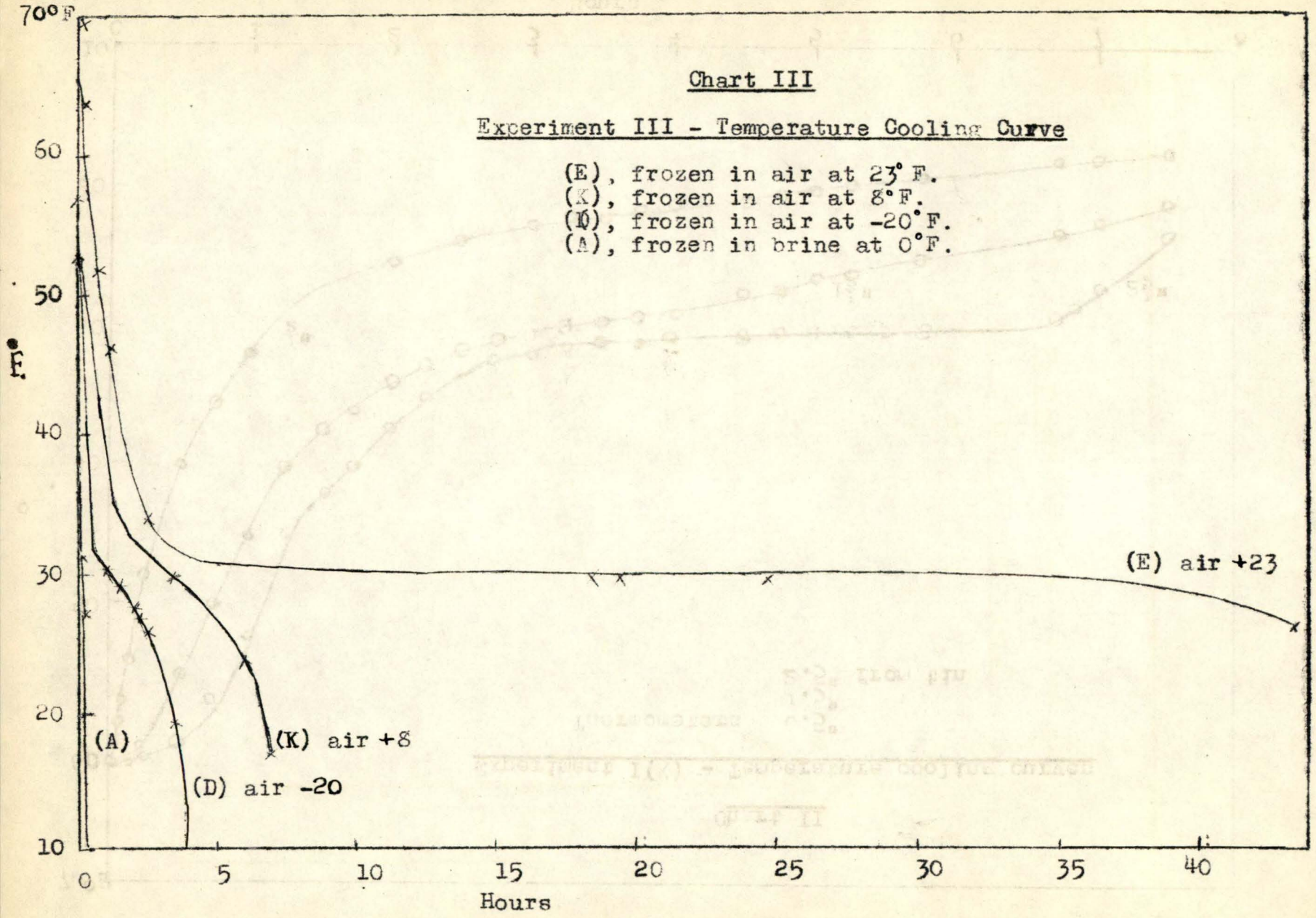


Chart III

Experiment III - Temperature Cooling Curve

(E), frozen in air at 23° F.
(K), frozen in air at 8° F.
(D), frozen in air at -20° F.
(A), frozen in brine at 0° F.



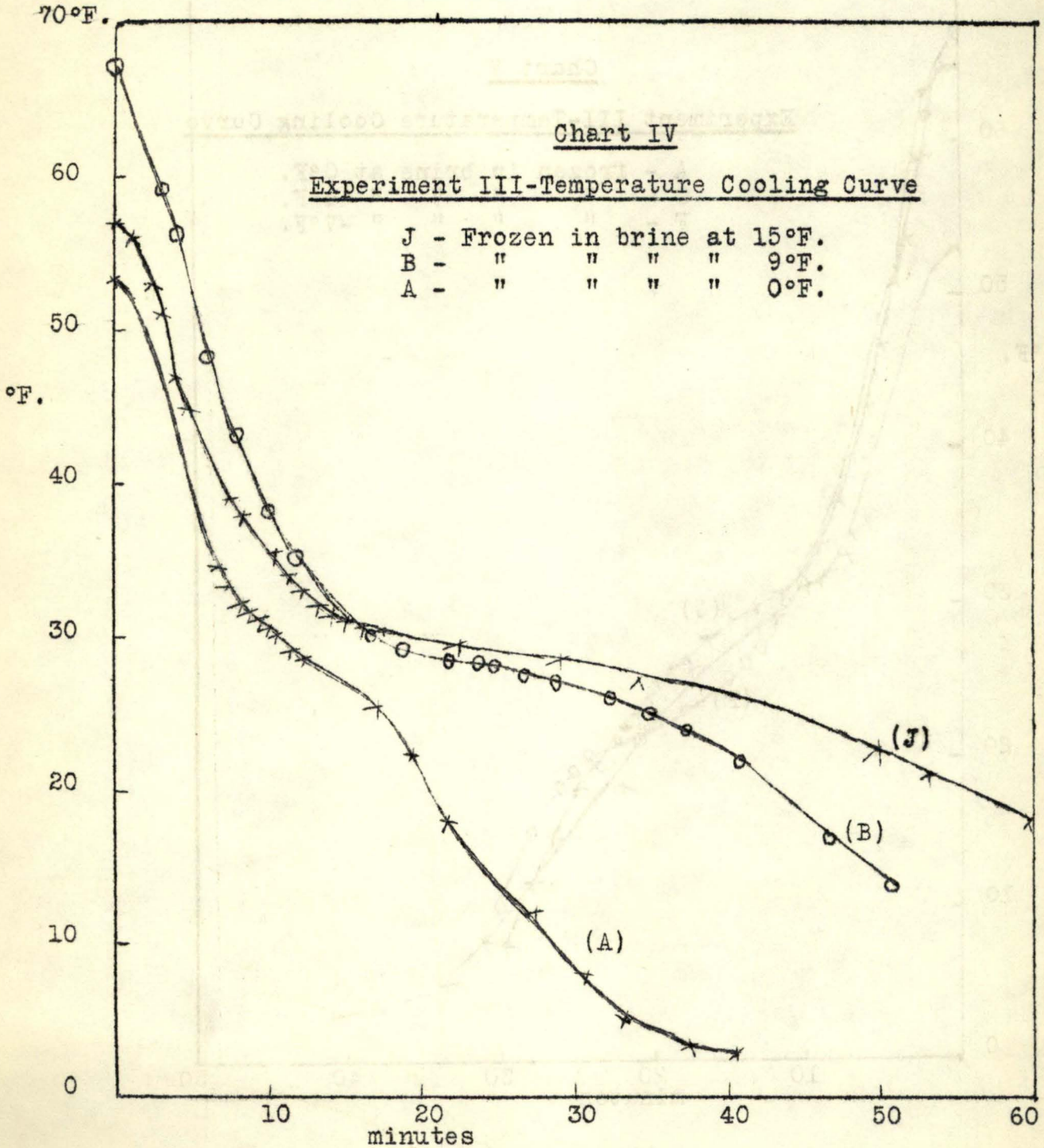


Chart IV

Experiment III-Temperature Cooling Curve

J - Frozen in brine at 15°F.
B - " " " " 9°F.
A - " " " " 0°F.

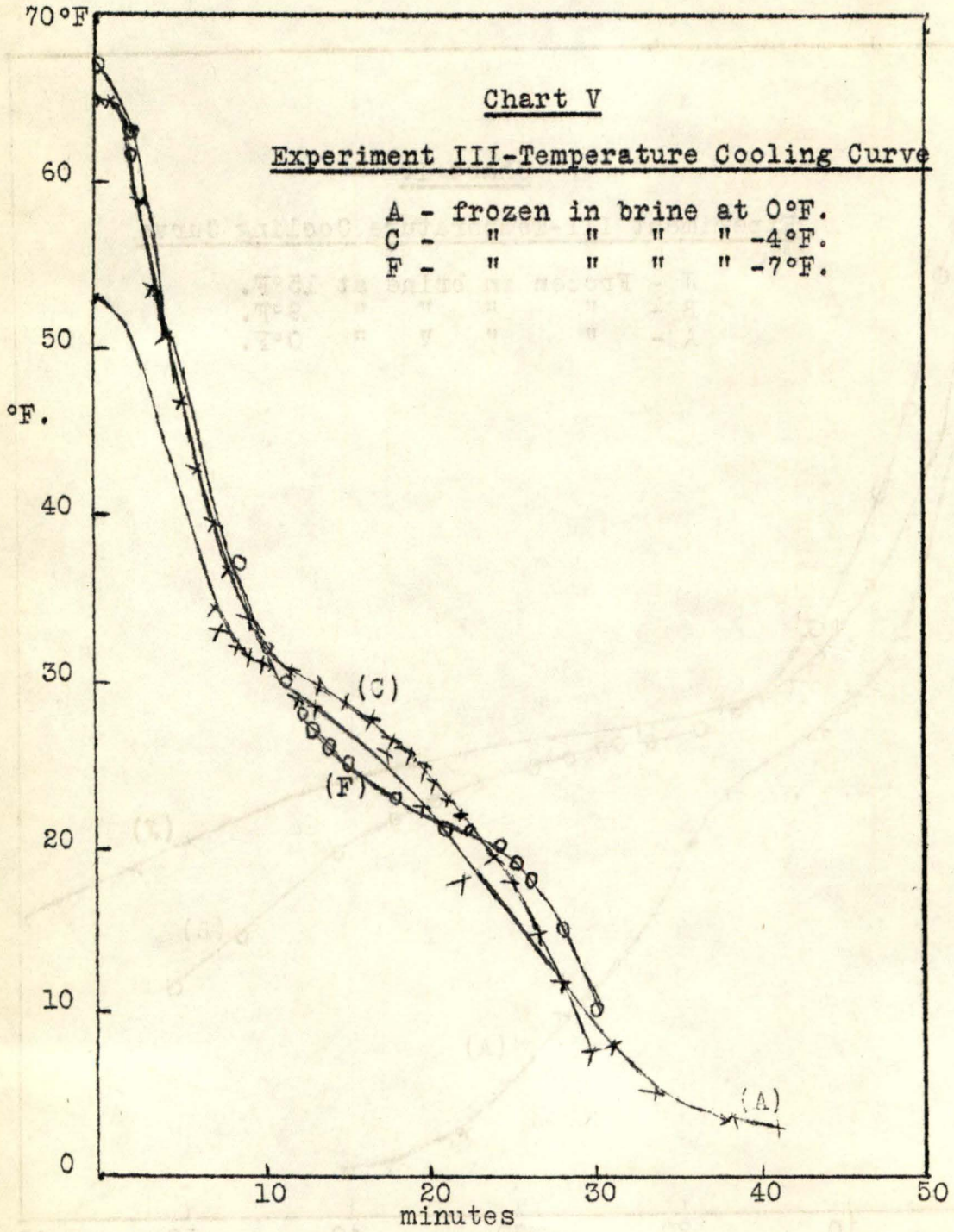


Chart V

Experiment III-Temperature Cooling Curve

A - frozen in brine at 0°F.
C - " " " " -4°F.
F - " " " " -7°F.

Chart VI

Experiment VIII-A - Freezing and Defrosting-Table XXV

Freezing - during freezing, in brine at 0°F.
A - defrosting in water bath at 100°F.
B - " " " " " 68°F.
C - " " " " " 50°F.
D - " " " " " 32°F.