A STUDY ON ICE CRYSTALS IN FROZEN MEAT

A thesis presented in partial fulfilment of the requirements for the degree Master of Technology in Food Technology at Massey University

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SUMMARY

A study on ice crystals in frozen beef has been made in an effort to gain more information on the process of ice formation in meat during freezing and to determine a quantitative relationship between the rate of freezing and ice crystal formation. As a measure of the rate of freezing the rate of ice formation was calculated from the slope of measured time/temperature cooling curves and eutectic data for meat, and by a calculation method based on a solution of the heat transfer equations by finite difference calculus. This latter method was found to be rather unsatisfactory as a result of the large changes in specific heat in the temperature range 27-30°F.

It was found that the rate of freezing and the physiological condition of the meat prior to freezing had a marked effect on ice crystal size and location, relative to tissue structures. These phenomena have been explained in terms of the water permeability characteristics of the major structural components of the tissue, and the water binding ability of the proteins. There was considerable variation in ice crystal size for any given rate of freezing and physiological condition. The variation was dependent on the rate of freezing and a relationship between rate of ice formation and maximum ice crystal size was established. Ice was found intracellularly, extracellularly within fibre bundles and extracellularly between fibre bundles at high rates of ice formation and only extracellularly at low rates.

"O le aso ma le matainatila, o le aso ma le filigaafa."

(Each day brings new problems, and new measures must be found to solve them.)

Samoan proverb

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INTRODUCTION

In 1924 in a paper delivered to the Fourth International Congress of Refrigeration T. Moran (1924) stated, "Probably nothing is more needed by the refrigerating industry than an adequate theory of the freezing of tissues, because on it must be based any important advance in practical procedure other than such as one of a purely engineering nature."

Addressing an advanced study institute held at the University of Strathclyde in September 1966, H.W. Symons said, "Important though it is, technology occupies a relatively minor position in the structure of the frozen food industry."

It is of interest then to consider just what technological advances have been made, particularly in the freezing of meat, since the advent of commercial food freezing. The most significant advance is the introduction of quick freezing techniques such as blast and plate freezing. With fruit and vegetable products there is an obvious improvement in quality to be gained by using these methods but with meat the improvement is not so obvious. The reason for adopting these techniques for meat freezing has been the economic pressure caused by ever-increasing production.

A. THE EFFECTS OF FREEZING ON MEAT

Probably the first detailed study of the effects of freezing on meat was made by Richardson & Scherubel (1908). They found from histological studies that water separates as ice from the meat fibres during freezing and that on thawing much of this water is lost from the meat as drip, particularly from freshly cut surfaces. Some of the nutritional value in the form of minerals and soluble protein was lost with the drip. Subsequent studies by Plank, Ehrenbaum & Reuter (1916) and Fearon & Foster (1922) demonstrated the existence of a critical rate of cooling. At cooling rates faster than this there was little drip loss on thawing and the thawed meat had similar organoleptic characteristics to fresh meat. Histological studies (Koonz & Ramsbottom, 1939, Ramsbottom & Koonz, 1939) showed that in meat frozen at speeds greater than the critical rate, ice crystals were located within the muscle fibres, or cells, so that the fibres were not dehydrated during freezing. Unfortunately the critical cooling rate has been considered too high to be practical in the commercial freezing of large meat cuts.

1. Freezing Rate and Drip Formation

The existence of a critical freezing rate, as demonstrated by the absence of drip after freezing beef by immersion in liquid air, was confirmed by Cook et al (1926) in an extensive study of the factors influencing drip formation from frozen beef. They found that the amount of drip increased with decreasing freezing rate. Also the age and type of animal, the condition of the beef before freezing and the thawing temperature, influenced the amount of drip formed. From studies of changes in juices extracted from minced unfrozen beef with freezing, it was concluded that changes in the sarcolemma (cell wall) rather than the plasma were causing the drip (Vickery, 1926).

The observation that the amount of drip increases with decreasing freezing rate was subsequently confirmed by a number of workers.

(Moran, 1932 & 1933, Du Bois et al, 1940, Moiseva & Piskareva, 1959, Hankins & Hiner, 1940, Hiner et al, 1945, Ramsbottom & Koonz, 1939, Hiner & Hankins, 1941).

However, it has been suggested (Emprey, 1933) that the rate of freezing has no significant effect on the amount of drip formed, but rather the amount of thaw drip was a function of the hydrogen ion concentration. It has been confirmed (Sair & Cook, 1938, Ramsbottom & Koonz, 1940) that pH could markedly influence the amount of drip on thawing. Ramsbottom & Koonz (1940) went further and showed that pH was not the only factor involved and that the condition of the meat prior to freezing was just as important, this was not a pH affect. They also demonstrated by histological studies that the lack of drip in pre-rigor meat was the result of intracellular ice formation rather than high pH. In post-rigor meat, for an equivalent rate of cooling, the ice formation was extracellular and tended to increase in size with ageing, an interesting observation as the amount of drip decreased with ageing. However, they did demonstrate that the amount of drip formed was dependent on the rate of freezing for each stage of ageing.

Extended studies on drip (Ramsbottom & Koonz, 1941) indicated that storage of the frozen meat greatly increased thaw drip and that the increase over a year was independent of storage temperature. The initial differences caused by different rates of freezing were still apparent after a year's storage.

2. Freezing Rate and Organoleptic Quality

Opinions on the influence of freezing on the organoleptic properties of meat tend to be rather more conflicting than those on dip formation. Generally there is an increase in tenderness when meat is frozen and the amount of increase is related to the rate of freezing, being greater with rapid freezing. (Hankins & Hiner, 1940, Tressler et al, 1932, Du Bois et al, 1940, Dansk Koleinstitut, 1944, Nicholas et al, 1947, Hiner & Hankins, 1941, Hiner et al, 1945, Moiseva & Piskareva, 1959). This gain in tenderness, however, is lost after about six months storage at $0^{o}F$ (Hiner & Hankins, 1941, Nicholas et al, 1947). Against this there is a considerable amount of evidence which can find no conclusive relationship between organoleptic properties and freezing (Paul & Child, 1937, Bray et al 1942, MaCoy et al, 1949, Moran, 1932 & 1933, Fin, 1933, Pennsylvania Agricultural Experiment Station, 1953, Valdecantos, 1963). A notable feature of the work done by Valdecantos is that he attempted to assess the difference in quality produced by the different rates of cooling within a single piece of meat. With the freezing conditions he used the differences in cooling rate were small and he was unable to detect any significant quality differences.

The colour of frozen meat is influenced by surface ice crystal size (Taylor, 1930 & 1931) being a darker colour with large ice crystals. The colour difference is lost on thawing so this is of little importance to the consumer who buys thawed meat. During storage the surface colour changes with the breakdown of oxymyoglobin to metmyoglobin, this change is not related to ice crystal size (Ramsbottom & Koonz, 1941).

The size of the surface ice crystals influences the type of freezer burn which develops (Kaess & Weidemann, 1962 & 1967). The finer the crystals the whiter and less visually acceptable the burn becomes.

In general ice crystals do not grow significantly during the storage of frozen meat when large intracellular ice crystals or extracellular ice is the predominant form (Ramsbottom & Koonz, 1941). When finer ice crystals are present and storage is at high temperatures (15-20°F) some regrowth is apparent (Moran & Hale, 1932).

3. Freezing Rate and Ice Crystal Formation

Much of the conflicting opinion on the way in which freezing affects the quality of meat has arisen from a lack of appreciation of all the factors which govern the rate of cooling in meat which makes comparison of findings difficult.

Moran (1931) was one of the first people to appreciate that the rate of freezing was not uniform throughout a piece of meat being frozen. He found from studies of ice formation in frozen meat that the largest crystals were formed at the centre and the smallest at the surface. Du Bois et al (1940) and Tressler & Du Bois (1940) found that ice crystal size in air blast frozen beef and chicken was related to air temperature and air velocity. Hiner et al (1945) conducted tenderness, drip and histological examinations on beef frozen in still air at various temperatures. They found a decrease in crystal size with freezer air temperature. Ramsbottom et al (1949) demonstrated that crystal formation varied with position relative to the cooling surface and that the freezing time as determined from measured cooling curves also varied with position. They observed both extracellular and intracellular ice within the one piece of meat. Koonz and Ramsbottom (1938) made a detailed histological study of the types of ice formation in frozen poultry meat, particularly of intracellular ice formation at high cooling rates. They observed that crystallisation was profoundly influenced by cooling rate but did not define the cooling rate in a way which could be quantitatively related to their observations.

More recent histological studies of frozen meat (Biro, 1962, Kaess, 1956, Chizhov & Kulmanova, 1966) suggest that the condition of the meat before freezing has a greater influence on the histological structure of the frozen meat than the rate of freezing. Kaess found that in pre-rigor frozen bovine muscle, ice crystals form predominantly intracellularly. In slow frozen post-rigor muscle, ice crystals appeared extracellularly, and in quick frozen post-rigor tissue both extracellularly and intracellularly. The number of ice crystals were found to increase and their sizes to decrease with increasing rate of freezing, independently of the physiological condition of the tissue.

Plank (1941) made an analytical study of the rate of ice front advance during freezing and suggested that this has some influence on ice crystal size. He determined the effects of freezing conditions and the shape and size of blocks of material being frozen on the rate of ice front advance and concluded that these factors could cause considerable differences in ice crystal sizes within a piece of frozen food. This he considered would be detrimental to the product as it would encourage recrystallisation by virtue of the fact that large ice crystals have a lower vapour pressure than small ice crystals. To avoid this he proposed freezing methods which would produce uniform freezing rates throughout the material. The implications of this work as regards ice crystal size and quality have been largely overlooked by subsequent workers in their attempts to relate ice crystal size or rate of freezing to quality changes in frozen foods. In order to make his analytical solution, Plank made certain assumptions which limit its application to real systems. However, more recently numerical solutions based upon the use of finite difference approximations to heat conduction equations have been used successfully to calculate cooling rates during the freezing of meat (Earle & Earl, 1966, Cullwick, 1967). By combining the cooling rate so obtained with eutectic data for meat it is possible to predict the rate of ice formation in terms of the rate of change of the fraction of freezable moisture frozen. In this way it is possible to express the rate of freezing in fundamental terms.

B. THE OBJECTIVES OF THIS STUDY

The object of this study was to evaluate ice crystal formation in frozen meat in terms of the rate of change of the fraction of freezable moisture frozen, to observe what effect the physiological condition of the muscle prior to freezing has on ice crystallisation with regard to the rate of freezing, and to determine the degree of variation in ice crystal formation within a single block of frozen meat.

From a fuller understanding of the process of ice formation in frozen meat it is hoped that it will be possible to determine with more certainty what influence the rate of freezing has on the initial quality of frozen meat and upon subsequent quality changes on storage.

CHAPTER I

THE PHYSICS OF ICE CRYSTAL FORMATION IN BIOLOGICAL MATERIALS

This chapter reviews and evaluates the literature on ice crystal formation and attempts to provide a coherent picture of present knowledge in this field.

Crystal growth depends on many factors, intrinsic and extrinsic to the material being frozen. Intrinsic factors are derived from the nature of the system itself; including water content, structure and shape, the nature and concentration of solutes and disperse phases, and the thermal properties which will be similar to those of water because of the high water content. Extrinsic factors include the ambient temperature, the thermal and physical properties of the environment and the nature of the interface between the environment and the freezing body.

A. EFFECT OF SHAPE AND THERMAL PROPERTIES ON ICE CRYSTAL FORMATION

In the introductory chapter it was observed that ice crystal size and type of ice formation were not uniform throughout a piece of frozen meat. Plank (1916) outlined the different histological pictures presented in a cross section of a cylinder of frozen meat and then went on to calculate the distribution of freezing rates in the cross section. The calculation of freezing rate distribution was later extended to systems of other geometries (Plank, 1941).

The intrinsic thermal properties which influence ice formation in frozen tissue are the specific heat and thermal conductivity of the tissue.

The value of these properties alter during the course of freezing and this has a marked effect on the shape of the cooling curve particularly through the zone of maximum ice formation. In meat it has been observed that the thermal conductivity is anistropic, being about 15% higher when the heat flow is parallel to the muscle fibres than when it is perpendicular to them (Lentz, 1961, Hill et al, 1967).

Extrinsic factors influence the rate of heat removal from the surface of the freezing material and hence the rate of cooling and ice formation within it. The extrinsic factors are the ambient temperature and the surface heat transfer coefficient. The surface heat transfer coefficient is influenced by packaging material, and where heat transfer is by convection, by the thermal and physical properties of the coolant fluid.

The action of some of these factors on the rate of freezing can be seen in the following expression (due to Plank) for the rate of freezing within a slab cooled from both sides in a plate freezer.

$$W = A\frac{dx}{dt} = \frac{T_f - T_a}{L} \quad \frac{1}{1/_h + {}^{Z1}{}^{o}/_{2K_o}} \qquad (1.1)$$

Where $W = A\frac{dx}{dt}$ is the rate of advance of an ice front of unit area, i.e. the volumetric rate of ice formation,

$T_f - T_a$ is the difference between ambient and product freezing point temperature, L is the volumetric latent heat of freezing,

h is the overall surface heat transfer coefficient,

$Z = \frac{2l}{l_o}$,

l_o is the slab thickness,

l is the thickness of the frozen layer,

K_o is the thermal conductivity of the frozen layer.

This expression assumes that all the water in the material is frozen at a fixed temperature (the freezing point) and that the product is uniformly at the freezing point before the commencement of freezing. By making these assumptions it follows that the specific heat of the frozen material is constant and the change in heat content during freezing is contained in the latent heat term; the thermal conductivity of the frozen material has a constant value and the specific heat and thermal conductivity of the unfrozen material can be ignored as there is no cooling of the unfrozen material prior to freezing.

For a cylinder the equation becomes:-

$$W = \frac{T_f - T_a}{L} \quad \frac{1}{\frac{1}{h} + \frac{r_o}{K_o} \ln \frac{r_o}{r}} \qquad (1.2)$$

Where r_o is the radius of the cylinder,

r is the radius of the unfrozen core.

For a sphere:-

$$W = \frac{T_f - T_a}{L} \quad \frac{1}{\frac{1}{h} + \frac{r_o^2}{K_o}\left(\frac{1}{r} - \frac{1}{r_o}\right)} \qquad (1.3)$$

Where r_o is the radius of the sphere,

r is the radius of the unfrozen core.

Solutions of these equations are illustrated in Figs 1, 2 and 3. From these graphs the relative importance of the shape factors included in equations (1.1), (1.2) and (1.3) becomes apparent.

Where the surface heat transfer coefficient is high the temperature of the freezing medium has a marked effect on the rate of ice formation, particularly at the surface. When it is low the influence of freezing medium temperature is not great. Slab thickness has less effect on the rate of ice formation when the surface heat transfer coefficient is low, causing a smaller range of ice formation rates between centre and surface. Shape can cause considerable changes in freezing rate, particularly when the surface heat transfer coefficient is high.

Analytical solutions for the general problem of heating or cooling of systems of simple geometry have been developed (Carslaw & Jaeger, 1959).

The general equations for cooling are:-

For a slab:-

$$Y = \sum_{n=1}^{\infty} \frac{2Nu \, Cos \, (\alpha_n \frac{x}{a})}{(Nu^2 + Nu + \alpha_n^2) Cos \, \alpha_n} \, e^{-\alpha_n^2 F_o} \qquad (1.4)$$

	h (BTU/ft²hr°F)	T_a (°F)	l_0 (ins)
1.	40	-40	2.5
2.	40	-40	5.0
3.	40	-10	2.5
4.	5	-40	2.5
5.	5	-40	5.0

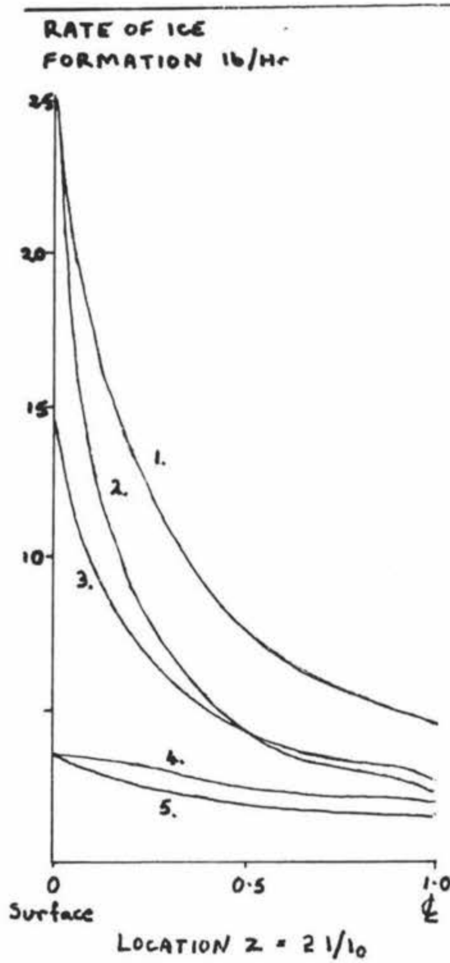


FIG. 1. RATE OF ICE FORMATION IN A SLAB OF MEAT COOLED FROM BOTH SIDES.
EQUATION (1.1) K = 0.9 BTU/ft.h.°F. L = 110 BTU/lb

	h (BTU/ft²hr°F)	T_a (°F)
1.	40	-40
2.	40	-10
3.	5	-40
4.	5	-40

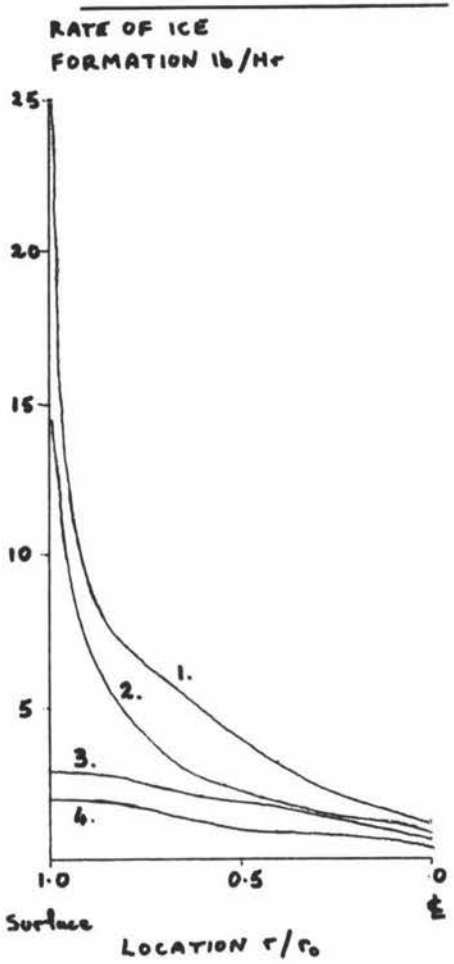


FIG. 2. RATE OF ICE FORMATION IN A CYLINDER OF MEAT DURING FREEZING.
EQUATION (1.2) r_0 = 2 ins.

	h (BTU/ft²hr°F)	T_a (°F)
1.	40	-40
2.	40	-10
3.	5	-40
4.	5	-10

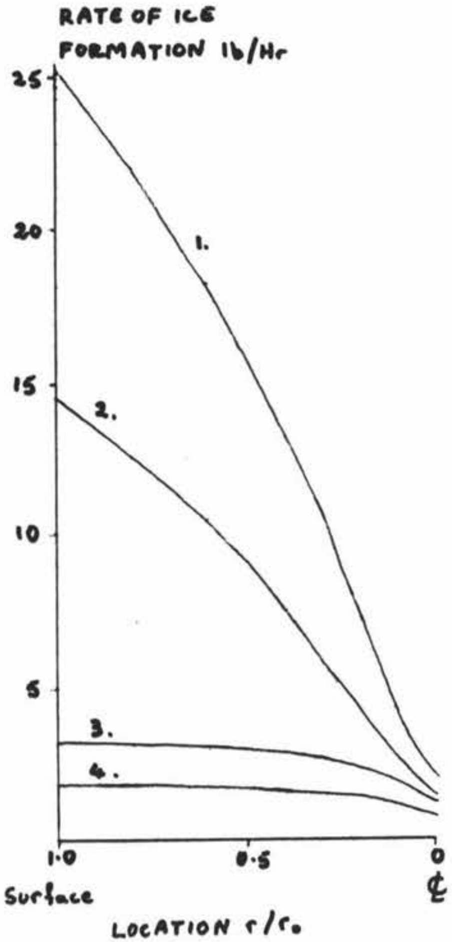


FIG. 3. RATE OF ICE FORMATION IN A SPHERE OF MEAT DURING FREEZING.
EQUATION (1.3) r_0 = 2 ins.

For a sphere:-

$$Y = \sum_{n=1}^{\infty} \frac{2Nu \text{ Sin } (\gamma_n \frac{x}{a})}{\frac{x}{a} (\gamma_n^2 + Nu^2 - Nu) \text{ Sin } \gamma_n} e^{-\gamma_n^2 F_o} \qquad (1.5)$$

For a cylinder:-

$$Y = \sum_{n=1}^{\infty} \frac{2Nu \, J_o \, (\beta_n \frac{x}{a})}{(\beta_n^2 + Nu) \, J_o \, (\beta_n)} e^{-\beta_n^2 F_o} \qquad (1.6)$$

Where Y is the unaccomplished temperature change as a fraction of the total possible change after a given time,

$\frac{x}{a}$ is a dimensionless expression for the location of the point under consideration,

a is the half thickness of the body,

x is the location of the point with respect to the centre,

$Nu = \frac{ah}{k_o}$ is the Nusselt number,

$F_o = \frac{K_o \theta}{a^2 \rho C_p}$ is the Fourier number,

θ is the time from commencement of cooling,

ρ is the density of the body,

C_p is the specific heat,

α_n is the nth root of the transcendental equation $\alpha \tan \alpha = Nu$,

β_n is the nth root of the transcendental equation

$\beta \, J_1 \, (\beta) = Nu \, J_o \, (\beta)$,

$J_o \, (\beta)$ and $J_1 \, (\beta)$ are Bessel functions of the first and second orders,

γ_n is the nth root of the transcendental equation

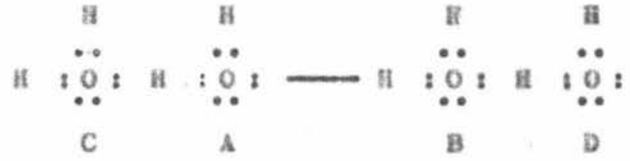
$\gamma \cot \gamma = 1 - Nu$.

These equations illustrate the complex nature of the process of heat removal. The process becomes further complicated when a change of state is involved as the intrinsic thermal properties of the body change as cooling proceeds. Solutions by numerical approximation which take this into account have been developed, such as the one outlined in Chapter IV.

B. THE MOLECULAR STRUCTURE OF WATER AND ICE

Liquid water has several unique properties indicating that there are fundamental structural differences between it and most other liquids. They include the high melting and boiling points, the unusually high heat capacity, the decrease in volume on melting and the subsequent contraction between 0^{o} and $4^{o}C$. These apparent anomalies are due to strong intermolecular interactions, mostly hydrogen bonding between molecules. The H_2O molecule can participate in four hydrogen bonds, two of them involving the two hydrogens of the molecule, and two the lone pair of electrons of the oxygen and the hydrogens of two neighbouring molecules (Fig. 4). This tetrahedrally directed bonding is involved in the crystal structure of ice.

In liquid water there is a resonance in the hydrogen bonding between molecules which may be explained as follows:-
(Franks, 1965)



A hydrogen bond has been formed between the molecules A and B. B is more basic than it would be in the unbonded state increasing its tendency to form another hydrogen bond by accepting a proton from D.

The δ- charge would then shift to D making D more basic. Similarly A is more acidic increasing its tendency to form a hydrogen bond with C by donating a proton to the electron pair of C resulting in a shift of the δ+ charge. The polarity of this large C - A - B - D unit confers additional stability to the A - B bond. Therefore once one bond forms there will be a tendency for a chain of bonds to proliferate. Such a process could be arrested by thermal fluctuation. Hydrogen bond formation is exothermic, the heat released by bond formation could break other bonds resulting in the unzipping of the structure as the smaller units so formed will be less stable. To minimise the unfavourable free energy change caused by the entropy loss associated with immobilising molecules by hydrogen bonding, tetra-bond formation rather than the linear process first described would make the clusters of hydrogen bonded molecules more compact and is therefore more likely to occur (Nemethy and Sheraga, 1962). This does not necessarily mean that the clusters will have the same ordered lattice structure as ice, which is only one of several possible tetra-bonded arrays.

The structure of water can be considered then, as one of clusters of hydrogen bonded molecules embedded in and in equilibrium with monomeric unbonded water. These clusters form and melt as hydrogen bonds form and break with local energy fluctuations. At a given temperature, the total number of hydrogen bonds existing in the liquid and the equilibrium between clusters and unbonded water are determined by the requirement that the free energy of the system should be a minimum.

On freezing the tetra-bonded structure becomes more ordered and proliferates as a fixed crystal lattice similar to the tridymite form of SiO_2 in which the silicon atoms form a hexagonal array. Fig. 5 (a & b) illustrates the molecular structure of ice.

If the transformation to ice is particularly rapid, a cubic lattice structure is formed. Amorphous ice has been formed by condensing water onto cold surfaces in a vacuum (Pryde & Jones, 1952, Blackman & Lisgarten, 1956).

C. ICE CRYSTAL NUCLEATION IN PURE WATER SYSTEMS

The first step in the conversion of water to ice is the formation of a nucleus around which the ice can grow. Nucleation occurs by the chance aggregation of a number of water molecules as a result of local energy fluctuations in the manner described in the previous section. When the number of water molecules in the cluster, or nucleus, becomes sufficiently large for the addition of one more molecule to decrease the free energy of the cluster it has reached critical size and may grow to form an ice crystal.

The number of nuclei in supercooled water, i.e. liquid water at a temperature below its freezing point, containing a given number of molecules is given by:-
(Turnbull et al, 1948)

$$Nc \propto e^{-\Delta G/kT} \qquad (1.7)$$

Where Nc is the number of nuclei,

T is the absolute temperature,

k is Boltzmann's constant,

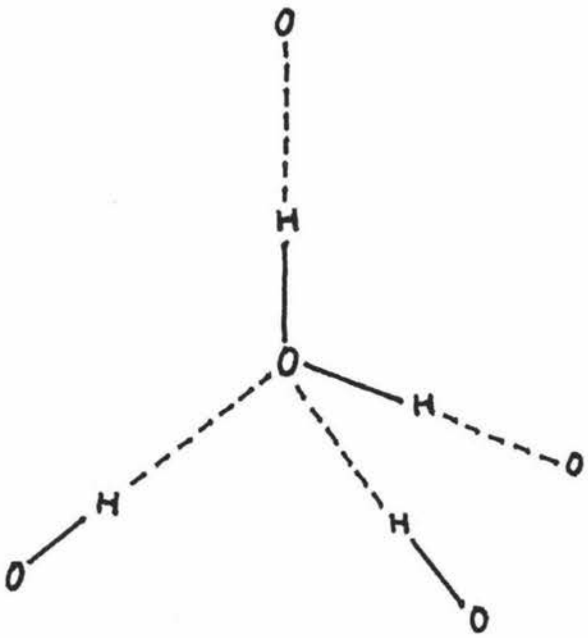


FIG. 4. HYDROGEN BONDING IN H_2O MOLECULE IN ICE

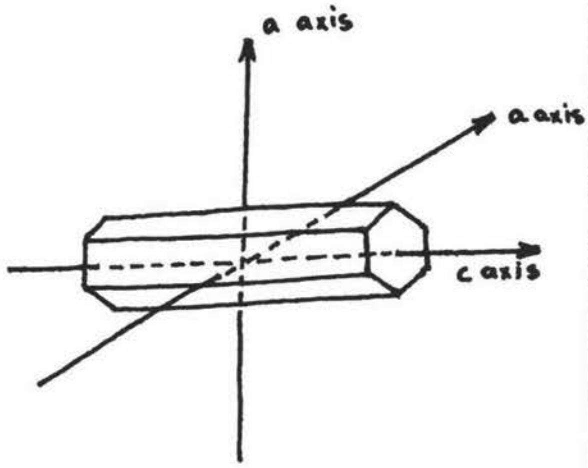
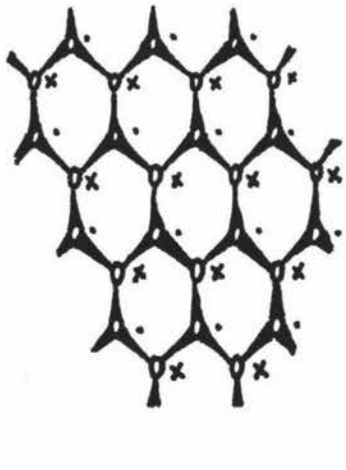


FIG. 5(a). ICE CRYSTAL UNIT

Crystal cross section showing molecular arrangement with in crystal unit.

Oxygen atoms labled x are in a lower plane than the paper and · in a higher plane.



Longitudinal section.

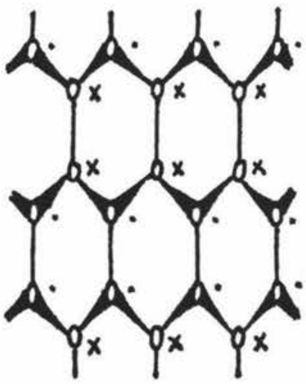


FIG. 5(b). MOLECULAR STRUCTURE WITHIN ICE CRYSTAL UNIT.

ΔG is the free energy of formation of a nucleus and:- (Stephenson, 1960b)

$$\Delta G = i(\mu_i - \mu_w) + b\,\sigma\, i^{2/3} \tag{1.8}$$

where μ_i is the chemical potential of ice,

μ_w is the chemical potential of water,

i is the number of molecules in the nucleus,

b is a shape factor,

σ is the surface free energy between ice and water.

Now if $\Delta G\,(i + 1) > \Delta G(i)$ the nucleus is subcritical in size and will generally decay, if $\Delta G\,(i + 1) < \Delta G(i)$ the free energy of the nucleus is decreased by the addition of a molecule so growth can occur.

Hence the critical size is given by:-

$$\Delta G\,(i + 1) - \Delta G(i) = 0$$

or approximately:-

$$\mu_i - \mu_w + \tfrac{2}{3}\, b\,\sigma\, i^{-\frac{1}{3}} = 0$$

Now $\mu_w - \mu_i = \Delta S\,(T_o - T)$

$= \Delta H\,(T_o - T) / T$

Where S is the entropy decrease on freezing,

H is the heat of freezing,

T_o is the equilibrium temperature between bulk solid and liquid.

$$\therefore\ \Delta G^* = \frac{4\, b^3\, \sigma^3\, T_o^{\,2}}{27\, \rho^2\, L_f^{\,2}\, (T_o - T)^2} \tag{1.9}$$

$$i^* = \left(\frac{3\, b\, \sigma\, T_o}{3\rho L_f\, (T_o - T)}\right)^3 \tag{1.10}$$

Where * denotes critical size,

ρ is the density of ice,

L_f is the latent heat of fusion.

Thus in supercooled water:-

$$N_c^* \propto e^{-\Delta G^*/kT} \tag{1.11}$$

This theory has been further extended to determine the net forward rate at which nuclei in an equilibrium distribution of critical nuclei become longer than critical, i.e. start to grow (Turnbull, 1949).

This is given by:-

$$\frac{dN}{d\theta} \doteq n_1 \frac{kT}{h} e^{-\left(\frac{\Delta g^* + \Delta G}{kT}\right)} \tag{1.12}$$

Where N is the number of critical nuclei starting to grow,

n_1 is the number of molecules per unit volume of liquid,

h is Planck's constant,

g* is the free energy of activation for diffusion across the water:ice interface,

θ is time.

Integration of this equation yields:-

$$N(\theta) = \int_0^\theta N' \left[T(\omega)\right] \left[1 - w(\omega)\right] d\omega \tag{1.13}$$

Where $N'\left[T(\omega)\right]$ is the rate of creation of nuclei longer than critical at temperature T and time ω,

$w(\omega)$ is the fraction of water which has been converted to ice at time ω,

$\left[1 - w(\omega)\right]$ is the aqueous fraction at time ω.

Including a location factor so that this expression is applicable to a point within a body of water cooled from its surface the equation becomes:-
(Stephenson, 1960a)

$$N(\theta, \vec{x}) = \int_0^\theta N' \left[T(\omega, \vec{x})\right] \left[1 - w(\omega, \vec{x})\right] d\omega \qquad (1.14)$$

Where $\vec{x}$ specifies the location of the point.

The amount of water available for new critical nuclei to form in will decrease as existing nuclei grow so from these equations it can be seen that the number of ice crystals per unit volume will depend on the rate of temperature decrease and the lowest temperature reached during the nucleation period.

D. NUCLEATION IN BIOLOGICAL SYSTEMS

Suspended particles in an aqueous system can provide sites for ice crystal growth. This results in less supercooling. The amount of supercooling possible in a system containing suspended particles is related to the particle size, being greater for the smaller the particle (Mallet, 1965). The chemical nature of the particle also influences its ability to provide a site for crystal growth.

It is thought that the action of the non-aqueous material could be to lower the surface free energy between the developing nucleus and the water (Knight, 1967). Fig. 6 illustrates the surface free energies acting in the nucleus, water, particle system.

Balancing surface free energies:-

$$\sigma_{ws} = \sigma_{wi} \operatorname{Cos} \theta + \sigma_{is}$$

$$\sigma_{wi} = \frac{\sigma_{ws} - \sigma_{is}}{\operatorname{Cos} \theta} \qquad (1.15)$$

Where σ_{ws} is the surface free energy between water and the particle,

σ_{is} is the surface free energy between ice and the particle,

σ_{wi} is the surface free energy between water and ice,

θ is the contact angle between ice and the substrate.

The smaller θ becomes with increasing affinity between ice and the substrate the smaller σ_{wi} becomes. From equations (1.9) and (1.10) it can be seen that for a given amount of supercooling the number of molecules in the critical nucleus, and the free energy of formation of a critical nucleus, will be lowered. The change in free energy of formation will be very much greater than the change in the number of molecules.

The free energy of activation for diffusion of molecules across the liquid/ice interface, Δg^*, will be greatly different in colloidal systems. For a mixed colloidal system the colloid which individually causes the greatest lowering of Δg^* could be considered as controlling value of Δg^* in the mixed system. Work by Rohatgi and Adams (1967a) on solutions containing a mixture of solutes indicates that ice crystal size is not controlled by the solute with the lowest diffusivity but rather by the solute present in the greatest concentration. When the solutes were in similar concentrations the effect was intermediate to the effects produced by pure solutes.

However, all of their solutes were electrolytes and their range of diffusivities was not great.

This indicates that the effect of mixed solutes on the activation energy for diffusion is complicated.

From equation (1.12) it can be seen that only a small difference in the value of $(\Delta g^{*} + \Delta G^{*})$ can cause a large difference in the value dN/dt. Thus minor changes in the composition of a colloidal system could cause major changes in the number of ice crystals formed.

Within a body being frozen, the cooling rate through the temperature zone of maximum ice formation generally declines with increasing distance from the cooled surface. At low cooling rates, crystal growth on to existing crystals will remove the water before the temperature has fallen enough to permit new nuclei to form. At high cooling rates, crystal growth is not rapid enough for this to happen so that new crystal nuclei will form. Thus in a body being frozen nucleation can only be expected to occur in a surface zone where the cooling rate is high.

Rindfret (1966), in studies on freezing erythrocytes in drops and cylindrical containers, observed a marked depression in cooling curves at the surface which could be interpreted as supercooling. This was only observed at the surface, indicating that deeper into the drop the site for ice growth is provided by the advancing ice front rather than by new nuclei.

The nature of nucleation sites in biological material has not been determined. It has been suggested that immobilised water structures associated with proteins may provide suitable sites for growth, but there is no conclusive proof of this.

Meryman (1963) demonstrated that more supercooling occurs in small sections of tissue than in larger pieces. He explains this by stating that the probability of finding a site for nucleation is greater in a large section. It could also be explained by his temperature sensing device being situated further from the nucleation site in a large section.

By extending the probability concept it is possible to explain an apparent preference for extracellular crystallisation. If each cell is considered as containing an isolated body of water with the extracellular fluid as a continuous body of water, then although the extracellular fluid is a small volume compared to the total volume of water, it is large compared to the volume contained within a single cell. Hence there is a greater probability of extracellular ice formation. Other theories explaining this phenomenon are; the freezing point of the extracellular material might be higher than the intracellular material and that the intracellular material might be deficient in nucleation sites.

These theories are speculative, and in view of the fact that the phenomenon of intracellular verses extracellular crystallisation is dependent on the rate of freezing, are of doubtful value.

It has been shown that where a surface has been provided as a site for nucleation that the nucleation temperature is dependent on the nature of the solute and its concentration (Lusena, 1955).

E. ICE CRYSTAL GROWTH IN BIOLOGICAL SYSTEMS

1. Effect of the Disperse Phase on Ice Crystal Growth

An analytical solution for the growth of nuclei of supercritical size does not appear to have been developed.

For the crystal to grow the heat of crystallisation must be removed from the advancing ice front. The problem of ice crystal growth is therefore one of both mass and heat transfer. While analytical solutions are available for unsteady state heat transfer or mass transfer to or from a moving phase boundary (Danckwerts, 1950) none have been developed for simultaneous mass and heat transfer.

(a) Freezing point depression and eutectic phenomena

Solutes modify the thermal behaviour of an aqueous system firstly by depressing the freezing point, then, as ice starts to form, the increasing solute concentration further depresses the freezing temperature until both the solute and remaining water crystallise out at the eutectic temperature. This means that the heat of fusion of ice will not be released at a fixed temperature as in a pure water system but over a temperature range, which explains the variation of the apparent specific heat with temperature in biological materials as they pass through the freezing zone. As ice has a much higher thermal conductivity than water, the thermal conductivity of biological materials will also change with temperature as the proportion of ice increases through the freezing zone.

In a single solute system there will be a small plateau on the cooling curve corresponding to a rise in specific heat at the eutectic temperature, the size of which will depend on the amount of solute present. In a system with mixed solutes where one solute is present in small amounts, as would frequently be the case in biological materials, the plateau will be correspondingly small. At the eutectic temperature the mixture may crystallise out as non hydrated solute and ice, as a solute hydrate and ice, or completely as the solute hydrate. The form of crystallisation can be explained in terms of the relative fluidity of the solute and its crystalline radius. For example, the ions F^-, NH_4^+, Ag^+, K^+, Rb^+, Cs^+, U^-, Br^- are all of similar size and fluidity to water molecules. Electrolytes composed of these ions would cause little disruption of the water structure. The ice lattice is less amenable to disruption and expels these ions in a non hydrated form.

Some organic solutes such as glycerol and dimethylsulphoxide do not crystallise out at all and form a vitreous solid (Meryman, 1966). With this type of solute there is no eutectic temperature determinable by electrical conductivity measurements or differential thermal analysis (Rey, 1960) although two eutectic values are given for glycerol in the International Critical Tables. In a system containing both a solute which normally has a eutectic temperature and glycerol, the solute does not crystallise out but becomes incorporated into the glass.

(b) Crystal morphology

Luyet (1960) has studied the effects of different types of solute on the morphology of ice crystals growing in thin films of solution frozen between glass slides. With pure water he found that the ice formed as hexagonal plates. In solutions, various modified forms were obtained depending on the rate of cooling and the concentration of the solute. At high cooling rates or solute concentrations, the hexagonal symetry became more irregular and gave way to plain spherulite disc formations in which the component needle-like ice elements were arranged radially around a centre.

At higher cooling rates the structure in the plain spherulite disc forms disappeared to form transparent evanescent spherulite discs. Examination under polarised light indicated that crystalline structure was still present. X-ray diffraction analysis suggested that cubic ice was present in varying proportions depending on the cooling rate, solute concentration and type of solute (Luyet, 1966a). On rewarming the cubic ice changed to the opaque hexagonal form.

The nature of the solute has a marked affect on the ice structure formed. The extent of the change compared with pure water is related to the size of the disperse molecules, particularly chain length. For example, unmodified hexagonal structures were not observed under any condition of concentration or cooling rate in gelatin solutions, which such structures could be observed in bovine albumin at slow cooling rates (Luyet, 1959). In gelatin the molecules form an extended gel network while in albumin the protein is globular. Similar observations can be made with non-protein material such as polyvinyl-pyrrolidone and glycerol. Beef muscle juice extracted by centrifuging severely macerated muscle behaved in a similar manner to bovine albumin (Luyet, 1959 & 1960).

This work provides a useful indication of the influence solutes can have on ice development but gives no indication of what happens at the ice front in the freezing system illustrated in Fig. 7.

(c) Solute concentration at the ice/water interface

Rohatgi et al (1968) have derived an expression for the concentration gradient developed in the liquid between ice crystals in an advancing ice front.

$$c = \frac{L^2}{8D} \bar{c} \frac{df_s}{d\theta} \qquad (1.16)$$

where c is the concentration difference as a function of the centre-to-centre spacing of adjacent ice crystals,

$\frac{df_s}{d\theta}$ is the freezing rate,

$\bar{c}$ is the initial solute concentration,

D is the solute diffusivity,

f_s is the fraction of freezable water frozen.

The concentration gradient is therefore dependent on the ice crystal population density, the rate of ice formation and the solute diffusivity, i.e. the velocity of the ice/liquid interface of adjacent crystals moving towards each other, and the solute diffusivity, Fig. 7. The rate of growth of adjacent crystals towards each other is dependent on the rate of ice front advance. The greater the speed of the ice front advance the more rapid this movement will be and the greater the concentration of solutes at the surface of the crystal.

Water must diffuse through this colloidal matrix at the ice surface if growth is to occur, so that it is likely that the matrix

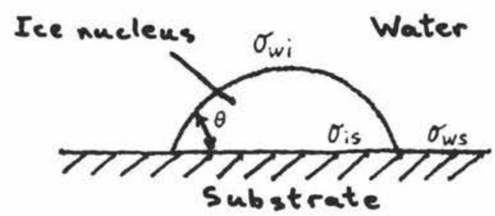


FIG.6. HETEROGENIOUS NUCLEATION

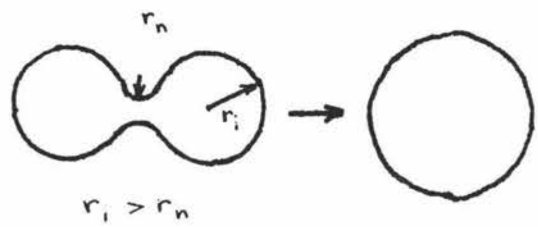


FIG.8. SINTERING OF ICE SPHERES

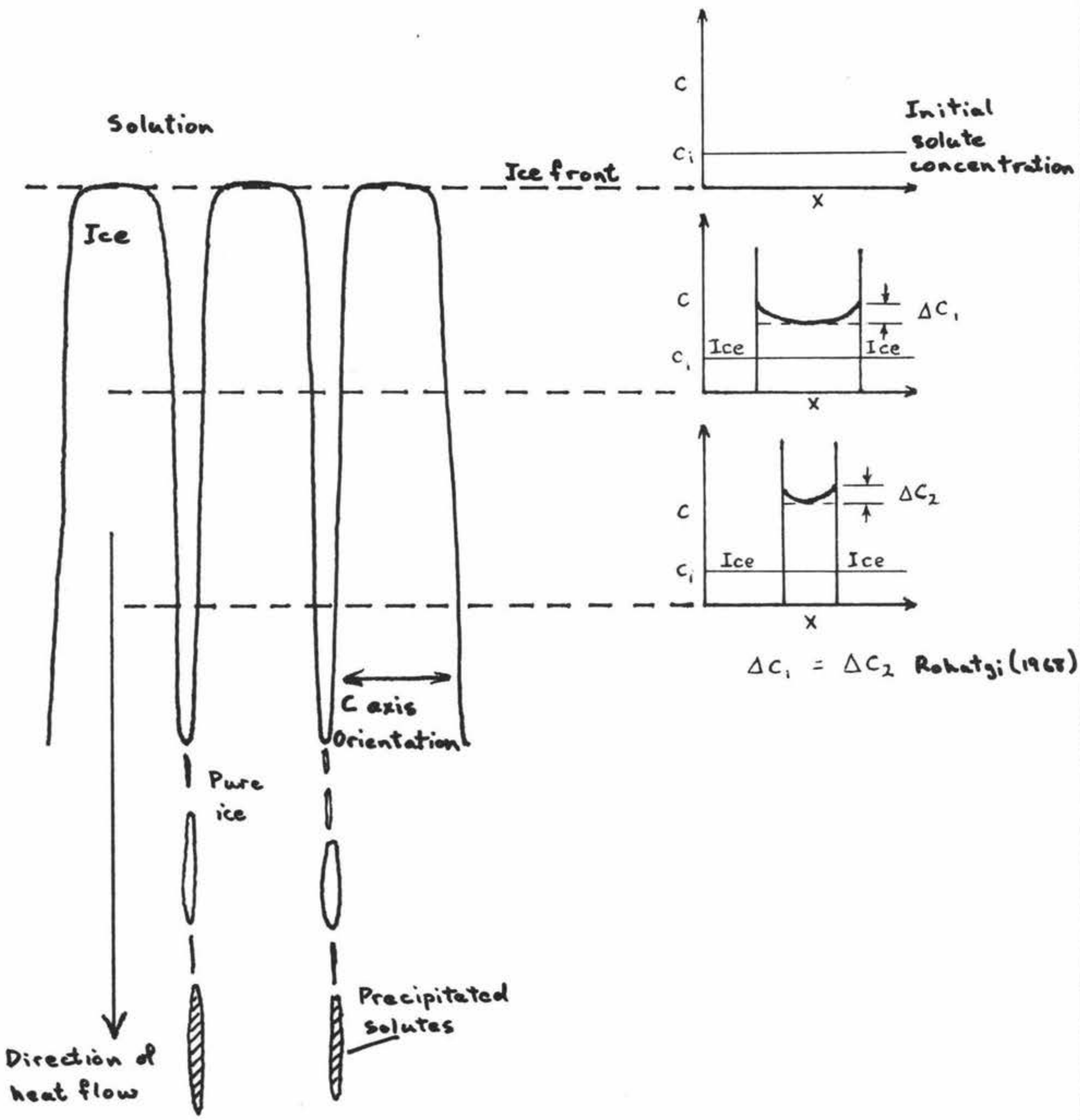


FIG.7. ICE FRONT MORPHOLOGY IN SOLUTIONS.

will influence the free energy of activation for diffusion of water molecules to the ice surface which is probably as an important factor in the growth process as it is in the nucleation process.

At the ice front (the commencement of freezing) the amount of ice which has to be formed to achieve a given increase in solute concentration or temperature fall is very much greater than behind the ice front (towards the end of freezing). The rate of solute rejection from the ice into the solution will be lower for a given speed of ice interface advance. For this reason the speed of the ice interface's advance will be high. Behind the ice front and between the crystals the solute concentration is higher so that less ice has to be formed for a given temperature fall and more solute has to be rejected. This is balanced by a lower speed of interface advance so the concentration build up at the surface compared to the average concentration between crystals tends to remain constant (Rohatgi & Adams, 1967a).

If the initial solute concentration is high the intercrystal spacing in the frozen material will be less than expected from consideration of the relative amounts of solute and water originally present. This is due to the formation of side branches from the crystals and the inclusion of pockets of solute into the sides of the crystals.

(d) Ice crystal numbers and distance from the cooled surface

The rate of ice formation decreases with increasing distance from the cooled surface so the velocity of transverse ice growth would be expected to decrease also. The resulting decrease in concentration gradient in the intercrystalline liquid enables the crystals to grow to a larger size before growth is hindered. This means that some ice crystals will grow larger while others will disappear, as there is insufficient water available at any given point to enable all of them to grow larger.

Aitken (1966) has shown that in frozen fish the c axis orientation of the ice crystals is always perpendicular to the direction of heat flow. He failed to detect any preferred orientation in the perpendicular plane but this could be because the range of cooling rates in his samples was not very great and the rates were probably high. Studies of lake ice (Knight, 1967) suggest that a preferred orientation in the perpendicular plane is likely. This preferred orientation could explain why the growth of some crystals is continued in preference to others. No explanation for a preferred orientation in the perpendicular plane has been given.

2. Effect of Cellular Structure on Ice Crystal Growth

The cell wall presents a barrier to ice crystal growth. Luyet (1959), studying the freezing of thin sections of meat with a cold stage microscope, found that the rate of growth down the length of the fibres was approximately twenty times the rate of growth across them. He claimed that the difference was because the ice encounters more cell walls in growing across the fibres which causes the difference in rate. The direction of heat transfer in his freezing system is predominantly perpendicular to the plane of the section so this phenomenon is unlikely to be related to the anisotropic thermal conductivity of meat which is determined by fibre direction.

The cell wall is not completely impermeable to ice growth. Meryman (1966) put forward two possible theories for the cell wall acting as a partial barrier to ice growth;

(a) that the growth of ice on one side of the cell wall continues until the cell wall becomes sufficiently disrupted to become permeable to ice growth and

(b) the cell wall properties do not alter, but the ability of ice to pass through the wall increases with decreasing temperature.

The first theory cannot be totally discounted as there is evidence to show that the cell wall can be damaged by freezing to the extent that it becomes completely permeable to crystal growth on subsequent freezing (Love, 1966). Mazur (1965) has made a thorough investigation of the second theory and found that it is at least part of the truth.

He found that as a suspension of cells in water is cooled below $0^{o}C$ both the extracellular and intracellular water at first remains unfrozen and supercools. At some temperature the extracellular water freezes which then initiates intracellular freezing provided that the temperature is sufficiently low and the cooling rate sufficiently high. He reasons that the volume of water within a given cell is tiny in comparison with the extracellular water so that nucleation within the cell is unlikely. As an alternative explanation for preferred extracellular crystallisation it is possible that the complex nature of the disperse phases within the cell reduces the nucleation rate at a given temperature in comparison with the nucleation rate outside the cell thus reducing the chances of nucleation within the cell at that temperature.

intracellular ice formation could be initiated by extracellular ice growing through water filled channels in the cell membranes. These channels are very small and for ice to grow throughthem the ice crystal must be correspondingly small; so small in fact that they are not much thicker than the diameter of the nucleus they were formed from.

From the equation for the radius of a critical cluster in water, (Stephenson, 1960b)

$$r^* \doteq \frac{500}{(T_o - T)} \text{ Å} \qquad (1.17)$$

where $T_o - T$ is the amount of supercooling, it can be seen that for a given channel size whether intracellular ice will form or not will depend on the amount of supercooling.

When extracellular water freezes, its vapour pressure drops below that of the supercooled water within the cells thus producing a tendency for water to flow out of the cell. If cooling is slow there is sufficient time for an appreciable quantity of water to leave the cell before it cools to a temperature low enough for intracellular freezing to occur. By the time that temperature is reached most of the water has left the cell so that the residual contents are incapable of freezing. With rapid cooling there is not time for sufficient water to leave the cell to prevent intracellular freezing when the temperature drops sufficiently.

The factors that influence the outflow of water are summarised in the following equations:-

$$\ln \frac{P_i}{P_e} = \frac{L_f}{R} \left(\frac{\Delta T}{T\,T_f}\right) + \ln \frac{x_i}{x_f} \tag{1.18}$$

and
$$\frac{dV}{d\theta} = \frac{k\,A\,RT}{v_1} \ln \frac{P_i}{P_e} \tag{1.19}$$

Where P_i is the intracellular vapour pressure,

P_e is the extracellular vapour pressure,

x_i is the mole fraction of intracellular water at freezing temperature T_f,

x_f is the mole fraction of intracellular water at temperature T_1,

θ is time,

k is the permeability content of the cell for water,

A is the cell surface area,

V is the volume of intracellular supercooled water,

v_1 is the molar volume of water,

L_f is the molar heat of fusion.

Equation (1.18) relates supercooling to the vapour pressure differential, and equation (1.19) the rate at which water leaves the cell to the vapour pressure differential. Knowing the cooling velocity, dT/dt, these equations can be combined to give the amount of supercooled water in the cell in relation to temperature:-

$$Te^{b(Tg-T)} \frac{d^2V}{dT^2} - \left[(bT+1)\,e^{b(Tg-T)} - \frac{ARK_g\,uT^2}{BV(V+nv_1)}\cdot\right] \frac{dV}{dT} = \frac{L_f A K_g}{B v_1} \tag{1.20}$$

Where B is the cooling velocity (assumed to be uniform),

b is the temperature coefficient of the permeability constant,

K_g is the known permeability constant at temperature Tg.

From this equation it can be seen that the important biological parameters are the surface to volume ratio of the cell and hence the size of the cell, and the absolute permeability of the cell to water as a function of temperature. The greater the surface to volume ratio and the higher the permeability, the higher the cooling rate required to produce intracellular ice; thus the cooling velocities required to produce intracellular ice vary considerably for different cells.

This theory was developed for isolated cells in suspension not in tissues, and whether the same conclusions can be drawn regarding nucleation and freezing of intracellular and extracellular fluids in tissues is not known. It is likely, though, that the same factors govern the growth of ice across tissue cell membranes.

3. Recrystallisation

Ice does not stop changing form once all the freezable water is frozen, but continues to change at an ever decreasing rate.

Two forms of recrystallisation are commonly recognised, irruptive recrystallisation or the transition of cubic to hexagonal ice, and migratory recrystallisation or grain growth.

Migratory recrystallisation is probably caused by vapour pressure differences between surfaces of differing curvature. In pure polycrystalline ice preparations where there is no obstacle to the transfer of water molecules between crystals recrystallisation can be rapid. The process is retarded by reduced temperatures.

Kingery quoted by Mazur (1966) has analysed the thermodynamics of sintering two small ice spheres of equal radius. See Fig. 8. He compares the chemical potential and vapour pressure differences between a planar surface and the convex surface of the spheres with the chemical potential and vapour pressure differences between a planar ice surface and the concave surface of the neck and concludes that the neck will grow in thickness to minimise the difference. He derives equations for the kinetics of various molecular transport mechanisms by which this could occur and by comparison with experimental results suggests that surface diffusion is the most probable mechanism.

In the systems containing non aqueous material between the ice crystals the diffusion process is hindered and the process of recrystallisation retarded considerably.

CHAPTER II

HISTOLOGICAL EXAMINATION OF FROZEN MEAT

In this chapter methods of examining and photographing ice crystal in frozen meat are discussed and the methods used in this study are outlined.

Histological examination of frozen meat is difficult, as the disruption of the tissue by ice formation is reversible on thawing. To overcome this difficulty the tissue must be examined in the frozen state or be fixed in some way so that the disruption is maintained on warming the tissue to room temperature. Even in the frozen state ice formations are not fixed, particularly at temperatures approaching the freezing point, so that it is important that temperatures be kept low during fixation and during observation of the frozen tissues.

A. TEMPERATURE AND ICE CRYSTAL STABILITY

Some idea of the temperatures necessary to reduce recrystallisation to an insignificant level for light microscopy can be gained from the work of Luyet (1962) on recrystallisation in rapidly frozen solutions of various organic solutes. By examining the frozen solutions under polarised light he was able to detect any major change in the ice crystallisation. His findings are summarised in the following two tables.

Table 1: Recrystallisation in solutions of albumin, gelatin, sucrose and glycerol rapidly frozen into evanescent spherulites.

Solute	Concen-tration	Freezing Bath Temp.	Stable Temp.*	Transition Temp.*
Albumin	30%	-40°C	-8°C	-5°C
Gelatin	30%	-40°C	-15°C	-10°C
Sucrose	30%	-40°C	-40°C	-30°C
Glycerol	26%	-90°C	-90°C	-60°C

Table 2: Recrystallisation in solutions of albumin, gelatin, sucrose and glycerol frozen at moderate rates into irregular dendrites.

Solute	Concen-tration	Freezing Bath Temp.	Stable Temp.*	Transition Temp.*
Albumin	10%	-20°C	-8°C	-3°C
Gelatin	10%	-20°C	-15°C	-5°C
Sucrose	5%	-40°C	-40°C	-15°C
Glycerol	10%	-70°C	-70°C	-50°C

* Stable means that no change was observed after 5 hours at this temperature.

* Transition temperature means that the change went to completion in one minute or less at this temperature.

He found that the transition temperature was affected only slightly by the concentration of solute. In mixtures of solutes the recrystallisation temperature was intermediate to those of the individual solutes. This also applied to mixed electrolyte and non-electrolyte systems.

The interference caused by solutes in muscle juice extracts has been shown to be greater than sucrose but not as great as gelatin (Rapatz & Luyet, 1959) so that the spherulite transition temperature could be expected to lie between -10 and -30°C and the irregular dendrite transition between -5 and -15°C. On this basis it was considered that for this study -30°C (-22°F) would be a safe temperature for histological examinations.

B. METHODS OF EXAMINATION

1. Formalin Fixation

The most frequently used method of tissue fixation (Richardson & Scherubel, 1908, Cook et al, 1926, Kaess, 1967) involved immersing pieces of the frozen meat in a 10% formalin solution chilled to its freezing point, -9°C. Apart from the temperature being rather high to be sure of avoiding recrystallisation, fixation tends to be uncertain due to inadequate penetration of the formalin (Koonz & Ramsbottom, 1938). Glycerol or certain salts could be added to the formalin solution to reduce its freezing point but this would not assist penetration.

2. Freeze Drying

Koonz and Ramsbottom used a modification of the Altmann freeze drying technique for cytological fixation in their studies. This involved freeze drying the frozen tissue at -32°F at an estimated 0.0003 mm mercury pressure for 24 hours. The dried pieces were removed from the drying chamber after it had been allowed to warm to room temperature and transferred directly into molten paraffin for embedding then sectioning and examination.

Simpson (1941) made an experimental study of the freeze drying technique for tissue preparation. He found that the drying temperature should be held below -30°C for adequate structural preservation and that some form of chemical fixation was necessary before embedding. To achieve this he soaked the tissue in absolute alcohol before embedding. He recommended embedding in celluloidin to avoid the heat damage encountered with paraffin embedding. Koonz & Ramsbottom (1941) apparently recognised the limitations of paraffine embedding as in their later studies they transferred their dried tissue pieces to alcohol and then embedded in celluloidin.

Modifications have been made to the freeze drying technique to enable simpler equipment to be used. Saravacos (1967) developed a laboratory freeze drying apparatus for studies on the freeze drying process in foods using 4Å molecular sieve desiccant to absorb the water vapour removed from the product. Data provided by the suppliers of the desiccant (Union Carbide Ltd.) indicated that it would be a satisfactory desiccant at -30°C so that its use in preparation for histology was possible.

Meryman (1959) developed a method of freeze drying for histological preparation which relied on the passage of a very dry stream of air over the section to dehydrate it. He recirculated the air through a bed of pelletised 4Å molecular sieve to remove the moisture picked up from the section. The time required to achieve desiccation by this method was too long for it to be used in the present studies.

A disadvantage inherent in all freeze drying methods is the number of phase changes involved with their attendant risk of histological distortion. They are:- ice : air : solvent : liquid : embedding medium : solid embedding medium.

In the present studies freeze drying was rejected on the grounds that mechanically it is a complicated technique and simpler methods were available.

3. Freeze Substitution

Kaess (1967) used a freeze substitution technique in which he soaked sections of the frozen meat in dry ethanol for several weeks at -78°C before staining with a Haemotoxylin Eosin stain and embedding in paraffin. This technique was developed by Simpson (1941) as a control method in his freeze drying studies.

Freeze substitution, like freeze drying, is used as a preparative technique for tissues in electron microscopy. At this level of magnification it yields superior results to freeze drying (Bullivant, 1968). One of the more dangerous phase changes in freeze drying (air/solvent) is eliminated by this technique. A serious disadvantage of this method is the time taken (two or more weeks depending on sample thickness) to achieve substitution of the ice by solvent.

4. Direct Observation

To avoid the problems associated with mechanical complexity and lengthy preparation time Hankins et al (1945) adopted a direct observation technique for histological studies. The frozen meat was sectioned with a sliding microtome and then the sections were examined in a -10°C cold room. To flatten the sections and make them stick to the microscope slides they warmed the section by placing their finger on it, a doubtful practice in view of the recrystallisation problem.

Rohatgi & Adams (1967) in their studies on ice formation in solutions used a technique based on metalurgical practice. Working in a -40°C atmosphere they cemented thick sections onto microscope slides with a drop of water then polished them down to the desired thickness with a series of emery papers of increasing fineness. After polishing with tissue paper the sections were examined and photographed at -40°C. The risks inherent in this method are; heat generated during polishing which could raise the temperature of the section to a dangerous level, and the possibility of slight thawing during cementing the section onto the slide.

C. METHODS USED IN THIS STUDY

1. Principal Method

In the present studies it was decided to use a method similar to that used by Rohatgi & Adams as a microscope and camera were available which, after lubrication with a temperature stable silicone grease, could be used in a cold room at -30°C.

The method used was to cut through the frozen sample at the position to be examined with a fine toothed fret saw then polish the cut surface on carborundum stones of different fineness grades. A microscope slide was cemented to the polished surface with a drop of water and the sample cut away from the slide leaving a thin section on the slide. In this way the section could be removed with a minimum of mechanical distortion. The section was then cut down with a coarse bastard file and polished on the carborundum stones to the requisite thickness for microscopic examination. It was found that a new coarse file cut the surface more cleanly with fewer deep scratches and showed less tendency to clog than a finer file. The clogging of the file gave some indication of surface melting caused by filing as the trouble was more prevelant if the room temperature was allowed to rise.

Generally the fine scratching left by the polishing operation masked the histological detail in the section. To overcome this difficulty the slide was flooded with 95% alcohol which quickly dissolved the ice and scratches without distorting the meat structure. Unfortunately the alcohol released the gases frozen out of solution from the meat and mounting water so that bubbles tended to be trapped in the section. Most of them could be removed by applying a vacuum of 25" Hg to the section but the handling of the slides into and out of the vacuum chamber tended to cause waves to form in the tissue section, making observation difficult.

No problems were encountered with the camera or film at -30°C except that care had to be taken when advancing the film as the perforations were liable to tear.* The principal advantages of this technique are simplicity and the short time involved in preparing and photographing the section. Its main disadvantage is that it is a fairly coarse technique and is not very satisfactory for examining crystals of less than 20 u thickness.

* The film used in this study was processed by the author and details of processing and general photographic technique are given in Appendix I.

2. Control Methods

Freeze substitution and a simple freeze drying method were used as control methods. Plates 1-6 show that all three methods give comparable results at the magnification used in this study. The tissue in freeze substitution and freeze drying pictures has been stained.

The freeze substitution method used was to hold the sections in about 50 times their volume of absolute ethanol at -20°F for about three months before warming to room temperature and embedding.

To freeze dry, the tissue sections were wrapped in cotton wool and buried in 1Å molecular sieve in a sealed container and held at -20°F for about three months before transferring to dry ethanol and warming to room temperature for embedding. To assist the penetration of ethanol a vacuum of 25" Hg was applied to the sections in ethanol to remove most of the air.

For the embedding medium it was decided not to use paraffin because of the attendant risk of heat damage. Instead it was decided to use one of the synthetic polymers used by electron microscopists in preparation of tissue sections for light and electron microscopy. The acrylic resin, methyl methacrylate, was rejected because of the risk of tissue distortion on polymerisation (Dickson, 1968, Kay, 1965), and the fact that it did not seem to be available in small enough quantities. Of the epoxy resins the Shell resin Epicote 812 was generally recommended over the CIBA resin Araldite M as the hardness was more easily controlled and generally better sections could be

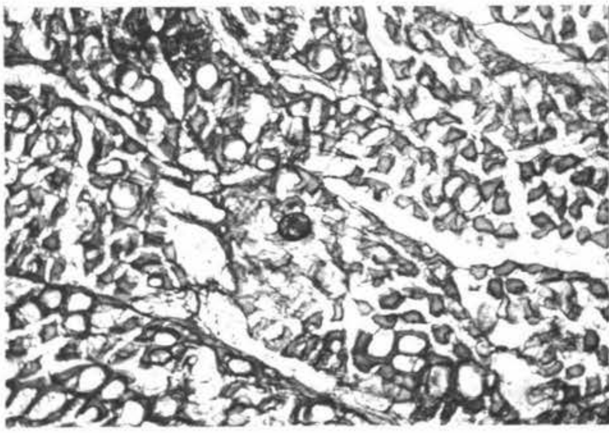


Plate 1

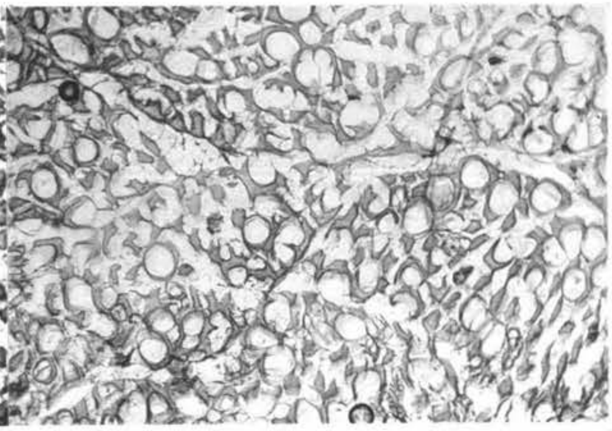


Plate 2

Photomicrographs prepared from freeze dried material

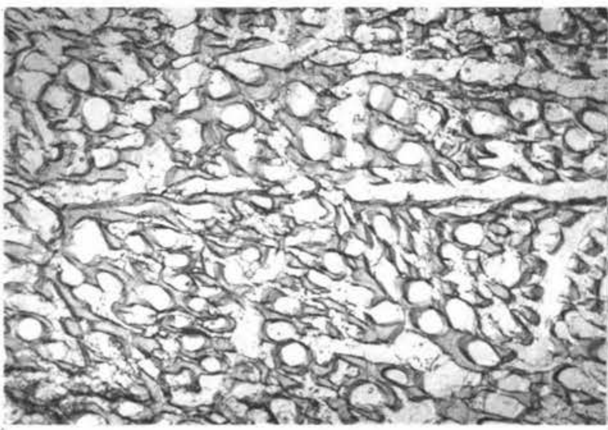


Plate 3

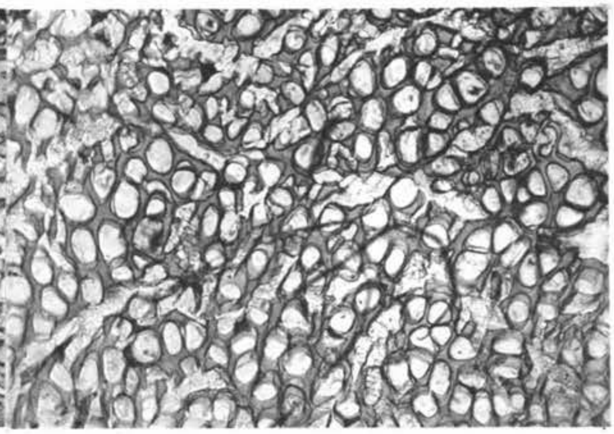


Plate 4

Photomicrographs prepared from freeze substituted material

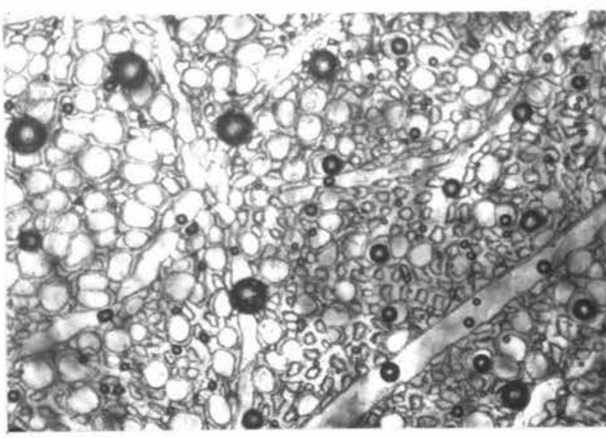


Plate 5

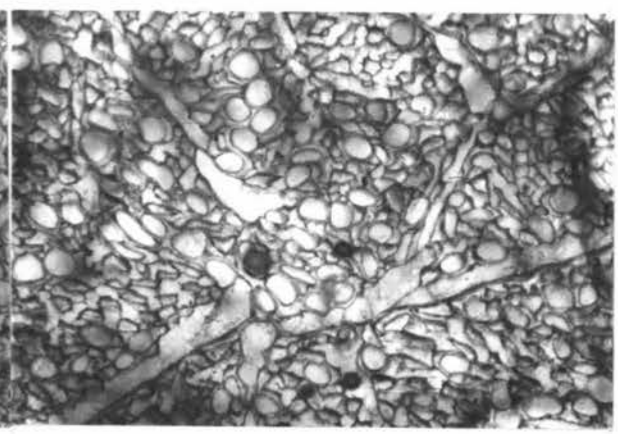


Plate 6

Photomicrographs of frozen meat by direct observation obtained (Dickson, 1968, Williamson, 1968). Unfortunately the Shell resin or hardeners could not be obtained in suitable quantities so Araldite M was used as it and the hardeners required could be brought readily.

The formulation recommended (Kay, 1965) was found to be too hard for sectioning with a steel bladed rocking microtome so it was modified to produce a block of suitable hardness. See Appendix I.

Once sectioned the tissue was stained with a safranin in 85% ethanol and mounted on a microscope slide in a drop of immersion oil for a temporary mount, or in epoxy resin for a permanent mount. By including a small amount of water in the stain, the tissue took it up more than the embedding resin thus improving the contrast between tissue and resin. The slight rehydration of the tissue did not alter the disruption caused by the ice which was fixed by the presence of the embedding medium. The slides so obtained were generally of greater clarity than could be obtained by paraffin embedding techniques, colour photographs of paraffin embedded material being available for comparison.

D. FREEZE ETCHING, AN ALTERNATIVE TECHNIQUE

Freeze etching was another technique which was examined as having possible application to this type of study. At present it is used in electron microscopy, particularly for the study of cell membrane surfaces and the surfaces of other cellular structures.

The method as used by electron microscopists involves treating the tissue sample with 20% glycerol to inhibit ice crystal formation, quenching it in liquid nitrogen cooled freon 22, then chipping the surface of the specimen away with a liquid nitrogen cooled blade until the point for observation is reached. The exposed surface is allowed to dehydrate slightly by sublimation, before a carbon film is deposited onto it.

The carbon film strikes the surface obliquely so that shadows are cast by the raised features. A film of inert backing metal is deposited over the carbon and the sample is etched off the carbon film by acid digestion to leave a relief map of the tissue surface which can be examined under the electron microscope (Hall & Bertand, 1968, Sullivant, 1966).

The method suggested for light microscope studies is to work in the cold room at -20°F using a microscope which observes the specimen by reflected rather than transmitted light. The frozen sample is quenched in liquid nitrogen then broken so that the fracture plane passes through the point for observation. The sample is mounted under the microscope so that the fracture surface is illuminated by low incident light. As the ice starts to sublime the meat will stand proud of the surface and catch the light making a clear cut distinction between ice and meat.

E. OBSERVATION OF ICE CRYSTALS DURING GROWTH

Some consideration was given to the possibility of observing the ice crystals as they grew. An account of this work and the proposed method of doing this is given in Appendix III.

CHAPTER III

DESIGN OF A FREEZING SYSTEM WITH UNDIRECTIONAL HEAT FLOW

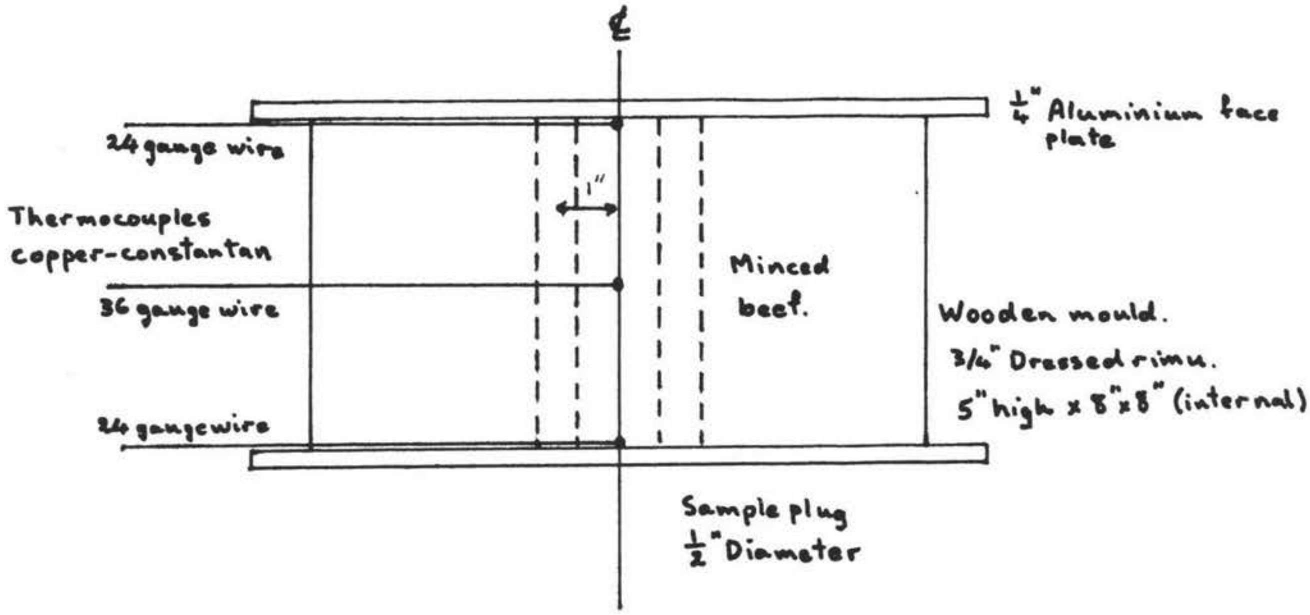
The problems encountered in developing an experimental freezing system with undirectional heat flow are discussed and the system used in this study is described.

A. UNDIRECTIONAL HEAT FLOW

By specifying undirectional heat flow one of the variables probably involved in ice crystal formation is eliminated and the calculation of the rate of ice formation at any given point is simplified considerably.

B. POLYSTYRENE MOULD SYSTEM

In the original design of the freezing system not only was it necessary to provide undirectional heat flow but also samples of meat had to be removed for examination during the freezing process. Firstly a freezing system similar to the one used by Cullwick (1967) in his one-dimensional freezing studies was considered. In this system sample plugs of diameter suitable for sectioning for microscopy were inserted into a slab of mince contained within a wooden mould and frozen in a plate freezer. Fig. 9. It was realised however that it would be difficult to remove the plugs, particularly when only partially frozen, when rapid removal would be essential. An alternative approach was sought.



Temperatures recorded by a 12 point Honeywell-Brown Automatic recording potentiometer, calibrated −100°F to +100°F.

FIG. 9. MINCED MEAT BLOCK FREEZING MOULD.

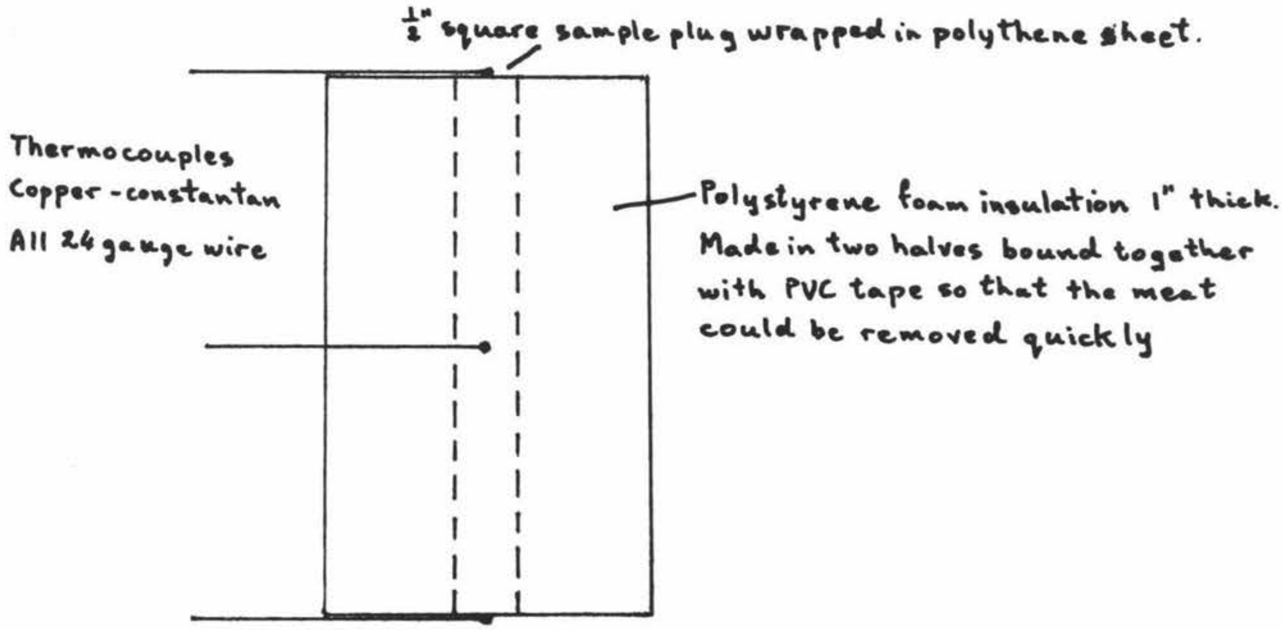


FIG. 10. POLYSTYRENE FREEZING MOULD.

The system developed was to freeze a stick of meat of suitable dimensions for sectioning for microscopic examination in a foam polystyrene mould which left the ends of the stick exposed to make contact with the freezer plates. Fig. 10. In practice, unfortunately after all the studies on ageing had been completed, it was found that the freezing times were very much shorter than those which could be expected from a one-dimensional freezing system. Subsequent calculations showed that the basis used for the original calculations was invalid and that freezing could only be considered one-dimensional at distance up to 0.5 inches from the plate surface.

C. MINCED MEAT MOULD SYSTEM

1. Description of the System

At this stage it was decided to reconsider the mince mould system as the idea of observing the partially frozen material had been abandoned. To minimise variation in surface temperature a ¼" thick aluminium plate was placed on top and bottom of the mould. Experience with the polystyrene moulds had suggested that there were considerable local temperature variations across the freezer plates. Four copper constantan thermocouples were attached to the meat side of each aluminium plate.

To overcome the difficulty in removing the sample plugs from the mince mould the sample plug was wrapped in polythene sheet then drawn into a varnished paper tube. The outside of the tube was coated with silicone grease and wrapped in polythene. The plug was then placed vertically in the mould and a copper constantan thermocouple made from 36 gauge wire inserted into the centre of the plug through a hole punched in its side. The mince was then packed into the mould and the thermocouple support wires removed. To remove the plug from the frozen mould it was necessary to drive it out using a piece of ½" steel rod and a heavy mechanic's hammer. This operation inflicted considerable damage to one end of the plug.

2. Verification of Undirectional Heat Flow

(a) Experimental

Cullwick (1967) found experimentally that the temperature of the centre of the plane parallel to the freezing plates in a 6" x 8" x 4" thick slab was uniform over much of its area during freezing and concluded that one-dimensional freezing was occurring over this area. This constant temperature plane extended to 1" from the centre of the slab. This finding was repeated for a 5" thick slab. The mould used in the present study was 8" x 8" x 5" thick with the sample plugs located at the corners of a square of side 2" about the centre so that each plug should have frozen one-dimensionally.

(b) By calculation

i. Minced meat mould:-

The calculation method used for the polystyrene mould was applied to this sytem and it was found that the system was approximately one-dimensional.

Briefly, the calculation method used was to find the thermal properties of a 5" slab of a hypothetical substance whose centre temperature would cool one-dimensionally from 50°F to -10°F in a plate freezer operating at -40°F in five hours. This is about the same time it would take to cool a slab of meat over the same temperature range under the same conditions. A surface heat transfer coefficient of 100 BTU/ft^{2}hr$^\circ$F was assumed.

A thermal conductivity of 0.6 BTU/ft^{2}hr$^\circ$F was chosen as this is the mean thermal conductivity of meat over this temperature range and from these values a specific heat of 1.75 BTU/lb$^\circ$F was determined. This is lower than the mean specific heat of meat over this temperature range. By assuming a surface heat transfer coefficient of 1.6 BTU/ft^2 hr$^\circ$F for the wooden side surfaces the temperature after five hours cooling at a point 1" from the centre in the centre plane parallel to the freezer plates could be calculated. It was found to be -15.5°F or 6% of the temperature range 50 to -10°F lower than for one-dimensional cooling. It would take a further 0.86 hours or 15% longer for the temperature to fall to this value in one-dimensional freezing.

ii. Polystyrene system:-

Cooling calculations for a block 2" x 2" x 5" high of polystyrene foam cooled in the plate freezer indicated that in the centre plane parallel to the plate, more heat was being conducted along it to the air than perpendicular to it to the plates and that the block cooled very much more quickly than a meat slab of 5" thickness would take to freeze. By including the thickness of polystyrene in the surface heat transfer term a surface heat transfer coefficient of 0.21 BTU/ft^2 hr$^\circ$F was calculated for the sides of the mould. On this basis it would take 0.6 hours for a stick of the hypothetical substance to cool to -10°F.

3. Allowance for Different Plate Temperatures

During freezing it was found that the top plate operated at a uniform and steady -11°F and the bottom plate at a steady and uniform -43°F. This performance was duplicated each time the mince block was frozen. The thermodynamic centre of the mould was determined by a trial and error solution of Plank's equation (Plank, 1913) for determining the freezing time of a one-dimensionally frozen slab.

$$\theta_f = \frac{L\rho}{T_f - T_a}\left(\frac{l_o}{2h} + \frac{l_o^2}{8K}\right) \tag{3.1}$$

Where θ_f is the freezing time,

L is the latent heat of fusion,

ρ is the density of frozen meat,

T_f is the freezing point of meat,

T_a is the plate temperature,

l_o is the slab thickness,

h is the surface heat transfer coefficient,

K is the thermal conductivity of frozen meat.

For this solution values of ρ = 67 lb/ft^3, T_f = 30°F, T_a = -43°F and -11°F, L = 110 BTU/lb, θ_f = 3.75 hrs and K = 0.8 Btu/ft hr°F were used. Values of $l_{ob}/2$ = 2.89", $l_{ot}/2$ = 2.13" and h - 121 BTU/ft^2 hr°F were obtained. Where l_{ob} is the thickness of a slab taking 3.75 hours to freeze, where both surfaces are at -43°F and l_{ot} is the thickness of a slab taking 3.75 hours to freeze when both surfaces are at -11°F.

An alternative method of solution using temperature profiles predicted by the method outlined in Chapter IV found the thermodynamic centre in the same place as the trial and error solution from Plank's

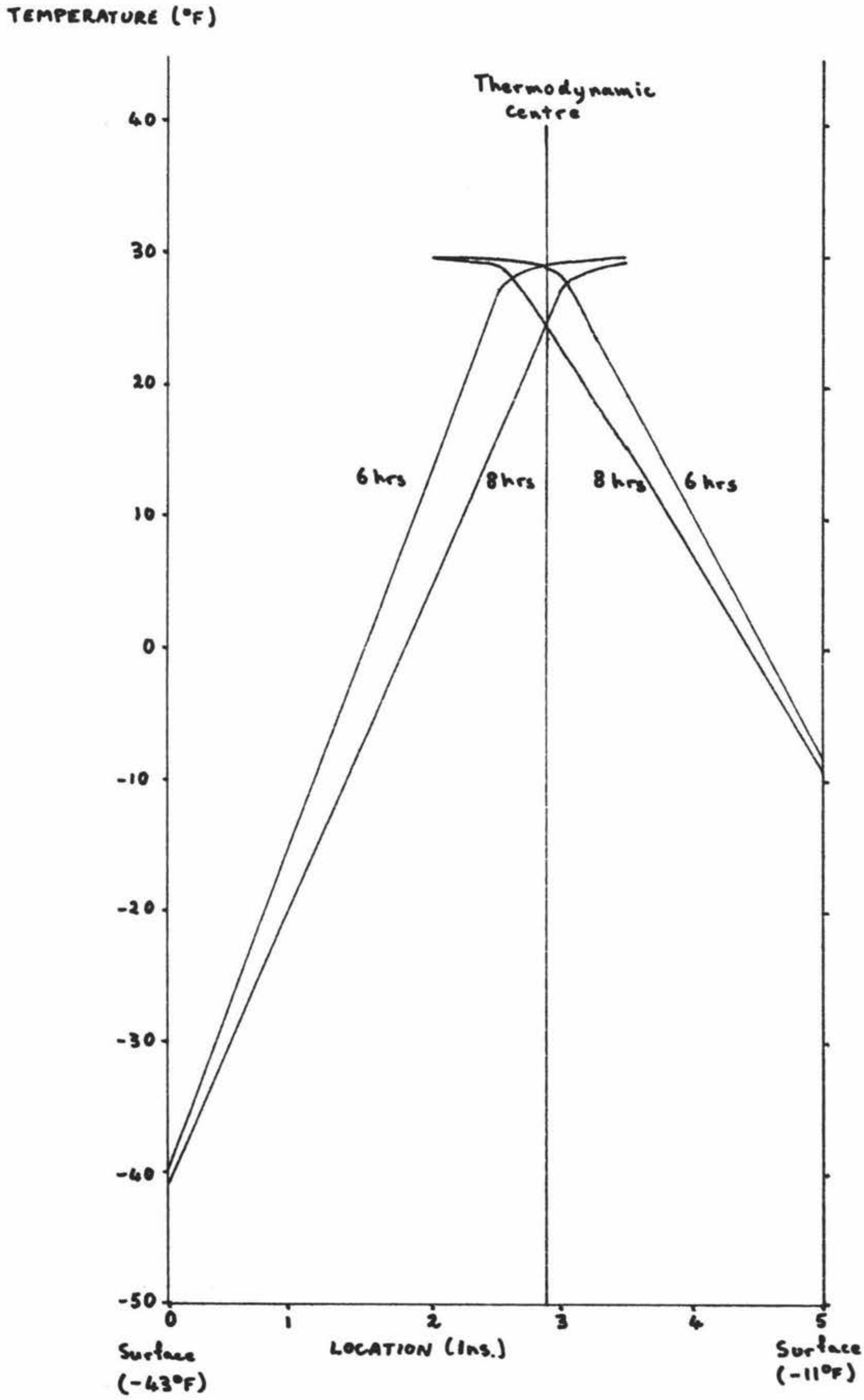


FIG 11 LOCATION OF THERMODYNAMIC CENTRE FROM CALCULATED TEMPERATURE PROFILES.

equation (Fig. 11). The method involved determining the temperature profiles in a 7" slab with a surface temperature of -43°F and a 6" slab with a surface temperature of -11°F then plotting profiles as in Fig. 11.

For calculation of ice formation profiles the experimental system was taken as being a slab of half-thickness 2.88", frozen in a plate temperature operating at -43°F.

This location of the thermodynamic centre was later confirmed by experiment.

CHAPTER IV

TEMPERATURE AND RATE OF ICE FORMATION PREDICTION

This chapter explains the development of a method of predicting the rate of ice formation using a digital computer and compares the predicted values with values derived from experimentally determined cooling curves.

A. PREDICTION OF RATE OF ICE FORMATION FROM FOURIER'S LAW

The freezing process involves unsteady state heat transfer. The relationship between temperature and time of cooling at any point within the body will therefore be governed by the Fourier unsteady-state law. For undirectional heat flow this may be expressed as:-

$$\frac{dT}{d\theta} = \alpha \frac{d^2T}{dx^2} \qquad (4.1)$$

where T is the temperature at point x and time θ,

θ is the time from commencement of cooling,

x is the location relative to the cooled surface of the point under consideration,

α is the thermal diffusivity,

$\alpha = \frac{k}{\rho C_p}$

K is the thermal conductivity,

ρ is the density,

C_p is the specific heat.

Difficulty arises in applying this equation to the freezing problem as the specific heat and thermal conductivity are temperature dependent.

B. ERROR FUNCTION SOLUTIONS

For a simple ice/pure water system, the specific heat and thermal conductivity can be considered to have constant values in water and different constant values in ice with a steep change in value between the two phases.

A particular solution for this problem is the Neumann method (Ingersoll et al, 1954) giving an error function type solution. Rohatgi & Adams (1967b) used this type of solution as a basis for their rate of ice formation calculations. Their experimental system consisted of a solute plus water which they froze starting from the freezing point temperature. They divided the freezing process into two zones of constant specific heat and thermal conductivity so that they could use this type of solution. By assuming constant thermal properties over the temperature range where they are changing most rapidly, deviations between the calculated and actual temperature histories could be expected.

In calculating the rate of ice formation the slope of the cooling curve at any given instant is considered so that errors in the calculated rate of ice formation could be expected as a result of these deviations. It appears that this has happened in their solution as they find that the rate of ice formation is inversely proportional to the square of the distance from the chill surface. From Plank's equation (1.1) it could be expected that with a high surface heat transfer coefficient, as is present in this system, the rate of ice formation would be inversely proportional to the distance from the chill surface.

C. SOLUTION BY FINITE DIFFERENCE CALCULATION

1. Infinite Surface Heat Transfer Coefficient

An alternative solution of the Fourier equation is by the calculus of finite differences. Expansion of equation (4.1) in terms of finite differences yields:-

$$\frac{T_{\theta+\Delta\theta,x} - T_{\theta,x}}{\Delta\theta} = \alpha \left(\frac{\frac{T_{\theta,x+\Delta x} - T_{\theta,x}}{\Delta x} - \frac{T_{\theta,x} - T_{\theta,x-\Delta x}}{\Delta x}}{\Delta x} \right) \qquad (4.2)$$

where $T_{\theta,x}$ is the temperature at time θ and point x,

$T_{\theta,x+\Delta x}$ is the temperature at time θ and point $x + \Delta x$,

$T_{\theta,x-\Delta x}$ is the temperature at time θ and point $x - \Delta x$,

$T_{\theta+\Delta\theta,x}$ is the temperature at point x and time $\theta + \Delta\theta$.

If a modulus M is defined as:-

$$M = \frac{\Delta x^2}{\alpha\Delta\theta} = \frac{\Delta x^2 \rho C_p}{k\Delta\theta} \qquad (4.3)$$

Solution of equation (4.2) for $T_{\theta+\Delta\theta,x}$ gives:-

$$T_{\theta+\Delta\theta,x} = \frac{T_{\theta,x+\Delta x} + (M - 2)\, T_{\theta,x} + T_{\theta,x-\Delta x}}{M} \qquad (4.4)$$

To use this equation for one-dimensional freezing calculations, such as a slab cooled from both sides, a number of calculation points are spaced across the slab along the direction of heat flow at equal spacings, Δx. When there is an infinite surface heat transfer coefficient the surface temperature is taken as being the same as the ambient temperature. Starting from time θ equation (4.4) is used to calculate the temperature at each point after time $\theta + \Delta\theta$.

In freezing a substance such as meat, where the thermal properties are temperature dependent, this means that for a constant value of Δx and $\Delta\theta$ the modulus M will vary (equation 4.3). The value of M in equation (4.4) determines the weighting applied to the present temperature at the point under consideration in calculating the new temperature. Cullwick (1967) found that $M \geqslant 4$ gave consistent time temperature relationships. Values < 4 lead to unstable calculations particularly in the early stages of freezing. Large values of M are undesirable as the value of $\Delta\theta$ must be reduced for given values of Δx, ρ, C_p and K which will prolong calculation as indicated in equation (4.3).

In the methods used by Earle and Earl (1966) and Cullwick (1967) and the method used in this study, the value of M is held constant and $\Delta\theta$ is allowed to vary as K and C_p vary. This means that the new time $\theta + \Delta\theta$ at each calculation point will vary according to its temperature. However it is necessary to bring each point to a common time before calculation can continue. This is achieved by a linear interpolation of the temperature change $T_{\theta,x}$ to $T_{\theta+\Delta\theta,x}$ to a temperature $T_{\theta+D\theta,x}$ where $D\theta$ is a constant time increment.

$$T_{x,\theta+D\theta} = \frac{D\theta}{\Delta\theta}\left(T_{x,\theta+\Delta\theta} - T_{x,\theta}\right) + T_{x,\theta} \qquad (4.5)$$

To avoid extrapolation which will induce instability during the initial stages of calculation $D\theta < \frac{\Delta x^2 \rho C_p}{KM}$. The minimum values of $\frac{\Delta x^2 \rho C_p}{KM}$ will occur when the ration C_p : K is smallest, i.e. when the meat is completely frozen at the point under consideration.

2. Finite Surface Heat Transfer Coefficient

To apply this calculation method to a system with a finite surface heat transfer coefficient another equation is required to calculate the surface temperature. Dusinberre (1949) proposed the following equation:-

$$T_{s,\theta+\Delta\theta} = \frac{N}{N+1} \cdot T_{a,\theta+\Delta\theta} + \frac{1}{N+1} \cdot T_{s+\Delta x,\theta+\Delta\theta} \tag{4.6}$$

where T_s is the surface temperature,

T_a is the ambient temperature,

$T_{s+\Delta x}$ is the temperature at a point Δx from the surface,

$N = \frac{h \Delta x}{\lambda}$ is the Nusselt modulus,

h is the surface heat transfer coefficient,

λ is the thermal conductivity of the body.

Expanding this equation:-

$$T_{s,\theta+\Delta\theta} = \frac{1}{N+1} \cdot T_{a,\theta+\Delta\theta} + \frac{1}{M(N+1)} \left(T_{s,\theta} + (M-2)\, T_{s+\Delta x,\theta} + T_{s+2\Delta x,\theta} \right) \tag{4.7}$$

When N is large, the surface temperature calculated by this equation will change almost instantaneously to the ambient temperature at the commencement of freezing.

To avoid this Mickley et al (1957) suggest using the following equation to calculate the initial surface temperature:-

$$T_{s,\theta+\Delta\theta} = \frac{1}{2} \left[T_{s,\theta} + \frac{N\, T_{a,\theta} + T_{s+\Delta x,\theta}}{N+1} \right] \tag{4.8}$$

Where θ is the time at commencement of cooling.

D. COMPUTER SOLUTION USING FINITE DIFFERENCE APPROXIMATIONS

Freezing temperature history calculations by the method outlined above can be most conveniently made using a digital computer. To this end a programme in Fortran II-D with Monitor I was developed for an IBM 1620 II computer. A programme developed by Cullwick (1967) was used as a basis in developing this programme.

The temperature programme was extended to calculate the rate of ice formation at each point. Eutectic data for meat as determined by Riedel (1957) was plotted in terms of the equilibrium fraction of freezable water frozen against temperature. This curve was differentiated to convert the data to give the fraction of freezable water frozen per unit temperature change (fractional solidity) at various temperatures. Table AII-1, Appendix II. The product of the fractional solidity and the slope of the cooling curve at any particular temperature is the rate of ice formation at that temperature expressed as change in fraction of freezable water frozen per unit time.

Having made this modification to the programme it was found that the rate of ice formation value was extremely sensitive to change in slope of the cooling curve. The large change in specific heat at 30^{o}F meant that the temperature calculation at each location had to be stopped when the calculated temperature dropped below 30^{o}F, and restarted at 30^{o}F to prevent the temperature passing through the zone of maximum ice formation, without calculating the rate of ice formation. This danger was particularly apparent at points near the surface, but the modification made negligible difference to the total freezing time.

A listing of the basic temperature programme is given in Appendix II. As an arithmetic check on this programme, cooling curves for a slab with constant thermal properties ($K = 0.8$ BTU/ft hr^{o}F, $C_p = 0.5$ BTU/lb ^{o}F) were calculated using two different values for $\triangle x$ (0.2 inches and 0.1 inches in a 2 inch thick slab). By halving $\triangle x$ the time for the centre temperature to cool from 38^{o}F to -10^{o}F (ambient temperature -43^{o}F, h = 240 BTU/ft^{2}hr^{o}F) was reduced slightly but both agreed to within 0.5% of the time calculated from equation (1.4). Similarly consistent results were obtained at each point during the entire cooling process.

When the thermal properties for meat were substituted in the same system, it was found that halving $\triangle x$ reduced the overall cooling time by 1% and the temperatures at given points in each block would lag and lead with respect to each other in a cyclic pattern. By using a constant value of unity for the fractional solidity the slope of the cooling curve could be calculated as the rate of ice formation with the ice formation programme. Repeating the calculations with the ice formation programme showed that 2-3 fold differences in the cooling curve slope were possible between the two calculations in the temperature range 30 to 20^{o}F. For this reason it was decided to calculate the mean rate of ice formation over the temperature range 30 to 20^{o}F as a measure of the rate of ice formation, rather than the rate at any particular temperature or the maximum rate. The mean calculation was not included in the programme and had to be calculated from the data printed out.

The discrepancy between calculations is probably a consequence of the large change in specific heat in the temperature range 30 to 27^{o}F.

The calculation method assumes constant thermal properties over a thickness Δx each side of the point under consideration and over the time $\Delta \tau$. The temperature range over this thickness may be large particularly near the surface so the assumption is not always valid. Decreasing Δx improves this assumption but at the same time it prolongs calculation.

To overcome this difficulty it was decided to change Δx during calculation as the ice front moved away from the surface. In changing Δx a new set of calculation points had to be set up at the new Δx spacing, which meant that the ice formation programme had to be modified to determine the temperatures at these points, by interpolation from the temperatures at the former calculation points. The interpolation system was designed so that both larger and smaller values of Δx could be catered for. This made it possible to start calculating with a small Δx value, then to enlarge Δx to reduce computation time as the temperature gradients within the slab decreased. Finally Δx was decreased as the ice front approached the centre to examine the ice development process at the centre more closely, as an increase in the rate of ice formation at the centre compared with points on either side of it appeared from the calculations. This may be a real phenomenon as rates of ice formation derived from experimental freezing curves (Meryman, 1966) and Fig. 12 show such an increase. A listing of the ice formation programme is given in Appendix II.

E. COMPARISON OF CALCULATED RATES OF ICE FORMATION WITH EXPERIMENTALLY DERIVED VALUES

The computed data has been plotted (Figs 12, 13) in the form:-

$$\text{Rate of ice formation} \propto \frac{1}{\text{position of the ice front}}$$

Where rate of ice formation is the mean rate of ice formation between 30 and 20°F,

position of the ice front is the distance of the ice front from the surface (location).

From Plank's Equation (1.1) this relationship would be predicted when $h \rightarrow \infty$.

Fig. 12 graphs the rates of ice formation across the experimental system, calculated with varying Δx (0.12 inches, 0.18 inches and 0.36 inches) and with constant Δx (0.36 inches), and compares them with values derived from differentiating the experimental time/temperature curves of the experimental system. It can be seen from this that by maintaining a constant value for Δx the rates of ice formation have been approximately halved towards the surface and so this throws considerable doubt on such a calculation technique as a method of predicting rates of ice formation. A series of calculations made with the temperature programme using decreasing values of Δx, suggested that the solution may have been approaching a limiting series of values. If this is so the rates of ice formation calculated using varying Δx values are likely to be more probable. Comparison of these values with the experimentally derived values, illustrates that towards the centre the values calculated using variable Δx values are closer to the experimental values than using constant Δx. There is, however, considerable difference towards the surface.

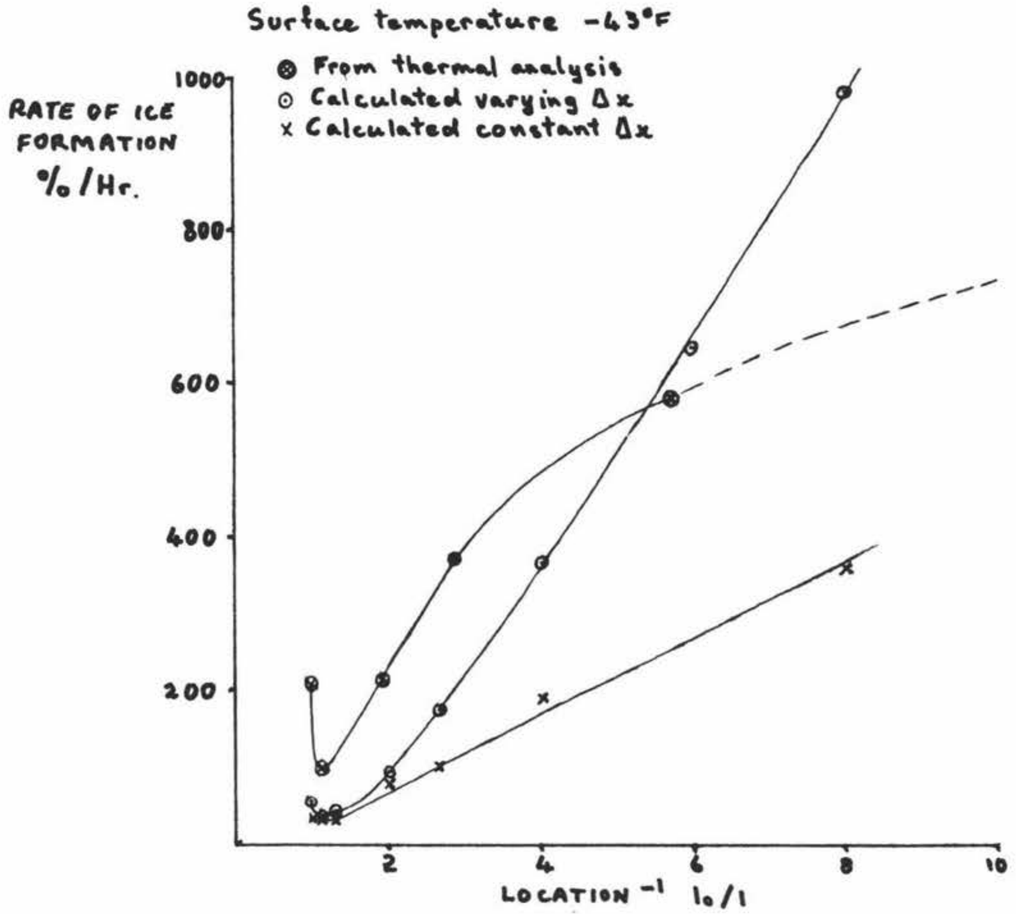


FIG.12. RATES OF ICE FORMATION IN THE MINCED MEAT MOULD EXPERIMENTAL SYSTEM.

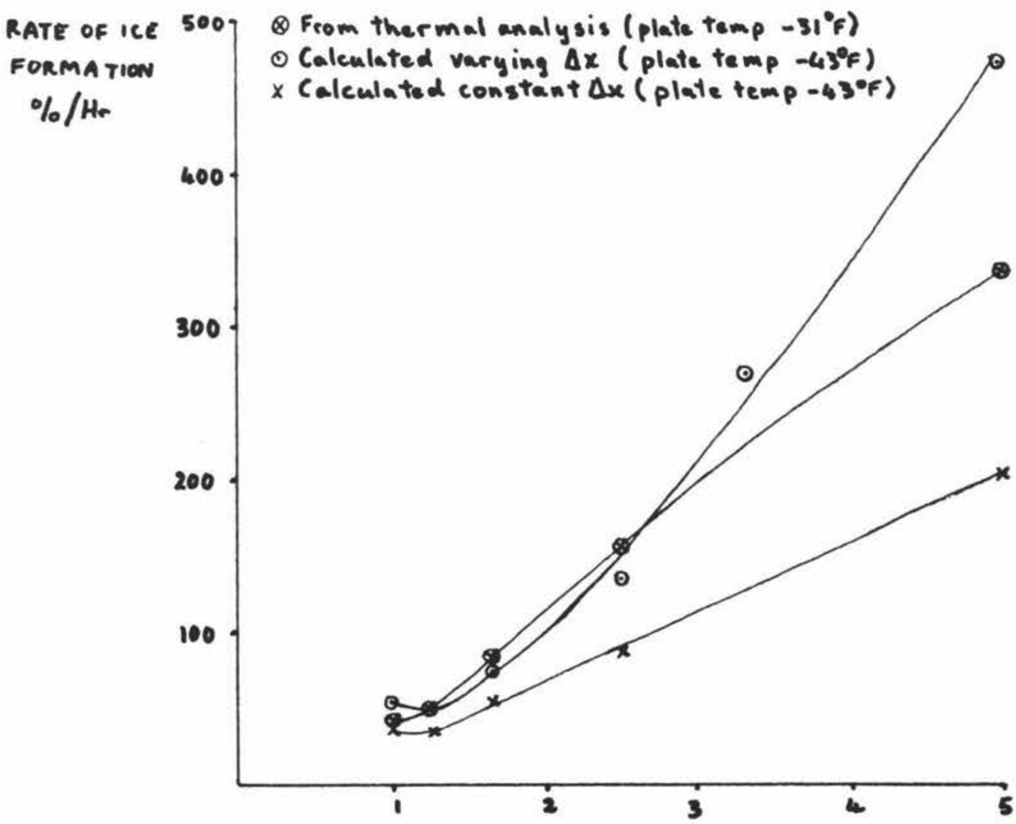


FIG.13. RATES OF ICE FORMATION IN 5" MINCED BEEF SLAB (DERIVED FROM EXPERIMENTS BY CULLWICK, 1967)

To check this finding rates of ice formation were determined by differentiating cooling curves measured by Cullwick (1967) in a 5" thick minced meat slab and compared with calculated rates of ice formation for this system, Fig. 13. The calculated rates were determined by constant Δx (0.5 inches) and varying Δx (0.125 inches, 0.25 inches and 0.5 inches). Comparison of Figs 12 and 13 shows that these results have a similar form.

The rates of ice formation were derived from the experimental systems by measuring the slope of the cooling curve at $1^{\circ}F$ intervals, in the temperature range 30 to $20^{\circ}F$, and multiplying the slope at each point by its corresponding fractional solidity value.

F. COMPARISON OF CALCULATED AND MEASURED FREEZING TIMES

Measured and calculated temperature histories at the geometric centre of the experimental system are compared in Fig. 14. In spite of the high surface heat transfer coefficient used in the calculation (240 $BTU/ft^2hr^{\circ}F$), the calculated freezing time (38 to $-10^{\circ}F$) is much larger. The measured freezing time is a little short compared with some freezing times found in commercial plate freezing practice, while the calculated freezing time is longer. This indicates that the experimental system may not be behaving in a strictly one-dimensional manner and also that the calculated system does not give an adequate description of the freezing process.

It was thought that the computer may not have been carrying enough significant figures during the interpolation time calculation. This would have meant that it would calculate a value for the interpolated

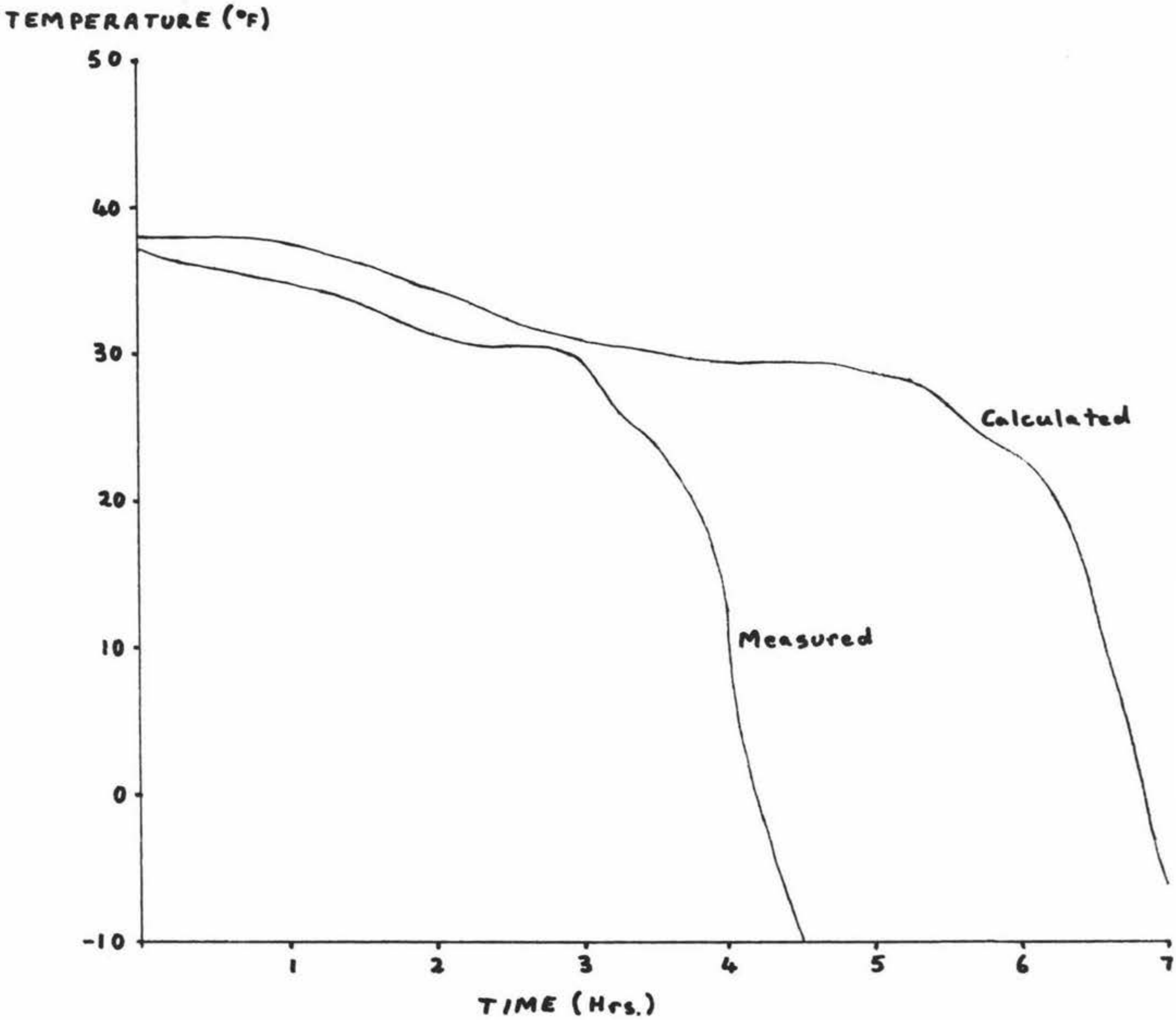


FIG. 14. COMPARISON OF MEASURED AND CALCULATED TEMPERATURE HISTORIES AT THE GEOMETRIC CENTRE OF THE EXPERIMENTAL SYSTEM.

temperature higher than it should have been, particularly in the temperature range 30 to 28°F. Altering the computer setting did not cause any significant change demonstrating that this was not the reason for the longer cooling time.

As a check on the freezing time, a combined numerical/analytical solution was employed using Plank's freezing time equation, equation (3.1), and the ice formation programme. Equation (3.1) was used to calculate the time taken for the ice front to reach the centre of the slab, then the ice formation programme was used to calculate the time taken for the centre temperature to fall to -10°F. It was assumed that once the ice front had reached the centre there would be a linear temperature distribution between the surface at -40°F (ambient temperature -45°F) and the centre at 30°F; the constant thermal properties of completely frozen meat were employed in the calculation. The freezing time calculated in this manner was 4.1 hours, which compares favourably with the measured experimental freezing time.

CHAPTER V

EXPERIMENTAL OBSERVATIONS ON ICE CRYSTAL FORMATION

The experimental observations on ice formation are presented and are related to rate of ice formation and the physiological condition of the meat. A relationship between maximum ice crystal size and rate of ice formation is derived.

A. ICE CRYSTAL FORM AND RATE OF ICE FORMATION

1. Quantitative Studies

(a) Selection of an index of crystal form

By examining sections taken from different locations in the experimental system, the influence of different rates of ice formation on the ice crystal structure in frozen meat could be seen. An attempt to find a quantitative relationship between rate of ice formation and ice crystal size was made and the results of this work are presented here.

The maximum width of an ice crystal (ice crystal width) was taken as being a suitable index of crystal size. The cross sectional area may have been a more suitable index as it would then be possible to obtain an estimate of the relative amounts of water in each type of ice formation. To make this measurement large prints would need to be made of each photomicrograph and the area of each crystal measured with a plananimeter. This was considered impractical for the present studies because of the large variation in crystal sizes for a given

FIGS 15-21. RATE OF ICE FORMATION STUDIES: CRYSTAL WIDTH FREQUENCY DISTRIBUTION

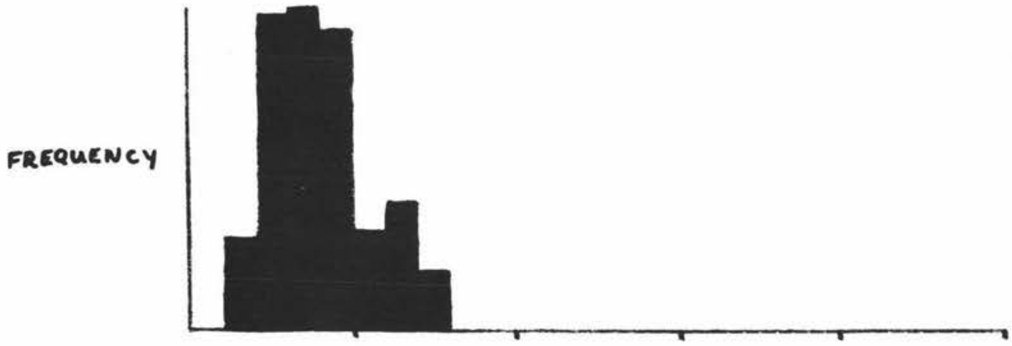


FIG. 15. RATE OF ICE FORMATION = 680 %/Hr

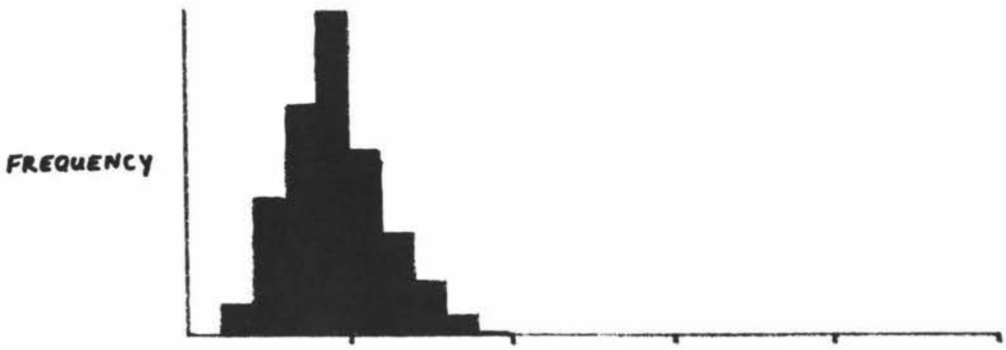


FIG. 16. RATE OF ICE FORMATION = 660%/Hr

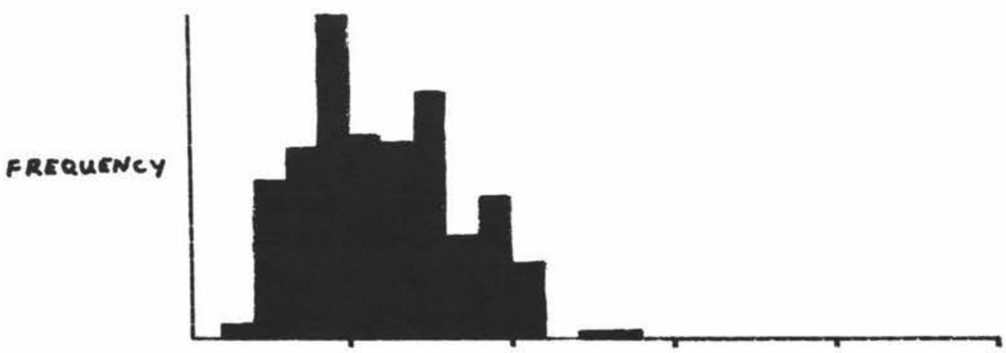


FIG. 17. RATE OF ICE FORMATION = 410 %/Hr

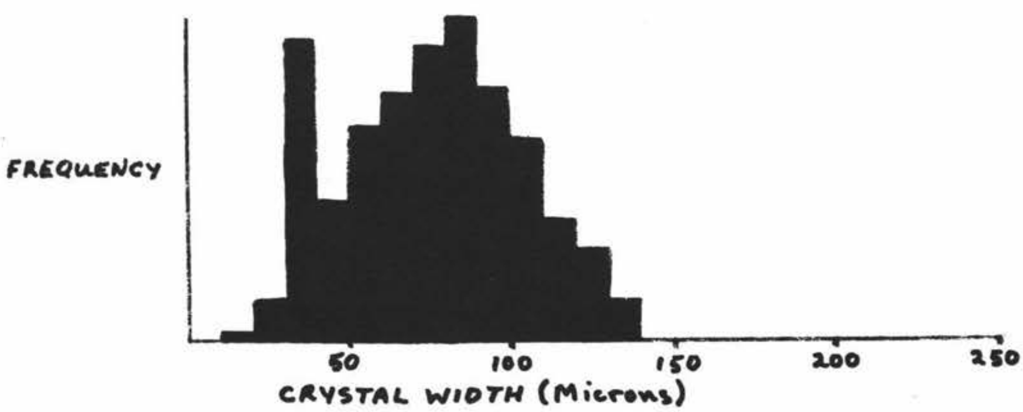


FIG. 18. RATE OF ICE FORMATION = 250 %/Hr

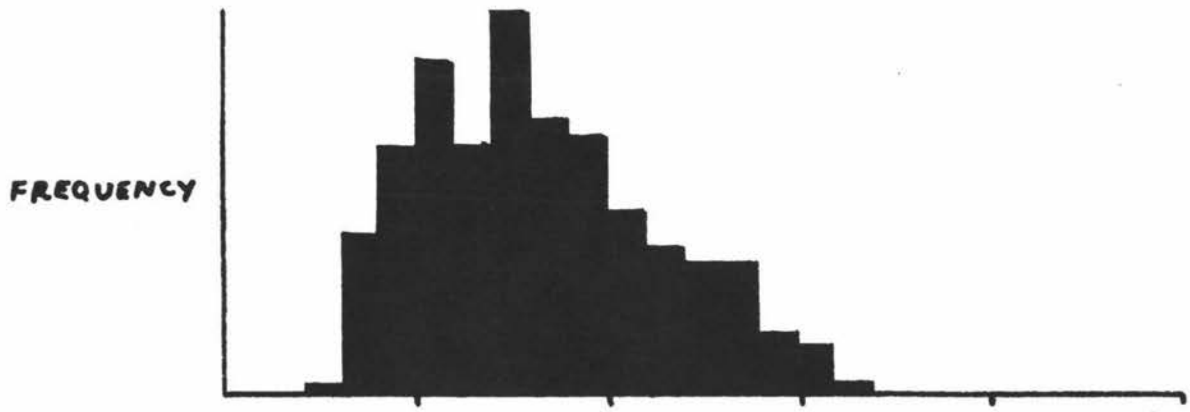


FIG. 19. RATE OF ICE FORMATION = 190 %/Hr

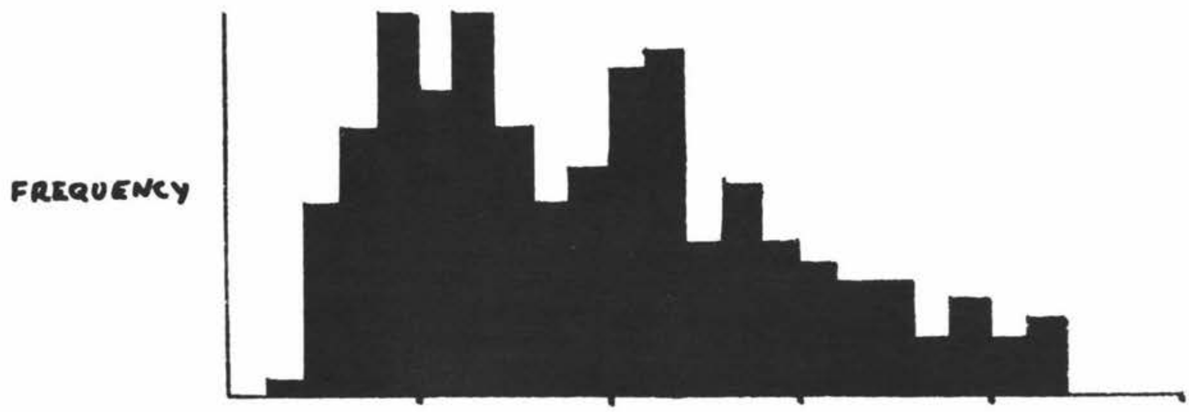


FIG. 20. RATE OF ICE FORMATION = 100 %/Hr

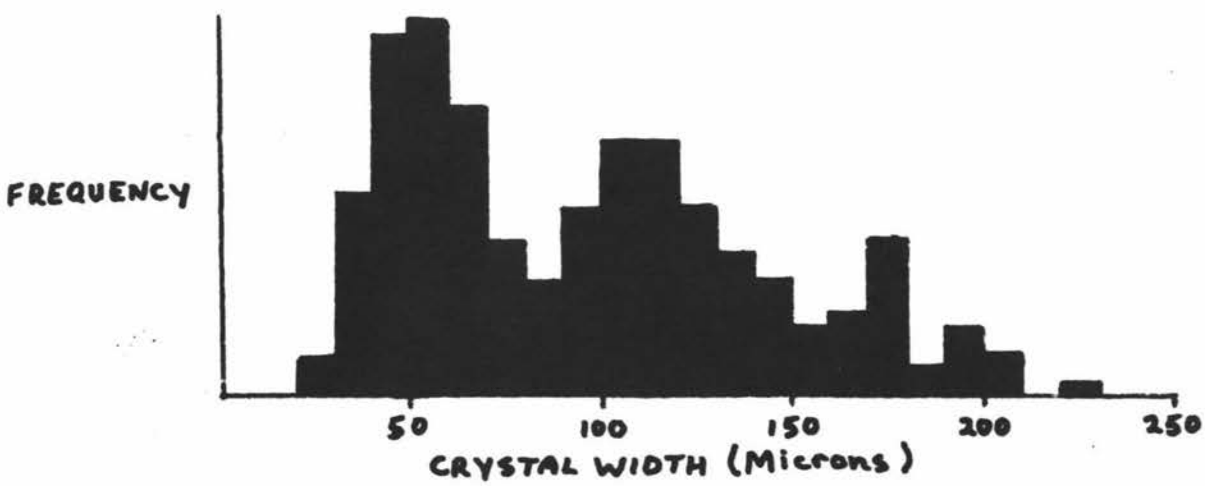


FIG. 21. RATE OF ICE FORMATION = 200 %/Hr
CENTRE OF SLAB.

rate of ice formation and the consequent numbers of crystals which would have to be measured to obtain statistically significant size parameters.

As it was not possible to measure the cross sectional area it was decided to square the crystal width to obtain an estimate of the crystal area and use this as an additional index of crystal size. The method used to measure ice crystal size is given in Appendix I.

(b) Analysis of crystal size data

As approximately 18,000 crystal width measurements were made a statistical method of analysing this data was obviously necessary. However, before attempting to use a statistical analysis to correlate crystal width to rate of ice formation, it was decided to study the frequency distribution of ice crystal widths at each location examined. A suitable computer programme for determining frequency distributions was available. The results from this programme were expressed as numbers of ice crystals in each width class, and as frequency distribution histograms. Each histogram bar was representative of a width class and its length was calculated as a fraction of the length of the bar in the most popular width class to which was assigned a fixed length. In this way it was possible to obtain a visual comparison between the frequency distribution at each location, and this is shown on Figs 15, 16, 17, 18, 19, 20, 21 and Plates 7, 7a, 8, 8a, 9, 9a, 10, 10a, 11, 11a, 12, 12a, 13, 13a.

The histograms illustrate that the width frequency distribution is approximately normal at the surface and becomes skewed as the rate

Plates 7-13 and 7a-13a Rate of Ice Formation Studies

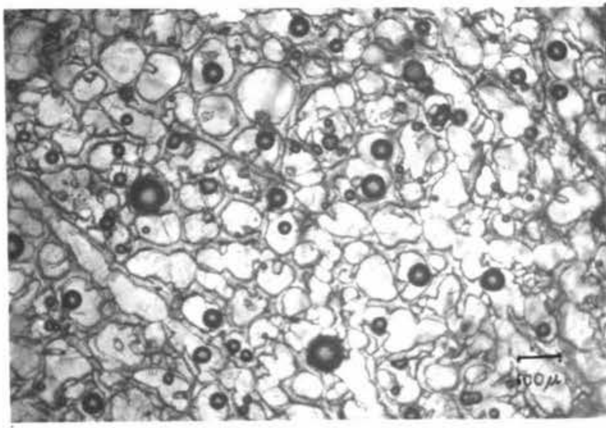


Plate 7: Fibre parallel to heat flow. Rate of ice formation 680%/Hr.

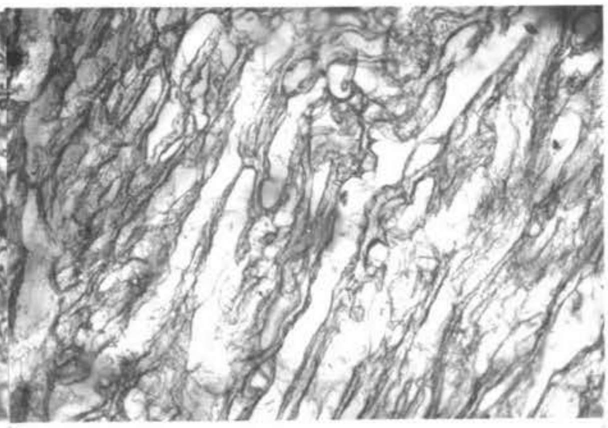


Plate 7a: Fibre perpendicular to heat flow. Rate of ice formation 680%/Hr.

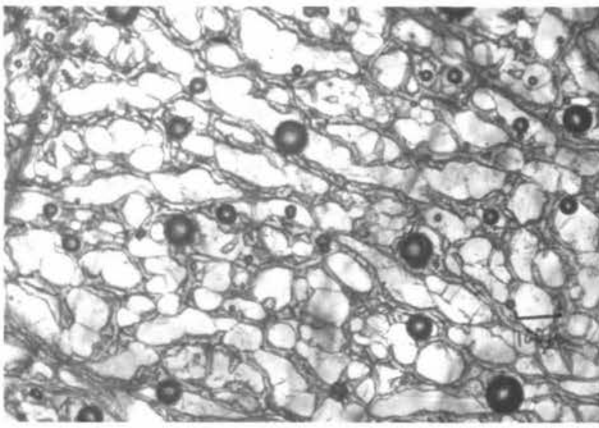


Plate 8: Fibre parallel to heat flow. Rate of ice formation 660%/Hr.

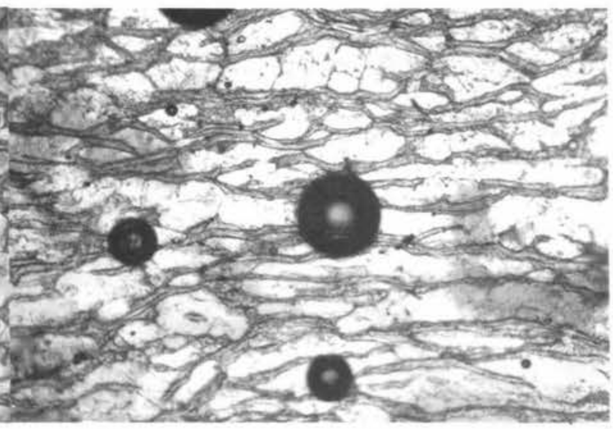


Plate 8a: Fibre perpendicular to heat flow. Rate of ice formation 660%/Hr.

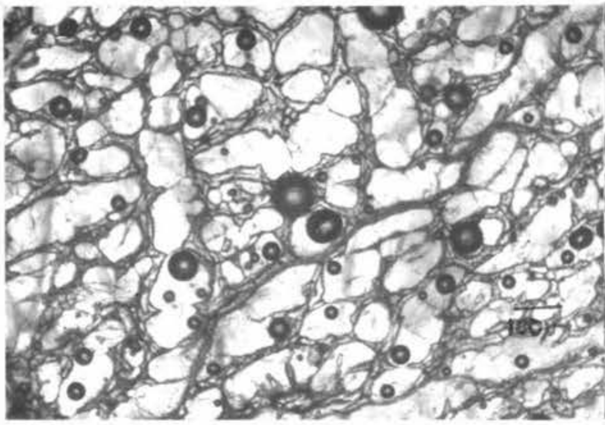


Plate 9: Fibre parallel to heat flow. Rate of ice formation 410%/Hr.

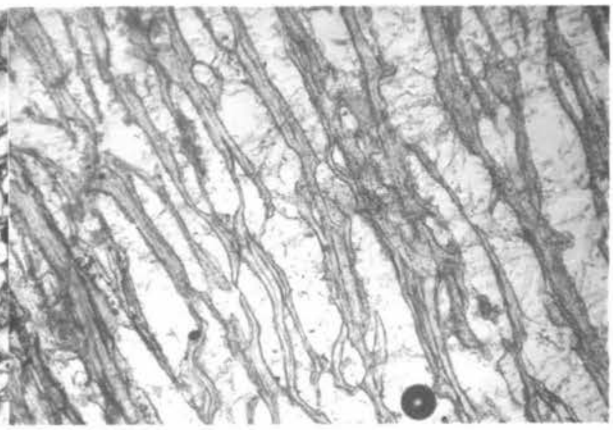


Plate 9a: Fibre perpendicular to heat flow. Rate of ice formation 410%/Hr.

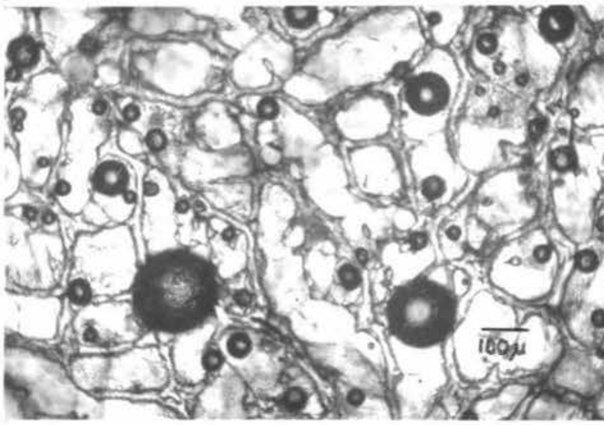


Plate 10: Fibre parallel to heat flow. Rate of ice formation 240%/Hr.

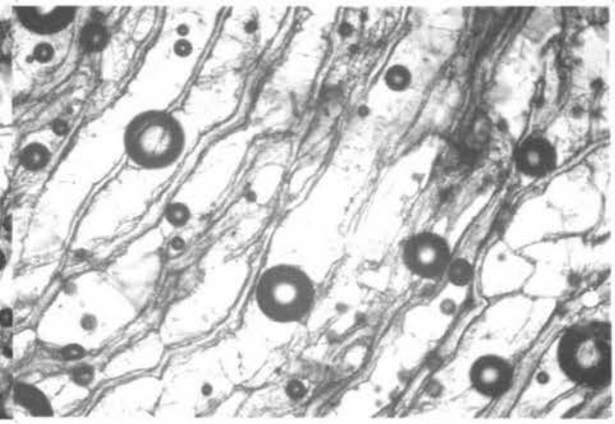


Plate 10a: Fibre perpendicular to heat flow. Rate of ice formation 240%/Hr.

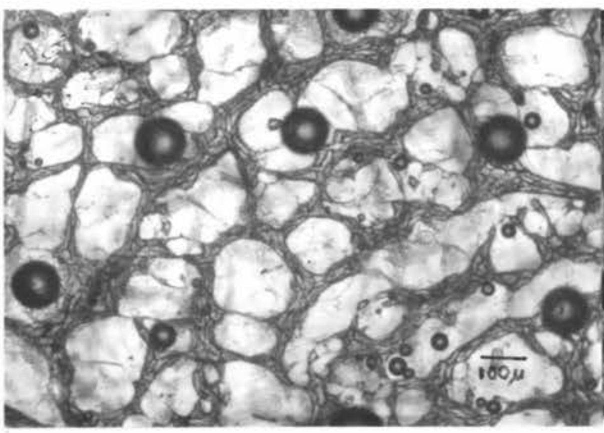


Plate 11: Fibre parallel to heat flow. Rate of ice formation 190%/Hr.

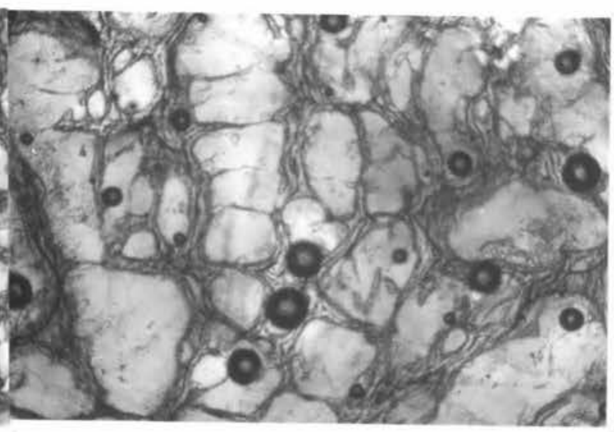


Plate 11a: Fibre perpendicular to heat flow. Rate of ice formation 190%/Hr.

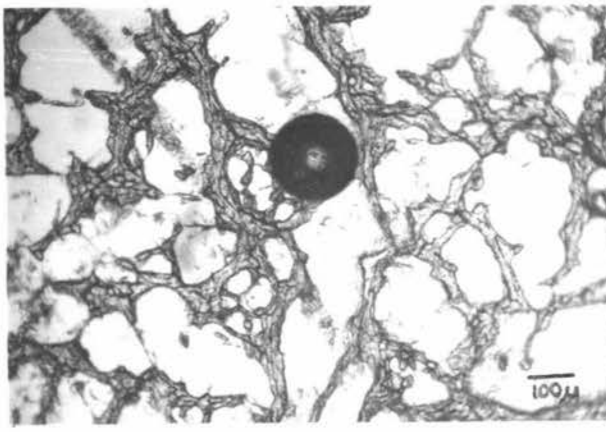


Plate 12: Fibre parallel to heat flow. Rate of ice formation 100%/Hr.

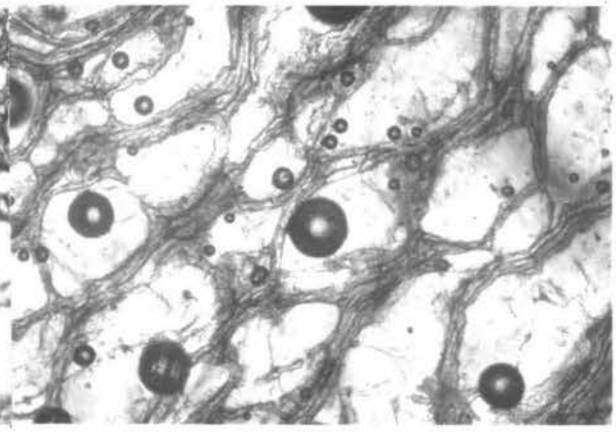


Plate 12a: Fibre perpendicular to heat flow. Rate of ice formation 100%/Hr.

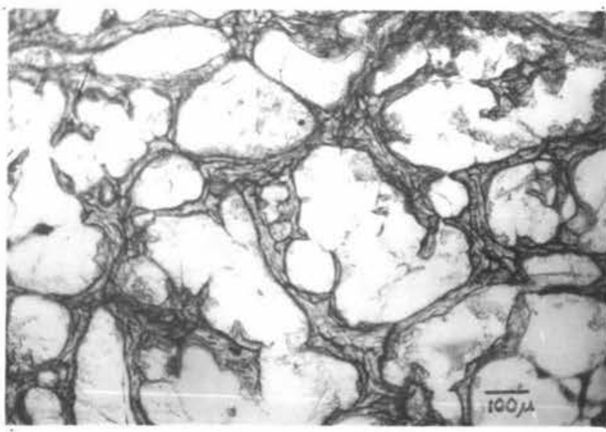


Plate 13: Fibre parallel to heat flow. Rate of ice formation 200%/Hr. Thermodynamic centre of block.

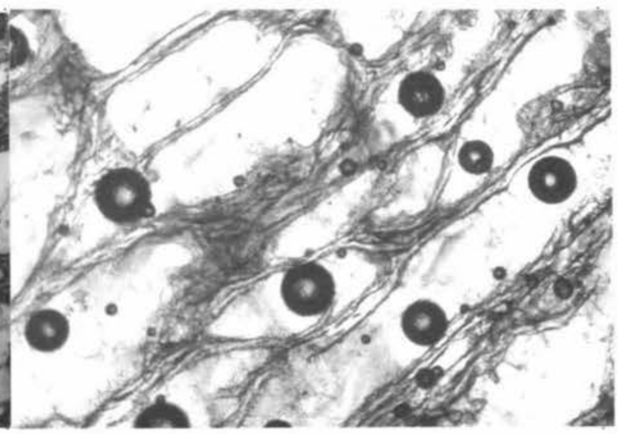


Plate 13a: Fibre perpendicular to heat flow. Rate of ice formation 200%/Hr. Thermodynamic centre of block.

of ice formation declines. To analyse this data statistically a normal distribution cannot therefore be assumed. While it might be possible to describe the distribution changes in numerical terms by fitting equations to the lines of best fit through the tops of the histogram bars (a computer programme which could have done this was available), it was felt that as there was no theoretical basis for expecting any given type of distribution, there was insufficient justification for doing this.

A scatter diagram comparing ice crystal width to location has been prepared from the frequency distribution histograms, Fig. 22. It demonstrates that the largest crystal width varies almost linearly with location between 1 and 2½ inches from the surface and could be related to rate of ice formation by a relationship of the form:-

$$\text{Largest ice crystal width} \propto \frac{1}{\text{Rate of ice formation} + C}$$

Where C is a constant.

Outside this range the relationship between largest crystal width and location is not linear and the largest crystal width is larger than would be expected from extrapolation of the linear portion. Fig. 12 shows that the rate of ice formation (experimental) is smaller than would be expected from extrapolation of the linear portion of this graph which is what would be expected from a reciprocal relationship between largest ice crystal width and rate of ice formation.

To check this hypothesis an equation relating ice crystal width and rate of ice formation over the linear portion of each graph was developed:-

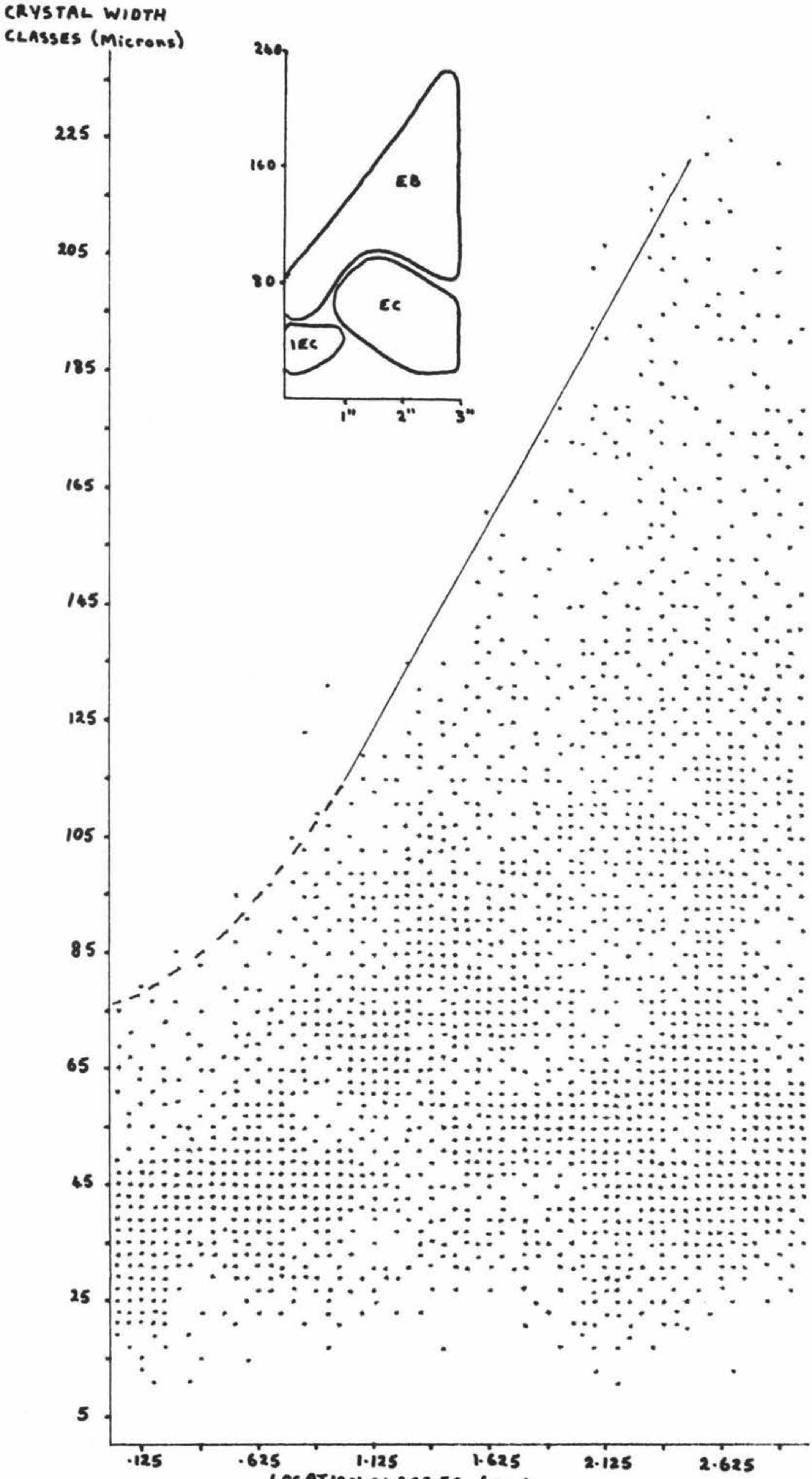


FIG.22. CRYSTAL WIDTH LOCATION SCATTER DIAGRAM

$$C_s = \frac{2.8 \times 10^4}{R_i + 80} - 42 \qquad (5.1)$$

Where C_s is the largest ice crystal width,

R_i is the rate of ice formation.

Fig. 23 graphs crystal widths determined by this relationship and compares them with experimental values. The graph demonstrates that this relationship is valid over the non linear portion of Figs 22 and 12. This further supports the contention that the calculation method is unsatisfactory for predicting rates of ice formation as the non linear portion of the rate of ice formation location curve is not predicted by calculation.

The significance of the darker zones in Fig. 22 is discussed later in this chapter.

(c) Crystal size distribution in the polystyrene mould experimental system

A scatter diagram was also prepared from frequency distribution histograms taken from the polystyrene mould experimental system, Fig. 24. Compared to the mince mould the distribution is uniform over the thickness of the slab, which confirms the theoretical prediction that the rate of ice formation would be determined by the heat removed from the sides of the plug as well as from the ends. The meat used in this study was frozen post-rigor without ageing. A cold storage study on this meat showed that recrystallisation after 4 months storage at -20°F was undetectable, confirming that -20°F was a safe temperature for preparing frozen meat for microscopy.

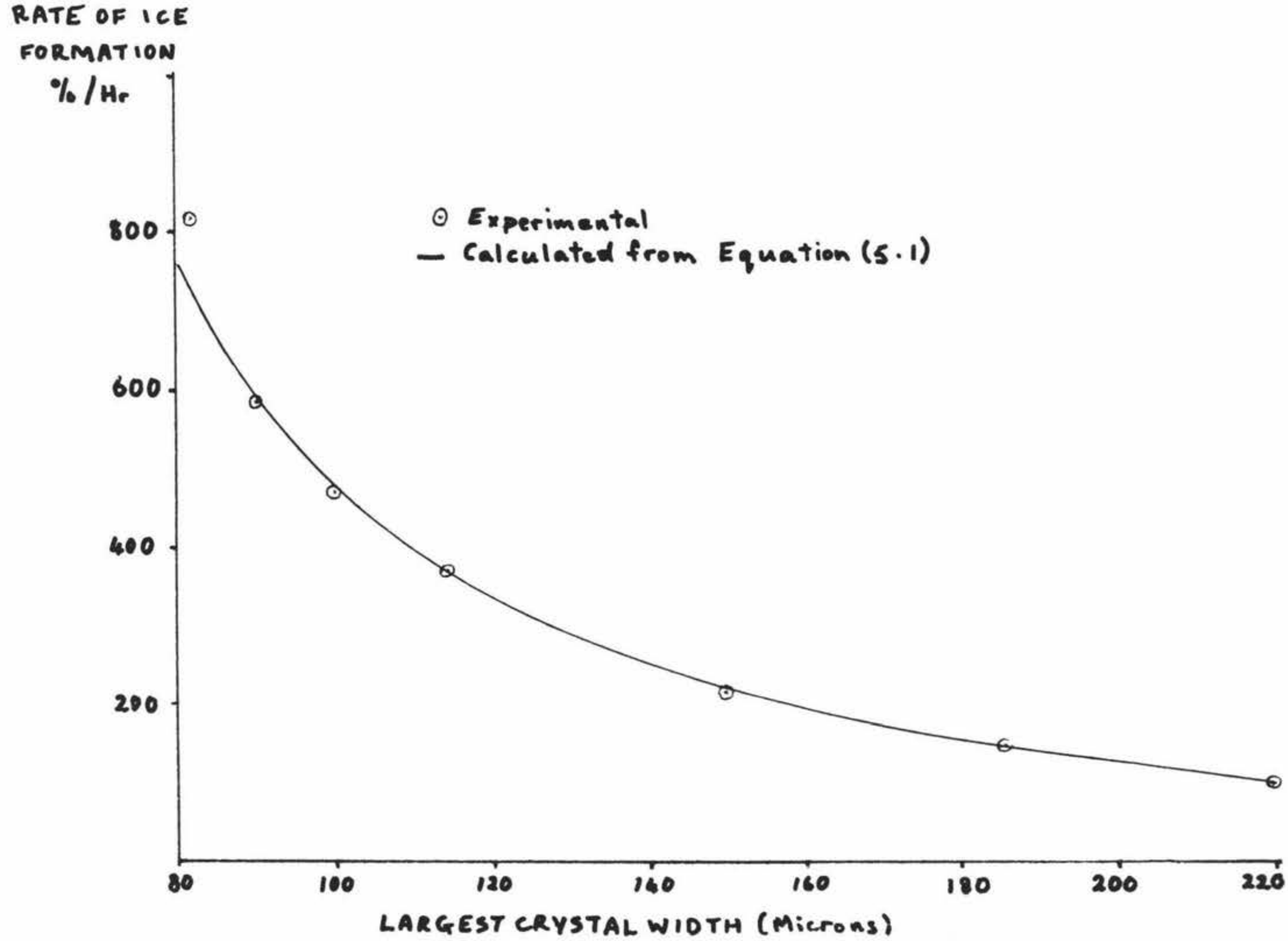


FIG. 23 RATE OF ICE FORMATION AND LARGEST CRYSTAL WIDTH.

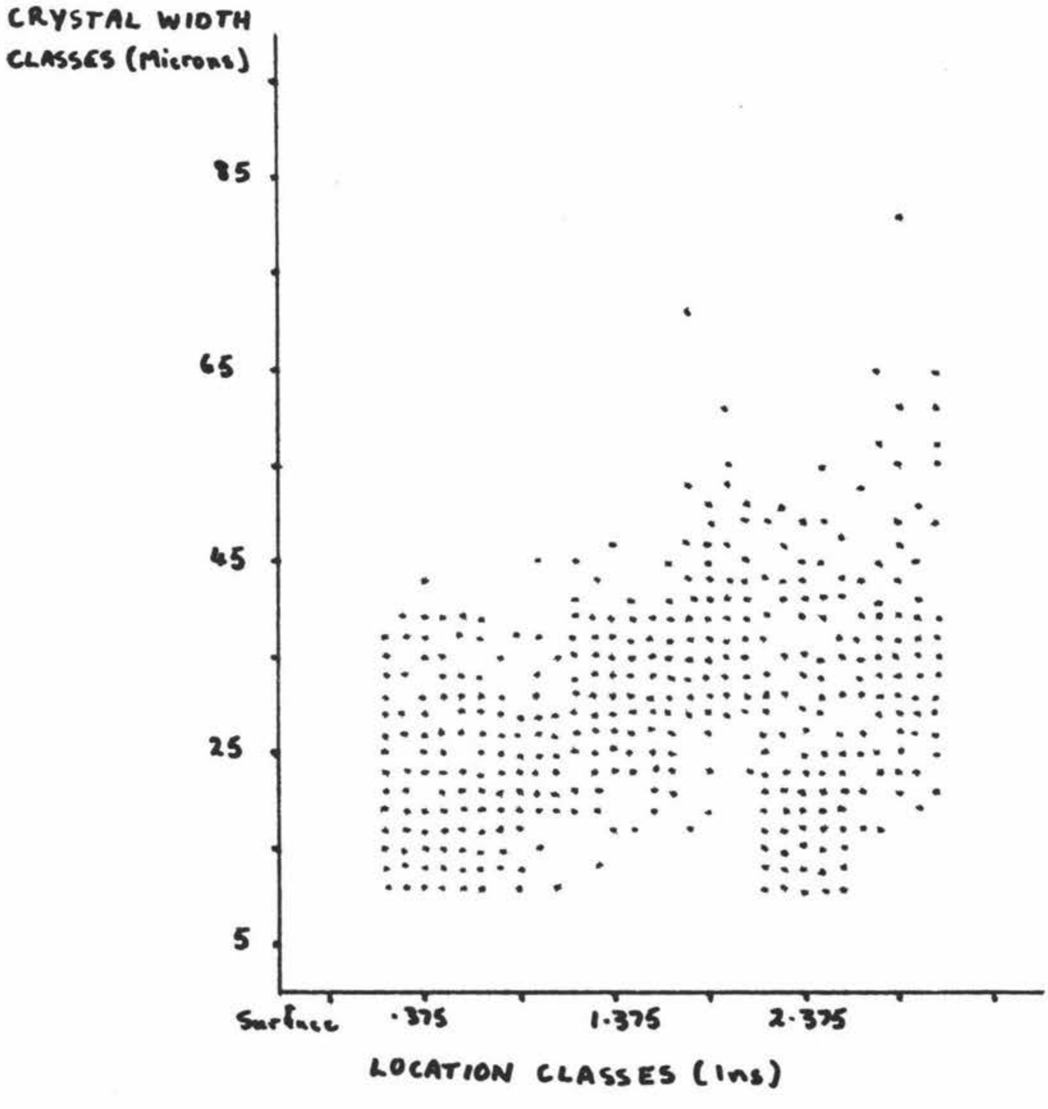


FIG. 24. POLYSTYRENE MOULD EXPERIMENTAL SYSTEM

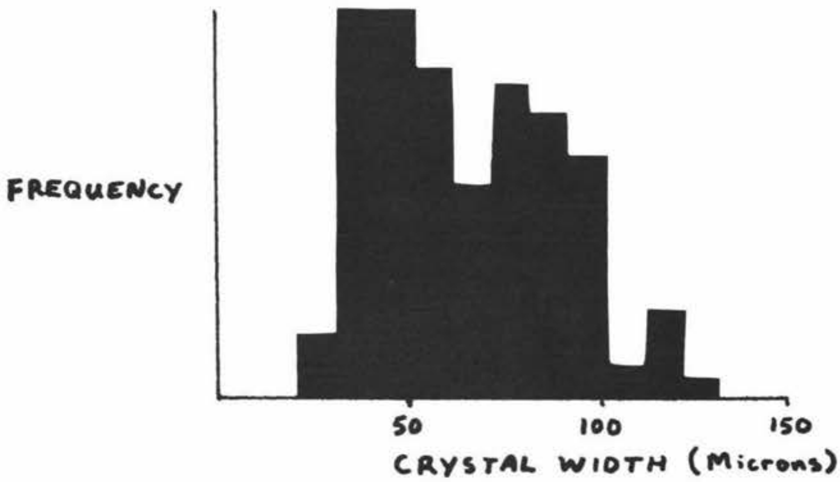
CRYSTAL WIDTH LOCATION SCATTER DIAGRAM

(d) Influence of muscle fibre direction

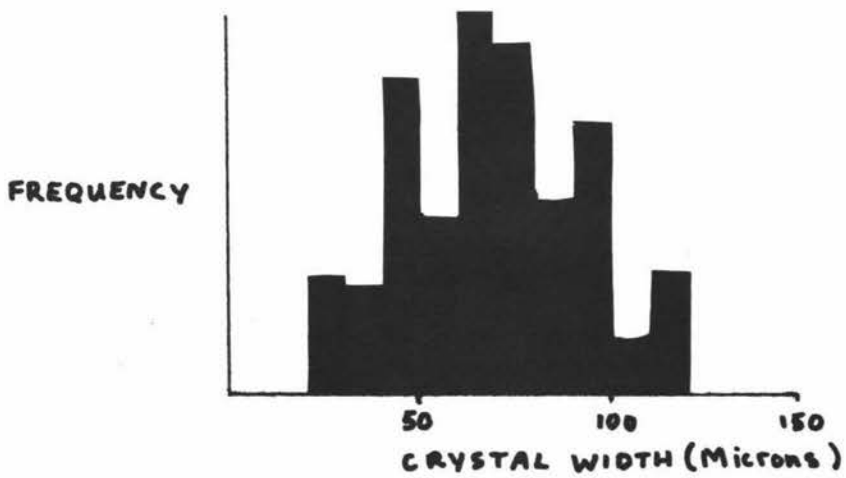
The longitudinal sections (Plates 7a - 13a) were taken from meat plugs in which the heat flow was perpendicular to the fibres. It is unlikely that this had any major effect on the rate of ice formation in the experimental system as the thermal properties of the mince surrounding the plug would tend to mask any differences in the thermal conductivity of the frozen meat plug due to fibre orientation. Some difference may be evident towards the centre, as the greatest difference in thermal conductivity occurs in the frozen meat. Fig. 25 compares frequency distributions taken from the same location in four plugs, two with fibres parallel and two with fibres perpendicular to the direction of heat flow. The two histograms indicate that there may be a higher proportion of small ice crystals in the meat frozen with perpendicular fibre orientation. This may be the result of a higher rate of ice formation but the similar widths of the larger ice crystals suggest that this is not so. A more likely explanation is that the longitudinal section does not pass through the widest part of all of the crystals.

(e) Analysis of estimated crystal cross sectional area data

The squared crystal width values were treated in the same way as the crystal width values and histograms at various rates of ice formation were obtained. Histograms from the same rates of ice formation as in Figs 15-21 are shown in Figs 26-32. By squaring the width values the skewness of the distribution has been exagerated and the distribution has been smoothed. In relation to the ice crystal width histograms, the class sizes for the smaller crystals are larger than for the larger crystals; this causes the increased skewness and



FIBRES PERPENDICULAR TO HEAT FLOW



FIBRES PARALLEL TO HEAT FLOW

FIG.25. INFLUENCE OF FIBRE DIRECTION ON CRYSTAL SIZE DISTRIBUTION

FIGS 26 - 32. RATE OF ICE FORMATION STUDIES: CRYSTAL WIDTH SQUARED FREQUENCY DISTRIBUTION

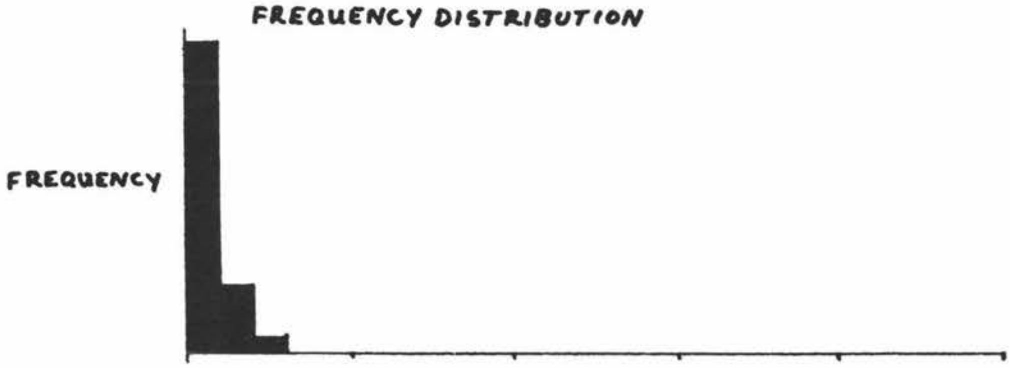


FIG. 26. RATE OF ICE FORMATION = 680% / Hr.

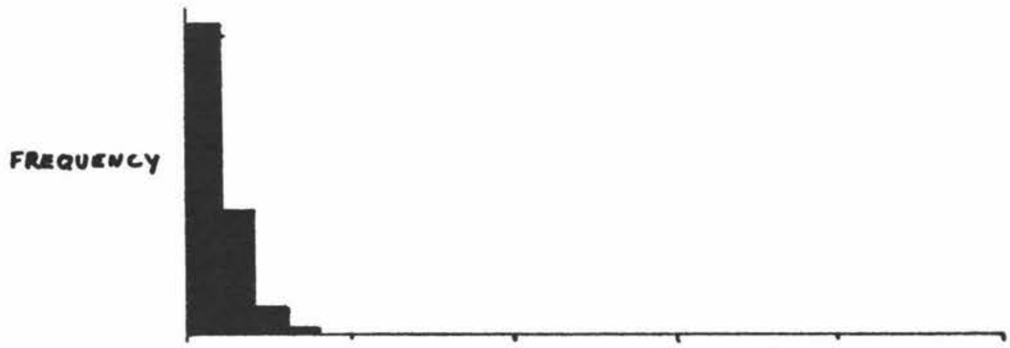


FIG. 27. RATE OF ICE FORMATION = 660 % / Hr.

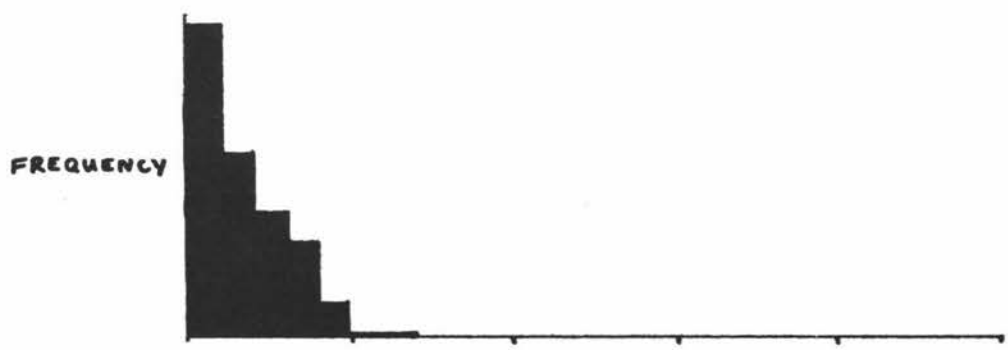


FIG. 28. RATE OF ICE FORMATION = 410 % / Hr

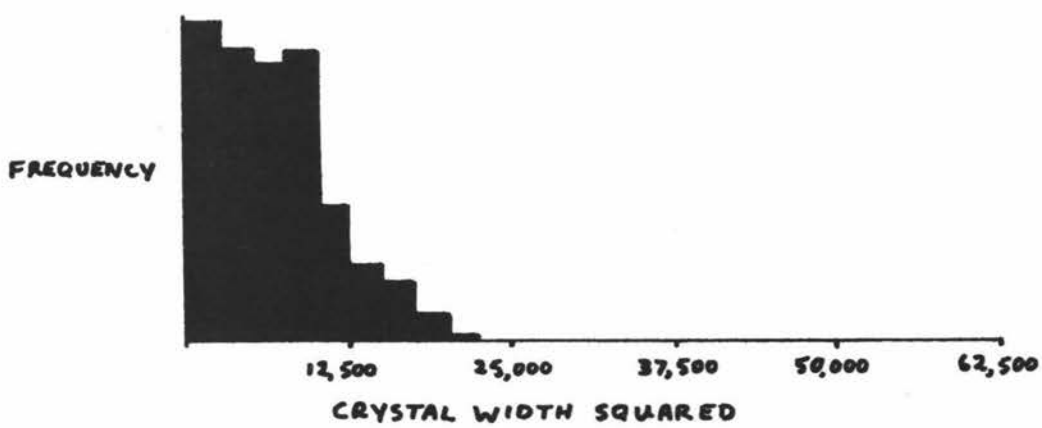


FIG. 29. RATE OF ICE FORMATION = 250 % / Hr

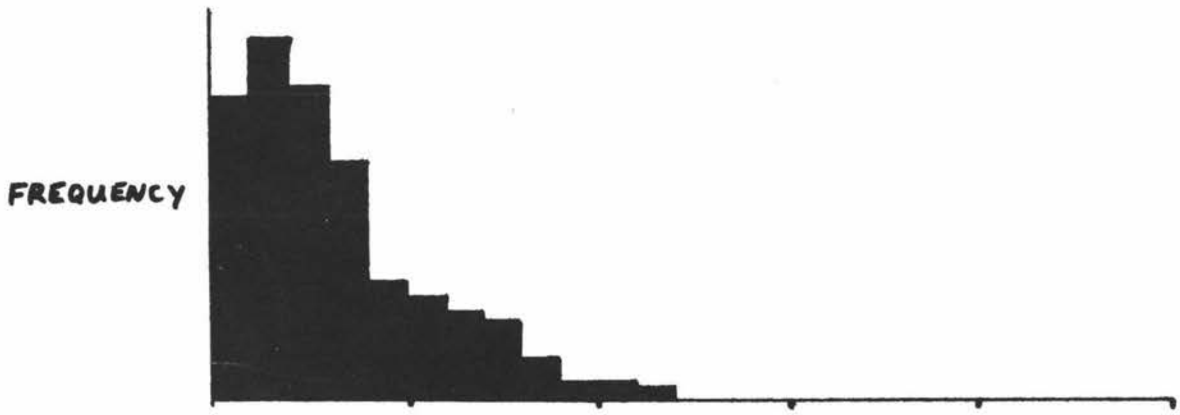


FIG. 30. RATE OF ICE FORMATION = 190 %/Hr

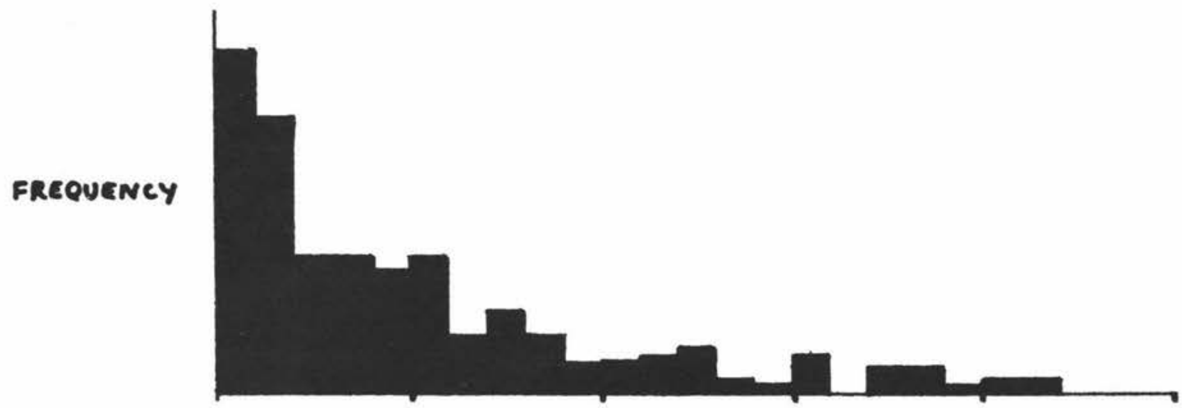


FIG. 31. RATE OF ICE FORMATION = 100 %/Hr

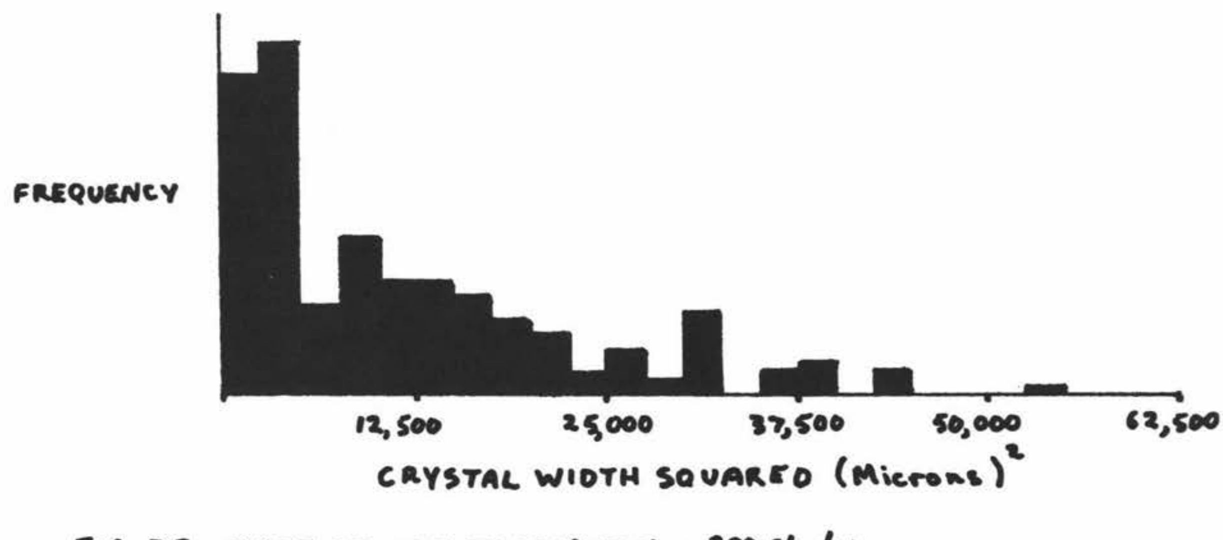


FIG. 32. RATE OF ICE FORMATION = 200 %/Hr
CENTRE OF SLAB.

fixes the highest observation frequency in the smallest class. This means that the range can be used as an index of skewness. The square root of the range gives the largest crystal width.

The total range is a difficult parameter to use as it has a rather indefinite value. For this reason it was decided to use the 95% confidence limit as a measurement of the range, i.e. the value of the class beyond which 5% of the total number of observations remain. These range values are plotted in Fig. 33 and compared with range values calculated from the largest crystal width predicted by equation (5.1). The experimental values parallel the predicted values indicating that the constant value 2.8×10^4 in equation (5.1) is too high when the 95% limit of the width-squared distribution is used as an estimator of maximum crystal size. From a plot of the square root of 95% range against location and Fig. 12 a new constant value of 2.33×10^4 was derived for use with this estimator of maximum crystal size.

(f) Limitations of crystal size as an index of crystal form

It is apparent from the photographs (Plates 7-13) that size alone, as measured by width or estimated area, does not adequately describe the changes in ice crystal form which take place with changing rate of ice formation. Three types of ice crystal formation exist; intracellular, extracellular between individual fibres, and extracellular between fibre bundles. The relative proportions of each type of ice vary with the rate of ice formation as does the size within each type of formation. The influence of the rate of ice formation on the overall crystal size can therefore be expected to be complicated.

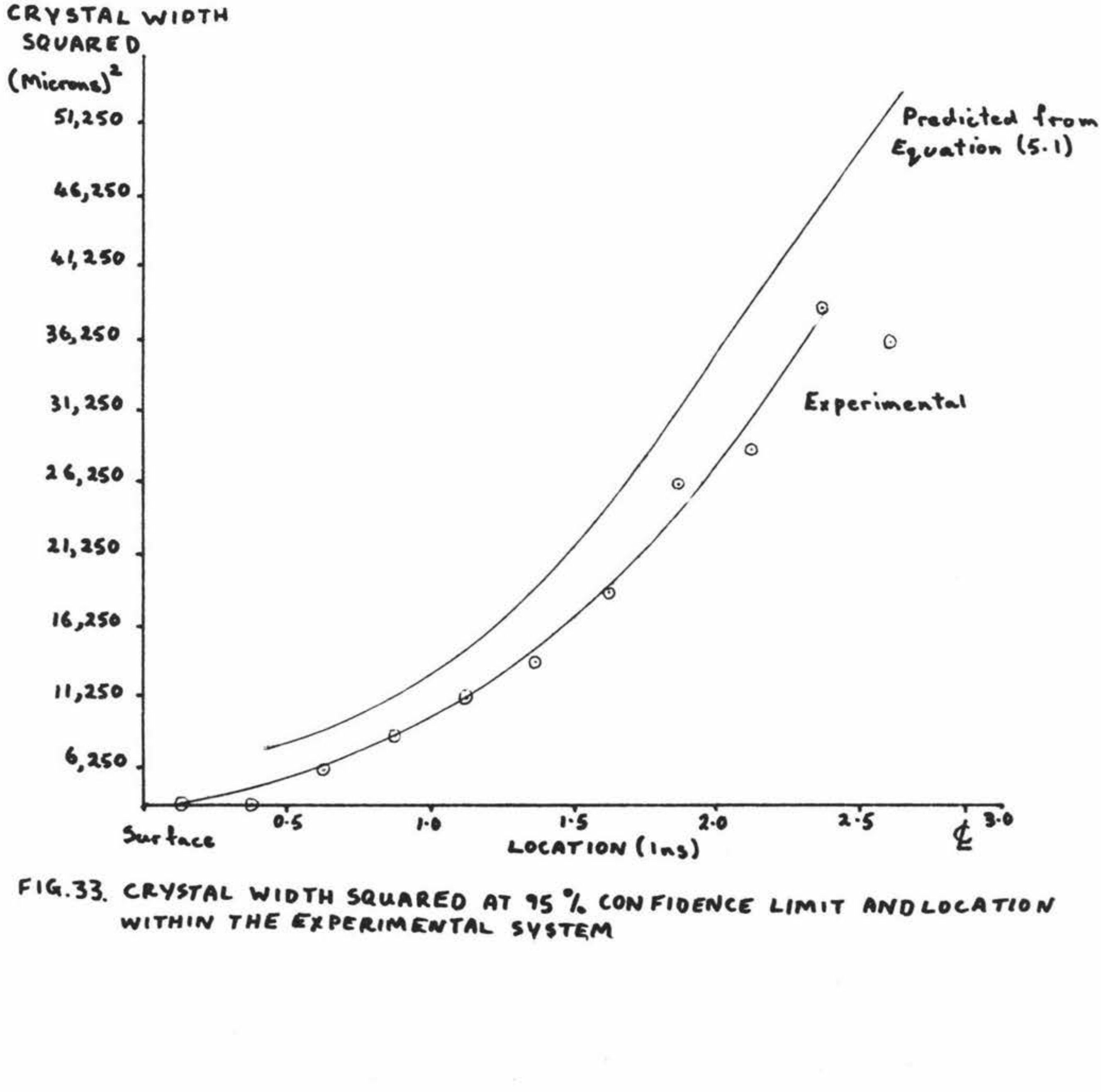


FIG. 33. CRYSTAL WIDTH SQUARED AT 95 % CONFIDENCE LIMIT AND LOCATION WITHIN THE EXPERIMENTAL SYSTEM

In this study no attempt was made to measure the influence of the rate of ice formation on crystal size in any one type of ice crystal. The size distribution near the surface, where the rates of ice formation are high, is almost normal. In this region extracellular ice crystals within fibre bundles and extracellular ice are of similar sizes so that a study such as this would provide evidence on the effects of rate of freezing on individual types of ice formati .

2. Qualitative Observations on Ice Crystal Size and Rate of Ice Formation

The following discussion describes the changes in crystal size with changing rates of ice formation.

(a) Intracellular ice

Fig. 34, Plate 14. Rate of ice formation ≑ 1000%/hr.
In the post-rigor meat used in this study there is a size limit of 20-30 μ on this type of crystallisation. The photomicrograph is of a section just below the surface and shows that intracellular and both types of extracellular ice are present. The size of each type of ice formation is approximately the same. The intracellular ice is present as a number of ice needles within each cell.

(b) Extracellular ice

Fig. 35, Plate 15. Rate of ice formation ≑ 800%/hr.
In this photomicrograph much of the intracellular ice has been excluded by extracellular ice. Only one ice crystal is present within each cell. The extracellular ice crystals between fibre bundles are not as large as the ice crystals within them.

CHANGES IN ICE CRYSTAL FORM WITH CHANGING RATE OF ICE FORMATION.

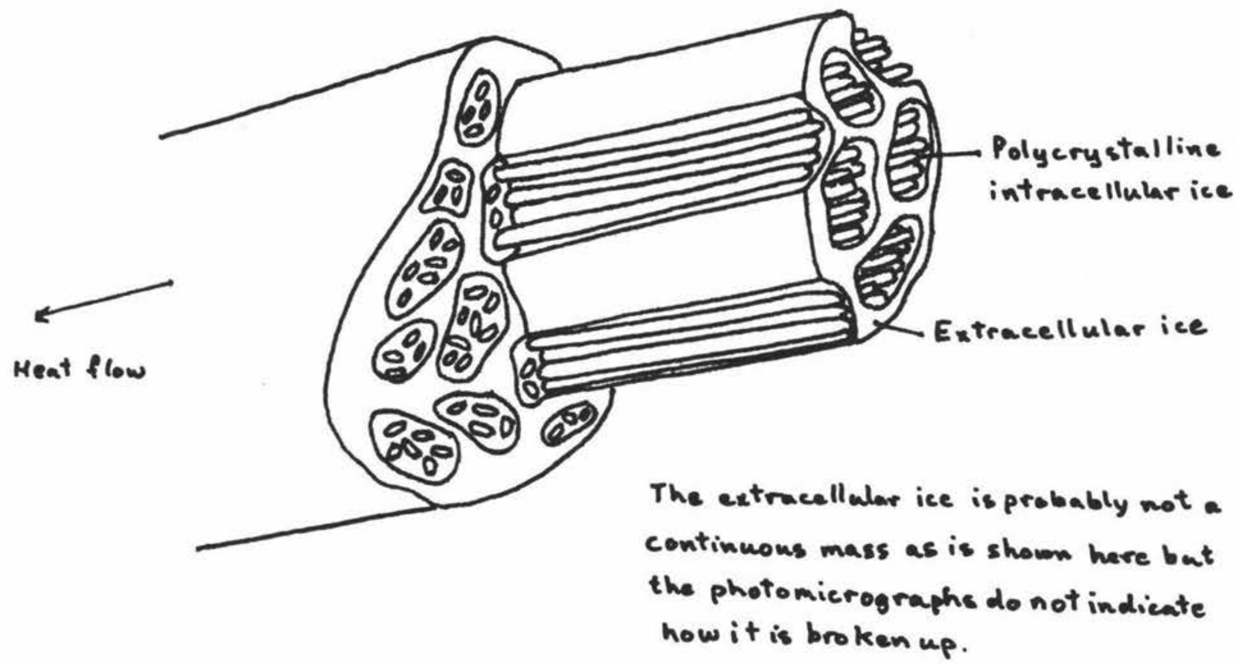


FIG. 34. POLYCRYSTALINE INTRACELLULAR ICE AND EXTRACELLULAR ICE BETWEEN FIBRES.

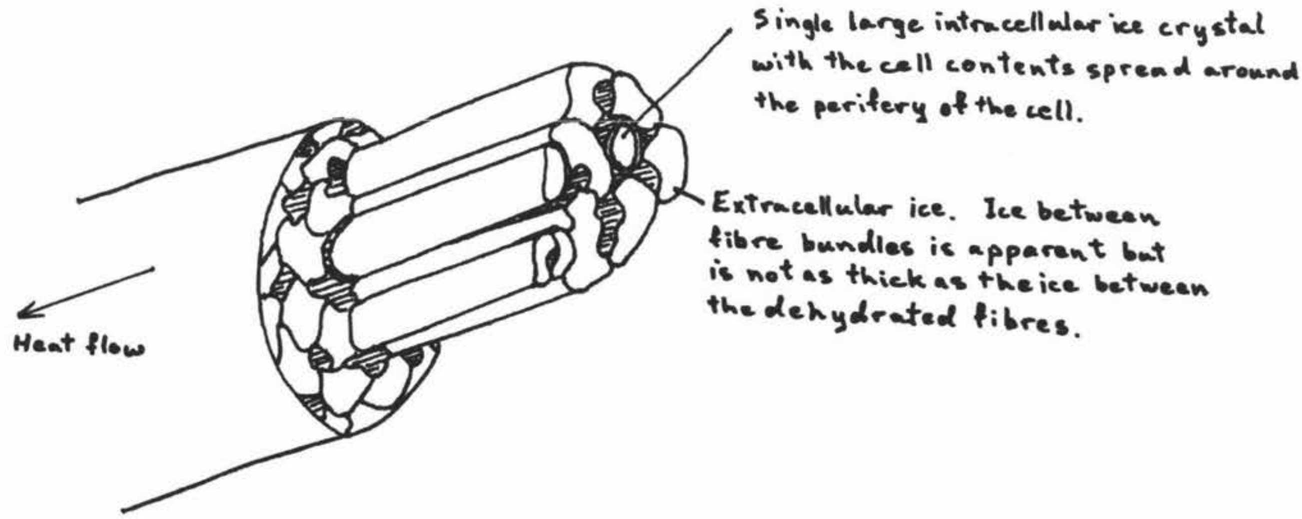


FIG. 35. SINGLE INTRACELLULAR ICE CRYSTALS AND EXTRACELLULAR ICE BETWEEN FIBRES.

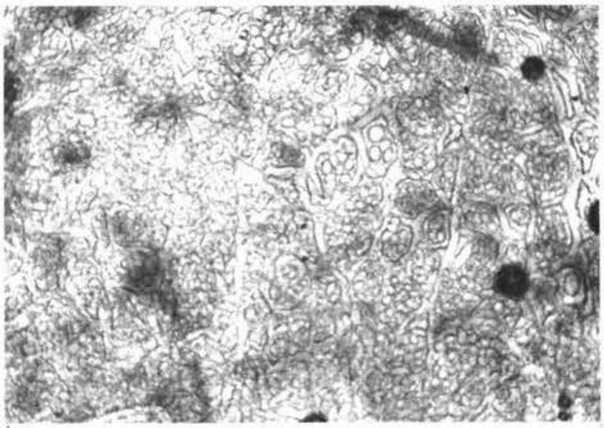


Plate 14: Polycrystalline intracellular ice.

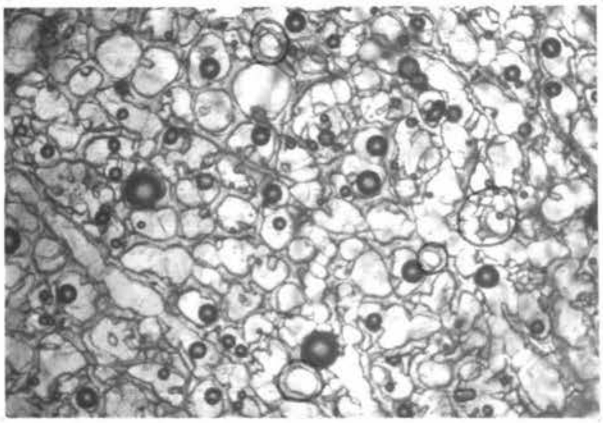


Plate 15: Extracellular and intracellular ice. (O)

Fig. 36, Plate 16. Rate of ice formation ≑ 400%/hr. The ice between the fibre bundles is of the same general size as the ice between fibres, 70-90 μ. Intracellular ice has been excluded completely.

Fig. 37, Plate 17. Rate of ice formation ≑ 100%/hr. The ice between fibre bundles has become thicker than the ice within them, and is forming at the expense of these crystals. The crystals between the fibres are becoming smaller and larger masses of dehydrated fibres are forming. Illustration of an advanced state of this condition is given in Plate 18 (rate of ice formation ≑ 50%/hr.).

The size distribution of ice crystals between fibre bundles is greater than for intracellular ice or ice between the fibres at any given rate of ice formation.

(c) Ice form and location

Zones of high crystal size frequency (darker areas) can be seen in the width location scatter diagram, Fig. 22. Zone IEC corresponds to intracellular and extracellular ice within the fibre bundles of the same general size. Zone EC corresponds to extracellular ice within the fibre bundles, and the decreasing size trend in this zone as the rate of ice formation becomes slower should be noted. Zone EB corresponds to ice outside the fibre bundles and demonstrates the increasing size distribution as the rate of ice formation decreases.

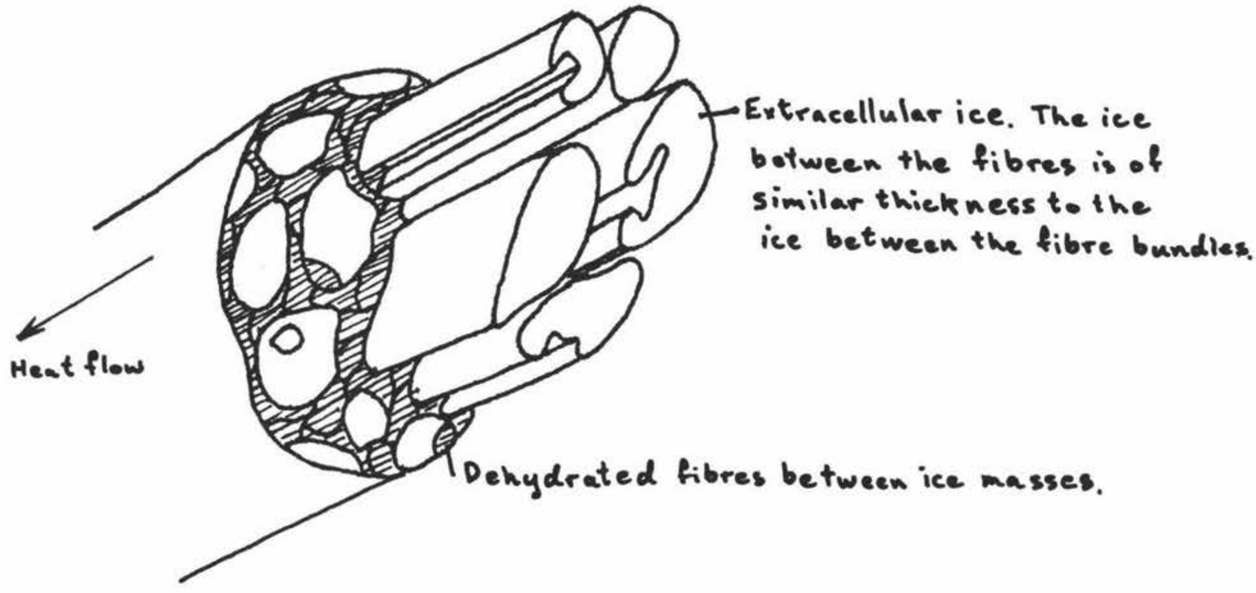


FIG. 36. EXTRACELLULAR ICE BETWEEN FIBRES.

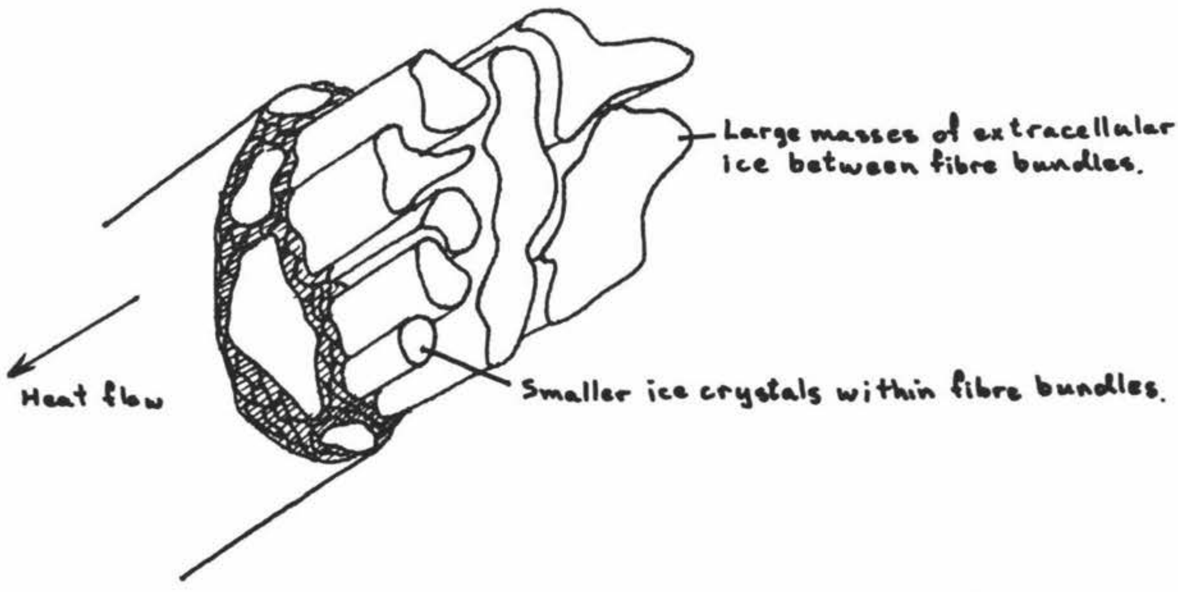


FIG. 37. EXTRACELLULAR ICE BETWEEN FIBRES AND FIBRE BUNDLES.

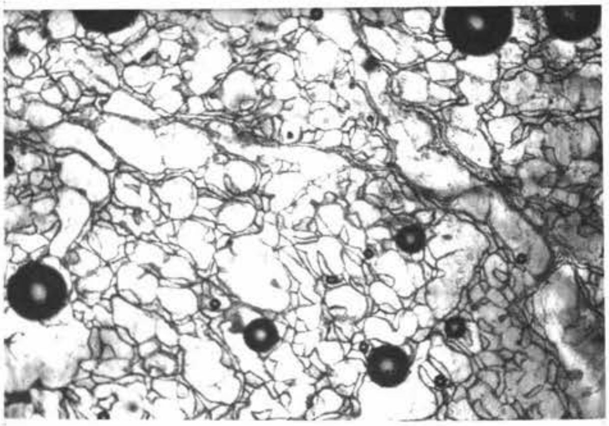


Plate 16: Extracellular ice.

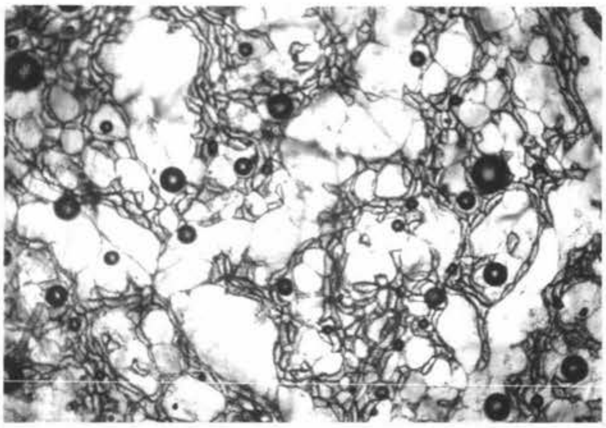


Plate 17: Extracellular ice within and between fibre bundles.

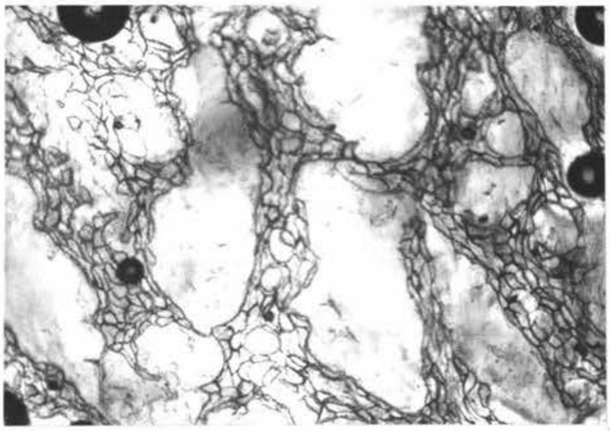


Plate 18: Extracellular ice between fibre bundles.

B. ICE CRYSTAL FORMATION AND THE PHYSIOLOGICAL CONDITION OF THE MEAT

Unfortunately, all of the ageing and meat condition studies were made using the polystyrene mould experimental system which prevented the assessment of the combined influence of condition and rate of ice formation on ice crystal form.

1. Ageing Studies

Width distribution histograms were prepared from crystal measurements taken from sections at ⅜ inch and ⅛ inch from the surface, in meat which had been aged 0, 5, 10, 15 and 21 days in a 35°F chiller and are shown on Figs 38, 39, 40, 41. Equal numbers of measurements from each location within each plug were used in the histogram calculations except for the five day histogram for which only ⅜ inch data was available. This histogram has been omitted. The histograms and a scatter diagram prepared from them, Fig. 42, fail to demonstrate any obvious trends with ageing, an observation which is confirmed by comparison of the photomicrographs of the tissue taken ⅜ inch from the surface, Plates 19, 20, 21. There is, however, some indication of a small increase in crystal size over the 21 day period.

2. Rigor Mortis Studies

There is considerable difference in crystallisation between meat frozen pre-rigor and meat frozen post-rigor. In pre-rigor meat there is a greater tendency for intracellular crystallisation which persists until the crystals reach a thickness of 60-70 μ. Extracellular ice forming between fibres at this stage is smaller than intracellular ice, while ice forming between fibre bundles is approximately the same size, Plates 22, 23, 24, Figs 43, 44.

FIGS 38-41. AGING STUDIES: CRYSTAL WIDTH FREQUENCY

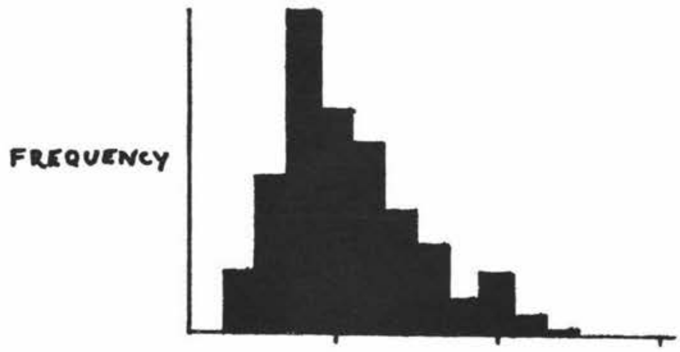


FIG. 38. UNAGED.

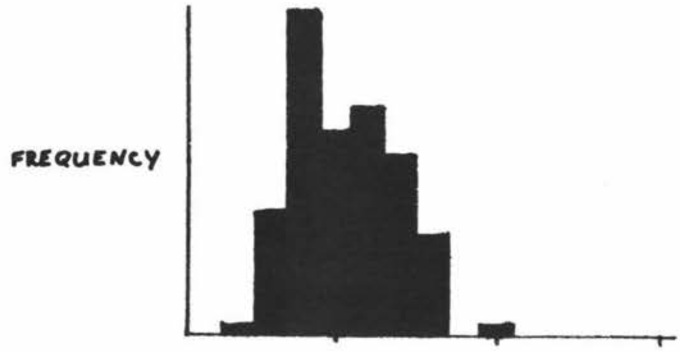


FIG. 39. 10 DAYS AGING AT 35°F.

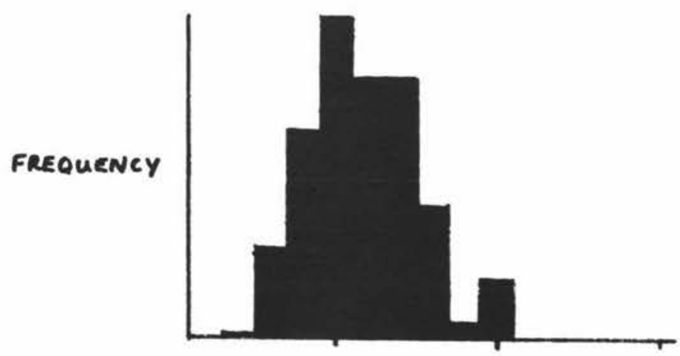


FIG. 40. 15 DAYS AGING AT 35°F.

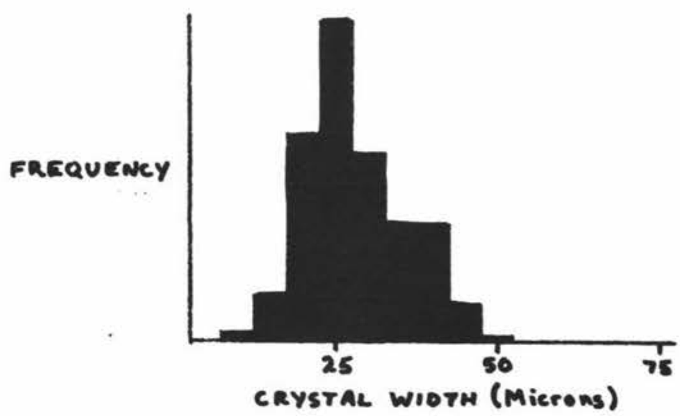


FIG. 41. 21 DAYS AGING AT 35°F.

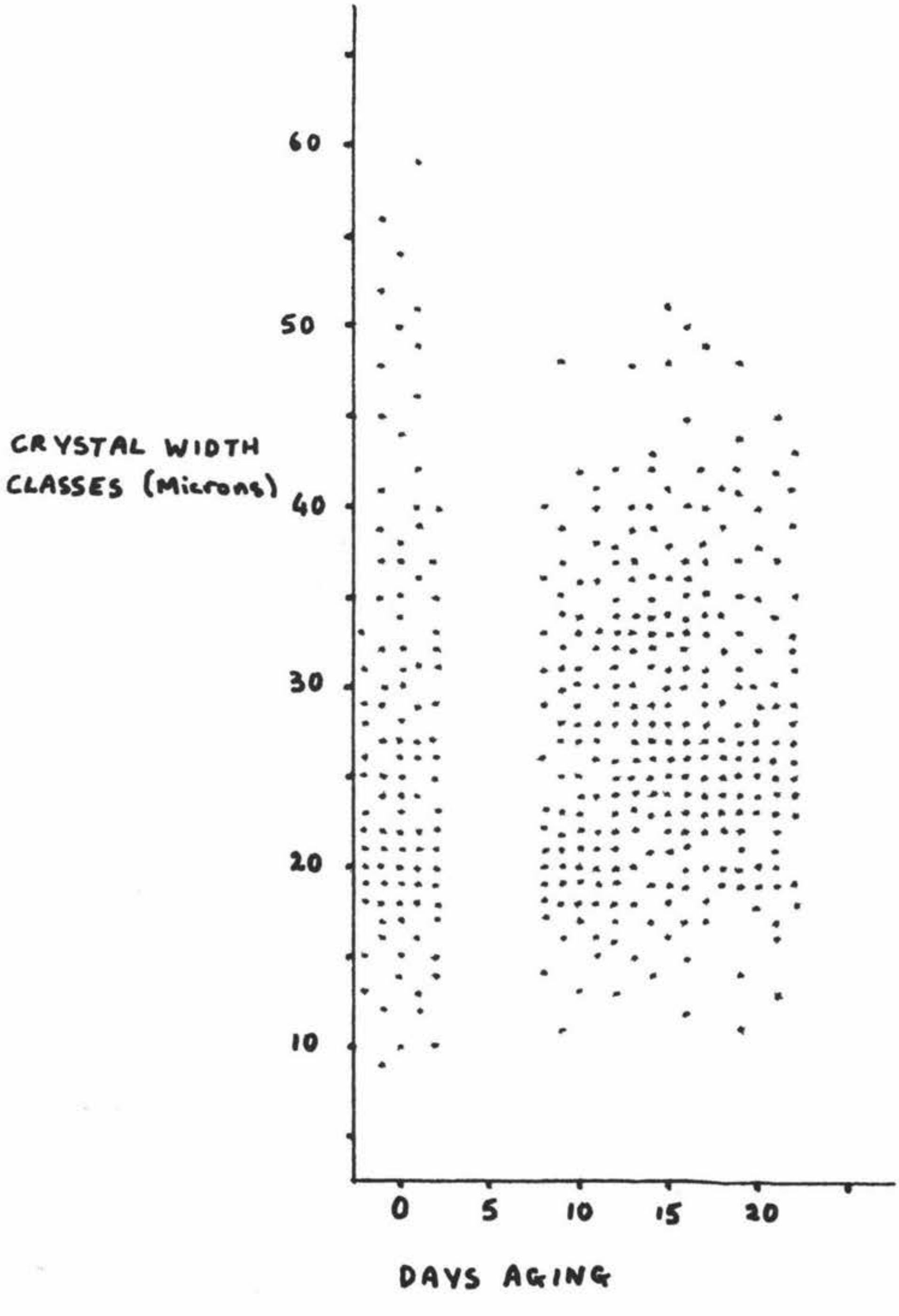


FIG. 42. CRYSTAL WIDTH AGING SCATTER DIAGRAM

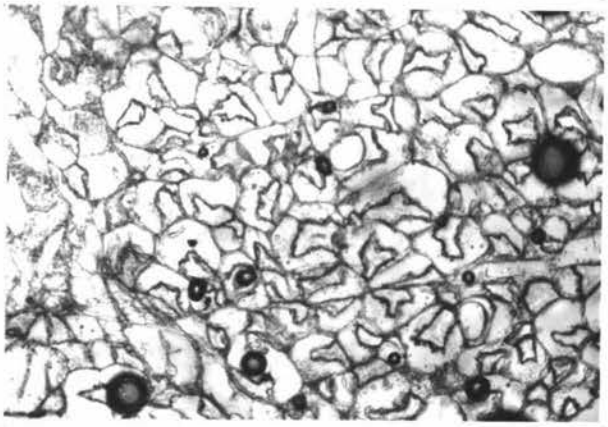


Plate 19: Unaged meat.

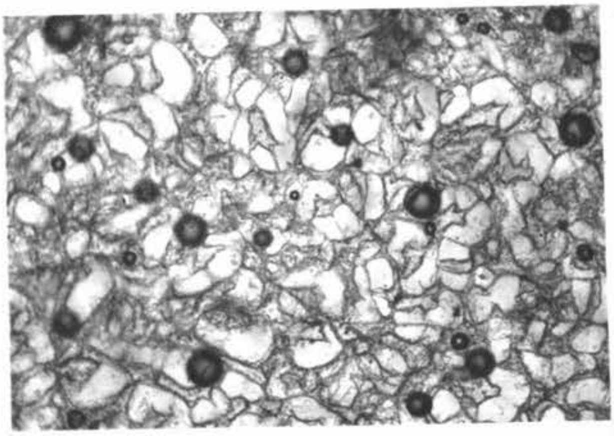


Plate 20: Meat aged 5 days at 35°F.

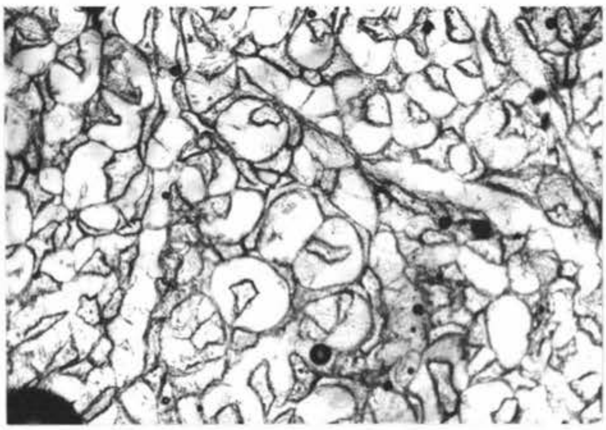


Plate 21: Meat aged 15 days at 35°F.

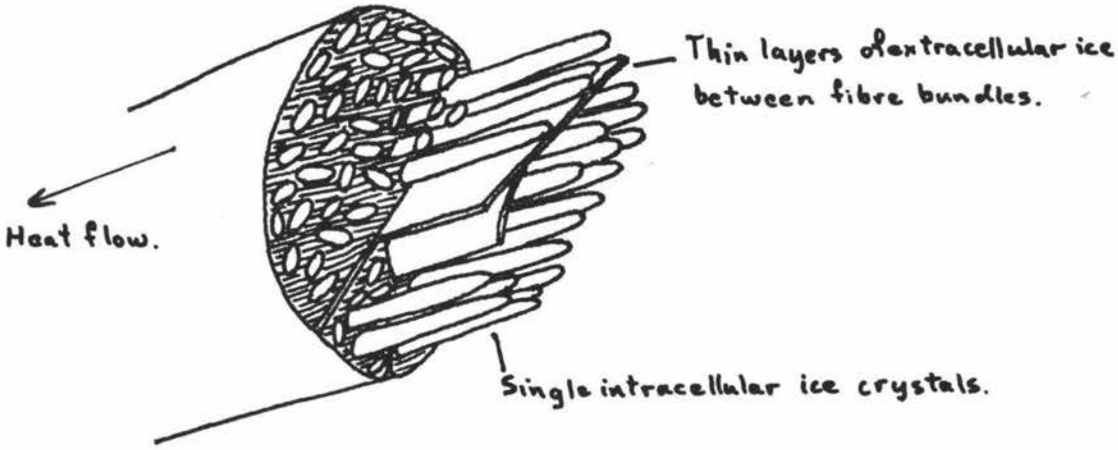


FIG. 43. INTRACELLULAR ICE IN PRE-RIGOR FROZEN MEAT.

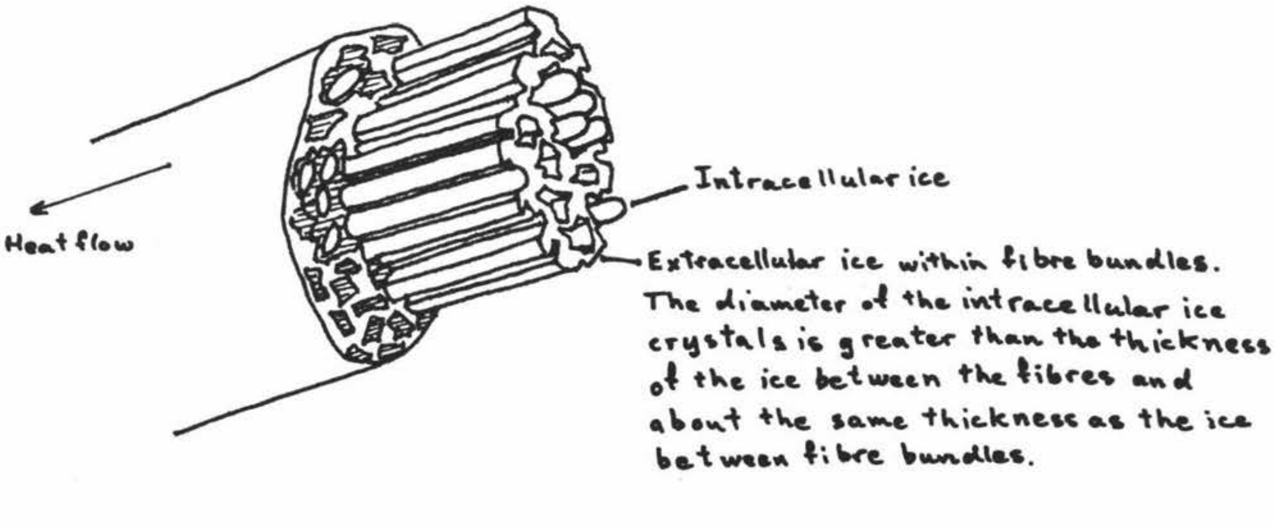


FIG. 44. INTRACELLULAR AND EXTRACELLULAR ICE WITHIN FIBRE BUNDLES IN PRE-RIGOR FROZEN MEAT.

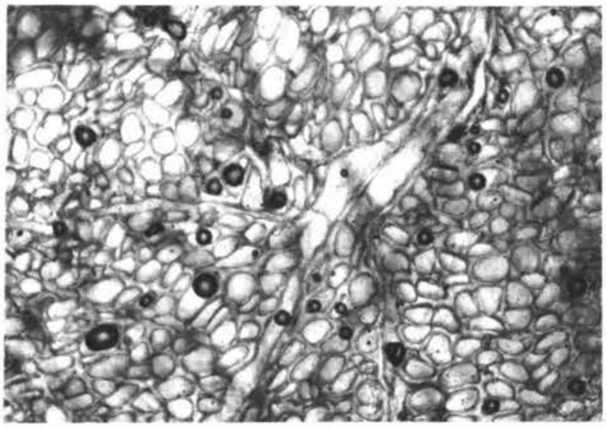


Plate 22: Single intracellular ice crystals in pre-rigor frozen meat.

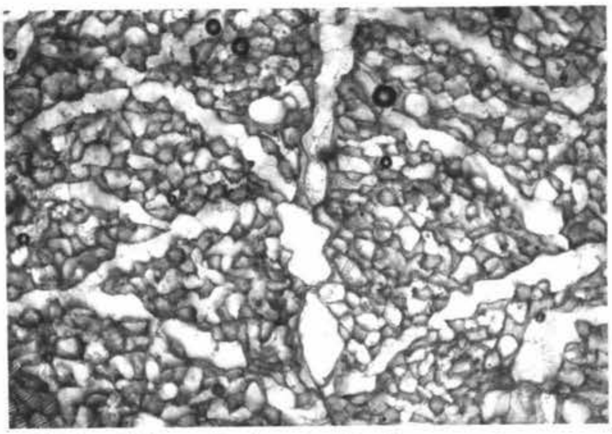


Plate 23: Intracellular and extracellular ice in pre-rigor frozen meat.

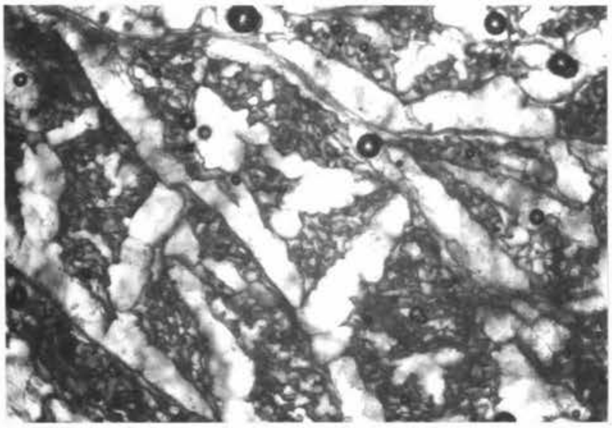


Plate 24: Extracellular ice mostly between fibre bundles in pre-rigor frozen meat.

3. Cold Shortening Studies

The changes in the cold shortened meat were intermediate between pre-rigor and post-rigor meat, tending to be more like the meat frozen pre-rigor, Plates 25,26. The crystal size distribution was smaller than in post-rigor meat for equivalent freezing conditions.

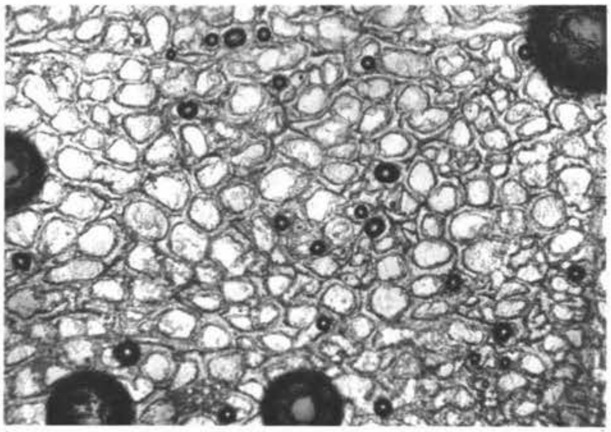


Plate 25: Intracellular ice in meat cold shortened before freezing.

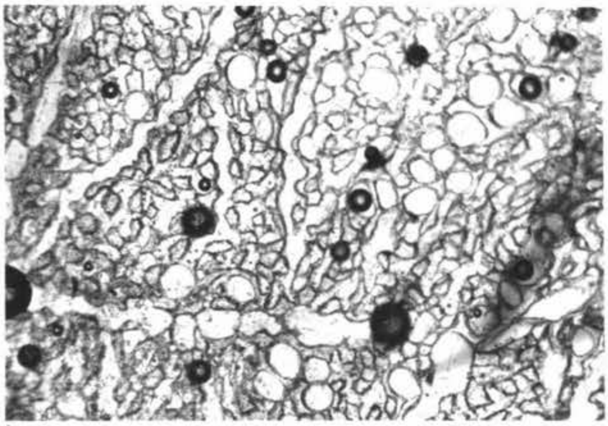


Plate 26: Extracellular ice in meat cold shortened before freezing.

CHAPTER VI

ICE CRYSTAL FORMATION IN MEAT

Discussion on the experimentally observed processes of ice crystal formation in meat, and possible practical consequences of these observations.

A. THE PROCESSES INVOLVED DURING ICE FORMATION

1. The Nature of the Disperse Phase

In Chapter I it was stated that ice crystal structure in frozen solutions was controlled by two factors, the rate of heat removal and hence the rate of ice formation, and the properties of the disperse phase.

The disperse phase in a tissue such as meat is complicated, consisting of the dissolved and suspended components of the sarcoplasm and other tissue fluids together with the structural elements of the tissue. The experimental observations have shown that there is a relationship between crystal size and ice formation rate. Apart from the maximum crystal size finding, the mathematical form of this relationship is not obvious and there is no theoretical basis for expecting any particular relationship. It is clear from these observations and from the influence of the physiological condition of the muscle, that the disperse phase plays a major role in determining ice crystal size and structure. It is worthwhile then to consider the possible role of the various tissue components in determining ice crystal form.

In meat, crystal size could be expected to be controlled by tissue membranes and the myofibrils. Their influence on water diffusion rates and the activation energy for diffusion is likely to be greater than for the sarcoplasmic proteins, mineral constituents and other tissue components in colloidal suspension or true solution. At high rates of ice formation these latter components could become important in the formation of polycrystalline intracellular forms. As this phenomenon was rarely observed this discussion will be confined to the influence of the structural components of the tissue.

2. The Influence of Tissue Membranes

The tissue membranes which are likely to influence ice crystal form are; the perimysium, surrounding the fibre bundles; the endomysium, continuous with the perimysium and surrounding each individual fibre; and the sarcolemma or cell wall. The function of the perimysium is to carry the larger blood vessels and nerves and to transfer mechanical forces originating in the myofibrils to the epimysium, the connective tissue sheath surrounding the muscle, and the tendons. A certain amount of fluid to lubricate the muscle, and associated with the lymphatic system, is likely to be found with the perimysium. The endomysium presumably carries finer blood vessels and nerves to the individual cells or fibres, and translates mechanical forces to the perimysium. Some fluid will be associated with this membrane also. One of the functions of the sarcolemma is to maintain the osmotic integrity of the cell so that as a living membrane it can control the process of water diffusion through it. This ability to control diffusion through the membrane is probably absent in the other membranes.

(a) Perimysium

Exactly where the extracellular ice forming between the fibre bundles forms in relation to the perimysium is not clear from the photomicrographs. The fact that this type of ice forms at all rates of ice growth, suggests that the free water normally associated with this membrane freezes in situ and then for further ice to form water has to diffuse from within the fibre bundle at the expense of other types of ice formation. When the rate of ice formation is low enough to allow this to take place the extracellular ice masses between the fibre bundles could be expected to be surrounded by a layer of dehydrated cells and beyond this layer there would be other ice forms, possibly with clumps of dehydrated fibres dispersed between them. Plates 16, 17, 18 show that this happens in practice. This type of ice forms independently of the physiological condition of the meat indicating that there is always some fluid associated with this membrane.

(b) Endomysium

The action of this membrane is probably to limit the diffusion rate which retards the formation of ice between fibre bundles. In Plates 7, 19, 21 there is evidence of ice having formed outside the cell. Fig. 45 and Plate 27 illustrate the diffusion processes involved in this type of ice formation. Ice will be formed first within the endomysium surrounding the cells which freeze extracellularly at high rates of ice formation. As the rate of ice formation falls, ice will grow within the endomysium surrounding these cells from water which diffuses through the endomysium from surrounding cells. Ice will not then form within the endomysium surrounding the neighbouring

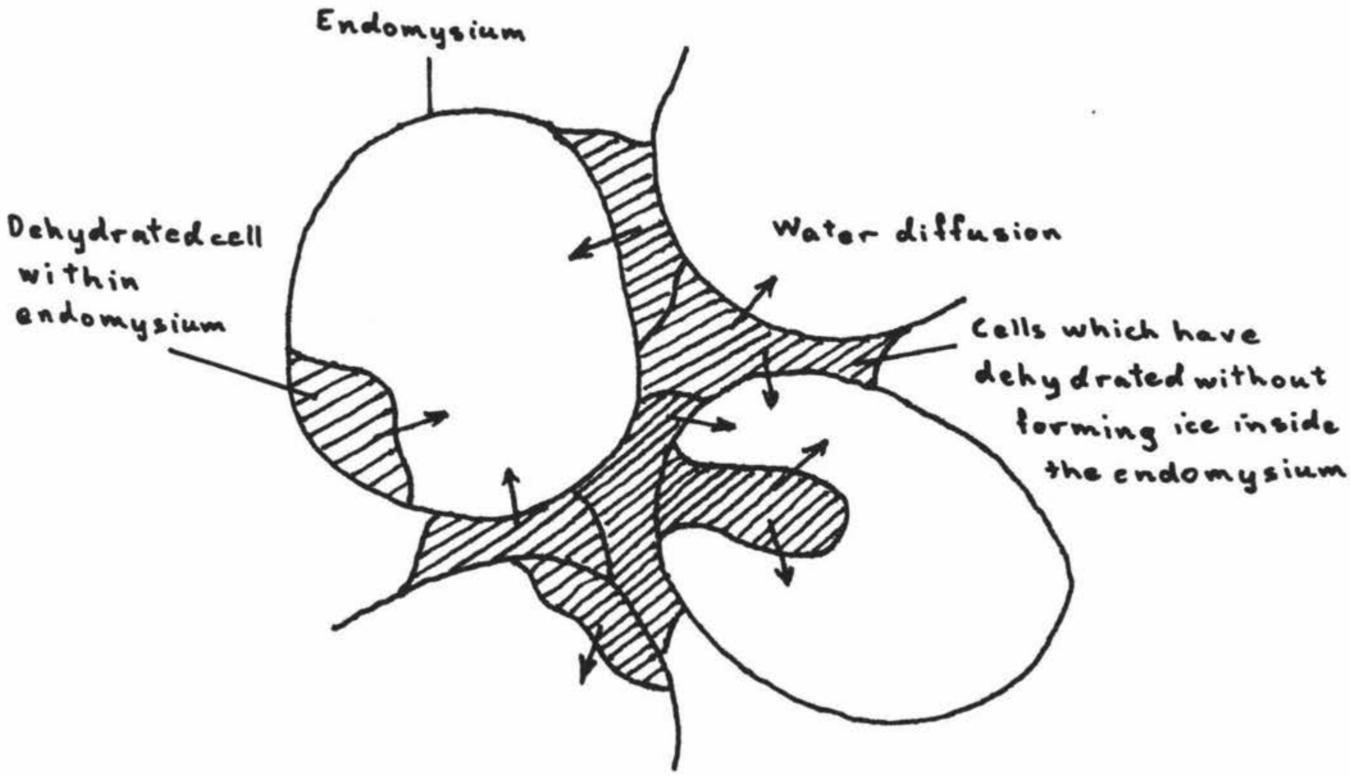


FIG. 45. EXTRACELLULAR ICE FORMATION BETWEEN INDIVIDUAL FIBRES.

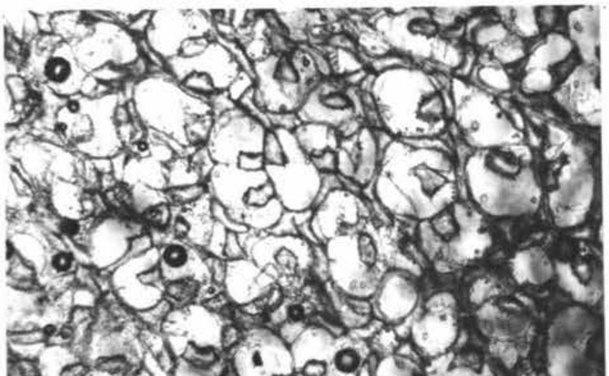


Plate 27: Extracellular ice formation within the endomysium.

cells. On reaching sufficient size the developing ice crystal may tear the endomysium, giving rise to irregular ice formations within fibre bundles at certain rates of ice formation, shown on Plates 8 & 9.

(c) Sarcolemma

As the living sarcolemma can exert some control over the diffusion of water through it, a dramatic change in ice formation could be expected when it dies or loses this ability. The loss of function by the sarcolemma probably partially expalins the change in type of crystallisation in pre-rigor and post-rigor frozen meat.

When ice develops extracellularly, water passes from within the cell by diffusion through pores in the sarcolemma. In pre-rigor meat this means that the water must diffuse against the sarcolemma mechanism to retain water inside the cell. This will inhibit the growth of extracellular ice. At the same time it will assist the formation of intracellular ice as the sarcolemma will tend to draw extracellular water into the cell to replace the water which is being converted into ice. Once all of the extracellular water has been drawn into the cell and frozen, if the ice crystal is to grow any larger water would have to be drawn into the cell from a neighbouring cell. To do this water has to diffuse against the neighbouring cell's water retention mechanism, and then through the endomysium and the cell's sarcolemma. For ice to develop extracellularly water has only to diffuse against the sarcolemma of one cell and possibly through the endomysium. The size of the ice crystal is controlled by the rate of ice formation as is the water flow rate to the developing ice front. Therefore more resistance to flow can be accommodated at low rates of ice formation and ice is able to develop extracellularly. Continued intracellular ice growth will cease at this critical point as the water flow resistance is higher than for extracellular growth. Once started, extracellular ice will continue to grow to the exclusion of intracellular ice even though at still lower flow rates continued intracellular growth could be accommodated.

In post-rigor meat the sarcolemma permits water to diffuse in either direction through it, providing the same amount of resistance in either direction. At high rates of ice formation the ice is formed intracellularly as most of the water was originally intracellular. The resistance to water diffusing out of the cell is smaller and to water diffusing into the cell is greater, relative to pre-rigor meat, so that ice will start to form extracellularly at higher rates of heat removal in post-rigor meat.

3. The Influence of the Myofibrils

During freezing the myofibrils are pushed aside by the developing ice crystals. The resistance of the myofibrils to displacement could influence ice crystal form in a manner analogous to a solute of low diffusivity. In pre-rigor meat they would offer less resistance to displacement than in post-rigor meat as the actin and myosin elements in the myofibril are free to slide over one another. Ice developing in pre-rigor meat will tend to displace the myofibrils to the perifery of the cell. No evidence of polycrystalline intracellular crystal formations was observed in pre-rigor meat. In rigor mortis the actin and myosin elements combine to form relatively rigid actomyosin. With ageing the myofibril breaks down by fission at the Z line reducing its resistance to displacement. The rate of ice formation required to produce polycrystalline intracellular ice could therefore be expected to increase with ageing. It is probably significant then that polycrystalline intracellular ice was observed with certainty only in unaged post-rigor meat.

Changes in the myofibrils could be expected to influence extracellular crystallisation, as collectively they determine the dehydrated fibres resistance to displacement. As meat ages, larger ice crystals would form for a given rate of ice formation. The ageing studies indicate that this effect is not particularly great although there is some suggestion of its existence. This is possibly because the endomysium and perimysium, which do not break down to any great extent with ageing, control extracellular ice crystal size.

4. The Influence of Chemical Changes

A chemical effect which may influence ice formation is the state of hydration of the protein structures. The impression gained from comparing the pre-rigor and post-rigor photomicrographs (Plates 7-21 and 22-24 respectively) is that less ice is formed in the pre-rigor meat. This could result from more water being chemically bound to the protein in the pre-rigor meat so that it is not available for ice formation. The hydration of proteins is pH dependent and the pH of meat falls during the onset of rigor mortis which would reduce water binding.

As the myofibrils and sarcoplasmic proteins are contained within the cell, their affinity for water above the isoelectric point, pH 5.4-5.5, could discourage the withdrawal of water from the cell in pre-rigor meat thus encouraging intracellular ice crystallisation. The onset of rigor mortis would not only increase the amount of water available for ice formation by precipitation of the sarcoplasmic protein and reducing the water binding of the myofibrils, but would also enable water to be withdrawn from the cell more easily.

The pH of meat rises slowly with ageing, with a concurrent increase in water holding capacity as some of the water binding ability is restored to the myofibrils. This increase in water binding may compensate for any increase in crystal size, due to increased flexibility of the myofibrils, by reducing the available water for ice formation.

B. PRACTICAL CONSEQUENCES OF ICE FORMATION

1. Consumer Acceptability

An experimental attempt to relate the different types of ice formation to changes in meat as a consumer product was not made. However, some observations can be made on the basis of this work about the rate of freezing and its possible influence on the consumer acceptability of the meat.

Some of the conflicting opinion over the extent to which freezing rate influences the quality of frozen meat may be due to ignorance of the variation in ice crystal size which is possible within a single piece of meat.

2. Drip Formation

This study has shown that there can be considerable differences in ice formation within a slab of frozen meat. Love (1958) and Love & Haraldsson (1961) have shown that in frozen fish certain types of ice formation favour drip formation. Similar phenomena are likely to occur in meat. In a block of meat frozen, thawed, and allowed to hang intact for a period after thawing (common practice in carcase meat handling), the escape of fluid from areas which have been subjected to rates of ice formation conducive to drip formation will be mechanically prevented by the surrounding tissue. This fluid is then able to be reabsorbed into the cells, and is not lost as drip. However, if a frozen carcase is dissected into cuts while still frozen (a common practice in the precut lamb trade) the cut surfaces will expose these areas so that on thawing the drip can escape freely without being reabsorbed. What effect this would have on palatability is not certain, but it would be unsightly to the housewife who thaws her meat before cooking.

3. Storage Deterioration

Ice crystal location may play some role in determining the type and extent of deterioration in frozen storage. Khan (1966) claims that the protein denaturation reaction involving disulphide bond interchange is catalysed by ice surfaces. This reaction does not normally occur at pH values as high as those found in meat. Denaturation from extracellular ice, where ice is in contact mostly with connective tissue protein, could result in a different type of denaturation to that from intracellular ice where it is in contact with the myofibrils. The denaturation of the connective tissue and cell walls may be favourable, enabling more rapid reabsorbtion of water into the cells on thawing. Denaturation of the myofibrilar protein would be undesirable, as it would release the water binding capacity of the protein and may induce toughening. This may explain the more rapid loss of tenderness in quick frozen meat during storage than in slow frozen meat. The water released by denaturation of the myofibrils would not form drip as it would be retained within the cell. If thawed frozen meat is to be minced for sausage or hamburger manufacture the denaturation of the myofibrilar protein would definitely be undesirable as the mincing would rupture the cell walls and reduce the free water within them. Treatment with polyphosphates to improve water retention is unlikely to be of much benefit because of the denatured state of the protein.

4. Tenderness

Extracellular crystallisation is unlikely to tenderise the meat as the ice dehydrates the fibres without any splitting of individual fibres. There may be some tenderising from tearing the endomysium in some types of extracellular ice formation, but the role of the endomysium in determining overall toughness is probably small compared to the fibres. Intracellular crystallisation may cause some tenderising by splitting the fibres.

5. Thaw Rigor

Ice crystal location may influence the increase in ATPase activity on freezing pre-rigor muscle. This would influence the shortening during the onset of thaw rigor and the amount of drip exuded. Lawrie (1966) suggests that the ATPase activity is dependent on the rate of freezing and considers that this is the result of localised salt concentrations. The location of the ice crystals would determine which components of the tissue would be bought into contact with high salt concentrations and could thus influence ATPase activity.

C. CONCLUSIONS

1. Ice crystal size in frozen meat depends on the rate of heat removal and hence the rate of ice formation. A relationship of the form:-

   $$\text{Maximum ice crystal size} \propto \frac{1}{\text{Rate of ice formation} + C}$$

   was found. The large size variation between ice crystals for a given rate of ice formation makes a complex statistical description of size necessary and with the present data it has not been possible to develop a complete relationship between crystal size and the rate of ice formation.

2. The location of ice crystals relative to tissue structures in frozen meat is dependent on the rate of ice formation.

3. The physiological condition of the muscle before freezing has a marked effect on the location and size of ice crystal formations. These effects can be explained in terms of the water permeability characteristics of the major structural components of the tissue and the water binding ability of the proteins.

4. The changes in ice formation observed could have important consequences in frozen meat quality and handling practice.

5. Further work is needed on the analytical study of thermal processes during freezing to evolve a more satisfactory method of prediction of rate of ice formation.

6. Little is understood of the processes involved in ice crystal formation, and endeavours to find a relationship between thermal processes and ice crystal formation are likely to show little advance until there is a better understanding of these processes.

EPILOGUE

In 1924 Moran (1924) put the question; "Can we avoid the evils of freezing, the loss of texture, etc., by altering the form of the cooling or thawing curves, or by restricting the temperature limits?" His answer was; "The theory of freezing of tissues is not sufficiently advanced to furnish a reply."

This study shows that we can alter the form of ice crystallisation by altering the form of the cooling curves and suggests that it may be possible to reduce the "evils" of freezing by doing this, so this study has at least gone some of the way in answering his question.

APPENDIX I

HISTOLOGICAL EXAMINATION METHODS AND MATERIALS

Microscopy

The microscope used in this work was an Olympus Laboratory microscope model E with photomicrographic attachment model PM-6 and built-in illuminator. Before the microscope could be used in the freezer all mechanical parts were dismantled and lubricated with Dow Corning silicone stopcock grease (temperature rating -40°F to +400°F). Aeroshell Grease II was considered as an alternative lubricant, (temperature rating -65°F to 250°F) but unfortunately it was not available in suitable quantities. In the camera only the eyepiece focussing and film advance mechanisms required lubrication. The shutter mechanism operated dry.

After reassembly the camera and microscope were calibrated with a stage micrometer by photographing the micrometer at 60x, 100x and 400x magnifications. Also the exposures which would probably be required were determined with an exposure meter attachment. This could not be used in the cold room as the circuitry in the meter was not sufficiently temperature-stable to give reliable readings at this temperature.

Before taking the microscope into the cold room it was packed into plastic bags containing silica gel to dry the air around and inside the microscope so that there would be no condensation onto the optical parts when it was taken into the cold room. One week was allowed for desication to take place. In the cold room the microscope was set up on a dense foam rubber pad to minimise vibration. Both the microscope and the camera gave satisfactory performance in the cold.

Polarising filters were made to fit the microscope but were not used as it was found that the meat exhibited birefractive properties which made distinguishing meat from ice difficult.

To examine the epoxy embedded material, the possibility of using phase contrast microscopy was considered so as to avoid having to stain the material. However, it was found that definition was little better than with conventional microscopy.

Photography

Film:- Ilford FP3 bought in bulk and cut and rolled to 20 exposure lengths. Exposure $^1/_{25}$ sec. for 100x and 60x magnifications.

Developer:- Ilford Microphen. Development time 6 min. at 68°F, after reusing once the time was increased to 6.6 min. and reused a further two times.

Stop bath:- 2% acetic acid.

Fixer:- Amphix. Fixation time 2.5 min. at 68°F.

Embedding

Materials supplied by CIBA N.Z. Ltd.

Resin:- Araldite M (Araldite CY212).

Hardener:- DY604, dodecenyl succinic anhydride.

Accelerator hardener:- HY964, tridimethylaminomethyl phenol.

Plasticiser:- Dibutyl phthalate.

The following formulation was found to give satisfactory sections with a conventional Cambridge rocking microtome.

5 ml Araldite M

0.4 ml DY604

4 ml HY964

2 ml Dibutyl phthalate

This is a modification of the following formulation recommended by Kay (1965) for electron microscopy.

10 ml Araldite CY212

10 ml HY964

0.5 ml DY064

1.0 ml Dibutyl phthalate

The amount of accelerator hardener is critical as it determines the amount of cross linking within the polymerised resin. Too much gives a dark coloured brittle block. This latter formulation is recommended as a permanent mounting medium for epoxy sections.

Embedding Schedule:-

1. The dehydrated ethanol soaked pieces of meat were soaked in xylene for 20-30 min. at room temperature.
2. The xylene was decanted off and replaced with a 50:50 mixture of xylene and the embedding medium. The bottle was shaken gently to mix the components and allowed to stand for 1 hour at room temperature.

3. An equal volume of embedding mixture was added and after mixing allowed to stand 3-6 hours or overnight at room temperature.
4. The xylene resin mixture was drawn off, replaced with the embedding medium and left to stand 24 hours at room temperature.
5. The tissue piece was then transferred to the embedding mould, covered with embedding medium, and transferred to a 45°C incubator to polymerise the embedding resin.
6. After polymerisation, which took 1-2 days, the block was allowed to cure at room temperature for about one week before sectioning.

The embedding mould consisted of pieces of soft ¼" plastic tube set in holes drilled in a wooden block. The mould was half-filled with resin which was partially polymerised before the tissue piece was placed in the mould so that the tissue would lie in a suitable plane for sectioning.

Microtomy

A set of wooden jaws were made to fit the specimen block chuck on the microtome so that the epoxy block could be held for sectioning. Before clamping in the microtome, the epoxy blocks were removed from the wooden mould and the plastic tube pared away with a scalpel to a level below the tissue piece. Some difficulty was encountered from the rather elastic nature of the block in controlling section thickness. The resulting sections would probably be between 10 and 20 μ thick. Further experimentation with the embedding medium formulation might have reduced this difficulty.

The epoxy sections were tough enough to be handled with a fine pair of tweezers, which simplified section transfer.

Staining

The sections were transferred to a spotting tile and flooded with safranin stain (1% safranin in 85% ethanol). After soaking for about 2 min. they were transferred to a piece of filter paper to draw off the surplus stain, and then onto a drop of immersion oil or mounting epoxy on a microscope slide taking care not to entrap air bubbles under the section. The section was covered with further oil or resin and covered with a cover slip. The sections could then be examined or photographed and the epoxy mounted material transferred to the 45°C incubator to polymerise.

Measurement of Crystal Size

Ice crystal size was determined by projecting the negatives of the photomicrographs onto a screen which had been marked out with a grid with markings equivalent to 10 μ spacing. The screen and projector were calibrated for each magnification using the photomicrographs of the stage micrometer. The calibrated screen was later abondoned in favour of a ruler calibrated at 10 μ spacings to facilitate measuring. Measurements were recorded on a recording adding machine so that a neat convenient record could be kept of sizes and mean ice crystal size. This method of recording proved particularly convenient when it came to transferring data to punched cards for computer analysis.

Meat Used in Experiments

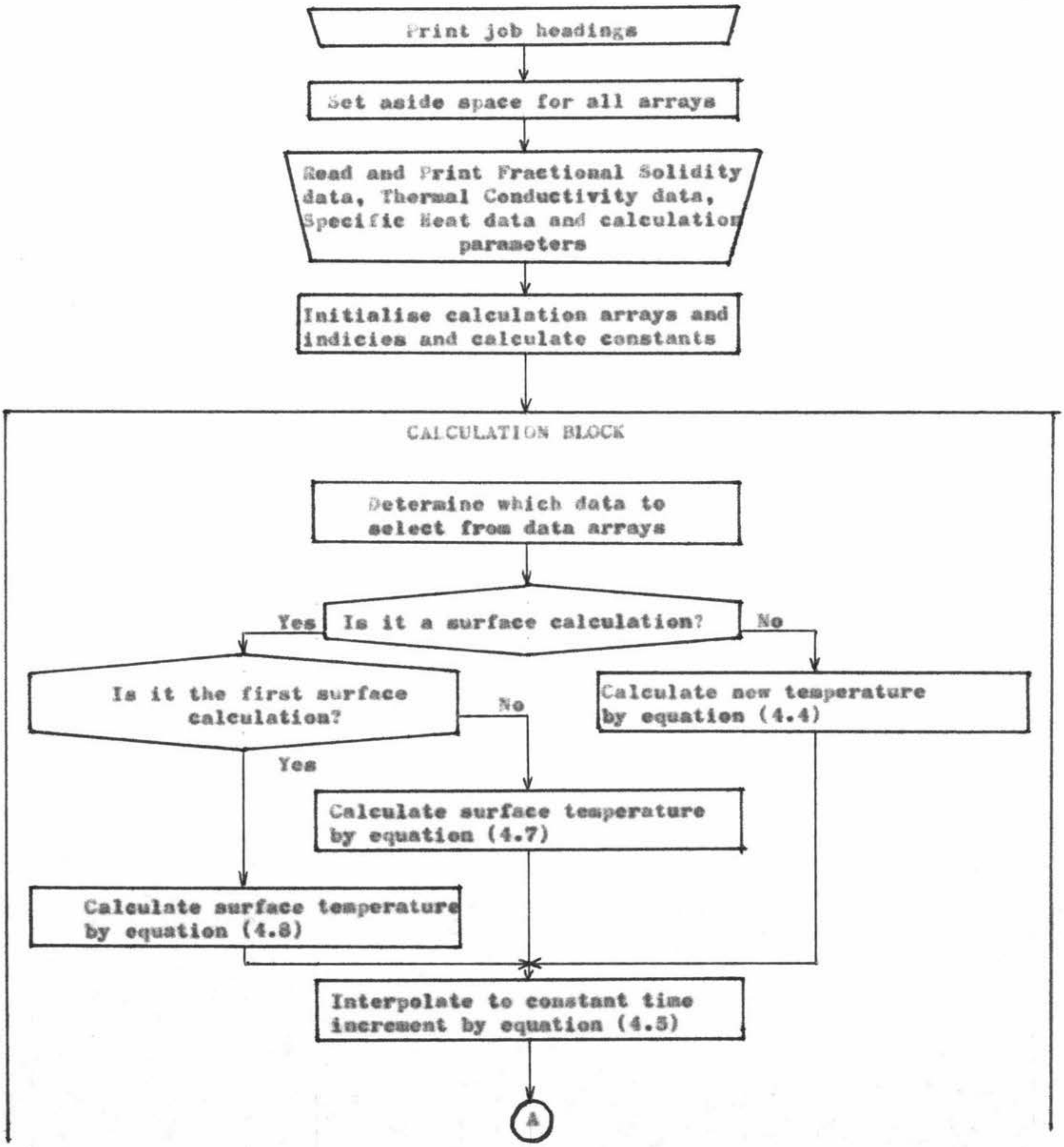
Lean beef was used in the mince mould system. This involved trimming off all visible fat before mincing. The details of the animal it came from were not known. The sample plugs were all prepared from Sterno-mandibularis muscles (neck muscles) removed from Aberdeen Angus beasts within 30 min. of slaughter. With the exception of the cold shortened and pre-rigor frozen muscle they were allowed to go into rigor mortis at room temperature (50-60°F). The meat was held at this temperature for 24 hours to enable this to occur. After this they were transferred to a 35°F chill room to age for the prescribed time. The meat used in the effect of rate of ice formation studies was allowed to age 3 days before freezing.

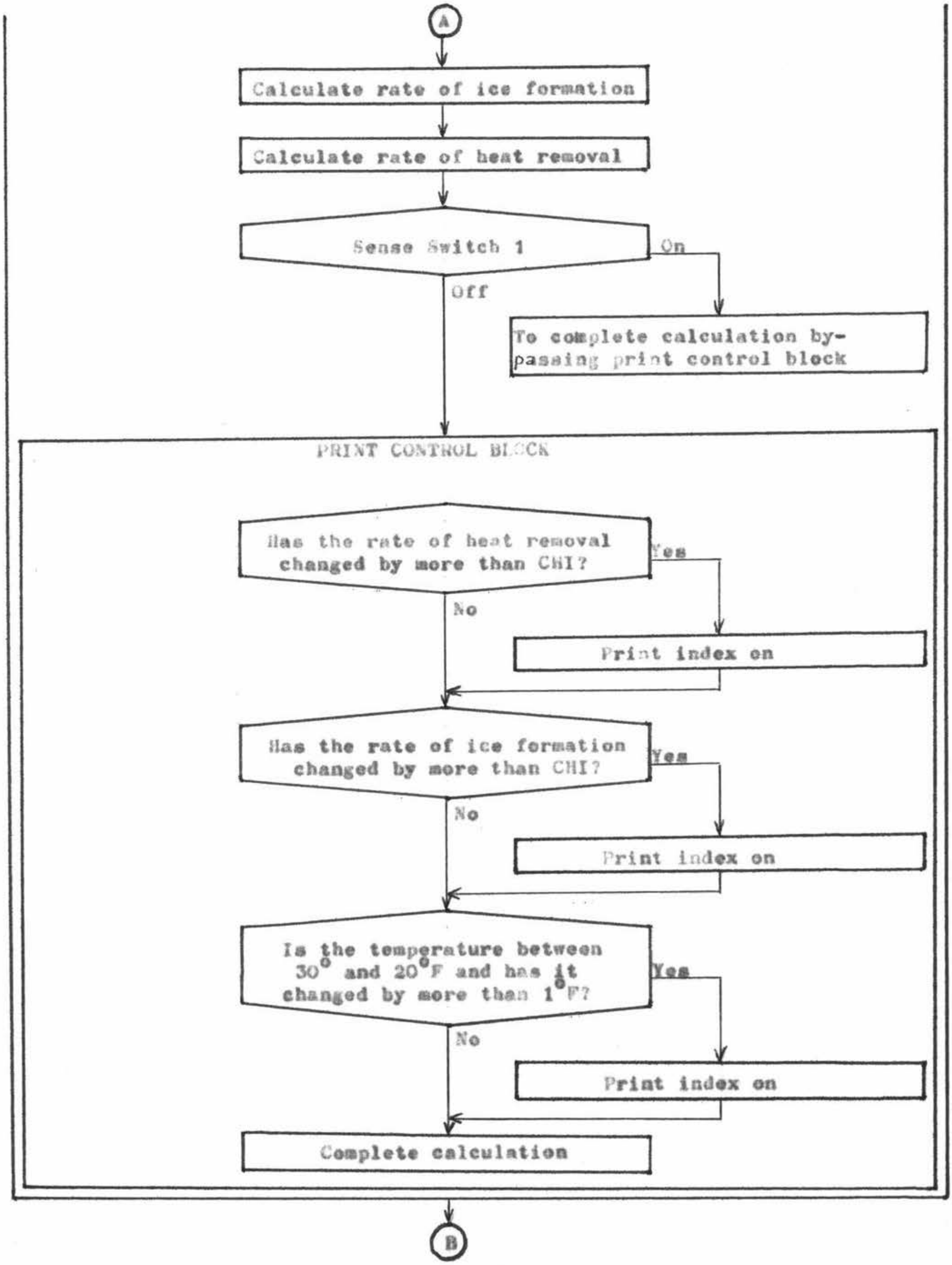
The meat frozen pre-rigor was in the freezer within 2 hours of slaughter. The cold shortened meat was transferred to the 35°F chiller within 1½ hours of slaughter and frozen 24 hours later.

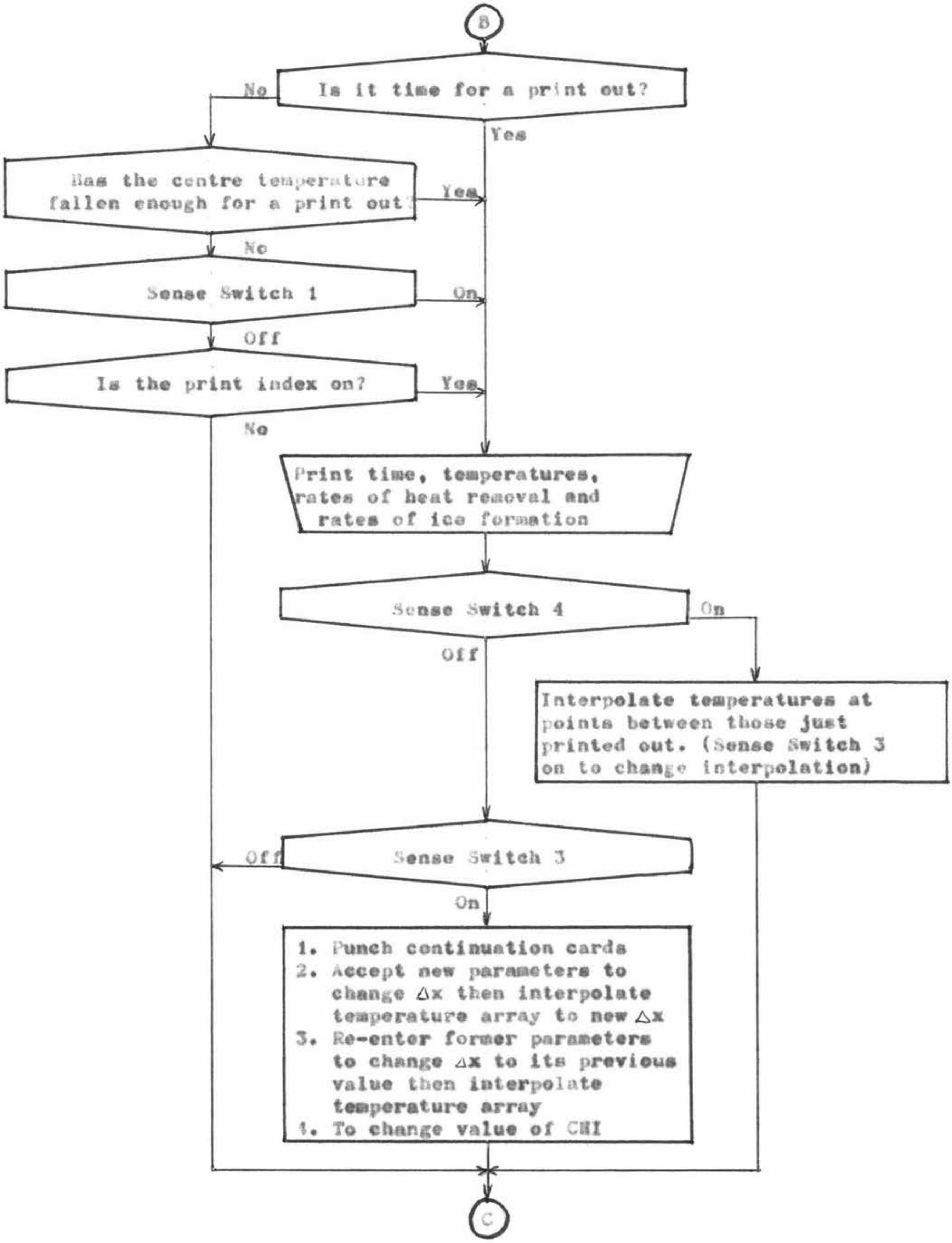
APPENDIX II

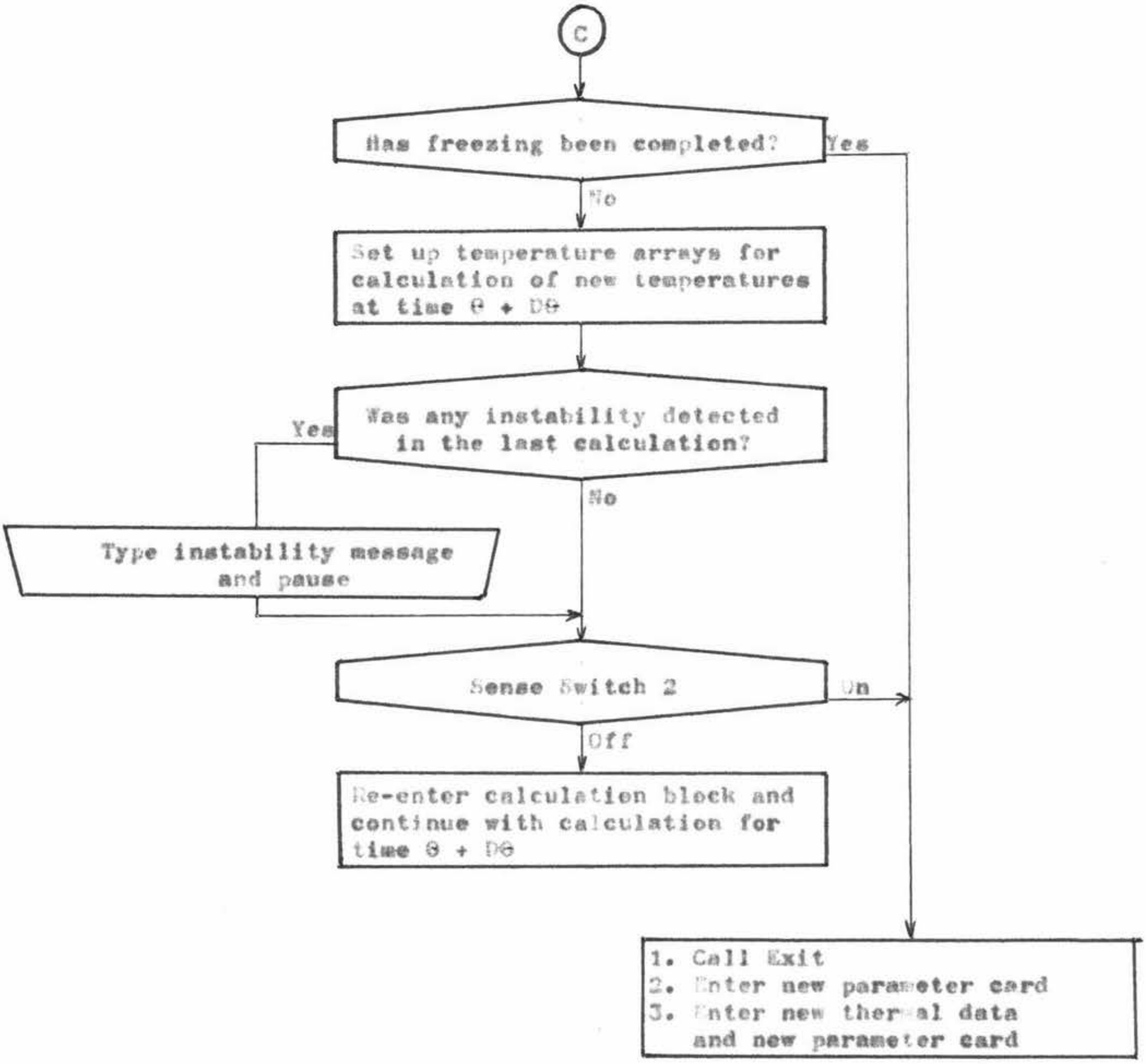
TEMPERATURE AND ICE FORMATION PROGRAMMES

Qualitative Logic Flow Diagram for Ice Formation Programme




A
Calculate rate of ice formation
Calculate rate of heat removal
Sense Switch 1
On
Off
To complete calculation by-passing print control block
PRINT CONTROL BLOCK
Has the rate of heat removal changed by more than CHI?
Yes
No
Print index on
Has the rate of ice formation changed by more than CHI?
Yes
No
Print index on
Is the temperature between 30° and 20°F and has it changed by more than 1°F?
Yes
No
Print index on
Complete calculation
B


B
Is it time for a print out?
No
Yes
Has the centre temperature fallen enough for a print out?
Yes
No
Sense Switch 1
On
Off
Is the print index on?
Yes
No
Print time, temperatures, rates of heat removal and rates of ice formation
Sense Switch 4
On
Off
Interpolate temperatures at points between those just printed out. (Sense Switch 3 on to change interpolation)
Sense Switch 3
Off
On
1. Punch continuation cards
2. Accept new parameters to change Δx then interpolate temperature array to new Δx
3. Re-enter former parameters to change Δx to its previous value then interpolate temperature array
4. To change value of CHI
C


C
Has freezing been completed?
Yes
No
Set up temperature arrays for calculation of new temperatures at time θ + Dθ
Was any instability detected in the last calculation?
Yes
No
Type instability message and pause
Sense Switch 2
On
Off
Re-enter calculation block and continue with calculation for time θ + Dθ
1. Call Exit
2. Enter new parameter card
3. Enter new thermal data and new parameter card

ICE FORMATION PROGRAM

```
C     MAXIMUM NUMBER OF DIVISIONS 27
      READ 111
      PRINT 111
  111 FORMAT (20H                    /,11H           /)
   36 PRINT 222
  222 FORMAT (53H0TEMPERATURE, HEAT REMOVAL AND ICE FORMATION PROFILES/,
     124H0ONE DIMENSIONAL COOLING//)
      TYPE 333
      DIMENSION T(28),TI(28),FRST(28),RHR(28),RFSE(20),CON(20),SPEC(20)
     1,RH(28),FR(28),TY(28)
      PRINT 444
  444 FORMAT (42H0RATE OF CHANGE OF FRACTION SOLID WRT TEMP/)
      DO 2 I=1,20
    2 RFSE(I)=0.
      READ 100,RFSE
      PRINT 100,RFSE
      PRINT 555
  555 FORMAT (21H0THERMAL CONDUCTIVITY/)
      READ 100,CON
      PRINT 100,CON
      PRINT 666
  666 FORMAT (14H0SPECIFIC HEAT/)
      READ 100,SPEC
      PRINT 100,SPEC
   37 READ 200, N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP,CHI
      PRINT 777
  777 FORMAT (11H1PARAMETERS/,74H  N    MOD     A TEMP I TEMP  DDT  X IN
     1C T INC  DEN    SHTC  TE INC FIN TE)
      PRINT 200,N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP,CHI
      DO 1 I=1,28
      RHR(I) = 0.
      RH(I) = 0.
      FRST(I) = 0.
      FR(I) = 0.
      T(I)=0.
    1 TI(I)=0.
      DZO = 0.
      NO = 0
      NN = 0
      MB = 0
      BZ = DZ*DZ*DEN/EM/144.
      BM = EM-2.
      MM = 0
      IF (B-999.999)59,60,60
   60 READ 700, TMM,TMP,TIM
      IX = 1
      READ 800,(TI(I),I=1,N)
      GO TO 29
   59 IX = 0
      NX = N+1
      DO 6 I=1,NX
```

```
  6 T(I) = B
    TIM = 0.
    TMP = B-TNP
    TMM = TMN
  7 M = 0
    DO 42 I=1,N
  8 L = T(I)/10.+4.
    IF (L)10,9,10
 10 IF (L-6)15,12,11
 11 IF (L-7)12,14,13
  9 L = L+1
    GO TO 15
 12 L = T(I)-11.
    GO TO 15
 14 L = T(I)/5.+13.
    GO TO 15
 13 L = L-2
 15 IF (I-1)16,16,19
 16 BNT = H*DZ/CON(L)/12.
    IF (IX)18,17,18
 17 TX = .5*(T(I)+(BNT*TS+T(2))/(BNT+1.))
    GO TO 20
 18 TX = (BNT*TS+(T(I)+BM*T(2)+T(3))/EM)/(BNT+1.)
    GO TO 20
 19 TX = (T(I+1)+T(I-1)+BM*T(I))/EM
 20 TI(I) = DDT/BZ*(TX-T(I))*CON(L)/SPEC(L)+T(I)
    IF (T(I)-30.)44,43,43
 43 IF (TI(I)-30.)45,44,44
 45 T(I) = 29.9999
    M = 1
    GO TO 8
 44 IX = 1
    IF (RFSE(L))21,21,22
 21 FRST(I) = 0.
    GO TO 23
 22 FRST(I) = RFSE(L)*(T(I)-TI(I))/DDT
 23 RHR(I) = SPEC(L)*(T(I)-TI(I))/DDT
    IF (RHR(I)-0.)63,64,64
 63 MM = 1
 64 IF (SENSE SWITCH 1)42,46
 46 IF (RH(I)-RHR(I)-CHI)47,48,48
 47 IF(RHR(I)-RH(I)-CHI)49,48,48
 48 M = 1
    RH(I) = RHR(I)
 49 IF (FR(I)-FRST(I)-CHI)53,51,51
 53 IF (FRST(I)-FR(I)-CHI)39,51,51
 51 M = 1
    FR(I) = FRST(I)
 39 IF (TI(I)-30.)40,40,42
 40 IF (TI(I)-20.)42,41,41
 41 IF (T(I)-TI(I)-1.)42,52,52
 52 M = 1
 42 CONTINUE
 24 TIM = TIM+DDT
 25 IF (TIM-TMM)26,27,27
 26 IF (T(N)-TMP)28,28,50
```

```
27 TMM = TMM+TMN
   GO TO 29
28 TMP = TMP-TNP
   GO TO 29
50 IF (SENSE SWITCH 1)29,54
54 IF (M-1)31,29,29
29 PRINT 300, TIM
   DO 30 I=1,N,13
   PRINT 400, TI(I),TI(I+1),TI(I+2),TI(I+3),TI(I+4),TI(I+5),TI(I+6),T
  1I(I+7),TI(I+8),TI(I+9),TI(I+10),TI(I+11),TI(I+12)
   PRINT 600, RHR(I),RHR(I+1),RHR(I+2),RHR(I+3),RHR(I+4),RHR(I+5),RH
  1R(I+6),RHR(I+7),RHR(I+8),RHR(I+9),RHR(I+10),RHR(I+11),RHR(I+12)
30 PRINT 500, FRST(I),FRST(I+1),FRST(I+2),FRST(I+3),FRST(I+4),FRST(I+
  15),FRST(I+6),FRST(I+7),FRST(I+8),FRST(I+9),FRST(I+10),FRST(I+11),F
  2RST(I+12)
   IF (SENSE SWITCH 4)67,68
67 MB = 1
   IF (NN)69,70,69
69 IF (SENSE SWITCH 3)70,71
70 TYPE 223
   ACCEPT 201,NN
   PRINT 201,NN
71 NSAVE = NO
   XSAVE = DZO
   NO = N
   N = NN
   DZO = DZ
   XM = NO-1
   XL = N-1
   DZ = DZO*XM/XL
   GO TO 76
68 IF (SENSE SWITCH 3)57,65
57 B = 999.999
   TYPE 999
   ACCEPT 201,K
   GO TO (66,73,72,91),K
72 NSAVE = N
   XSAVE = DZ
   TSAVE = DDT
   N = NO
   DZ = DZO
   DDT = DDTO
   NO = NSAVE
   DZO = XSAVE
   DDTO = TSAVE
   GO TO 74
73 NO = N
   DZO = DZ
   DDTO = DDT
   ACCEPT 202,N,DDT
   XM = NO-1
   XL = N-1
   DZ = DZO*XM/XL
74 PRINT 777
   PRINT 200,N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP,CHI
76 TY(1) = TI(1)
   I = 1
```

```
      MU = 2
      DZI = DZ
      IF (DZO-DZ)77,29,82
   77 DO 81 J=2,N
   78 IF (DZI-DZO)80,80,79
   79 I = MU
      MU = MU+1
      DZI = DZI-DZO
      GO TO 78
   80 TY(J) = TI(I)+(TI(MU)-TI(I))*DZI/DZO
   81 DZI = DZI+DZ
      GO TO 85
   82 DO 84 J=2,N
      TY(J) = TI(I)+(TI(MU)-TI(I))*DZI/DZO
      DZI = DZI+DZ
      IF (DZI-DZO)84,84,83
   83 I = MU
      MU =MU+1
      DZI = DZI-DZO
   84 CONTINUE
   85 IF (MB)88,88,86
   86 PRINT 400,(TY(I),I=1,N)
      DO 87 I=1,N
   87 TY(I) = 0.
      MB = 0
      N = NO
      DZ = DZO
      NO = NSAVE
      DZO = XSAVE
      GO TO 65
   88 DO 89 I=1,NO
      RHR(I) = 0.
      RH(I) = 0.
      FRST(I) = 0.
      FR(I) = 0.
      T(I) = 0.
   89 TI(I) = 0.
      DO 90 I=1,N
      TI(I) = TY(I)
   90 TY(I) = 0.
      BZ = DZ*DZ*DEN/EM/144.
      BM = EM-2.
      MM = 0
      GO TO 29
   66 PUNCH 200,N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP,CHI
      PUNCH 700, TMM,TMP,TIM
   58 PUNCH 800,(TI(I),I=1,N)
      GO TO 34
   91 ACCEPT 203,CHI
   65 IF (TI(N)-TTP)34,34,31
   31 DO 33 I=1,N
   33 T(I) = TI(I)
      T(1) = TI(N-1)
      IF (MM-1)56,55,55
   55 TYPE 888
      MM = 0
      PAUSE
```

```
 56 IF (SENSE SWITCH 2)34,7
 34 TYPE 221
    ACCEPT 201,K
    GO TO (38,37,36),K
 38 CALL EXIT
100 FORMAT (5X,5F8.2)
200 FORMAT (I3,F8.3,2X,2F8.3,F7.4,F6.3,F5.2,F5.1,F7.1,3F7.2)
300 FORMAT (5HOTIME,F9.4)
400 FORMAT (5H TEMP,13F10.2)
500 FORMAT (5H RCFS,13F10.2)
600 FORMAT (5H RHR ,13F10.2)
700 FORMAT (3F10.4)
800 FORMAT (8F10.4)
201 FORMAT (I3)
202 FORMAT (I3,F7.4)
203 FORMAT (F5.1)
888 FORMAT (20HTEMPERATURE INCREASE/,57HSENSE SWITCH 2 ON TO ABANDON C
   1ALCULATION, OFF TO CONTINUE//)
221 FORMAT (19HTO CALL EXIT TYPE 1/,29HTO READ NEW PARAMETERS TYPE 2/,
   129HTO READ NEW THERMAL DATA TYPE 3/,10HFORMAT(I3)//)
223 FORMAT (73HSENSE SWITCH 3 OFF THEN TYPE TEMPERATURE INTERPOLATION
   1VALUE, FORMAT (I3)//)
333 FORMAT (52HTO PRINT RESULTS OF EACH ITERATION SENSE SWITCH 1 ON/,4
   11HTO INTERUPT CALCULATION SENSE SWITCH 3 ON/,40HTO ABANDON CALCULA
   2TION SENSE SWITCH 2 ON/,61HTO INTERPOLATE TEMPERATURE BETWEEN DIVI
   3IONS SENSE SWITCH 4 ON/,60HTO ENTER INTERPOLATION SUBDIVISION SENS
   4E SWITCHES 3 AND 4 ON//)
999 FORMAT (47HTO PUNCH CONTINUATION CARDS TYPE 1, FORMAT (I3)/,60HTO
   1MODIFY N AND INTERPOLATION TIME TYPE 2 THEN MODIFICATIONS/,16HFORMA
   2T (I3,F7.4)/,59HTO RE-ENTER PREVIOUS N AND INTERPOLATION TIME VALU
   3ES TYPE 3/,57HTO CHANGE HEAT AND ICE PRINT CONTROL TYPE 4 THEN NEW
   4VALUE/,13HFORMAT (F5.1)//)
    END
```

TEMPERATURE PROGRAM

```
C     MAXIMUM NUMBER OF DIVISIONS 27
      READ 111
      PRINT 111
  111 FORMAT (20H                    /,11H          /)
   36 PRINT 222
  222 FORMAT (53HOTEMPERATURE, HEAT REMOVAL AND ICE FORMATION PROFILES/,
     124HOONE DIMENSIONAL COOLING//)
      DIMENSION T(28),TI(28),FRST(28),CON(20),SPEC(20)
      PRINT 555
  555 FORMAT (21HOTHERMAL CONDUCTIVITY/)
      READ 100,CON
      PRINT 100,CON
      PRINT 666
  666 FORMAT (14HOSPECIFIC HEAT/)
      READ 100,SPEC
      PRINT 100,SPEC
   37 READ 200, N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP
      PRINT 777
  777 FORMAT (11H1PARAMETERS/,74H  N     MOD      A TEMP I TEMP  DDT  X IN
     1C T INC  DEN     SHTC  TE INC FIN TE)
      PRINT 200, N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP
      DO 1 I=1,28
      T(I)=0.
    1 TI(I)=0.
      BZ = DZ*DZ*DEN/EM/144.
      BM = EM-2.
      IF (B-999.999)59,60,60
   60 READ 700, TMM,TMP,TIM
      IX = 1
      MM = 0
      READ 800,(TI(I),I=1,N)
      GO TO 29
   59 IX = 0
      NX = N+1
      DO 6 I=1,NX
    6 T(I) = B
      TIM = 0.
      TMP = B-TNP
      TMM = TMN
    7 MM = 0
      DO 42 I=1,N
    8 L = T(I)/10.+4.
      IF (L)10,9,10
   10 IF (L-6)15,12,11
   11 IF (L-7)12,14,13
    9 L = L+1
      GO TO 15
   12 L = T(I)-11.
      GO TO 15
   14 L = T(I)/5.+13.
      GO TO 15
```

```
 13 L = L-2
 15 IF (I-1)16,16,19
 16 BNT = H*DZ/CON(L)/12.
    IF (IX)18,17,18
 17 TX = .5*(T(I)+(BNT*TS+T(2))/(BNT+1.))
    GO TO 20
 18 TX = (BNT*TS+(T(I)+BM*T(2)+T(3))/EM)/(BNT+1.)
    GO TO 20
 19 TX = (T(I+1)+T(I-1)+BM*T(I))/EM
 20 TI(I) = DDT/BZ*(TX-T(I))*CON(L)/SPEC(L)+T(I)
    IF (T(I)-30.)44,43,43
 43 IF (TI(I)-30.)45,44,44
 45 T(I) = 29.9999
    GO TO 8
 44 IX = 1
    IF (TI(I)-T(I))42,42,53
 53 MM = 1
 42 CONTINUE
 24 TIM = TIM+DDT
 25 IF (TIM-TMM)26,27,27
 26 IF (T(N)-TMP)28,28,50
 27 TMM = TMM+TMN
    GO TO 29
 28 TMP = TMP-TNP
    GO TO 29
 50 IF (SENSE SWITCH 1)29,31
 29 PRINT 300, TIM
    PRINT 400,(TI(I),I=1,N)
    IF (SENSE SWITCH 3)57,65
 57 B = 999.999
    PUNCH 200,N,EM,TS,B,DDT,DZ,TMN,DEN,H,TNP,TTP
    PUNCH 700, TMM,TMP,TIM
 58 PUNCH 800,(TI(I),I=1,N)
    GO TO 34
 65 IF (TI(N)-TTP)34,34,31
 31 DO 33 I=1,N
 33 T(I) = TI(I)
    T(I) = TI(N-1)
    IF (MM-1)56,55,55
 55 TYPE 888
888 FORMAT (9HTEMP RISE/49HSENSE SWITCH 4 ON TO ABANDON JOB, OFF TO CO
   1NTINUE)
    PAUSE
 56 IF (SENSE SWITCH 4)34,7
 34 PAUSE
    IF (SENSE SWITCH 2)35,38
 35 IF (SENSE SWITCH 3)36,37
 38 CALL EXIT
100 FORMAT (5X,5F8.2)
200 FORMAT (I3,F8.3,2X,2F8.3,F7.4,F6.3,F5.2,F5.1,F7.1,2F7.2)
300 FORMAT (5HOTIME,F9.4)
400 FORMAT (5H TEMP,13F10.2)
700 FORMAT (3F10.4)
800 FORMAT (8F10.4)
    END
```

THERMAL DATA ARRAYS

FRACTIONAL SOLIDITY DATA

0.00	0.00	0.10	0.40	0.50
0.00	0.00	0.00	1.00	1.20
1.50	2.00	2.50	4.00	5.00
8.00	20.00	32.50	0.00	0.00

* THERMAL CONDUCTIVITY DATA

0.96	0.93	0.89	0.83	0.77
0.28	0.29	0.30	0.70	0.69
0.67	0.65	0.62	0.58	0.53
0.46	0.36	0.32	0.27	0.27

* SPECIFIC HEAT DATA

0.50	0.50	0.50	0.90	1.30
1.00	1.00	1.00	1.80	2.00
2.30	2.50	3.10	3.30	4.60
6.80	27.00	23.00	1.00	1.00

PARAMETER CARD

17 4.000 -43.000 999.999 .0025 .180 .25 67.0 240.0 5.00 -10.0

* According to Cullwick (1967)

Notes on Temperature and Ice Formation Programmes

Listings of the ice formation and temperature programmes are given on pages AII-5 to AII-11. A qualitative logic flow diagram for the ice formation programme is given on pages AII-1 to AII-4.

Control of the ice formation programme is achieved by sense switches and typewriter input to computed GO TO statements. Fullest use has not been made of the Monitor 1 features incorporated into Fortran II-D with Monitor 1 as the programme had been almost fully developed in Fortran II-D when it was introduced. Monitor 1 allows the use of free input formats, a feature which would have been particularly useful for the parameter input as it would have offered greater flexibility in the number of significant figures and size of numbers which could have been punched into the parameter card. Monitor 1 also provides a calculation trace which could be operated at any stage of computation by turning Sense Switch 4 on if a trace card had been included in the source deck. Because Sense Switch 4 was used in this programme the operations between statements 68 and 76 could not be traced in this way. However, the diagnostic features of Monitor 1 proved invaluable in tracing programming errors.

The time required for calculation was lengthy (about 5 hours for the calculation of the experimental system with varying $\triangle x$) so that provision had to be made to ensure that sufficient relevant rate of heat removal and rate of ice formation data was printed out to avoid wasting time by having to go back over the calculation because important data had been missed. Manual print control by sense switch proved inadequate, as it was not known how the rate of ice formation would change during freezing, and so a print control routine was added.

The print-out control value CHI represents the change in rate of heat removal or ice formation rate which was considered large enough to warrant recording. As this control operates at every calculation point too much data could be printed out, especially when there were many calculation points, so provision was made to change this value during calculation. The temperature print control in the 30 to 20°F range was intended to ensure that any gradual changes in ice formation rate were recorded, but it is doubtful if it ever functioned as a 1°F fall in one iteration is unlikely in this temperature range.

The time and temperature print controls punched into the parameter card were intended to control the print-out outside the range 30 to 20°F.

The long calculation time meant that some provision had to be made to interupt calculation then pick it up at a later date. Punching continuation cards was the most convenient method of doing this. It also provided a means for inserting non-uniform initial temperature data and going back over portions of the calculation with different values for Δx.

The reasons for providing for changes in Δx during calculation are given in Chapter IV. Provision for temperature interpolation during calculation was made so that the moment to change Δx could be chosen more precisely.

It was found that calculation instability could be induced by punching errors in the parameter card, or by too large an interpolation time value which caused the interpolation equation to extrapolate rather than interpolate. If the instability was allowed to develop, the computer was addressed out of storage in a very short time which meant that the whole programme had to be cleared out and restarted. To avoid this, provision was made for any negative rate of heat removal (temperature rise in the temperature programme) to stop computation and type out an instability message. The calculation could then be abandoned or continued as small negative values did not always lead to instability.

To reduce the amount of storage occupied by thermal data arrays, the data was condensed into the arrays listed on page AII-12. The routine bounded by statements 8 to 13 selects the appropriate values from these arrays. Comparison of these arrays with Table AII-1 shows how the data is arranged.

The values on the parameter card shown on page AII-12 are, from left to right:-

N the number of subdivisions in the half thickness of the slab plus 1, i.e. the number of temperature calculation points,

EM the calculation modulus, M,

TS the plate temperature, Ta (^{o}F),

B the initial temperature, Ti. 999.999 is punched in this column if continuation cards or non-uniform starting temperature data are to be entered (^{o}F),

DDT the constant time increment, DΘ (hrs),

DZ the subdivision thickness, $\triangle x$ (inches), TMN the time print-out value (hrs),

DEN the density of the meat (lb/ft^3),

H the surface heat transfer coefficient ($BTU/ft^2hr^{\circ}F$),

TNP the centre temperature print-out value ($^{\circ}F$),

TFP the end of freezing temperature ($^{\circ}F$)

CHI (not shown on page AII-12) the rate of ice formation and heat removal print control.

The ice formation programme types out operating instructions during execution.

No instructions are included in the temperature programme, as there is no provision for changing Δx during computation, and the print-out is controlled manually and by the time and centre temperature values.

Table AII-1: Thermal Properties and Fractional Solidity of Minced Lean Beef

Temperature	-40	-30	-20	-10	0	10	20	21	22	23	24	25
Specific Heat	0.50	0.50	0.50	0.50	0.90	1.30	1.80	2.00	2.30	2.50	3.10	
Thermal Conductivity	0.96	0.96	0.93	0.89	0.83	0.77	0.71	0.69	0.67	0.65	0.62	
Fractional Solidity	0.00	0.00	0.00	0.10	0.40	0.30	1.00	1.20	1.50	2.00	2.50	

Temperature	25	26	27	28	29	30	35	40	50	60	79
Specific Heat	3.30	4.60	6.80	27.00	23.00	1.00	1.00	1.00	1.00	1.00	
Thermal Conductivity	0.58	0.53	0.46	0.36	0.32	0.27	0.27	0.28	0.29	0.30	
Fractional Solidity	4.00	5.00	8.00	20.00	32.50	0.00	0.00	0.00	0.00	0.00	

APPENDIX III

A Method for Observation of the Freezing Process as it Proceeds

The techniques described in Chapter II all examine the completely frozen material which limits the amount of information which can be gained from histological study. A considerable amount of thought was given to the possibility of overcoming this limitation and a possible technique was evolved. Unfortunately the time available for this study was insufficient to develop it into a working technique so it had to be abandoned. In this Appendix the thought which went into developing this technique is outlined.

A. By Direct Observation

Originally the possibility of observing the process of ice development in the tissue during the freezing process with a cold stage microscope was examined. The approach was to cool a section of meat on the microscope stage so that its temperature history closely resembled the temperature history of any given point within a slab of meat being frozen. A number of workers have used cooled microscope stages to examine freezing processes (Luyet & Rapatz, 1957, Rapatz & Luyet, 1957, Rey, 1957, Asahina, 1962, Smith et al, 1951). With the exception of Asahina who cooled the whole microscope, all of these workers used either dry ice or liquid nitrogen as a heat sink and circulated a heat transfer fluid from the sink to the stage at controlled rates to control the stage temperature. Rey could also heat the stage with an electric element to obtain closer control. In this study some attention was given to using the thermoelectric effect between dissimilar metals to cool the stage, this being a more controlable method of reducing the stage temperature. The method was abandoned, however, when it was realised that the freezing conditions in a small section on a microscope stage could not be representative of the situation within a slab of meat.

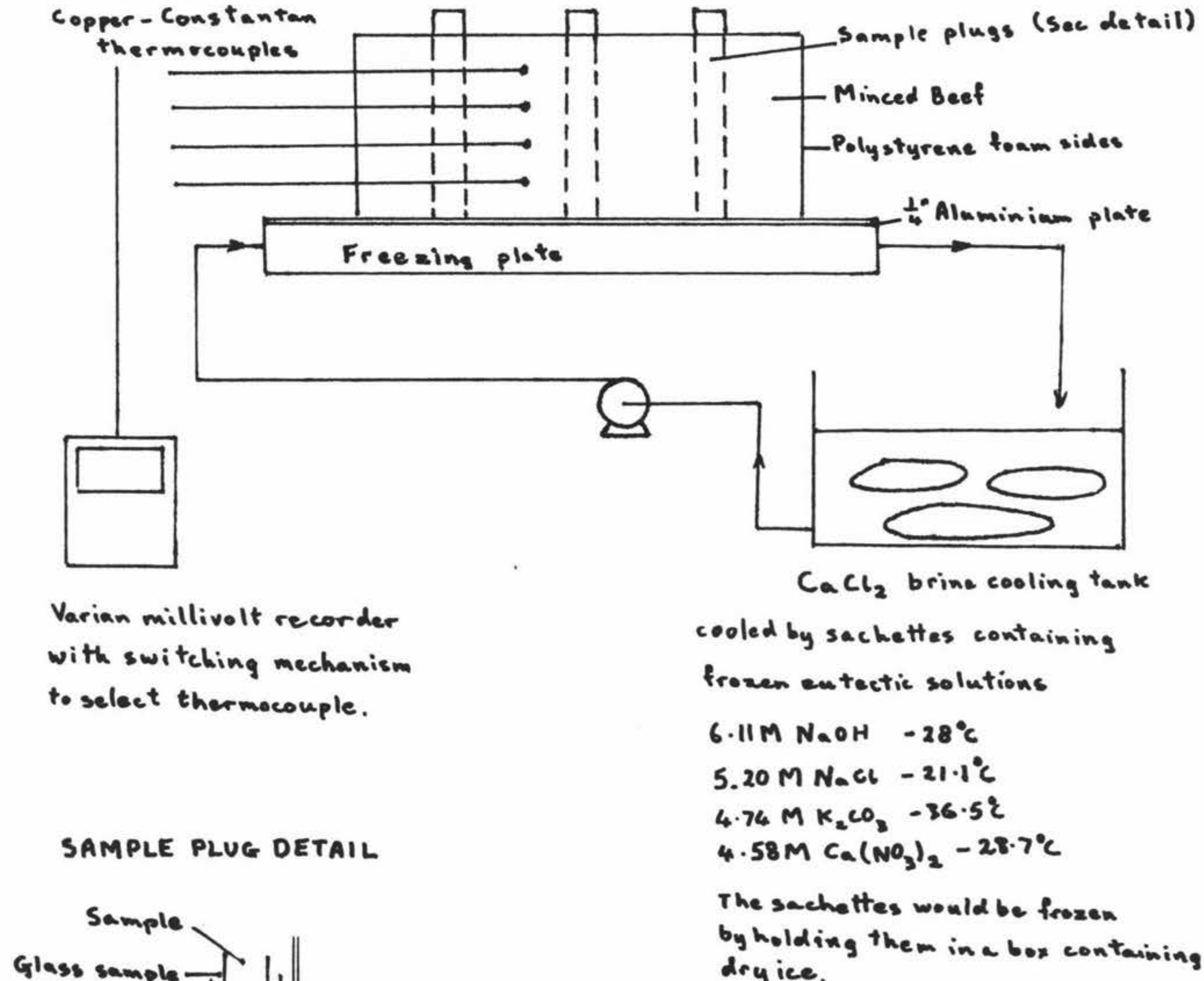
In a large piece of meat, except in the initial stages of freezing, an advancing ice front provides the site for ice crystal growth. In a small section of meat on the microscope stage it would be difficult to simulate this condition. A nucleus would first have to be formed or provided, and in either case it would be difficult to initiate growth without supercooling. It would be difficult to obtain approximately the undirectional heat flow on a microscope stage that there would be within a slab of meat.

Even if these difficulties could be overcome it would not be possible to obtain a thin enough section of meat for observation without considerable disruption of the cellular structure, which would mean that the meat would lose many of its ice development inhibition properties.

B. By Examining the Partially Frozen Material

The possibility of "stopping" the freezing process and then examining it was also considered. This could be achieved by freezing the partially frozen material rapidly enough to freeze the unfrozen water into submicroscopic ice crystals, and then preparing the tissue by freeze substitution or freeze drying for microscopic examination. An outline of the method is given in Fig. AIII-1.

FREEZING SYSTEM



SAMPLE PLUG DETAIL

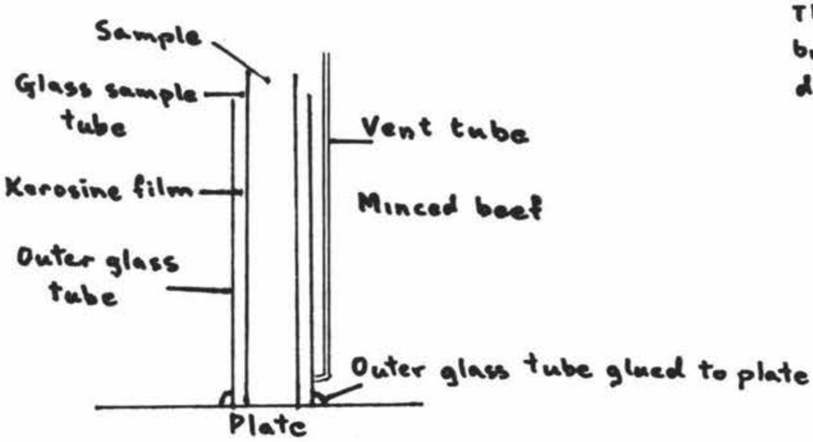
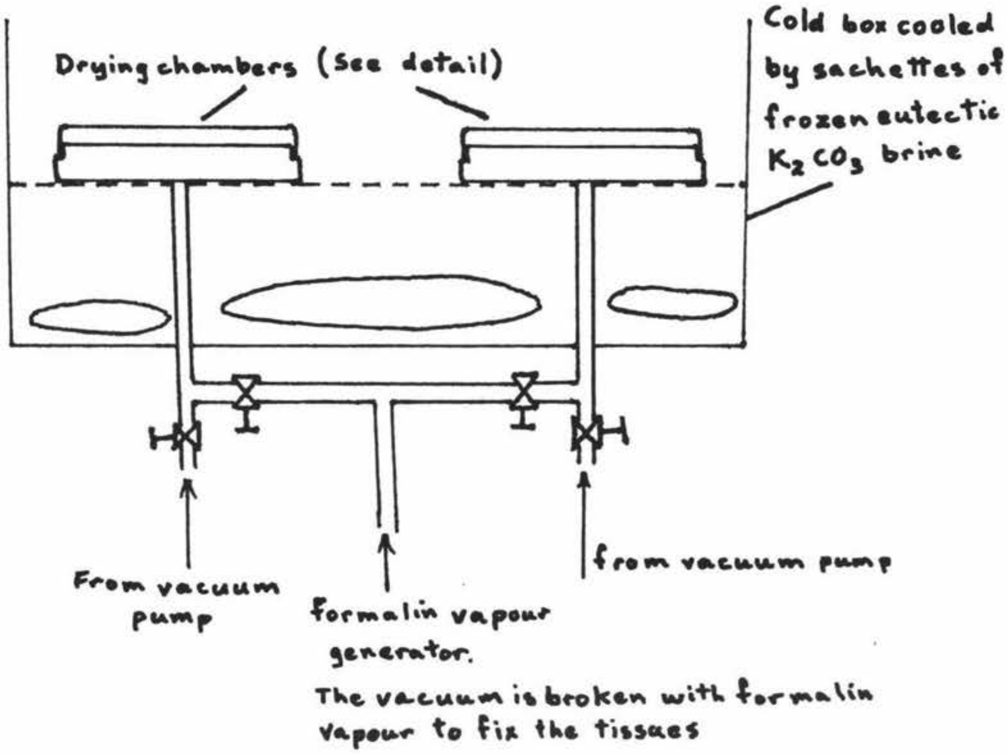


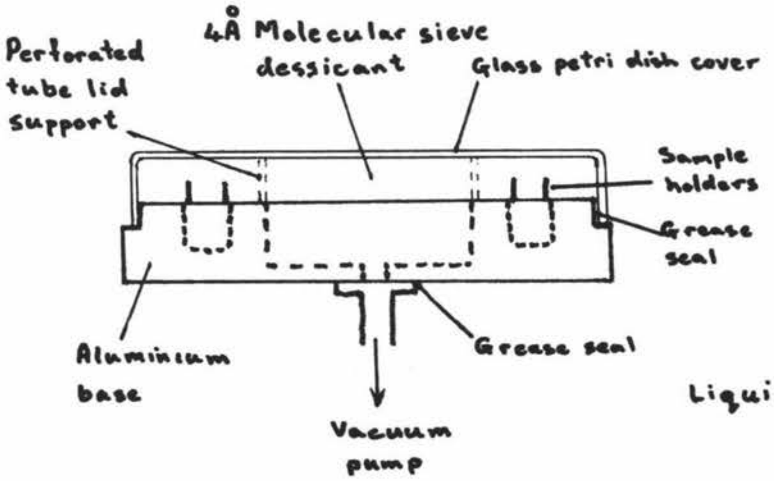
FIG. AIII-1. PROPOSED METHOD FOR EXAMINING THE ADVANCING ICE FRONT

All equipment is held in a 30-35°F room.

FREEZE DRYING SYSTEM



DRYING CHAMBER DETAIL



QUENCHING BATH

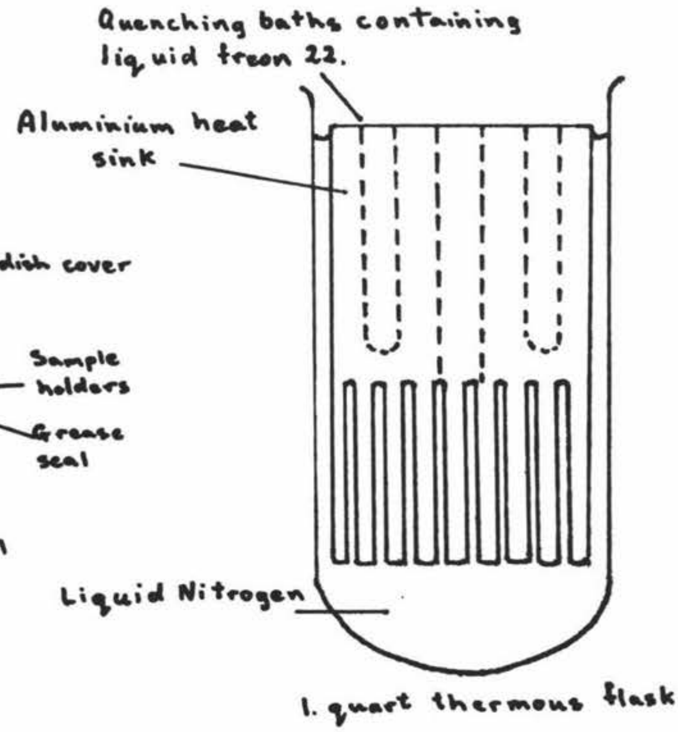


FIG. A III-1 (cont)

Freezing rapidly enough to produce submicroscopic ice crystals could be obtained by quenching the partially frozen meat in liquid nitrogen cooled liquid propane or, for safety, freon 22. Calculated cooling rates for this system indicated that sufficiently rapid cooling rates could be obtained with sticks of meat 3 mm in diameter.

In developing this method there was much debate as to whether freeze substitution or freeze drying would be the most suitable preparation technique for embedding. The possibility of using the water soluble acrylic resin, ethyleneglycol monomethyacrylate, as the freeze substituting fluid (Bartl, 1962, Leduc et al, 1963) was considered. This would avoid the necessity for a separate embedding process. The resin unfortunately was not readily available and its synthesis was not simple (Weichterle & Lim, 1960, Caldwell, 1950). In preparation of tissues for electron microscopy it is customary to use a crystallisation inhibitor, such as glycerol, which also reduces recrystallisation of small ice crystals during the time taken for substitution. In the method under consideration, it is not possible to use such an inhibitor so that the risk of recrystallisation is high during the long substitution period.

For this reason it was proposed to use a low temperature freeze drying process. By using a number of small vacuum chambers connected to a common vacuum pump the holding time before drying would be minimised and a succession of freezing runs could be handled at the same time, reducing the disadvantage of the long drying time involved with low temperature drying.

The background to the freezing system which might be used in this method is given in Chapter III. The cores of meat, 3 mm in diameter, would be enclosed in thin wall glass tubes which could be withdrawn and plunged into the quench bath at any stage during freezing. The minced meat block would be frozen from the bottom surface only leaving the top surface exposed to enable the withdrawal of the cores. By maintaining the air above the block at slightly above the freezing point, conditions at this surface would be similar to those experienced at the centre of a slab cooled from both sides.

To enable easy withdrawal of the glass sample tubes they might be inserted into another glass tube which would be glued to the freezing plate and a film of kerosine maintained between the two tubes. This film would need to be thin to minimise convection currents. On quenching the glass sample tube would either shatter if the meat inside was unfrozen, or the frozen meat would contract at a greater rate than the glass tube enabling the meat to be removed for drying. During freezing in the mould it is hoped that freezing would be slow enough to permit expansion along the length of the sample tube.

Using this approach it should be possible to determine with more certainty the morphology of the ice front in meat. Freeze etching could provide useful information in this type of study as the sublimation rate from the large ice masses formed in the freezing process is probably higher than from the fine ice engrained into the cellular material formed during the quenching process. If this is so it would be possible to obtain relief pictures of the ice front as it penetrates the meat.

Quantitative information on ice development could be obtained by measuring the area of the ice voids in sections taken at various stages during freezing, and comparing it with the area of the ice voids in completely frozen meat so as to obtain a measure of the fraction of the freezable water frozen.

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