HEAT CAPACITY
OF
GELATIN GELS

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A Thesis

by

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TABLE OF CONTENTS

EXPERIMENTAL (Continued)

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GENERAL SUMMARY

The heat capacities of gelatin gels, prepared from ash-free gelatin, of concentrations ranging from 9% to 100% gelatin and over the range of temperature between -180° and 25°C., have been measured. The adiabatic calorimeter used permits a much higher degree of accuracy than has been previously attained in such measurements. Such previous measurements as have been made on gelatin gels cover a much more restricted range of temperature and concentration, and have not been made on pure materials.

The investigation was undertaken in order to obtain some information concerning the relationship which exists between gelatin and water in gelatin gels. Estimates made by other methods of the amount of water 'bound' by the gelatin show very wide discrepancies. Certain limitations in the method used by other workers for the calculation of unfrozen or 'bound' water in gels from calorimetric data are discussed and a new equation for this purpose is derived. The amount of water remaining unfrozen in a gel is found to be dependent both on the concentration of the gel and the temperature.

The evidence obtained indicates that water exists in these gels in three states, namely, water which

cannot be frozen out at -180°C.; water which is held in the gel by forces of varying intensity so that the amount frozen depends upon the temperature; and water which is held within capillary spaces in the gel, its freezing point being lowered by pressure.

The heat capacity of the Monel metal container used for the gels, was determined and an equation is derived for the specific heat of Monel metal between -183° and 25°C.

INTRODUCTION

I Water relationships in colloidal systems.

Colloidal systems have been classified broadly into two types, namely, lyophobic and lyophilic colloids. The behaviour of lyophobic systems is reasonably well understood and their properties have been explained fairly successfully. In such systems there appears to be no close relationship between the disperse phase and the dispersion medium, and the properties of the system are found to differ but very little from those of the dispersion medium. The nature and behaviour of lyophilic systems, however, are still the subjects of much controversy, and, although an immense amount of information concerning them has been accumulated, we are still at a loss to account for the general factors which are effective in governing the structure and behaviour of such systems.

In contrast to lyophobic systems, the properties of those of the lyophilic type are found to differ quite markedly from the properties of the dispersion medium. A close examination of the properties of these systems has led to the conclusion that the cause of this lies in the fact that some sort of association exists between the disperse phase and the dispersion medium. Indeed it is this property which characterises lyophilic systems and serves to distinguish them from those of the lyophobic type.

In order to realize that a close relationship must exist between the component parts of a lyophilic system it is only necessary to compare calculations of the viscosity of lyophobic systems made by means of the simple Einstein viscosity equation (1,2) with those of lyophilic systems made in the same way. Einstein's equation is derived mathematically for the viscosity of an ideal system of a suspension of rigid spheres of uniform size, in which the radius of the particles is small compared with the mean distance between them. It is expressed thus:

$$\lambda_s = \lambda_m(1 + 2.5\theta)$$

where,

λ_s = the viscosity of the system

λ_m = the viscosity of the medium

and θ = the fraction of the total volume occupied by the dispersed particles

The value of θ is calculated from the known values of the weight and normal density of the dry material.

Calculated values for the viscosity of lyophobic systems are found to agree very well with those obtained experimentally. For lyophilic systems, however, the equation is quite inadequate. The calculated values of λ_s are much smaller than the experimental values. In order that the two may agree it is found that the value of θ would have to be several hundred times greater than the value calculated from a knowledge of the density and weight of the dry material. In other words the volume of the particles in the system is much greater than in the dry material. But the only substance present which can contribute to this increase in volume is the liquid medium. Evidently, therefore, some sort of association must exist between the colloidal particles and the liquid medium. Hatschek (3) first suggested that this association might be such that the solid particles are surrounded by a rigid layer of the medium thus making the value of θ very large.

Colloidal systems with water as the dispersion medium are more commonly known than any other. The majority of colloidal systems which occur in nature, e.g., in biological and plant structures, are systems dispersed in a water medium. It is not surprising therefore that more interest has been displayed in, and more energy devoted to, the investigation of these than of any others. The present work is concerned with the system gelatin-water. Consequently the discussion will be confined to water systems.

Living systems also are generally comprised of lyophilic colloids dispersed in water. Up to comparatively recently a knowledge of the composition of these systems had been considered sufficient and very frequently discussions are found which make reference to analyses carried out on the 'dry basis'. The present point of view is that a knowledge of the form in which the water exists in the system is of far greater importance than a knowledge of the amount (4). In a recent review of the subject Gortner (5) has expressed the belief 'that many of the reactions characteristic of living processes have to do more with the water relationships of the organism than with any other single factor', and that when these problems are solved 'there will have been inaugurated a new day in biochemistry, physiology, and medicine, for the water relationships in the living organism lie at the foundation of problems concerning both health and disease.'

II Free and bound water in hydrophilic colloids.

With the application of physico-chemical methods to the study of hydrophilic colloids it has been discovered that not all the water present in the system possesses the properties which are characteristic of free water in bulk. Thus, for example, it has been found that even when the system is exposed to extremely low temperatures, part of the water remains unfrozen. Also, the depression of the freezing point of sucrose solutions is found to be increased considerably when hydrophilic materials are present. This increase corresponds to a concentration greater than that given by the amount of sucrose and water known to be present. This is interpreted to mean that the hydrophilic material produces some change in part of the water such that that portion can no longer act as a solvent for the sucrose.

Such discoveries have led to the differentiation between 'free water' and 'bound water'. 'Free water' is relatively easy to define. It is that portion of the water which possesses more or less the properties of ordinary water. A definition of 'bound water', however, presents considerable difficulty. Although we have ample evidence for the existence of bound water, we are as yet almost entirely ignorant as to the exact nature and intensity of the forces causing the binding. It is quite probable that these forces vary both in nature and intensity with the result that there are different types or degrees of binding. Investigations so far seem to support this view but as yet there is no method which will enable us either to distinguish between different kinds or degrees of binding or to draw any distinct line of demarcation between bound and free water. It is quite likely that the gradation from bound to free is a continuous one. All the methods which have been developed for determining bound water are based upon the fact that the process of water-binding is accompanied by certain physical and chemical changes in the colloidal system. The result is, according to Briggs (6), that 'there are nearly as many definitions of bound water as there are methods for determining it'.

Various attempts have been made, however, to give a comprehensive definition of bound water. Briggs (6) has defined bound water as 'that portion of the water in a system containing colloid and crystalloid, which is associated with the colloid together with those ions which form a part of the colloid complex'. Essentially this is a more or less specific form of the definition offered by Hardy (7), who has published a theoretical discussion of the phenomenon of water-binding. Hardy's definition of bound water is 'that water which is more strongly attracted by the particles of the disperse phase than by all other forms of matter which may be present'. Kuhn (8), on the other hand, has expressed the view that, in a gel, all the water is to be regarded as bound to the colloid by forces of varying degrees of intensity. He considers that the various physico-chemical methods which have been employed for the determination of bound water give only an estimate of the intensity of the binding forces.

III Evidence for the existence of bound water.

Evidence for the existence of bound water can be obtained outside the domain of colloid chemistry. The phenomenon of the hydration of ions and of molecules, for example, is well known (9). In the case of the ions of the alkali metals it is to be expected that the lithium ion should possess a mobility considerably greater than that of the caesium ion since its atomic volume is much smaller. The reverse is found to be the case however, and it is believed to be due to the fact that the lithium ion is strongly hydrated. The smaller the ion, the more intense is the electric field surrounding it, and hence its tendency to unite with molecules of the solvent.

The hydration of sucrose has been proved by separate and distinct experiments. Thus Philip (10), Findlay (11), and Scatchard (12) have shown that six or at least five molecules of water are associated with each sucrose molecule. In this case as well as in that of the lithium ion the water associated with the non-aqueous substance can be considered as bound water.

Finely divided substances such as carbon black and platinum black have the power of adsorbing water so strongly that the adsorbed water may possess properties, such, for example, as vapour pressure and density, which are quite different from those of ordinary water. Filby and Maass (13) have shown that water adsorbed on cellulose possesses a density considerably greater than that of pure water. Water adsorbed up to 1.6%, for example, has an average apparent density of 2.62, and with increasing amounts of water adsorbed it was found that the average apparent density decreases until it reaches a value of 1.05 at 14.00% moisture. In each of these cases also the water associated with the solid material can be described as bound water. Filby and Maass put forward the hypothesis in the case of cellulose that the first amounts of water adsorbed actually enter into chemical combination with the cellulose. It is possible that a similar union occurs in the formation of colloidal gels.

Considerable evidence for the existence of bound water can be obtained from studies upon silica gels. Thus Vanzetti has found (14,15) that even when the gel is exposed to a temperature of -200°C, some of the water remains unfrozen. Further, Neuhausen and Patrick (16) have shown that the water content of a silica gel cannot be reduced below 3.8% even by heating at 300° for a period of six hours in a vacuum. Ewing and Spurway (17) have measured the density of water adsorbed on silica and have found it to be greater than that of pure water for quantities up to 4.36%. They also found that the vapour pressure of this water at 25°C is extremely low. Fells and Firth (18) have found that tha concentration of a sodium chloride solution in a silica gel prepared from water glass and hydrochloric acid decreases with the age of the gel. In other words the amount of water available for the solution of the sodium chloride increases with time. They have attributed this to the fact that the water is present in two forms which they call 'fixed' and 'free'. The 'fixed' water is considered to be definitely associated with the silica so that it cannot act as solvent. As the age of the gel increases some of the 'fixed' water is believed to be converted into 'free' as a result of a rearrangement of the SiO_2 molecules.

Foote and Saxton (19,20) have investigated the freezing of inorganic hydrogels of silica, alumina, and hydrated ferric hydroxide. They used a dilatometer method for this purpose and from their results they concluded that in these gels some of the water remains unfrozen at temperatures as low as -30°C.

IV Bound water in gelatin gels.

In the case of systems of gelatin and water there are a number of phenomena which serve as indications of water-binding. When gelatin swells in water a considerable amount of heat is evolved. This is known as the heat of swelling. A similar phenomenon is observed when powders are wetted. In the latter case the evolution of heat is due apparently to a decrease in the free energy of the water, such as would result from the adsorption of the water by the powder, since the heat effect is found to be proportional to the surface area of the powder. It is possible that a similar state of affairs occurs with gelatin except that the process may not be merely adsorption. According to Rosenbohm (21) the heat evolved when dry gelatin takes up water is nearly proportional to the amount of water up to about 0.25 to 0.3 gm. water per gm. of dry gelatin. At this point the curve bends rather sharply and the heat change accompanying further imbibition of water is small. Evidently the free energy of the first amounts of water which come into contact with the gelatin is greatly decreased. In other words, the water probably becomes adsorbed, or, to use a more general term, bound, by the colloid.

Another phenomenon which accompanies the swelling of gelatin is that of a volume contraction. The volume of the swollen gel is found to be less than the sum total of the volumes of water and gelatin from which it was formed. Measurements on gelatin have been carried out by Ludeking (22) and by Neville and his co-workers (23,24). The phenomenon is quite common with substances which swell in water and it is attributed to a hydration of the colloidal material.

Vapour pressure measurements upon gelatin gels have been made by Katz (25) and, more recently, by Briggs (26). Katz found that the vapour pressures of gels of concentrations given by 4.6 gm. water per gm. of dry gelatin and greater, are practically equal to the vapour pressure of water. For amounts of water smaller than this the relative vapour pressure of the gel is considerably less than 1, becoming smaller as the amount of water is decreased. Evidently the first amounts of water which come into contact with the gelatin become associated with it in such a way that it no longer exhibits the vapour pressure of pure water. In other words, the water is bound to the gelatin.

These observations have been substantiated by studies upon the freezing of gelatin gels. Jones and Gortner (27) have studied the process of freezing by means of a dilatometer. They found that all the water which could freeze was converted into ice at about -6°C. if sufficient time were allowed for equilibrium to be reached. Some water remained in the gel unfrozen and lowering the temperature to about -50°C. failed to produce any further effect. Moran (28) found by separating the ice which forms entirely on the exterior of a thin disk of gel when it is slowly frozen, that the unfrozen portion contained 45.7% water at -3°C. and the concentration increased to 34.5% (corresponding to

0.53 gm. water per gm. gelatin) at -19°C., after which further lowering of the temperature produced no further changes. Moran confirmed this observation by immersing a gel containing 65.5% gelatin in liquid air. It remained clear and transparent showing that no ice had been formed.

V The nature of bound water.

It has already been pointed out (pages 6 and 7) that our knowledge of the state of water in the bound condition is very limited. Various hypotheses as to the nature of the forces causing the binding have been proposed but as yet there is insufficient evidence to permit of a choice among them. In this section some of these hypotheses are briefly reviewed.

The classical work of Tamman and Bridgeman (29,30) has shown that the lowest temperature at which ordinary water can exist in the liquid state is -22°C. At this point the liquid exists under a pressure of 2115 kgms. per sq. cm. and raising or lowering the pressure causes the immediate formation of ice. It has already been pointed out that bound water can exist without freezing at temperatures down to -180°C. Evidently the bound water is in a state which is very different from that of ordinary free water.

It is possible to picture various ways in which water may be held, or bound, by colloidal materials. Kuhn (8), for example, enumerates seven possible types of binding which he describes as: water of occlusion, adsorbed water, capillary water, colloidally bound water, chemically bound water, osmotic water, and 'complexly bound' water (Komplex gebundenes Wasser). Which or how many of these forms actually exist in a given system is unknown. In all probability it would be very difficult to differentiate them one from another.

Haller (31) in a recent review of the problem of hydration (or more generally solvation), has examined in detail the molecular fine structure of lyophilic colloids with the idea that such knowledge should lead to a possible explanation of the complex nature of hydration. A consideration of the work of Staudinger (32), and of Meyer and Mark (33) shows that lyophilic colloids consist of long, thin, thread-like molecules which are flexible and can exist in bent and twisted forms. Haller considers that such forms offer two possibilities for binding and he distinguishes between static binding, i.e., adsorption, and spatial or mechanical binding which he calls 'Immobilisierung'. Static binding is caused by surface forces and occurs with a diminution of internal energy, i.e., it is exothermic. Spatial binding or 'Immobilisierung' he ascribes to the action of certain primary free forces and the process of binding in this case is endothermic. Both forces are considered to act together and from a mathematical consideration of the mechanism of each, Haller has derived equations for the swelling pressure and viscosity of lyophilic colloids which agree with the results of experiment.

Kraemer and Williamson (34) entertain a somewhat similar point of view. Thus Kraemer has made the statement (35): 'Although solvation (in a specific chemical sense) is undoubtedly a primary factor in determining the swelling and aggregation of particles, it is probable that high relative viscosities in low concentrations are usually directly due to a mechanical immobilization of the dispersion medium by the presence of jelly-like particles or bulky aggregates permeated with the solvent, or by greatly elongated particles or macromolecules that increase resistance to shear through mutual entanglement and interference, but without ordinary flocculation.'

Gortner (5) has offered two hypotheses of the nature of bound water: (1) the orientation of water molecules at the interface, and (2) the selective adsorption of orientated layers of hydrogen and hydroxyl ions to form a shell surrounding the micelle. The first case is simply that of orientated adsorption at an interface and it is probable that the adsorbed layer would be polymolecular. Gortner points out that our knowledge of water in orientated adsorption films is not sufficient to enable us to state whether or not this is the type with which we have to deal in hydrophilic colloidal systems. The second hypothesis is based on the work of Boswell and Dilworth (36). These investigators found that water adsorbed on aluminium oxide cannot be entirely removed by heating under atmospheric pressure at 500°C. for 20 hours. They attribute the greate stability of the film of water which remains on the oxide to the presence of the water in the form of adsorbed and orientated hydrogen and hydroxyl ions. Gortner makes no attempt to choose between these two hypotheses. In fact he states that a choice at the present time would probably be premature.

Kruyt (37) also supports this view of orientated adsorption. He has suggested that that part of the water which is not free to dissolve sucrose probably exists in the form of orientated dipoles, the degree of orientation diminishing as the distance from the solid particle increases. He recognizes two types of envelopes, a compact one composed of fully orientated molecules and a diffuse one where there is relatively little orientation. He has expressed the view that the former cannot be removed while the latter can. Sheppard and Houck (38) have also suggested, quite independently, that binding may take place by dipole orientation.

Katz and Derksen (39), who have developed a method of studying gels by means of X-rays, have been able to distinguish between two kinds of bound water in gels of gelatin and agar-agar. These types are termed 'intermicellar' and 'intramicellar'. Intermicellar bound water lies in layers around the colloidal micelle. Intramicellar bound water is held inside the micelle.

Neville and Theis (40) have attempted to distinguish between hydration and swelling. They prefer to regard hydration as the adsorption of water by residual valencies. Talmud and Suchowolskaja (41) have interpreted the phenomenon of water-binding as being simply hydration by polar groups.

Two points of view which differ somewhat from those outlined so far are those of Moran (42) and Marinesco (43). Moran distinguishes between two regions of binding in gelatin gels. He approaches the problem from the point of view of the activity of the water in the gel. A change of activity from zero to about 0.8 corresponds to a water content of from 0.0 to 0.5 gm. water per gm. of dry gelatin. This change is rather sudden and Moran terms it Region I. He suggests that the water up to this amount is chemically combined to the gelatin. The change of activity from 0.8 to 0.995 is accompanied by a change in the water content from 0.5 to 1.44 gm. per gm. of gelatin and this he calls Region II. This water is considered either to be adsorbed in polymolecular layers or to form a solid solution with the gelatin. Thus his results for the amount of water in Region II can be explained on the basis that gels are one-phase systems.

Marinesco (43) has made some very interesting studies upon the dielectric polarization and structure of colloids. In his paper he has devoted a section to the consideration of the physical structure of water in hydrophilic colloids. His conclusion is expressed in his own statement: 'La micelle hydrophile est formée, par conséquent d'un noyau solide, entourné d'une couche d'eau diélectriquement saturée de glace VI se trouvant en contact direct avec l'eau libre, liquide du systeme. He quotes his previous work (44) on organic colloids and tissues in support of this view.

VI General methods for the quantitative determination of bound water.

The methods which have been developed and used for the estimation of bound water depend usually upon the determination of the free water by some physico-chemical method. Knowing the total amount of water present, the bound portion is obtained by difference. Since the amount of bound water is usually only a small fraction of the total water present, and since the methods for the determination of both the total amount and the free portion are not very accurate, the error in values for the bound portion is likely to be large.

(1) The Cryoscopic Method.

This method was devised by Newton and Gortner (45) in 1922. It is based upon the hypothesis that bound water is associated with the colloidal material in such a way that it will not dissolve substances which are soluble in ordinary water.

The first step in a determination is to determine the freezing point of the colloidal system. Exactly .01 mol. of sucrose is then added and allowed to dissolve. A second determination of the freezing point is then made. From the depression of the freezing point, the amount of water available for the solution of the sucrose can be calculated from the molecular depression of the freezing point. The total amount of water having been determined previously by drying to constant weight, the bound portion, or that part which will not act as solvent, is obtained by difference.

The method is rapid and widely applicable, and it gives reproducible results. Kruyt and Winkler (37) have repeated some of Gortner's work and have obtained the same results. Sayre (46) has shortened the laboratory procedure by using a refractometer to measure the exact amount of sucrose added. The method has been used extensively for the determination of bound water in plant structures (47 to 53).

Grollman (54) has pointed out that Newton and Gortner have failed to consider the hydration of the sucrose which is added to the system. The effect of this hydration is to increase the freezing point depression. Consequently a correction must be introduced into the formula used by Newton and Gortner. According to

Grollman this correction affects the results quoted by these authors quite appreciably.

(2) The Vapour Pressure Method

This method has been devised by Hill (55). It corresponds to Gortner's method except that depressions of vapour pressures are measured instead of depressions of freezing points. It consists in measuring the depression of the vapour pressure caused by the addition of some suitable, soluble material to the particular system being studied, and comparing this value with that caused by the addition of the same substance to an approximately isotonic salt solution. The construction and use of the myothermic apparatus employed for this purpose have been described in detail by Hill (56), and by Downing and Hill (57). It has been used by Hill chiefly for the study of bound water in muscle and tissue.

Briggs (26) has made vapour pressure measurements upon isoelectric casein and upon various sodium and calcium caseinates, using the isotenoscope devised by Smith and Menzies (58) for the study of the vapour pressures of liquids. Briggs uses the relation between the relative vapour pressure and the activity to determine how much water in a colloidal system corresponds to a given activity.

(3) The Method of Jensen and Fischer.

Jensen and Fischer (59,60) have made quantitative determinations of bound water in muscle by plotting cooling curves and calculating the heat absorbed from the area under the curves. A comparison of the results with those obtained with solutions of sodium chloride is believed to allow the calculation of the amount of bound water. Rubner has objected to this method on the basis that the thermal conductivity of muscle may not be the same as that of a sodium chloride solution.

(4) The Dilatometric Method.

This method is based upon the fact that, as the temperature of pure water is lowered, an expansion takes place at the freezing point, followed by a uniform contraction in volume of the ice formed. It was introduced in 1916 by Foote and Saxton (19,20), who used it to study the behaviour of inorganic hydrogels at low temperatures. The method is based upon the assumption that bound water will not freeze.

The method consists of sealing the colloidal material in a dilatometer which is then immeresed in constant temperature baths at various low temperatures. Readings on the dilatometer scale are plotted against temperatures. From the sudden expansion due to freezing at or near zero, the amount of water frozen can be calculated from a knowledge of the specific volumes of ice and water. Again knowing the total water content of the system as determined by drying to constant weight, the amount of bound water is obtained by difference.

Foote and Saxton have used this method to study the behaviour of mixtures of lampblack and water upon freezing (62). In 1917 Bouyoucos (63) applied it to the study of soils. McCool and Millar (64) have used it in studies of plant relationships and Rosa (65) has applied it to studies of winter-hardiness. Moran (28) has used it to study gelatin gels and more recently Jones and Gortner (27) have made extensive use of it in studies of free and bound water in elastic gels such as those of gelatin and egg-white, and in non-elastic gels such as silica and hydrated ferric hydroxide.

(5) The Calorimetric Method.

This method is also based upon the assumption that bound water does not freeze. It was first used by Muller-Thurgau (66,67) on apple and potato tissue. Rubner in 1922 (61) and Thoenes in 1925 (68) have used it to study colloids and animal tissue. Rubner's procedure has been improved by Robinson (69 to 72), who has used the method to study winter hardiness in insects. In a recent paper Robinson (73) has published complete experimental details of his procedure. St. John (74) has applied the method to the study of bound water in egg white.

The method consists of cooling the material to some initial temperature, about -20°C., and then plunging it into a water calorimeter at some known temperature. The fall in temperature of the water is measured, and, knowing the amount and the water equivalent of the calorimeter, the heat required to warm the sample from its initial temperature up to the final temperature of the calorimeter, is obtained. The process of warming up involves the following steps:

(a) warming the sample from the initial to the final temperature,

(b) warming the ice from the initial temperature up to the freezing point,

(c) melting the ice at the freezing point.

Thus we have:

$$H = MS(T_2 + T_1) + 0.5T_1x + 80x$$

or

$$x = \frac{H - MS(T_2 + T_1)}{80 - 0.5T_1}$$

where

H = the measured quantity of heat,

x = the weight of water frozen at T_1,

M = the weight of the sample,

S = the specific heat of the sample,

and T_1 and T_2 are the initial and final temperatures, the sign of T_1 being neglected. This is the equation given by Thoenes (68) and used in slightly modified form by Robinson (73) and St. John (74). A discussion of its limitations is reserved for a later section.

In the present work the calorimetric method has been extended and improved. A new equation for the calculation of bound water is derived.

VII Quantitative results for bound water in gelatin gels.

An estimate of the amount of bound water in gelatin gels can be made from an examination of the curves for the vapour pressure and the heat of swelling of gels of varying water content. Rosenbohm (21) has shown that when gelatin takes up water, the amount of heat evolved reaches a constant value when 1 gm. of dry gelatin takes up 0.5 gm. water corresponding to a gel concentration of about 66.6% gelatin. The vapour pressure determinations of Katz (25) show that the relative vapour pressure - water content curve approaches 1 asymtotically at a gel concentration of about 66.6% gelatin. These results are of course only approximate, but they serve in a general way as an indication of the extent of the binding by gelatin.

For the amount of water bound by 1 gm. of gelatin as determined by direct methods, different investigators have obtained different results. Adair (75) has carried out some direct osmosis measurements upon gelatin gels, from which he concludes that 1 gm. of gelatin binds 0.6 gm. water as water of hydration at 0°C. Experiments on swelling show much less bound water. Svedberg (76) has measured the volume contraction during gel formation of gelatin at different concentrations and the curve constructed from his values shows a sharp bend at 0.08 gm. water per gm. of gelatin. The equilibrium between gelatin gels and dried acetone as well as the curve for the velocity of drying of such gels leads to values of less than 0.1 gm. water bound by 1 gm. of gelatin. Marinesco (43), from his studies upon the dielectric polarization of gelatin gels gives a value of 0.013 gm. water associated as Ice VI with 1 gm. of gelatin in a 0.3 to 4.0% solution.

Moran (28) observed that when gelatin gels of 12 to 40% concentration, in the form of thin disks, were frozen slowly at -3°C., ice formation occurred entirely on the surface. The outer layer of ice could be removed from the core of partly dehydrated gel and the concentration of the latter determined. On lowering the temperature further, water passed from the unfrozen core into the outer layer of ice and the concentration of the unfrozen gel increased from 54.3% at -3°C. to a maximum of 65.5% at -19°C. corresponding to 0.53 gm. water per gm. of dry gelatin. Moran called this the bound water and designated the water which separated between -3° and -19°C., 'interstitial' or 'capillary' water. Later measurements by the same method (42) gave closely concordant results with three different samples of ash-free gelatin, for gels of various concentrations within the limits of 12 to 40% and for widely different rates of freezing. The values obtained by Moran for the equilibrium concentrations in these samples at various temperatures, as determined by the method outlined above, are given in Table I. In order to determine the effect of concentration in the case of any one sample, four gels A, B, C, D were prepared of concentrations 13.7, 23.3, 30.9 and 35.1% ash-free gelatin respectively. The equilibrium concentrations obtained are given in Table II.

TABLE I

Freezing Temperature	Equilibrium Concentrations Coignet (1)	Coignet (2)	Eastman
-0.5	40.9		
-0.9	47.1	48.0	
-3.0	55.8	57.1	56.3
-6.0	60.0		
-18.0	64.6	66.3	63.6

TABLE II

Temperature	A	B	C	D
- 3	57.1	57.2	57.5	57.8
-10	64.2	65.7	65.8	66.1
-18	65.8	67.2	67.6	67.5

According to Moran bound water is apparently independent both of gel concentration (within the limits stated) and of temperature (below -19°C.). In support of the latter he found that three days immersion in liquid air caused no further separation of ice from a sample which had been frozen first at -20°C.

From the gel concentrations tabulated in Table I, Moran has calculated the total quantity of water bound per gm. of dry material at the equilibrium temperatures and from the equation of Lewis and Randall (77),

$$\log a_{H_2O} = -0.004211\theta - 0.0000022\theta^2$$

he has also calculated the activity of this water at these temperatures. The results as given in his paper (42) are reproduced in Table III.

TABLE III

Temperature	Activity	Quantity water per gm. dry material
- 0.5	.995	1.443
- 0.9	.991	1.120
- 3.0	.971	.793
-6.0	.943	.666
-18.0	.8345	.547

It is seen that the amount of bound water increases rapidly as the activity of water, 1, is approached.

Moran (78) has also found an average value of 0.56 gm. water bound per gm. of gelatin from experiments on purified isoelectric gelatin kept for 44 days at -18°C.

According to Briggs (6), measurements upon frozen gels should yield a result for bound water which depends on the temperature of the frozen material but which is independent of the amount of water originally present in the gel. He says: 'Bound water is not a fixed quantity of water associated with the colloid but will vary with the activity of the water in the system in a manner consistent with the vapour pressure isotherm of the colloid.' Jones and Gortner (27), on the other hand, found, by their dilatometer method, that if sufficient time were allowed for equilibrium to be established at the temperature where freezing first began, about -6°C., no more ice appeared to be formed even when the temperature was brought as low as -50°C. They also found that the amount of water bound by the gelatin was dependent on the concentration of the gel. Their results taken directly from their paper are given in Table IV. The relation between bound water and gel concentration was found to be a logarithmic one from which they infer that the binding of water by gelatin is an adsorption process. To account for the fact that the amount of water remaining unfrozen was apparently independent of the temperature, they have suggested that there is an increase of 'adsorption pressure' as the temperature is lowered which is just sufficient to counterbalance the decrease in the vapour pressure of the ice.

TABLE IV

Gel Concentration %	Gms. water bound per gm. dry gelatin -10°C.	-30°C.
2	4.675	4.675
8	1.888	1.899
16	1.010	1.051
32	0.643	0.701

In the foregoing results no mention is made of the effect of the pH of the gels. In most cases this is not even mentioned. Presumably isoelectric gelatin has been used. Thoenes (68) has made some measurements upon gelatin gels of different pH using the calorimetric method. His results are given in Table V. They must be considered to be of uncertain value because his method of calculation is based upon somewhat doubtful assumptions and, moreover, takes no account of the effect of the inorganic matter present. He himself states that his results are not absolute but only relative.

TABLE V

Total Water	pH	Water bound per gm. dry gelatin
92.4%	5.5	2.40 gm.
87.0	5.3	1.86
86.4	4.2	1.92
87.1	3.0	2.14

It is seen that there are considerable discrepencies in the results reported for bound water in gelatin gels even when only those methods involving freezing of the gel are considered. Evidently there is room for much further research to settle the questions of the effects of concentration and temperature and also of pH. In the latter field very little work appears to have been done.

EXPERIMENTAL

I Method

In the present work the method of investigating the behaviour of gelatin gels on freezing and of determining the amount of bound water in these gels, is a calorimetric one based upon the fact that, upon freezing a gel, only part of the total water present in the system can be frozen. Fundamentally it is exactly the same as that used by Mennie (79) in his studies upon the freezing of water in fish muscle and in gelatin.

The method involves the determination of the amount of heat required to warm a sample of gel from various initial temperatures up to the final temperature of the calorimeter. All determinations are corrected to the same final temperature, 25.0°C., and plotting heat capacities as ordinates, and temperatures as abscissae, a heat capacity curve is constructed. The determinations extend over the whole temperature range from -183°C. to 25.0°C.

In all cases where the desired initial temperature was above -78.5°C., the gel was first rapidly frozen in solid carbon dioxide and ether and wasbthen allowed to come to the desired temperature in a constant temperature bath in which it was kept for 1 to 1.5 hours before introduction into the calorimeter. This procedure eliminated the possibility of supercooling and made certain that equilibrium had been established. It provided for uniform freezing conditions in every determination. The necessity for this is indicated by Moran's (28) observation that when gelatin gels are frozen slowly (in air at -3°C.) the water which freezes comes out of the gel and forms an ice layer on the surface. With rapid freezing, as used in the present work, no such segregation of the components of the gel occurs.

It was found that a whole series of determinations could be made on a single sample of gel which had been sealed in the container. Mennie (79) found no evidence that repeated freezing and thawing produced any permanent change in the gel, which would prevent repition of results with the same sample. In the present work this was confirmed with a freshly prepared gel by making three consecutive runs on a 24% gel at -78.5°C. The values obtained for the total heat capacity of the container and gel were 2648.4, 2653.1, and 2644.8 cals. At the end of a long series of determinations on this sample, a fourth run was made at -78.5°C. giving a value of

2635.9 cals. This is somewhat low compared with the earlier figures. However, as Barnes and Maass (80) point out, the difficulties inherent in the method of transferring the container from the bath to the calorimeter tend to give low results, especially at low temperatures. If the difference were due actually to a change in the gel, it would indicate a decrease in the amount of free water, a sufficiently improbable result, which is not supported by any other measurements on the same gel.

II Description of the apparatus.

The adiabatic calorimeter used was that designed by Barnes and Maass (81) and used by them to measure the heat capacity of ice (80). Since a detailed description of the apparatus and of the method of operation has already been published by these investigators (81), it will be sufficient here merely to point out some of the particular features involved in its construction and to consider the precision with which calorimetric measurements can be made with it.

The calorimeter is an adiabatic one and differs essentially from all other forms of adiabatic calorimeters in that a radiation thermel is employed to detect temperature differences between the inner and outer baths. The radiation thermel consists of eight pairs of copper-constantan junctions arranged in the form of a helix around the inner calorimeter vessel, in such a way that eight of the junctions make thermal contact with the inside wall of the outer jacket while the others are supported in air upon small ebonite forks at a distance of about 3 mm. from the side of the inner calorimeter vessel and receive heat from it by radiation. The junctions which make thermal contact with the inside of the outer calorimeter jacket are insulated electrically from the surface of the jacket by thin strips of mica.

The construction of the calorimeter showing the arrangement of the thermel junctions is illustrated diagramatically in Fig. 1. L represents the outer jacket with the cover removed and M is the inner calorimeter vessel. I, I are ebonite chimneys through which pass the wires to the inner stirrer (see below, pp. 39-40), and J is the ebonite chimney through which the substance under investigation is introduced. A, B, C, D, E, F, G, and H are the pairs of junctions of the radiation thermel.

The method of supporting the thermel junctions is shown on a large scale in the inset, Fig. 1. S indicates

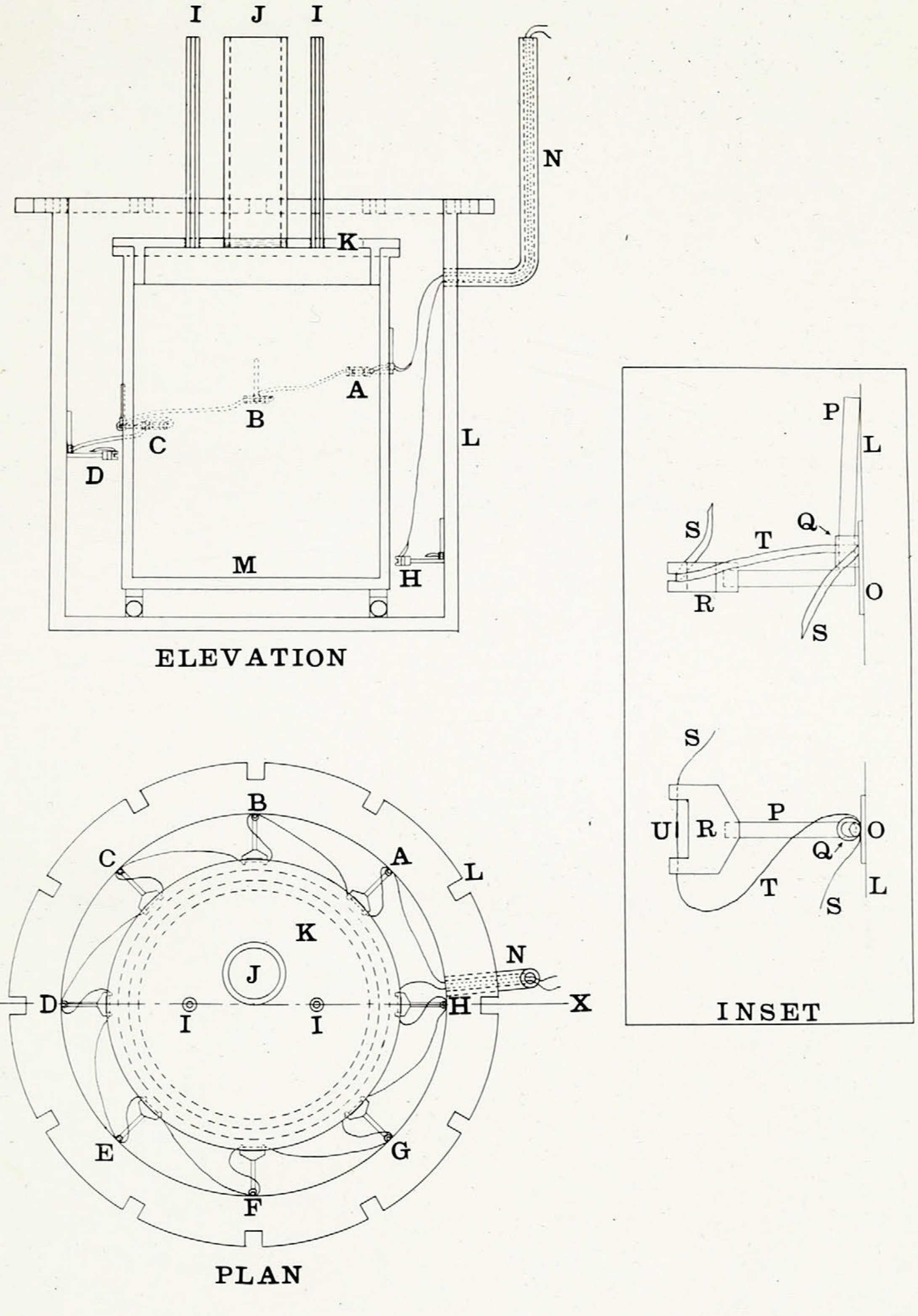
I
J
I
N
K
A
B
C
D
L
M
H
ELEVATION
P
L
S
T
Q
R
O
S
S
U
R
P
O
Q
T
L
S
INSET
B
C
A
L
K
N
J
X
D
I
I
H
X
E
G
F
PLAN

FIG. I

copper wires and T constantan. One junction, U, is suspended upon an ebonite fork R as shown and the other passes between the light ebonite collar Q and the thin strip of mica O. The ebonite fork R is supported upon a light copper wire P which is soldered to the inner wall of the outer jacket L.

This radiation thermel possesses a number of distinctive features. It was found (81) by the designers to be about twice as sensitive as the ordinary type of thermel consisting of two sets of copper-constantan junctions, one immersed in the inner and the other in the outer bath of the calorimeter. The use of the radiation thermel eliminates all errors due to inaccuracies in accounting for the heat capacity of the junctions, which, in the original form, were immersed directly in the water in the inner calorimeter vessel. It also permits of closing off the water in the inner calorimeter vessel from contact with the outside air, thus eliminating errors due to evaporation, etc.

The water in the outer bath of the calorimeter is stirred by means of four stirrers driven by an electric motor, each stirrer having two propellers. Stirring of the water in the inner calorimeter vessel is effected by means of a circular piece of lead suspended horizontally by means of very fine copper wires passing through the ebonite chimneys I, I (Fig. 1). This stirrer, when in operation, possesses an up and down motion transmitted to it by means of a series of electrically driven pulleys and an eccentric. A large circular hole is cut in this stirrer to permit the passage of the container, in which is sealed the material under investigation, when it is introduced into the calorimeter.

The apparatus is made very sensitive to small differences of temperature between the inner and outer baths by placing the galvanometer scale about three metres away from the mirror. In this way a temperature difference between the two baths of 0.00015° can be detected.

Temperature equilibrium between the two baths is maintained constant over a period of time by means of different sizes of jets for hot and cold water.

III Tests on the calorimeter.

In order to be sure that the calorimeter was functioning properly, the following tests were carried out:

(1) Comparison of the temperatures of the inner and outer baths.

Any difference in temperature between the inner and outer baths, when the galvanometer deflection was zero, was tested for by inserting into each bath, Beckmann thermometers which had been compared previously. The calorimeter was then put in operation for about two hours exactly as in an actual determination. The deflection of the galvanometer was kapt at zero on the scale by running in hot or cold water as required, and the temperature readings were made on each thermometer at five minute intervals.

It was found that the outer bath was always at a temperature of about 0.001° greater than that of the inner. Since this is the limit of precision with which a Beckmann thermometer can be read, it is neglected.

(2) Sensitivity.

The sensitivity of the thermel was tested by keeping the temperature of the outer bath greater than that of the inner by a constant amount. This was accomplished by allowing a fine, carefully regulated stream of hot water to drip slowly into the outer bath. It was found that 1 cm. on the galvanometer scale corresponds to a temperature difference between the two baths of about 0.003°. Since a deflection of about 0.5 mm. on the galvanometer scale can be noticed, this means a temperature difference of 0.00015° can be detected.

Also with careful adjustment the spot of light can be kept within 1 mm. of zero on the scale. Hence the two baths can be maintained at the same temperature to within 0.0003°.

(3) Trial determination.

As a final check on the performance and accuracy of the apparatus, a test run was made on ice using exactly the same platinum container which had been used by Barnes and Maass (80).

A comparison of the results obtained in these tests with those obtained by Barnes and Maass is given in Table VI. The H at -78.5°C. is the heat capacity of ice at that temperature.

TABLE VI

	Barnes and Maass	This work
Sensitivity	0.00014°	0.00015°
Diff. in temp. between outer and inner baths	0.002°	0.001°
H at -78.5°C.	138.2 cals.	138.1 cals.

IV Standardisation of Beckmann thermometer.

A Beckmann thermometer was used to obtain the temperatures of the outer bath of the calorimeter. This thermometer had been calibrated by the Bureau of Standards at Washington, and all readings made were corrected according to the certificate supplied by them.

The thermometer was standardised by comparing it with a standard mercury thermometer at nine different points. Readings on the Beckmann (corrections being made) were then plotted against readings on the standard. The straight line obtained was extrapolated to zero reading on the Beckmann. In this way the Beckmann zero was found to correspond to 23.65°C. The readings are given in Table VII.

TABLE VII

Beckmann	Standard
0.050	23.70
0.080	23.72
0.123	23.77
0.450	24.10
0.480	24.14
0.511	24.17
0.859	24.52
0.899	24.56
0.945	24.60

V The constant temperature bath.

This bath was used to bring the gels to a known initial temperature before introduction into the calorimeter. It consisted merely of a wide-mouthed Dewar flask. For 0°C. an ice-water mixture, and for -78.5°C. solid carbon dioxide moistened with ether or acetone were used. Liquid air was used to give the lowest temperature, -183°C. Points between -78.5°C. and 0°C. were obtained by cooling ether or acetone to the desired temperature and maintaining it at a constant value by dropping in small lumps of solid carbon dioxide at regular intervals.

The arrangement of the bath for temperatures near -130°C. is illustrated in Fig. II. The bath liquid for these temperatures was obtained by distilling gas machine naptha and collecting the fraction boiling below 30°C. This was brought to the desired temperature and maintained at this point by means of liquid air blown at regular intervals into a very thin copper tube P suspended in the bath liquid. A number of thin copper vanes were soldered to this tube in order to make heat conduction as rapid as possible. In all cases stirring was effected by means of a stream of dry air blown into the bath liquid.

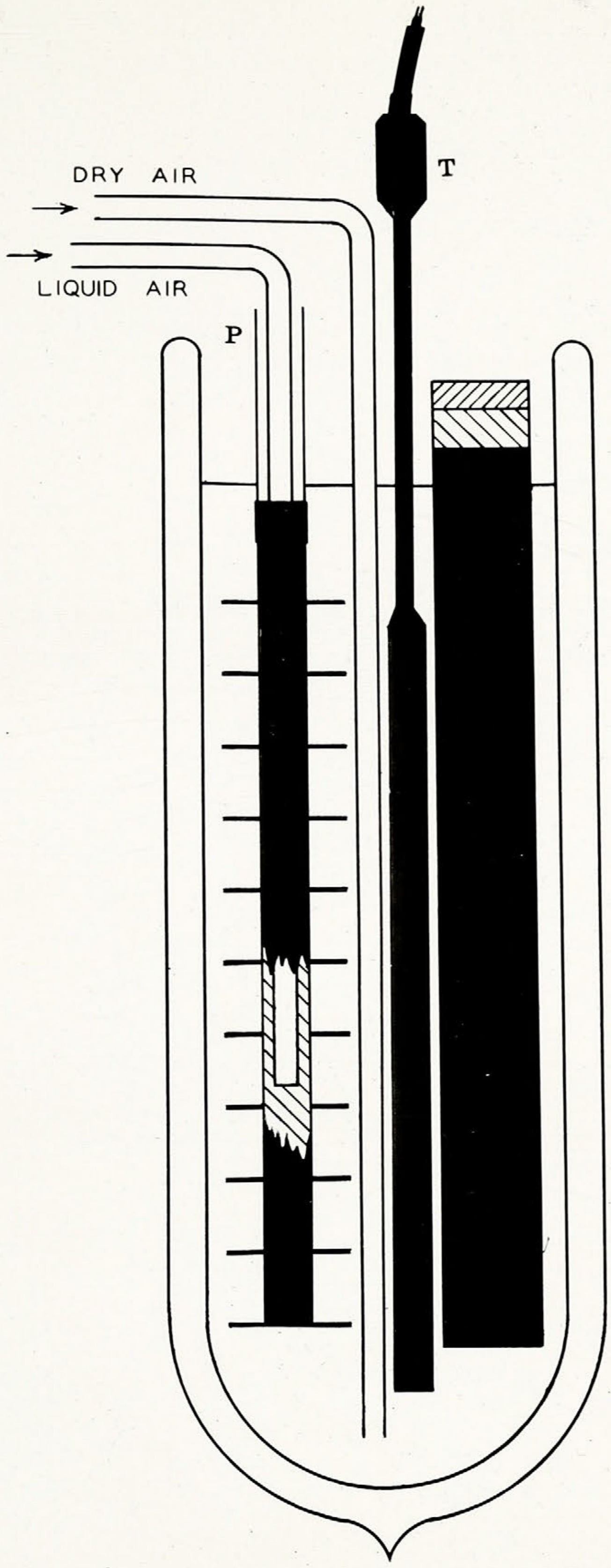

FIG. II

It is estimated that, with careful manipulation, the temperature of the thermostat could be controlled to about 0.2°C. at -130°C., and easily to 0.1°C. at temperatures above -78.5°C.

The temperatures of the bath were obtained by means of a calibrated platinum resistance thermometer (T in Fig. II) which was read by the usual Wheatstone bridge arrangement using a plug-type resistance box in series with a calibrated mercury resistance of the type designed by Maass and Mennie (82), and the same galvanometer used in the thermel circuit.

VI Calibration of the platinum resistance thermometer.

The platinum resistance thermometer was carefully calibrated by determining its resistance at three fixed points. The fixed points chosen were those given by ice-water (0°C.), solid carbon dioxide (-78.5°C.), and liquid air (-184.5°C.).

Since the boiling point of liquid air depends upon its composition, a sample was collected in a gas burette, diluted with a known volume of air, and a gas analysis made on it in the usual manner using pyrogallol as the absorbing reagent.

From the analysis the amount of oxygen present was calculated and the corresponding temperature obtained from Claude (83). Since the value for the normal boiling point of oxygen as given by Claude is -182.0°C. whereas the International Critical Tables (84) give -183.00°C. as the best value, Claude's values were raised by 1° to make them agree with the more accurate values. The barometric pressure was neglected since a change of 10 mm. only affects the temperature by about .01°.

The carbon dioxide temperature was corrected for barometric pressure by means of Henning's formula (85) as recommended in the International Critical Tables (84).

The constant θ of the Callendar-Barnes formula was calculated as follows:

The temperature given by the platinum thermometer when its resistance is R is defined by the equation:

$$t_{pt} = \frac{R - R_0}{R_{100} - R_0} \cdot 100 \qquad (1)$$

where,

t_{pt} = the platinum temperature corresponding to a resistance R,

R_{100} = the resistance at 100°C.

and R_0 = the resistance at 0°C.

The relation between the temperature on the platinum scale and that on the absolute gas scale is given by the Callendar-Barnes formula:

$$t_{pt} = t - \theta A \qquad (2)$$

where,

t = the temperature on the absolute gas scale,

θ = a constant,

and

$$A = \left(\frac{t}{100}\right)^2 - \left(\frac{t}{100}\right)$$

Now equation (1) can be written:

$$t_{pt} = a(R - R_0)$$

by putting

$$a = \frac{100}{R_{100} - R_0}$$

Hence substituiting for t_{pt} in equation (2), we get:

$$a(R - R_0) = t - \theta\left\{\left(\frac{t}{100}\right)^2 - \left(\frac{t}{100}\right)\right\}$$

This equation contains two unknowns, a and θ. We need θ only. R_0 is determined and values of R are obtained et the two fixed points. Hence we have two equations in which everything is known except a and θ. Hence we can calculate θ. It was found to have a value of 1.419.

This device of putting $a = \frac{100}{R_{100} - R_0}$ eliminates the necessity of determining R_{100}, so that the thermometer, which is to be used for low temperatures only, need not be heated above room temperature.

Resistances of the thermometer were now calculated for 10° intervals and a curve plotted to cover the range over which the experiments were carried out.

VII Preperation of the gels.

As it was desirable to make measurements upon gels of concentration greater than 20% gelatin, and since the usual method of preparing gels by allowing the gelatin to stand in contact with water for some time before melting was not suited for this purpose, owing to the relatively large volume of gelatin compared with the small volume of water, some preliminary experiments were tried in order to find a standard procedure which would be suitable for making gels of almost any desired concentration. This was done with a certain amount of success.

The gelatin used was very pure isoelectric gelatin obtained from the Eastman Kodak Company. The moisture and ash content were specified respectively as 11.04% and 0.04%. These were checked shortly after the gelatin had been received and were found to be correct.

The rate at which gelatin takes up water was first determined. The method of doing this was to take a weighed strip of gelatin about 3 in. long and 1 in. wide and immerse it in water at room temperature. By removing and weighing the strip at regular intervals the amount of water taken up in a given time was found. Before each weighing the strip of gelatin was carefully and quickly dried between two strips of filter paper in order to remove any loose water. The strips were weighed in a covered petri dish to avoid losses by evaporation as much as possible.

From these results the time of immersion of the gelatin in water to produce a gel of approximately the concentration required could be obtained. The method has proved very suitable for obtaining gels of concentrations from about 20% to 60%.

To prepare a gel of approximately the concentration desired, the following procedure was used. An amount of sheet gelatin which would give about 20 gms. of the gel was immersed for the required time in distilled water which had been boiled previously and allowed to cool. It was then removed, placed in a test tube and kept at about 50°C. for half an hour or more in order to produce uniformity of composition. In the case of the more concentrated gels, which are very viscous, the water-soaked material was pressed into the container (Section VIII) and the heating at 50°C. done in this. The exact composition of the more dilute gels was obtained by pouring a portion of the molten gel into a petri dish, weighing it quickly and drying to constant weight in an electric oven at 105°C. This process usually required at least 48 hours. In order to obtain the exact composition of the more concentrated gels, portions were cut from various parts of the sheets immediately they were removed from the water. These portions were used for the analysis. In all cases duplicate determinations were made and the average taken as the concentration of the gel.

For the gel containing 87.5% gelatin, the sheet gelatin as it was stored was used. It was cut into small pieces about 5 mm. square and packed into the container.

Dry gelatin was obtained by cutting the sheet form into small pieces, drying first in an electric oven at 105°C. for 24 hours and then to constant weight in a vacuum over phosphorus pentoxide for about two weeks. The latter procedure was adopted in order to avoid any possibility of decomposing the gelatin and prolonged heating at 105°C. would very probably cause this.

VIII Container for the gels.

Several trial experiments were made using a pyrex glass container. It was found to be unsatisfactory and was replaced by one made of Monel metal which served the purpose excellently.

This container was specially designed to give an air-tight joint between the container and the cover and also to eliminate capillary spaces into which water might creep. A cross-section of the container is given in Fig. III (not drawn to scale). The external dimensions were about 3 in. x 1 in.

The container was made entirely of Monel metal

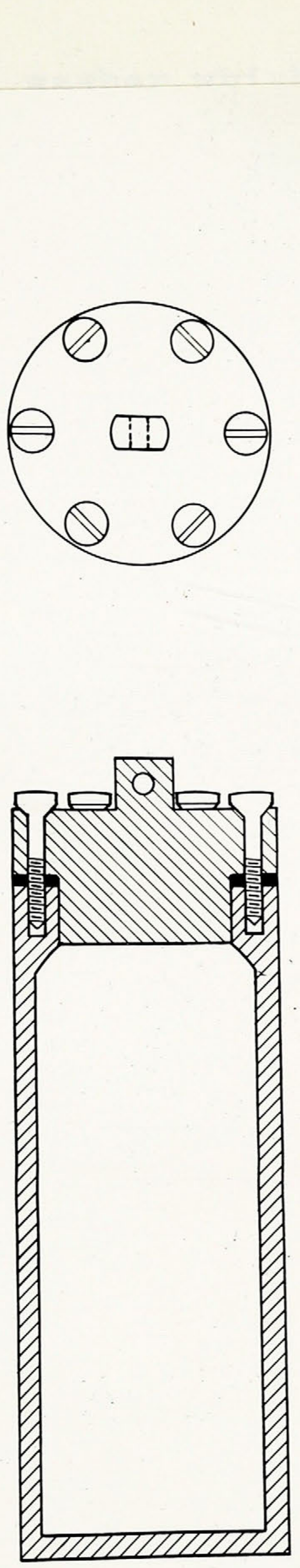

FIG. III

except for a thin lead washer which was placed between the container and the cover. Capillary spaces in the neighbourhood of the screws in the cover were avoided by countersinking the holes for the screws and machine-turning their heads so that there was a bevelled edge which fitted the countersunk hole exactly. When the screw was tightened into place, a very efficient water-tight joint was obtained.

The lead washer was of practically pure lead and hence was quite soft, so that, when the cover was pressed tightly upon it, all possible fine openings were filled.

This container was found to hold ether very well under a vacuum.

IX Filling the container.

Two points had to be considered in filling the container. In the first place since there is water present which will expand and exert tremendous pressure on freezing, the container must not be filled completely. Sufficient space must be allowed for this expansion.

The second point is concerned with the rate of transfer of heat into the gel on introducing it into the calorimeter. As the thermal conductivity of gelatin solutions is only about 0.00133 (86), this is of prime importance since a rapid heat transfer within the calorimeter is desirable.

Both these difficulties were overcome in the following manner. After the gel had been kept in the sol form for about one-half an hour at about 50°C., it was poured into the container until the latter was about three-quarters filled. Immediately the cover was screwed on as tightly as possible and the container rotated in a horizontal position at about 300 revolutions per min. until the gel had set. Preliminary experiments showed that this rotation caused the gel to set around the sides of the container, thus exposing as much surface as possible to aid in any transfer of heat, and also leaving a cylinder of air in the center of the gel which permitted of any expansion resulting from freezing.

This method of filling the container was found to be very satisfactory for gels of concentrations up to 45% gelatin. In the cases of the 45% and more concentrated gels the samples were heated at 50°C. in the container rather than in a test tube because these gels are very viscous when in the sol form and will not flow readily. The container was filled by pressing the water-soaked samples as tightly as possible into it. Since in gels of these concentrations there is little or no water which will freeze, practically no expansion takes place on freezing. Hence in these cases the procedure of spinning the gels was omitted. It has already been pointed out (pp. 51 and 52) that the samples of the 87.5% gel and of dry gelatin were packed into the container in the form of small pieces.

X Heat capacity measurements on gels.

(a) Procedure for dilute gels up to 45% gelatin.

The molten gel prepared as described in Section VII was poured into the Monel metal container, which had been weighed previously, until it was about three-quarters filled. The cover of the container was then hurriedly placed into position. Immediately following this portions of the gel were poured into two weighed petri dishes, covered and weighed as quickly as possible. The petri dishes were placed in an electric oven at 105°C. and left to dry to constant weight. The cover of the container was then tightened securely into place and the container rotated in a horizontal position for about 1 hour in order to ensure sufficient time for setting. At the end of this time the weight of the container and gel was determined. Subtracting the weight of the container gave the weight of the sample.

Between experiments the container was always kept in a desiccator over calcium chloride to be sure it remained dry on the outside.

The method of making a heat capacity determination is described in the following section. All heat capacity calculations are made to 25°C. by assuming that the specific heat of the gel remained constant over the range from 0°C. to 25°C.

Upon the completion of a set of runs on a gel, the container was opened and the concentration of the sample checked.

(b) Procedure for concentrated gels (58 to 67% gelatin). The water-soaked sheets of gelatin were dried between strips of filter paper to remove traces of water adhering to the surface. Samples for analysis were taken by cutting sections from various parts of these sheets. The sheets were then pressed tightly into the container and the cover placed into position. The analyses for the water content were carried out exactly as described above. After screwing the cover of the container tightly into place, it was placed in a closely fitting copper tube (described below) and heated for about half an hour at 50 to 60°C. It was then dried and weighed in order to obtain the weight of the sample.

(c) Procedure for the 87.5% gel.

The gelatin as it existed in storage was used to give this gel concentration. The method of filling the container has already been pointed out (p. 56).

Since the gelatin had been in storage for some time another determination of its moisture content was made. An average value of 87.5% gelatin was obtained.

(d) Procedure for dry gelatin.

The dry gelatin prepared as described in Section VII was packed into the container and the whole then placed in an oven at 105°C. for about 6 hours in order to remove any traces of moisture which might have been picked up during this process.

XI Method of making a determination.

It has already been mentioned that (p. 34) the gels were first of all rapidly frozen in a solid carbon-dioxide-ether mixture. This procedure was adopted for all runs at temperatures between -78.5°C. and zero, but was omitted for the zero runs. In the case of runs at liquid air temperature or at -130°C., initial freezing at -78.5°C. was unnecessary since, according to Moran (28), freezing at these temperatures should produce the same effect as freezing at -78.5°C. After freezing at -78.5°C. the gel was transferred to the constant temperature bath (Sect. V) where it was kept for about 1.5 hours.

The container (plus contents) was prepared for immersion into the constant temperature bath by suspending it from a cork by a silk thread of appropiate length and placing it in a closely fitting copper tube which was closed at one end and which had been dried thoroughly. The cork was sealed securely to the tube by means of adhesive tape to prevent moisture or ether creeping in. A piece of cotton wool was also introduced into the tube above the container as an additional precaution against this. The arrangement is shown in Fig. IV.

The copper tube was immersed in the carbon dioxide-ether mixture for 10 to 15 mins. During this time the constant temperature bath was prepared. The

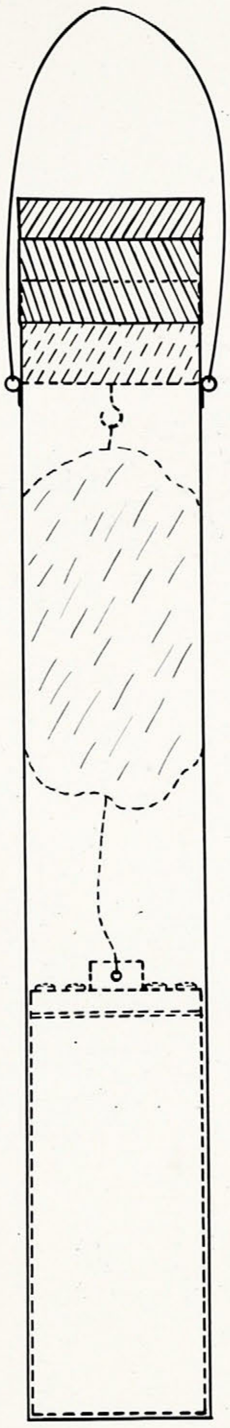

FIG. IV

ether was cooled to approximately the temperature desired by dropping in lumps of solid carbon dioxide. The current of dry air for stirring was then turned on.

When the preliminary freezing of the gel was completed, the copper tube was transferred to the constant temperature bath. Some time was allowed for temperature equilibrium to be reached and then the temperature of the bath was kept at a constant value in the manner already described (p. 44).

During this process the calorimeter was prepared and put into operation. The inner calorimeter vessel was dried and about 800 ccs. of water weighed into it. The flange and collar of the cover were greased with vaseline. The wires for the lead stirrer were pulled through the chimneys I, I(Fig. I) and the cover then placed in position. The calorimeter vessel was placed into position in the jacket as shown in Fig. I. Vaseline was smeared over the flange of the cover of the jacket, which was then screwed into place. The large chimney J was closed with a rubber stopper.

The outer bath was next filled with water and both the inner and outer stirrers set in operation. Hot water was added to the outer bath until its temperature was slightly greater than that of the inner one. It was kept at this point until about 30 min. previous to transferring the container.

At the appointed time the temperature of the outer bath was adjusted until the galvanometer registered zero deflection. Since the working of the inner stirrer produces a continuous heating effect in the inner bath, a steady stream of hot water from a adjustable jet was allowed to run into the outer one in order to maintain the two at the same temperature. When this stream had been adjusted so that a steady state was reached, temperatures registered on the Beckmann thermometer in the outer bath were recorded. These were taken at 5 min. intervals over a period of about 20 mins.

The container was next transferred from the constant temperature bath into the calorimeter. This was done as rapidly as possible by removing the adhesive tape from the copper tube and lifting the container out by the cork. The cotton wool fell away when the container was removed. The actual transfer took but a few seconds. The time at which it was effected was noted exactly.

Immediately the container was introduced into the calorimeter, ice-cold water was run into the outer bath through a very large jet until the galvanometer deflection was again zero. This required less than 1 min. From this point on the more gradual fall in temperature was followed by running cold water into the outer bath through an adjustable jet and one slightly larger controlled by a pinch clamp. In this way the galvanometer deflection could be kept at zero.

About 20 min. was required for equilibrium to be reached. At the end of this time temperature readings on the Beckmann thermometer were again recorded for about 20 to 30 mins. until a uniform rise in temperature was observed.

At the end of the run the container was removed, dried with a clean towel and placed in a desiccator over calcium chloride until the next run.

The weight of the container was checked after the first run with any one sample and thereafter at frequent intervals to be sure no water was creeping in.

An example of the method of calculation is described in Section XIII.

XII The water equivalent of the calorimeter.

This was obtained by calculation. All sundry parts and supports of the inner calorimeter vessel as well as the vessel and cover were taken into account. In certain cases approximations were made according to the procedure outlined by Barnes (81,87). Thus, for example, only one-half the length of the fine copper wires supporting the lead stirrer in the inner bath was taken into account. The same approximation was used in the case of the bakelite posts and copper studs which formed the three legs of the inner calorimeter vessel. In the case of the bakelite chimneys I, I, and J, only the heat capacity of those portions given by one-half the length of the chimney from the top of the cover of the inner vessel to the bottom of the cover of the outer jacket was used. Added to this was a small amount due to those parts of the chimneys projecting below the top of the cover of the inner vessel.

The various parts were weighed on an analytical balance and the following specific heats were used: copper, 0.0923 (88); bakelite, 0.339 (89); lead, 0.0305 (90).

Unfortunately the original chimney J was accidently broken. It was found necessary to have a new one of this particular size made to order, so a chimney made of celluloid was used temporarily. This was made from a strip of celluloid by rolling it into the form of a cylinder and sealing the ends and edges with a paste made by dissolving celluloid in acetone. This same paste was used to attach the chimney to the cover of the inner vessel. An approximation was made for the heat capacity of part of this chimney in the same way as mentioned above. The specific heat of celluloid was taken as 0.36 (91).

When the new bakelite chimney was obtained it was found that, in order to make it fit snugly into the hole provided for it in the cover of the inner vessel, a copper collar was necessary. This collar fitted snugly around the chimney and could be screwed into the hole in the cover of the vessel.

In this final form the water equivalent of the calorimeter was calculated to be 43.14 cals.

XIII A sample calculation.

The method of calculating the result of a run is illustrated by the following figures for a 20% gel at -134.1°C.

The following data were recorded:

Wt. of container + gel = 147.4488 gm.

Wt. of inner calorimeter + water = 1090 gm.
Wt. of inner calorimeter = 247 '
Wt. of water = 843 '

Temperature of constant temperature bath = -134.1°C.

The following readings on the Beckmann thermometer were recorded at the times indicated.

Time in mins.	Beckmann readings.
0	4.640
5	4.643
10	4.646
Transfer effected at exactly 12 mins.	
20	.813
25	.767
30	.770
35	.773
40	.776

These readings were plotted (Fig. V) and the straight lines obtained extrapolated to cut the time axis at 12 mins. Temperatures were read from this time axis and the difference gave the fall in temperature of the water in the inner vessel if the transfer of heat into the container and contents had been instantaneous and there

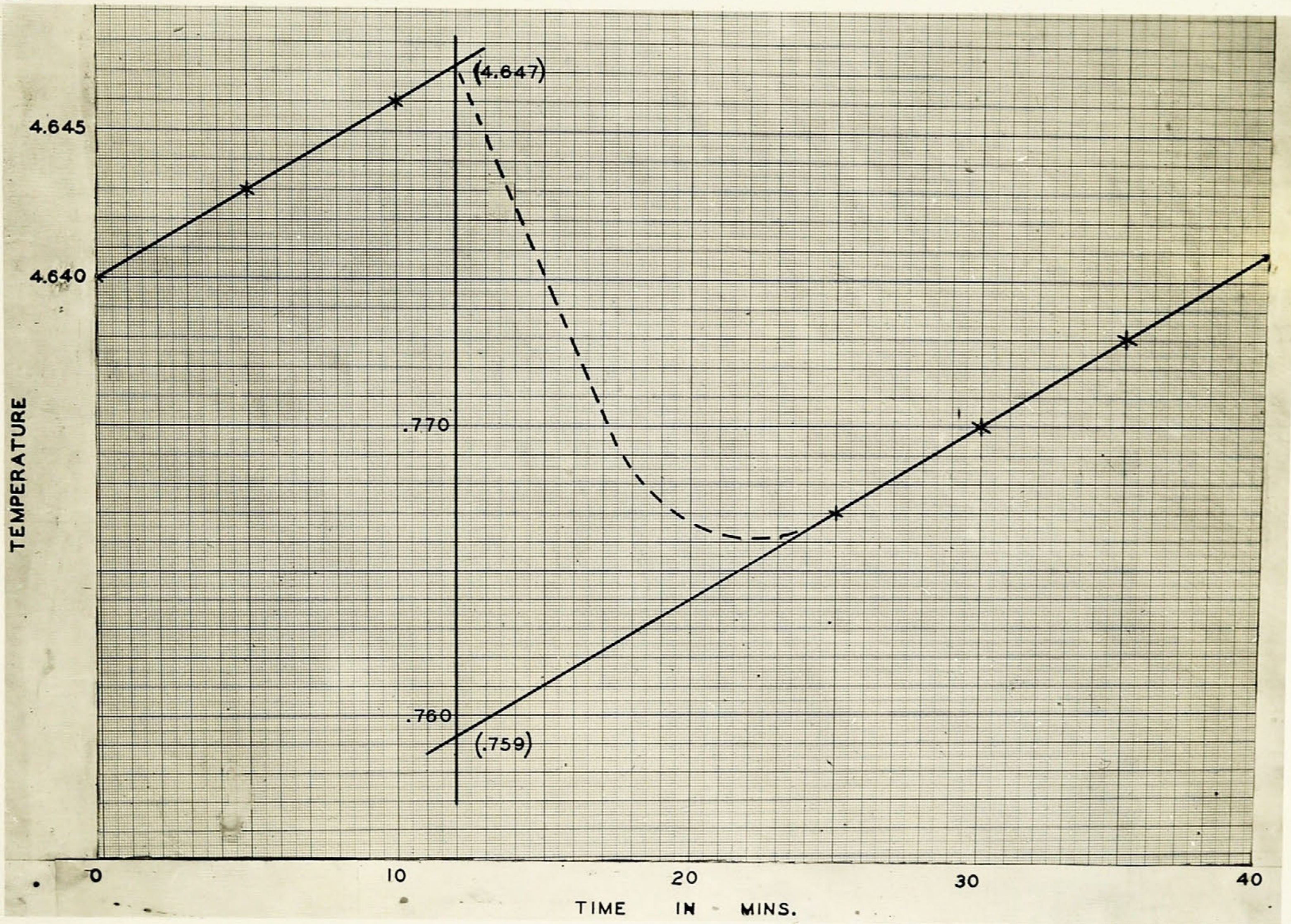
4.645
4.640
TEMPERATURE
(4.647)
.770
.760
(.759)
0
10
20
30
40
TIME IN MINS.

FIG. V

had been no heating effect due to the action of the inner stirrer. These values give the initial and final temperatures.

Initial temperature = 4.647
Correction = -0.002
Corrected temperature = 4.645

Final temperature = .759
Correction = -.001
Corrected temperature = .758

Temperature difference = 4.645 - .758

= 3.887°

Final temperature of calorimeter = 23.65 + .76

= 24.41°C.

Wt. of water in inner vessel = 843
Correction to brass weights in vacuo = .89
Corrected weight = 843.89

Water equivalent of calorimeter = 43.14

Hence total heat capacity (H) to 24.41°C.

$$= (843.89 + 43.14) \times 3.887 \qquad (1)$$

Average H for container plus contents between 0.0°C. and 25.0°C. = 604.3 cals.

Hence H per degree over this range $= \frac{604.3}{25}$

= 24.17 cals.

It will be seen that the number of calories which have to be added to the total H given by equation 1 to give the total H calculated to 25.0°C. is given by:

$$24.17 \times (25.00 - 24.41) \text{ cals.}$$

Hence the total H to 25.0°C. is given by:

$$(843.89 + 43.14) \times 3.887 + (24.17 \times .59)$$

$$= \underline{3455.4 \text{ cals.}}$$

RESULTS

Heat capacity measurements have been made upon gelatin gels containing 9, 20, 45, 58, 61, 67, 88 and 100% gelatin over the temperature range from -183°C. to 25°C. and upon a 24% gel over the range from -78.5°C. to 25°C. The experimental values for the total heat capacity of the container plus gel are given in Tables VIII to XVI. At the top of each table are given the exact concentration and the weight of the sample of gel used in each case. Table XVII gives the values obtained for the heat capacity of the container alone over the whole temperature range of the experiments. The values for the 24% gel in Table X represent the average of at least two determinations. All the values recorded for the other gels except those at 0.0°C. are single determinations. The values at 0.0°C. are also the average of two or more determinations. Experiments were repeated only when the behaviour of the apparatus was erratic and gave reason to doubt the accuracy of the result, or when the transfer of the container from the constant temperature bath into the calorimeter was not very successful. All these values were plotted and gave smooth curves as is shown in Fig. VI. The curves for the 24% and 67% gels have been omitted to avoid confusion.

From these curves the values for the heat capacity of each of the gels at different temperatures were obtained by subtracting the value for the container at the corresponding temperature. The weight of the sample of gel being known in each case, the heat capacity per gm. of gel at different temperatures was calculated. These calculated values are given in Tables XVIII to XXVI. and are plotted in Fig. VII (excepting the 24% gel). All values of the heat capacity used in subsequent calculations were read from these smooth curves.

TABLE VIII

Total Heat Capacity of Container + 9% Gel

Wt. sample = 12.9730 gm. Concn. = 9.0% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 647.3
- 1.0	-1471.6
- 4.8	-1600.0
- 10.0	-1712.5
- 18.0	-1874.2
- 28.9	-2079.9
- 52.6	-2514.8
- 78.5	-2968.5
-124.8	-3673.2
-193.2	-4441.8

TABLE IX

Total Heat Capacity of Container + 20% Gel

Wt. sample = 11.9608 gm.   Concn. = 20.9% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 604.3
- 1.2	-1113.0
- 5.0	-1306.5
- 22.6	-1668.1
- 10.7	-1438.8
- 51.7	-2183.8
- 78.5	-2651.5
-134.1	-3455.4
-191.2	-4068.8

TABLE X

Total Heat Capacity of Container + 24% Gel

Wt. sample = 12.2848 gm.   Concn. = 24.0% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+25.0	0.0
0.0	- 607.0
- 0.6	- 839.3
- 0.6	-1008.1
- 0.8	-1087.4
- 1.3	-1129.1
- 5.3	-1293.6
-15.2	-1516.7
-27.9	-1760.0
-38.3	-1941.5
-44.5	-2062.7
-54.2	-2234.2
-63.8	-2397.5
-78.5	-2645.6

TABLE XI

Total Heat Capacity of Container + 45% Gel

Wt. sample = 15.1890 gm. Concn. = 45.3% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 636.9
- 1.1	- 705.5
- 3.2	- 902.2
- 6.1	-1062.8
- 11.6	-1212.4
- 25.6	-1510.9
- 36.3	-1728.7
- 45.0	-1884.9
- 53.9	-2047.1
- 78.5	-2490.5
-123.6	-3213.8
-184.7	-3926.1

TABLE XII

Total Heat Capacity of Container + 58% Gel

Wt. sample = 9.1576 gm. Concn. = 58.1% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 482.3
- 2.2	- 526.3
- 3.0	- 544.1
- 5.0	- 596.0
- 8.5	- 694.5
- 12.0	- 776.3
- 25.6	-1011.4
- 26.1	-1022.7
- 47.4	-1379.6
- 78.5	-1882.8
-127.0	-2540.7
-184.3	-3128.0

TABLE XIII

Total Heat Capacity of Container + 61% Gel

Wt. sample = 8.4173 gm. Concn. = 61.4% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 459.3
- 2.1	- 493.6
- 4.0	- 533.6
- 5.0	- 571.6
- 9.4	- 672.3
- 15.0	- 780.8
- 32.8	-1075.1
- 55.4	-1437.8
- 78.5	-1798.7
-130.4	-2494.9
-183.9	-3041.1

TABLE XIV

Total Heat Capacity of Container + 67% Gel

Wt. sample = 10.0525 gm. Concn. = 67.7% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 501.4
- 4.9	- 587.5
- 5.2	- 599.5
- 9.2	- 671.0
- 9.4	- 672.0
- 23.3	- 928.5
- 39.5	-1221.5
- 78.5	-1849.9
-123.6	-2467.4
-184.1	-3113.0

TABLE XV

Total Heat Capacity of Container + 88% Gel

Wt. sample = 9.7415 gm. Concn. = 87.5% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 414.4
- 29.2	- 900.1
- 30.5	- 905.8
- 52.4	-1238.6
- 78.5	-1613.6
-129.6	-2263.2
-183.5	-2781.6

TABLE XVI

Total Heat Capacity of Container + Dry Gelatin

Wt. sample = 7.6590 gm. Concn. = 100.0% gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 389.5
- 20.2	- 687.0
- 34.9	- 909.4
- 56.2	-1207.2
- 78.5	-1523.8
-131.6	-2166.6
-184.5	-2678.0

TABLE XVII

Heat Capacity of Container

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.0
0.0	- 335.6
- 26.7	- 686.4
- 57.8	-1077.9
- 78.5	-1339.5
-132.6	-1925.5
-184.2	-2371.7

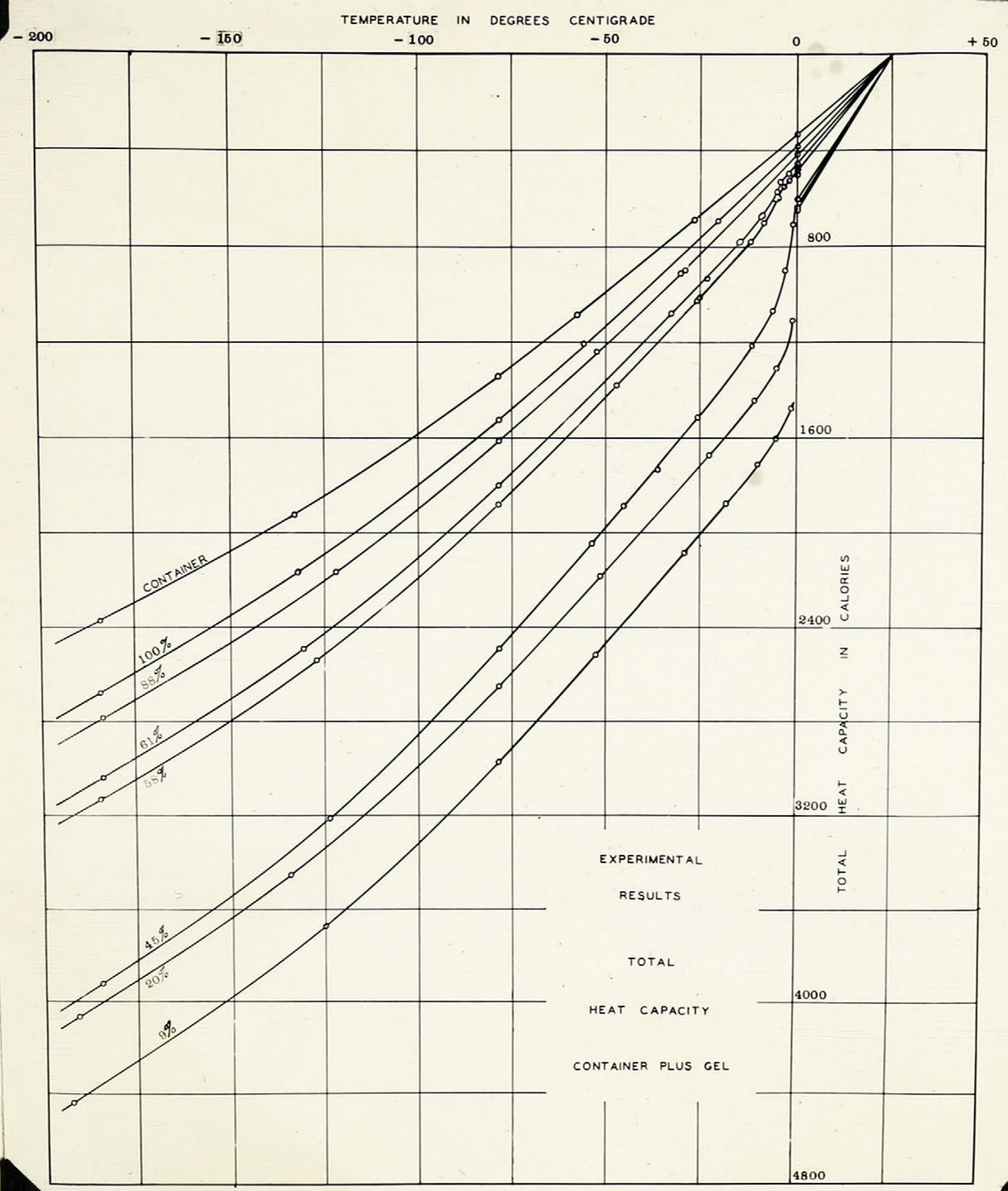
TEMPERATURE IN DEGREES CENTIGRADE
- 200
- 150
- 100
- 50
0
+ 50
800
1600
2400
3200
4000
4800
TOTAL HEAT CAPACITY IN CALORIES
CONTAINER
100%
88%
61%
58%
45%
20%
9%
EXPERIMENTAL
RESULTS
TOTAL
HEAT CAPACITY
CONTAINER PLUS GEL

FIG. VI

TABLE XVIII

Heat Capacity per Gram of 9% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 24.05
- 1.0	- 86.64
- 2.0	- 88.96
- 3.0	- 90.65
- 5.0	- 92.73
- 10.0	- 95.97
- 20.0	-101.3
- 40.0	-110.0
- 80.0	-126.3
-120.0	-139.2
-180.0	-151.5

TABLE XIX

Heat Capacity per Gram of 20% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 22.51
- 1.2	- 63.56
- 2.0	- 68.22
- 3.0	- 71.48
- 5.0	- 75.66
- 10.0	- 80.18
- 20.0	- 85.36
- 40.0	- 93.80
- 80.0	-110.4
-120.0	-123.0
-180.0	-135.4

TABLE XX

Heat Capacity per Gram of 24% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 22.09
- 0.6	- 40.40
- 0.6	- 54.14
- 1.0	- 61.54
- 3.0	- 68.54
- 5.0	- 71.88
- 10.0	- 76.38
- 20.0	- 82.50
- 40.0	- 91.49
- 60.0	- 99.84
- 78.5	-106.3

TABLE XXI

Heat Capacity per Gram of 45% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 19.88
- 1.0	- 22.65
- 2.0	- 27.78
- 3.0	- 33.38
- 5.0	- 40.42
- 10.0	- 46.68
- 20.0	- 52.34
- 40.0	- 61.49
- 80.0	- 76.50
-120.0	- 89.60
-180.0	-101.4

TABLE XXII

Heat Capacity per Gram of 58% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	-16.08
- 3.0	-18.45
- 5.0	-21.18
- 10.0	-29.16
- 20.0	-34.73
- 40.0	-43.35
- 80.0	-60.28
-120.0	-71.74
-180.0	-81.90

TABLE XXIII

Heat Capacity per Gram of 61% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	-14.77
- 3.0	-16.16
- 5.0	-20.20
- 10.0	-25.66
- 20.0	-31.36
- 40.0	-39.92
- 80.0	-55.36
-120.0	-67.95
-180.0	-79.24

TABLE XXIV

Heat Capacity per Gram of 67% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	-15.92
- 10.0	-21.39
- 40.0	-37.00
- 80.0	-51.53
-120.0	-61.87
-180.0	-73.31

TABLE XXV

Heat Capacity per Gram of 88% Gel

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 8.15
- 10.0	-11.29
- 40.0	-19.81
- 80.0	-28.54
-120.0	-36.03
-180.0	-42.29

TABLE XXVI

Heat Capacity per Gram of Dry Gelatin

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.00
0.0	- 7.12
- 10.0	- 9.27
- 40.0	-15.67
- 80.0	-24.42
-120.0	-30.94
-180.0	-39.17

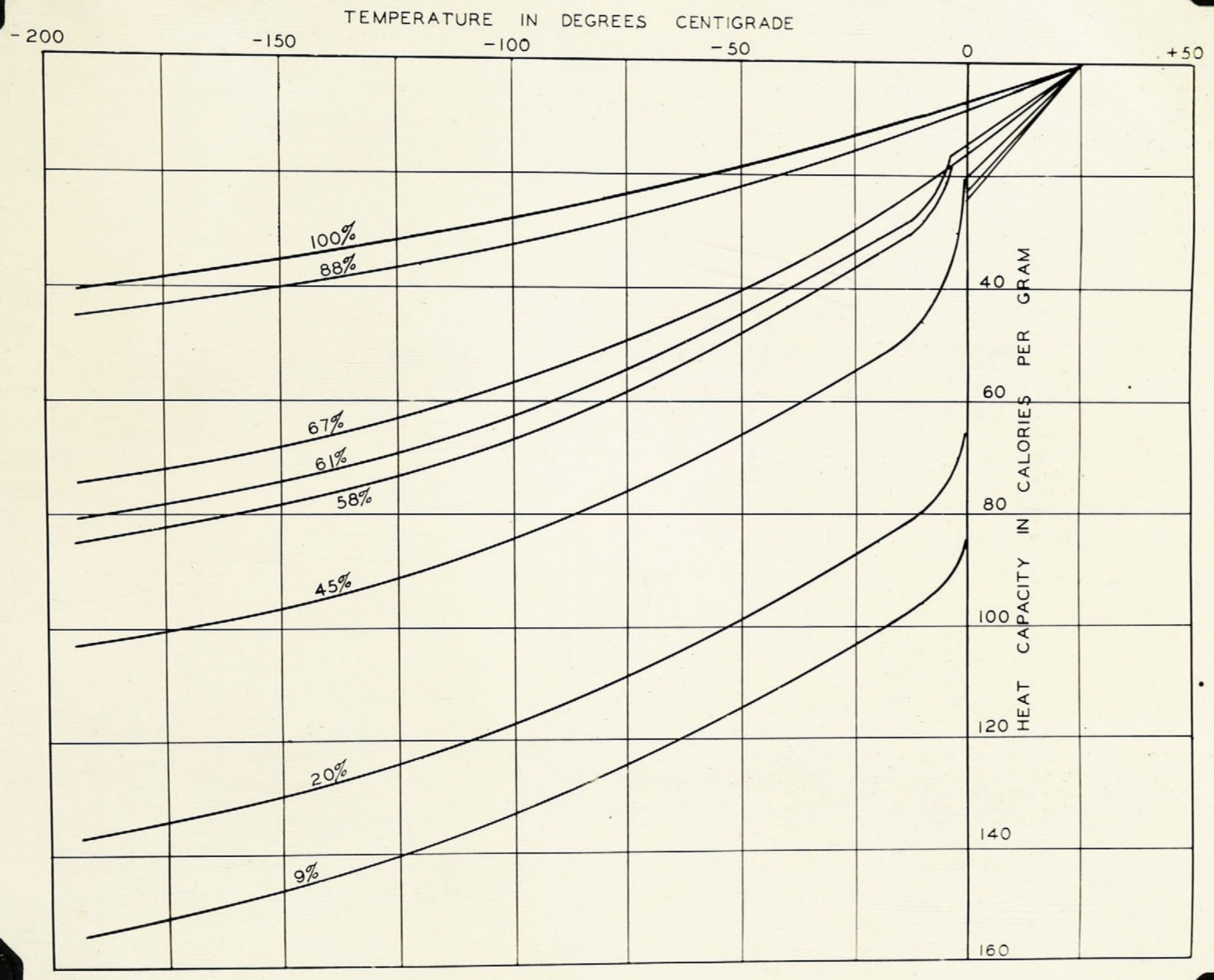
TEMPERATURE IN DEGREES CENTIGRADE
-200
-150
-100
-50
0
+50
HEAT CAPACITY IN CALORIES PER GRAM
40
60
80
100
120
140
160
100%
88%
67%
61%
58%
45%
20%
9%

FIG. VII

DISCUSSION OF RESULTS

I Freezing in gels.

From the general appearance of the heat capacity curves in Fig. VII it is evident that freezing occurs in gels up to and including 61% gelatin. This is indicated by the fact that, at some temperature just below zero, there is a definite break in the heat capacity curve for each of these gels. The absence of such a break in the cases of the 67 and 88% gels apparently means that, in these, there is no freezing at any temperature.

It should be possible to detect the formation of ice in gelatin gels at low temperatures by means of X-ray photographs of the gels. Several photographs of samples of gels at -78.5°C. were taken through the courtesy of Dr. W. H. Barnes and Mr. A. V. Wendling. In the photograph of a 60% gel at this temperature, lines were found which correspond to those of ice. In the case of a 70% gel, however, under the same conditions, only those lines given by gelatin could be detected. It is unlikely that the ice lines in the case of the more dilute gel are due to moisture condensed on the surface of the sample because no such lines appeared with the

70% sample although the time of exposure (about 3 hrs.) was the same in each case. These results are in agreement with those deduced from the heat capacity curves. However, they cannot be considered as definite proof that there is no freezing in a 70% gel. It is possible that, in this gel, any quantity of ice formed would be too small to show up in the photograph.

The sharp break near zero in the curves for the 9, 20, and 45% gels is evidently due to the latent heat of fusion of the water which is frozen in these gels. Between about -1° and -25°C. the direction of these curves changes very rapidly. This gradual change in direction is observed in all cases up to 61% gelatin. Evidently with gels up to this concentration there is a gradual freezing of water taking place between these temperature limits. The amount of water frozen appears to be some function of the temperature. The curves are similar to those which would be obtained with a solution of a crystalloid of concentration less than that given by the eutectic composition over the range between the freezing point and the eutectic temperature (92). One cannot conclude, however, that the mechanism of the freezing point lowering is the same in the two cases.

In the gel the interstitial water is apparently held by forces which vary in intensity, so that the amount of this water which is frozen at a given temperature depends on how much of it can be freed from these forces at that temperature. It is well known that when gelatin swells in water there is an enormous contraction in the total volume of gelatin and imbibed water (22, 23, 24). To cause such a contraction in the volume of the water alone would require a tremendous pressure. It is possible, therefore, that the forces acting upon the water are pressure forces. That water which is under greatest pressure would freeze only at the lower temperatures and the temperature at which any portion of the water could be frozen would be dependent on the pressure acting upon it.

We may distinguish three possible states in which the water in dilute gels may exist. Firstly there is that water which cannot be frozen out of the gel even at temperatures as low as -180°C. This water may be chemically combined with the gelatin or, at least, must be very strongly adsorbed. Then there is water which is held in the colloidal micelle by forces of varying intensity which permit it to freeze out gradually as the temperature is lowered. Finally there is that water which is held within the capillary spaces between the gelatin micelles, its freezing point being lowered by pressure. There is no sharp line of demarcation between these different states, that is, we are dealing not with 'bound' and 'free' water, but with water held in different degrees of 'binding'. Unless indeed we can distinguish definitely a portion of the water as being united stoichiometrically with the gelatin to form a gelatin hydrate.

The behaviour of water in gels on freezing is shown more clearly by calculating the heat capacity at various temperatures of the water associated with 1 gm. of dry gelatin. This is done by multiplying the figures read from the smooth curves of Fig. VII (p. 82) by the weight of gel which contains 1 gm. of dry gelatin, and subtracting the heat capacity of the dry gelatin. The results are shown in Table XXVII and are plotted in Fig. VIII in the form of a family of isothermals in which the weight of water associated with 1 gm. of dry gelatin is plotted against the heat capacity of this water.

Beyond 0.6 gm. water per gm. dry gelatin the isothermals are straight lines. The slope of each gives the heat capacity per gram of the water in excess of

TABLE XXVII

Heat Capacity of Water Associated with 1 gm. Gelatin

% Gel	Wt. Water (gms.)	Heat Capacity(cals.)					
		0°C.	-10°C.	-30°C.	-50°C.	-100°C.	-180°C.
9.0	10.11	260.7	1058.0	1165.2	1257.0	1449.4	1650.2
20.9	3.79	101.2	374.8	418.8	455.8	532.0	609.4
24.0	3.18	85.9	310.4	351.5	382.5		
45.3	1.21	37.4	94.2	111.9	126.4	155.0	186.7
58.1	.72	21.5	41.2	54.3	64.7	84.4	103.9
61.4	.63	17.5	32.9	45.2	54.5	72.5	90.4
67.7	.48	16.6	22.8	34.0	42.6	56.3	70.1
87.5	.14	2.9	4.0	5.8	6.9	8.6	10.3

0.6 gm. per gm. dry gelatin to the corresponding temperature. As shown in Table XXVIII these correspond to the heat capacity per gram of ice (including latent heat of fusion) between the temperatures indicated and 25°C. The figures for the heat capacity of ice were read from the smooth curve obtained by plotting the measurements of Barnes and Maass (80). These go only as low as -78.5°C. and the curve was extended to -180°C. by employing the results of Maass and Waldbauer (93) which agree exactly with those of Barnes and Maass at the higher temperatures.

Below 0.6 gm. water per gm. dry gelatin the points deviate from the straight line. The heat capacity appears to be greater than the amount indicated by the

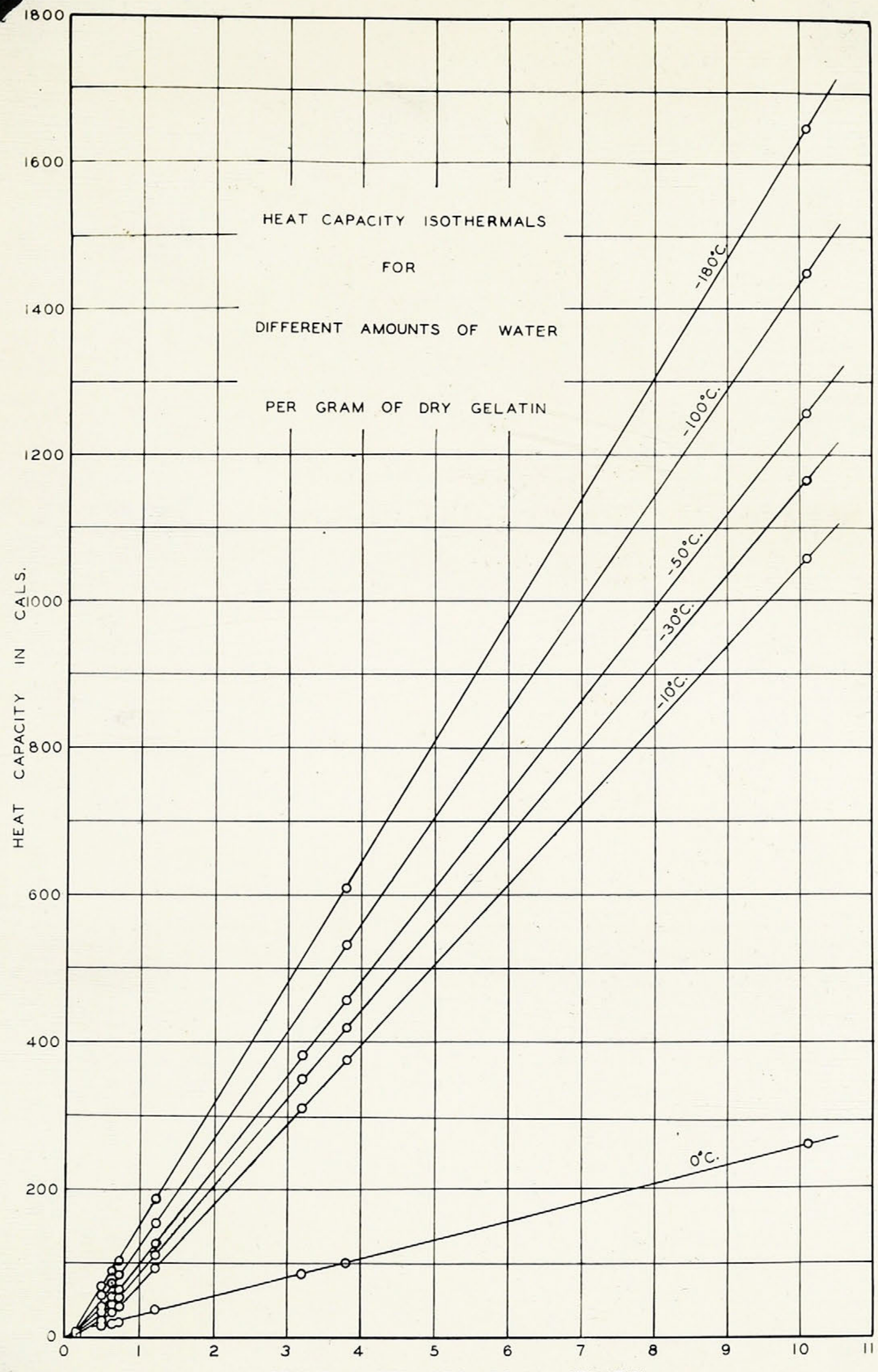
HEAT CAPACITY ISOTHERMALS
FOR
DIFFERENT AMOUNTS OF WATER
PER GRAM OF DRY GELATIN
-180°C.
-100°C.
-50°C.
-30°C.
-10°C.
0°C.
HEAT CAPACITY IN CALS.
0
200
400
600
800
1000
1200
1400
1600
1800
GRAMS WATER PER GRAM GELATIN
0
1
2
3
4
5
6
7
8
9
10
11

FIG. VIII

weight of water present. For reasons which will be made evident later, further measurements on gels containing less than 0.6 gm. water per gm. gelatin are necessary before this anomaly can be interpreted satisfactorily.

TABLE XXVIII

Comparison of Slopes of Isothermals
with
Heat Capacity of Ice

Temperature(°C.)	Slope of Isothermal	Heat Capacity of Ice
0	25.23	25.05
-10	108.3	109.3
-30	118.3	118.6
-50	127.0	127.3
-100	145.3	146.2
-180	164.5	166.3

II The calculation of unfrozen water.

Other investigators who have used the calorimetric method (pp. 24 and 25) have calculated the unfrozen water by more or less modified forms of an equation derived by Thoenes (68).

This equation may be most simply expressed as:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5T_1} \qquad (1)$$

where x is the weight of water frozen at T_1, H is the heat absorbed when the sample is warmed from T_1 to T_2, (signs taken into account), M is the weight of the sample and S its specific heat. The amount of unfrozen water is obtained by subtracting x from the total water present.

The meaning of this equation can be discussed most conveniently by reference to Fig. IX. ABC is a typical heat capacity curve similar to that for the 9% gel shown in Fig. VII (p. 82). The slope of AB gives the heat capacity per degree Centigrade (the quantity MS) of the unfrozen material. Extrapolation of AB to the temperature T_1 gives the point D. The heat capacity corresponding to D is evidently $MS(T_2 - T_1)$. The length of the line CD then represents $H - MS(T_2 - T_1)$. This is the heat liberated if the frozen portion of the water is melted isothermally at T_1. The weight of water frozen is obtained by dividing this quantity by the latent heat of fusion per gram <u>at the temperature T_1</u>.

From the Kirchoff equation (94) for the rate

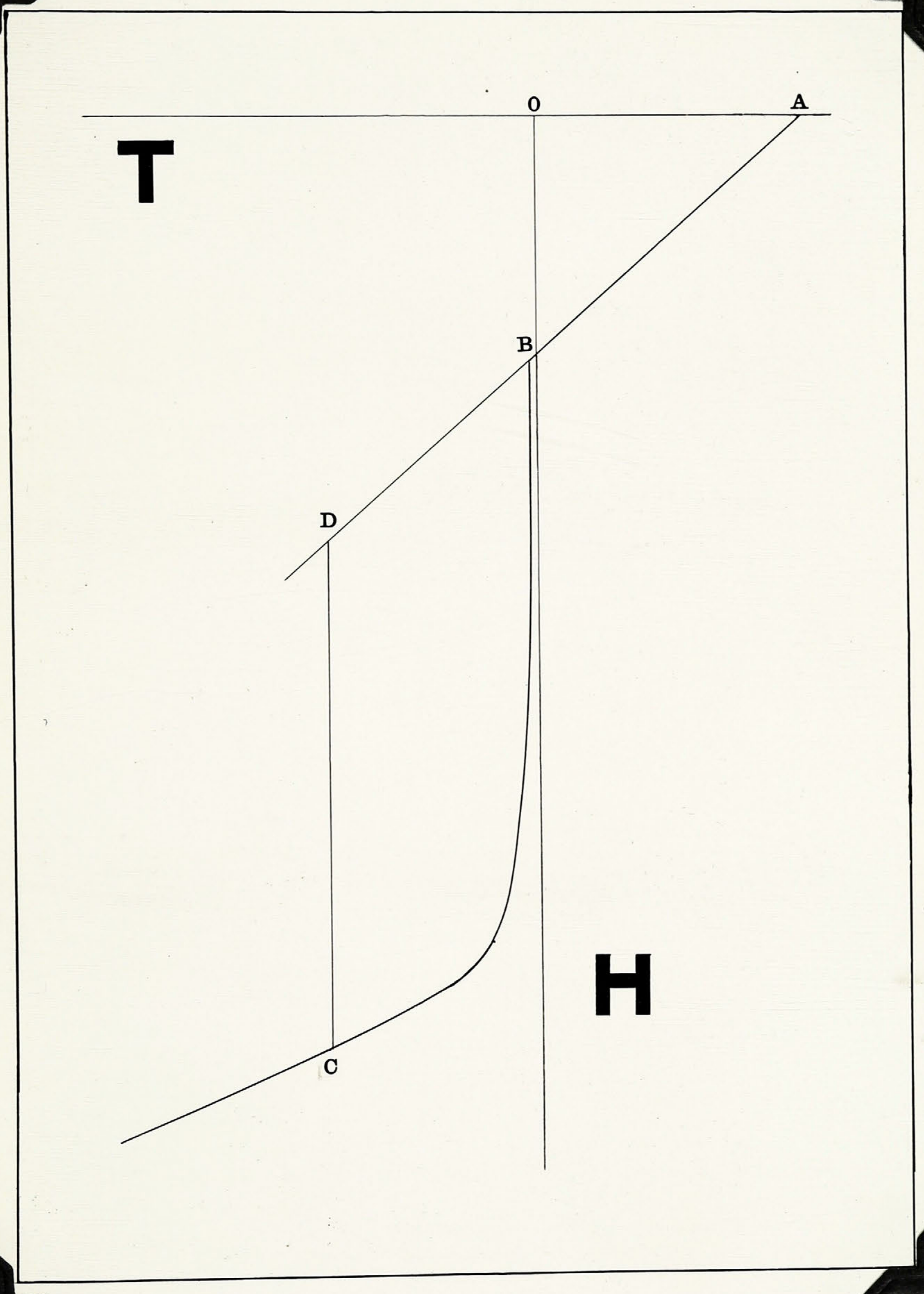

FIG. IX

of change of latent heat with temperature,

$$\frac{dL}{dt} = C_w - C_i ,$$

the latent heat at T_1 is given by:

$$L_{T_1} = L_0 + T_1\frac{dL}{dt}$$

$$= L_0 + T_1(C_w - C_i)$$

where C_w and C_i are the average specific heats between 0°C. and T_1, of water and ice respectively. If C_w is taken as 1 and C_i as 0.5 and L_0 as 80 cals. per gm., then

$$L_{T_1} = 80 + 0.5T_1,$$

corresponding to Thoenes' equation.

St. John (74) derives the equation in a slightly modified form which, using the foregoing notation, would be:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5(T_2 - T_0)} \qquad (2)$$

where T_0 is the observed freezing point of the material. From the above discussion it is evident that this modification is incorrect. The actual error in St. John's derivation was the use of 80 cals. for the heat of fusion at T_0. If L_{T_0} is inserted in place of 80, the equation becomes identical with that of Thoenes.

The equation is an approximation at best. The specific heats of water and of ice are not independent of the temperature. The specific heat curve of water is known only to about -5°C. (95,96,97) and it is questionable how far below that temperature it may legitimately be extrapolated. Extrapolation to -20°C. gives a mean value of 1.03 between 0° and -20°C. Taking the recent accurately determined values of Barnes and Maass (80) for the latent heat and specific heat of ice, the equation becomes:

$$x = \frac{H - MS(T_2 - T_1)}{79.40 + 0.55T_1} \qquad (3)$$

The numerator still depends on the extrapolation of the line AB (Fig. IX) i.e., on assuming a constant specific heat continuing far into the metastable or unstable region below the freezing point. From the appearance of the curves in Fig. VII (p. 82) it seems probable that the extrapolated line should bend slightly toward the temperature axis. If so, the true value of CD would be slightly greater. Since the unfrozen water is found by subtracting the values of x from the total water content and since this difference is usually quite small, a slight error in the value of x may have a considerable effect on the amount of unfrozen water found. Hence the equation gives values for the unfrozen water which are likely to be appreciably high. In any case the extrapolations on which it depends, even if approximately valid as low as -20°C., the initial temperature used by Thoenes, can scarcely be relied on at temperatures any lower.

It is necessary then to find some other basis for the calculation of the unfrozen water, which may be used over the whole temperature range covered by these experiments.

When a gel at a temperature T_1 is warmed to T_2, the free water which is frozen at T_1 is melted and reabsorbed into the gel. The total heat change involved in this process is independent of the path by which the process occurs. It will be the sum of the heat effects which accompany any set of intermediate steps that may be assumed. The mechanism implied in Thoenes' equation is the isothermal melting of the ice and reabsorption of the water at T_1 followed by warming of the gel to T_2. The possibility of a heat effect accompanying the reabsorption of the water is disregarded.

The gel might be assumed to be warmed to 0°C. without melting, the frozen portion of the water to be melted isothermally at 0°C., the whole to be warmed to T_2 and the water reabsorbed isothermally at T_2. The heat capacity, H, of 1 gm. of gel between T_1 and T_2 would then be the sum of the heat effects involved in the individual steps, or:

$$H = aH_g + xH_x + (1 - a - x)H_i - \lambda \qquad (4)$$

In this equation x represents the weight of bound or unfrozen water per gram of gel and a is the weight of dry gelatin. H_g is the heat capacity per gram of dry gelatin, H_x the average heat capacity per gram of bound water and H_i the heat capacity per gram of ice (including latent heat of fusion), all between T_1 and T_2. Total heat capacities rather than specific heats are used in this equation because they are the quantities actually measured experimentally. The quantity λ refers to the heat effect accompanying any change in the gelatin-water relationship in the gel when it is warmed from T_1 to T_2. It appeared that λ should represent heat liberated when $(1 - a - x)$ gm. of water absorbed by a gel which already contains a gm. of dry gelatin and x gm. of water. The value of λ will depend on the value of x. According to Rosenbohm (21) the heat evolved when dry gelatin takes up water is nearly proportional to the amount of water up to about 0.25 to 0.3 gm. water per gm. of dry gelatin. Moran (28,42) estimates the bound water to be 0.53 gm. per gm. dry gelatin and a similar figure was obtained by Mennie (79) from earlier calorimetric measurements, using Thoenes'equation. It was expected that if these estimates could be accepted, the value of λ should be zero or negligible.

This being the case, the amount of bound or unfrozen water should be given by

$$x = \frac{aH_g + (1 - a)H_i - H_{obs.}}{H_i - H_x} \qquad (5)$$

H_g, the heat capacity of the dry gelatin, is obtainable from the data of Table XXVI (p. 81). Values of H_i are given by Barnes and Maass (80) to -78.5°C. and by Maass and Waldbauer (93) to -180°C. It was thought that an estimate of the average heat capacity of bound water could be obtained from measurements on a gel which showed no break in the heat capacity curve, and in which, consequently, no freezing presumably occurred. For instance, subtracting the heat capacity of 0.875 gm. dry gelatin from the heat capacity of the 87.5% gel would give the heat capacity of 0.125 gm. water bound in this gel. It was realized that if there are different stages or degrees of binding, the heat capacity of 1 gm. of bound water in an 87.5% gel would not necessarily be the same as the heat capacity of 1 gm. of water in a more dilute gel. But it was thought that from measurements on a gel containing nearly the maximum amount of non-freezable water should give a value for the average heat capacity of the water which could be applied to the unfrozen portion of the water in more dilute gels.

This was based on the assumption that the water, bound in such a way as to remain unfrozen, would remain similarly bound at the final temperature of the calorimeter, 25°C. A consideration of the specific heats of the gels above the freezing point revealed a flaw in this assumption. The specific heat of the bound portion of the water should be less than 1 since bound water presumably possesses fewer degrees of freedom than does free water. Hence the average specific heat of all the water contained in the gel should be less than 1. This inference was verified for the 87.5% gel, but in the 67% gel and also in the more dilute gels, the average specific heats of the water portion of the gel between 0° and 25°C. proved to be, not less than 1, but greater than 1. This is shown in Table XXIX where the average specific heats given were calculated from the data of Tables XVIII to XXVI (pp. 78 to 81). Evidently the heat capacity at zero

TABLE XXIX

Average Specific Heat of Water in Gels between 0°C. and 25°C.

Per-cent Gel	Average Specific Heat
9.0	1.03
20.9	1.07
45.3	1.24
58.1	1.19
61.4	1.11
67.7	1.18
87.5	0.80

of a gel is greater than the sum of the heat capacities of the water and gelatin. In other words the process of cooling a gel from 25°C. to zero results in some change in the gelatin-water relationship which is accompanied by the evolution of heat.

This change is probably an increased binding of water as the temperature is lowered. That this is Probable has been shown by Neville and his co-workers (23). These investigators have found that the volume contraction of gelatin gels during the process of swelling increases with lowering of the temperature. Such an increased volume contraction apparently means greater hydration or firmer binding. This process would certainly be accompanied by the evolution of heat since the imbibition of water by gelatin is an exothermic process. Upon warming the gel from 0° to 25°C. the converse takes place, namely, the absorption of heat. Hence the measured values for the heat capacities of these gels at 0°C. include this effect and this explains the unexpectedly high values for the specific heat of the water in these gels.

The average specific heat of the water in a 67% gel is found to be greater than 1 down to -80°C. This may be due entirely to the increase in intensity of binding continuing throughout this range of temperature or it may be due to a gradual freezing of part of the water. From the shape of the heat capacity curve (Fig. VII, p. 82) for this gel, it is impossible to state definitely that there is no freezing. Certainly no break occurs. But there is the possibility of a gradual freezing similar to that which appears to take place in the more dilute gels between the temperature where freezing commences and about -25°C.

Some evidence that no freezing occurs was obtained by measuring the heat capacity of this gel at -10°C., first by previous freezing at -78.5°C., and then by cooling slowly to -10° from room temperature when supercooling rather than freezing should occur. All experimental results fell on the smooth curve for this gel. Furthermore, the X-ray photographs taken by Dr. Barnes and Mr. Wendling showed no evidence of freezing at -78.5°C. in a 70% gel.

Another anomalous result of the measurements on the 67% gel is that its heat capacity at 0°C. proves to be greater than that of the 61% gel. This may be seen by reference to Fig. VII and Tables XXIII and XXIV. The following is suggested as a possible explanation. It has already been pointed out that a lowering of the temperature from 25° to 0°C results in increased intensity of binding accompanied by the evolution of heat. The amount of heat evolved during this process will depend upon the intensity of the binding forces brought into play (21). In a 67% gel at 25°C. all the water is presumably more or less bound by the gelatin but as it is cooled to 0°C. an increase in the intensity of binding occurs with the evolution of a considerable amount of heat. If binding is pictured as a surface phenomenon occurring at the boundary of the gelatin particles, it may be assumed that not all the available surface forces are being employed by water molecules at 25°C. As the gel is cooled additional surface forces are brought into play. Now if we assume that when more water is added at 25°C. to form a more dilute gel, these additional surface forces are brought into action at that temperature, it follows that the increase in intensity of binding on cooling the gel to 0°C., and the consequent evolution of heat, will be less than in the case of the more concentrated gel. (Compare Filby and Maass (13), p. 176). This is in accord with the experimental observations.

From the above considerations two conclusions may be drawn with reference to the application of equation (4). In the first place it appears that λ represents, not heat evolved when the melted ice is reabsorbed into the gel, but heat taken up in loosening the binding of water to gelatin as the gel is warmed. The data are insufficient to make an estimate of the value of λ. Rosenbohm's measurements (21) already mentioned, were made with a Bunsen ice calorimeter and hence refer to 0°C. If they are to be relied on, it follows that, provided 0.4 - 0.5 gm. water per gram dry gelatin is firmly bound at 0°C., the thermal effect of the binding of any further amount of water is negligible. As a basis for calculation, the heat capacities between T_1 and 0°C. have been taken. These are obtained from the experimental values for the gels, dry gelatin and ice by subtracting the respective heat capacties between 0° and 25°C.

In the second place, the measurements on the 67% gel do not provide a basis for the estimation of the heat capacity of bound water. Consequently, values of H (T_1 to 0°C.) have been calculated from the 87.5% gel. These are shown in Table XXX. It must be admitted that they represent a small difference between relatively large quantities and hence the experimental error is probably fairly high. Consequently no very great accuracy can be claimed for the calculations of bound (unfrozen) water made by means of equation 5 (p. 96), which are shown in Table XXXI. For the purpose of comparison calculations made according to Thoenes' method using equation 3 (p. 93) are shown in Table XXXII.

TABLE XXX

Heat Capacity per Gram of 'Bound' Water in 87.5% Gel

Temperature(°C.)	Heat Capacity to 0°C.(cals.)
0.0	0.0
- 1.0	- 0.7
- 3.0	- 2.3
- 5.0	- 3.8
- 10.0	- 7.6
- 20.0	-14.7
- 40.0	-25.6
- 60.0	-32.4
- 80.0	-37.2
-100.0	-40.9
-120.0	-44.2
-180.0	-53.0

TABLE XXXI

Amount Water 'Bound' by 1 gm. Gelatin in Gels of Different Concentrations at Different Temperatures

Temp.	Amount 'Bound' Water (gms.)						
(°C.)	9%	20%	24%	45%	58%	61%	67%
- 1	1.42		1.13	1.14			
- 3	1.00	.92	.81	.87			
- 5	.84	.72	.69	.70	.67	.55	
- 10	.72	.59	.57	.59	.54	.49	.44
- 20	.62	.55	.49	.55	.50	.46	.41
- 40	.53	.47	.41	.50	.45	.40	.34
- 80	.48	.37		.40	.34	.30	.24
-120	.47	.32		.33	.28	.24	.21
-180	.45	.30		.24	.22	.18	.16

TABLE XXXII

'Bound' Water Calculated by Equation 3

Temp.	Amount 'Bound' Water (gms.)					
(°C.)	9%	20%	24%	45%	58%	61%
- 3	1.00	.91	.82	.90		
- 5	.89	.72	.70	.72	.65	.59
-10	.78	.62	.61	.66	.57	.52
-20	.67	.62	.56	.68	.58	.52

III Comparison of results with those of other investigators.

Three different points of view are held by Moran (28,42), Jones and Gortner (27) and Briggs (6) as to the freezing of water in gels. Moran concludes from his observations that, at -20°C., all the water in the gel which can be frozen is converted into ice and that further lowering of the temperature produces no further effect. In other words the amount of water frozen is constant below -20°C. Jones and Gortner have concluded from their dilatometric measurements (p. 24) that the amount of water remaining unfrozen increases with the total water present per gram of dry gelatin, but that it is independent of the temperature once freezing is complete. Their results have been shown in Table IV (p. 32). Briggs, on the other hand, maintains that the amount of water associated with, or bound by, a given quantity of colloid is determined by the activity of the water, and hence that the amount of bound (unfrozen) water should depend on the temperature of the frozen material but should be independent of the gel concentration.

It is evident from the results of the present investigation given in Table XXXI, that the amount of water in the gel, which possesses the same heat capacity as the water in an 88% gel, is not constant. It varies with both the temperature and the gel concentration. If the water in the 88% gel is described as bound, which presumably it is, then the amount of bound water decreases with lowering of temperature in a gel of any given concentration. It appears however, especially in the dilute gels, to reach a fairly constant value between -80° and -180°C. The variation of the amount of bound water with concentration at any one temperature appears to be that of a decrease with decrease in amount of water present, in qualitative agreement with the results of Jones and Gortner.

IV Suggestions for further work.

It is evident from the preceding discussion upon the calculation of unfrozen water that an evaluation of λ in equation 4 (p. 95) is desirable. This would involve making more extensive and more accurate determinations of the heat of swelling of gelatin gels than those given by Rosenbohm (21). As already mentioned Rosenbohm's measurements were made with a Bunsen ice calorimeter and refer only to one temperature, 0°C. An estimate of the temperature coefficient of the heat of swelling from measurements at various temperatures would be of great interest. It is probable that measurements such as these would throw some light upon the anomalous behaviour of the 67% gel when it is cooled from 25° to 0°C. The rotating adiabatic calorimeter designed by Lipsett, Johnson and Maass (98,99) for measuring surface energies and heats of solution would be very suitable for these investigations.

The influence of the rate of freezing on the amount of water unfrozen should be investigated. All the results in the present work are for gels which are rapidly frozen at -78.5°C. previous to making a determination. A similar investigation using slow freezing as employed by Moran (28) should afford an interesting comparison. The effect of pH upon the amount of water frozen could also be studied by the method of investigation outlined in the present work.

Further X-ray analyses of frozen gels might be made to yield interesting information regarding the nature of bound water.

Finally there remains heat capacity measurements upon inelastic gels such as silica. These have been investigated by the dilatometer method (19,20,27) but no calorimetric measurements appear to have been done on them.

APPENDIX

THE SPECIFIC HEAT OF MONEL METAL BETWEEN -183° AND 25° C.

From the values for the heat capacity of the container given in Table XVII (p. 76) a calculation of the specific heat of Monel metal has been made over the temperature range from -183°C. to 25°C. The container is made entirely of Monel metal except for a thin lead washer placed underneath the cover. Subtracting from the value for the heat capacity of the container at any temperature that portion due to the lead washer gives the heat capacity for the weight of Monel metal in the container. From this, the heat capacity per gram of the metal is obtained. All values for the heat capacity of the container used in these calculations were read from the smooth curve obtained upon plotting the experimental values given in Table XVII. The weight of the lead washer was 3.0309 gm. and for the purpose of calculation the value 0.0300 obtained by Richards and Jackson (100) for the average specific heat of lead between -180° and

20°C.,;was used. The weight of Monel metal was 132.9050 gm.

Values for the heat capacity per gram of Monel metal calculated for different temperatures as outlined above are given in Table XXXIII. Upon plotting these values a smooth curve was obtained and from this curve the equation for the heat capacity of Monel metal was found to be:

$$H = -2.503 + 0.09948t + 0.00001684t^2 - 0.0000004165t^3$$

Differentiation of this equation leads to the following expression for the specific heat of Monel metal over the temperature range from -180° to 25°C.

$$C_p = 0.09948 + 0.00003368t - 0.000001250t^2$$

Values for the specific heat calculated from this equation are given in Table XXXIV. They are found to be in good agreement with tangents drawn to the heat capacity curve.

Unfortunately no sample of the Monel metal used in the container was available for analysis and its exact composition is not known. A typical analysis of Monel metal, supplied by the International Nickel Company, is as follows: nickel, 68%; copper, 29%; iron, 1.45%; carbon, 0.13%; manganese, 1.04%; silicon, 0.03%; sulphur, 0.008%. Taking the specific heats at 20°C. (101) of nickel as 0.1064, copper 0.0915, iron 0.1078, manganese 0.1211, the value calculated from the above analysis for the specific heat of Monel metal at 20°C. is 0.1017, assuming that the specific heats are strictly additive. This is in reasonably good agreement with the value here found, 0.0997. The average specific heat of Monel metal between 20°C. and 1270°C. has been reported as 0.128 calories per gram (102).

The results for the specific heats at the lower temperatures are submitted with some reserve. The method of carrying out a heat capacity determination involves the transfer of the container from the thermostat into the calorimeter. Ordinarily this process takes less than three seconds and when the temperature is not too low the heat loss during transfer can be neglected. At lower temperatures however, as Barnes and Maass (80) have pointed out, it is very unlikely that the heat loss during transfer is negligible owing to the large difference between the temperature of the container and that of the surrounding air. Moreover, rapid manipulation is more difficult at very low temperatures. Consequently the values given for the heat capacity at liquid air temperatures are probably somewhat too small.

It may be mentioned that this source of error is of much less importance in the measurement of the heat capacity of materials enclosed within the container. The latter involves the subtraction of the heat capacity of the container at a given temperature from that of the container and contents at the same temperature. Since the heat losses involved in the transfer in each case are small and very nearly equal they tend to cancel.

TABLE XXXIII

Heat Capacity per Gram of Monel Metal

Temperature(°C.)	Heat Capacity(cals.)
+ 25.0	0.000
0.0	- 2.503
- 40.0	- 6.412
- 80.0	-10.143
-120.0	-13.461
-140.0	-14.899
-160.0	-16.200
-180.0	-17.434

TABLE XXXIV

Specific Heat of Monel Metal

Temperature(°C.)	C_p
+ 20.0	0.0997
- 10.0	0.0990
- 40.0	0.0962
- 70.0	0.0910
-100.0	0.0836
-130.0	0.0740
-160.0	0.0621

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