OSMOTIC RELATIONSHIPS IN THE HEN'S EGG, AS DETERMINED BY COLLIGATIVE PROPERTIES OF YOLK AND WHITE

By EVELYN HOWARD

(From the Physiological Laboratory, School of Medicine, The Johns Hopkins University, Baltimore)

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The osmotic relationships between the yolk and white of the hen's egg have recently aroused considerable interest because of the fundamental bearing of the observed results on the general problem of cellular energetics. Straub (25) first called attention to the fact that to maintain the observed freezing point difference between the yolk and white, the yolk must in some way produce an osmotic pressure difference of nearly 2 atmospheres, since, in spite of the difference in freezing points, the yolk membrane is freely permeable to water. This delicate membrane could obviously withstand no such mechanical pressure, so that it was assumed that the difference in pressure must be maintained by the constant application of metabolic energy at the membrane in some unknown manner. Straub assumed that the cell must derive the required energy from oxidative processes at the cell membrane. The problem of how such a steady state might be maintained would be of great interest in view of its bearing on water exchanges and secretion in living systems generally.

Hill (10) promptly confirmed Straub's observation of an osmotic pressure difference by means of thermal measurements of vapor pressures. However, Hill found that the observed difference was still present after the eggs had been kept for 29 days in hydrogen, and concluded that hence the source of the energy which maintained this non-equilibrium condition or steady state could not be oxidative in nature.

The question has also been studied in a series of papers by J. and D. Needham, Stephenson, Smith, and Shepherd (18–20, 23, 24). These authors find that without bacterial contamination the anaerobic lactate production of the hen's egg is so low that it would be necessary

to use all of the energy evolved to maintain the supposed osmotic difference. The isolated vitelline membrane showed no tendency to maintain osmotic non-equilibrium when separating saline solutions. From this and other lines of evidence the authors concluded that their results pointed against the idea that thermodynamic work was done by the membrane, and that no evidence could be obtained in favor of the alternative of an impermeability of the vitelline membrane to water.

The actual validity of the observation of an osmotic pressure difference was denied by Grollman (8) who showed that the true freezing point of the egg yolk was not easily obtained, and, finding that dialysates from yolk and white were in osmotic equilibrium, concluded that the observations of Straub and Hill were experimental artifacts and that no osmotic difference actually existed between yolk and white.

Meyerhof (17), on the other hand, on the basis of dialysis experiments, supported the view that an osmotic pressure difference existed; however, his experiments using the vitelline membrane do not agree with those using collodion membranes, but indicate a considerably smaller freezing point depression for the yolk. To explain this he invokes an idea previously used by Needham, that the outer yolk layer had a lower osmotic pressure than the inner. There is no adequate evidence that such a layer exists, and in any case it would be difficult to explain how such a situation might arise.

The disagreement on this important point indicated that a further study would be of interest. Not only are there two views as to the existence of the osmotic pressure difference between yolk and white, but among those who conclude that it exists, there is no agreement as to its significance or as to the method by which it is maintained.

Accordingly the osmotic relations between the yolk and white of the egg have been studied by means of freezing point and vapor pressure determinations, and by dialysis experiments. Certain previously unrecognized sources of error are described. It has been found that there is no osmotic pressure difference between the yolk and white of the hen's egg. This is in accordance with Grollman's observation and, furthermore, explains the failure of Needham and collaborators to detect the performance of any osmotic work by the yolk membrane.

The interest in the supposed action at the yolk membrane lay in the

fact that it appeared to be an easily accessible biological system with a single non-cellular membrane which was analogous to secretory epithelial tissues in being able to use metabolic energy to maintain or create an osmotic pressure difference. Although the egg yolk-white system does not possess this property, many cellular tissues do, and the mechanism of such tissue action remains a problem of fundamental interest.

The Direct Determination of the Freezing Point of Egg Yolk

Straub (25), and previously Bialascewicz (2) and others (22, 24) have reported the freezing point of egg white as -0.43 to -0.46, that of the yolk -0.60 to -0.56°C. None of these authors give any detailed discussion of the technique used in their freezing point determinations, or of the range of experimental error involved. They also all appear to have overlooked a paper by Atkins (1) who gives the freezing point of the hen's egg as -0.454°, and states that "no difference was found in the freezing point of the white and yolk of the same egg, and a mixture of white and yolk gave the same depression." Atkins describes his technique in detail, having worked with a minimum of supercooling and having approached the plateau of the freezing point from both higher and lower temperatures. Furthermore, Grollman, using an electrometric method, reports experiments in freezing the yolk in which he failed to obtain a clear-cut plateau in the temperature-time curve (8) such as should be obtained at the freezing point, and showed that very variable temperature maxima were obtained according to the degree of supercooling. He concluded that due to its high viscosity and low water content the true freezing point of the yolk could not be determined directly by the usual Beckmann technique or by the thermoelectric method which he used.

However, by the use of a technique recently described by Johlin (11) it has been possible to freeze egg yolk and to obtain reproducible plateaus of temperature which can be maintained for 10 to 30 minutes, and coincide with the values similarly obtained for egg white. The method consists essentially of efficient stirring and strictly limited supercooling of not over 0.4°C.

The method, as described by Johlin, was closely adhered to except that in order to make stirring possible about 10 cc. of yolk was used in a larger tube (17 mm.

inside diameter) instead of 1 cc. of solution, as used by him. The apparatus consists essentially of a small tube surrounded by a double air jacket in a cooling bath. Cooling mixture may be sucked up into the inner air jacket and then allowed to recede when the desired cooling has been attained. Seeding and local supercooling are brought about by adding small rings of nichrome wire cooled in solid CO_2 On exposure to air the rings become covered with a layer of frost, which acts as a small amount of finely dispersed centers for ice formation. The amount of water added is negligible, as determined by Johlin, but the local supercooling caused by the cold wire makes these rings a very efficient method of starting ice formation. Stirring was accomplished by moving the thermometer vigorously up and down a distance of a cm. or less. The stirring was more efficient than with the usual Beckmann technique, and this is an advantage for a mixture such as egg yolk which freezes with difficulty.

It has been stated that vigorous stirring may give too high a freezing point, but this could only be so if the temperature plateau was not determined as such. Stirring undoubtedly adds heat to the mixture, but in the presence of ice and solution, addition or loss of heat should change the volume of the ice phase, not the temperature. If an efficient mixing is maintained, a prolonged temperature plateau could only occur at an actual invariant point, unless the rate of heat addition exactly balanced the rate of heat diffusion. Stirring was done by hand and was somewhat irregular, so that it was impossible that the heat added could have balanced the heat lost for any appreciable time and hence a plateau determined over a fair range of time could not have been shifted significantly.

The thermometer zero point was determined in a mixture of ice and water. Determinations on NaCl and KCl solutions checked values given in International Critical Tables to within 0.002°C. The egg white freezes very readily, and no difficulty in obtaining a constant temperature was encountered when using fresh eggs. The yolk is inclined to give deceptive apparent freezing points at various inconstant temperatures below the freezing point of the white, which could account for the results reported by previous investigators. However, by avoiding too great supercooling, with vigorous stirring and repeated seeding constant temperature plateaus may readily be obtained at the freezing point of the white. These plateaus with the yolk check each other within a few thousandths of a degree, whereas the lower pseudoplateaus are not reproducible. Pseudoplateaus do not always occur, sometimes the yolk goes promptly to its true freezing point.

Examples of actual determinations on yolk are shown in Fig. 1.

Eggs were obtained from Tancred hens (a white Leghorn breed), and were used within 20 to 40 hours after laying. Freezing points of the yolks and whites from six eggs are given in Table I. The values for the yolk represent the mean of three plateau values concordant to within a few thousandths of a degree. The plateaus were each fol-

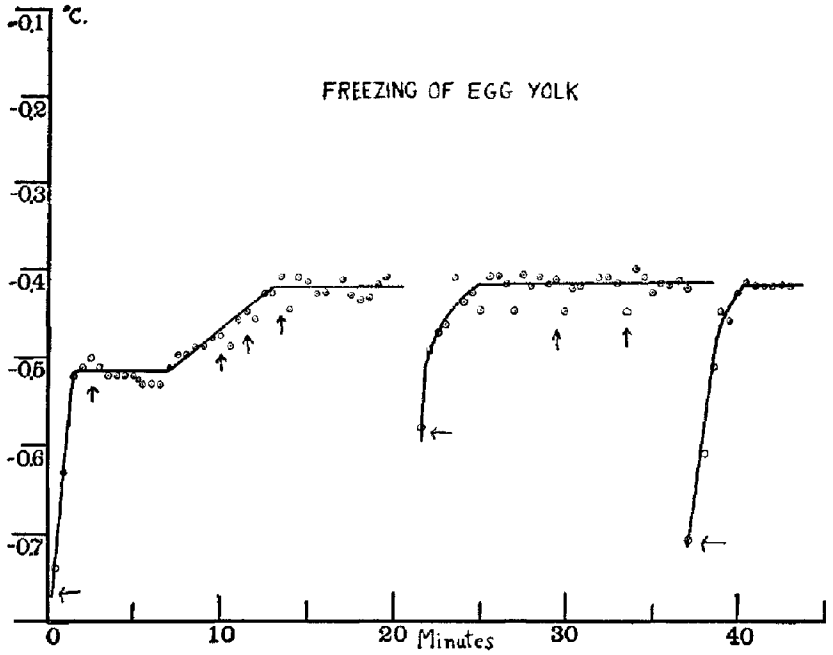

FIG. 1. Typical determination of the freezing point of a sample of egg yolk. Ordinates are the temperatures in degrees Centigrade, and abscissae are time in minutes. The first curve shows an initial rapid rise of temperature with the appearance of an apparent plateau which is followed by a gradual rise of temperature to a final plateau representing the freezing point. The second and third curves show repetitions of the process in which the final plateau appears without evidence of any secondary plateau. The arrows mark the points where the yolk was seeded.

lowed for 3 to 10 minutes and the value for the plateau is the average of readings taken every 30 seconds. These individual readings had a probable variation within 1 to 2 hundredths of a degree, dependant somewhat on the rate of stirring. The readings for the white were

taken usually from one or two plateaus, as no difficulty in obtaining the true freezing point was encountered with the white. The observed average values for the yolk and white differ by only 0.001°C., as given in Table I.

It is concluded that there is no significant difference in the freezing point depression of the yolk and white of the hen's egg. The yolk is difficult to freeze not only because of its high viscosity, low water content (48 per cent), and high content of fat globules which have low heat conductance, but also probably because it contains a dialyzable material which decreases the rate of ice crystal formation, as will be described in the next section.

TABLE I

Freezing Points of Yolk and White of Hens' Eggs

Egg No.	Yolk	White	Difference
	°C.	°C.	°C.
1	−0.422	−0.434	−0.012
2	0.452	0.438	+0.014
3	0.424	0.426	−0.002
4	0.416	0.414	+0.002
5	0.419	0.419	0.000
6	0.418	0.412	+0.006
Average	0.425	0.424	

The value of −0.425°C. found in the freezing point of egg white is in agreement with the results of Rice and Young, but is somewhat higher than that reported by previous investigators (Straub, −0.46, Atkins, 0.454, Grollman, 0.456, Smith and Shepherd, 0.452). This might be accounted for by either of the following reasons. The value 0.425 was obtained from eggs which were used within 20 to 40 hours after laying. Eggs of this lot which had been kept at 1°C. for 6 weeks still tasted perfectly fresh but had a freezing point of −0.46 for yolk and white, an increase which is probably due chiefly to evaporation of water. Table eggs bought at another store and apparently perfectly fresh had a freezing point of 0.447. Mature yolks removed from the ovaries of two hens had depressions of 0.408 and 0.420 respectively, so that the lower values are to be preferred as representing the conditions

in freshly laid eggs. The use of a larger freezing tube similar to that used in the Beckmann apparatus gave somewhat greater values (0.03°) for the freezing point depression of white than were obtained in Johlin's apparatus, a difference probably due to the more efficient stirring obtained in the latter. The freezing of the white was performed in a tube of 10 mm. diameter containing a thermometer of 9 mm. diameter, so that very efficient stirring was produced by moving the thermometer. We might thus attribute the slightly higher freezing points of egg white observed by previous authors to the use of older eggs or to an inefficient stirring during the determination. Eggs of different breeds according to Rice and Young vary slightly as regards their freezing points.

The present findings of the same freezing point for the yolk and the white are in agreement with the early observation of Atkins (1), and it is concluded that the observations of Straub, and others (2, 22, 24, 25), were in error due to their failure to recognize that the determination of the true freezing point of egg yolk is beset with technical difficulties.

The Osmotic Pressure of Egg Yolk as Determined by Dialysis

In order to confirm the direct determination of the freezing point of egg yolk just described, observations were made on the freezing points of solutions which were allowed to come into equilibrium with the yolk across collodion membranes. Freezing point determinations were made by the same method used for egg yolk, except that volumes of only 2 cc. were used, in a tube of appropriate dimensions. The greater fluidity of the dialysate permits a much more efficient stirring than is possible with egg yolk.

It was found that it was often more difficult to obtain the actual freezing point of the dialysate than of the yolk itself. In following the temperature-time curve, after seeding the supercooled solution, an initial rapid rise of temperature in the first $\frac{1}{2}$ minute is followed by a marked change in the freezing rate and a very slow attainment of the final equilibrium temperature.

Typical results obtained in freezing dialysates are shown in Figs. 2 and 3. In Fig. 2, Curve B represents the freezing of a dialysate in which, after the initial rapid rise of temperature during the first half minute after seeding, there succeeded a slight but steady rise of temper-

ature for 10 minutes or more, to a moderately well defined plateau terminated by an abrupt temperature rise. Curve C shows the same type of behavior except that an apparent plateau appears during the first 2 minutes, after which the temperature gradually rises to the final plateau. The freezing of a 1.00 per cent NaCl solution is shown in Curve A, where it is seen that a plateau is reached after about 3 minutes, which is more stable than that of the dialysates, and is terminated

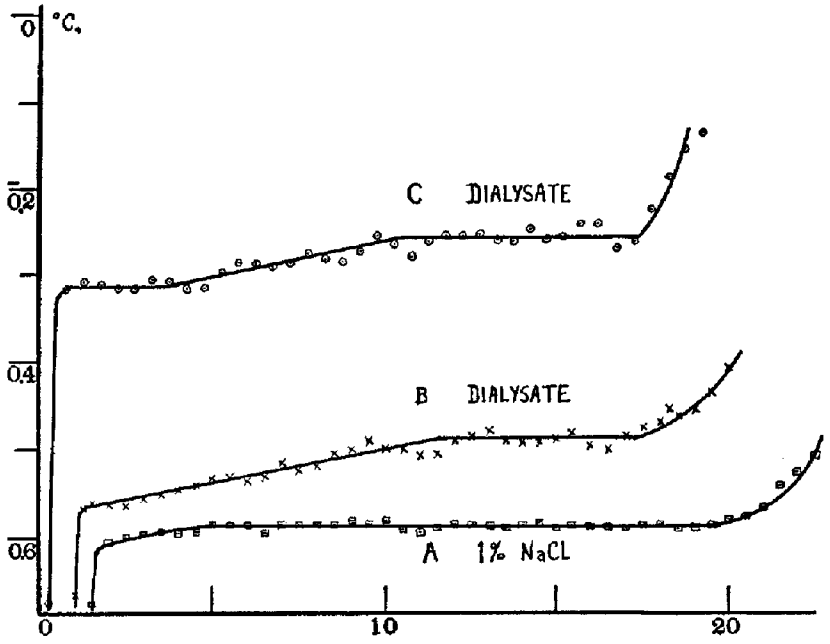

FIG. 2. The freezing of egg yolk dialysates compared with a 1 per cent NaCl solution. Ordinates are temperatures in degrees Centigrade: abscissae, time in minutes. The three curves have the same origin, but are displaced laterally for convenience in representation. It will be seen that, following the rise in temperature after seeding the supercooled solutions, a constant temperature is attained more rapidly by the NaCl solution than by the dialysates.

in the same way by an abrupt temperature rise. In the case of the NaCl solution the heat liberated by freezing results in a prompt rise to a temperature at which equilibrium is maintained between the ice and the solution. Heat is continuously added by the friction of stirring, which gradually melts the ice, and when the ice phase is completely

melted a further rise in temperature ensues. In the case of the dialysates it is evident that the attainment of thermal equilibrium between the ice and the original solution is delayed. It is obvious from these curves that the true freezing points of the dialysates are less easy to determine with accuracy than is that of the NaCl solution.

Fig. 3 shows an even more anomalous type of freezing, which was frequently encountered with dialysates. It is seen that the change in

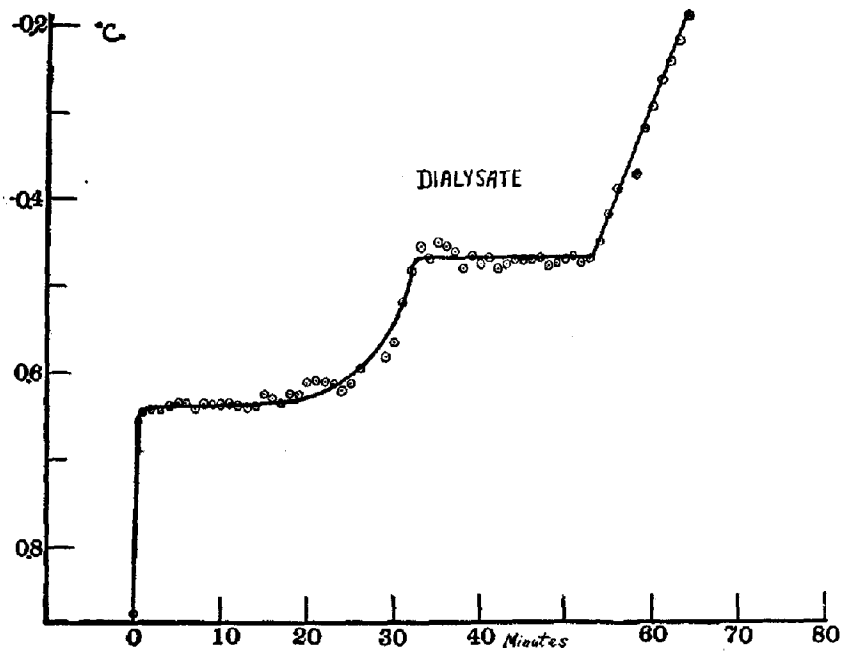

FIG. 3. The freezing of an egg yolk dialysate in which a secondary low plateau appears before the true freezing point is reached. (Ordinates, temperature in degrees Centigrade, abscissae, time in minutes after the initiation of the freezing process.) In freezing dialysates there were obtained various types of temperature-time curves similar to or intermediate between those shown in this figure and in Fig. 2.

freezing rate which occurs after the first $\frac{1}{2}$ minute is followed by a plateau which is later succeeded quite abruptly by a second plateau at a higher temperature. Various curves intermediate between those of Figs. 2 and 3 are obtained, so that often a distinct initial plateau is

succeeded gradually by the final plateau which is terminated by the disappearance of the ice.

The lower plateau varies, in dialysates of different concentrations, according to the temperature of the final plateau, so that it cannot represent a eutectic or other invariant point. Such a secondary inflection has never been observed in freezing NaCl or KCl solutions.

The lower temperature plateau cannot represent the true freezing point because it is followed by a higher plateau before the ice phase has disappeared. The lower plateau is associated with the presence of large amounts of ice, which would concentrate the solution, and hence lower the temperature at which the liquid phase is in equilibrium with ice. The form of the curve is such that one might be misled and consider the initial plateau as representing the true freezing point if one did not follow the temperature until the final disappearance of ice. If one does follow the temperature until the ice disappears, one finds that the plateau which is presented just before the ice goes is never attained promptly, but only after a gradual rise of temperature during which at times a lower plateau appears. As long as ice is present in a solution, and stirring is adequate to maintain thermal equilibrium, the temperature of the solution cannot exceed its true freezing point.

It is only when a small amount of ice is present that the concentration of the solution is not affected, and it is only under these conditions that the true freezing point is obtainable. This state of affairs occurs in the region of the second plateau just before the abrupt rise following the melting of the last ice crystals. Hence one must take the true freezing point of a solution determined by this method to be the highest temperature which is maintained at a constant level in the presence of ice.

The freezing behavior of the dialysate suggests that a substance is present which slows the rate of ice formation. This has been shown to be the case in the following manner. Direct measurements of the rate of formation of ice crystals have been carried out according to a procedure due to Gernez (5), and previously applied to aqueous solutions by Walton, Brann, and Judd (26, 27, 3). Two U-tubes containing saline and dialysate respectively were immersed in a cooling bath, and after seeding at one end, the times were obtained at which the ice crystal boundary had moved a distance of 40 cm. In making a

determination, the tubes of saline and dialysate were always seeded simultaneously. The velocities are a function of temperature, and were obtained at several temperatures. The results are given in Table II, the velocities being expressed in cm. per second. It will be seen that the crystallization velocity in the yolk dialysate is less than in an NaCl solution of the same freezing point, with an average difference of 25 per cent. Whether this decrease in crystallization velocity is the only factor responsible for the anomalous freezing of the dialysates cannot be decided at present. It is, however, interesting to note that $MgCl_2$ solutions, which according to Brann slow the crystallization velocity of water, show an inflection in the temperature-time curve when freezing which is quite similar to the inflection of the dialysate curves.

A change of crystallization velocity after the first $\frac{1}{2}$ minute has been described by Marc (12–16) in the crystallization of a series of salts in water solutions. The first $\frac{1}{2}$ minute of freezing was characterized by a greater velocity constant than the remainder of the process. Marc attributes this to changes at the crystal solution interphase which acquires certain constant properties after the first $\frac{1}{2}$ minute. Marc (14–16) adduces evidence that crystallization velocities are affected by means of the adsorption of inhibiting substances at the crystal surface. Brann, on the other hand, related the effect to solvation of the solute in a series of salts; whether the mechanism is essentially the same in the two cases is not clear at present.

In the light of the above experiments, it is probable that anomalous freezing behavior, such as shown by the egg yolk dialysate, is a phenomenon of fairly general occurrence. The true explanation of this anomaly must probably await a more extensive knowledge of the mechanism of crystallization from solutions.

In the present determinations, it was found that repeated freezing of the same dialysate gave values for the final plateau (determined by averaging the individual readings) which checked to within 0.01°C. The approach to the plateau varied in the same dialysate between the two extreme types of curves shown in Figs. 2 and 3. The point of initial inflection of the curve was constant to within about 0.02°C. so that this anomaly is a fairly constant characteristic of a given solution. What substance in the yolk is responsible for this effect has not been

determined. It is present in dialysates which are clear and colorless. The anomalous freezing is not affected by boiling the dialysate. At-

TABLE II

Crystallization Velocities in Cm. Per Sec. of Egg Yolk Dialysate and Isosmotic NaCl Solution

Bath temperature	0.71 per cent NaCl	Dialysate
°C.		
6 mm. tube		
−2.12	0.228	0.173
1.3	0.049	0.041
1.9	0.192	0.155
10 mm. tube		
2.3	0.286	0.270
1.8	0.153	0.105
2.0	0.198	0.106
1.9	0.176	0.109
Average.....	0.182	0.137

TABLE III

The Freezing Point of Egg Yolk Calculated from That of Its Dialysate

Weight yolk	Membrane weight	Volume dialysate	△ Dialysate observed	△ Yolk calculated
gm.	*gm.*	*cc.*	°C.	°C.
A. Against 1 per cent NaCl: membranes soaked in 0.71 per cent NaCl				
19.6	0.9	10	−0.503	−0.420
24.2	0.35	4	0.467	0.426
23.8	0.3	4	0.456	0.410
26.2	0.3	7	0.491	0.437
27.7	0.5	7	0.487	0.434
B. Against H_2O: membranes soaked in H_2O				
25.1	0.4	5	0.304	0.440
27.4	0.52	5	0.298	0.423
Average.....				0.427

tempts to adsorb the material on carbon and cotton seemed to accelerate the freezing somewhat, but were not effective enough to remove the inflection in the freezing curve. The interfering substance was present

in dialysates after using the following types of membranes: cellophane, a synthetic parchment, gold-beaters' skin, and collodion membranes made by drying to various degrees with or without ethylene glycol. The interfering substance is not evident in the white of fresh eggs, but in the white of eggs which had stood for 6 weeks the anomalous freezing may be very marked.

When the freezing temperature was determined by following the temperature-time curve with constant stirring until the abrupt rise of final ice disappearance occurs, the dialysate freezing points shown in Table III were obtained. For the experiments in Table III collodion membranes were made following, in principle, the method of Pierce (21).

Merck's C.P. collodion to which was added 1 per cent ethylene glycol was poured into test-tubes (15 × 1.8 cm.) which were rotated slightly and dried overnight. Membranes were then washed for 50 hours in running water, and placed in 0.71 per cent NaCl (where indicated) overnight. The membrane with included solution was weighed just before adding egg yolk, and the weight × 0.8 (membrane water content as determined by drying) taken as the weight of contained solution in computing the freezing point of the yolk from the value of the dialysate. The water content of the yolk was found to average 48 per cent. Dialysis against 1 per cent NaCl was allowed to continue for from 15 to 20 hours, with gentle shaking, at room temperature. No difference in the results was obtained after 6 hours dialysis. In dialyzing against water, experiments were run for 50 hours at 1°C. No significant differences in the osmotic pressure of the yolk at the two temperatures were observed.

The average value of the freezing point of the yolk calculated from that of the dialysate agrees with that obtained by direct measurement with a difference of only 0.002°C.

This dialysis evidence that yolk and white are in osmotic equilibrium is in agreement with Grollman's dialysis experiments and in opposition to Straub's and Meyerhof's conclusions from dialysis. It is worthy of emphasis that the true freezing points of egg yolk dialysates are not always obtained without difficulty, and unless this source of error is recognized one may obtain erratic results.

Vapor Pressure Measurements

In order to further verify the conclusions based upon freezing point data a series of vapor pressure determinations on egg yolk were per-

formed. In this way freezing points of egg yolk could be calculated from data based on an entirely different type of experimental technique. For this purpose the dynamic method of Washburn and Heuse (28) was utilized, as used by Grollman (6) for the determination of the vapor pressure of dog's blood. I am indebted to Dr. Grollman for performing these determinations of vapor pressure.

The method as used in these experiments consists essentially of passing a stream of gas over the material to be investigated with which it comes into equilibrium. The gas saturated with the vapor from the unknown solution is then led over an absorber which removes the water vapor. The dried gas is next led through a similar train in series with the first where it becomes saturated with water vapor from a known solution, and it then gives up this water vapor to a second absorber. By weighing the absorbers before and after the experiment a differential measure of the vapor pressure of the unknown solution is obtained.

The saturators were of the type described by Washburn and Heuse (28). They consisted of a train of glass tubes placed on a rocking table, and were about half filled with liquid. In the absorbers the gas was passed through a condensation bulb, an H_2SO_4 train, and Dehydrite. The gas (air or oxygen) was led first over a presaturator containing 1 per cent NaCl, before being introduced into saturators containing solutions to be tested, to avoid concentration by drying of the solutions. The equilibration temperature was 20°.

Several technical difficulties were encountered in carrying out determinations on egg yolk which rendered the results more erratic and open to greater error than that encountered by Grollman in his study of blood. The viscosity of egg yolk prevents an efficient shaking of the material and hence tends to prevent true equilibrium occurring between the gas and the yolk. To avoid this occurrence a slower passage of the gas was required. The passage of the gas was continued for about 20 hours, but was not extended further because of the danger of putrefaction. The tendency of egg white to foam rendered necessary the insertion of a trap at the end of the saturator containing this substance, to minimize any carrying over of small droplets of solution into the absorbers. Both of these sources of error would lead to obtaining too high values for the freezing point depression of egg yolk.

The freezing points were calculated from the vapor pressure data by means of the simplified equation of Callendar (4) which is sufficiently accurate when the freezing point depressions are less than 1.0°C.

$$\log e \frac{p_o}{p} = \frac{LF_o \Delta T_F}{RT_o^2} = 0.0097\ \Delta T_F$$

where ΔT_F is the difference in the freezing points of the two solutions, LF_o is the molar heat of fusion of the pure solvent at its freezing point, T_o the absolute freez-

ing point of the known solution, and p_0 and p the vapor pressures of the known and unknown solutions respectively.

In this calculation of freezing point data from vapor pressure differences determined at temperatures other than the freezing point, it is assumed that we can neglect the change in the heat of dilution of the solution with temperature and the change in the activity with temperature. As discussed by Grollman (6) these factors are negligible for NaCl up to 1 M concentration, and we have no knowledge that they are otherwise for the constituents of egg yolk.

The results are given in Table IV. There is considerable experimental error as indicated in the range of the values, but the average calculated freezing point of −0.45°C. shows quite satisfactory agree-

TABLE IV

The Freezing Point of Egg Yolk Calculated from Its Vapor Pressure

Experiment No.	Solution against which yolk was measured	Freezing point of solution assumed	Difference between freezing point of yolk and Column 2	Calculated freezing point of yolk
		°C.	°C.	°C.
1	Egg white	−0.43	−0.05	−0.38
2	" "	0.43	+0.06	0.49
3	" "	0.43	+0.03	0.46
4	0.75 per cent NaCl	0.44	+0.05	0.49
5	" " " "	0.44	−0.04	0.40
6	H_2O	0.00	+0.47	0.47
Average				0.45

ment with the value obtained directly. It is slightly high, but the chief sources of error discussed above would tend to give too great a freezing point depression.

These results are in opposition to those obtained by Hill (9) using a vapor pressure method (10) which depends on the measurement of the differences in the heat of condensation of vapor in two solutions. The method appears to be admirable for dilute aqueous solutions, as shown by Grollman (7), but the disparity between Hill's determinations on the egg and the various lines of evidence presented herewith leads me to believe that Hill's method cannot be applicable to a concentrated viscous mixture such as egg yolk, especially since the present determinations show that the expected osmotic equilibria between egg yolk

and white are fulfilled, whereas Hill's results require an explanation *de novo*.[1]

SUMMARY

The osmotic pressure of the yolk and white of the hen's egg have been shown to be identical, by means of direct freezing point determinations, dialyses, and vapor pressure measurements.

Dialysates of egg yolk slow the rate of ice formation compared with NaCl solutions. They also show a marked change of freezing rate as the freezing point is approached. The anomalous freezing behavior of this material may lead to errors in the determination of the true freezing point which would tend to make the value for the yolk erroneously low.

The postulate of a vital activity at the yolk membrane maintaining an osmotic pressure difference is thus shown to be unnecessary, since a simple osmotic equilibrium exists between the yolk and the white.

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[1] While this paper was in press an article appeared (Bateman, J. B., 1932, *J. Exp. Biol.*, **9,** 322) purporting to confirm Hill's vapor pressure determinations on the egg. In view of the present experiments it is impossible to accept Bateman's measurements as representing the true vapor pressure difference between yolk and white, and consequently I suspect that some unknown factor produces a pseudoequilibrium of heat quantities in the thermopile experiments—probably conditions at the yolk-air interphase are not identical with those in the body of the material. Significantly, Bateman's findings of the vapor pressure of mixtures of yolk and white are in disagreement with his own determinations of the yolk vapor pressure, but agree with the value of approximately −0.42°C. freezing point depression of both yolk and white.

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