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# Denaturation of egg proteins. I, Effect of heat treatments on viscosity of liquid egg products

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# NOTE TO USERS

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# DENATURATION OF EGG PROTEINS

## I. EFFECT OF HEAT TREATMENTS ON VISCOSITY OF LIQUID EGG PRODUCTS

by

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# INTRODUCTION

The preheating of liquid egg prior to spray-drying is now recognized as an improvement in the processing. This investigation of some of the problems involved in the preheating of liquid egg was carried out as a project of the National Coordinated Dried Egg Research Program. The results obtained in this study now form the basis of the preheating treatment required by the Quartermaster Corps Tentative Specification for Dried Egg C. Q. D. No. 117A, 28 January 1944.

In the processing of dried egg, preheating prior to spray-drying offers several advantages. Preheating ultimately improves the keeping quality of the dry product. It is a well established fact that viscosity and surface tension decrease with rise in temperature. This decrease in viscosity and surface tension brought about by preheating results in a greater ease of drying. Preheating the liquid egg permits the use of lower inlet-air temperatures in the spray-drying process, since approximately 10% of the total heat required can be obtained from the liquid egg itself. Other advantages offered by preheating are: a more rapid attainment of a high temperature in the egg particle, greater ease of atomization, and a more rapid drying. It has been shown that the degree and uniformity of atomization can be satisfactorily controlled in certain types of dryers by preheating the liquid egg.

The use of a process involving the application of heat to a product of such a complex composition as liquid egg presents many difficulties. The main problem was to determine to what extent preheating could be safely

employed without causing a very marked change in the physical and chemical characteristics of the liquid egg. Obviously, the effect of preheating would be the denaturation of the proteins by heat. Since denaturation is a function of the time and temperature of heating, the study involved the following problems:

A. To devise a process of heating whereby:
    1. The liquid egg can be heated to the desired temperature within the shortest time possible.
    2. Every particle of the sample can be held at the desired temperature for varying periods of time.
    3. The sample can be completely cooled within a short time.

B. To employ a sensitive method that can be used with convenience and ease of reproducibility to measure the progress of denaturation of the liquid egg proteins preheated at different temperatures for varying periods of time.

## REVIEW OF LITERATURE

Any treatment of naturally occurring biological materials such as foods comes under the realm of colloidal phenomena. Proteins are classified as hydrophilic colloidal systems. As such, their properties are a function of the type, the degree of dispersion, the shape, and the degree of solvation of the particles of the disperse phase. Thus any agents or conditions that alter these factors will result in a change in the properties of the protein.

A review of the literature has been made on the particular proteins known as corpuscular proteins as they are affected by certain colloidal phenomena. A great deal of attention is paid to the effect of heat on the properties of the corpuscular proteins. It is common knowledge that protein solutions are coagulated by heat. Early work on heat coagulation by Chick and Martin (18) showed that heat coagulation takes place in steps. Investigators in the field of protein chemistry have accepted the view that heat coagulation involves two distinct processes: (a) denaturation and (b) precipitation or the formation of an insoluble coagulum. Bull (14), however, states that denaturation consists of three steps: (a) denaturation proper, which is an intramolecular rearrangement, (b) flocculation of the denatured molecule preparatory to coagulation, the $\zeta$-potential on the particles being the stabilizing factor and (c) formation of an insoluble coagulum. However, the use of the term "denaturation" by most investigators is limited to what Bull refers to as "denaturation proper."

The term denaturation is commonly applied to the phenomenon that occurs when certain protein solutions are acted upon by agents such as heat, acid, alkali, alcohol, acetone, urea, ultraviolet light, X-rays, high pressure, shaking, and other means. Denaturation is now generally believed to be a physical change in the protein molecule and not a definite chemical reaction as was formerly supposed. The physical change involved is an intramolecular rearrangement (14,47) such that after denaturation, the protein exhibits properties different from those of the native protein. Changes are observed in the physical, chemical, and biological properties. Physical changes are reflected in the solubility, crystallizability, and viscosity of the protein solution. Thus a denatured protein, at the isoelectric point, becomes insoluble in reagents in which it was previously soluble (14,18,47). An increase in viscosity is observed in protein solutions after denaturation (1,15,44). The protein which is crystallizable in the native state loses this property after denaturation (47). The work of various investigators, notably Mirsky and Anson (49), has shown that the chemical changes during denaturation involve the exposure of certain groups in the protein molecule, namely, the disulfide, sulphydryl, and phenol groups. The changes in specific biological properties are reflected in the loss of enzymatic and immunological properties (47).

## A. Theory of Denaturation

Various theories of denaturation have been proposed, since Chick and Martin performed their experiments on the heat coagulation of proteins.

The most generally accepted interpretation today is that denaturation is the disorganization of the natural protein molecule, the change from the regular arrangement of a rigid structure to the irregular diffuse arrangement of the flexible open chain (16,47). Later studies have elaborated on this view; denaturation has been explained by the loss of the uniquely defined configuration of the native molecule (50), the uncoiling or unfolding of the native protein molecule into a more elongated form (3, 44,57), loss in structure or increase in randomness (22).

The protein molecule is composed of polypeptide chains which hold the amino acid residues. These polypeptide chains exist parallel to each other and may or may not exhibit a folded arrangement depending on the type of protein. The 'grid' arrangement has been proposed by Astbury (5) on the basis of X-ray studies. Various types of bonds may join these polypeptide chains, namely,hydrogen bonds (57), salt bridges (22), and disulfide linkages (22). The salt linkages occur between the charged amino and charged carboxyl groups and give rise to the zwitter ionic structure (16). Bull (16) suggests that the second type of bonds are N-H-O hydrogen bridges between the neighboring loops of the same chain or between the neighboring peptide chains in the molecule, and cites the work of Buswell, Krebs, and Rodebush as furnishing experimental evidence for such linkages in proteins.

A survey of the literature reveals that there is as yet no agreement on the particular bonds that are broken during denaturation. There is, however, some agreement that the loss of the uniquely defined configuration

of the native protein molecule is due to the breaking of non-peptide protein bonds. It was suggested that denaturation involved the breaking of hydrogen bonds (50), but this view has been questioned on the grounds that the loss of hydrophilic properties as a result of protein denaturation is exactly the opposite of what one would expect if there were rupture of hydrogen bonds, since the ruptured bonds would be available for waterbinding and therefore enhance the hydrophilic properties (14). From studies on the energy of activation, Eyring and Stearn (22) came to the conclusion that denaturation involved the breaking of two kinds of bonds: (a) salt linkages which are insensitive to pH changes, and (b) homopolar bonds which are sensitive to pH changes, which they considered to be disulfide linkages. By the breaking of these bonds, solvent is eliminated and the solubility decreases.

The unfolding or uncoiling of the protein molecule on denaturation has been inferred from studies on protein denaturation by means of X-ray diffraction studies, and measurements of the energy of activation, entropy, and the number of SH groups.

Lewis (41) found the increment of the energy of activation to be excessively great, of the order of 100,000 calories per mole, in the neighborhood of the neutrality point. He is of the opinion that an energy term of this magnitude suggests as the most significant alteration in the protein unit, an actual physical distension or opening up of the structure, possibly accompanied by the breaking of bridge-like linkages in the original undenatured unit.

Another evidence which provides support of this view of protein denaturation is the large increase in entropy which has been observed to

accompany denaturation (48), and which is taken as indicating that for a denatured protein, there is a multitude of available configurations. Eyring and Stearn (22) picture denaturation as the simultaneous breaking of weak bonds in the molecule, with a resulting loss of structure, or a large increase in randomness.

Astbury and co-workers (3,4,5) give a structural interpretation of denaturation based on the study of the X-ray diffraction patterns of proteins. Heat denatured egg albumin was found to show an X-ray photograph similar to that produced by β-keratin. They suggested that denaturation of egg albumin involves a change in the molecular configuration from the corpuscular into the fibrous (β-keratin) form, and that on coagulation, these chains aggregate into parallel bundles (3). Recent studies by Lundgren (46) and by Palmer and Galvin (59) not only substantiate this view but also furnish evidence that a corpuscular protein (egg albumin) can be converted into a fiber of the β-keratin type. The X-ray pattern of these fibers indicated that they are composed of "parallel bundles of polypeptide chains running parallel to the fiber axis." The remarkable agreement shown by the X-ray photographs of steam stretched egg albumin fiber and β-keratin is proof that they have the same molecular configuration (59). Likewise the X-ray photographs of heat denatured egg albumin and that of an egg albumin fiber after removal of detergent were found to be identical. Furthermore, they found that the average tensile strength of the fiber is dependent upon the degree of molecular orientation; molecular orientation was first detected at 100% elongation; further elongation improved the orientation, reaching an optimum at 400% elongation.

From studies on the viscosity of solutions of native and denatured egg albumin, Bull (15) presents evidence that even urea denatured egg albumin (which was found to have an asymmetry of 7.3:1; heat denatured 5.8:1; native 1.2:1) still has considerable structure, and is not simply a polypeptide chain in the β-keratin form; he states that the egg albumin molecule, if it existed as a β-keratin chain, should be over 1000 Å units long and about 10 Å wide, which would yield an asymmetry greater than 100:1. He concludes that even in a denatured globular protein, the peptide chain is greatly folded and collapsed.

## B. Criteria of Denaturation

Certain chemical and physical changes which occur in the native protein as a result of denaturation (16) are: (a) decreased solubility, (b) increased viscosity of solution, (c) exposure of oxidizing and reducing groups (sulphydryl and disulfide linkages among others), and (d) a large loss of specific biological properties, among which may be mentioned (47) loss or impairment of immunological and enzymatic properties, and inability to form crystals.

### 1. Chemical changes.

Certain chemical changes that occur as a result of denaturation are employed in the quantitative characterization of denaturation. These chemical changes are: the increase in the number of detectable sulfhydryl, disulfide, phenol groups, and peptide bonds (47). Block (11) states that

denaturation may bring about the liberation of previously masked SH groups, or a reduction of S-S groups; in some cases it brings about an exposure of S-S groups (47). Block (11) classifies cystine-containing proteins into four categories: (1) natural proteins which give a positive nitroprusside test for SH (example muscle, thymus histone), (2) proteins (egg albumin) which give a positive SH reaction after denaturation, (3) denatured proteins (serum albumin) which give a positive sulphydryl test after treatment with cyanide, and (4) those proteins which do not give a positive reaction (globin, ovomucoid, brain proteins, etc.).

Mirsky (47) states that the appearance of SH groups is not due to reduction of pre-existing S-S groups. "In some proteins, as in serum, only S-S groups are detectable; in some proteins, as the crystalline lens, only SH groups are detectable; and in other proteins, as egg albumin, both SH and S-S groups are detectable." There are various methods in the quantitative estimation of these detectable groups, among which may be mentioned the ferricyanide, nitroprusside, tetrathionate, porphyrindin, iodosobenzoate tests, etc. In the estimation of the SH groups of denatured egg albumin, reagents which oxidize or reduce the SH groups are used. The use of urea, guanidine hydrochloride or detergents (2) as denaturing agents is considered an improvement in the method because these reagents both denature the protein and keep the denatured protein in solution. The amount of a reagent such as porphyrindin, ferricyanide or tetrathionate required to oxidize the SH groups gives a quantitative estimation of the SH groups present. Since aggregation and precipitation interfere with the oxidation of the SH groups by ferricyanide, the advantage of using those denaturing agents that keep the denatured protein

in solution lies in the fact that it enables the oxidation of all the SH groups present. Other reagents used are p-chloromercuribenzoate, iodosobenzoate and uric acid. The amount of p-chloromercuribenzoate that combines with the SH, and the amount of iodosobenzoate or uric acid reduced is used in estimating the number of SH groups. Cold 1 M potassium iodide is employed in the determination of SH groups in native egg albumin. The amount of reagent reduced by the native egg albumin indicates the number of SH groups present. The ferricyanide method is also applicable to egg albumin denatured by heat and by shaking if it is allowed to oxidize the SH groups during the denaturation. Thus aggregation and precipitation of the denatured protein cannot interfere with the reaction.

Estimation of the SH groups is also done by allowing an excess of cystine to react with the protein (52). The reaction of the SH linkages of the denatured protein and the S-S linkages of the cystine:

$$RSH + Cy - S-S - Cy \equiv RS - SCy + CySH$$

results in the decomposition of the cystine. From the quantity of cystine decomposed, the number of reactive SH groups of the denatured protein is estimated.

Mirsky and Anson (49) found a precise and quantitative correlation between the formation of denatured egg albumin, using insolubility as a quantitative criterion, and the appearance of SH groups.

Further evidence that denaturation is an unfolding process is given by Mirsky (48) in the determination of the number of SH groups of egg albumin. Sulfhydryl groups become detectable as a result of denaturation. Mirsky suggests two possible explanations for the appearance of SH groups

on denaturation. One explanation is that when the configuration of the protein molecule becomes more extended and open, reagents such as ferricyanide are able to reach the groups located within the compact, native protein molecule (48). According to this view the SH groups in the native protein molecule like behind a barrier formed by the atoms in a closely folded arrangement of peptide chains; the unfolding of the peptide chains, and the consequent extension thereby, expose the SH groups to the reagent. The other explanation, advanced by Linderstrom-Lang and Jacobsen (56), is that the SH groups are not present in the native egg albumin, but are actually formed during the denaturation process. Another possibility has been suggested by Neurath (56). He points out that the estimation of SH groups by such reagents as nitroprusside, ferricyanide, etc., involves the oxidation of SH to S-S, and that two cysteine residues are required for this reaction to proceed. In the native protein, it may so happen that individual cysteine residues are not close enough to form a disulfide bond, hence the reagents will be ineffective in testing for SH groups. There is a possibility that as the molecule assumes a much looser and random arrangement upon denaturation, even distant cysteine residues may come, at least temporarily, into close contact. Mirsky (48) found that the number of SH groups liberated when egg albumin is denatured by heat is less than the number found in egg albumin denatured by urea, guanidine hydrochloride or Duponol. However, in a urea denatured albumin solution in which the urea had been diluted by the addition of water, the number of SH groups was found to be the same as that found in heat denatured egg albumin. It was also found that in the denaturation of egg albumin by

urea, removal of the denaturing agent (dilution of the urea) caused some of the SH groups to disappear. On this evidence, Mirsky postulates the possibility that in heat denatured egg albumin, removal of the denaturing agent (cooling the solution) also causes disappearance of the SH groups. To prove this, experiments were run in which the SH groups were determined while the albumin was being denatured by heat. The number of SH groups found, under these conditions, was the same as in the albumin denatured by urea or guanidine hydrochloride. By the use of the ultracentrifuge, it was found that the extent of aggregation of solutions of heat denatured egg albumin and that of solutions of egg albumin denatured by urea and subsequently diluted with water are the same. On this evidence, Mirsky (48) concludes that in egg albumin, denaturation causes an unfolding of the protein molecule with liberation of SH groups; withdrawal of the denaturing agent is accompanied by protein aggregation and disappearance of some SH groups. In heat denaturation, he states that there is an unfolding sufficient to uncover all SH groups, followed by a polymerization which covers some of the exposed SH groups.

2. Physical changes.

a. Solubility. The changes that occur in the native protein have been employed as satisfactory criteria in the study of protein denaturation. In their classical papers on denaturation, Chick and Martin (18) showed that the change in solubility can be used as a satisfactory criterion of denaturation. Under conditions in which the original native protein is soluble, the denatured protein is insoluble at its isoelectric point

but soluble on either side of it (47). Bull (16) states that the loss of solubility is probably the reflection of at least two changes in the native molecule. The first change involves the polar groups of the protein molecule. In the native protein, these polar groups are available for water binding. On denaturation, they are linked to other polar groups in the protein molecule itself and are, therefore, no longer available for water binding. The result of this change is decreased solubility. This assumption is based on the work of Neurath and Bull (57) who showed that the water of hydration of heat denatured and of surface denatured egg albumin is less than that of the native protein.

Meyer (52) explains the reduction in solubility in terms of the exposure of hydrophobic groups brought about by the unfolding of the molecule. In the native state, these hydrophobic groups may be situated within the compact molecule and mutually saturate each other. When the molecule unfolds, these hydrophobic groups may be set free and may contribute to the linkage with other molecules. The unfolding may also expose ionisable groups which may form a salt-linkage with groups of other molecules, and so bring about precipitation as a multivalent salt (52).

According to Bull (16) the second change that is reflected in the loss of solubility is the increased asymmetry of the protein molecule. From viscosity measurements Bull (15) estimated the asymmetry of the native egg albumin molecule to be 3.9:1; of heat denatured 7.4:1; and of urea denatured 9.2:1. Considering the effect of hydration, the asymmetry was found to be 1.2:1 for native, 5.8:1 for heat denatured, and 7.3:1 for urea denatured egg albumin. The increased asymmetry of the denatured protein

molecules results in a greater surface area, greater ease of cohesion and, therefore, decreased solubility.

b. Viscosity. One of the changes observed on denaturation of protein solutions is the increase in apparent viscosity. The explanations that have been offered (14) for such a phenomenon are listed as: (a) increased hydration of the particles, (b) association or aggregation of particles, occluding water, and (c) increased asymmetry of the protein molecule.

(1) Hydration phenomena and viscosity. In the classical papers of Chick and Martin (18), they state that heat coagulation of proteins involves two distinct processes: (a) the reaction between water and protein, and (b) the precipitation of the protein. They studied the heat coagulation of solutions of crystalline hemoglobin and crystalline egg albumin, both without water, and with the addition of water. They came to the conclusion that the "complete solubility of both proteins after exposure to dry heat at high temperatures (110°-150°C.) indicates that heat coagulation of protein solutions is not a pure temperature effect, but a reaction between water and protein."

Loughlin and co-workers (44) made viscosity measurements on 3% solutions of egg albumin before and after heat denaturation. The mean value of the relative viscosity of a 3% solution of undenatured egg albumin was found to be 1.168; that of a 3% denatured solution, 1.303. This increase in relative viscosity was found to be constant and independent of pH, for any given concentration of protein. Using a modified Einstein mathematical treatment of the viscosity of a disperse system, the value of $\frac{\Phi \text{ undenatured}}{\Phi \text{ denatured}}$ was found to be $\frac{1}{1.8}$. ($\Phi$ is the aggregate volume of spheres in unit volume of the sus-

pension). They interpreted these results to indicate that on denaturation at the isoelectric point, the volume of the dissolved protein unit increases, the increase being of the order of 80-100%. At this point, they recognized two possibilities: (a) that the protein units in swelling, imbibe part of the continuous medium, and (b) that the protein units are impermeable to this medium. If the latter case be true, they reasoned that the increase in volume would be transmitted to the total volume of the system, that is, the process of denaturation would be accompanied by an increase in the net volume of the protein solution. By dilatometric measurements, they found no appreciable expansion on denaturation, so they concluded that as the protein units expand on denaturation, "the continuous medium flows in to such an extent that there is no change in the total volume of the solution". This finding was considered sufficient explanation for the observations of Chick and Martin (18) that denaturation will not occur in the absence of water.

Bull (14) rules out increased hydration as the explanation for the increase in apparent viscosity of protein solutions, on the evidence that the hydration of heat denatured albumin is less than that of native albumin (15).

(2). Aggregation phenomena and viscosity. Anson and Mirsky (1) showed that the gross increase in apparent viscosity on denaturation is due to the formation of aggregates which occlude water, but that a small part of the increase in viscosity is due to ordinary hydration. By the use of the Kunitz equation (37) it was found that the amount of water contained in the solute is greater than can reasonably be attributed to hydration, so they assumed that the solute is not dispersed into molecules of ordinary size.

but that it has a structure consisting of aggregates occluding water. However, they further showed that although the gross increase in apparent viscosity is due to aggregation, part of it is due to ordinary hydration.

Bancroft (6) pointed out that the increase in the apparent viscosity of protein solutions on denaturation may be due to a number of factors. The degree of dispersion of the particles may be an explanation. He cited, as an example, Oden's results (6) in which an approximately 50% greater viscosity was found in sulfur sols whose particles had a diameter of about 10 mμ than with sols in which the sulfur particles had a diameter of 100 mμ. Hatschek (28) attributed this phenomenon to the existence of an adsorbed film of liquid around the sulfur particles, according to Bancroft (6), a phenomenon which was not considered when the formula was deduced. Bancroft (6) criticized the Einstein and Hatschek formulas as follows: in both of these equations, the viscosity of the solution depends only on the volume of the dispersed phase, and the degree of dispersity does not enter in as a factor. Hatschek (28) calculated the thickness of the adsorbed film of liquid around the sulfur particles to be about 0.87 mμ, on the assumption that the thickness of the adsorbed film is independent of the size of the particles; an adsorbed film of this thickness on particles having a diameter of 10 mμ was calculated to correspond to a 62% increase in the total volume of the particles. A decrease in the thickness of the film brings about increased fluidity, i. e. decreased viscosity.

Other possible explanations offered by Bancroft (6) for the increase in apparent viscosity of colloidal solutions are: aggregation of particles into chains; and loose agglomeration of particles into spherical masses

occluding water.

Loeb's experiments (42) on the effect of pH on the apparent viscosities of gelatin and of egg albumin solutions, led to the following conclusions: (a) "since the viscosity measurements of solutions of crystalline egg albumin and of gelatin agree fairly well with the Einstein and Arrhenius formulas respectively, it seems that the viscosity of solutions of proteins is primarily a function of the relative volume occupied by the protein in solution; (b) since the measurements were made at (or near) the isoelectric point of the two proteins, the difference in the viscosity of the two protein solutions cannot be ascribed to differences in the degree of hydration of the individual protein ions, since at the isoelectric point, the protein is practically not ionized." He explains the difference in the order of magnitude of the viscosity of the two protein solutions as due to the fact that gelatin forms gels which occlude relatively large amounts of water, while egg albumin solutions do not exhibit gel formation. The high viscosity shown by gelatin solutions is, therefore, due to structure. However, recent work by Meyers and France (53) showsthat egg albumin solutions can form systems "which have any degree of viscosity or which have gel structures of any rigidity," by simply varying the protein concentration, the time interval since the mixing of the components, the amount of solvation of the polypeptide chain, and the concentration and valence of salt. The increase in viscosity is explained as being due to the elongation of the polypeptide chain, and to the increased degree of solvation (53).

(3). Molecular symmetry and viscosity. The use of viscosity measurements in the study of molecular shapes is a recent development.

Various experimental studies have shown that viscosity is a most sensitive index of asymmetry (20). These studies, while not directly made on protein solutions for the study of denaturation, may be used to explain the increase in the apparent viscosity of denatured protein solutions. Assuming that denaturation involves the unfolding and distension of the compact native protein molecule due to the breaking of nonpeptide protein bonds, it is easy to imagine that the process of denaturation involves a change in molecular shape, from the spherical or nearly spherical (in the case of globular proteins) to that of an ellipsoid (elongated ellipsoid or flattened ellipsoid).

Kraemer (34) reflected the new trend by the suggestion that the high viscosities exhibited by intrinsic colloids might be caused "by greatly elongated particles or macromolecules that increase resistance to shear through mutual entanglements and interference, but without ordinary flocculation." Lauffer (38) gives an excellent review of the experimental facts which form the basis of this new idea.

From the empirical approach, Lauffer (38) reviews the work of Staudinger and collaborators on the relationship of viscosity of chain-type compounds and polymers and their molecular weights. He presents the data of numerous other investigators on linear molecules and linear polymers. After an examination of all these data, Lauffer derives a general relationship which suggests strongly that there is actually some fundamental connection between the size of the chain-type molecule and its solution viscosity. For all the cases examined, the intrinsic viscosity can be expressed at least as linear functions of molecular weights, or chain lengths.

On examination of the data on proteins, no correlation of any significance was found between intrinsic viscosity and molecular weight; however, a real correlation was observed between intrinsic viscosities of protein solutions and the axial ratios of the rod-shaped ellipsoids assumed to represent the protein particles. In general, the shape factors derived from two types of measurement (viscosity and sedimentation-diffusion) agree well. Both sets of calculations were made on the assumption that the proteins are not hydrated. The ratio $f/f_o$ is called the dissymmetry constant (56) and is a function of the deviation of the molecules from spherical shape, if all effects of hydration are neglected. The molecular dissymmetry is estimated by comparing the measured frictional resistance with that to be expected for a spherical molecule of equal size, by the use of methods based on measurements of resistance to sedimentation, diffusion, or viscous flow.

The increased asymmetry of the protein particles has been considered as tending to the formation of either an elongated ellipsoid, a flattened ellipsoid, or an array of spheres (38,56,62). Lauffer (38) states that the choice of the rod shape over the plate shape as a model for all protein particles is not entirely justifiable. According to Bull (17), "No one knows what form the asymmetries of protein molecules assume; i. e., are they cylinders, disks, prisms, prolate ellipsoids, oblate ellipsoids, etc.?" Simha (62) states that "as long as these idealized molecules do not change their shape under the influence of the shear gradient, the exact shape will not be important, and will show up only in slight changes of numerical factors, if the molecules are long enough, or more exactly, if their ratio

between longest and shortest axis is large enough. Due to this asymmetrical shape, we may expect a considerable dependence of the results on the degree of orientation in the solution, as for instance, measured by the birefringence of flow. The dependence is the more pronounced the greater the asymmetry."

For a clearer comprehension of the effect of asymmetry of the molecules on viscosity, it is necessary at this point to give an explanation (20,35,38) of the forces influencing the orientation of the particles in the system. In the measurement of viscosity, there are two forces which affect the orientation of the protein particles, (a) that produced by the shearing force in the liquid, and (b) the rotatory diffusion constant which is associated with the Brownian movement and whose magnitude is determined by the absolute dimensions and degree of asymmetry of the particles (35). The shearing force tends to orient the particles in the direction of flow. This orientation is opposed by the Brownian movement (rotation of the particles about their axes, arising from thermal agitation) whose effect is to cause random distribution of the particles (20). One force tends toward complete orientation, the other toward complete disorder. Thus a greater viscosity is shown by a system in which the rods are oriented at random than by a system in which the rods are oriented parallel to the direction of flow. The degree of orientation of the protein particles will depend on the magnitude of these two opposing forces. Depending on the magnitude of these two forces, an intermediate state is gradually achieved. For systems which exhibit intermediate Brownian motion, the viscosity apparently decreases as the shearing force is increased. Predominance of the shearing force causes an increased orientation of the particles in the direction

of flow, and results in decreased viscosity. This is one explanation (35) for the anomalous viscosity exhibited by protein solutions. A molecule with a greater asymmetry will be less easily oriented in the direction of flow and will contribute more to the viscosity of the system.

Eirich and collaborators (38) established a definite correlation between viscosity of solution and axial ratio of the dispersed particles in their experiments on the viscosity of models of silk fibers of different axial ratios suspended in a mixed solvent. Lauffer (38) is of the opinion that when these data and those on proteins are considered together, a good case can be established for the existence of a relationship between the intrinsic viscosity and the axial ratio of rigid particles.

According to Simha (61), "It is possible today to formulate quantitative relations between shape factors of the solute molecule and viscosity increment, in a linear concentration range, as long as appreciable orientation effects are absent. This can be done in ascribing to the molecule an effective shape corresponding to an ellipsoid, or as done by Kuhn and Huggins, to an array of spheres, which cannot change their shape to a considerable extent while moving in the viscosimeter. In these cases it is possible to undertake a comparison with sedimentation data. Orientation due to shear as well as flexibility tends to decrease the viscosity, the latter factor presumably to a lesser degree."

Another factor to be taken into account is the solvation effect (62). According to Simha (62) deviations from the laws found for spherical suspensions may be due to two causes: (a) solvation, and (b) asymmetry. However, it is not possible to separate these two effects on the basis of

viscosity measurements alone (62), so that a combination of different independent methods such as sedimentation and diffusion measurements, or measurements of dielectric dispersion must be used.

## C. Composition of Eggs.

A review of the literature on protein denaturation shows that most of the work has been done on relatively dilute solutions of pure, crystalline proteins. Food processors, instead of using pure, dilute solutions, must work with natural products. In this study, an attempt is made to apply the results of theoretical studies on heat denaturation to a workable point on liquid egg, which is a natural colloidal material of complex composition.

The composition of whole egg, egg white, and egg yolk is given by Cruickshank as follows:

| | Whole Egg | Egg White | Egg Yolk |
|---|---|---|---|
| Water | 73.7% | 87.77% | 49.0% |
| Protein | 13.4 | 10.0 | 16.7 |
| Fat | 10.5 | 0.05 | 31.5 |
| Ash | 1.0 | 0.82 | 1.5 |

Egg white which makes up 58% of the whole egg (21), is a viscous material containing a dispersion of proteins, traces of carbohydrates, and some mineral salts. The yolk makes up 31% (21) of the total weight of the egg; it is a more concentrated solution of colloidally dispersed proteins, emulsions of fat containing lecithin, and mineral salts.

### 1. Protein.

The protein content of the egg white has been shown to consist of

different proteins. Their proportions are given by Cruickshank (21) as: ovalbumin, 70%; conalbumin, 9%; ovoglobulin, 7%; ovomucin, 2%; and ovomucoid, 13%. Studies on the electrophoretic patterns of egg white (45) reveal the presence of seven components in the following proportions: egg albumin, 60%; conalbumin, 13.8%; ovomucoid, 14%; globulin, $G_1$, 2.8%; globulin, $G_2$, 4.6%; globulin, $G_3$, 4.3%; and a mucin whose accurate estimation could not be made because of its insoluble nature.

The proteins of the yolk are ovovitellin and livetin (21,33). Ovovitellin is present in larger amounts, the proportion of ovovitellin to livetin being about 4:1 (33). Vitellin is a phosphoprotein, and its phosphorus content constitutes one third of the total phosphorus in the yolk (21). Livetin contains a little phosphorus, but has a high percentage of sulfur, containing half of the total sulfur of the yolk (21,33).

2. Fat.

Cruickshank (21) gives the following information on the fat content of eggs. Except for traces in the white, the fat occurs as an emulsion in the yolk. The two types of fat in the yolk are: (a) neutral fats or glycerides, and (b) phosphorized fats or lecithins. The fatty acids present are palmitic and stearic acids, oleic acids, linoleic acid and linolenic acid. Arachidonic acid is present in very small quantities. Cholesterol may occur in yolk either in the free form or in combination with fatty acids.

3. Carbohydrate.

A portion of the carbohydrate content of whole egg is found in combination with the egg white proteins (54) and is reported to be present in the following proportions: albumin, 1.7% mannose; conalbumin, 2.8% of a 5:1 mixture of mannose and galactose; mucoid, 9.2% of a similar mixture; globulin, 4% mannose; mucin, 14.9% of a mixture of mannose and galactose. A free glucose content of 0.45% is also present (29). Neuberger (54) isolated the carbohydrate component of egg albumin and found it to have "a molecular weight of about 1200 and to be composed almost entirely of four molecules of mannose and two of glucosamine together with an unidentified nitrogenous constituent."

## D. Heat Denaturation and Coagulation of Eggs.

For the purpose of this study, the chief interest was not in the behavior of a single protein, but in the over-all conduct of the various constituents of liquid egg as they are affected by the application of heat. Only one reference (26) has been found in the literature concerning the effect of heat on liquid whole egg. The review of the literature on heat denaturation and coagulation is necessarily confined to the behavior of egg albumin.

1. Coagulation of egg white and egg yolk.

In cookery, it has been generally observed (45) that coagulation of egg white may start at 52°C. if given enough time; it coagulates to a

jelly-like consistency at 60°C.; around 64-65°C., a firm coagulum is formed.

The yolk has been observed (45) to start thickening at 65°C., and at 70°C. it ceases to flow.

2. Temperature coefficient.

That the rate of heat coagulation increases with increasing temperature is shown by the high temperature coefficient of coagulation calculated by different investigators (18,40). Chick and Martin (18) found the temperature coefficient of coagulation of a 1% egg albumin solution to be 1.9 per 1°C. rise in temperature, or about 635 for a 10°C. rise in temperature. They conclude that "heat coagulation is a reaction with a high temperature coefficient, the reaction velocity of which varies considerably with different proteins and according to the acidity and saline content of the solution." Lepeschkin (40) confirmed the data of Chick and Martin. He showed that at a certain temperature the coagulation of denatured protein proceeds more rapidly than the denaturation at the same temperature, provided the protein solution contains a sufficiently great amount of salt.

3. Effect of pH.

Chick and Martin (18) studied the effect of acidity on the rate of coagulation of a solution of egg albumin, and found that acid accelerates the coagulation rate, but that it does not hasten the rate of denaturation. The mean coagulation rate is not proportional to the hydrogen-ion concentration; the reaction velocity increases more slowly at first, and then more quickly than the hydrogen-ion concentration. The denaturation rate was

shown to increase with the increase in the concentration of hydroxyl ions, but agglutination does not occur in alkaline solution (18). Lepeschkin (40) observed that acid strongly accelerates the rate of coagulation of denatured albumin, while alkali strongly diminishes it. The decrease of coagulation velocity was not proportional to the hydroxyl-ion concentration. This is in accord with the results of Chick and Martin.

4. Effect of salts.

Coagulation is greatly influenced by the presence of neutral salts (18) and this is explained as being due to: (a) a shift in pH, and (b) neutralization or increase of the electric charge of the protein particles. Lepeschkin (40) demonstrated that the presence of salt is essential to the coagulation process. Egg white that had been dialyzed in order to decrease the salt content, did not coagulate on heating. Furthermore, he observed that the effect of salts on denaturation depends on the concentration and valence. Denaturation is accelerated by the addition of a small concentration of salts; it is diminished by a large concentration, and it is not affected by an intermediate amount of salt.

5. Effect of water content.

Barker (7) states that a decrease in water content diminishes the rate of heat denaturation of crystallizable egg albumin. The temperature of heat denaturation, which he defines as "the temperature at which half of the protein becomes insoluble in distilled water after a definite time of heating" was found to be a "linear function of the relative humidity with

which the protein is in equilibrium." According to Chick and Martin (18) denaturation is a reaction between protein and water, and egg albumin is completely soluble after exposure to dry heat.

6. Change in pH during denaturation and coagulation.

Chick and Martin (18) reported a decrease in the free acid concentration of a solution of egg albumin as coagulation proceeds. For a definite quantity of protein, the decrease in the amount of free acid is at first proportional to the concentration of free acid present, but as this concentration is increased, the amount of acid "fixed by coagulation" is no longer proportional. They suggest that the phenomenon is one of adsorption. Chou and Wu (19) studied the titration curves of natural and heat denatured egg albumin and found an increase in alkalinity which they explain as being partly due to a change in the albumin itself and partly to a liberation of ammonia. Hendrix and Wharton (30) found little change in acid-binding and base-binding power of egg albumin after heat denaturation, but found a marked decrease in the combing power after coagulation. That heat denaturation does not affect the number of titrable groups of egg albumin is reported by Booth (12).

7. Heat denaturation and viscosity.

Relatively very little information in the literature has been found on the use of viscosity as an index of the heat denaturation of egg albumin. The investigations of Anson and Mirsky (1), Loughlin, Lewis and McCullagh (44), and Bull (15) constitute the only references available. Anson and

Mirsky (1) found an increase in viscosity of an albumin solution after denaturation. Loughlin, Lewis and McCullagh (44) compared the viscosity of 3% solutions of undenatured egg albumin with that of denatured but unflocculated solutions and found that on denaturation, the viscosity of the solution increased. The increase in viscosity was found to be the same at all pH values for any given concentration of protein. They confirmed Loeb's results which showed that the viscosity is independent of pH. Bull (15) determined the viscosity of dilute (less than 1%) solutions of denatured and of native egg albumin and used the viscosity values in calculating the asymmetry of the native and denatured egg albumin molecules. The specific viscosities obtained were: 5.5 for native; 9.3 for heat denatured; and 12.3 for completely urea denatured.

Greco (26) employed viscosity measurements in studying denaturation of liquid whole egg as a function of the time and temperature of heating, within the temperature range 56-68°C. His results indicate the following: (a) at any one temperature, the rate of denaturation is a linear function of time, (b) the rate is greater at higher temperatures, (c) the rate of denaturation of aged egg is slower than that of fresh egg.

## E. Measurement of Viscosity

The choice of viscosity as a criterion of denaturation in the present study necessitates the inclusion of some fundamental background material involved in its measurement.

The concept involved in any work on the measurement of viscosity of

solutions is based upon hydrodynamic considerations; hence it might be in order to give a brief explanation of the hydrodynamic viewpoint. As stated by Lauffer (38) "....the hydrodynamic viewpoint is that, in a pure liquid undergoing plane laminar flow, infinitesimal layers of the liquid glide over one another, each layer moving with a velocity slightly less than that of its neighbor on one side. In this process energy is dissipated, accounting for the viscosity of the liquid. If a rigid, solid object, large compared to the infinitesimal layers of the liquid, i. e.,large compared to the dimensions of the fluid particles, is placed in such a flowing system, some layers of liquid will move faster than the particle and some slower. Hence liquid will have to flow around the obstruction. This disturbance in the motion of the fluid results in an added dissipation of energy by the system, that is, in increased viscosity."

The viscosity of a liquid is a measure of its resistance to shearing stress (20). The definition of viscosity according to Maxwell (10) is as follows: "...the viscosity of a substance is measured by the tangential force on a unit area of either of two horizontal planes at unit distance apart required to move one plane with unit velocity in reference to the other plane, the space being filled with the viscous substance."

The coefficient of viscosity is explained by Bingham (10) as follows: "consider two parallel planes A and B, $s$ being their distance apart. If a shearing force F per unit area give the plane A a velocity $v$ in reference to B, the velocity of each stratum, between A and B, as was first pointed out by Newton, will be proportional to its distance from B. The rate of

shear $\underline{dv/ds}$ is therefore constant throughout a homogeneous fluid under the above conditions. ...Since the force F is required to maintain a uniform velocity, this force must be opposed by another which is equal in amount due to the internal friction. The ratio of this force to the rate of shear is called the coefficient of viscosity and is usually denoted by the symbol $\eta$

$$\eta = \frac{F\ s}{v}$$

The dimensions of viscosity are $ML^{-1}T^{-1}$ ." The unit of viscosity is the "poise", and the submultiple of this unit which is one hundredth as large is known as the "centipoise" (10).

The above equation was first derived by Newton who made the assumption that the viscosity coefficient is independent of the velocity gradient of the flowing liquid. Most pure liquids have been shown to follow this rule over a wide range of velocity gradients.

There are two methods (20) of major importance for measuring the viscosity of liquids: (a) the capillary viscometer, and (b) the Couette type viscometer. This discussion will be confined to the first type. The capillary method depends on the rate of flow of the liquid through a capillary of known tube radius and length and under a known pressure. Below a certain critical velocity of flow, that is, if the flow is not too rapid, the liquid flows through the capillary in concentric cylindrical laminae, the velocity being uniform throughout any lamina. The general assumption is that the layer of liquid immediately adjacent to the wall of the capillary adheres to the wall and does not move (20).

1. Poiseuille's law.

The simple law of Poiseuille was first discovered experimentally, after which its theoretical deduction was made (10). Poiseuille investigated the effects upon the rate of flow in capillary tubes, of changes in: (a) pressure, (b) length of capillary, (c) diameter of capillary. Briefly, Poiseuille's law (10) may be stated as follows:

a. Law of pressures. For tubes of very small diameters and of sufficient length, the quantity of liquid which transpires in a given time and at a given temperature, is directly proportional to the pressure, or:

(1) $$V = K p$$

in which K is a constant, V the volume, and p the pressure head, causing the flow through the tube.

b. Law of lengths. The quantity of liquid passing through a tube of very small diameter at a given temperature and pressure varies inversely as the length, and we have:

(2) $$V = \frac{K' p}{l}$$

where l represents the length of the tube.

c. Law of diameters. The quantity traversing the tube is proportional to the fourth power of the diameter; the formula becomes:

(3) $$V = \frac{K p d^4}{l}$$

## 2. Modifications of Poiseuille's law.

From theoretical deductions made by Hagenback, Neumann, Jacobson, Helmholtz, Stephan, Mathieu, Stokes, and Bingham (10), the law of Poiseuille becomes:

$$V = \pi \frac{g\, p\, R^4\, t}{8\, l\, \eta} \qquad (4)$$

$$\eta = \pi \frac{g\, p\, R^4\, t}{8\, V\, l}$$

where g is the gravitational constant, p the pressure in grams per square centimeter, R the radius of the capillary in centimeter, l the length of the tube, V the volume of liquid flowing in time t.

According to Bingham (10), there is a considerable amount of data for which the simple law is not sufficient. He states that the law may be given greater usefulness by adding certain correction terms. Bingham (10) discusses in detail the possible sources of error, and from his considerations, it appears that under proper conditions, the only correction that is necessary to make the simple law of Poiseuille is that for the kinetic energy of the fluid as it leaves the capillary,

$$\frac{m\, \rho\, V}{8\, \pi\, l\, t}$$

in which m is a constant whose value is taken as 1.12 and $\rho$ is the density of the liquid. Bingham considers the other sources of error in detail, and comes to the conclusion that they may all be eliminated by using long, narrow capillaries, with a low velocity of flow. Thus taking into account

the loss in kinetic energy, the formula of Poiseuille becomes:

$$\eta = \frac{\pi g p R^4 t}{8 V l} - \frac{m \rho V}{8 \pi l t} \tag{5}$$

When used for a given viscometer, this formula may be written in the form:

$$\eta = C p t - \frac{C' \rho}{t} \; ; \; C = \frac{\pi g R^4}{8 V l} \; ; \; C' = \frac{M V}{8 \pi l} \tag{6}$$

in which C and C' are the constants of the instrument.

## S. The viscometer.

a. Determination of the constants of the instrument. The constant C' may be estimated by the formula:

$$C' = \frac{0.446 V}{l} \tag{7}$$

The term $\frac{C'}{t}$ of equation (6) is the kinetic energy correction, which, according to Bingham should never exceed 5% of the value of C p t. For this reason, the value of C' and of the density, $\rho$, need be known with an accuracy of 2% only in order to allow viscosity determinations to be made with only an error of 0.1%.

b. The rate of flow. Bingham (10) distinguishes the kinds of flow under three regimes: (a) viscous, linear or laminar flow, (b) turbulent flow, and (c) plastic flow. Bingham states that "it is characteristic of viscous or linear flow that the amount of deformation is directly proportional to the deforming force, and the ratio of the latter to the former gives a measure of viscosity. It has been questioned at times whether this ratio is truly constant, but it appears that only one qualification is necessary. In very viscous substances time may be necessary for the flow to reach a steady

state, aside from any period of acceleration, because with substances like pitch the viscous resistance develops slowly, so that the above ratio gradually increases when the load is first put on, but even in this case the ratio finally reaches a value which is independent of the amount of load. As, however, the deforming force is steadily increased, a point may be reached where the above ratio suddenly decreases. At this point the regime of turbulent or hydraulic flow begins. There are substances, on the other hand, for which the value of the above ratio increases indefinitely as soon as the deforming force falls below a certain minimum. These substances are said to be plastic. In plastic flow it is generally understood that a definite shearing force is required before any deformation takes place. But whether this is strictly true or not has not been established."

The rate of flow should be such that the liquid flow is laminar, not turbulent, since the Poiseuille formula does not apply to the ordinary flow of liquids in pipes. It is important to know under what conditions (the limits of velocity and dimensions of instrument) the transition occurs so that the necessary precautions may be taken to guard against it. A clear picture of the phenomenon connected with the transition from linear to turbulent flow has been given by Reynolds and is summarized by Bingham (10) and by Barr (8). Reynolds reasoned from the equation of motion (defined by the average velocity v and the diameter of the tube) that the ratio between the forces due to viscosity and those due to acceleration depend on the value of the quotient:

$$R = \frac{V d \rho}{\eta}$$

Barr (8) points out that according to this formula, the length was supposed to be without effect. The transition from laminar to turbulent flow should, therefore, take place when this quotient has a certain definite value. Since R is dimensionless, its value is the same in any consistent set of units (8). Reynolds found that Poiseuil e's law does not hold at a velocity such that

$$R = \frac{V\, d\, \rho}{\eta} = 2000$$

4. Flow-pressure relationships of colloidal systems.

Various studies have shown that for a large number of hydrophilic colloidal systems, the flow-pressure relationships do not obey Newton's law. Unlike true liquids which exhibit a linear relationship, the rate of flow of hydrophilic colloidal systems is not proportional to the pressure, but increases more rapidly than the pressure (20, 24,28,36). Hence the corresponding viscosity coefficients decrease with increasing rates of shear. It has, therefore, become the practice to refer to these values as "apparent viscosities" or "apparent fluidities" (8,28,31), "anomalous viscosity" (39), or "structural viscosity" (24). Hatschek (28) sees no reason for calling the variable resistance to shear exhibited by colloidal systems "plasticity" or "consistency" as suggested by Bingham (10). Hatschek (28) suggests that the coefficient of viscosity of these systems be defined by reference to the velocity gradient, since "single measurements at arbitrary and unknown shear gradients have no theoretical value."

Sinha (62) and Heymann (31) classify systems which exhibit non-Newtonian

flow as transitional between Newtonian liquids and plastic materials. It is immaterial whether the flow shown by colloidal systems be called "apparent viscosity", "anomalous viscosity", or "structural viscosity", as long as there is agreement that systems which show non-Newtonian flow, and which exhibit no "yield value", are transitional between Newtonian liquids and plastic materials.

Barr (8) states that Ostwald "adduces evidence to show that the complete velocity-pressure gradient function for the flow of sols through a capillary is S-shaped; the curve is convex towards the pressure axis near the origin and concave at pressures so high as to produce turbulent flow, while between the two portions there may be a straight line corresponding with normal Poiseuille flow. He regards the lower curved portion as indicating a 'structural viscosity', the structure being gradually broken down with increasing rate of shear. In some sols a definite break occurs in this part of the curve which Ostwald ascribes to 'structural turbulence", since it appears at a critical velocity much lower than would be indicated for an apparent viscosity calculated by the usual formulae. Such turbulence has been demonstrated by Hatschek and Jane and by Andrade and Lewis ....At a higher velocity the structure viscosity seems to disappear and a log $\underline{Q}$-log $\underline{P}$ diagram ($\underline{Q}$ is the rate of flow) may indicate a transition to normal viscous flow; but Haller and Trakas.... point out that since the velocity gradient is zero at the axis of a capillary tube, some structure viscosity should persist until the turbulent hydraulic regime is established."

Kraemer (36) summarizes the various proposed substitutes for Newton's

law and states that "efforts to compare these suggestions with experimental observations have been neither very numerous nor highly successful." Hatschek (28) states that as the viscosity coefficient varies with the velocity gradient, it is obvious that the behavior of the liquid cannot be defined by equations containing a single coefficient.

Bingham (10) suggested extrapolation of the straight portion of the curve to zero rate of flow to obtain a "yield value", indicated by the intersection of the pressure axis on the positive side of the origin. The "yield value" represents the pressure below which no flow would occur if there were no change of regime. Barr (8) states that Bingham's experiments were made not on colloidal solutions but on relatively coarse and concentrated suspensions, and that actually the points representing the lowest velocities observed lay very definitely off the straight line, indicating that the curve might really pass through the origin. Bingham tentatively ascribed this divergence to a change of regime - to seepage or separation of the phases. Barr adds that it has not been found possible to fit the approximately linear relation, between Q and P (Q is rate of flow) to all the data obtained for colloidal solutions especially the lyophilic sols, and that no definite "yield value" of the required order of magnitude has been observed in such systems. Farrow, Lowe and Neale (23) likewise find no evidence to satisfy the "yield value" hypothesis that no flow takes place below a certain value of the applied stress. They observed that flow took place at stresses far below those which would be assigned as "yield values." Thus for a 6% starch solution, a "yield value" of 400 dynes/cm$^2$ was calculated by extrapolation; flow was actually observed at a stress of

2.87 dynes/cm$^2$. Another objection against the "yield value" hypothesis is that the extrapolation is too arbitrary, and that it depends upon capillary size as well as the nature of the sol (13).

# EXPERIMENTAL

## Methods

### 1. Preparation of sample.

For this study, day-old pullet eggs, secured from the College poultry farm, were incubated at 30°C. for seven days and then refrigerated until ready for use. Incubation was done in order to simulate the condition of eggs used in commercial freezing and drying. The slower rate of denaturation of aged eggs as compared to fresh eggs (26) seemed more desirable for the purpose of the study.

a. Whole egg. The eggs were broken and mixed in a Waring blender for two seconds at a time using two second intervals, giving a total blending time of five minutes. The short-time blending was done to reduce foaming to a minimum. This procedure was found essential for the purpose of breaking up the structure of the egg, thus insuring a homogeneous sample for viscosity measurement. The blended egg-melange was placed in the refrigerator for a few hours and the small amount of foam skimmed off the surface.

b. Egg white. The whites were separated from the yolks, and mixed in a Waring blender one second at a time using two second intervals for a total blending time of five minutes. The sample was allowed to stand in the refrigerator for a few hours and the foam skimmed off.

c. Egg yolk. The yolks were separated from the whites, and each yolk

carefully washed with running water to remove all traces of white. The yolk was then transferred from one hand to the other, each time wiping the hand dry on a towel; this served to remove the excess water from the washing procedure. It was then transferred to a Petri dish and allowed to stand for a few minutes before picking off the chalazae with a pair of forceps, breaking the yolk membrane and pouring the supposedly pure yolk (free of membrane) into fruit jars. To insure uniformity of yolk samples, the percentage total solids was determined for each batch and it was found to vary from 50.5 to 51% total solids. Homogenization of the sample was accomplished by the use of the Waring blender as in the whole egg sample.

2. Treatment of samples.

a. Instantaneous heat treatment. The prepared sample (at 25°C.) was brought up to the desired temperature nearly instantaneously by the use of a special heat exchanger arrangement (Fig. 1). Water used for heating the sample was pumped from a constant-temperature water bath into the condenser. The egg sample, in a one liter suction flask, was driven into the spiral part of the condenser by the use of air-pressure at 60 centimeters as registered by a mercury manometer. A copper-constantan thermocouple connected to a Leeds and Northrup (378961) potentiometer was inserted by the use of a T-tube at the outlet of the heating condenser to record the final temperature attained by the sample during the heat treatment. For whole egg, it was noted that a temperature differential of only 1.5°C. (between the temperature of the water bath and the final temperature of the sample) was required to bring the temperature of the sample nearly instantaneously from 25°C. to the desired temperature. For whites, a temperature differential of 1.5°C. was required,

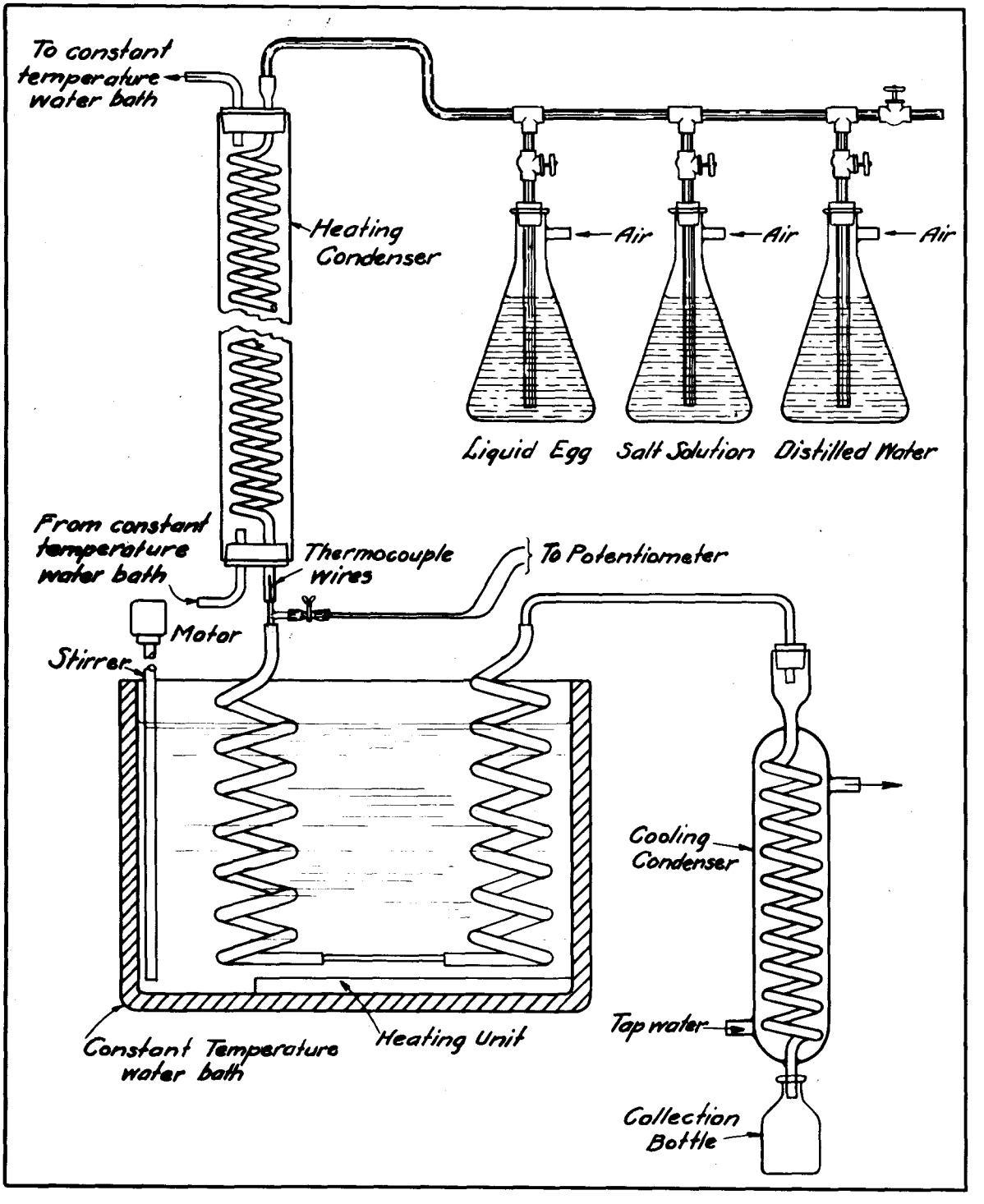


Fig. 1 EXPERIMENTAL PASTEURIZER

and for yolks, 2°C. The time required for the heat treatment varied for the three products as follows: whole egg, 8.5 ± 0.5 seconds; egg white, 6.2 ± 0.5 seconds; egg yolk, 21 ± 1.5 seconds.

In order to determine the highest pressure effective in bringing the temperature of the sample from 25°C. to the desired temperature, in the shortest time, a study was made of the temperature attained in the sample by the use of varying pressures. As a result of this study, it was decided that a pressure of 60 centimeters mercury would be the most effective.

It was not known whether the sample reached the final temperature before it reached the outlet of the condenser where the thermocouple was placed. The total heating time required, as recorded by a stopwatch as the sample passes into and out of the heat-condenser, may indicate one of two things: (a) time required for the sample to just reach the final temperature, or (b) time required for the sample to reach the final temperature plus some seconds held at that temperature.

b. Holding periods. After the temperature of the sample was brought up to the desired temperature as indicated by the thermocouple, the sample was held at that temperature for varying periods of time by continuous flow through different lengths of rubber tubing immersed in a constant temperature water bath. The time of holding was recorded by a stopwatch. In order to keep the rubber tubing immersed in the bath, individual 12 feet lengths were coiled around frames of mesh screen, and successive lengths joined by glass tubing.

c. Cooling. The heated samples were cooled to about 19°C. by running

them directly into a water condenser. The cooled samples were collected in prescription bottles, stoppered tightly and stored in the refrigerator overnight for viscosity measurements.

3. Measurement of viscosity.

Viscosity was chosen as a quantitative index of denaturation in preference to solubility and the determination of the number of disulfide and sulfhydryl groups, for several reasons. It seems to be the most suitable method that can be employed in following the progress of denaturation of a mixture of proteins, by virtue of its being a physical measurement of the total effect of heat on the system as a whole. Chemical methods seem to be more adaptable to pure protein solutions. Viscosity is a sensitive criterion; it enables the detection of denaturation at the early stages. It is easily reproducible, and it is relatively simple and convenient to measure.

a. Capillary viscometer. A viscometer patterned after the one devised by Bateman and Sharp (9) was employed (Fig 2). It consisted of a convenient length of thermometer tubing which served as capillary tube, a piece of Pyrex tubing into which the sample was poured, and a centrifuge tube which served as a receiver. Rubber stoppers, metal sheets, bolts, and wing nuts were used to hold them in place. This whole set-up was placed in a 25°C. water bath provided with a stirrer and a thermo-regulator and connected to a manometer which registered the amount of pressure applied. The pressure was regulated by a reducing valve set between the pressure unit and the tank.

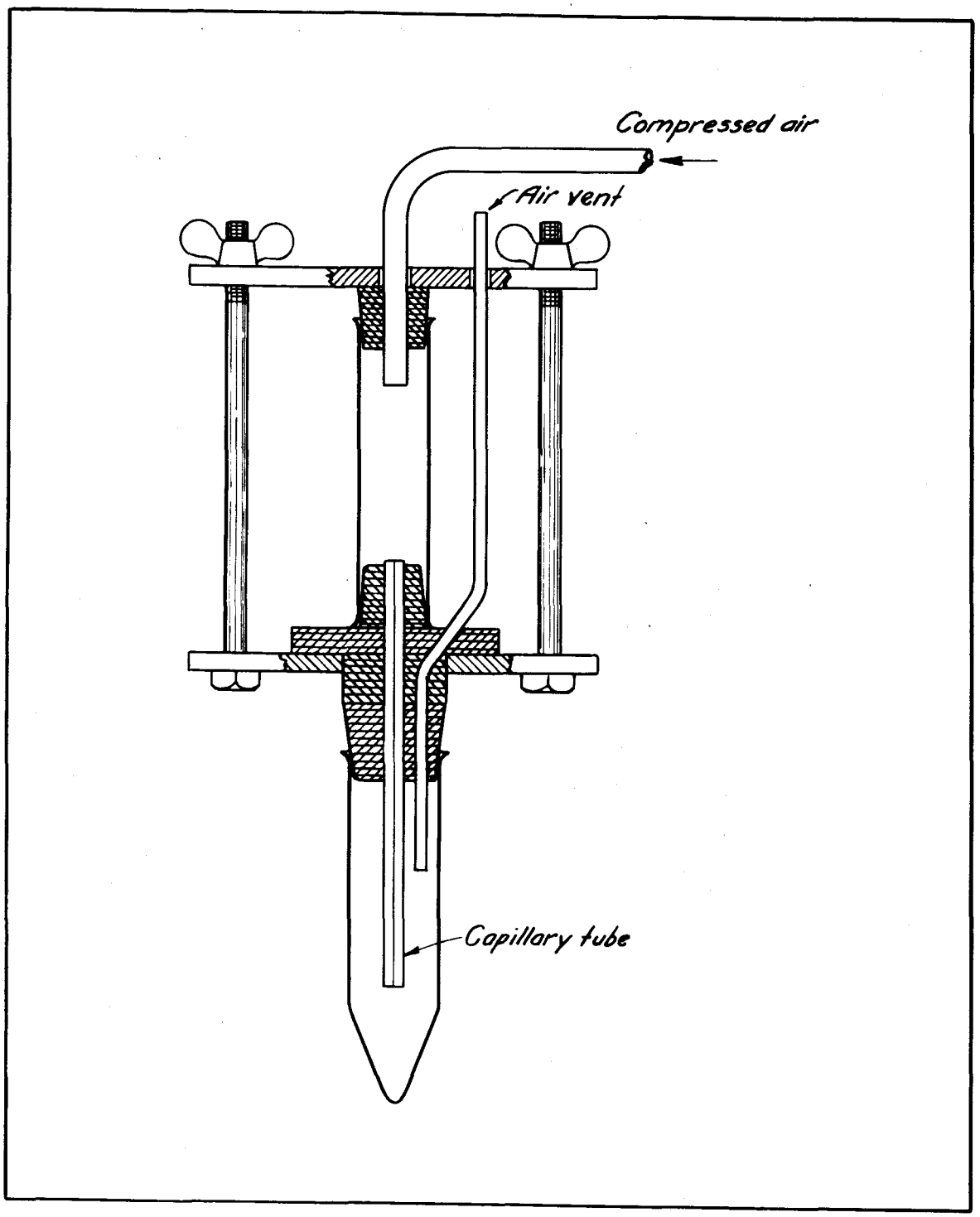


Fig. 2. CAPILLARY VISCOMETER

b. Selection and calibration of capillary tube. Detailed directions for the calibration of the capillary tube as given by Bingham (10) and by Barr (8) were followed. The methods used were: (a) the microscopic method and (b) the mercury thread method. Having determined the radius, the length of the capillary tube was calculated. Barr (8) and Bingham (10) agree that in the design of a capillary viscometer, there should be a certain ratio between the bore and the length of the capillary tube in order to minimize the error due to kinetic energy. Barr suggests the use of a capillary tube whose length is at least 300 times its radius, while Bingham suggests a length of at least 500 radii.

c. Viscosity data. The sample (in 250 cubic centimeter centrifuge bottles) was centrifuged in an International Size 2 Centrifuge at a speed of 1600 r. p. m. for thirty minutes. The supernatant liquid was decanted and its viscosity determined. Thirty cubic centimeters of the liquid at 25°C. were poured into the viscometer, the apparatus set in position and connected to the manometer. A convenient number of drops was counted, and the time recorded. Preliminary work showed that recording the time for a certain number of drops was more accurate and gave more easily reproducible results than recording the time for the flow of a definite volume of liquid, since it was very difficult to see the meniscus of the liquid egg. The weight of a specific number of drops (to make nearly one cubic centimeter) was recorded. The density of the sample was determined at 20°C. by the use of a pyknometer; from the density of the sample and the weight of the drops, the volume was calculated.

(1) Calibration of the viscometer with sucrose solution. In order to insure the absence of any peculiarities of the apparatus, the viscometer was calibrated by the use of 40% and 60% sucrose solutions. The calculated viscosities of the sucrose solutions were found to check with the figures as given by Bingham (10).

(2) Pressure corrections. The pressure (in grams per square centimeter) to be used in calculating the coefficient of viscosity was corrected according to specifications by Bingham (10). The corrections made were: (a) for temperature, and (b) for the hydrostatic head exerted by the material.

# RESULTS

The apparent viscosity obtained by the use of the capillary viscometer is expressed in centipoises, although objection will be found in expressing the apparent viscosity of a non-Newtonian system in terms of the absolute unit of viscosity, the poise. However, Robinson (60) gives some justification for this. He states, "In the commonly accepted sense, such a quantity is not a viscosity coefficient at all, and to emphasize this, it is usually called an 'apparent viscosity'. It will still, however, be a measure of the rate of dissipation of energy in the system per unit volume and (velocity gradient)$^2$. Since this is what a viscometer actually measures, there may be less objection to calling it a viscosity than is often supposed."

## A. General Observations

### 1. Liquid whole egg.

In the heat treatment of liquid whole egg, very few visible changes were observed. A small quantity of a somewhat "powdery" sediment was obtained after the sample was centrifuged, prior to measuring its viscosity. This sediment was observed in the native as well as in the heated whole egg. However, in the heated sample, a small amount of gelatinous precipitate was obtained in addition to the sediment.

2. Liquid egg white.

Interesting observations were noted in the instantaneous heat treatment of liquid egg white at several different temperatures and at several periods of heating. At least three stages were observed, namely, turbidity, gel formation, and precipitation. In the study of denaturation as a function of the temperature of heating, turbidity was observed in the sample heated to 62.5°C. At 63°C., the liquid white was clear, with a gelatinous mass that collected on the top of the liquid. Above 63°C., increased turbidity and precipitation occurred up to 66°C., at which temperature the liquid became clear again and a mass of gelatinous material collected on the top. Beyond 66°C., turbidity became evident again, increasing progressively until a white coagulum was formed. At 70°C., the heat treated white gave off a "cooked egg" odor, and the consistency of the coagulum was similar to the white of a soft-boiled egg.

In the time study, the same stages were observed. After holding the sample at 62.5°C. for a period of about 20 seconds, no turbidity was observed, but a trace of gelatinous precipitate was obtained after centrifugation. After holding the sample for about 45 seconds, turbidity was evident. Loose gelatinous flocs were formed occupying a large portion of the volume of the liquid. After a holding period of 70 seconds, an increase in the volume of the gel was observed. Even after centrifugation, it was very difficult to pipette out the supernatant liquid, as the loose gelatinous flocs occupied almost the whole volume of the liquid. On further holding (90 seconds) there were less flocs, and the turbidity of the supernatant liquid was less

than those of the samples held at 46 and 70 seconds. At a holding period of 145 seconds, a transition was observed. The gel became slightly opaque and started to differentiate into segments, which on further holding formed into particles similar to swollen rice grains. With still longer holding at 62.5°C., the gel became progessively opaque, reaching a maximum whiteness at a holding period of 445 seconds.

In general at 62.5°C., the region of gel formation occurs between 46 and 145 seconds holding time. Within this range, the volume of the translucent gel became greater with increased periods of holding. After 145 seconds, a transition from the gel form to a coagulum was evident from the nature of the precipitating material. A slight opaqueness was first observed at this point, and with further holding the opaqueness increased until a maximum quantity of white coagulum was formed at 445 seconds.

3. Liquid egg yolk.

No visible changes occurred in the heat treatment of liquid egg yolk. On centrifugation of the samples, no sediment nor gelatinous material collected at the bottom of the centrifuge bottle.

## B. Flow-pressure Relationships

A study was made of the effect of pressure on the rate of flow of liquid whole egg, egg white, and egg yolk in the capillary viscometer. The results are shown in Fig. 3 and Table 1. It will be noticed that the rates of flow are linear with pressure, for the pressures employed.

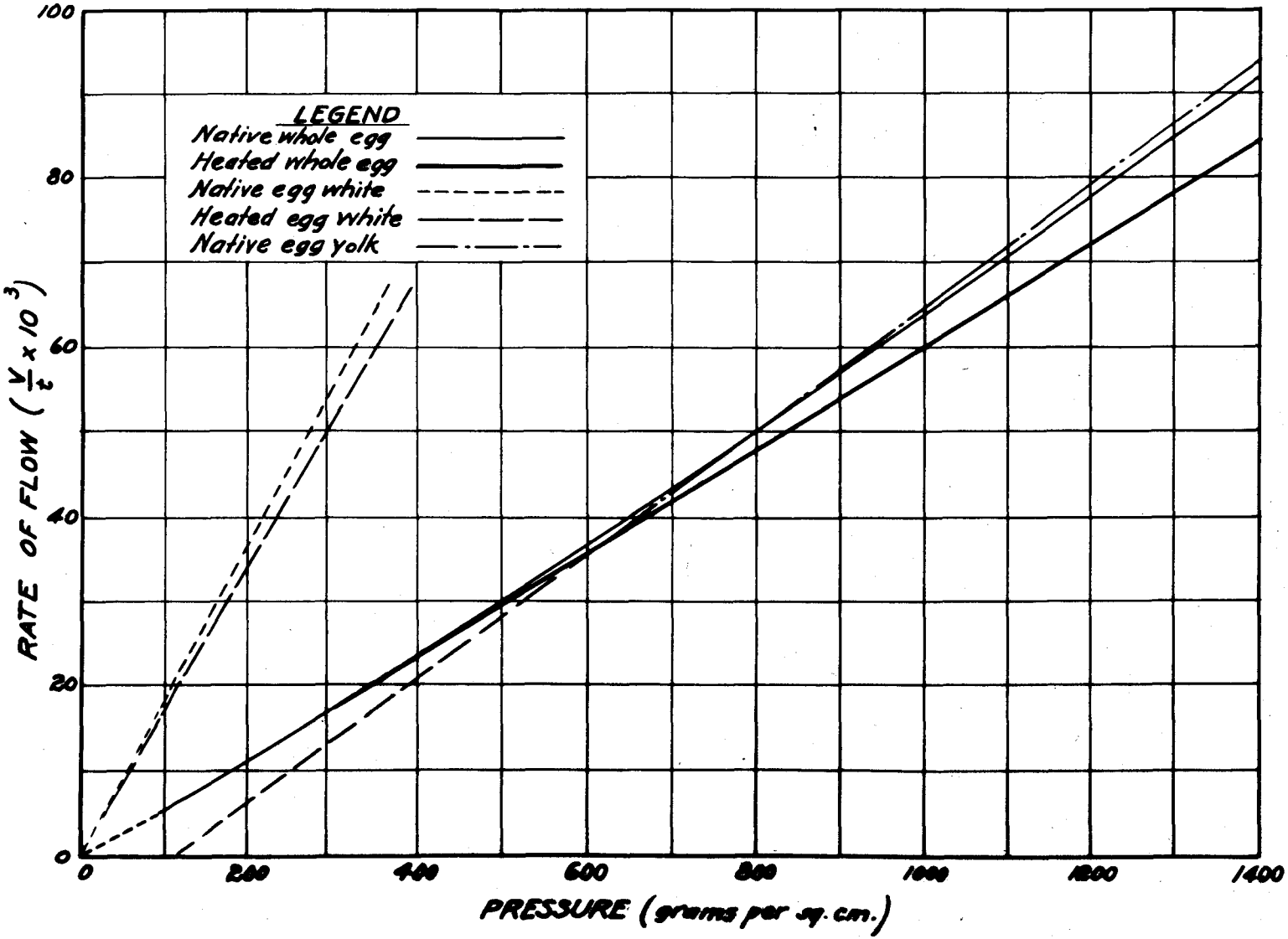


Fig. 3 Effect of pressure on the rate of flow of liquid whole egg, egg white and egg yolk.

Table 1

Rate of Flow of Liquid Whole Egg, Egg White, and Egg Yolk
in Viscometer at Various Pressures

| Pressure gms/sq.cm | Rate of Flow $V/t \times 10^3$ Whole Egg | | Pressure gms/sq/cm | Rate of Flow $V/t \times 10^3$ Egg White | | Pressure gms/sq.cm | Rate of Flow $V/t \times 10^3$ Egg Yolk |
|---|---|---|---|---|---|---|---|
| | Native | Heated | | Native | Heated | | Native |
| 90.1 | 5.2 | | 90.1 | 16.2 | 15.6 | 566.2 | 35.5 |
| 157.7 | 9.0 | | 157.7 | 29.2 | 26.0 | 836.9 | 53.5 |
| 225.5 | 13.4 | | 295.1 | 54.5 | 51.0 | 1107.5 | 71.7 |
| 293.1 | 17.4 | 17.0 | | | | 1310.4 | 90.0 |
| 428.3 | 26.0 | | | | | | |
| 563.7 | 34.1 | 32.8 | | | | | |
| 685.5 | 41.8 | | | | | | |
| 834.4 | 51.3 | 49.9 | | | | | |
| 969.6 | 62.0 | | | | | | |
| 1105.0 | 71.4 | 66.8 | | | | | |

This linear relationship may not hold at extremely high pressures, since it has been shown by numerous investigators that colloidal systems exhibit a non-Newtonian flow. However, for systems of low concentration, the decrease in apparent viscosity with increasing velocity gradient is usually insignificant (35). It will be seen that by extrapolation to zero pressures, all the lines pass through the origin except the line representing liquid yolk, which intercepts the pressure axis to the right of the origin, showing a "yield value". However, since the intercept made on the pressure axis was obtained by extrapolation alone, it may not represent a true "yield value". It seems likely that at extremely low pressures, the linear relationship may not hold and that the line curves toward the origin, and therefore, would indicate no "yield value".

It was not considered important to attempt to establish the existence of a "yield value" for egg yolk, since the main interest was not in the viscosity as such, but only in its employment in following the detection and progress of denaturation as a function of the time and temperature of heating.

### C. Denaturation as a Function of Temperature of Heating

The employment of a nearly instantaneous method of heating the samples was devised in an effort to separate the effect of the temperature of heating from that of the time element. It was hoped that by this method, the process of heat denaturation could be followed as a function of the temperature of heating alone, since the sample was heated to the desired temperature within

a very short period of time. The viscosity of the samples that were heated to the desired temperatures are plotted against the temperatures reached in passing through the heating condenser.

1. Liquid whole egg.

For whole egg Figure 4 and Table 2 show that the viscosity increases with rise in temperature of heating until 66°C. is reached. Then a drop in viscosity is observed with further rise in temperature. A considerable amount of precipitate was centrifuged out of the sample which showed a decrease in viscosity. At 67°C., the viscosity increases again, and continues to increase with rise in temperature until coagulation sets in, as indicated by the dotted lines, above 73°C.

2. Liquid egg white.

The viscosity of egg white is linear with temperature of heating up to 62.5°C. (See Figure 5 and Table 2). Above 62.5°C., the curve becomes irregular. This irregularity of the viscosity-temperature curve indicates the rise and fall of viscosity which is observed when precipitation of the protein occurs. Notice that viscosity is greatest at 63°C. and at 66°C. At these two temperatures, no turbidity was observed in the liquid white; however, a gelatinous mass collected on the top of the liquid. Evidently, at these two points, the viscosity is higher than that at other temperatures of heating because of the absence of considerable precipitation. The difference in the effect of heat on the precipitation of each of the egg

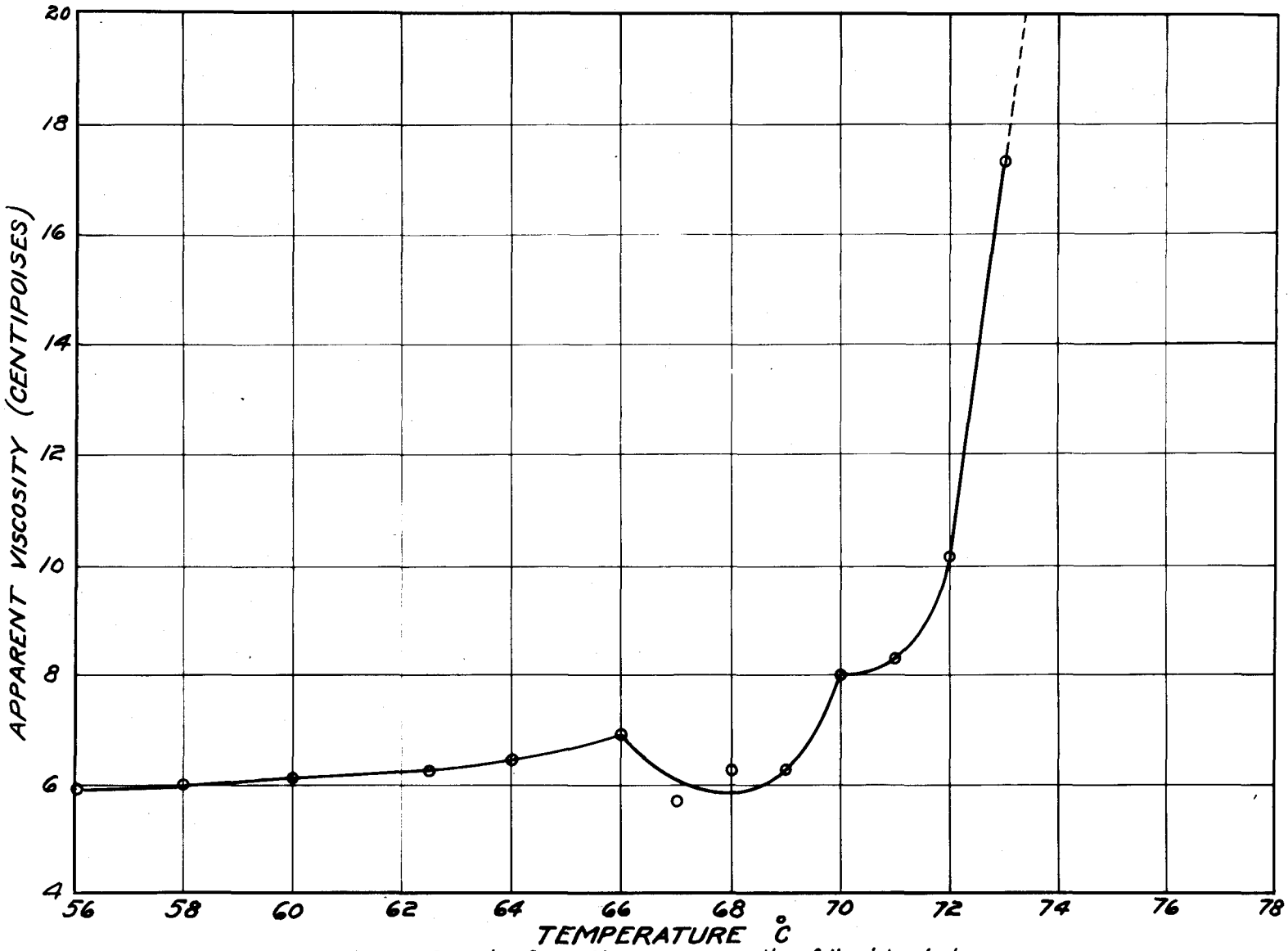


Fig. 4. Effect of heating on viscosity of liquid whole egg.

Table 2

Apparent Viscosity (in centipoises) of Liquid Whole Egg, Egg White and Egg Yolk as a Function of Temperature of Heating

| Temperature °C. | Apparent Viscosity (centipoises) | | |
|---|---|---|---|
| | Whole Egg | Egg White | Egg Yolk |
| 56 | 5.98 | | |
| 58 | 6.06 | 2.48 | |
| 60 | 6.16 | 2.5 | |
| 62.5 | 6.26 | 2.5 | 832.4 |
| 63 | | 2.8 | |
| 64 | 6.46 | 1.45 | |
| 65 | | 1.59 | 840.0 |
| 66 | 6.79 | 2.58 | 864.3 |
| 67 | 5.70 | 1.75 | |
| 68 | 6.32 | 1.24 | 943.4 |
| 69 | 6.32 | 1.3 | |
| 70 | 8.02 | | 1108.0 |
| 71 | 8.26 | | |
| 72 | 10.2 | | |
| 73 | 17.36 | | |

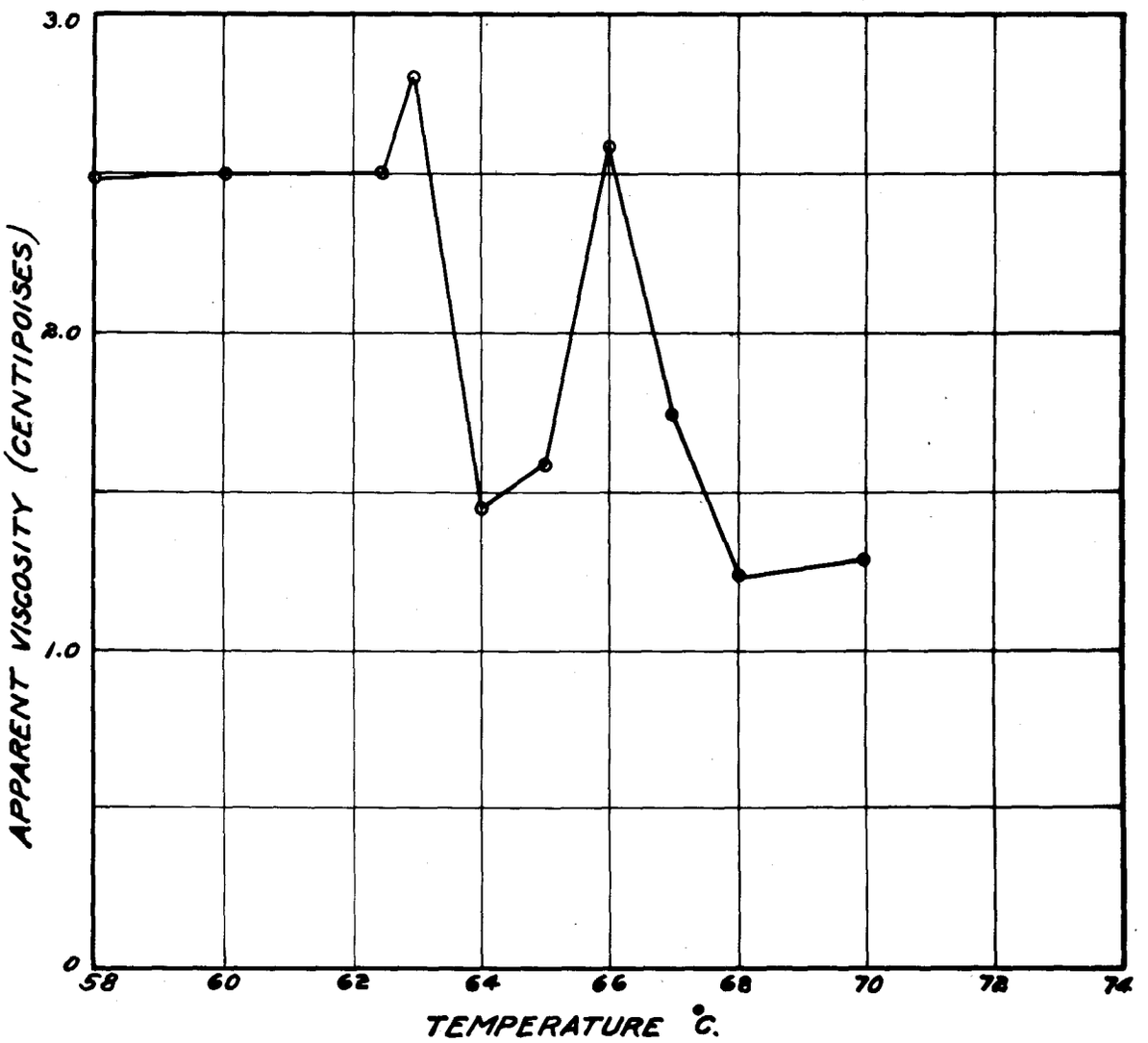


Fig. 5 Effect of heating on viscosity of liquid egg white.

white proteins probably accounts for the irregular character of the viscosity-temperature relationship.

3. Liquid egg yolk.

The viscosity of egg yolk as it is affected by the temperature of heating is shown in Figure 6 and Table 2. As in whole egg and in egg white, the effect of rise in temperature is to increase the viscosity of the yolk. However, yolk did not show any drop in viscosity before the onset of coagulation, possibly due to the different nature of the proteins in the yolk. The increase in viscosity became greater at the higher temperatures, until a maximum viscosity was reached at 70°C., above which the sample coagulated in the heating condenser, as indicated by the dotted lines.

## D. Denaturation as a Function of Time of Heating

1. Liquid whole egg.

The effect of the time of heating at 62.5°, 64.5°, and 66°C. on the viscosity of liquid whole egg is shown graphically in Figure 7 and Table 3. The increase in viscosity is linear with time for the temperatures studied. The slopes of the lines indicate that the increase in viscosity with time of heating is greater at the higher temperatures. As shown in Table 3 no viscosity change was detected at 56°C.

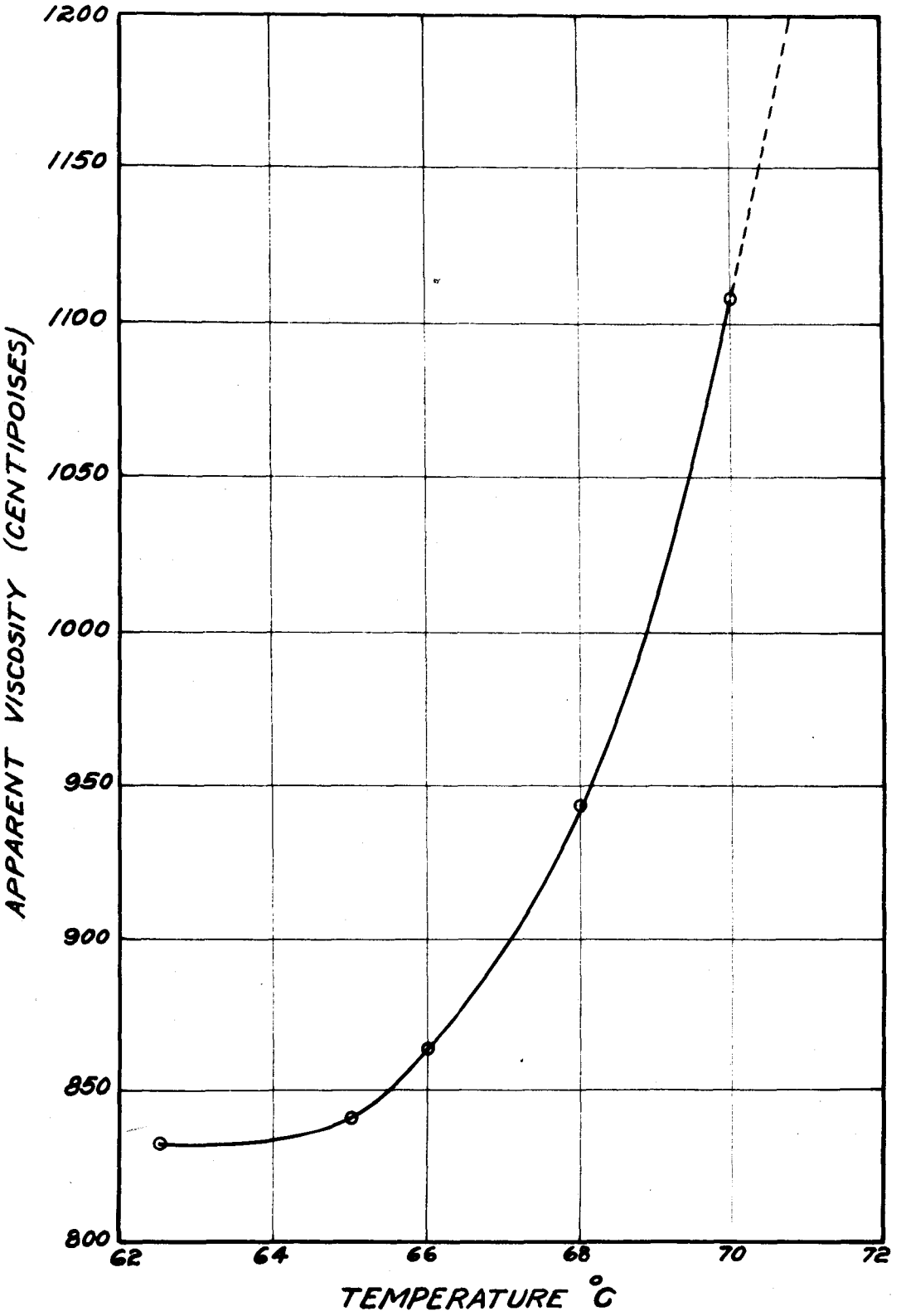


Fig. 6 Effect of heating on viscosity of liquid egg yolk.

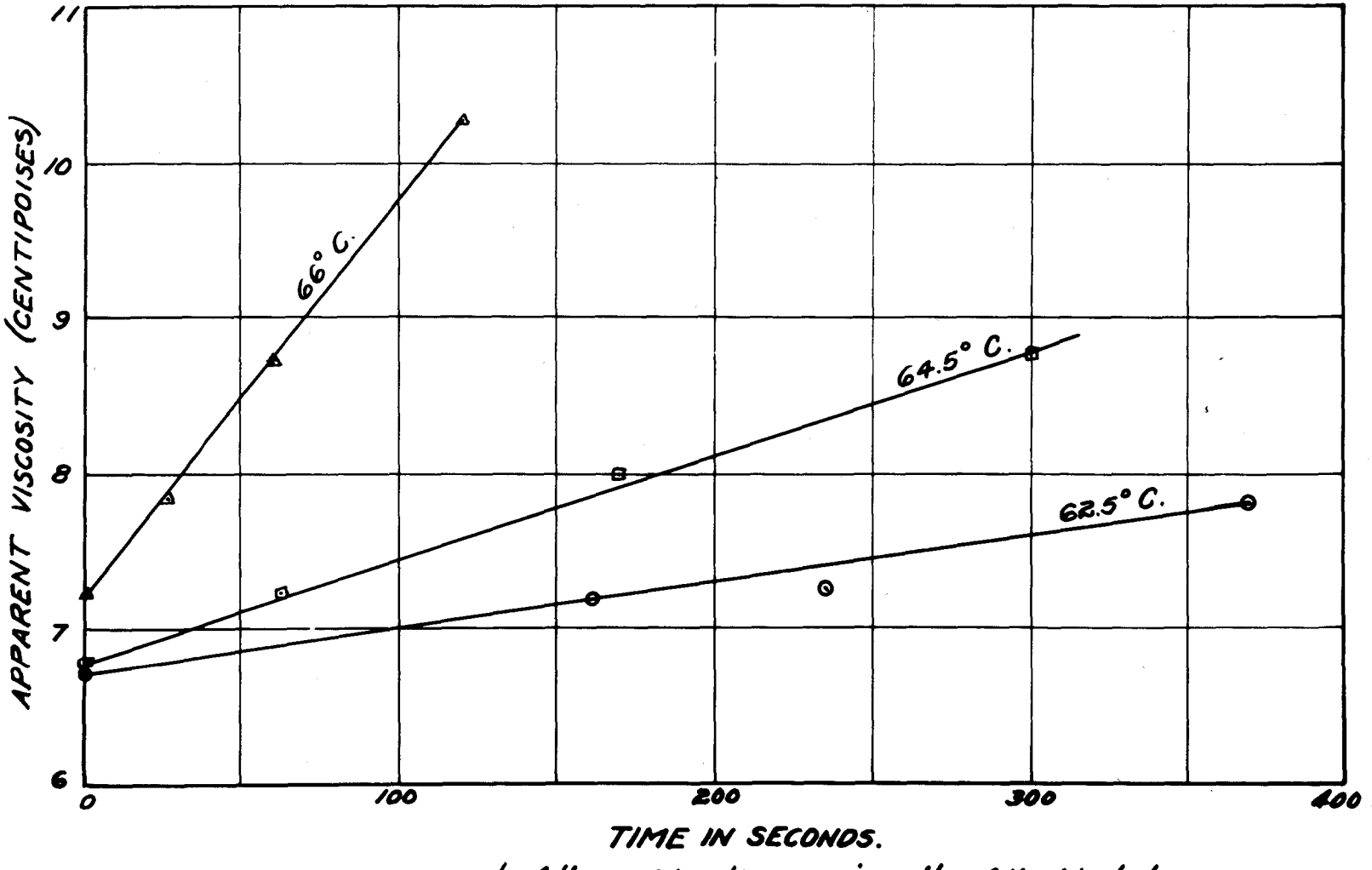


Fig. 7 Effect of time of heating on viscosity of liquid whole egg.

Table 3

Apparent Viscosity (in centipoises) of Liquid Whole Egg as a Function of Time of Heating at 56°, 62.5°, 64.5°, and 66°C.

| Time of Heating (seconds) | Temperature of Heating °C. | | | |
|---|---|---|---|---|
| | 56°C. | 62.5°C. | 64.5°C. | 66°C. |
| 0 | 6.43 | 6.68 | 6.69 | 7.23 |
| 28 | | | | 7.83 |
| 61 | | | | 8.74 |
| 63 | | | 7.26 | |
| 119 | | | | 10.2 |
| 163 | | 7.20 | | |
| 170 | | | 8.00 | |
| 236 | | 7.26 | | |
| 300 | | | 8.75 | |
| 370 | | 7.82 | | |
| 2400 | 6.63 | | | |
| 3300 | 6.5 | | | |
| 5100 | 6.67 | | | |

Since the viscosity-time relationships are linear (Figure 7), the rate of denaturation was calculated for each temperature as the increase in viscosity per unit time held at that temperature. The rate of denaturation

$$\frac{\Delta \eta}{\Delta t} \times 100$$

was obtained by calculating the slope of the line (Figure 7) for each temperature used. These values were then plotted against temperature, as shown in Figure 8 and Table 4. It will be noticed that an increase in viscosity is

Table 4

Rate of Denaturation $(\frac{\Delta \eta}{\Delta t} \times 100)$ of Liquid Whole Egg at 56°, 62.5°, 64.5°, and 66°C.

| Temperature °C. | | | |
|---|---|---|---|
| 56°C. | 62.5°C | 64.5°C. | 66°C. |
| 0 | 0.3 | 0.76 | 2.49 |

observed above 56°C.; the rate is slow at first, but with rise in temperature, the increase in the rate becomes disproportionately greater, reaching a maximum at 66°C.

These results are to be expected. The temperature coefficient of coagulation of egg albumin was found by Chick and Martin (18) to be 1.8 per 1°C. rise in temperature.

2. Liquid egg white.

The effect of the time of heating on the viscosity of egg white is shown

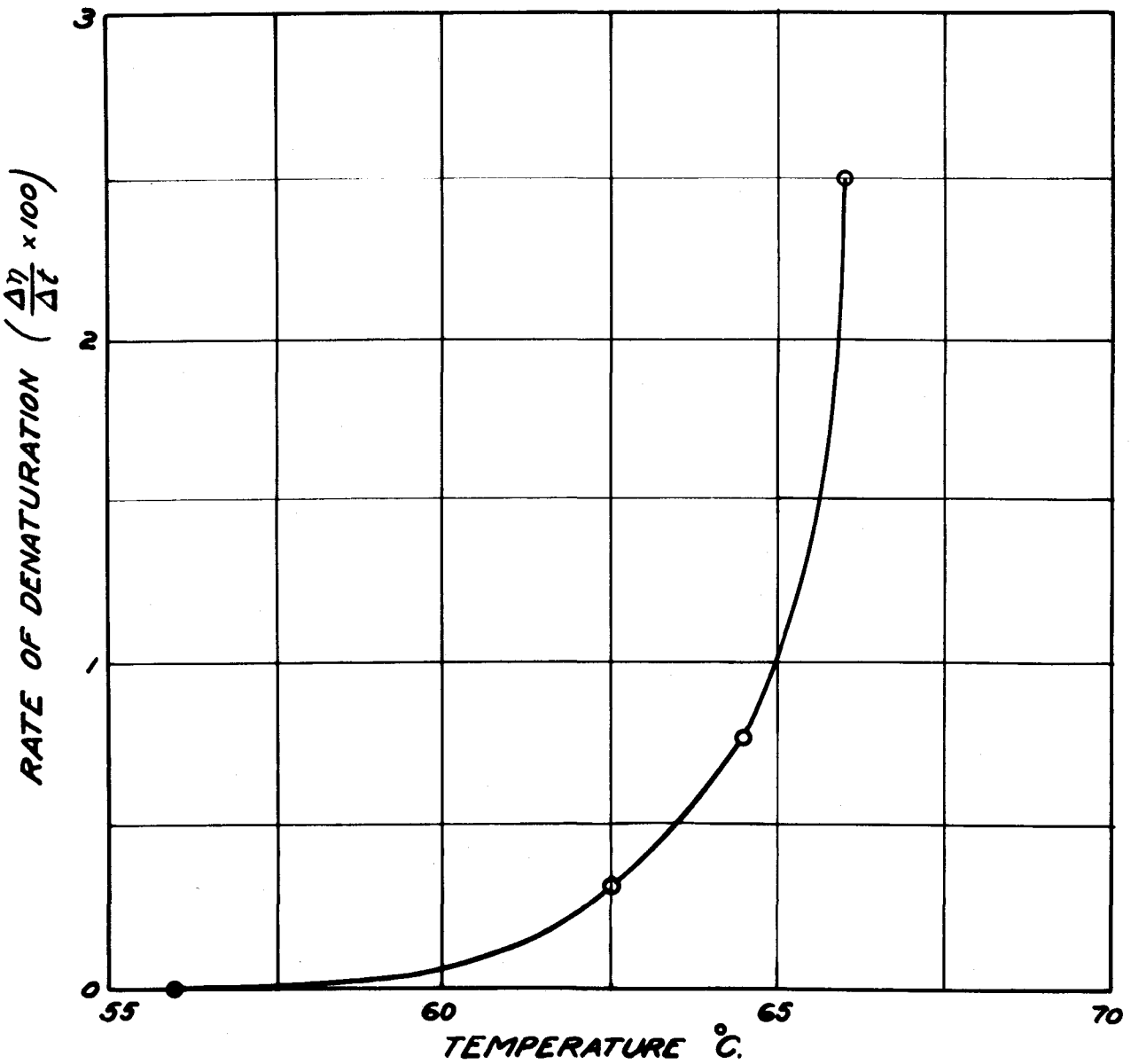


Fig. 8. Effect of heating on rate of denaturation of liquid whole egg.

graphically in Figure 9 and Table 5. It will be noticed that no change in viscosity was detected at 58°C. At 60°C., the rate is quite slow, but it increases with time. At 62.5°C., the rate of denaturation is more rapid than at 60°C. Maximum viscosity is reached at a heating time of 46 seconds. With further holding, viscosity decreases. As described earlier, the drop in viscosity is accompanied by turbidity, flocculation and coagulation of the egg white proteins. These stages are indicated in the curve (Figure 9) as follows: A corresponds to the time of holding at which turbidity becomes evident; flocs of gelatinous material were also observed. At B, there was an increase in the volume of the flocculent material, such that it occupied almost the whole volume of the liquid. At C, the flocculent material became slightly opaque and started to separate into segments. At point D, the coagulum formed into particles similar to swollen rice grains, and the supernatant liquid was decidedly more fluid. At E, the coagulum had a slightly firmer consistency.

The drop in viscosity may be explained by the fractional precipitation of the various proteins at different periods of the heating. When the heated samples were centrifuged, prior to the measurement of viscosity, a gelatinous precipitate separated. This portion was not used in the viscosity determination. Unless this gelatinous mass imbibed a considerable amount of water, it would seem that the precipitation of the protein would decrease the protein content of the system, and therefore, decrease the viscosity. It has been shown (15,38) that viscosity is a function of protein concentration. This phenomenon may indicate the end of denaturation and the beginning

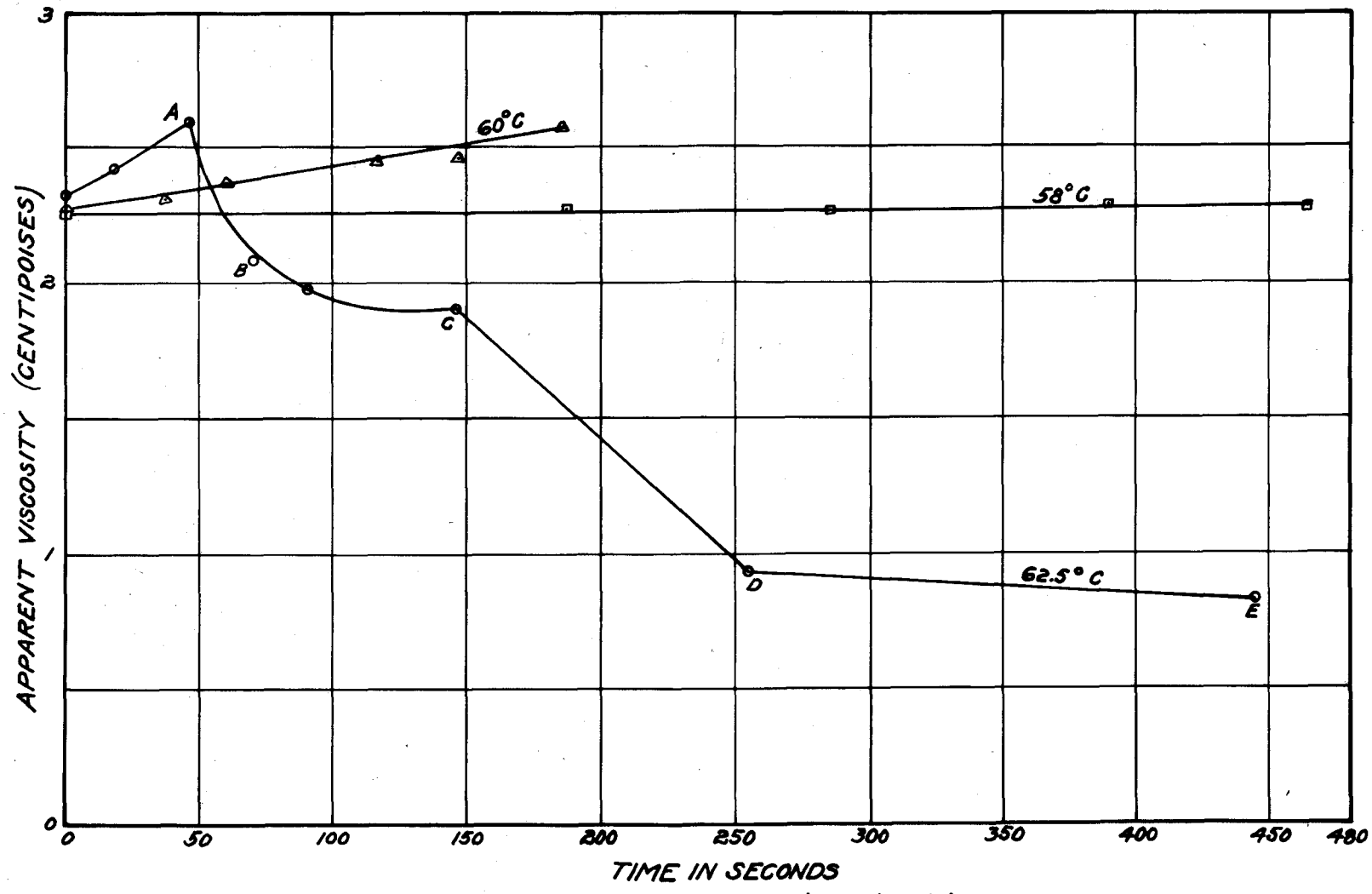


Fig. 9. Effect of time of heating on viscosity of liquid egg white.

Table 5

Apparent Viscosity of Liquid Egg White as a Function of Time of Heating at 58°, 60°, and 62.5°C.

| Time of Heating (Seconds) | Temperature of Heating °C. | | |
|---|---|---|---|
| | 58°C. | 60°C. | 62.5°C. |
| 0 | 2.48 | 2.53 | 2.53 |
| 8 | | | |
| 17 | | | |
| 19 | | | 2.63 |
| 32 | | | |
| 36 | | 2.53 | |
| 46 | | | 2.80 |
| 70 | | 2.60 | 2.29 |
| 76 | | | |
| 90 | | | 2.18 |
| 116 | | 2.71 | |
| 146 | | 2.67 | 2.11 |
| 186 | | 2.80 | |
| 187 | 2.48 | | |
| 256 | | | 1.14 |
| 285 | 2.48 | | |
| 390 | 2.48 | | |
| 445 | | | 1.03 |
| 464 | 2.48 | | |

of precipitation, the second step in the process of heat coagulation. Hydrophilic micelles are stabilized by the existence of electric charges on the surface of the particles, and by a shell of water surrounding the micelle, called the water of solvation. Precipitation will not occur until the electric charges are neutralized and the micelles are desolvated, possibly by the electrolytes present in egg white. Gortner (25) states that solvation is easily followed by changes in viscosity. He cites as an example the very rapid fall in viscosity which accompanies the addition of traces of inorganic salts to an acidulated flour-water suspension. He explains this fall in viscosity as follows: "It would appear as if a large fraction of the dispersion medium had been oriented on the surface of the protein micelles, by electrical forces, and that this oriented water shell had been released by the neutralizing effect of the inorganic salt."

A similar mechanism may occur in the liquid egg white. The process of denaturation may be accompanied by changes in pH, due to any number of causes (reviewed under heat denaturation). The shift in pH may cause desolvation of the micelles by the salts present in the system, and thus cause a decrease in viscosity. The view that decrease in viscosity is due to decrease in hydration of the micelles seems to fit in as an explanation for this drop in viscosity.

Meyers and France (53) explain the increase in viscosity (emptying periods) of egg albumin solution in terms of the degree of unwinding of the polypeptide chains and the degree of solvation. The maximum viscosity corresponds to (a) the highest degree of randomness in the configuration

of the polypeptide chain, and (b) maximum solvation. Thus a decrease in the degree of solvation may result in "coagulated systems of low viscosities".

Jirgensons (32) followed the heat denaturation and degradation of various globular proteins (egg albumin included) viscometrically and found that viscosity increases at first until a maximum is reached, and then decreases. He explains the increase in viscosity as being due to the increase in the degree of asymmetry of the protein particle brought about by the unwinding of the molecular ball. On decomposition, hydrolysis of the chief valence chains occurs, thus causing a decrease in the degree of asymmetry, and decreased viscosity.

3. Liquid egg yolk.

The viscosity of native liquid egg yolk, containing 49-49.5% water, was found to be about 800 centipoises at 25°C. This value is approximately 100 times as large as the viscosity of liquid whole egg. Moran (51) calculated the viscosity of fresh yolk with a water content of 47-47.7% to be approximately 2500 centipoises, at 18-19°C. Comparison of Moran's value with that obtained in this study reveals some discrepancy. This may be explained as being due to the temperature at which viscosity was measured, and the water content of the yolk sample used. Moran (51) found the viscosity of yolk to be a function of the water content. From Moran's graphical illustration of the effect of water content on the time of outflow, it was found that an increase in water content from 47.7% to 49.5% corresponds to an increase of about 54% in the outflow time, which would indicate a smaller viscosity value for the sample containing more

water. Thus a yolk sample of 49.5% moisture would have a viscosity of about 1150 centipoises. The difference in the temperature of measurement and the limitations of the method used probably account for the rest of the discrepancy. A liquid exhibits increased fluidity or decreased viscosity with rise in temperature.

The change in viscosity of liquid egg yolk with time of heating at 62.5 and 65°C. is shown in Figure 10 and Table 6. It is evident that the viscosity of yolk increases with the period of time held at one temperature, until a maximum is reached. Further holding at that temperature results in a progressive decrease in viscosity. At 62.5°C., the yolk was not held long enough at that temperature for coagulation to occur. At 65°C., it was found that 477 seconds was the longest period of time that the yolk could be held; with further holding, no sample could be collected, as the rate of flow became very slow until it finally ceased and coagulation occurred. It will be noticed that the rate of change in viscosity is much greater at 65°C. than it is at 62.5°C.

The decrease in viscosity shown by liquid egg yolk as a function of the period of heating probably indicates a decrease in the degree of stability of the micelles of the disperse phase. An explanation of this phenomenon will be attempted on the basis of the behavior of the constituents of the liquid yolk, namely, the yolk proteins, the fat present as an emulsion, and the lecithoprotein, which is the emulsifying constituent of egg yolk (45), as they are affected by heat.

The stability of a protein in colloidal dispersion depends on the

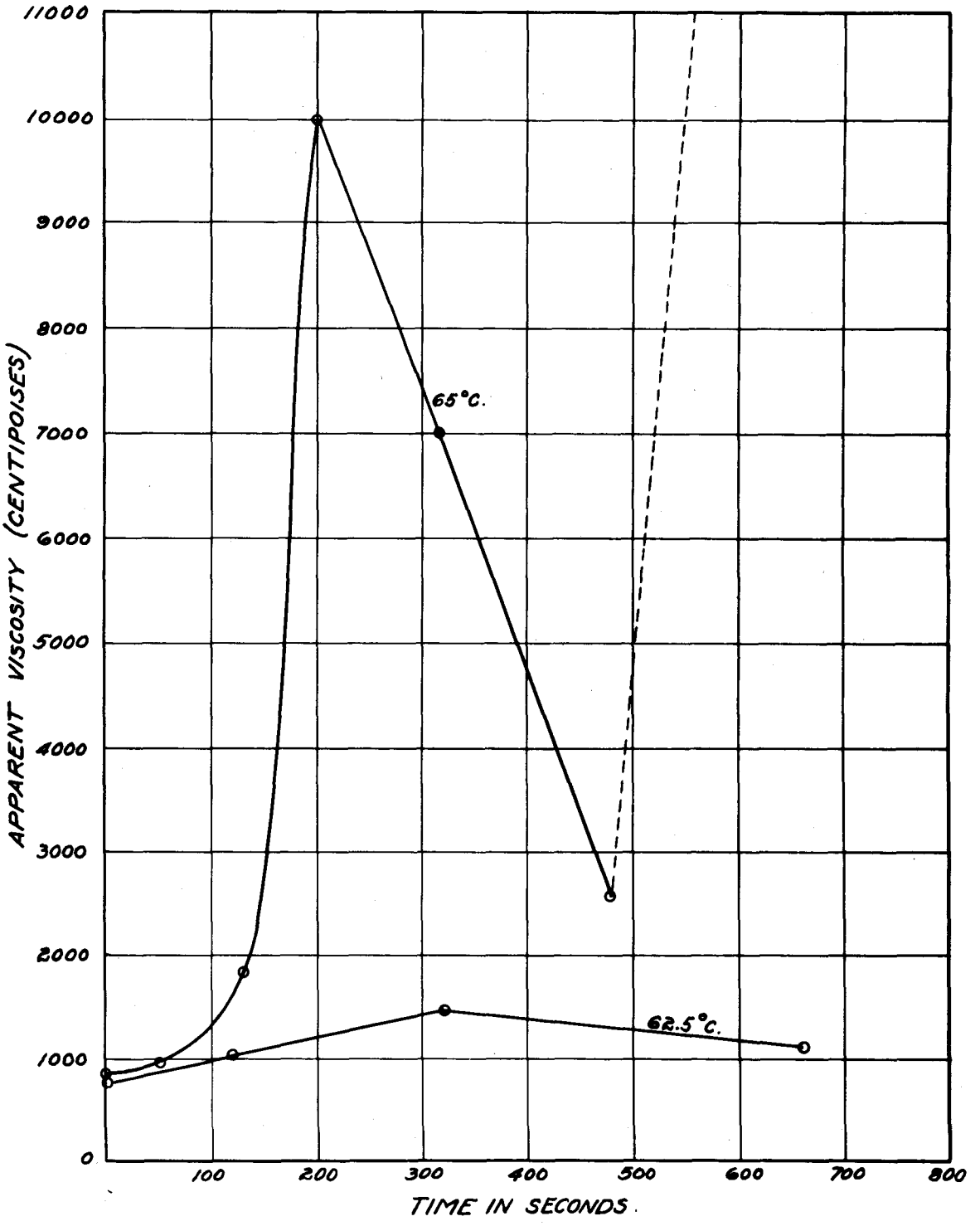


Fig. 10 Effect of time of heating on viscosity of liquid egg yolk.

Table 6

Apparent Viscosity (in centipoises) of Liquid Egg Yolk as a Function of Time of Heating at 62.5° and 65°C.

| Time of Heating (Seconds) | Temperature of Heating °C. | |
|---|---|---|
| | 62.5°C. | 65°C. |
| 0 | 832.4 | 887.1 |
| 50 | | |
| 117 | 1063.3 | 1531.3 |
| 180 | | 10043.8 |
| 308 | | 7067.9 |
| 321 | 1445.3 | |
| 477 | | 2573.9 |
| 658 | 1186.7 | |

degree of solvation of the micelles. The degree of solvation is known to decrease with increasing temperature (35), accounting for the increased fluidity at higher temperatures. The proteins of the liquid yolk possibly become irreversibly desolvated on exposure to heat, the degree of desolvation probably being a function of the time of heating. The protein micelles probably remain in the partly desolvated state even when cooled to 25°C., at which temperature the viscosity was determined. Hence, a drop in viscosity is observed. With increased periods of heating, a further decrease in viscosity may indicate an increasingly greater degree of desolvation of the micelles, so that coagulation occurs almost instantaneously with an increase in heating time.

As mentioned earlier, egg yolk contains 31.6% fat (21) in the form of an emulsion. The stability of this emulsion may be affected by heating. During the heating period, the fat globules probably increase in size and become unstable. Stiffer mayonnaise, as indicated by viscosity or penetrometer tests, have smaller spheres of oil, as shown by photomicrographs (45). Heating of the yolk possibly affects adversely the emulsifying properties of the lecithoprotein, resulting in a coalescence of the fat globules, and hence, the decreased viscosity of the system. With further heating, separation of the phases may be brought about, and this is reflected in a progressive drop in viscosity, until coagulation occurs.

## DISCUSSION

Within the limitations of the methods employed, this study has shown that liquid egg products may be successfully preheated within a time and temperature range which is specific for each of the products studied.

In liquid whole egg, the region of denaturation occurs within the temperature range 56° - 66°C. Above this range, precipitation of the proteins occurs, such that at 73°C. the liquid whole egg coagulates almost instantaneously. The time study shows that the rate of denaturation increases rapidly with rise in temperature of heating. The choice of the periods of heating at the temperatures used are based on the results obtained by Greco (26) in his study of the pasteurization of liquid whole egg. He showed that a 99% kill (microorganism) was obtained at the following temperatures and periods of heating: at 56°C., about 85 minutes; at 62.5°C., about 6 minutes; at 64.5°C., about 2.8 minutes; and at 66°C., about 1.6 minutes. The present study has shown that preheating at the temperatures and periods of heating to obtain a 99% kill, as reported by Greco, can be safely applied to liquid whole egg.

The findings on the behavior of liquid egg white during preheating indicate that the temperature (58°-62.5°C.) and time range (0-46 seconds at 62.5°C.) in which pasteurization may be employed are limited, due to the occurrence of fractional precipitation of the proteins above the temperature and time ranges used.

In liquid egg yolk, only two temperatures were studied. From the

results obtained, it seems possible to preheat liquid egg yolk at 62.5°C. within the time range, 0 - 321 seconds. At 65°C, the time range is very short, 120 seconds possibly being the maximum time that the sample can be held at that temperature. The very rapid increase in viscosity that occurs beyond this period of heating limits the applicability of the time of pasteurization to a maximum of 120 seconds.

The findings of this study may be applied to the preheating of liquid egg products prior to freezing and dehydration. Freezing and dehydration are carried out for the preservation of food for human consumption. Hence, to be of value, preheating should accomplish the purpose for which it is used, yet without markedly affecting the culinary properties of the product.

The study has been confined to the basic fundamental aspects of heat denaturation as shown by viscosity, but it may be expanded for greater usefulness by a series of studies dealing with specific applications. Studies on the culinary behavior of the preheated liquid egg products are needed so as to be able to determine to what extent preheating can be employed without adversely affecting the functional properties of eggs in cookery. If the objective is to preheat the product for the purpose of pasteurization, and hence prolongation of its storage life, it is obvious that the ultimate judge of the product will be the consumer. Will the preheated product serve its purpose in cookery as efficiently as the unheated product? How will preheating affect the flavor and general cooking performance? These are some of the problems on which work may be done.

Likewise, studies may be made on the effect of certain substances

on the rate of heat denaturation of liquid egg. These substances may be ingredients that are used in cookery, as salt and sugar. What effect will the addition of varying amounts of these substances have on the rate of denaturation? Is it possible to prolong or delay the onset of coagulation by the addition of certain substances and thus make pasteurization more efficient?

From the bacteriological angle, a considerable amount of work can be done. This field has been explored partly by some investigators in this laboratory.

An attempt to evaluate the findings of this investigation reveals that more data could have been obtained on the maximum periods of heating possible at the different temperatures, before the onset of coagulation, particularly on whole egg. The effect of pH on the rate of denaturation might have been explored. A considerable amount of other data may have been obtained on various aspects of the problem. However, if the investigation is of any value at all, it is felt that it lies chiefly in the technique devised for "flash pasteurization" or instantaneous heat treatment, and in the set-up for holding every single particle of the sample at the desired temperatures for varying periods of time. Thus, overheating was avoided and the progress of heat denaturation could be followed accurately at the different stages. It is hoped that this study may at least start the stimulus for the investigation of various aspects of the problem which will ultimately be of value to food processors and consumers alike.

# CONCLUSIONS

Under the experimental conditions employed and the limitations imposed by the variation of biological materials, the following conclusions may be made:

1. Viscosity is a sensitive index of denaturation, so that the rate of denaturation and degradation of liquid egg products can be followed viscometrically.

2. Denaturation of liquid egg products is a function of the temperature and time of heating.

3. Liquid egg products can be preheated within a temperature and time range that is specific for each of the systems studied.

4. For the three systems studied, denaturation progresses at unequal rates, starting at lower temperatures for whole egg and for egg white than for yolk. The temperature range for whole egg was found to be 56°- 66°C.; for egg white, 58°- 62.5°C.; and for egg yolk, 62.5° - 70°C.

## SUMMARY

A study has been made of the effect of heat treatments on the denaturation of liquid egg products, using viscosity as a criterion.

The apparatus devised for the nearly instantaneous heat treatments was found to be of value in studying separately the effect of the temperature and the time of heating on the progress of heat denaturation of liquid egg products.

Viscosity, as determined by the capillary viscometer, was found to be a sensitive index of denaturation.

Denaturation of liquid egg products is a function of the temperature and time of heating.

### Temperature of Heating

For liquid whole egg, the region of denaturation, as determined viscometrically, occurs within the temperature range 56°- 66°C. At temperatures above this range, fractional precipitation of the proteins and a general break down of the system are indicated by an irregular viscosity-temperature relationship. Above 75°C. whole egg coagulates almost instantaneously.

In liquid egg white, denaturation occurs in the temperature range 58°- 62.5°C. As in whole egg, the irregular character of the viscosity-temperature relationship above 62.5°C. indicates the region at which fractional precipitation of the proteins occurs.

The denaturation of liquid egg yolk within the temperature range 62.5° - 70°C. is characterized by an increasing rate of denaturation as the temperature rises until a temperature of 70°C. is reached, above which, coagulation occurs almost instantaneously.

## Time of Heating

In liquid whole egg, viscosity increases as a linear function of the time of heating, the rate being more rapid at the higher temperatures.

A linear viscosity-time relationship is shown by liquid egg white at 58°C. and at 60°C., but at 62.5°C., the region of denaturation is very short. Beyond a certain period of heating (46 seconds) at 62.5°C., the fractional precipitation of the proteins is indicated by a drop in viscosity of the liquid white.

At 62.5°C., viscosity of liquid egg yolk is a linear function of the time of heating. However, beyond a certain period of heating (300 seconds), a drop in viscosity is observed. At 65°C. viscosity was not linear with time; it increased very rapidly until a maximum viscosity at 200 seconds was reached. Beyond this point, a sharp drop in viscosity was observed before coagulation occurred.

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