Abstract:
1. No appreciable quantity of ammonia could be detected when edeatin was coagulated by heat, 2. When edestin which has been dissolved in ten per cent sodium chloride solution was boiled (boiling point 95° C.) for thirty minutes it was only partially coagulated, 3. Heat coagulation is not an instantaneous process.
CHEMISTRY OF THE HEAT COAGULATION
OF PROTEINS

No. I

HEAT COAGULATION OF EDESTIN
FROM HEMP SEED

by

SAMI WAFA DAIJANI

A THESIS
Submitted to the Graduate Committee in
partial fulfillment of the requirements
for the Degree of Master of Science
in Chemistry at Montana
State College

Approved:

B. L. Johnson
In Charge of Major Work

Oden E. Sheppard
Chairman Examining Committee

F. B. Cotner
Chairman Graduate Committee

Bozeman, Montana
March 1933
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Part</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I.</td>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Part II.</td>
<td>Review of the Literature on Heat Coagulation</td>
<td>3</td>
</tr>
<tr>
<td>Part III.</td>
<td>Principles Governing the Selection of Material for Study of Heat Coagulation</td>
<td>9</td>
</tr>
<tr>
<td>Part IV.</td>
<td>Heat Coagulation of Edestin</td>
<td>19</td>
</tr>
<tr>
<td>Part V.</td>
<td>Remarks on Immunological Specificity and Chemical Identity of Proteins</td>
<td>23</td>
</tr>
<tr>
<td>Part VI.</td>
<td>Serological Study of Edestin and of the &quot;Unknown&quot;</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Summary</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Literature Cited</td>
<td>43</td>
</tr>
</tbody>
</table>
Part I

Introduction

Heat coagulation of protein substances is a phenomenon of frequent occurrence. It plays an important part in our daily life, in our industry and in our agriculture. The literature on the heat coagulation of proteins is very conflicting and investigators in this field even vary widely in their interpretation of the few experimental data which are considered definitely establish. The only point of agreement among such investigators is that heat coagulation of a protein occurs in two stages: (a) denaturation, and (b) agglutination, or the separation of the denatured protein in particulate form. Of these two phases, the one indicated by the term agglutination is most easily explained in terms of modern chemical formulation. Very diverse opinions on the other hand are held at present with respect to the essential chemistry of the denaturation phase.
Part II.

Review of the Literature on Heat Coagulation.

Heating solutions of many proteins causes, as the temperature rises, an irreversible change—the so called "heat coagulation"—to take place. Chick and Martin (1) believed that the heat coagulation of proteins consists of two distinct processes (a) "denaturation", or the reaction between protein and hot water, and (b) "agglutination", or the separation of the altered protein in particulate form. They point out that in acid solution agglutination occurs at a rate very greatly in excess of denaturation because if the protein is coagulated and the filtrate separated from the coagulum it does not show any change (formation of coagulum) if set aside for some time. The denaturation process, therefore, becomes the limiting factor and the rate of the heat coagulation of a protein can be studied without much complication. The heat plays only the part of accelerator. Chick and Martin showed that the temperature coefficient of heat coagulation is very high. The velocity constant increases nearly fifteen times for a ten degree rise in temperature between 60 and 70 degrees. The velocity constant is found from the equation

\[ K = \frac{\log C_0 - \log C_t}{t} \]

where \( C_0 \) is the initial concentration of albumin and \( C_t \) is the concentration at time \( t \). The velocity constant at 60° C, for
egg albumin is 0.0094 and at 70.4° C, it is 0.15 for the same material. However, when egg albumin is heated in alkaline solution agglutination does not occur, but may be induced by subsequent acidification or saturation with sodium chloride. They found that the reaction velocity varies considerably with different proteins and it also varies according to the acidity and the saline content of the solution. The presence of acid permits heat coagulation at a lower temperature than when acid is not present. If an acid were added in equal quantities to a solution of albumin, the first additions produce a small fall in coagulation temperature, but the acid effect becomes greater and greater with each addition, till a point is reached where the velocity of denaturation becomes so rapid as to make it difficult to determine the velocity constant. Thus the coagulation rate is not directly proportional to the hydrogen ion concentration. As, however, most of the acid added to a protein combines with protein molecules to form salts it may be that the whole or part of the effect of acid upon reaction rate is due to these protein salts reacting with water more rapidly than the protein itself reacts and that the more acid combined with the protein the more the coagulation rate is increased. The free acid in a solution of egg albumin diminishes, and even disappears as coagulation proceeds. The quantity of acid fixed by the coagulation of a definite quantity of protein is at first nearly proportional to the
concentration of free acid, but as the concentration increases the amount fixed falls more and more out of proportion. The phenomenon is probably one of adsorption inasmuch as washed coagulated protein adsorbs acid from solution. Thus Chick and Martin (1) found that egg albumin, precipitated by heating in nearly neutral solution, removed acid from 0.003 normal hydrochloric acid solution.

The denaturation rate of egg albumin by heat in alkaline solutions has been shown by Chick and Martin (1) to increase with increasing concentration of the hydroxyl ions exactly as was stated previously relative to the increase in the rate of heat coagulation in acid solution. In the neighborhood of the neutral point the change in the hydrogen or hydroxyl ion concentration is less effective in modifying the denaturation rate than in regions further removed. Increase in alkalinity from $10^{-3.8}$ normal to $10^{-2.7}$ was accompanied by an increase in average denaturation rate of over a hundred fold. They also showed that the hydroxyl ion is continuously removed as denaturation proceeds in alkaline solution in the same manner as in acid solution. In alkaline solution, however, agglutination does not occur due to the electric charge given to the particles. If the electric charge is neutralized and the protein becomes isoelectric agglutination occurs. In order to bring about agglutination of the protein which has been denatured by heat, the samples tested by Chick and Martin (1) were acidified by the addi-
tion of one tenth normal acetic acid in slight excess of the amount of alkali originally added. Enough pure sodium chloride was then added to saturate the solution. After standing for 24 hours, the coagulum on boiling was determined in a measured volume of the filtrate. This method was found to be useful in securing complete precipitation of the denatured protein in cases where the reaction was not correctly adjusted for most complete agglutination.

The progress of the reaction of protein denaturation by heat as ordinarily carried out conforms to no known simple law. Thus the reaction velocity does not remain proportional to the concentration of the residual protein, but departs more and more from the theoretical value as the experiment proceeds. This is due, according to Chick and Martin (1) to the progressive diminution in the alkalinity which is taking place in the solution during the progress of heat coagulation at constant temperature. The attempt was then made to keep the alkalinity constant during the process of denaturation by means of the presence of excess of solid magnesium oxide, a solution of which possesses a hydroxyl ion concentration of about $10^{-4}$ normal. When the hydroxyl ion concentration was thus maintained constant the denaturation rate of heat coagulation of egg albumin at constant temperature was found to follow a first order reaction. Thus if the concentration of the residual albumin in solution at any time is plotted against time a straight line is obtained. In the same
way, if the acidity is kept constant during the process of heat coagulation of egg albumin by the means of the presence of excess solid boracic acid, a saturated solution of which possesses a hydrogen ion concentration of $10^{-3.1}$ normal, coagulation proceeds as a reaction of the first order and the coagulation rate at any moment is proportional to the concentration of the albumin in solution. Chick and Martin (1) thus believed that heat coagulation is a first order reaction between protein and water, with temperature, salt concentration and hydrogen ion concentration functioning merely as accelerators.

As shown by Chick and Martin (1) heat coagulation does not occur in the absence of water below 120° C. These investigators heated egg albumin in the absence of moisture at 120° C. for five hours after which period of heating they found no evidence of denaturation as denoted by no change in solubility in water. The agglutination or the separation of the denatured protein in particulate form, is influenced greatly by the presence of neutral salts. In the case of egg white, agglutination of denatured protein is assisted by the presence of electrolytes and the hydrogen ion concentration. In the case of solutions of other proteins, however, agglutination is hindered by presence of salts.

Chick and Martin (1) pointed out that the coagulation temperature which has been almost universally regarded as a physical characteristic of the particular protein, is subject to
variations according to the conditions of the experiment; and, therefore, it is entirely misleading to regard this temperature as a constant characteristic of the protein unless the conditions of the experiment are specified. Thus the coagulation temperature of serum globulin was found to range from $72^\circ$ to $75^\circ$ centigrade. The variation apparently depends upon the rate of heating, on the concentration of salt, and on the concentration of the protein. A fourth factor which exerts a profound influence on the coagulation temperature is the addition of even minute amounts of acid or alkali. It was found in the case of serum albumin that neutralizing an alkaline solution to litmus lowered the coagulation temperature from $80^\circ$ to $78^\circ$ C., and that by the successive addition of small quantities of acid, it fell as low as $53^\circ$ C.

Hycraft and Duggan (8) pointed out that the act of coagulation takes a certain time and that the temperature of coagulation rises as the concentration of the protein diminishes.

T. B. Robertson (14) believes that the phenomenon of heat coagulation is a phenomenon of dehydration and not of hydration as suggested by Chick and Martin (1). He points out that the base combining capacity of casein diminishes with the rise in temperature due to the fact that it undergoes some measure of polymerization which in turn is due to the dehydration of the end amino and carboxy groups. He believes that denaturation
corresponds to the dehydration of the protein molecule, and that agglutination corresponds to the simultaneous polymerization of these anhydrides.

Wu and Wu (16) claim to have shown that when egg albumin is coagulated by heat tyrosine splits off.

Mastin and Rees (9) repeated Wu and Wu's experiments but found no evidence that tyrosine is split off when albumin is coagulated by heat. The method they used in the estimation of the amount of tyrosine was the Weiss modification of the Millon's reaction which could be used to detect one part of tyrosine in 500,000 parts of solution.
PART III
Principles Governing the Selection of Material for Study of Heat Coagulation
Preparation and Properties of Edestin

My early work on the study of heat coagulation was done with crystalline egg albumin. Crystalline egg albumin was prepared in the usual way, recrystallized several times and was dialyzed until free from ammonium sulfate. Toluene was used as a preservative during the process of dialysis. Mastin and Rees (9) reported the fact that when egg albumin is heated for a period of 15 to 30 minutes at 60° or 70° C, 0.5% of the nitrogen present originally remains in solution. This nitrogen seems to decrease on further prolonged heating. The experimenter suggested that this nitrogen might arise from the ammonium sulfate which was used in the precipitation of albumin, and which was not removed by dialysis. They, however, did not attempt to prove it experimentally. My interest in the source of this nitrogen led me to choose a protein which could be extracted and purified without the use of nitrogenous substances. Edestin from hemp seed proved such a substance.

When dialyzed egg albumin was dissolved in water and heated, a minute amount of ammonia was eliminated. It was thought that the source of this ammonia was most probably ammonium sulfate which had been used in the precipitation of egg albumin. To test this supposition, it was planned to determine whether ammonia is given
off when a protein like edestin (in the extraction of which no nitrogenous substance need be used) was heated.

Edestin conforms strictly to the definition of globulin: it being insoluble in water but soluble in sodium chloride solution. Edestin has the useful property of being much less soluble in cold brine than in warm. This property makes it possible to obtain edestin of high purity.

**Definition of Globulin:** Globulins are defined as simple proteins soluble in neutral salt solutions of the salts of strong acids and bases, but are insoluble in water. They are usually heat coagulable.

**Occurrence:** Globulins occur in animals and plants. In the case of plants globulins are to be found in seeds and buds as storage material. In animals they occur in the blood.

**The Animal and Plant Globulins:** Globulins obtained from animal sources differ in certain properties from those obtained from plant sources. Animal globulins are precipitated by saturating their solutions with MgSO₄, but many of the vegetable globulins can not be thus precipitated. Animal globulins are precipitated by half saturation with ammonium sulfate but many plant globulins are precipitated at less or considerably more than half saturation. Saturation with Na₂SO₄ at 33⁰ precipitates both animal and plant globulins.

**Preparation of Plant Substance Before Extracting Protein:** The seed or part of plant from which the protein is to be extracted
must be finely ground before the process of extraction is ef-
fected to insure securing maximum yield and to effect complete
extraction in the shortest possible time. In case the ground
plant substance is oily it must be extracted several times with
ether until entirely free from oil. The method employed in the
present investigation is a modification of the Soxhlet method.

**Solvents and Methods Used for Extraction of Plant Proteins:** The
solvents usually employed for the extraction of proteins from
plants are water, neutral saline solutions, 70\% to 80\% alcohol
and very dilute acids and alkales.

Some common proteins extracted from plants by means of
water are albumins and proteoses. When this aqueous extract is
saturated with (NH₄)₂SO₄ the proteins are precipitated.

In the case of the extraction of plant substance with solu-
tions of neutral salts a 10\% NaCl solution is a solvent fre-
quently employed. Osborne (11) states that "the general plan
followed in conducting an extraction with sodium chloride
solution is to treat the ground seed with a sufficient proportion
of the solvent so that it will subsequently yield an extract of
which so much can be filtered clear as to be equal to about three-
fourths of the volume of the solvent applied to the meal. The
proportion necessary to attain this end depends not only on the
amount of the insoluble residue but also on the proportion of
water with which it combines. It also depends to a large extent
on the character of the solution which the soluble constituents
of the seed yield, for some varieties of seeds contain substances which produce extremely gummy, viscid solutions which render filtration extremely difficult. No general statement can be made as to the proper proportion of solvent, or the methods to be employed in filtering the extracts, as each kind of seed requires special treatment. A filtered extract may be subjected at once to dialysis and the dissolved globulin thus separated. The extraction may also be made with warm dilute saline solutions and the globulin precipitated by cooling the filtered extract as, in most cases, the globulins are much less soluble in cold solutions than in warm. This method of treatment often results in the production of crystalline preparations which can be afterwards recrystallized from warm dilute saline solutions and brought to a high state of purity. Other salts than NaCl which may be employed for extracting proteins are: NH₄Cl, BaCl₂, CaCl₂, KBr, KI, MgSO₄, ammonium oxalate, potassium ferro cyanide and organic salts—ammonium benzoate.

The amount of protein extracted by alkalies and acids is usually greater than that extracted by water or neutral salts. This is due to the fact that alkalies and acids dissolve proteins enclosed in places inaccessible to salt solutions. This method is disadvantageous because alterations in the proteins may result by the action of acids or alkalies if their concentrations exceed certain limits.

Edestin a typical globulin: Edestin conforms strictly to the
definition of globulin and it is one of the most typical and abundant of vegetable globulins. The reason for selecting edestin for the study of the phenomenon of heat coagulation have been already mentioned. It was first prepared by Barbies from squash seed and then it was prepared in a crystallized form by Grubler. Ritthausen (13) extracted it from hemp seed, castor bean and sunflower. T. B. Osborne (11) extracted edestin from the seeds of flax, cotton, wheat, rye, barley and maize.

Edestin is soluble in 10% sodium chloride solution. Its solubility, however, is much more pronounced in warm brine solution than in a cold one. The solvent effect of different quantities of salts on edestin were determined by Osborne (11) and I. F. Harris as follows: "Portions of two grammes each of pure crystallized edestin were suspended in sufficient water to make a final volume of 20 c. c. with the different quantities of molar solutions of the several salts which were afterwards added, the edestin being in each case in excess of the amount dissolved. After agitating for some time, the solutions were filtered, nitrogen was determined in 10 c. c. of each, and the amount of edestin dissolved was calculated from the nitrogen in solution. It was thus found that the amount of dissolved edestin was closely proportional to the concentration of the salt solution. Its solubility in solutions of sodium, potassium and calcium chlorides was nearly the same. In solutions of magnesium, calcium, strontium, or barium chloride its solubility was twice as great as it was in solutions of the chlorides of the monovalent
bases, with the exception of the lithium chloride, in solutions of which it did not dissolve as abundantly as in those of the chlorides of the other monovalent bases. The sulphates of potassium, sodium, lithium and magnesium had a solvent power corresponding closely with that of the chlorides of the bivalent bases. Bromides and iodides did not behave like chlorides, for sodium and potassium iodides had a solvent power twice as great as that of the corresponding chlorides, agreeing in this respect with the chlorides of the bivalent bases. The bromides were less energetic solvents than the iodides but more energetic than the chlorides. Barium and calcium bromides were equal to one another in solvent power but this was less than that of sodium or potassium iodide and greater than that of sodium or potassium bromide, the two latter being somewhat less powerful solvents than the corresponding chlorides.

Solubility of edestin in acid and bases: Edestin is a compound which is amphoteric in character and which for this reason combines with both acids and bases.

Pure edestin when free from combined acids or bases is insoluble in water. If this edestin is suspended in pure water and hydrochloric acid is added in the proportion of two mols. of hydrochloric acid to one mol. of edestin, it was found by Osborne (11) that no solution of edestin takes place until one-half of the amount of the acid has been added. On adding the second half of the acid, solution takes place at a rate proportional to
the amount of acid added. The acidity of the solution obtained with the second half of the acid increases at twice the rate at which the acid is added. When all the second half of the acid is added edestin dissolves completely. If one ceases to add acid before all edestin dissolves and if one examines the solution and the insoluble parts, one will find that the acidity of the part insoluble in water is equal to that of a compound of one molecule of edestin to one molecule of hydrochloric acid, while the acidity of the part soluble in water is equal to a compound of two molecules of hydrochloric acid to one molecule of edestin. Edestin, therefore, forms salts corresponding to edestin monochloride and edestin bichloride. Edestin also functions as an acid. Thus it dissolves in alkali in molecular proportions forming alkaline salts. When edestin is extracted from plants by neutral salts the product thus obtained was shown by Osborne (11) to consist not of the free edestin but of a mixture of its salts. Thus edestin contains chiefly edestin chlorides when crystallized from sodium chloride solution and sulfates when crystallized from solutions containing ammonium sulfate. That edestin contains chiefly edestin chlorides when extracted by NaCl solution is shown by the fact that when the thus extracted protein is treated with pure water a part of it goes into solution. The part that goes into solution is twice as acid as the insoluble part. The soluble protein is edestin bichloride.

Properties of edestin monohydro-chloride: According to Osborne
(11) Edestin monohydro-chloride is wholly insoluble in water but dissolves in 10% sodium chloride solution and it is readily thrown out from solution by adding water. On warming this solution the precipitate dissolves and appears on cooling. Edestin monohydro-chloride conforms to the definition of globulin.

Properties of esterin bihydro-chloride: According to Osborne (11) edestin bihydro-chloride is soluble in water and it is precipitated from this solution by addition of small quantities of alkali salts. It is insoluble in one or two per cent sodium chloride solution.

Precipitation of edestin from edestin monohydro-chloride and edestin in bihydro-chloride: When edestin monohydro-chloride or edestin bihydro-chloride is suspended or dissolved in water and this made neutral to phenolphthalein edestin separates out completely. When this solution is filtered and filtrate evaporated to dryness and analyzed it will be found to consist of the chloride salt of the alkali used in neutralization. If the edestin was originally crystallized from ammonium sulfate rather than sodium chloride the product will be potassium sulfate.

Precipitation of edestin from 10% sodium chloride solution: Edestin dissolved in 10% sodium chloride is precipitated completely when saturated with sodium sulfate at 34°. It is partially precipitated when saturated with sodium chloride and is precipitated more completely when saturated with magnesium sulfate.

Composition, formula, and molecular weight of edestin: When edestin is dried at 110° and analyzed, its composition is found
by Osborne (11) to be as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>51.55%</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>6.92%</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>18.67%</td>
</tr>
<tr>
<td>Sulphur</td>
<td>0.90%</td>
</tr>
<tr>
<td>Oxygen</td>
<td>21.9%</td>
</tr>
</tbody>
</table>

Edestin has a molecular weight of 7,138, twice which is 14,276. The formula being carbon 61.4, hydrogen 9.8, nitrogen 19.0, sulphur 4, oxygen 19.6.

EXPERIMENTAL

Extraction of edestin from hemp seed: The method followed for the extraction of edestin from hemp seed is that outlined by Morrow (10) with minor modifications which were found desirable to apply in order to get edestin of high purity.

750 c. c. of 10% sodium chloride solution were heated to 65° C. and, while stirring continuously, 250 grams of the ground ether extracted and air dried hemp seed were added in small amounts at a time and then allowed to stand in a water bath at 70° for an hour. The substance was stirred frequently during this time. A piece of clean cloth was then moistened with hot 10% sodium chloride solution and a portion of the mixture was then placed in it and squeezed by the hand until all the liquid was wrung out in a clean beaker placed in a water bath at 70°. This process was repeated with new portions of the mixture until
all the solution was squeezed out of all the mixture. The solution or extract thus obtained, when heated to a temperature of 65° C., was filtered through a Buchner funnel which was previously prepared as follows: A Buchner funnel which was fitted to a suction flask which, in turn, was connected to a suction pump, was fitted with filter paper. A hot 10% sodium chloride solution was poured on the filter paper while the suction pump was in operation, thus causing it to adhere closely to the funnel. Decolorizing carbon was then placed on the filter paper and hot 10% sodium chloride solution was poured into the funnel until a completely moistened mat of decolorizing carbon was formed. The Buchner funnel was kept warm by a simple but effective device. This device consisted of winding as many times as possible a rubber tubing connected at one end to a steam source. When steam was allowed to pass through the rubber tube the temperature of the content of the funnel was easily kept at 60° C. The reason for using the decolorizing carbon was to decolorize the extract which was slightly colored when obtained as mentioned above. Some edestin was lost by being adsorbed by the decolorizing carbon, but this was sacrificed for a purer product. The extract was filtered through the above mentioned Buchner funnel and the clear filtrate obtained was cooled to room temperature and then placed in a refrigerator for a day or two. When the edestin had crystallized out it was filtered through another Buchner funnel. The edestin crystals were then washed several
times with cold ten per cent sodium chloride solution and then was returned to a clean flask, dissolved in ten per cent sodium chloride at 65°C, filtered, cooled to room temperature and placed in the refrigerator as before. This process was repeated several times and it yielded edestin of high purity. The edestin was then washed with 50% ethyl alcohol, 95% ethyl alcohol and absolute ethyl alcohol and finally with ether. The edestin was then dried and bottled for use.
PART IV.

Heat Coagulation of Edestin

Inasmuch as compounds which are known to contain amide groups are decomposed by water when such compounds are heated in certain aqueous media, it was thought advisable to investigate the possibility of amide groups of proteins being decomposed by water and thus give rise to ammonia during heat coagulation. Egg albumin can not be used for such a study due to the fact that ammonium salts are used in its extraction. Indeed when dialyzed egg albumin solution is heated it gives rise to some ammonia. The source of such ammonia is, most probably, ammonium sulfate which is used in the extraction of albumin. It is necessary, therefore, to choose a protein in the extraction of which and in the purification of which no ammonium compound is used.

Preparation of Edestin

Edestin was prepared in the same manner as outlined before and precautions were taken so that the materials used for its extraction and purification were free from ammonia. Thus edestin was prepared in an atmosphere which was tested and found to be free from ammonia. The sodium chloride used was of high purity and ammonia free. The water employed was conductivity water which was shown to be free from ammonia.

Apparatus Designed for the Detection of Ammonia and Carbon Dioxide
The apparatus devised for the estimation of ammonia eliminated during the heat coagulation of edestin consisted essentially of vessels in which air was scrubbed for its carbon dioxide and ammonia content, of vessels in which suitable tests for these constituents could be made, of a vessel in which the heat coagulation could be carried out, and of a vessel in which the ammonia, if it were to be given off, could be caught. The system was suitably connected by means of glass tubes, which were connected with rubber tubing, to permit the entire system to be swept with a stream of ammonia and carbon dioxide free air. Figure I is a photographic picture of the apparatus used in this study.

The important parts of the apparatus are lettered in the figure and the names assigned to them are given in the following legend:

(A) Carbon dioxide (air) absorber
(B) Ammonia (air) absorber
(C) Carbon dioxide test tube
(D) Ammonia test tube
(E) Lime water funnel
(F) Dilute sulfuric acid funnel
(G) Coagulating chamber
(H) Foam collector
(I) Ammonia absorber
(J) Concentrated sulfuric acid driers
The carbon dioxide absorber (A) consisted principally of a large salt mouth bottle two-thirds full of fifty per cent sodium hydroxide solution. A glass combustion tube was fitted into the rubber stopper which closed this bottle. Into this tube was packed by means of glass beads another tube of much smaller diameter and shorter in length to increase the circulation of the alkali during the passage of air through the system. The inside tubing extended beyond the larger tube in the alkali solution and thereby caused the air, as the level of the alkali in the bottle was lowered when the air pressure forced the alkali to flow up the combustion tube, to ascend in the tube between the glass beads. Besides the combustion tube which served as a scrubbing tower, the stopper which closed the absorber bottle, carried two other tubes to permit entrance of air, and to permit release of air in case the pressure became too great. The scrubbed air left the absorber at the upper end of the scrubber column.

The carbon dioxide free air then passed into the ammonia absorber (B). It consisted of a bottle containing concentrated sulfuric acid.
The purified air, which now was free from carbon dioxide and ammonia, next passed into bubler tube (O) and then into carbon dioxide test tube (C) where it bubbled through lime water. The presence of any carbon dioxide in the stream of air was revealed by the formation of a white precipitate of calcium carbonate in this tube. Accessory parts (N), (M), and (E), with their accompanying tubes and connections, permitted tube (C) to be cleaned without air from the laboratory entering the system.

The stream of air then passed into ammonia test tube (D), which contained dilute sulfuric acid for the retention of air ammonia. This tube, like tube (C), had accessory parts whereby it could be cleaned and recharged without permitting laboratory air to enter the system. The nitrogen content of the acid solution of this tube must be nil before the protein is heat coagulated. The stream of air then passed into the coagulating chamber where it picked up any ammonia given off during coagulation. In later experiments the air did not bubble through the solution of the protein but was allowed to play upon the surface of the solution. This procedure prevented undue foaming. From this point, Figure I is self explanatory. The last absorber (K) is a carbon dioxide absorber, whereby it was hoped to demonstrate whether carbon dioxide was eliminated during heat coagulation. The studies directed toward this end were not sufficiently conclusive to warrant reporting them in this thesis.

Results of the Experiment

When highly purified edestin, which was prepared under such
conditions that the materials used for its extraction and purification were free from ammonia, was tested for the elimination of ammonia during heat coagulation in the specially constructed heat coagulation tester illustrated in Figure I., no measurable quantities of ammonia were found to be eliminated. From this it was concluded that the ammonia which was eliminated during the heating of egg albumin solutions and of solutions which had been prepared by dissolving a cruder edestin than was used in performing the last tests, had its origin in ammonium salts.
PART V.

Remarks on Immunological Specificity and Chemical Identity of Proteins

When a small quantity of protein is introduced into a rabbit or any animal it incites the formation of antibodies which combine specifically with the protein. Such a substance is called an antigen. Not all foreign substances introduced into an animal's body are antigenic. Examples of substances which are not antigens are alcohol and sugar. Proteins are generally antigenic if they are soluble in the plasma of the animal but if they are insoluble in the plasma they can not reach sites of antibody formation and, therefore, they can not show antigenic properties. Wells (15) states that as yet we do not know to what the proteins owe their antigenic properties. The antigenic properties of proteins are lost when hydrolyzed to amino acids. The antigenic activity of a protein is measured by the speed with which it incites the production of antibodies for this speed varies with different proteins. The protein native to an animal can not stimulate antigenic properties in that animal but when this protein is chemically altered as for instance by treating it with formaldehyde, nitrous acid or iodine it may behave as an antigen and give rise to specific antibodies when injected into the animal's body. Thus, it must be remembered that the specificity of the artificially made protein compounds is different from the specificity of the original protein. It is not
known what antibodies are chemically. We only recognize their existence through their function. Thus they function as what we call precipitins in precipitin tests, as agglutinins in agglutinin tests and as anaphylactins in anaphylaxis. It is a general belief that antibodies are formed in a certain part or parts of the animal body but all investigations to determine such a place or places are not conclusive. Since antibodies are constituents of blood it is logical to conclude that they are formed where some of the constituents of blood are made. Antibodies are inactivated by heat and the temperature at which they are inactivated by this method varies with different sera. The inactivation temperature is usually between $65^\circ$ and $85^\circ$.

Chemical Properties of Proteins and Immunological Specificity:

H. G. Wells (15) declares that "the extensive acquaintance with the composition of the protein molecule and the application of more refined methods to its study, have suggested that immunological differences between proteins are usually, and as far as now known always associated with and presumably dependent upon chemical differences which can be detected by chemical and physical methods". It is interesting to note that immunological methods are highly capable of detecting the slightest differences between proteins even if differences are so minute as to be non-detectable by any other method. Because I have been working with edestin which is a vegetable protein I shall illustrate the above statements with examples from vegetable proteins.
Jones and Wells (15) found that "globulins from seeds of cantaloupe and of squash are chemically, crystallographically and immunologically identical. On the other hand, one finds that two chemically different proteins from the same seed are readily distinguished by immunological reactions. Thus, the highly soluble seed proteins designated as "proteoses" are usually quite distinct from the other proteins of the same seeds. The proteins from seeds, indeed, offer a particularly favorable material for the study of specificity, because being merely storage protein for nourishment of the embryo plant they are set aside in relatively pure form and of limited variety in the same seed. They also often offer unusual readiness of crystallization or peculiar solubilities which facilitate their separation in pure form. By investigating such purified materials rather than complex mixtures such as serum or tissue extracts, much more exact information may be obtained. For example, the alcohol-soluble protein of wheat, gliadin, shows no recognizable chemical difference from the gliadin of rye, and these two proteins react immunologically as if identical, despite their derivation from plants of different species."

Does protein specificity depend on the entire protein molecule? According to Wells (15) the bulk of the protein does not determine the character of the immunological reactions of protein, but these characteristics are developed by certain radicals of the protein molecule and a single protein molecule may contain
two or more of such groups. The protein molecule acts as an antigen but these groups determine the specificity.

The Precipitin test: If an antigen is injected into an animal and an appropriate period of time is allowed to elapse (7-30 days) it shows a remarkable hypersensitivity to this antigen so that if it is injected a second time with the same protein the animal will be intoxicated severely and death might follow. This phenomenon is called anaphylaxis. On the other hand, when the originally injected protein is followed by several injections into the animal, at 4 or 5 days interval until a quantity of 0.25 gram or more of the foreign protein has been introduced into its body, its blood serum acquires the property of precipitating that particular protein. Such a reaction is called a precipitin reaction. Here the antibody or antibodies formed are called precipitin and the antigen is called precipitinogen. Wells (15) states that "the precipitin appears in the blood generally about six days after injection of the protein, but disappears after injection of each subsequent dose of protein, to reappear again after a somewhat shorter lapse of time."

The hydrogen ion concentration has no influence upon a precipitin reaction within a \(pH\) range of 4.5 to 9.5 but if the \(pH\) is outside this range no precipitate forms. If the precipitate is treated with a hydrogen or hydroxyl ion concentration outside this range it usually dissolves. Electrolytes have a decided influence on precipitin reactions for such a reaction does not
occur in the absence of electrolytes. This is substantiated by the fact that no precipitin reaction occurs when the immune serum and precipitinogen are dialyzed free from salts before mixing, but if a small quantity of sodium chloride is added to such mixture precipitation occurs quickly. The amount of precipitation that occurs is much modified by the amount of inorganic salts present. According to Downs and Gottlieb (2) the following salts in molar concentration markedly inhibit the formation of a precipitate when antigen and antibody are mixed: 

\[ \text{LiCl, NaCl, KCl, NaNCS, KCNS, Mg(NO}_3\text{)}_2, \text{Sr(NO}_3\text{)}_2, \text{Al(NO}_3\text{)}_3, \text{Al}_2(\text{SO}_4)_3, \text{Fe(NO}_3\text{)}_3, \text{FeCl}_3 \].

On the other hand the following salts show no inhibition: 

\[ \text{NaBr, KBr, KI, NaNO}_3, \text{Li}_2\text{SO}_4, \text{MgCl}_2, \text{CaCl}_2, \text{BaCl}_2, \text{MgSO}_4, \text{Ba(NO}_3\text{)}_2. \]

Some remarks on the chemistry of precipitin: According to Wells (15): "Neither the precipitin nor the antigen seems to be altered appreciably by the reaction, since when either is separated from the precipitate it retains its original properties. The amount of precipitation obtained is much modified by the amount of inorganic salts present, and, according to Freidmann (5 and 6) there is a general resemblance between the precipitin reactions and the precipitations occurring when colloids precipitate one another, i.e., when an amphoteric colloid reacts with either an acid or a basic colloid." Ramsdall (12) states that, "as there is no change observed in the surface tension during the precipitin reaction, it probably is not associated
with any marked denaturation of the protein. As the precipitate is said to be able to sensitize anaphylatically, both actively and passively, it would seem that it must contain both the antibody (which confers passive sensitization) and antigen (to cause active sensitization)." The precipitate seems to contain no other protein than those of the antigen and antibody. Wells (15) has summarized the physical chemistry of colloidal precipitation and the precipitin reaction as follows: "Proteins are regarded, generally, as amphoteric electrolytes, that is substances which are able to unite with acids as well as alkali. The hydrogen ion concentration of the surrounding solution determines whether they unite with the one or the other. When the hydrogen ion concentration exceeds a critical point which is known as the iso-electric point of the protein, the protein combines with acid to form a salt which, disassociating, gives rise to a protein cation carrying a positive electrical charge and an acid anion with a negative electrical charge. When the hydrogen-ion concentration of the solution is on the alkaline side of the iso-electric point of the protein, the protein combines with the metals to form a salt such as sodium proteinate which disassociates into negatively charged protein ions, and positively charged ions of the metal. That precipitation of a colloid bearing an electric charge of one sign is accomplished by the addition of a colloid bearing an opposite charge, and that both colloids are carried down in the precipitate, is generally recognized.
The precipitin reaction on mixing an antigen in solution with its homologous immune serum probably belongs to the same group of reactions. Blitz suggests the following rules regarding the precipitation of one colloid by another when mixed together quickly and uniformly:

"If to a given colloidal solution, one of the opposite sign is added in small proportion, there is no precipitation. As the quantity of the second increases, the coagulative action follows parallel until a proportion is reached which causes immediate coagulation. As the amount is still further increased, coagulation ceases; that is, there is an optimum precipitation for certain proportions, and when these favorable proportions are exceeded on either side, no precipitation occurs." The precipitin reaction is analogous to the precipitation of one protein by the other but differs from it in the phenomenon of specificity. The phenomenon of specificity, therefore, indicates that colloidal reactions are not the only changes that are involved in precipitin reactions. Wells (15) points out that the bulk of the precipitin obtained in a precipitin reaction comes from the protein of the immune serum and comparatively little from the antigen. Freidmann and Michaelis (7) laid down the following rules governing the formation of a precipitate when a precipitin acts on an antigen: "(a) With a given quantity of precipitinogen the amount of precipitate increases with the increase in the quantity of precipitin.
(b) With a given quantity of precipitin the amount of precipitate increases at first with addition of precipitinogen, then decreases, and with a certain excess of the latter approaches zero, this being the "zone phenomenon."

(c) With constant amounts of both, the quantity of precipitate decreases as the amount of fluid in which the reaction takes place increases.

It is of exceeding importance to remember that the amount of precipitate is greater when the precipitin reaction takes place between homologous, antiserum, and antigen, than when it takes place with closely related heterologous antigens, so that the quantitative measurement of the precipitate is of value in applying this reaction to determine the nature of the protein solutions. According to Wells (15) the zone phenomenon is quantitatively specific, for only the specific antigenic protein has the full inhibiting effect. Two closely related antigens might react with the specific antiserum (the serum from the blood of the immunized animal) of one of them but the inhibition affected by one antigen occurs at a different dilution from that of the closely related antigen. Wells (15) claims that "in the reaction between isolated serum globulin and a specific antiserum for this globulin, the precipitate is more readily redissolved by the same serum globulin than by serum albumin from the same species or by serum globuline from another species. When the inhibition is complete it can not be overcome by further addition of antigen, but further addition of antiserum will do so."
PART VI

SEROLOGICAL STUDY OF

EDESTIN AND OF THE "UNKNOWN"

Any method leading to an elucidation of the chemistry of heat coagulation must of necessity have included in it heating of protein material either in the moist or in the dry condition. Edestin, the protein which was selected for the study of heat coagulation for reasons already mentioned, was, therefore, heated while in the dissolved form in ten per cent sodium chloride solution for thirty minutes or more at a boiling temperature (95°C, altitude 4800 feet). The resulting coagulum was in each case removed by filtration through #42 Whatman filter paper. The clear filtrate, which henceforth will be referred to as the coagulation filtrate, was investigated for the presence of soluble products which might have been formed during the heat coagulation of Edestin.

PROPERTIES OF THE COAGULATION FILTRATE

This filtrate was shown to contain a soluble nitrogenous substance which when investigated with a saccharimeter caused the plane of polarized light to turn. The filtrate responded positively to a Millon's test and to a Heller's ring test. When the filtrate was diluted with several volumes of cold distilled water a white, voluminous, and finely divided precipitate formed. This precipitate dissolved in NaCl solutions either in part or in whole, and the extent of its solubility depended apparently upon
the concentration of sodium chloride and the quantity of solution with which it was in contact.

The precipitate which resulted when the filtrate was diluted was caused to settle by allowing the containing vessel and its contents to stand in a refrigerator over night. The clear supernatant liquid was then siphoned off. The precipitate, which was now in contact with only a small volume of solution, was concentrated further by centrifugation and again siphoning off the supernated liquid. The complete removal of the mother liquor was effected by repeating this process a number of times, each time suspending the substance in distilled water.

It was recognized that a study of this substance might lead to a better understanding of heat coagulation of proteins. For the sake of convenience, this substance will be referred to hereafter as the Heat Coagulation Unknown No. 1 or more simply, as far as this thesis is concerned, as the "Unknown".

What is this "Unknown"?

Is it a by-product that splits off when edestin, which has been dissolved in ten per cent sodium chloride solution, is denatured by heat? Is it an impurity that accompanies edestin in the course of its extraction and its purification? Is it edestin itself? These are the questions that I have sought next to answer.

Reasons for Selection of Experimental Method.

The problem now is to determine the most effective method of
attack upon this problem.

One might, for instance, attack this problem by comparing the ratios of the amount of nitrogen to sulfur in the "Unknown" and in the original edestin. But such datum is not conclusive evidence in solving a problem of this kind for the following reasons: If such a ratio in the "Unknown" were to be found different than the one in edestin it would only prove that the "Unknown" would be different from the original edestin. But this finding would not prove whether the "Unknown" is an impurity which accompanies edestin during the processes of its extraction and purification, or whether it is a by-product that splits off when edestin, which has been dissolved in ten percent sodium chloride solution, is heat coagulated.

If, on the other hand, one were to find that the ratios, when obtained as outlined above, are identical, one has no right to conclude that the "Unknown" is edestin. These ratios are the same even though the "Unknown" is not edestin. Thus, if the "Unknown" were to result from polymerization of edestin during heat coagulation, or if it were to result from some slight, or profound, internal rearrangement of the constituent parts, which would involve neither gain nor loss in the edestin molecule, the ratio of sulfur to nitrogen in the two substances would be the same. Indeed the ratio of carbon to hydrogen in five different compounds having the formula C6H14 is the same.

The viewpoints just cited emphasize the necessity to answer the three questions relative to the nature of the "Unknown".
Such a way has been found in the study of the immunological specificity of proteins. Thus, if immunological differences between the edestin and the "Unknown" were to be noted, the conclusion would be that there is a chemical difference between them. But if there are no immunological differences between them they are chemically identical. More explicitly, if a solution of the "Unknown" gives no precipitin reaction with the specific antiserum of edestin in the inference would be that the unknown is chemically different from edestin, and that it is a by-product which splits off when edestin, in ten per cent sodium chloride solution is coagulated by heat. On the other hand, if the solution of the "Unknown" gives a precipitin reaction with the specific antiserum of edestin then there are three possibilities.

1. The "Unknown" is an impurity.
2. The "Unknown" is edestin itself.
3. The "Unknown" is a by-product of the heat coagulation of edestin and that it is probably closely related chemically to edestin itself.

The first of these conclusions is probable, for if the "Unknown" is an impurity it will produce specific antibodies when injected with edestin into the body of the rabbit. When we add the solution of the "Unknown" to the specific antiserum of edestin we are at the same time adding the impurity to its specific antiserum or antisera thus causing the precipitin reaction to occur.

The second conclusion is also possible, for, if the "Unknown"
is edestin itself a precipitin reaction would then occur since when we add the solution of the Unknown to the specific antiserum of edestin, we are merely adding edestin solution to its specific antiserum.

The third conclusion is also possible for a protein might give a qualitative, but not quantitative precipitin reaction, when added to the antiserum of a protein closely related to it.

If the quantitative concentration at which the solution of the unknown inhibits precipitin reaction is the same as the quantitative concentration of the original edestin at which it also inhibits precipitin reaction, when both are tested against edestin antiserum, then the conclusion reached is that the "Unknown" is identical with edestin.

If, on the other hand, the concentration of the unknown in the solution which barely inhibits the precipitin reaction is different than that at which edestin inhibits the precipitin reaction when it is tested with its own specific antiserum, the conclusion would be that the unknown is either an impurity contaminating edestin or a by-product of edestin when it is heat coagulated.

Overcoming the Difficulties

There are three major difficulties which have to be overcome when performing precipitin tests on edestin and the unknown. The first of these difficulties arises from the fact that edestin and also the unknown dissolve in sodium chloride solutions in amounts which not only depend upon the temperature
but upon the concentration of salt. It is necessary, therefore, when determining the dilution at which the edestin or the unknown inhibits precipitin tests, that the temperature of solutions of these substances be kept constant. This temperature must be chosen so that it will comply with the following conditions: First, it must not be high since antibodies are inactivated at higher temperature (65° - 85°). Secondly, the temperature must be high enough to permit an appreciable quantity for at low temperatures the amount of edestin dissolved is not. appreciable enough to give a strong precipitin test. Thirdly, the higher the temperature the less the concentration of sodium chloride that can be used. The temperature chosen was 40°C.

The second of the difficulties arises from the fact that edestin and the unknown are insoluble in water but are slightly soluble in dilute salt solution and are very soluble in ten percent sodium chloride solution. Molar sodium chloride solution inhibits precipitin tests and therefore it becomes necessary to determine the lowest concentration of sodium chloride which will dissolve an appreciable amount of edestin or the unknown to give a strong precipitin test.

Such a concentration of salt need only be determined roughly at first and the procedure was as follows: A 3.5 per cent sodium chloride solution of edestin was placed in a thermostat at 40°C, a sample of which was tested against edestin antiserum and which
gave a positive precipitin test. The solution was then diluted with pure water at 40° C., filtered clear and samples of the filtrate were tested against edestin antiserum. This process was repeated until a dilution was reached such that edestin gave but a faint precipitin test. A known volume of this solution was then analyzed for its sodium chloride content.

The actual concentration of sodium chloride maintained in the course of the precipitin tests was two per cent. This concentration of sodium chloride was very nearly equal to the one experimentally determined. This concentration had two advantages. It permitted enough edestin to be dissolved so that the concentration of edestin, as determined by a micro-Kjeldahl analysis, was easily demonstrated with a minimum of error and also so that a strong precipitin test was afforded.

The third of these difficulties arises in determining exactly the concentration of edestin and of unknown in their respective solutions which will just inhibit the precipitin test. Such a determination can be made in either of two ways.

1. An amount of edestin or the "Unknown" may be dissolved in two per cent sodium chloride solution, which should be kept at 40° C., and the concentration of each of these substances in the filtrates obtained from these solutions after they have been filtered, determined by a micro-Kjeldahl analysis. These filtrates may then be diluted with successive portions of two per cent sodium chloride solution of which the temperature
Should be 40° C. After each dilution the intensity of the precipitin test may be observed upon samples taken after each dilution, and when the precipitin test is just inhibited, the proper dilution will have been reached. Knowing the concentration and the volume of edestin in the original solution and the volume of two per cent sodium chloride added each time a dilution was made, the concentration of edestin which inhibits the precipitin test may be easily calculated after applying the necessary corrections.

II. An unknown quantity of edestin which has been dissolved at 40° C. in two per cent sodium chloride solution may be further diluted with two per cent sodium chloride solution which also must be at 40° C. until a dilution which just inhibits the precipitin test is reached. In order to obtain the proper dilution, the original solution must be diluted with successive volumes of two per cent sodium chloride solution and the precipitin test made upon samples taken after each dilution. The concentration of protein in the solution which finally inhibits the precipitin test by the micro-Kjeldahl method may then be determined.

Serological Demonstration of the Identity of Edestin and of "Unknown"

Two half grown rabbits were immunized by injecting, at five day intervals, increasing doses of edestin suspension. When six days had elapsed after the last dose, blood was with-
drawn from a vein in the rabbit's ear and the serum was found to be immune since it responded positively to precipitin test when placed in contact with edestin solution, but responded negatively when placed in contact with a serum immunized to a protein other than edestin, or when placed in contact with a dilute sodium chloride solution.

A series of precipitin tubes of about 3 mm. in inside diameter were supported by racks placed in a thermostat at 40° C. A beaker containing a clear solution of edestin in two per cent sodium chloride at 40° C., another containing a solution of the unknown also in two per cent sodium chloride solution at 40° C., and a third beaker containing only two per cent sodium chloride solution were placed in the same thermostat.

A quantity of the immune serum was placed in a precipitin tube by means of a pipette and then a quantity of the edestin solution was introduced into the precipitin tube in such a way that the two liquids did not mix and that the surface of contact between them was well defined. This precipitin tube was then replaced on the rack in the thermostat. A number of other precipitin tubes containing edestin antiserum were treated with two per cent sodium chloride solution under the same conditions and found to respond negatively to precipitin test. The precipitin tubes containing edestin antiserum and edestin solution were observed and found to have responded positively to precipitin
test. The edestin solution in the beaker was then diluted with two per cent sodium chloride solution which was at 40° C. The edestin solution which was thus obtained reacted positively when tested with edestin antiserum. In accordance with the second of the two methods outlined previously, this edestin solution was diluted with two per cent sodium chloride solution repeatedly until non-occurrence of the precipitin test.

Nine cubic centimeters of this solution were analyzed by the micro-Kjeldahl method whereby the amount of nitrogen per c.c. was found to be 0.0000139 grams. This corresponds to a concentration of 0.000074 grams of edestin per c.c. of the edestin solution which just failed to give the precipitin test.

The "Unknown" was then tested with edestin antiserum in the same manner as all the edestin solutions were tested. The solution of the "Unknown" which finally also inhibited the precipitin test was likewise analyzed for its Kjeldahl nitrogen content in exactly the same manner as the final edestin solution was analyzed. The amount of nitrogen found was the same as that which was found in the case of the edestin solution which had been similarly tested.

The details with respect to the determination of the nitrogen content of the solutions mentioned above are as follows: Nine cubic centimeters of this solution of the "Unknown", which just causes the precipitin test to fail to appear, were placed in a Kjeldahl flask, and were treated according to the specifications of the
Kjeldahl method, and the amount of ammonia liberated was absorbed in twenty cubic centimeters of 0.1074 normal sulfuric acid. The sulfuric acid which now contained the ammonia was transferred quantitatively to a 500 c.c. volumetric flask, and the volume of the solution was made to 500 c.c. Four cubic centimeters of this solution were nesslerized in the usual way. The color which developed had the same intensity as a standard solution which contained 0.000003 gram of nitrogen. This color also had the same intensity as the color which was obtained when the edestin solution which just failed to give the precipitin test was analyzed for its nitrogen content in the same way.

The reagents used in the above mentioned tests were also analyzed for their nitrogen contents by means of the micro-Kjeldahl method. The nitrogen in the quantities of reagents used, when calculated to the same basis as for the edestin and the unknown, was 0.000002 gram of nitrogen.

When this amount of nitrogen is subtracted from the amount obtained in the course of the tests with edestin and the unknown 0.000001 gram of nitrogen are obtained. The amount of nitrogen per c.c. that corresponds to the edestin and to the unknown in their respective solutions which inhibit the precipitin test is obtained when this value is multiplied by $\frac{500}{459}$. This amount of nitrogen is 0.000014 gram or 0.00000714 gram of edestin per c.c.
DISCUSSION OF RESULTS

I. Inasmuch as the "Unknown", when dissolved in sodium chloride solution, responded qualitatively to edestin antiserum, and inasmuch as the nitrogen concentration of the solution of the "Unknown" which just failed to give the precipitin reaction is exactly the same as that of the edestin brine solution which also just failed to give precipitin reaction under the same conditions, I conclude that the "Unknown" is identical with edestin, and that edestin which is dissolved in ten per cent sodium chloride solution is only partially coagulated when such a solution is boiled for one-half hour (boiling point 95° C.).

II. I also conclude that the heat coagulation of edestin is not an instantaneous process but a reaction which proceeds measurably with time.
SUMMARY

1. No appreciable quantity of ammonia could be detected when edestin was coagulated by heat.

2. When edestin which has been dissolved in ten per cent sodium chloride solution was boiled (boiling point 95° C.) for thirty minutes it was only partially coagulated.

3. Heat coagulation is not an instantaneous process.


19:37 (1930)

(3) Downs and Goodner. J. Infectious Diseases. 38:240 (1925)


(5) Friedmann and Friedentall. X. Exp. Path. Therap. 3:73 (1906)

(6) Fleischmann and Michealis. Biochem. Z., 3:425 (1907)


(9) Morrow, C. A. Biochemical Laboratory Methods for Students of Biological Science (1927) page 123.


Amer. Chem. Journal 14:671

Journal American Chem. Society 18:609; 21:488 (1899);

24:26 (1902)

(12) Ramsdall. J. Exp. Med. 48:615 (1928)


(14) Robertson, T. B. The Physical Chemistry of the Proteins. (1924) page 307


(16) Wu and Wu. J. Biol. Chem. 64:369 (1925)

*Only abridgement of reference cited.
Chemistry of the heat coagulation of proteins.