SULFHYDRYL GROUPS AND THE COAGULATION OF PROTEINS AS INFLUENCED BY REDUCING SUGARS

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CONTENTS

INTRODUCTION

The study of proteins and the factors influencing their behavior has been greatly stimulated due to the gradual realization that a great many substances posessing remarkable physiological activities appear to be proteins. Certain enzymes, hormones, and viruses as well as toxins, antitoxins, antigens, and antibodies appear to be proteins.

The terms denaturation, flocculation, and coagulation are confused in the literature on proteins. In this paper I will use these terms as outlined by Bull (1).. "Protein denaturation consists of an over-all process of three reactions, and these reactions can under appropriate conditions, be separated and studied individually. The first reaction in the series is that of denaturation proper, which apparently is an intermolecular rearrangement whereby certain chemical groups which were not detectable in the native protein are rendered so in the denatured product. Denaturation is a necessary but not sufficient reaction in the production of coagulated egg albumin. The second reaction consists in the flocculation of the denatured molecules preparatory to coagulation. The flocculation reaction is the only one of the series that is reversible. The third and last step is the formation of an insoluble coagulum."

The coagulation of proteins was one of the first changes noted in connection with the stability of proteins. Coagulation changes the properties of proteins greatly and was regarded as a factor in life processes. In 1929 Beilinnson

(2) reported that in the presence of sucrose, protein solutions were stabilized towards heat coagulation. Protection not nearly so great was reported for glycerol. Duddles (3) studied the effect of sugars and mannitol upon coagulation of egg albumin. He found that not only sucrose but glucose, mannose, fructose, and mannitol showed a definite inhibiting effect towards coagulation by heat and ultra-violet light. Newton and Brown (4) worked with plant saps and found that sucrose and glucose prevented coagulation of plant proteins by freezing.

In a large number of cases when proteins denature sulfhydryl groups appear (5). It is thus possible to use their detection as a measurement of denaturation. It is known that in a great many instances sulfhydryl groups are closely associated with the physiological and chemical activities of proteins (26).

In the present work I will attempt to show how certain factors influence the liberation of sulfhydryl groups as well as the estimation of these groups in native, partially coagulated, and completely coagulated egg albumin.

HISTORICAL

In reviewing the literature on this problem I will consider the following points.

1. The occurence of sulfhydryl groups in proteins.
2. Methods for estimating sulfhydryl groups in proteins.
3. Possible protein and carbohydrate combinations.

THE OCCURENCE OF SULFHYDRYL GROUPS IN PROTEINS

In 1901 Embden (7) reported that he had isolated cysteine from a protein hydrolasate. In the same year Mörner (8) showed that this amino acid had been formed during the process of hydrolysis and separation and was in reality an artifact. Later Patten (9) was able to make it clear that cystine and not cysteine was the primary amino acid formed when proteins were hydrolyzed in the manner employed by Embden.

Harris (10) cited work by Sieber and Schonbenko who obtained methyl and ethyl mercaptans by the fusion of proteins with alkali. He also refered to work by Rubner who obtained the same products by dry distillation and Drechsel who reported that during acid hydrolysis of certain proteins, mercaptans were formed and believed that they were derived from some basic body present in the original protein.

Mörner (8) first attempted to determine quantitatively the sulphur groups in proteins. He tested proteins for thiolactic acid formerly isolated from horn protein by Friedmann, according to Harris (10), thioamino succinic acid believed by

Baumann and Schmitz (11) to be a protein constituent, cystine, and cysteine. From the results obtained using egg albumin Mörner concluded that cystine was the only one of these acids present in the native protein. Using a quantitative method based upon the blackening of lead acetate paper Mörner (8) reported that of the total sulphur present in ovalbumin only one third to one half of it could be accounted for by cystine. He noted also that in the case of ovalbumin an unidentified sulphur compound was volatilized and this represented about one third of the total sulphur. Suter (12) used a method similar to that used by Mörner and reported analagous results on ovalbumin and on other proteins.

Pick (13) working with primary proteoses derived from fibrin noted that all of the sulphur was given off as hydrogen sulfide when treated with alkali. He interpreted this as indication that some of the sulphur present was in a form other than cystine. Johnson and Burnham (14) suggested that this sulphur, other than cystine might be in the form of thiopolypeptides and their derivatives. Johnson and Burnham suggested that oxygen in peptide linkages may be replaced by sulphur.

Arnold (15) worked on a large variety of protein material with the aid of the nitroprusside reaction. He found that tissue extracts, certain coagulated proteins, and native proteins gave a positive test. He believed that these positive tests were in all cases due to the presence of cysteine. Later Hopkins (16) was able to show that in tissue extracts the positive nitroprusside test was not due to free cysteine but to glutathione which he at that time believed was a dipeptide containing cysteine and glutamic acid.

Using the nitroprusside test of Arnold's with Hopkins modification, Harris (10), tested egg albumin in various states for cysteine. He found that raw egg white was non-reactive with nitroprusside but upon heat coagulation it gave a positive test. Furthermore he found that albumin precipitated by ammonium sulfate and weak alcohol gave a negative test but that albumin precipitated with hydrochloric acid gave a positive one. This showed that precipitation by hydrochloric acid was not simply a precipitation as had formerly been believed. The white feathery precipitate resulting from ultra-violet light treatment gave a negative test with nitroprusside. The observation formerly made by Young (17) that this precipitate could be readily redissolved by shaking was confirmed. Somewhat different results were reported by Mirsky and Anson (5) who found that protein coagulated after irradiation with ultra-violet light gave a positive test for sulfhydryl groups and the quantity was the same as when the coagulum was further treated with strong acid.

Meldrum (19) stated that denatured proteins could be divided into three groups on the basis of their reaction with nitroprusside.

1. Those which gave a positive test for sulfhydryl groups Egg albumin was an example.
2. Those which gave a positive test for the disulfide group. Serum proteins were examples.
3. Those which did not give a reaction for either free sulfhydryl or disulphide groups. Ovomucoid (10) and globin (19) examples.

Mirsky and Anson (18) did not believe that globin belonged in group three because they obtained positive tests for both sulfhydryl and disulfide groups. Meldrum (19) repeated this work and pointed out that the conclusions drawn by Mirsky and Anson were erroneous. However, Mirsky and Anson (20) in a later paper refuted Meldrum.

In a series of papers Mirsky and Anson (20) (21) (5) (22) brought out the following points. The number of sulfhydryl and disulfide groups that can be detected in an unhydrolyzed coagulated protein was equivalent to the total amount of cysteine and cystine that could be determined in the hydrolyzed protein. The appearance of sulfydryl and disulfide groups when proteins coagulated was the only known chemical change that occured in the protein molecule. Some native proteins contained detectable sulfhydryl and disulfide groups and the number measurable was always less than the number found when the protein was coagulated. The effect of denaturation was to shift the range of activity so that the sulfhydryl and disulfide groups could react at a lower pH. When coagulation was reversed the sulfhydryl and disulfide groups that became detectable upon coagulation were no longer active, furthermore the behavior of these groups indicated that coagulation was reversible. The activity of sulfhydryl and disulfide groups was affected by coagulation, pH, temperature, and the nature of the protein. Coagulation increased the number of detectable sulfhydryl and disulfide groups. The effect that temperature had on the activity was not stated by the authors although they did say that it had a definite effect.

Mirsky (23) pointed out that an increase in the activity of sulfhydryl groups without shifting the pH is the criterion of denaturation.

Anson (24) believed that native egg albumin contained free sulfhydryl groups, but that these groups were relatively unreactive. By using iodine and iodoacetamide he was able to detect these groups even though they were unreactive towards ferricyanide, porphyrindine or nitroprusside.

The fact that the properties of certain proteins were altered when placed in urea or other amide solutions, often with an increase in the number of detectable sulfhydryl groups was first reported by Hopkins (25). In a series of papers Greenstein and Coworkers (26) (27) (28) pointed out that when proteins are placed in solutions of urea, guanidine hydrochloride, and related substances the number of detectable sulfhydryl groups varied widely. For all of the proteins investigated by Greenstein the maximum number of these sulfhydryl groups was found in solutions of guanidine hydrochloride. The percentage of the sulfhydryl groups that could be detected was independent of the protein concentration and depended upon the concentration of the guanidine hydrochloride.

Blumenthal and Clarke (29) stated that there was ample evidence in some proteins that sulphur existed in forms other than cystine, cysteine, and methionine. Ashley and Harrington (30) believed that part of the sulphur in zein was present as thiolhistidine and Zahnd and Clarke (31) have detected thioglyoxaline in fractions of hydrolyzed egg white.

METHODS FOR ESTIMATION OF SULFHYDRYL GROUPS IN PROTEINS

The earliest attempts to estimate quantitatively the sulfhydryl groups in proteins made use of the nitroprusside method described by Arnold (15). This reagent was considered rather specific for sulfhydryl groups and developed a pink color in the presence of these in alkaline solution. However, the color faded rather rapidly and several modifications have been suggested in an attempt to stabilize the color. Hopkins (16) used an excess of ammonium sulfate instead of aqueous ammonia. Walker (32) obtained better results by using sodium cyanide or potassium cyanide. Zinc salts intensified and stabilized the color according to Giroud and Bulliard (33). Saturated solutions of sodium sulfate and magnesium sulfate were used to accomplish the same purpose by Mentzer (34). By varying the pH at which the protein and nitroprusside are allowed to react the reaction can be made more specific according to Zimmet and Perrenoud (35).

Two methods and their applications for the determination of sulfhydryl groups were described by Mirsky and Anson (5). The so called direct method in which the sulfhydryl groups are oxidized by cystine which is thereby reduced to cysteine. Consequently the number of sulfhydryl groups oxidized can be estimated by determining the amount of cysteine found. The authors stated that cystine oxidized all of the sulfhydryl groups and no other groups in the protein. In the indirect method oxidizing agents or iodoacetate were used to eliminate the sulfhydryl groups and the reagent added was then removed.

The protein was then hydrolyzed and its total cysteine content compared with the cysteine content of the untreated protein. The decrease in the cysteine content was equal to the number of sulfhydryl groups which reacted with the reagent. Cystine and cysteine in the hydrolasate were determined colorimetrically with phospho-18-tungstic acid. Cysteine but not cystine gave a blue color in the absence of sulfite while cystine gave a blue color in the presence of sulfite.

Kuhn and Desnuelle (36) introduced the use of the blue dye porphrindine for the estimation of reducing groups including sulfhydryl groups. This dye a powerful oxidizing agent reacts rapidly and stoichiometrically with sulfhydryl groups in the cold. Greenstein and co-workers (27) (6) (37) have used this reagent extensively for the determination of sulfhydryl groups in proteins. They noted that its application was based upon two assumptions. (1) That a positive nitroprusside test in a protein solution is given only by sulfhydryl groups and (2) that porphyrindine was reduced in a neutral protein solution at room temperature within one to two minutes by sulfhydryl groups only. Greenstein also pointed out that the carbonyl group gave a positive test but the tint of the color so developed was in marked contrast to the rapidly fading color given by sulfhydryl groups. Tyrosine formed an orange color that developed slowly and could be readily distinguished. Perez and Sandor (38) have reported satisfactory results using porphyrindine. Objections against the use of this reagent have been raised by Anson (39) on the grounds that it was hard to prepare, was unstable, and was a

dangerously strong oxidizing agent for use on proteins.

Todrick and Walker (40) reported a method which consisted in measuring the amount of the oxidation-reduction indicator phenolindo-2-6-dichlorophenol (2-6 dichlorobenzenoneindophenol) reduced by a known weight of protein. The indicator was first standardized in terms of cysteine hydrochloride. A series of test tubes containing the same amount of protein were prepared and varying quantities of the indicator were added. The contents were then allowed to react and the tubes that were decolorized within twenty minutes were noted. A further series of tubes were then set up containing a closer range of quantities of indicator. The authors claimed that under the conditions described by them the reaction was specific for sulfhydryl groups and accurate to two or three percent.

Dickens (41) showed that under certain conditions of pH and temperature, halogenated acetic acids reacted readily with sulfhydryl groups forming the corresponding thio ethers and hydrogen halide. That iodoacetic acid can react with amino groups but slowly if at all under these conditions had been shown by Michaelis and Schubert (42). They pointed out that in the absence of an excess of iodoacetic acid the reaction between sulfhydryl groups and iodoacetic acid was much more rapid and the amino group was not affected.

Smythe (43) has measured the rate of reaction of iodoacetic acid with various sulfhydryl compounds by measuring the carbon dioxide liberated from a carbon dioxide--bicarbonate buffer as a result of the hydriodic acid formed.

Rapkine (44) first showed that iodoacetic acid reacted not only with sulfhydryl groups of relatively simple molecules

such as cysteine and glutathione but also with proteins. Based upon this observation was the method described by Rosner (45) for the determination of sulfhydryl groups in proteins. In this method the hydriodic acid formed was treated with hydrogen peroxide and the color that developed was read on the photelometer which had previously been standardized in terms of cysteine hydrochloride.

Mirsky and Anson (5) and Anson (22) found that ferricyanide in a neutral solution oxidized sulfhydryl groups to disulfide groups. They stated that cysteine was the only amino acid which was known to react stoichiometrically with dilute ferricyanide. These same authors concluded that concentrated ferricyanide will oxidize tyrosine and tryptophane but these reactions were indefinite. To estimate sulfhydryl groups by this method ferricyanide was added to the protein solution, the sulfhydryl groups reduced the ferricyanide to ferrocyanide which was then estimated as prussian blue. These workers have obtained best results when the protein was dissolved in Duponal P. C. solution.

Flatow 47 has estimated the glutathione content of protein solutions by using an excess of ferricyanide and back titrating with indigosulfonic acid.

Methods involving the use of iodine have been used by a number of workers; Okuda and Masayoshi (48), Tunnicliffe (49), Thompson and Voegthin (50), and Woodward and Fry (51). Iodine is believed to react with sulfhydryl groups forming disulfide groups and hydriodic acid. The hydroidic acid may then be titrated or oxidized and determined colorimetrically. This method has had rather wide applications but Mirsky and

Anson (5) believed it to be inaccurate for sulfhydryl groups at low concentrations.

Anson (46) has stated that no reagent is available to determine without question all the sulfhydryl groups and no others in denatured egg albumin.

POSSIBLE PROTEIN AND CARBOHYDRATE COMBINATIONS

The retarding effect of carbohydrates on protein coagulation may be due to peptization (52) or to a more definite chemical combination (53).

According to Meyer (54) protein and carbohydrate combinations are divided into two main classes. The first, composed of the various mucopolysaccharides occur in nature either as free polysaccharides or as protein salts. The second group contains the glycoproteins; proteins or polypeptides containing hexose amines and other sugars in an unknown combination.

Chondroitinsulfuric acid a mucopolysaccharide first isolated from cartilage by Mörner, according to Meyer (54), was noted by this author to co-precipitate with gelatin upon acidification. Meyer and co-workers (55) made use of this observation and prepared protein chondroitinsulfuric acid complexes. After purifying these complexes by reprecipitation they were analyzed for hexosamine. The results indicated that the inorganic cation in the chondroitinsulfuric acid had been replaced by the basic amino group of the protein. These workers came to the conclusion that these combinations were of a salt like nature. Compounds of a similiar type were prepared using mucoitinsulfuric acid but they differed from the above in that they were more stable.

Fränkel and Jellinek (56) were able to separate a non-reducing carbohydrate fraction from egg white. From this fraction they isolated glucosamine and mannose. The same compounds were isolated from egg white and ovomucoid by Levene and Rothen (57) and Levene and Mori (58). These workers believed that the non-reducing carbohydrate was a glucosamine dimannoside.

Fränkel and Katchalsky (59) studied the reactions between mixtures of sugars and amino acids and polypeptides by following the change in pH occuring as the free amino groups disappeared. They were able to detect a reaction when aldose sugars were used; a reaction depending entirely upon the alpha amino group of the amino acid. Reactions were detected over a pH range of 4.5-11.0 and an optimal pH for reaction was noted. In a later paper Fränkel and Katchalsky (60) reported that glycine would combine with aldehydic sugars but would not combine with non-aldehydic sugars.

A definite crystalline acid was obtained by Genevois and Cayrol (61) by allowing a dilute cysteine solution of pH greater than 3.0 and formaldehyde to react mole for mole. Compounds were also formed using alanine and formaldehyde but the resulting acid was less stable. Slower reactions between cysteine and other aliphatic aldehydes were noted but reactions between cysteine and ketones were reported only when the ketones were present in large excess. The fact that thioglycolic acid and thiomalic acids did not react was explained by these workers as being due to the absence of an amino group in a beta

position to the sulfhydryl group.

Schubert (62) by analysis of the crystalline compounds formed between cysteine and d-arabinose, d-xylose, d-glucose, d-mannose, d-galactose, and lactose showed that cysteine combined with these sugars in a mole to mole ratio with the elimination of a molecule of water. These compounds were formed at room temperature in a neutral solution and were rather easily soluble in water giving solutions that were acid to litmus. The fact that these compounds did not give positive nitroprusside tests under conditions where cysteine gave a strong test indicated that the combination was through the sulfhydryl group in cysteine. Schubert was not able to isolate any compound from mixtures of fructose and cysteine indicating he believed that the linkage in the sugar is through the aldehyde group.

Przylecki and Majmin (63) (64) were able to form complexes using proteins and various so called polyglucides. They found that below their isoelectric points, albumins formed salt like complexes with phosphorylated glucides. Above their isoelectric point the complexes did not appear to be salt like. These workers presented evidence showing stoichiometric combinations of myosin with various polyglucides.

In a later paper Przylecki (53) stated that three kinds of combinations between carbohydrates and proteins were possible, covalent, molecular and salt like. He also listed twelve ways in which carbohydrates might react with proteins through the free groups of the amino acids present in proteins. Przylecki was able to form compounds between maltose and ovalbumin, crystallized seralbumin, casein, and clupein.

In preparing these compounds which he called "symplexes", mixtures of the protein and carbohydrate were allowed to stand at low temperatures for several days at a rather high pH. A parallelism between the lysine content of the protein and the amount of sugar that would combine with the protein was noted. No symplex was obtained using sucrose indicating that the reducible group of the sugar must be free for a reaction to take place.

EXPERIMENTAL

The experimental work on this problem was divided into three parts as outlined below.

1. The effect of sugars and mannitol on the liberation of sulfhydryl groups.
2. The effect of sugars on the coagulation of egg albumin.
2. An attempt to show protein--carbohydrate combination.

THE EFFECT OF SUGARS AND MANNITOL ON THE LIBERATION OF SULFHYDRYL GROUPS

Egg albumin was prepared by the method of Kekwick and Cannan (65) in which sodium sulfate was used to precipitate the albumin. A propeller type stirrer as suggested by Westfall (66) was used to dissolve the sodium sulfate and precipitate the egg albumin. The sodium sulfate was dialyzed out of the solution under reduced pressure until the test for the sulfate ion was negative. The nitrogen content of the egg albumin solution was determined in duplicate by a Micro-Kjeldahl method (67). In this procedure 2 ml. aliquots of the protein solution were pipetted into Micro-Kjeldahl flasks, 2 ml. of concentrated sulfuric acid, 0.1 gm. of potassium sulfate and a few crystals of copper sulfate were added. The mixture was heated with a micro burner until it became light brown; a few drops of thirty percent hydrogen peroxide were then added and the mixture again heated. This treatment with peroxide was repeated until the mixture was light blue. The distillation was then carried out in a Micro-Kjeldahl distillation apparatus using boric

acid in the receiving flask. Titrations were carried out with 0.01 N hydrochloric acid.

To study the effect of sugars on the liberation of sulfhydryl groups the photelometer was standardized in terms of cysteine by the method of Rosner (45). Standard solutions of cysteine hydrochloride containing from 0.1 to 0.5 of a mg. of cysteine per 1.5 ml. were prepared. To these were added 4.5 ml. of distilled water, the mixture then heated at 70° C. for fifteen minutes and then allowed to cool. These mixtures were then treated with 2 ml. of potassium dihydrogen phosphate--sodium hydroxide buffer, pH 7.4, 2 ml. of ~~2.5~~ 0.1 N iodoacetic acid, previously neutralized with potassium hydroxide, and then allowed to stand for thirty minutes. At the end of that time 0.25 ml. each of a solution ten percent in respect to both trichloracetic acid and concentrated sulfuric acid were added. This mixture was allowed to stand for five minutes and then filtered. To the filtrate was added 0.25 ml. of three percent hydrogen peroxide and the mixture made up to 10 ml. with distilled water. At the end of thirty-five minutes the color was read on a photelometer using a blue filter with a maximum absorption range of 4,500 A°--5,000 A°. The results of the standardization are shown in Figure 1. This standardization was repeated using sugar solutions in place of the distilled water giving identical results.

An egg albumin solution was then adjusted to pH 4.8 with 0.1 N hydrochloric acid. One and one half ml. aliquots of this solution containing 6.5 mg. of soluble nitrogen per ml. were pipetted into test tubes and 4.5 ml. of the sugar solution added. These mixtures were then treated exactly as in

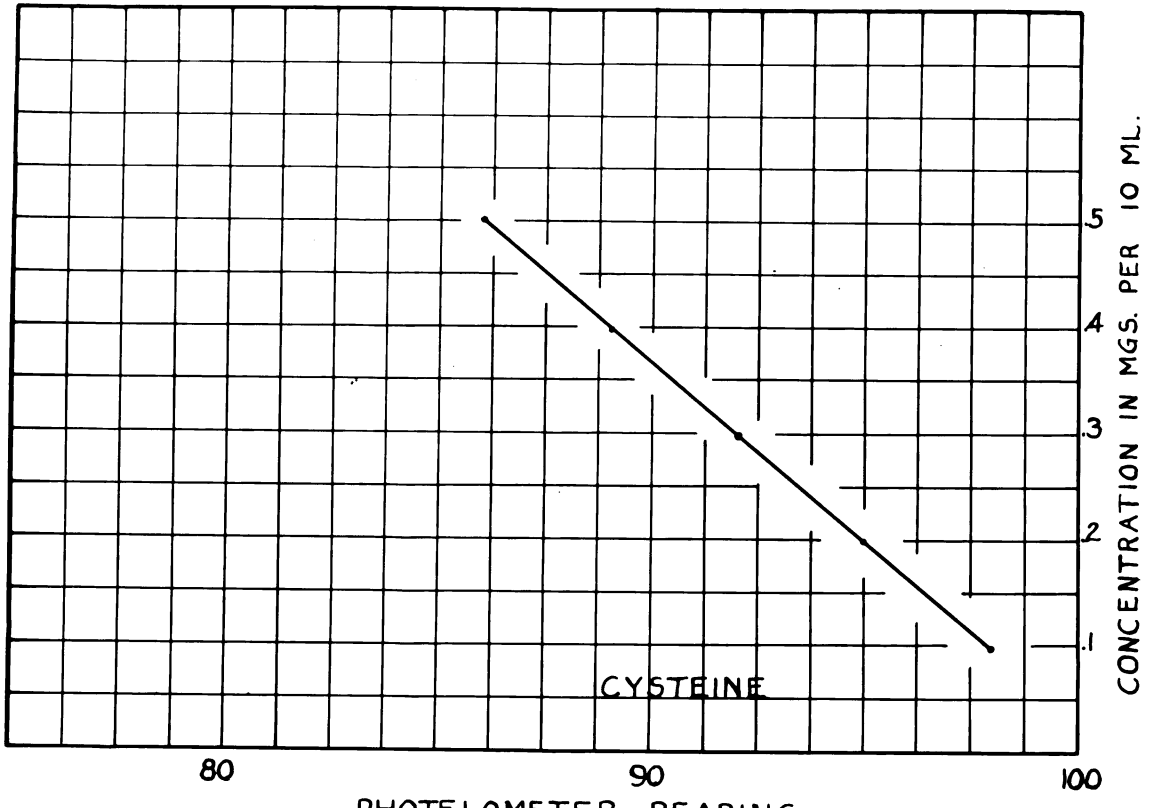
.5
.4
.3
.2
.1
CONCENTRATION IN MGS. PER 10 ML.
CYSTEINE
80
90
100
PHOTELOMETER READING

FIG. I

the standardization procedure and the color that developed was read on the photelometer. The mgs. of cysteine corresponding to the color that developed was then read from the standardization curve. The results obtained using d-glucose are given in Table 1. Each value in all of the following tables represents duplicate determinations.

The effect of d-fructose, d-mannose, the closely related alcohol mannitol, and the pentoses l-arabinose and d-xylose were studied following the same procedure used with d-glucose. The results are shown in Tables 2, 3, 4, 5, and 6.

In order to study further the effect of the sugars and alcohol an attempt was made to check the above results using a different method. Inasmuch as the protein used in this work was only partially coagulated, certain of the usual methods are not applicable because the reagents and methods cause further denaturation and coagulation of the protein. It was believed that the method of Todrick and Walker (40) using phenolindo-2-6-dichlorophenol would be applicable. In order to use this method the indicator was first standardized in terms of cysteine. A weighed amount of cysteine hydrochloride was first neutralized in an atmosphere of nitrogen and then dissolved in 2 ml. potassium hydrogen phthalate--sodium hydroxide buffer pH 4.8. This solution was then made up to 10 ml. using boiled distilled water and titrated also in an atmosphere of nitrogen using a one-tenth percent solution of the indicator. One tenth milligram of cysteine was found to be equivalent to three ml. of the indicator.

TABLE 1

Effect of Glucose on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of varying quantities of d-glucose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.590%, equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Glucose		$\frac{M}{2}$ Glucose		$\frac{M}{10}$ Glucose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.230	.377	.300	.493	.335	.550
.230	.377	.300	.493	.335	.550
.197	.324	.300	.493	.335	.550
.230	.377	.265	.434	.350	.575
.197	.324	.265	.434	.350	.575
.197	.324	.300	.493	.350	.575
.230	.377	.300	.493	.335	.550
.230	.377	.265	.434	.335	.550
.230	.377	.300	.493	.350	.575
.230	.377	.300	.493	.350	.575

TABLE 2

Effect of Fructose on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method.

Egg albumin solution (9.75 mgs. N. per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of varying quantities of d-fructose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.590%, equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Fructose		$\frac{M}{2}$ Fructose		$\frac{M}{10}$ Fructose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.230	.377	.300	.493	.350	.575
.265	.434	.300	.493	.350	.575
.265	.434	.300	.493	.350	.575
.265	.434	.282	.463	.350	.575
.230	.377	.282	.463	.350	.575
.265	.434	.300	.493	.355	.550
.230	.377	.300	.493	.350	.575
.265	.434	.300	.493	.350	.575
.230	.377	.300	.493	.350	.575
.265	.434	.300	.493	.335	.550

TABLE 3

Effect of Mannose on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method.

Egg albumin solution (9.75 mgs N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of d-mannose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.590% equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Mannose		$\frac{M}{2}$ Mannose		$\frac{M}{10}$ Mannose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.230	.377	.335	.550	.350	.575
.265	.434	.335	.550	.350	.575
.265	.434	.300	.493	.365	.600
.265	.434	.300	.493	.350	.575
.230	.377	.300	.493	.350	.575
.265	.434	.335	.550	.365	.600
.265	.434	.300	.493	.350	.575
.265	.434	.300	.493	.350	.575
.265	.434	.300	.493	.350	.575
.265	.343	.300	.493	.350	.575

TABLE 4

Effect of Mannitol on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs egg albumin) coagulated in the presence of mannitol by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.590%, equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Mannitol		$\frac{M}{2}$ Mannitol		$\frac{M}{10}$ Mannitol	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.300	.493	.350	.575	.366	.606
.300	.493	.350	.575	.366	.606
.265	.434	.366	.606	.366	.606
.265	.434	.350	.575	.350	.575
.300	.493	.350	.575	.366	.606
.300	.493	.350	.575	.366	.606
.300	.493	.666	.606	.350	.575
.300	.493	.350	.575	.366	.606
.300	.493	.350	.575	.366	.606
.300	.493	.366	.606	.366	.606

TABLE 5

Effect of Arabinose on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of l-arabinose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.590 % equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Arabinose		$\frac{M}{2}$ Arabinose		$\frac{M}{10}$ Arabinose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.426	.300	.493	.350	.575
.250	.409	.315	.516	.365	.606
.260	.426	.315	.516	.335	.550
.260	.426	.315	.516	.350	.575
.250	.409	.300	.493	.365	.606
.250	.409	.300	.493	.365	.606
.230	.377	.282	.463	.350	.575
.230	.377	.282	.463	.350	.575

TABLE 6

Effect of Xylose on the Cysteine Content of Altered Egg Albumin as Determined by the Iodoacetic Acid Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml. equivalent to 60.93 mgs. egg albumin) coagulated in the presence of d-xylose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of of distilled water 0.590% equivalent to .359 mgs. cysteine.

$\frac{M}{1}$ Xylose		$\frac{M}{2}$ Xylose		$\frac{M}{10}$ Xylose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.426	.300	.493	.350	.575
.260	.426	.300	.493	.350	.575
.282	.463	.315	.516	.365	.606
.260	.426	.315	.516	.365	.606
.282	.463	.315	.516	.365	.606
.260	.426	.300	.493	.350	.575
.260	.426	.315	.516	.365	.606
.250	.409	.315	.516	.365	.606

One and one half ml. aliquots of an albumin solution containing 6.5 mgs. of soluble nitrogen per ml. were then pipetted into test tubes. To these were pipetted 4.5 ml. of the sugar solution 2 ml. of the buffer pH 4.8 and 2 ml. of boiled distilled water. Varying quantities of the indicator were then added and the tubes heated at 70° C. for fifteen minutes. The tubes that were decolorized within that time were noted and a further series of tubes set up containing a closer range of quantities of indicator. The originators of this method claim to be able to distinguish between 0.01 ml. amounts of the indicator but in this work 0.5 ml. appeared to be the limit. The results using d-glucose, d-fructose, and d-mannose are shown in tables 7, 8, and 9.

THE EFFECT OF SUGARS ON THE COAGULATION OF EGG ALBUMIN

The inhibiting effect of pentoses on the liberation of sulfhydryl groups has been shown above but whether the pentoses would as expected effect the amount of coagulation was thought worth studying inasmuch as pentoses or pentosans are prominent in plant tissues. The effect of pentoses on the coagulation of egg albumin was studied in the following manner.

One and one half ml. of an egg albumin solution containing 6.5 mgs. of soluble nitrogen per ml. were pipetted into test tubes. Four ml. of the buffer pH 4.8 and 4.5 ml. of the sugar solution were added. The solutions were then heated at 70° C. for fifteen minutes, filtered and the nitrogen in a 2 ml. aliquot of the filtrate determined using the Micro-Kjeldahl technique previously described. The results are

TABLE 7

Effect of Glucose on the Cysteine Content of Altered Egg Albumin as Determined by the Indicator Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of d-glucose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.570%, equivalent to .343 mgs. cysteine.

$\frac{M}{1}$ Glucose		$\frac{M}{2}$ Glucose		$\frac{M}{10}$ Glucose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
2.13	.373	.281	.493	.333	.584
2.00	.350	.297	.521	.343	.601
2.20	.386	.271	.475	.330	.579
2.30	.403	.282	.493	.320	.592
2.16	.379	.282	.493	.320	.561

TABLE 8

Effect of Fructose on the Cysteine Content of Altered Egg Albumin as Determined by the Indicator Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of d-fructose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.570%, equivalent to .343 mgs. cysteine.

$\frac{M}{1}$ Fructose		$\frac{M}{2}$ Fructose		$\frac{M}{10}$ Fructose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.456	.290	.508	.308	.540
.264	.463	.280	.492	.305	.537
.259	.455	.296	.519	.311	.548
.261	.458	.288	.505	.321	.564
.254	.445	.284	.498	.326	.570

TABLE 9

Effect of Mannose on the Cysteine Content of Altered Egg Albumin as Determined by the Indicator Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 60.93 mgs. egg albumin) coagulated in the presence of d-mannose by heating at 70° C. for 15 minutes. Percent cysteine in egg albumin coagulated in the presence of distilled water 0.570% equivalent to .343 mgs. cysteine.

$\frac{M}{1}$ Mannose		$\frac{M}{2}$ Mannose		$\frac{M}{10}$ Mannose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.456	.307	.539	.345	.605
.255	.447	.320	.561	.335	.589
.251	.440	.293	.514	.333	.583
.248	.435	.305	.535	.325	.570
.265	.464	.285	.500	.343	.601

TABLE 10

Effect of Arabinose on the Coagulation of Egg Albumin Solution as Determined by the Residual Nitrogen Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 1.95 mgs. N. per 2 ml. filtrate) coagulated in the presence of l-arabinose by heating at 70° C. for 15 minutes. Percent nitrogen in filtrate from egg albumin coagulated in the presence of distilled water 8.42%.

$\frac{M}{1}$ Arabinose		$\frac{M}{2}$ Arabinose		$\frac{M}{10}$ Arabinose	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.826	42.35	.560	28.71	.280	14.36
.812	41.64	.620	20.81	.333	17.08
.834	42.76	.616	31.59	.371	19.06
.756	38.76	.588	30.10	.336	17.23
.854	43.78	.539	27.64	.315	16.15
.784	40.20	.532	27.28	.294	15.08
.792	40.61	.662	33.95	.315	16.15
.756	38.76	.588	30.16	.273	14.00

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Egg albumin solution (9.75 mgs N per 1.5 ml., equivalent to 1.95 mgs. N per 2 ml. of filtrate) coagulated in the presence of d-xylose by heating at 70° C. for 15 minutes. Percent nitrogen in filtrate from egg albumin coagulated in the presence of distilled water 8.42%.

$\frac{M}{1}$ Xylose		$\frac{M}{2}$ Xylose		$\frac{M}{10}$ Xylose	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.672	34.46	.423	21.69	.210	10.77
.714	36.61	.402	20.66	.182	9.33
.750	38.46	.476	24.41	.196	10.05
.761	39.03	.482	24.73	.185	9.48
.711	36.46	.406	20.82	.238	12.21
.731	37.49	.479	24.57	.210	10.77
.694	35.59	.386	19.79	.246	12.61
.724	37.13	.406	20.82	.704	10.46

TABLE 12

Effect of pH on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml. equivalent to 1.162 mgs. N per 2 ml. filtrate) coagulated in the presence of distilled water by heating at 70° C. for 15 minutes.

Distilled water at pH 4.8		Distilled water at pH 8.6	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.098	8.43	.266	45.95
.112	9.63	.294	50.60
.112	9.63	.308	53.10
.084	7.22	.226	45.95
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.098	8.43	.280	48.02
.084	7.22	.273	36.99
.098	8.43	.280	48.02

TABLE 6

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$\frac{M}{1}$ Xylose		$\frac{M}{2}$ Xylose		$\frac{M}{10}$ Xylose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
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One and one half ml. aliquots of an albumin solution containing 6.5 mgs. of soluble nitrogen per ml. were then pipetted into test tubes. To these were pipetted 4.5 ml. of the sugar solution 2 ml. of the buffer pH 4.8 and 2 ml. of boiled distilled water. Varying quantities of the indicator were then added and the tubes heated at 70° C. for fifteen minutes. The tubes that were decolorized within that time were noted and a further series of tubes set up containing a closer range of quantities of indicator. The originators of this method claim to be able to distinguish between 0.01 ml. amounts of the indicator but in this work 0.5 ml. appeared to be the limit. The results using d-glucose, d-fructose, and d-mannose are shown in tables 7, 8, and 9.

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$\frac{M}{1}$ Glucose		$\frac{M}{2}$ Glucose		$\frac{M}{10}$ Glucose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
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2.00	.350	.297	.521	.343	.601
2.20	.386	.271	.475	.330	.579
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$\frac{M}{1}$ Fructose		$\frac{M}{2}$ Fructose		$\frac{M}{10}$ Fructose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.456	.290	.508	.308	.540
.264	.463	.280	.492	.305	.537
.259	.455	.296	.519	.311	.548
.261	.458	.288	.505	.321	.564
.254	.445	.284	.498	.326	.570

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$\frac{M}{1}$ Mannose		$\frac{M}{2}$ Mannose		$\frac{M}{10}$ Mannose	
Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine	Mgs. Cysteine in 1.5 ml.	% Cysteine
.260	.456	.307	.539	.345	.605
.255	.447	.320	.561	.335	.589
.251	.440	.293	.514	.333	.583
.248	.435	.305	.535	.325	.570
.265	.464	.285	.500	.343	.601

shown in Tables 10 and 11.

Since Pryzlecki (53) has shown thatsymplexes between proteins and sugars are formed by allowing a mixture of the two to stand for several days at a low temperature and a rather high pH it was decided to see if standing under these conditions had any effect on the inhibtion of heat coagulation of proteins by sugars.

In making this study separate aliquots of an egg albumin solution were coagulated in the presence of distilled water and the proper buffer at pH 4.8 and at pH 8.6 to determine the effect of pH on coagulation. To show this effect one ml. aliquots of an egg albumin solution containing 5.81 mgs. of soluble nitrogen per ml. were pipetted into test tubes. Four ml. of the buffer pH 4.8 and 5 ml. of distilled water were added. The mixture was then heated at 70° C. for fifteen minutes then filtered and the nitrogen in 2 ml. aliquots of the filtrate determined by the Micro-Kjeldahl procedure. A boric acid--potasium chloride--sodium hydroxide buffer of pH 8.6 was then used in place of buffer pH 4.8. In this case after the mixture had been coagulated, 10 ml. of buffer pH 4.8 was added. The mixutre was then filtered and the nitrogen in 4 ml. aliquots of the filtrate was determined. The effect of pH on the coagulation of egg albumin is shown in Table 12.

The effect of d-glucose and also d-fructose at these pH values were then studied by substituting 5 ml. of 0.3 M sugar for the distilled water in the above procedure. The results are shown in Tables 13 and 14.

TABLE 10

Effect of Arabinose on the Coagulation of Egg Albumin Solution as Determined by the Residual Nitrogen Method.

Egg albumin solution (9.75 mgs. N per 1.5 ml., equivalent to 1.95 mgs. N. per 2 ml. filtrate) coagulated in the presence of l-arabinose by heating at 70° C. for 15 minutes. Percent nitrogen in filtrate from egg albumin coagulated in the presence of distilled water 8.42%.

$\frac{M}{1}$ Arabinose		$\frac{M}{2}$ Arabinose		$\frac{M}{10}$ Arabinose	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.826	42.35	.560	28.71	.280	14.36
.812	41.64	.620	20.81	.333	17.08
.834	42.76	.616	31.59	.371	19.06
.756	38.76	.588	30.10	.336	17.23
.854	43.78	.539	27.64	.315	16.15
.784	40.20	.532	27.28	.294	15.08
.792	40.61	.662	33.95	.315	16.15
.756	38.76	.588	30.16	.273	14.00

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Egg albumin solution (9.75 mgs N per 1.5 ml., equivalent to 1.95 mgs. N per 2 ml. of filtrate) coagulated in the presence of d-xylose by heating at 70° C. for 15 minutes. Percent nitrogen in filtrate from egg albumin coagulated in the presence of distilled water 8.42%.

$\frac{M}{1}$ Xylose		$\frac{M}{2}$ Xylose		$\frac{M}{10}$ Xylose	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.672	34.46	.423	21.69	.210	10.77
.714	36.61	.402	20.66	.182	9.33
.750	38.46	.476	24.41	.196	10.05
.761	39.03	.482	24.73	.185	9.48
.711	36.46	.406	20.82	.238	12.21
.731	37.49	.479	24.57	.210	10.77
.694	35.59	.386	19.79	.246	12.61
.724	37.13	.406	20.82	.704	10.46

TABLE 12

Effect of pH on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml. equivalent to 1.162 mgs. N per 2 ml. filtrate) coagulated in the presence of distilled water by heating at 70° C. for 15 minutes.

Distilled water at pH 4.8		Distilled water at pH 8.6	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.098	8.43	.266	45.95
.112	9.63	.294	50.60
.112	9.63	.308	53.10
.084	7.22	.226	45.95
.098	8.43	.273	46.99
.098	8.43	.280	48.02
.084	7.22	.273	36.99
.098	8.43	.280	48.02

TABLE 13

Effect of Glucose on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml., equivalent to 1.162 mgs. N. per 2 ml. filtrate) coagulated in the presence of d-glucose by heating at 70° C. for 15 minutes.

.3 M Glucose at pH 4.8		.3 M Glucose at pH 8.6	
Mgs. N in 2 ml. Filtrate	% original N in filtrate	Mgs. N in 2 ml. filtrate	% original N in filtrate
.140	12.04	.336	57.70
.126	10.67	.329	56.62
.126	10.67	.343	59.03
.196	16.86	.350	60.24
.140	12.04	.338	58.31
.134	11.56	.333	57.34
.151	12.99	.343	59.03
.168	14.45	.338	58.31

TABLE 14

Effect of Fructose on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml., equivalent to 1.162 mgs. N per 2 ml. filtrate) coagulated in the presence of d-fructose by heating at 70° C. for 15 minutes.

.3 M Fructose at pH 4.8		.3 M Fructose at pH 8.6	
Mgs. N in 2 ml. Filtrate	% original N in filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.140	12.04	.294	50.60
.126	10.67	.294	50.60
.134	11.56	.266	45.78
.162	13.97	.266	45.78
.173	14.94	.252	43.37
.136	11.75	.259	44.58
.154	13.25	.273	46.98
.140	12.04	.280	48.19

In order to study the effect of standing at low temperatures samples were prepared as in the previous experiment and allowed to stand in a refrigerator at 5° C. for 96 hours. At the end of this time the samples were coagulated and filtered as above and the nitrogen in the filtrates determined. The results of this experiment are shown in Tables 15 and 16.

AN ATTEMPT TO SHOW PROTEIN CARBOHYDRATE COMBINATION

In an attempt to investigate the cause of the protective effect of sugars against protein coagulation, egg albumin was coagulated first in the presence of glucose and then in its absence. The coagulum was then hydrolyzed and the hydrolasate analyzed for reducing substances.

The photelometer was first standardized in terms of glucose using a green filter with a maximum absorption range of 5,200 A°--5,800 A°. Known concentrations of glucose ranging from 0.05 to 0.5 mgs. per 2 ml. were prepared and treated according to the modified Benedict method for blood sugars (68). The color that developed was read on the photelometer and a standard curve prepared as shown in Figure II.

Five ml. of an egg albumin solution containing 60.5 mgs. of soluble nitrogen per ml. were heated at 70° C. for twenty minutes in the presence of 5 ml. of the buffer pH 4.8. The mixture was then filtered and the coagulum was then hydrolyzed with two per cent hydrochloric acid by heating in an oil bath at 120° C. for eight hours. At the end of this time the hydrolasate was treated with neutral lead acetate solution to remove any unhydrolysed protein. The mixture was then filter-

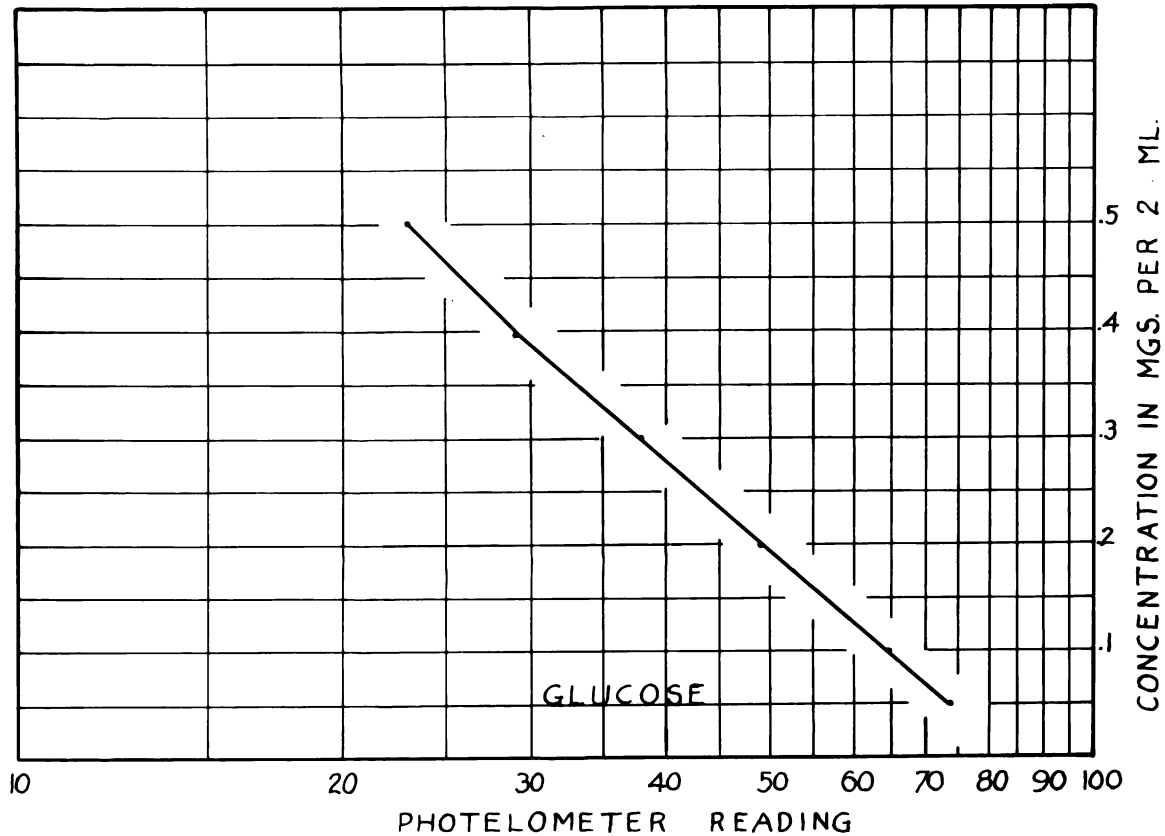
GLUCOSE
10
20
30
40
50
60
70
80
90
100
PHOTELOMETER READING
.1
.2
.3
.4
.5
CONCENTRATION IN MGS. PER 2 ML.

FIG. II

TABLE 15

Effect of Glucose on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml., equivalent to 1.162 mgs. N in 2 ml. filtrate) coagulated in the presence of d-glucose by heating at 70° C. for 15 minutes after stand- for 96 hours at 5° C.

.3 M Glucose at pH 4.8		.3 M Glucose at pH 8.6	
Mgs. N in 2 ml. Filtrate	% orginal N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.140	12.04	.364	62.65
.126	10.67	.371	63.85
.126	10.67	.308	53.01
.098	8 .43	.315	54.21
.168	14.45	.315	54.21
.182	15.66	.350	60.24
.150	12.04	.357	61.53
.126	10.67	.343	59.03

TABLE 16

Effect of Fructose on the Coagulation of Egg Albumin as Determined by the Residual Nitrogen Method.

Egg albumin solution (5.81 mgs. N per 1 ml. equivalent to 1.162 mgs. N in 2 ml. filtrate) coagulated in the presence of d-fructose by heating at 70° C. for 15 minutes after standing for 96 hours at 5° C.

.3 M Fructose at pH 4.8		.3 M Fructose at pH 8.6	
Mgs. N in 2 ml. Filtrate	% original N in Filtrate	Mgs. N in 2 ml. Filtrate	% original N in Filtrate
.098	8.43	.280	48.19
.098	8.43	.273	46.98
.112	9.63	.266	45.78
.126	10.67	.266	45.78
.154	13.25	.259	44.58
.140	12.08	.244	50.60
.162	13.97	.266	45.78
.112	9.63	.273	46.98

ed and potassium oxalate added to remove the excess lead acetate. The mixture was then diluted to 25 ml. with distilled water and the precipitate of lead oxalate filtered off. The reducing substances in two ml. aliquots were determined as described for the standardization.

Five ml. of the same albumin solution was then coagulated by heating at 70° C. for twenty minutes in the presence of 5 ml. of the buffer pH 4.8 and 5 ml. of 2 M glucose. The coagulum was then treated as described above and the amount of reducing substances in the hydrolasate compared with the amount in the albumin coagulated in the absence of glucose. The results are shown in Table 17.

TABLE 17

Effect of Presence of Glucose, at Time of Coagulation, on the Reducing Sugar Content of Hydrolysed Egg Albumin.

Egg albumin solution (302.5 mgs. N per 5 ml.) coagulated in the presence and absence of d-glucose by heating at 70° C. for 20 minutes. The Coagulum was hydrolyzed and the reducing substnces in the hydrolasate determined by the modified Benedict's method for blood sugar.

Egg Albumin Coagulated in absence of glucose		Egg Albumin Coagulated in presence of glucose	
Photelometer Reading	Mgs. glucose 2 ml. hydrolasate	Photelometer Reading	Mgs. glucose 2 ml. hydrolasate
60	1.25	59	1.31
62	1.17	62	1.17
60	1.25	60	1.25
59	1.31	59	1.31
58	1.37	59	1.31
59	1.31	59	1.31
60	1.25	55	1.62
60	1.25	58	1.37
59	1.31	62	1.17
60	1.25	60	1.25
62	1.17	60	1.25
59	1.31	59	1.31
Average	1.268		1.301
Average Deviation from Mean	±.056		±.072

DISCUSSION

It is apparent from the results shown in Table 1 that d-glucose has an inhibiting effect on the liberation of sulfhydryl groups from egg albumin by heat treatment. As the strength of the glucose solution was increased the percentage of cysteine detectable by the iodoacetic acid method decreased. The influence of d-fructose as shown in Table 2 was to decrease the number of sulfhydryl groups liberated from egg albumin. The amount of inhibition was not as great as in the case of glucose although it was greatest in the strongest fructose solution. From these results it might be concluded that aldose sugars are more effective in this respect than ketose sugars. However, the results obtained using d-mannose (Table 3) show that the amount of inhibition was less than in the case of fructose. This may indicate that the spatial configuration has a greater effect on the inhibition of denaturation than the carbonyl group. Mannitol was used in an attempt to verify this supposition and as shown by the results in Table 4 a slight but definite inhibiting effect increasing with the concentration of the mannitol was demonstrated. It appears, therefore, that the carbonyl group is not necessary for inhibition but it may increase the amount. According to some workers in this field fifteen minutes heating at 70° C. causes one hundred percent denaturation and subsequent coagulation of egg albumin (40). If this be true the percentage of cysteine in denatured coagulated egg albumin as measured by the iodoacetic acid method is 0.590%.

This value compares favorably with other values reported in the literature. The percentage cysteine in denatured egg albumin has been reported by various workers (26) (36) (40) to be 0.500%, 0.580%, and 0.630%. If 0.590% cysteine represents the value for completely denatured egg albumin it is possible to calculate the amount of denaturation on the basis of the percentage cysteine detectable. On this basis since the percentage of cysteine calculated from the sulfhydryl groups was 0.361%, the percentage denaturation of the egg albumin in the presence of $\frac{M}{1}$ glucose would be:-

$$\frac{.361}{.590} \times 100 = 61.18\%$$

On the same basis the amount of denaturation in the presence of $\frac{M}{1}$ fructose where the average percentage of cysteine is 0.411% would be:-

$$\frac{.411}{.590} \times 100 = 69.66\%$$

The percentage of cysteine in egg albumin denatured in the presence of $\frac{M}{1}$ mannitol is 0.481% and the amount of denaturation would be:-

$$\frac{.481}{.590} \times 100 = 81.52\%$$

The results obtained using the indicator method are in fair agreement with those obtained by the previous method. The percentage of cysteine in egg albumin denatured and coagulated in the presence of glucose is in the latter case slightly greater than in the former one. Again it will be noted that glucose showed a definite inhibiting effect. Fructose and mannose also show inhibiting effects but the inhibition is less then when measured by the iodoacetic acid

method. By the indicator method the percentage cysteine in completely denatured and coagulated egg albumin is 0.570% assuming fifteen minutes heating causes complete denaturation. The percentage denaturation may, therefore, be calculated from the average percentages of cysteine found when egg albumin is heated in the presence of $\frac{M}{1}$ glucose and $\frac{M}{1}$ fructose where the average values for cysteine in the presence of these sugars were respectively 0.378% and 0.455%. The percentages denaturation would be :-

$$\frac{.378}{.570} \times 100 = 66.13\%$$

and

$$\frac{.455}{.570} \times 100 = 79.82\%$$

The percentages denaturation in these cases are slightly greater and the percentage protection therefore slightly less than in the cases of the same sugars measured by the iodoacetic acid method. Because of the inability to differentiate between small amounts of the indicator this method was not considered very reliable.

The inhibiting effect shown by the pentoses, l-arabinose and d-xylose, can again be used to calculate the percentage denaturation. The average percentage cysteine found in the presence of $\frac{M}{1}$ arabinose was 0.406% by the iodoacetic acid method and the percentage denaturation would be:-

$$\frac{.406}{.590} \times 100 = 68.81\%$$

In the case of $\frac{M}{1}$ xylose the percentage cysteine found averaged 0.433% and the calculated percentage denaturation would be:-

$$\frac{.433}{.590} \times 100 = 73.38\%$$

The inhibiting effect of the pentoses is slightly less than the inhibiting effect of the hexoses when measured by the same method.

The pentoses l-arabinose and d-xylose show a definite inhibiting effect against coagulation measured by the residual nitrogen method as shown in Tables 10 and 11. Arabinose showed a greater amount of inhibition than xylose but both showed definite inhibiting effects that were proportional to the concentration.

To bring a substance into colloidal solution is called peptisation. Lloyd and Shore state that, "a worse term than peptisation, with its suggestion of peptic digestion, would have been hard to find." (70) The assumption of Bancroft and Rutzler (52) that protein coagulation is merely a physical aggregation of the colloidal particles of protein, which can be repeptised by addition of sugar was not entirely verified in the present work. According to Bull (1) before a protein can be coagulated it must be denatured and the denaturation process apparently causes certain changes in the protein molecule such as the liberation of sulfhydryl groups. Bancroft and Rutzler do not consider the denaturation process in their explanation of the prevention of coagulation by sugars. This present work shows that both denaturation and coagulation are inhibited by sugars and mannitol.

If this inhibiting effect is due to combinations of a more stoichiometric nature further experiments of the type Pryzlecki carried out might have offered an explanation (53).

Under certain conditions of time, temperature, and pH Pryzlecki has been able to obtain "symplexes" of a stoichiometric nature between proteins and carbohydrates. The conditions under which the influence of sugars on the coagulation of egg albumin were studied as previously described in this present work would not favor the formation of symplexes according to Pryzlecki. The same would be true of the work carried out by Duddles (3).

If the formation of symplexes does inhibit coagulation it should have been possible to demonstrate the effect by running simultaneous experiments under the conditions used by Pryzlecki and also as previously described in this work. The percentage nitrogen remaining in the filtrate from samples of egg albumin coagulated at pH 4.6 and at pH 8.6 as shown in Table 12 make it clear that a high pH causes less coagulation of egg albumin. Since the isoelectric point of egg albumin is approximately 4.8 a coagulum readily appear at this pH while at pH 8.6 a precipitate forms but it does not form a typical coagulum. If this mixture is then adjusted to pH 4.8 by the addition of a suitable buffer a coagulum appears. The percentage nitrogen in the filtrate from the latter case is much greater than in the former. This indicates that not only coagulation but denaturation as well is inhibited at higher pH values an observation fully described by Sørensen (69). Table 12 shows the inhibiting effect of glucose at both high and low pH. The amount of inhibition can be calculated at both pH values from the average percentage nitrogen in the filtrate.

The average percentage nitrogen in the filtrates coagulated at pH 4.8 is 8.42% but in the presence of .3 M glucose at pH 4.8 the average percentage nitrogen in the filtrate is 12.66% therefore the percentage inhibition would be:-

$$\frac{12.66 - 8.42}{8.42} \times 100 = 51.50\%$$

When egg albumin was coagulated in the presence of .3 M glucose at pH 8.6 the average percentage nitrogen remaining in the filtrate was 58.38% therefore the percentage inhibition would be:-

$$\frac{58.38 - 48.20}{48.20} \times 100 = 21.10\%$$

It appears that glucose shows a greater inhibiting effect at the lower pH. The influence of fructose can be calculated in the same manner referring to Table 14. At pH 4.8 the average percentage nitrogen remaining in the filtrate when albumin is coagulated in the presence of .3 M fructose at pH 4.8 is 12.52% and the percentage inhibition would be:-

$$\frac{12.52 - 8.42}{8.42} \times 100 = 48.60\%$$

At pH 8.6 the average percentage nitrogen remained in the filtrate was 45.96% which is less then the average value found for the albumin alone at this pH from these results it appears that fructose does not show an inhibiting effect at the higher pH. Other workers (60) (61) and (62) have reported that non-aldehydic sugars would not combine with proteins, amino acids or polypeptides and this may have some connection with this present work. However, Pryzlecki (53) believes that "symplexes" are formed when the sugar contains a reducible group.

The influence that d-glucose and d-fructose have, under the conditions specified by Pyrzlecki as shown in Tables 15 and 16, seems to indicate that standing for considerable periods of time causes no greater inhibition. Since standing under these conditions should permit the formation of "symplexes" according to Pryzlecki it would seem that the formation of these "symplexes" does not increase the inhibition of protein coagulation by these sugars. This present work does not indicate therefore, that the formation of these so called "symplexes" is the mechanism of the prevention of coagulation.

The results obtained in the attempt to show a combination between egg albumin and sugars as shown in Table 17 indicate that the sugar and the protein do not combine. The average result for the reducing substances found when egg albumin was coagulated in the absence of glucose is slightly higher than the average result obtained when egg albumin was coagulated in the absence of glucose. The results being 1.301 mgs. in the presence of glucose and 1.268 mgs. in the absence of glucose or an increase of 2.60% in the presence of glucose. However, variations as great as will be noted in individual successive trials and the difference in probably not significant. Since the coagulum in the above work was washed with distilled water in an attempt to remove any absorbed glucose it should not be concluded that no combination occured. An unknown labile combination of a very readily hydrolysable type that could readily be decomposed by washing with distilled water may have resulted. This experiment under these conditions did not show such a combination.

CONCLUSION

1. Denaturation of egg albumin by heat was inhibited by the presence of d-glucose, d-fructose, d-mannose, mannitol, l-arabinose and d-xylose.
2. The amount of inhibition was roughly proportional to the amount of sugar.
3. This denaturation can be measured by the determination of sulfhydryl groups both by the iodoacetic acid method and less satisfactorily by the use of 2-6 dichlorobenzeneindophenol.
4. The coagulation of egg albumin by heat is inhibited by l-arabinose and d-xylose.
5. At a lower pH d-glucose shows an increased inhibition to both coagulation and denaturation. Fructose does not show this.
6. The formation of Pryzlecki's "symplexes" does not appear to be the cause of this inhibition.
7. No stable combination between the protein and the carbohydrate was indicated.

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