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A Study of the
Proteids of Flesh

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A STUDY

OF THE

PROTEIDS OF FLESH

BY

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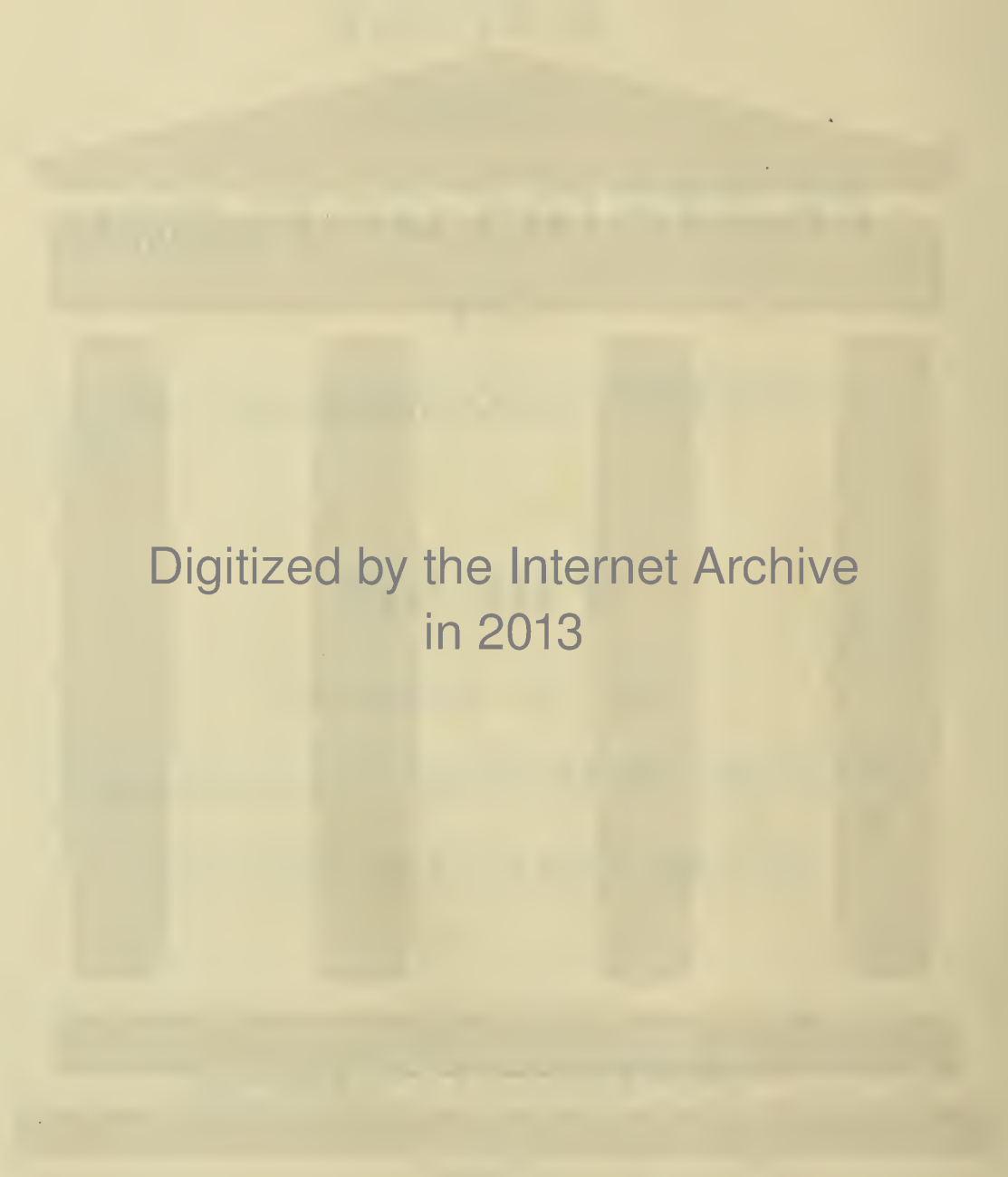

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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OF Master of Science,

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A STUDY OF THE PROTEIDS OF FLESH.

Introduction.

The present study is partly an outgrowth of some of the work which has been done upon meats at the University since the spring of 1898. It was originally undertaken with the idea of finding out the exact chemical changes which are brought about in the cooking of meat. In the very beginning of the work it was found that the study would be so involved, and so difficult that it was decided to limit our efforts to the study of the proteids bodies in both raw and cooked meat. After all it is only by the thorough study of these bodies, both before and after cooking, that the chemical changes brought about during the process of cooking, can be ascertained. Heat as ordinarily applied to meat during cooking brings about but few chemical changes outside of those induced in the proteid bodies. The field is such a large one, and in many respects it has been so little explored,- probably on account of the difficulties in the way, that even with the amount of work which we have done, the present paper can be considered as little more than a preliminary study of the subject.

Object of the study.

The object of the study is then an effort to get at all the available facts bearing upon the subject of the proteids of flesh. To arrive at this end we made as thorough a study as possible of all the available literature bearing upon the subject. All the text books, journals, periodicals, and indexes, that could lead to any facts touching upon the study were consulted. A review of this work will be g

en in this report. From the experimental side, the analysis of two samples of raw meat and one of cooked meat by methods which will be described later were made. The different proteids in the cold water extract from meat by fractional precipitation with $(NH_4)_2 SO_4$, and in the residue of meat insoluble in cold water, by treatment with 10% NaCl solution were also studied. The testing of all the methods, and their comparison, comprises an important part of the work.

The importance of meat products in American dietaries.

A study of the proteids of flesh is of both scientific and economic value. In the first place it is of importance to physiologists to know what the exact constituents of flesh are, in order to properly understand the functions of the various organs of the body, and to devise means for combatting disease when that becomes necessary. In the second place or from the economic standpoint with which this paper is more concerned we find that flesh foods form a large percentage of the total food products consumed in American homes. On an average of 185 dietary studies made in different parts of the United States, it has been found that flesh of all kinds including meat and poultry, fish and shellfish forms 20 per cent of the total food.

The table which follows gives the returns of the census of 1900 for the wholesale slaughtering and meat packing industry in the United States for this year. To these immense values is to be added the meat and meat products which pass through the hands of the butchers and other retailers, whose values are not included in the table, and also that which the farmer and other consumer slaughters for his own use.

Wholesale slaughtering and meat packing in U. S. for 1900.	
Number of establishments	920
Capital	$186,681,264
Wage-earners average number	67,908
Total wages	$33,144,013
Miscellaneous expenses	$23,991,312
Cost of materials used	$676,907,177
Value of products	$779,147,433

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The Bureau of Statistics of England published a statement in 1890 on the consumption of meat in the various civilized countries according to which Australia ranks first, the United States second, and Great Britain third, with 111.6, 54.4, and 47.6 kilograms per capita, respectively.

FOOD VALUE OF PROTEIDS.

(a) Use to body.

Nitrogen is essential for the life of all living matter, although the exact part which it plays is still largely a matter of conjecture with us. Substances which contain nitrogen in a form available to our bodies are of first importance. Some of these as egg albumin are so like human protoplasm that they need only to be disolved in order to be at once assimilated by the body. Others as the vegetable proteids principally, have to undergo a greater change before being available, while still others as gelatine assist in protecting the tissues of the body from waste. The proteid bodies then

supply the building material which the body needs. They can also serve as fuel in the body after they have served as building material. Still another important use of proteids to the body is their ability to form fats and carbohydrates.

The commonly accepted view of physiologists is that the body requires a certain amount of all the different food constituents in order that its various functions may all be properly carried out. It is commonly believed that an axcess of lean meat or other proteid substance in a diet, overloads the system with organic waste products, such as creatin, urea, and uric acid, which have deleterious effects when accumulated in the system.[3] In order to show that our knowledge on these questions is far from complete, the cases which follow may serve as examples.

Herschell[4] cites the case of the cannibals from Terra del Fuego who live entirely upon a meat diet. They live in a cold climate, eat little fat, no farinaceous foods, and drink a great deal of water. They are perfectly normal in health, with the pecularity of being inclined to sleep a great deal. It is commonly believed that gout results somewhat from the use of too much lean meat, and lack of exercise while gout is entirely unknown amongst these people.

J.W. Good[5] tells about the employees of the Hudson Bay Company in the Mackenzie River region, who live entirely upon fresh meat and fish, and yet who enjoy the best of health. They have been known to live upon such a diet for periods of twenty or thirty years, and yet during all this time they have retained the full possession of their bodily and mental vigor. Scurvy is unknown amongst them.

Masterman[6] had been confined in prison during the Brazillio-Paraguayan war, and for two months was fed on nothing but lean meat

and water. When rescued at the end of that time he was in perfect health although very lean.

From the standpoint of the physician we find lean meat recommended for a rather large number of ailments. It is frequently used to reduce obesity, and also in cases of dyspepsia, and for the absorption, and removal of tumorous growths.

(b) Differences in nutritive value.

The nutritive value of different albuminoids varies greatly. Such bodies as keratin, elastin, etc. commonly classified along with the proteid bodies would never support life. As a rule those that are most easily soluble, are the ones that have the greatest nutritive value.

Gelatin which finds such a large use in American households has been made the subject of a great deal study and discussion. The work of Voit[2] along this line is probably the most exhaustive that has been done. His experiments were conducted largely with dogs. He fed dogs on gelatin and on gelatin + fat, and then found the urine to contain more nitrogen than was injected in the body. By adding a certain proportion of gelatin to a ration containing insufficient nitrogen to cover the body losses, then the animal did not excrete more nitrogen than he took in. From these experiments he concluded that gelatin can take the place of but a part of an albuminoid ration. In other words it helps to protect the tissues of the body from waste, as already stated.

The nutritive value of many of the other albuminoids is established from the fact that they enter so largely into the food of man. The acid and alkali albumins and peptones to which reference will be made further on, also have a great food value since they are

amongst the products of digestion. It has been found that animals fed upon these substances will thrive very well.

(c) Fuel value.

Food contains energy in the latent form, and when consumed, the body has the power of changing this energy into the more active forms. The quantity of energy which any given food contains can be measured experimentally by means of the calorimeter. The energy is commonly calculated in terms of mechanical work expressed in heat units or calories. One Calorie (1000 calories) being the amount of heat necessary to raise the temperature of one kilogram of water one degree centigrade, or if expressed in terms of mechanical work would enable one ton to be lifted 1.53 feet.

The fuel values of the different classes of nutrients vary greatly, and also the fuel values of different substances in the same classes. So far, so few of the proteid bodies have been isolated in a pure condition, and a study made of their fuel values, that we are unable to state just what differences exist in this great class. An important part of future studies of the proteids of flesh should be the estimation of their fuel values. Experiments are beginning to show more and more that within practical limits we are safe in using the heat of combustion of any substance as the controlling measure of its food value. Of course in proteid bodies the nitrogen which they contain is what makes them of such great ualue, and in making up a diet it is of importance, as generally accepted, to have plenty non-nitrogenous foods. The commonly accepted ratio of nitrogenous to non-nitrogenous food is one to three or at least one to five.

At the present time we accept the following fuel values for the different classes of nutrients:-

1 gram proteid = 5.5 large calories, 8.4 foot tons.

1 " fat = 9.2 " " 14.2 " "

1 " carbohydrates = 4.1 large calories, 6.2 foot tons.

In calculating the available fuel value of proteid bodies the following value is commonly used:- 1 gram proteid = 4.1 large calories, 6.3 foot tons, or in other words the same value as that used for carbohydrates. This is owing to the fact that the final decomposition products of proteids in the body are not completely oxidized substances like carbondioxide and water as in the case of fats and carbohydrates, but they are certain organic bodies like urea, uric acid,creatin, ect. which all have a definite fuel value of their own. The last factor has been established by means of a large number of experiments.

(d) The digestibility of proteid bodies.

In order to know intelligently what the nutritive value of a certain food is, one must know what the digestibility of each nutrient in the food is. No study of this kind would be complete unless something definite was learned concerning the relative digestibility of the different proteid bodies. Here again we will be likely to find considerable differences between the different bodies,- perhaps especially between those of animal and vegetable origin. Results of this kind would help to throw some light on the process of digestion, and at the same time enable the practitioner to prescribe a given diet in certain cases.

If sufficient amounts of the various proteids can be separated in the pure state, then it will be possible to determine their relative digestibility by means of artificial pepsin solution with no little degree of accuracy. When that stage is reached we may be able

to speak as definitely about the digestibility of various proteids, as we can now about the fuel value of some of them.

From the work which has been done at the University of Illinois since the summer of 1899 in studying the digestibility of meat both by natural digestion experments in which healthy, robust men were the subjects, and also by artificial pepsin solution in which certain valuable improvements were made in the methods; it has been found that the proteids of beef, are very thoroughly digestible. To be more definite it has been generally found from a large number of experiments that from 95 to 100 per cent of the proteids of beef are digestible.

Atwater (9) gives a list of about ten common vegetable foods in which the digestibility of their protein content ranges from 75 to 85 per cent. Later than this Woods and Merrill (10) at the Maine Station confirmed Atwaters published statements by finding about 85 per cent of the protein of white bread to be digestible. Snyder (11) found the digestibility of the protein in bread made from standard patent flour, entire-wheat flour and graham flour to vary a great deal. By treatment of the bread crumbs with artificial pepsin solution for four hours he found the following coefficients of digestibility:- Bread from standard patent flour, 82 per cent; from entire wheat flour, 76 per cent; from graham flour 58 per cent. He obtained comparable results in his natural digestion experiments with men.

Aside from these differences in the total digestibility, we are confronted with the important question of the time elements involved in the process of digestion. From work done in this laboratory as well as from the work done by other investigators we find that the protein of animal origin is more readily digestible than that of vegetable origin. In a general way we can state that as a result of a very

large number of artificial digestion experiments with meat, carried on in this laboratory in connection with the meat investigations; that of those run for a proteid of four hours the coefficient of the digestibility of the meat was found to be from about 10 to 35 per cent higher than that for bread as obtained by Snyder (11) when treating it for the same length of time as we did the meat.

It is self evident that there is considerable significance in these facts, as both classes of food enter so largely into the food supply of our American people. The chemist is at once confronted by the question,-why these differences? The elementary composition is very much alike, and there are other striking similarities. We believe that a study can be made thorough enough, to solve these questions in part, or with time even in full.

Historical.

Animal flesh has formed part of the food supply of man at least as far back as human knowledge goes. The beliefs of the ancients and of the mass of people even down to the time of Lavoisier concerning the uses of foods and the part which they played in the body, were as ludicrous as they were unfounded.

Like practically all the chemical facts which hold today, we find that nearly all our knowledge of foods has been worked out upon a scientific basis since the beginning of the modern era of chemistry, which had its birth towards the close of the eighteenth century.

Egg albumin has been known for a very long time. Vegetable albumin was discovered much later by Fourcroy. (12) Beccaria (13) discovered gluten in flour, and Braconnot (14) found legumine in beans and peas.

To Mulder is given the credit of having first applied the term "proteid" to the albuminoids in plants, and of having pointed out the similarity of these to animal albumin. The general term now covers both classes of bodies. The word "protein" comes from the Greek and means "pre-eminence" or "holding the first place". Mulder advanced the theory that proteids contained a complex group to which he gave the name "protein", and which he supposed contained sulphur, phosphorous, etc. His views have been proved to have been incorrect.

It is the illustrious Justus Liebig who first distinguished between the different constituents of foods, and who showed their various uses to the body. With but few exceptions the definitions which he construed, and the theories which he advanced, have stood the searching tests of succeeding investigators. The methods which he originated for studying these bodies have been modified, and yet their general principles remain unchanged.

His researches on "the constituents of the fluids of flesh" and similar ones by his pupils Schlossberger, Scherer, Strecker, and Städeler prepared the way for later work by other investigators along these lines. It is Liebigs work on the composition of flesh and on the study of the processes which go on in the body that give birth to physiological chemistry.

Since Liebigs time the study of proteid bodies has been approached from many different standpoints. Kuhne contributed considerably to our knowledge by his work on muscle plasma by which he tried to explain rigor mortis.

Kühne has been followed by Chittenden, one of his pupils. In England, reference must be made to the long line of researches by Halliburton which will be referred to later on. Some of the work of

Stewart and Sollman was started with Halliburton and completed in this country. Von Furth in some instances confirmed, and in others flatly contradicted some of Halliburtons conclusions.

Schutzenberger (1892) studied for fifteen years the decomposition products of the albuminoid molecule with $Ba(OH)_2$ in order to arrive at the constitution of proteid substances. Arnaud also published a valuable paper along these lines. Other investigations will be refered to in our chapter devoted especially to this subject.

Even a brief summary like this would be incomplete if reference were not made to the elaborate, pain-taking researches of Osborne at the Connecticut station on a large number of vegetable proteids and even on a few of the animal proteids. His work has been more thorough probably than that of any other investigator, and he has enriched the science of chemistry with a large number of valuable facts.

The study of the proteids of flesh is a more difficult one than that of the proteids of plants on account of the decompositions to which flesh is so susceptible, and yet it is probable that the only way that the problem can be solved will be by the use of methods similar to those which Osborne used. Some modifications will be necessary, but the general principles will probably have to remain as they are. A careful study of his valuable work will be of first importance as a preliminary part of similar work on flesh.

CLASSIFICATIONS OF PROTEID BODIES.

Even a precursory study of the classification of proteid bodies as given by different authors reveals many striking differences. Such differences in what may be considered as the very foundation plan

of the subject, are bound to lead the student to no small amount of confusion, and at the same time present added difficulties to an already difficult subject. An attempt will be made in the following pages to present in a brief way the classifications which are at present in most common use, and to which the student has to constantly refer.

Watts in 1894 presented a classification containing six groups.

1. Albumins. These are proteids which are soluble in water and not precipitated from their solutions by saturation with NaCl or $Mg\ So_4$. They are coagulated by heat. Amongst the more important members of the group are,- egg albumin, serum albumin, and lactalbumin.

2. Globulins. These proteids are insoluble in water; soluble in dilute solutions of neutral salts; and are precipitated in an uncoagulated condition by saturation with NaCl and $Mg\ So_4$. They are coagulated by heat. The most important members of this group are,- serum globulin, fibrinogen, myosin, crystallin and globin.

3. Albuminates. This name is applied to the metallic compounds of proteids, and also to acid albumin or syntonin and to alkali albumin. If the term is restricted to the two latter substances, then they may be defined as proteid bodies insoluble in water and in neutral salt solutions, but readily soluble in dilute acids or alkalies. They are not coagulated by heat.

4. Proteoses. These are not coagulated by heat, but most of them are precipitated by saturation with certain neutral salts. They are formed from other proteids by proteolytic ferments, being intermediate products in the formation of peptones. They are found in certain animal and vegetable tissues. The best representatives

of this group are the albumoses.

5. Peptones. These are the proteids which are not precipitated by heat, nor by HNO_3, and are very soluble in water. Peptones are further subdivided into hemipeptones, those which yield leucine and tyrosine as the further result of pancreatic digestion, and the antipeptones, those which do not.

6. Insoluble proteids. This class includes a number of proteids varying in their reactions which cannot be included in any of the foregoing classes, but which resemble one another by their extreme insolubility in various reagents. This class includes fibrin, coagulated proteid, lardacein, antialbumid, and gluten.

Thorpe's[30] classification contains seven groups:-

1. Albumins. These are soluble in water, in neutral salt solutions, in dilute acids and dilute alkalies. The representatives of this group which he gives are egg albumin and serum albumin as given by Watts, and also crystallin found in the lens.

2. Globulins. These are insoluble in water, but are soluble in neutral salt solutions, in dilute acids and in dilute alkalies. Watts calls crystallin a globulin while Thorpe has called it an albumin. In addition to other typical globulins quoted by Watts, he gives vitellin.

3. Derived albumins. Same as Watts' class 3 called albuminates.

4. Fibrin which is insoluble in water, and is soluble with difficulty in neutral salt solutions, and in dilute acids and in dilute alkalies.

5. Coagulated albuminoids. These are soluble in water, in neutral salt solutions, dilute acids and dilute alkalies. They

are substances produced by the action of heat or of strong alcohol upon many of the albuminoids.

6. Lardacein or amyloid is an albuminoid substance occuring in diseased liver, spleen, etc. Its most characteristic features are that it is not converted into peptones by artificial gastric juice, and that it is colored violet or blue with strong H_2SO_4 and I.

7. Peptones. Nearly the same as Watts.

(31) Gamgee's classification(1880)is very similar to that given by Thorpe. Some of the groups are described a little more in detail, and some differences are made on their relative importance.

(32) Hammarsten in 1890 published a classification which in many respects is different from all others. He uses but three main divisions which are all further subdivided more or less.

1. Albuminoid bodies. This division is subdivided into (1) albumins, which include serum albumin, ovalbumin and lactalbumin;(2) globulins, which include serum globulin fibrinogen, myosin, and musculin; (3) nucleoalbumins, which include casein, pyin and others; (4) albuminates which comprise acid and alkali albuminates; (5) albumoses and peptones; (6) coagulated fibrin, albumin coagulated by heat and others.

2. Proteids. This group is subdivided into, (1) mucin which comprises pure mucine and mucoide or mucinoide; (2) haemoglobin.

3. Albumoides or albuminoides. This group is composed of keratin, elastin, and collagen.

It is evident that any student who would follow such a classification as this, would be at loss when he came to using those in more common use.

(33) Charles uses a classification adopted by Hoppe-Seyler and

in all important particulars, it is very much like that given by Thorpe. He goes into some of the details of the preparation and of the properties of the various bodies, which will be taken up later on in the work.

Novy (34) book published in 1898, retains the old classification of Wroblewski which is similar in its general grouping to that given by Hammarsten.

A radical departure from any of these forms, is the classification originated by Dreschel. (35) He studied the various decomposition products, and from such observations he divided proteid bodies into two great groups. (1) Substances that produce aromatic compounds upon decomposition. Amongst these he gives,- albumins, globulins, fibrins, coagulated albuminoid bodies, amyloids, acid albumin, alkali albumin, albumoses, peptones, and albumoids. Among the decomposition substances he includes,- tryosine indol, phenol, etc. (2) Substances that do not yield aromatic decomposition products as,- gelatin and analogous substances and spongine.

However since Maly (36) studied albumin and gelatin it has been decided that Drechsel's distinctions no longer hold. Maly found that although gelatin did not yield tyrosine, that it yielded benzoic acid, and hence a classification on the basis indicated had no reason for existing.

In encyclopedia chimique (37) we find a classification which divides the proteid bodies into thirteen different groups, principally with regard to their solubility in water or in salt solutions, or to their heat coagulation. The groups are as follows:-

1. Albumins soluble in water e. g. egg albumin and serum albumin.

2. Globulins. Substances insoluble in water, but soluble

in neutral salt solutions, such as NaCl, KCl, NH_4Cl and $MgSO_4$. They are also coagulated by heat. Examples of this group are,- vitellme, myosin, serum globulin and fibrinogenous matter.

3. Fibrins. Bodies insoluble in water; swelled up by solutions of neutral salts, and especially by dilute acids; and coagulated by boiling water. The most common example is that of blood fibrin from the blood of various animals.

4. Coagulated albuminoid matter. Insoluble in water and salt solutions; slightly swelled by salt solutions and dilute acids, and not colored by iodine.

5. Amyloid substances. Insoluble in water; in salt solutions; in dilute acids; in dilute alkalies; colored reddish brown to violet by iodine.

6. Acid albumin. Insoluble in water; in dilute salt solutions, and in hot or cold alcohol. When freshly ppted they are easily soluble in dilute acids or in dilute alkalies, and when mixed with an emulsion of $CaCo_3$ in water they remain insoluble.

7. Alkali albumin. Very slightly soluble in water or in salt solutions, and slightly soluble in hot alcohol. When mixed in water with $CaCo_3$ they dissolve, and at the same time expel the Co_2.

8. Albumoses or propeptones. These are like acid albumin in many respects. They are soluble in dilute NaCl solution; in cold HNO_3 with resolution of the precipitate upon heating.

9. Peptones. These bodies are very soluble in water; are not coagulated by heat; are not precipitated by an excess of acetic acid and KCN, and neither by excess of NaCl in presence of acid.

10. Proteids. He applies the term that we use as a general name, t o substances which upon decomposition give rise to two dif-

ferent classes of bodies, one of which is albuminous in character while the other is not. Examples of these substances are,- haemoglobins, onyhaemoglobins, nucleoalbumins, nucleins, mucins, etc.

11. Albuminoids. Substances generally insoluble, and not dissolved by the digestive fluids. They are found especially in organs that protect and sustain other tissues. Examples of these are keratin, elastin and fibroine.

12. Gelatinous substances of which gelatin is the principal type. These dissolve in hot water without undergoing any change.

13. Substances analogous to spongine. Soluble in hot water with change in composition.

The latest classification which has come to our notice has been that of Halliburton published in 1899. His classification is different again than any which we have so far considered.

He divides the proteid bodies into only five general classes.

1. Albumins. These are soluble in water; in dilute salt solutions; and in saturated solutions of NaCl and $MgSo_4$. They are precipitated by saturating their solutions with ammonium sulphate; and are coagulated by heat, usually from 70 to 73 degrees.

2. Globulins. These bodies are insoluble in saturated solution of NaCl, and $MgSo_4$, in one half saturated solution of ammonium sulphate. He gives the solubility of the two classes to be as follows:-

Reagent	Albumin	Globulin
Water	soluble	insoluble
Saturated solution am. sulphate	insoluble	insoluble
Dilute NaCl solution	soluble	soluble
Half saturated solution am. sulphate	soluble	insoluble
Sat. solution NaCl or $MgSo_4$	soluble	insoluble

3. Albuminates which are proteid bodies produced by action of acid or alkali on the other two groups.

4. The products of proteolysis, i. e. proteoses and peptones.

5. Coagulated proteids. This class includes the proteids in which coagulation has been produced by heat, and those in which coagulation has been induced by ferment action such as fibrin, myosin, casein, and anti-albumid,- an insoluble by-product formed in gastric digestion.

It is evident enough from the nine classifications, whose substance has been given or to which reference has been made, that some reform along these lines would be very welcome to the student and to the chemist. One great difficulty in the way to attaining such an end, is the fact that our knowledge of these problems is rather fragmentary in many places, and as long as the gaps are not filled we cannot hope to have a complete and uniform classification. A large part of what has been said, has been concerning the animal proteids. The numerous vegetable proteids either fall in groups along with the animal proteids, or on account of special property it may be found necessary to place them in some special groups.

Another source of considerable confusion comes from the great differences in the nomenclature of similar bodies. In English works the term proteid is used; first as a general designation for the entire group, i. e. in place of protein; second to denote one of the subgroups, namely, the albuminous bodies. On the other hand German writers use it to designate the more complex albuminous bodies. Some substances have been studied by a number of different investigators, and it frequently happened that each one assigned a different name to them. As an example reference need be made simply to one,-

namely, serum globulin. This body is also known as paraglobulin, or fibrinoplastin, or serum casein, or plasmine. Unless this fact is known the reader is sometimes at a loss to know what a certain author may be talking out.

To conclude this paragraph then we would say that present conditions call for a uniformity both in the classification of proteid bodies, and in their nomenclature. As soon as sufficient facts have been collected steps should be taken to bring about these ends. Reforms of this character would not only assist the general student of the subject, but the thorough investigator as well.

GENERAL PROPERTIES OF PROTEID BODIES.

(a) Physical properties.

Proteid bodies are usually amorphous; a few of the vegetable proteids have been found crystallized in their native state, and more recently (39) some of the animal albumins have been crystallized in the laboratory.

Some are very soluble in water; others not at all. Amongst the latter class are several which dissolve readily in neutral salt solutions. All those insoluble in water, but fibrin are soluble in dilute acids or in dilute alkalies, and form with acids or bases compounds which are analogous to salts.

(b) Indiffusibility.

Solutions of proteids are nondiffusible. Peptones, and to a less extent the albumoses are diffusible through animal membrane. This property of proteids is sometimes taken advantage of in precipitating them from salt solutions.

(c) Action of polarized light.

Proteids are all characterized by their power of turning the plane of paralized light to the left. The determination of this power is of great value in distinguishing between the members of this class.

(d) Heat coagulation.

Nearly all the native proteids are coagulated by heating their solutions. This fact is especially true in the case of the albumins and of the globulins. The process changes them to substances which are insoluble in weak solvents. The precipitation commences sometimes at 40 degrees, and it is influenced by the acidity or by the alkalinity of the solution, and by the presence of certain salts.

(e) Action of certain salts.

Proteid bodies are very often precipitated from their solutions by means of certain salts, without undergoing any chemical change. If allowed to remain together for too long a time compounds, of the salt with the proteid may result which areusually insoluble. Sometimes a new proteid may result from the action of the reagent.

PROPERTIES OF INDIVIDUAL PROTEIDS,- PRINCIPALLY THOSE COMPOSING FLESH.

Egg albumin.

Egg albumin forms the chief part of the organic substance of the white of bird eggs. It may be separated from the globulin also found in the white by precipitating the latter with acetic acid or by saturation with $MgSo_4$. After filtration the liquid is dialysed to remove salts as much as possible, when the albumin separates out.

Dried albumin is a pale yellow transculent mass, easily ground up to a fine, white powder. It is tasteless, and inordorous. Not readily soluble in water unless a little alkali is present. At

(i) Heat conduction.

(j) Action of osmotic pressure.

140° it loses four per cent of water but still remains soluble.

It is coagulated in its solution at 70° to 73° C. Its specific rotation is -35.5°. It is insoluble in alcohol and ether. Large amounts of alcohol precipitate it in the insoluble state, while smaller quantities give a precipitate that is entirely soluble in water.(40)

Bechamp(41) isolated three different substances from the white of hen's eggs. He used the polariscope largely in assisting him with the work.

Serum albumin.

The serum is that portion of the blood which remains after the fibrin has been removed. It differs from the blood in having lost its fibrinogen, and perhaps in having gained some paraglobulin or serum globulin. Blood serum is best prepared by means of the centrifical machine.

Serum albumin remains in solution after the removal of the serum globulin by methods which will be indicated later on. It is the most abundant of the proteids of the serum. When serum is heated to about 60° it becomes opaque, and coagulation occurs at 75°. Meanwhile the liquid increases in alkalinity .

Serum albumin is precipitated by alcohol when added in the proportion of two volumes of alcohol to one volume of the serum. The precipitate is at first soluble in water but prolonged contact with the alcohol renders it insoluble.

Solutions of serum albumin are not precipitated by CO_2, acetic acid, orthophosphoric acid, or ether. Most neutral salts as $HgSO_4$, $Pb(C_2H_3O_2)_2$ precipitate it readily. It is completely precipitated by saturation with ammonium sulphate.

Its properties are much the same as those of egg albumin,

but its rotatory power for polarised light is very different,- i. e. from 56 to 62.

Crystallin.

Crystallin is the name given to an albumin obtained from the crystalline lens.

It is obtained by triturating the crystalline lens with water, evaporating the filtrate thus obtained, removing some impurities by alcohol when a yellow mass is obtained.

Crystallin is soluble in water and is coagulated by heat. Bechamp concluded that crystallin was made up of two bodies, one whose rotatory power was -80° and the other -41°.

MYOSIN.

Voluntary muscles are composed of a fibrous sheath, which encloses a semifluid substance which is called muscle plasma. After death, when rigor mortis has set in, this plasma yields a liquid, and a clot to which the stiffness of the muscle is due.

Kühne [43] was the first to show these facts. He worked with the muscle of frogs. His theories have been largely confirmed by the work of other investigators.

The separation of myosin is hindered by cold. It 0° the coagulation proceeds very slowly while at 40° it is almost instantaneous. Dilute acids and also 10 to 20 per cent solutions of NaCl cause its coagulation.

Another method of preparing myosin is based on the solubility of the coagulated myosin in weak solutions of NaCl. Muscle is washed with water until nothing further dissolves. It is then thoroughly rubbed up with NaCl and diluted with water until the sol-

ution contains ten per cent of NaCl, or the well washed muscle may be treated with 10% NaCl solution direct. The mixture in either case is set aside for about 24 hours, pressed through linen, and then filtered through paper. The myosin is then obtained either by dialysing or by pouring the solution into a considerable bulk of water.

When a solution of myosin in gradually heated it begins to get turbid at 55° and deposits flakes of proteid at 60°. It is precipitated in a saturated solution of NaCl.

Myosin is neutral in reaction. It is insoluble in water, alcohol and ether. It is readily soluble in dilute acids and in dilute alkalies, and like fibrin it decomposes hydrogen peroxide.

Halliburton found that by washing myosin clot too long in distilled water it became insoluble in salt solutions. If washed but three or four times its solubility in salt solution was altered but very little. Washing it from ten to twenty times rendered it increasingly insoluble. Danielewski had observed this fact in the case of its solubility in ammonium chloride. He found this to be due to the removal of salts,-more especially those of Ca. The addition of small quantities of calcium salts to myosin renders it again soluble.

Myosinogen.

Myosinogen is the term applied to the forerunner of myosin in the muscle. According to Halliburton it can be prepared in several ways. First, myosin can be redissolved, and the precipitate which forms at 47° filtered off. The myosinogen remains in solution in the filtrate. Second, by fractional precipitation with $MgSO_4$. He took 100 c.c. of muscle plasma and added 50 grams of $MgSO_4$. This precipitated paramyosinogen. Myosinogen can be precipitated by adding 94 grams to 100 c.c. of the muscle plasma. This separates myosinogen

from myoglobulin which is not precipitated until the liquid is fully saturated.

Myosinogen is precipitated by strong mineral acids, and by dilute acetic acid. It coagulates at 56° in the form of a sticky precipitate.

PARAMYOSINOGEN.

Paramyosinogen was first shown to be in muscle plasma by Kühne. B.Demant [46] also studied it. He extracted it from muscle by means of water, and found the muscles of dogs, rabbits, and pigeons to contain about .5% of it.

It coagulates between 45 and 50. The coagulum is finely flocculent, quite unlike that of myosinogen which is rather sticky. The method of fractional precipition by salts gives it in a form best suited for study. With the use of $MgSo_4$ Halliburton found that paramyosinogen began to precipitate when the blood plasma contained 37 grams to each 100 c.c. and that the precipitation was complete with 44 grams. He was thus enabled to separate it from myosinogen.

It is not precipitated from salt solution by acetic acid, nor does it coagulate when solution is diluted with water. It is precipitated by dialysis.

Halliburton's work has been confirmed by Steward and Sollman, but Von Furth [24] [25] found a body in muscle plasma which coagulated at 40 and which he called soluble myofibrin.

MYOGLOBULIN.

Myoglobulin is precipitated by completely saturating muscle plasma with $MgSo_4$ or NaCl. Itcan be precipitated by dialysing its

solutions. It has the property of not being made insoluble either by dialysis, or by continued washing with saturated salt solution. It coagulates at about 63. It takes no part in the formation of muscle clot.

MYOALBUMOSE.

Myoalbumose is characterised by not being coagulated by heat. Proteids which are coagulated by heat are filtered and it remains in solution. It is completely precipitated by saturation with ammonium sulphate or sodio-magnesium sulphate. It is not precipitated by $MgSo_4$ or by NaCl in neutral or in slightly acid solution. Alcohol precipitates it, but does not convert it into coagulated proteid. It closely resembles the deuteroalbumose described by Kühne and Chittenden.[47]

SERUM GLOBULIN.

Serum globulin is also known as globulin or fibrinoplastic substance (Schmidt), or paraglobulin (Kühne).

It is best prepared by diluting fresh serum with ten times its bulk of cold water, and then subjecting it to a stream of Co_2. It is completely precipitated by saturation with $MgSo_4$. Its heat coagulation temperature is about 75°.

As precipitated it is in microscopic, colorless, amorphous granules. It is insoluble in water, alcohol and ether, but dissolves in water saturated with oxygen. It is soluble in dilute acids and dilute alkalies. When suspended in water, or when dissolved in neutral salt solution, it coagulates on heating at 70°.

FIBRINOGEN.

When blood serum which has been diluted with ten to fifteen times its volume of cold water, and has been freed from paraglobulin by a long continued stream of Co_2, is further diluted and again treated with Co_2 a second precipitate forms which resembles serum globulin, but yet possesses some marked distinctions. This substance is called fibrinogen which term indicates that it is supposed to be one of the precursors of fibrin.

It is insoluble in water, but soluble in water containing oxygen. When blood clots it is converted into fibrin.

Hammarsten devised a method for separating fibrinogen from serum globulin on the basis of their solubility. Both are soluble in 5 to 8 per cent solution of NaCl. When salt amounts to 12 to 16 per cent, fibrinogen is precipitated while serum globulin remains in solution. The quantity of NaCl must amount to more than 20 per cent before any appreciable quantity of serum globulin is precipitated.

FIBRIN.

Fibrin is that proteid formed from fibrinogen, when the latter undergoes coagulation probably under the influence of a ferment. Fibrin composes the clot formed when fresh blood coagulates. It is supposed that the ferment is derived from the white corpuscles in the blood.

Fibrin can be prepared from fresh blood by whipping the blood with twigs. It can be washed free from other proteids by means of water, and freed from fat by means of alcohol or ether. When prepared in this way fibrin is a white elastic body. It is insoluble in water, partially soluble in dilute salt solutions. The freshly prepared fibrin is soluble in 6 per cent KNO_3 if digested with it for

some time at 30 to 40. It dissolves similarily in NaCl and in 10 per cent $MgSo_4$ solutions. It has the power of liberating oxygen from solutions of hydrogen peroxide. Fibrin swells in cold .1 per cent HCl, and dissolves in a warm solution of the same strength. It is readily digested by pepsin or trypsin with the formation of albumoses and peptones. Hasebroek [41] states that the first product in the digestion of fibrin is a product which resembles fibrinogen in many of its properties.

The proteoses.

The term proteose includes a large number of bodies which are intermediate in the formation of peptones from other proteids. Similar substances are formed by both peptic and tryptic ferments. These various substances resemble each other in their origin, and vary slightly in their percentage composition.

The proteoses formed from egg albumin or blood fibrin are called albumoses, and are the bodies which have been studied the most. Kuhne and Chittenden worked extensively with these bodies. Most of Chittenden's work has been published in the Yale University series.

The chief albumoses are perhaps the three which follow,- (1) Proto-albumose, soluble in distilled water and in weak salt solutions, partly precipitated by saturating its acidified solution with NaCl. (2) Hetero-albumose, insoluble in distilled water, soluble in weak salt solutions, and completely precipitated therefrom either by dialysis or by saturation with NaCl. (3) Deutero-albumose is the albumose most nearly allied to peptones. It is soluble in distilled water and in salt solutions and is not precipitated by saturation with NaCl. It can be separated from peptones by saturation with ammonium sulphate.

The deutero-albumose is completely precipitated while the peptones remain in solution. The deutero-albumoses are in all cases the intermediate products between proto- and hetero-albumoses and peptones.

Peptones.

The peptones are the final decomposition products of proteids when acted upon by either peptic or tryptic ferments. It is also claimed thatthey are formed by the prolonged action of dilute acids at high and of moderately strong acids at medium temperatures, and further by the action of water at very high temperatures and pressure. Peptones possess in a feeble degree the character of acids, but at the same time they seem capable of forming combinations with acids. Solid peptones are amorphus, yellowish white, somewhat transculent, inodorous, very soluble in water, particularily when warmed and to a less extent in dilute alcohol. They are insoluble in absolute alcohol, ether and cloroform.

In neutral or slightly acid solutions, peptones are precipitated by mercuric chloride, mercuric nitrate, silver nitrate, lead acetates, tannic,picric, biliary, phosphotungstic and phosphomolydic acids.

They are not precipitated by heat; by mineral acids; nor by acetic acid and potassium ferrocyanide, $CuSo_4$, $FeCl_3$, or NaCl, nor by neutralization of their acid or alkaline solutions.

Their solutions diffuse readily through animal membranes, but their osmotic power is feeble. Their power of diffusing is especially characteristic, and explains in a measure the great part that they play in the support of the body.

Peptones turn the plane of polarized light to the left,but

the degree of polarisation in not the same with all peptones. The peptone from egg albumin polarizes more power-fully than that from fibrin.

The different investigators are greatly divided on the question of the differences between proteoses and peptones, and many conflicting views are found published. Maly, Herth and Henninger all claim that the digestion of fibrin gives rise to a single albumose-hemialbumose or propeptone while Kuhne and his followers believe that there is a decomposition of the fibrin molecule with the formation of a series of albumoses. The two theories have not yet been reconciled. Herth and Harnburger account for the large number of bodies separated by Kuhne by taking into consideration such factors which influence the behavior of albumoses as the presence of certain salts, the quantity of water, the acidity or the alkalinity of the solution, and the temperature.

This question need not be gone into any further at this time. Suffice it to indicate that a very valuable line of research would be a thorough study of these problems, especially from a quantitative standpoint. As far as could be learned no work has been done in the way of making a study of the products of the digestion of beef.

Coagulated albuminoid matter.

Certain proteids like albumins, globulins, alkali-albumins, fibrins, etc. are changed into insoluble modifications by the action of heat in the presence of water,alcohol or ether. The nature of this change is not yet known. According to Commaille a little H_2S is liberated during the coagulation of egg albumin.

Coagulated albuminoids are insoluble in water, alcohol, and solutions of neutral salts. Mineral acids sometimes swell them up.

Alkalies change them into alkali-albumin. Concentrated acids dissolve them, and form acid-albumin.

Mucin.

Mucin apparently forms a small proportion of all connective tissue, and it is especially abundant in embryonic connective tissue.

Rollett prepared mucin by treating finely divided tendons with large quantities of distilled water with the object of removing all water soluble bodies. The tendons are then digested for many days in lime or in baryta water. The mucin is then precipitated by means of acetic acid. It is somewhat gelatinous when freshly precipitated, and forms an opaque liquid with water without going into solution. It is insoluble in .1 to 1.0 per cent HCl. That precipitated by acids is insoluble in NaCl.

Mucin is not digested by pepsin solution, but it is dissolved by an alkaline solution of trypsin. It is precipitated by lead acetate from neutral or slightly alkaline solution. When boiled with dilute H_2SO_4 for twenty to twenty five minutes it is decomposed with the formation of acid-albumin, and a body which has the power of reducing copper salts. This body has no action on polarized light and appears to contain nitrogen, so it cannot be a sugar.

Mucoids or mucinoids.

Mucoids or mucinoids are mucin-like substances which differ from true mucins either in being non-precipitable from alkaline solution by acetic acid or in being readily soluble in excess of acetic acid.

Hammarsten has contributed most of the knowledge that we

have concerning these bodies. He divides the class into seven different groups,- (1) mucin from vitreous humour, (2) mucin from cartilage, (3) mucin from cornea,- cornea mucoid,(4) pseudo-mucin, the colloid-like substance found in the ovarian fluids; (5) a similar mucoid sometimes found in ascitic fluid; (6) ovomucoid, a mucoid found in the white of eggs first studied by Neumeister who called it pseudo-peptone, then by Salkowski and finally by Morner who indentified it as a mucoid; (7) paramucin, a substance sometimes found in ovarian cysts, and differing from pseudomucin in reducing Fehlings solution without previous treatment with acids . Its nature is still very uncertain.

Gelatin.

When white muscular fibers are subjected to the action of boiling water, or of water heated under pressure, or to the long continued action of dilute acids at ordinary temperatures, they dissolve. The solution contains a body called gelatin. Gelatin may be similarily prepared from bones.

Pure gelatin is commonly prepared from commercial gelatin by soaking the latter in distilled water for several days to remove the salts, and then dissolving the residue in hot water, and finally filtering while hot into 90 per cent alcohol. The gelatin separates in the form of white thready masses which can be further dried.

Gelatin is a colorless, amorphous, and transculent substance which swells without dissolving in cold water. It contains considerable ash, the removal of which lessens its power of gelatinizing. It dissolves readily in hot water, and upon cooling it sets into a jelly or gelatinises. This property is possessed by solutions containing as little as 1 per cent . It loses the power of gelatinising

by prolonged boiling, or by heating to 140° in sealed tubes.

Gelatin is precipitated by saturating its solutions with neutral salts like $ZnSo_4$, $MgSo_4$, or $(NH_4)_2So_4$. This is also true of gelatin which has lost the power of gelatinising. It is not precipitated by acetic acid and K_4FeCN_6, nor by most of the heavy salts that precipitate proteids. It is precipitated by $HgCl_2$, and by tannic acid.

Strong reagents like H_2So_4 on putrefaction, decompose gelatin, and form glycocine, leucine, various fatty acids, glutamic acid, carbon dioxide, and ammonia.

Collagen.

Collagen is the mother substance of gelatin. It is the material of which the white fibers of connective tissue are made, and it is the principal constituent of the organic substratum of bone. The collagen in bone is called ossein. In cartilage the material called chondrigen is collagen mixed with the mucinoid material of the cartilaginous matrix.

As already noted under gelatin, collagen can be converted into gelatin by boiling it with water, especially if faintly acid. On the other hand gelatin can be reconverted into collagen by heating it to 130°. Collagen and gelatin according to Hofmeister are probably related to each other as shown in the following equation.

$$\underset{\text{Gelatin}}{C_{102}H_{151}N_{31}O_{39}} - H_2O = \underset{\text{Collagen}}{C_{102}H_{149}N_{31}O_{38}}$$

Collagen is insoluble in water, in alcohol, in salt solutions, and in dilute acids and alkalies. Its decomposition products are the same

as those of gelatin.

Elastin.

Elastin is the material yielded by the yellow fibres of connective tissues. When connective tissues are boiled, the collagen of the white fibres is dissolved while the elastin remains as a net work of elastic fibres.

Elastin offers great resistance to reagents. So far as is known it is not soluble in any liquid without undergoing decomposition. It is soluble in boiling solution of KOH, in cold concentrated H_2So_4, and in concentrated HNO_3. It gradually dissolves when digested with pepsin and with trypsin.

Reticulin.

The fibres of reticular tissue are not histologically different than those of areolar tissue. It was Mall (15) who first stated that they were chemically different.

Seigfried (11) obtained reticulin from the mucous membrane of the intestine by digestion with trypsin and alkali. The residue undissolved was washed and extracted with ether; again subjected to tryptic digestion, and then washed with alcohol and ether. The collagen was removed by hot water. It is believed that reticulin is a constituent of all white connective tissue.

Keratin.

Keratin is the horny material of which the horny layer of the epidermis, hair,wool, nails, hoof, horns, etc. are composed. It is prepared by successively boiling the tissues with ether, alcohol,

water and dilute acids. The insoluble residue is keratin.

Keratin is not digested by either gastric or pancreatic juice. By heating with water to 150° to 200° it dissolves forming a turbid solution. It dissolves more readily in alkalies; the solution containing alkaline sulphides, and substances of the proteose class, called keratinoses by Krukenburg.(67)

Skeletins.

This term is applied by Krukenberg(68) to a number of substances found in the skeletal tissues of invertebrates. They are characterised by great insolubility, and are probably all amido-derivatives of carbohydrates. Under the term are included chitin, conchiolin, spongin, cornein, fibroin, and sericin.

Chondrigen.

Chondrigen is the substance in the matrix of cartilage which upon prolonged boiling with water yields a body called chondrin that will be described a little further on.

Chondrigen is unacted upon by cold water, and swells very slightly in acetic acid. It is dissolved by concentrated mineral acids and caustic alkalies. It dissolves by heating in water at 120° for three or four hours, and the solution thus formed contains chondrin.(69)

Chondrin.

Chondrin is the substance obtained by boiling cartilage. Hot aqueous solutions of chondrin gelatinise upon cooling like those of gelatin. When dry it presents the appearance of a hard, trans-

parent mass, devoid of smell and taste.

Chondrin is insoluble in cold water, in alcohol, ether, or chloroform. It is soluble in hot water, and is precipitated by the following reagents which have no such action upon gelatin:- acetic acid, silver nitrate, copper sulphate, and lead acetate.

Various investigators have studied the properties of chondrin but wide discrepancies have been found especially in the elementary analysis. These differences have led many to believe that in all probability chondrin is not a chemical individual. In an investigation of the chemical properties of this body Morochowitz (70) has arrived at the conclusion that the fibres of the ground substances of cornea consists of collagen and that the supposed chondrin is a mixture of gelatin and mucin. Gamgee (17) strongly inclines to the same belief.

The nucleins.

Brunton (71) described the nuclei of the red corpuscles of birds as consisting of a mucin-like substance. It was later found that although it gave many of the mucin reactions, that it was not mucin since it contained a high percentage of phosphorous. A similar substance was separated from various organic bodies. These varied largely in their solubility and composition, and hence it was soon surmised that nuclein is not a single body. During recent years Kossel has done most to advance our knowledge of the nucleins.

Kossel (73) divides the nucleins into two groups. The first is that of the true nucleins. These are obtainable from nuclei, and upon decomposition they yield the xanthine bases,- hypoxanthine, adenine, and other substances of the same group. The second class includes what are called the pseudo-nucleins, and include those obtain-

able from milk, egg-yolk, etc.

Hoppe-Seyler's classification of the nucleins is the following:- (1) Nucleins like those found in spermatozoa, which contain no proteid, but consist only of nucleic acid.

(2) The true nucleins, those found in cell nuclei. They yield proteid, xanthine or alloxuric bases, and form numerous links in the chain that passes insensibly into the group of nucleo-proteids.

(3) The para-nucleins or pseudo-nucliens which are obtainable from nucleo-proteids like caseinogen and vitellin. They yield no nitrogen bases, but only proteid and H_3PO_4 on boiling with water or dilute acids.

Schafer says that this subject is very complicated, and much yet remains to be discovered especially regarding the nuclein acids.

Nucleo-proteids.

The nucleo-proteids are the compounds of nuclein with proteids. The amount of proteid matter is large, and these bodies give the reactions of proteids, and in their solubilities approah very nearly to the globulins. The nuclein contained in nucleo proteids is unaffected by gastric digestion, but dissolved by pancreatic digestion.

Hammarsten divides the nucleo-proteids into two classes; the first to which he restricts that name yield true nuclein on gastric digestion; the second class which he calls nucleo-albumins yield pseudo-nuclein on gastric digestion and include caseinogen and vitellin.

Nucleo-proteids, using the term in the widest sense are obtainable from the protoplasm of cells.

Many of the nucleins and nucleo-proteids contain iron and

according to Bunge (71) constitute in foods the normal supply of iron in the body. If the iron is in organic union, as it appears to be, the nucliens that contain it must be among the most complex organic compounds known, consisting as they do of seven different elements. The exact method in which the iron is combined is however like the constitution of nucleins, still unknown .

Lecith-albumins.

Liebermann (77) has given the name of lecith-albumins to certain compounds of lecithin and proteid which he obtained from the kidneys, mucous membrane, lungs, spleen and liver. The lecithin is not removable from these by simple extraction with water. They yield no phosphoric acid and no xanthine bases on decomposition. According to Liebermann they play an important part, by virtue of their acidity, in the separation of HCl acid in gastric juice, and in decomposing alkaline salts of blood plasma. These important properties however cannot be definitely ascribed to them until they will have been much more thoroughly investigated.

Acid-albumin.

The term acid albumin was originally applied to the substance obtained by the action of dilute HCl upon albumin, and which could be reprecipitated from the liquid again by means of neutral NaCl or Na_2SO_4. At the present time the term has a much wider application. The term syntonin which originally covered only the acid-albumin from muscles now sometimes includes the entire series of acid-albumin.

Acid-albumin is formed by the action of mineral acids, preferably HCl, upon albumin, globulin, fibrin, coagulated albuminoids

or other proteids. It is also formed in the first stages of the digestion of proteids by pepsin and HCl acid.

It is precipitated by the careful addition of dilute alkali with the formation of a new albuminoid matter. It is almost insoluble in water, and in salt solutions, but soluble in dilute acids or alkalies. It does not dissolve in $CaCo_3$ and water nor does it liberate Co_2.

It is insoluble in strong mineral acids, and hence its precipitation is brought about by the use of these. HNO_3 has been found to be the best precipitant. A precipitate of acid-albumin brought down by strong acid will dissolve readily when put into large excess of water.

Solutions of acid-albumin in dilute acids are not coagulated unless heated beyond 100°. In alkali they are not coagulated at all. The quantity of alkali necessary to bring about solution has converted the acid albumin into alkali albumin. If precipitated by strong acid, and redissolved in alkali then it coagulates at a little over 100° which proves that it is alkali albumin.

Acid albumin behaves very much like alkali-albumin in its ability to be precipitated by salts, as by saturating its solutions with NaCl or with $MgSo_4$. The presence of an excess of acid hinders the precipitation a great deal, however less than does a like amount of alkali.

Alkali-albumin.

Alkali-albumin or as it is called by some authors alkaline albuminate is obtained by the action of the dilute alkalies upon proteids. As a result of the action it has been found that ammonia and a little sulphur are sometimes liberated. Johansson[78] found that

some ammonia and sulphur are liberated even with .2% alkali.

By carefully adding dilute acid, an alkaline solution of alkali-albumin gives a new proteid body that results from the action of the alkali on the original albumin.

It is probable that to each individual albuminoid there is a corresponding alkali-albumin. The composition of alkali-albumin from a given proteid may vary considerably according to the action of the alkali. Soxhlett has observed that by redissolving four or five times the albumin separated by acid, there is each time, a little separation of sulphur. Nevertheless the solutions from all the various products present about the same properties.

Alkali-albumin have marked acid reaction. They are not entirely insoluble in water. Solution of NaCl has no more solvent action than the pure water. They are easily soluble in alkali, in Na_2HPo_4, in Na_2Co_3 and in dilute acids. They are different from acid-albumin in that they do not precipitate upon neutralization in the presence of the alkaline phosphates.

A solution of alkali-albumin in the least possible amount of KOH still presents an acid reaction. Likewise in making the precipitation by means of acids, the solution has to be distinctly acid before a precipitate results. This has been found to be due to the formation of an acid salt which is more insoluble than the neutral salt and hence is precipitated. By continuing the addition of acid there comes a stage where the alkali-albumin is totally deprived of its base, and hence precipitated as the alkali-albumin which comes to the top of the liquid that has in the meantime become neutral again.

Alkali-albumins dissolve in alkali carbonates with the liberation of Co_2. Solutions are coagulated by heat at a little over

100°. They are coagulated at ordinary temperatures by NaCl. Excess of NaCl brings about complete precipitation of albumin especially if the solution is slightly acidic. Excess of alkali makes the precipitation incomplete or even prevents it altogether.

Oxyhaemoglobin.

Haematin was originally supposed to be the source of the coloring matter in the blood, but this is now known to be but the product of the decomposition of true blood coloring matter, namely haemoglobin, or as it is called when loosely combined with oxygen as it always is in the blood-oxyhaemoglobin.

The principle involved in the separation of oxyhaemoglobin is the bringing into solution of the haemoglobin of the red corpuscles either in the serum or in the water added to the separated corpuscles; and then by the addition of alcohol or by cold or by both conjointly, to cause the crystals to separate out.

Oxyhaemoglobin may be dried in vacuo over H_2So_4 at 0° without undergoing decomposition. It then still retains three to four per cent of water which can be removed by heating to 110°.

Solutions of oxyhaemoglobin can be kept many months without undergoing any change. On account of this fact standard solutions of haemoglobin can be prepared, and by their means a quantitative estimation can be made of the amount of coloring matter in samples of blood.

Oxyhaemoglobin is soluble in weak solutions of caustic alkalies, without undergoing change. Excess of alkali induces decomposition . All acids and all salts having acid reaction decompose it with the formation of haematin.

K_2Co_3 precipitates haemoglobin without decomposition if the temperature is low. Solutions of haemoglobin are not precipitated by $Pb(C_2H_3O_2)_2$, nor by $AgNO_3$ though these decompose it.

When a solution of haemoglobin is boiled, the solution becomes turbid and a coagulum of a reddish brown color separates out.

The haemoglobin has been decomposed, and has yielded two bodies insoluble in water,- one a proteid; the other haematin.

Very little information is possessed concerning this proteid body. [81] According to Hoppe-Seyler it behaves as a normal proteid in reference to bases and acids, yielding alkali and acid albumin. Preyer has described it as a body free from inorganic matter, insoluble in water, and which swells in NaCl and NaOH without dissolving. He has called it globin.

Haematin.

When blood is treated with acetic acid it undergoes change of color from red to brown which indicates decomposition of the haemoglobin and the formation of haematin. If the mixture be treated with ether, then the coloring matter is dissolved, and can in that way be removed. Haematin has been found to have the following composition,- C=64.3, H=5.50, N=9.06, Fe=8.82, and O=12.32 per cents.

Haematin is sparingly soluble in alcohol holding H_2So_4. Upon heating it with fuming HCl to 160° it forms $FeCl_2$, and a body free from iron called haematoporphyrin. According to Hoppe-Seyler if reduced haemoglobin is decomposed in the absence of oxygen, instead of haematin; there is produced a body to which he gives the name haemochromogen, whose solutions are purple. It is converted into haematin when oxygen comes into contact with it.

Proteids of the Nervous Tissues.

All what has been said before this has been concerning the proteids found in the muscular tissues and in the blood. To these classes must be added a short description of another very important class of proteids,- namely those which compose the nervous tissues. Perhaps less work has been done upon these, from a purely chemical standpoint, than upon any of the other bodies which have been described, and yet they are of first importance in helping to carry out the functions of the body.

Halliburton (92) believes that there are three proteid bodies in the brain. (1) a globulin coagulated by heat at 47° C., and analogous to cell globulin derivable from nearly all cellular tissue. (2) And most abundant is a nucleo-proteid. It may be prepared by precipitating a water extract of brain with acetic acid. It is coagulated at 56° to 60° and contains.5 per cent of phosphorous. It gives all the nucleo-proteid reactions. (3) Another globulin precipitated by heat at 70° to 75°.

Protagon.

In 1865 Liebreich (83) separated from the brain a material that he called protagon. It can be prepared as follows:- The brain is digested with alcohol at 45° C; the extract is filtered warm , and then cooled to 0° C. The protagon comes down as a white precipitate mixed in with cholesterin, which latter can be dissolved out with ether.

DECOMPOSITION PRODUCTS OF PROTEIDS.

In addition to the properties which have already been at-

tributed to the proteids either as individual or as classes, we find a number of reactions which are very characteristic of these bodies, and which have been studied very thoroughly by many different investigators. For convenience we will group these reactions under this heading, namely, the decomposition products of the proteids.

Decompositions in the body.

As already alluded to more or less, in the alimentary canal, the proteids are converted into proteoses and peptones. This change is supposed to be due to simple hydration. The pancreatic ferment acts further upon a certain class of peptones, called by Kühne the hemi-peptones and forms leucine, tyrosine, aspartic acid, ammonia, and protein chromogen,- a substance colored purple by bromine. Some putrefactive changes are brought about by bacteria, in the small intestine, and as a result of these indole, skatole, phenol and oxyacids are produced.

After absorption from the alimentary canal they become assimilated by the tissues, and there undergo combustion or metabolism the chief final products of decomposition being water, carbon dioxide and urea. Such intermediate products occur as glycocine, leucine, creatin, and ammonium carbonate. Proteids can also be converted into fats as has already been shown.

Action of water.

When heated with water in sealed tubes the proteids are in part dissolved. The solution afterwards undergoes decomposition, it being found to contain H_2S and according to Gautier a number of complex bodies, some of which are soluble in alcohol and ether.

Action of heat.

When subjected to dry distillation the proteids furnish the oily liquid long known as Dippel's oil,(84) which contains (1) ammoniacal salts of the fatty acids, as ammonium butyrate, valerate, and caproate; (2) amines derived from the monatomic alcohols, as methylamine, propylamine, butylamine; (3) aromatic compounds as benzene, aniline, and phenol; (4) picoline and lutidine.

Action of putrefaction.

When exposed to the combined influences of air and moisture, especially at a high temperature, the proteids yield ammonia, ammonium sulphide, carbon dioxide, volatile fatty acids, lactic acid, amines, leucine, and tyrosine.

Action of acids and alkalies.

Long continued boiling with H_2SO_4 and with HCl, and fusion with caustic alkalies gives rise to products of which the chief are the same in both cases,- namely, leucine, tyrosine, aspartic acid and glutamic acid, and ammonia.

Action of HCl and $SnCl_2$.

When proteids are heated with these reagents there is formed ammonia, aspartic acid, glutamic acid, leucine and tyrosine.(85)

Action of oxydising agents.

When proteids are oxidized by means of the various agents as MnO_2 and H_2SO_4, $K_2Cr_2O_7$ and H_2SO_4, they furnish bodies

belonging to the aromatic and fatty groups. About a dozen different bodies have been described in this connection. Among the more important ones may be mentioned several aldehydes, several cyanides, and acids of both the fatty and aromatic series. By the use of other oxidizing agents as nitric acid, chlorine and bromine, various other decomposition products are obtained.

CONSTITUTION OF THE PROTEID MOLECULE.

Many theories have been advanced by many different investigators, [illegible] to explain the constitution of the proteid molecule. Liebig believed that each molecule contained from 500 to 600 atoms. Dumas had previously compared albumins to amides. A. Bechamp obtained urea by oxidizing albuminoids. He thought that this was brought about by the splitting up of the albumin molecule, and that the molecule of urea was contained in the original albumin molecule. He advanced the theory that albuminoids would thus be very complex bodies. He believed that the synthesis of albumin would be brought about by bringing into combination, the products of decomposition either as obtained from the albumin itself or as made in the laboratory. By merely substituting one substance for another of similar composition, he believed that different proteid bodies could be obtained, whose elementary composition were nearly alike. Gerhardt was of the opinion that all the proteids are identical in constitution or in molecular arrangement, but differ from one another in the nature of the mineral substance with which they are associated.

Gautier states that proteids behave as the amides of the higher homologues of lactic and tartaric acids, and residues of arom-

atic acids; hence when proteids are oxidised there is a simultaneous production of fatty acids, of aromatic compounds, and doubtless of bodies analogous to urea. The decomposition products furnished by different proteids are not always obtained in the same proportion. From this it follows that the different radicals differ not only in their arrangement, but in their relative proportion and sometimes in some of their properties.

Pfluger (91) calls attention to the fact that one of the most striking features about proteids is the difference that exists between non-living proteid matter like the white of egg, and that which forms the part of living protoplasm. The former may be kept for years without undergoing decomposition; while the latter is continually decomposing, undergoing intermolecular changes, and lives by breathing oxygen. He believes that the assimilation of proteid food is a combination of a molecule of living proteid with the elimination of water. He believes that nitrogen in the food or non-living proteid is in combination as amides, while that in the living proteid is combined as cyanogen.

O. Loew (91) has found that living proteids reduce $AgNO_3$ while non-living proteids do not. He suggests that albumin is a condensation product of aspartic aldehyde.

Latham (92) regards albumins as compounds of cyan-alcohols united to a benzene nucleus. The cyan-alcohols resemble proteids in readily undergoing condensation and intermolecular changes.

Schutzenberger (93) studied the decomposition products of the albuminoid molecule, by means of $Ba(OH)_2$ for fifteen years. Among the important conclusions which he drew that had a bearing on the constitution of the albuminoid molecule may be mentioned the following:-

(1) Proteid matter when hydrating under the action of baryta

at over 100° use about as many molecules of water as they contain atoms of nitrogen.

(2) A part of the total nitrogen varying with the substance employed from one fourth to one fifth is separated as ammonia. Carbon dioxide and oxalic acid are liberated in the proportion of one molecule of the dibasic acid to each molecule of ammonia or in the proportion necessary to form urea or oxamide.

(3) All the decomposition products are amides corresponding generally to the formula $C_aH_{2a}N_2O_4$.

(4) The mixture of amides is composed of two series of compounds,- derived amides like leucine, and oxyacid amides.

Schützenberger tried to effect the synthesis of albumin. He dehydrated by means of P_2O_5 at 125°, a mixture of amides of the general formula $C_mH_{2m-1}NO_2$ and $C_nH_{2n-1}NO_2$, and about 10 per cent of urea. He obtained a product which was amorphous, soluble in water, and precipitated by alcohol in white caseous flakes. It gave the Biuret test, and was precipitated by tannic acid, picric acid, $HgCl_2$, etc. It was not precipitated by KCN and acetic acid.

Pavy regards all the common proteids with the exception of casein, as having the constitution of glucosides. He insisted that a carbohydrate could be obtained by the hydrolytic decomposition of proteids, and this fact has been confirmed by other writers. K. Morner obtained a reducing substance by treating serum globulin with HCl, but failed to get such a substance from purified myosin, vitellin, crystallin, serum albumin or egg albumin. H. Weydemann (14) also studied this question and confirmed most of Pavy's work.

Many of the theories or perhaps hypotheses which have been advanced may have but very little actual foundation, and yet they are

of great value in the way of stimulating research along these lines.

METHODS OF SEPARATION AND OF IDENTIFICATION OF PROTEID BODIES.

The methods in use for separating and for identifying proteids, are based largely upon a consideration of their various properties as already given at some length. These different methods can be discussed briefly at this point, and some of their applications shown.

Method of heat coagulation.

The method of heat coagulation for the quantitative estimation of those proteids which are coagulated by heat has been in use since these bodies were first studied, and it has met with widely varying degrees of approval.

Bechamp[95] concluded from a large series of experiments in testing the method,- (1) that, not all albuminoids are coagulated by heat. (2) The temperature of coagulation varies according to the concentration of the solution. He found that if a solution was sufficiently diluted, its heat coagulable albumin can be made incoagulable. (3) The purer the albumin the more difficult becomes the coagulation. (4) Different salts affect the temperature of coagulation. Too dilute solutions may be made precipitable by adding salts.

He believed from his work that the heat coagulation method has practically no value in distinguishing between different proteids.

Halliburton[96] has done a great deal of work at different times in testing the heat coagulation method in connection with his work on muscle plasma and muscle serum. He developed very largely the method of fractional heat coagulation, which consisted as the name implies

in the separation by filtration of those fractions of a total precipitate which were precipitated at different temperatures. He probably attached greater importance to this method than it really deserves.

Stewart and Sollman (91) concluded that the method of heat coagulation can be usefully employed in the quantitative estimation of muscle proteids, but believed that it was open to certain important sources of error.

One important objection to the method for certain kinds of investigation is the fact that proteids which are coagulated by heat are thus rendered practically insoluble in the ordinary solvents of those bodies. This fact would preclude the possibility of any further chemical study of the heat coagulated bodies.

Method of dialysis.

The fact that most proteids except peptones and to some extent proteoses, do not dialyse through parchment or animal membrane, is taken as a basis for their separation. By this method the elimination of the salts is never complete. However long the dialysis is continued, the proteid still retains some ash. In many cases however it has been found that this is the only method which could be practicably employed.

Precipitation by means of alcohol.

In the precipitation by alcohol, a measured volume of the liquid is mixed with certain proportions of alcohol, and the precipitate which is formed is allowed to settle, and finally filtered off, washed and weighed or the amount of nitrogen in it is determined by the Kjeldahl method. (15) The liquid may be neutralized with acetic acid,

and mixed with ten times its volume of strong alcohol, boiled, and the precipitate treated as before.

Precipitation by means of neutral salts and other reagents.

Proteids are capable of being precipitated by a large number of reagents. Some of these reagents precipitate large numbers of the proteids while again others precipitate only a very limited number.

The albumins are precipitated from their solutions, (1) by concentrated mineral acids, especially HNO_3 and metaphosphoric acid; (2) by mixture of KCN and acetic acid, and by platinocyanhydric; (3) by certain organic acids in the presence of certain salt solutions as NaCl and Na_2SO_4; (4) by acetic acid solution of tannin; (5) by phosphotungstic and molybdic acids in the presence of free mineral acids; (6) by the double iodides of K and Hg or of K and Bi in the presence of free HCl; (7) by a large number of the salts of heavy metals as Cu, Pb, Ag, and Hg; (8) by alcohol; (9) by chloral, trichloracetic acid, phenol, picric acid, and by taurocholic acids.

Globulins are most easily precipitated by the largest number of salts. They are completely precipitated by $MgSO_4$, $NaNO_3$, $NaC_2H_3O_2$ and Na_2CO_3. They are not completely precipitated by NaCl. Mehu called attention to $(NH_4)_2SO_4$ as a proteid precipitant. Since then its value as a precipitant has been very fully studied. Kühne has fully shown that it causes complete precipitation of all proteids, but the peptones. It has been used extensively in making fractional precipitations of the proteids. 23 grams per 100 c.c. of blood serum completely precipitates the globulins; 35.6 begins to precipitate albumins

which are entirely precipitated with 47.2 grams per 100 c.c.[101]

Halliburton[96] tried a large number of salts, and found in addition to what has already been said, (1) that $KC_2H_3O_2$ and K_3PO_4 completely precipitate all the proteids of serum, (2) that $CaCl_2$ precipitates the proteids in the insoluble form, and (3) that double saturation with certain salts like Na_2SO_4 and $MgSO_4$, precipitate all the globulins that can be separated by heat coagulation.

He calls attention to the fact that a liquid cannot be completely saturated with a salt, especially $MgSO_4$, by simply stirring with the hand. Schäfer mixed the liquid and the salt and shook it by machine for three hours. Hammarsten also points out the above fact.

The method of precipitation by means of neutral salts is perhaps of the greatest value of any of the methods in use in making separations of the different proteid bodies. By its use it is possible to get these bodies in a condition suitable for further chemical study. In some cases as noted in the discussions of some of the individual proteids, it has been found that continued contact of the salt with the proteid gives rise to an insoluble modification, and likewise sometimes the repeated washing of the precipitated proteid with distilled water also renders it insoluble.

The polarimetric method.

The polarimetric method for the identification of different proteids has been largely used by a number of investigators among whom may be mentioned Hoppe-Seyler[107] and Bechamp.[95]

The simplest method of determining the rotatory power of a substance consists in weighing a definite amount of the substance, dissolving it in a known weight of water, taking the polariscope read-

ing, and from these values of calculating the rotatory power of the substance.

One method of finding the weight of the substance used, consists in drying to constant weight at 100° a certain volume of the solution, and then in determining the amount of ash in the residue. The rotatory power is calculated by means of the formula $[\alpha]_j = \frac{v \alpha j}{lp}$, in which v = volume of solution taken; αj = observed reading; l = length of tube; and p = weight of the substance.

The proteids which are insoluble in water are sometimes dissolved in very dilute solutions of NH_4OH, $(NH_4)_2CO_3$, Na_2CO_3, acetic acid, or HCl acid. The rotatory power is usually a little higher in ammoniacal than in acids or neutral solutions. Bèchamp used about .02% acid. The solutions were filtered, and the total solids and ash determined in portions of the filtrate.

Bechamp used a monochromatic polariscope which he found superior to one in which day light was used as the solutions that he worked with were often colored. He sometimes had to resort to the use of the blast lamp in order to get a light that was intense enough.

Proteid bodies are very often mixed with other substances which would interfere with the polariscope reading. It is also frequently the case that one solution may contain a considerable number of different proteids. Bèchamp devised methods for analysing such solutions, and by their use he found a much larger number of different proteid bodies than was commonly supposed to exist. A brief summary of the methods which he employed would be of value at this time.

Because of the chemical changes which active reagents bring about in the proteid molecule, he turned his attention to the use of

neutral salts to bring about precipitation. He precipitated the proteid with lead acetate and removed the lead by means of CO_2. H_2S is objectionable as it colors the filtrate, and it sometimes becomes impossible to remove all the sulphur. If heated, some of the albumin is coagulated and a loss results in that way. The last traces of PbO can can be removed by adding a drop or two of H_2SO_4.

Sometimes CO_2 fails to decompose lead albuminate, in which case $(NH_4)_2CO_3$ is used. The liberated albumin is sometimes rendered insoluble in the $(NH_4)_2CO_3$ or in the water, so that the problem becomes a very difficult one.

The liquid tends to remain opalescent and it is found impossible to remove the last traces of lead by ordinary filters. Bèchamp used a specially prepared filter paper. He took a smooth, heavy filter, saturated it with $BaSO_4$ and moistened it with water just before using. Solution holding $BaSO_4$ in suspension was poured over the filter until there was a smooth, even layer of the salt about one half m.m. thick. The albuminoid liquid is then poured through the filter, and almost immediately a clear filtrate results.

The $BaSO_4$ filter cannot be used when the solution contains alkali. Instead of the $BaSO_4$ in such cases $BaCO_3$ or $CaCO_3$ can be used with good results. In any case the filtration is very slow.

In the decomposition of the lead compound by CO_2, some acetic acid is liberated. This has to be neutralized carefully with dilute ammonia. This neutralisation may produce a second precipitation of albumin.

He concluded from his extensive investigations that the determination of the rotatory power, gave a sure means of distinguish-

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ing between different albuminoid bodies. He believed that the rotatory power of a substance is a much more constant value than its temperature of coagulation as the latter is so readily influenced by a large number of factors.

From his studies of the optical properties of proteids, he concluded that there were a very large number of different proteid bodies. He studied the proteids of eggs, of casein, of vegetable albumin, of gluten, of serum albumin from ox blood, of fibrin from ox and hog blood and the albuminous substances from meat. Attention will be called to some of these bodies in the next section of this paper.

OUR PRESENT KNOWLEDGE OF THE PROTEIDS OF BLOOD AND MUSCLE.

Proteids in blood plasma.

In the living blood the red blood corpuscles float about in a fluid called liquor sanguinis or plasma. When blood coagulates it does so as a result of a separation from the plasma of a proteid substance called fibrin which has already been described at some length. Coagulation occurs almost as soon as the blood is removed from the body, unless prevented by outside means. The immediate lowering of the temperature as soon as the blood is drawn, or the introduction of neutral salts, both tend to retard or even to completely prevent the coagulation for considerable lengths of time.

The reaction of the blood plasma, and of the serum separated from it by coagulation is alkaline. The serum is found to be more alkaline than the plasma from which it separated.

Halliburton (103) concludes that the phenomena of blood coagulation is influenced by a number of causes as evidenced by all the known

facts.

(1) The coagulation of blood requires for its consummation the interaction of a nucleo-proteid called prothrombin and soluble lime salts; and the consequent production of a ferment called thrombin.

(2) Either the nucleo-proteid is not present in appreciable amount in the plasma of circulating blood, or the interaction in question is prevented from occuring in the blood vessels by some means not yet understood.

(3) The nucleo-proteid appears and the interaction occurs as soon as the blood is drawn and is allowed to come into contact with a foreign substance. The source of the nucleo-proteids is principally the leucocytes.

(4) That under certain circumstances and conditions, either the nucleo-proteid does not appear, or it does appear, in drawn blood plasma, and the interactionbetween it, and the lime salts is delayed.

The proteids of blood plasma according to Halliburton are:-

(1) One or more closely allied albumins. These can be separated from the solution after the removal of the globulins, by saturation with $(NH_4)_2SO_4$. He found the precipitate to be composed of three separate albumins which he termed α, β and γ respectively. They differ from one another in the temperature of their heat coagulation. α Albumin coagulates at 70° to 75°, β at 77° to 78° and γ at 83° to 86°. In the plasma of horse, ox and sheep blood, α albumin is absent; in man and in other mammals investigated by Halliburton all three are present; in reptiles only the α albumin is present.

(2) Two globulins termed serum globulin and fibrinogen which have already been described.

(3) A nucleo-proteid or nucleo-proteids. Beyond the mere fact of its presence or of their presence, and that it appears to be an essential factor in the form-ation of fibrin, very little is known about it .

Blood contains normally neither albumose nor peptone (104). All the proteids are completely precipitated by saturating the plasma with $(NH_4)_2SO_4$. The globulins and nucleo-proteids are completely precipitated in half saturated $(NH_4)_2SO_4$ or in completely saturated solution of $MgSO_4$. Fibrinogen is precipitated by half saturating plasma with NaCl.

In addition to the above proteids the blood normally contains the corpuscles to which it owes its color. The coloring matter of the corpuscles is the oxy-haemoglobin which has already been described. This body is of a distinct proteid character.

The proteids of muscle plasma.

When yet living, and rapidly frozen muscle is subjected to pressure, there is expressed from the interior of the fibrin, a viscous but perfectly liquid substance, called muscle plasma by Kühne (43), and known by that name since his time. Muscle plasma sets as a soft jelly as soon as the temperature is suitable. This phenomenonof coagulation has been made the subject of a great deal of study.

The reaction of muscle plasma.

The flesh of dead animals however fresh in the ordinary sense, has an acid reaction. Berzelius who first discovered this fact concluded that the acidity was due to lactic acid. This lactic acid was shown by subsequent researches to differ from the lactic acid produced by fermentation. Liebig first denied, but afterwards af-

firmed its presence in flesh. All early chemists thought that lactic acid was normally present in the tissues during life, since they always found it in the flesh of recently killed animals. At that time the conception had not been formed that when death sets in, certain processes start that give rise to new bodies which are products of decomposition of the flesh. This conception was due to Du Bois-Reymond.[106] He showed the importance of distinguishing between a tissue which is yet living, though it may be separated from the living body of which it once formed a part, and one which has ceased to manifest the phenomena which it possessed during life. There is a change in chemical and physical properties as soon as these phenomena cease,- in warm blooded animals almost instantly. The belief now is that when a muscle is alive it possesses a neutral reaction; when it dies it becomes acid. In warm blooded animals the change occurs so quickly that it is almost impossible to determine the normal reaction. In cold blooded animals the acidification goes on so slowly as to permit of its careful study.

Nasse[107] believed that the acidity was due to the decomposition of glycogen in the muscle. Since then most observers have come to believe that proteids are its source. Böhm[108] found the same amount of glycogen in putrefied and rigorized muscle as in the fresh, and hence concluded that it could not be the source of the acid.

The formation of acid almost simultaneously with coagulation, shows in a measure that the acidity comes from the proteids. In addition to the lactic acid, and perhaps to some of the other organic acids, some of the acidity is due to the acid phosphates formed from the neutral or alkaline phosphates by the development of new phosphoric anhydride from lecithin.

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There seems to be considerable conflict of authority in regard to the matter of an increase in the acidity of plasma or of muscle when coagulation occurs. Halliburton[44] found a considerable increase in the acidity, and his conclusions in this respect have been confirmed recently by Stewart and Sollman.[97] On the other hand von-Fürth[25] found that clotting was not accompanied by an increase in the acidity of the solution. Gamgee[114] states that muscle serum from which the clot has been removed at a low temperature has a neutral or fainting alkaline reaction.

The four last named investigators have done the most to advance our knowledge of the proteids of muscle plasma and of muscle serum. Halliburton's studies in this field were probably second to Kühne's both in time and in importance. The work of Stewart and Sollman, and also in some measure that of von Fürth was a repetition of Halliburton's work. In some cases they were able to use more improved methods, so that for that reason their work has added value.

By fractional heat coagulation and by their varying solubilities in different salts, Halliburton was able to separate four different proteids in muscle plasma.

(1) A globulin precipitated by heat at 47, and which is analogous to cell globulin found in most protoplasmic structures. He calls it paramyosinogen, and Hammarsten calls it musculin.

(2) Aglobulin precipitated by heat at 56 C. This is the proteid that is especially acted upon by myosin ferment, by which it is converted into myosin. Both it and the paramyosinogen enter into the formation of the muscle clot.

(3) A third globulin precipitated at 63° C which he calls

myoglobulin. This body is found in the serum.

(4) Small quantities of an albumin called myoalbumin which is similar in its properties to serum albumin, is also present.

In addition to this in the case of red muscles there is haemoglobin, and if muscles have been kept warm and acidity has developed, there are small quantities of proteoses and peptones formed by self-digestion.

Von Furth reduced the number of proteids in the plasma to three. Paramyosinogen called by him myosin forms from 77% to 83% of the total; and in addition to that small traces of albumin. Von Fürths work is of added value because it is quantitative. J.H.Milroy has also made quantitative estimations of the various proteids coagulated at different temperatures.

Stewart and Sollman have decreased the total number of proteids in dead muscle by one, i.e. they have concluded that dead muscle contains but two proteids. The first of these is a true globulin,- which coagulates at about 45° to 50°, and an atypical globulin,- myosinogen coagulation at about 50° to 65°. The latter readily passes into a modification, similar if not identical with the former.

Both paramyosinogen and myosinogen pass into an insoluble modification which they term as Halliburton does,- myosin. Their quantitative determinations of these bodies differed very widely from those of von Furth. They found that in general paramyosinogen seems to be more abundant in dead muscles than myosinogen, or at least more of it is extracted by salt solution. They found no marked difference in the quantitative amount of the two bodies in the muscles from different animals.

Bechamp believed that ox blood and ox flesh contained the following proteids as shown by their different rotatory powers,-

In ox blood serum:-

(1) An albuminoid precipitated by lead acetate $[\alpha]j = 71.9^\circ$

(2) An albuminoid precipitated by ammoniacal lead acetate $[\alpha]j = 65^\circ$

(3) Hemazymase $[\alpha]j = 55.7^\circ$

In fibrin from ox and hog blood:-

(1) Fibrine (solution in HCl) $[\alpha]j = 72.5^\circ$

(2) Fibrinine $[\alpha]j = 66^\circ$

(3) Fibrimine $[\alpha]j = 80^\circ$

In ox flesh:-

(1) Ox muscle (solution in HCl) $[\alpha]j = 70^\circ$

(2) Flesh albumin (not coagulated by alcohol) called carnisine $[\alpha]j = 42^\circ$

(3) Carnalbumin $[\alpha]j = 90^\circ$

Other flesh proteids.

Not very much is known chemically about the muscle fibers which remain after pressing out the muscle plasma. Liebig thought that when muscle is placed in dilute HCl (.1%), the greater part of the proteid matter is dissolved, to be reprecipitated again when the solution is neutralized. Liebig thought this to be a special body that he called muscle fibrin, but which we now know as acid-albumin or syntonin. We do not know at the present time, and in fact the weight of the evidence that we have would indicate that that residue of muscular fiber is not one distinct chemical individual

even though it would all be soluble in dilute HCl. This is a property which is common to a great many proteids.

Halliburton states further that our chemical knowledge of the involuntary muscles is of a fragmentary nature.

Gamgee in speaking of the proteids of the nervous tissues, says that although more than one half of the solids contained in the gray matter, and about one fourth of the white matter of the nervous centres consist of proteid bodies, that yet our knowledge of these is but very scanty. A short account of those bodies which are known has already been given. The work of Petrowsky published in 1880 is perhaps still the best work that we have upon the proteids of the brain.

Our need of more definite knowledge as shown by the above study.

In closing this preliminary part of the thesis, some stress may be laid upon the matter of showing, in the way of a summary, the present need of more definite knowledge concerning the entire subject of the various proteids of flesh. Although much is already known about this important subject, yet after a careful study it must be admitted that there are a great many gaps yet to be filled. Many of the most prominent authorities on the subject, conflict in certain important particulars, and again in many cases, definite facts are entirely wanting. It is in view of these facts, that even the most painstaking research would be fully warranted and justified if thereby the sum of our knowledge of the proteids of flesh could be increased.

II

EXPERIMENTS CARRIED ON IN CONNECTION WITH THE PRESENT STUDY.

The experimental part of this study consisted of the following work:-

(1) The analysis of one sample of raw, lean beef round in duplicate. The meat was first treated with cold, distilled water until practically nothing further dissolved. The filtrate was made up to a definite volume, and the following determinations then made:- total solids, ash, total nitrogen, syntonin, albumins and globulins, proteoses and gelatin, peptones precipitated by Almen's tannin reagent, precipitate by phosphotungstic acid, and finally the amount of nitrogen still remaining in the solution.

The residue from the water extraction was then treated repeatedly with 10 per cent NaCl solution until no more proteid dissolved. The filtrate was made up to a definite volume, and the following determinations then made:- total solids, ash, total nitrogen, globulins by saturation with NaCl, globulins by heat coagulation, precipitate formed by adding dilute H_2SO_4 to the filtrate from the heat coagulation, proteoses and gelatin, peptones, precipitate by Almen's tannin reagent precipitate by phosphotungstic acid, nitrogen still in solution after treatment with phosphotungstic reagent, nitrogen in filtrate from the precipitate of globulins by the saturated NaCl solution, and also the precipitation of the globulins by dialysis.

The residue from the NaCl extraction was washed free from NaCl by means of cold water, and the filtrate was tested for nitro-

gen. The NaCl free residue was now repeatedly treated with .15% HCl solution until the final extraction contained but .02% of protein. The filtrate was made up to a definite volume, and the following determinations then made:- total solids, ash, total nitrogen, precipitate formed by neutralization with $\frac{N}{10}$ NaOH, nitrogen in the filtrate from this precipitate, precipitate formed by $ZnSO_4$; by Br, by Almen's tannin reagent, by phosphotungstic acid, and lastly the amount of N still unprecipitated.

The residue from the extraction with .15% HCl with cold water, and the filtrate thus obtained was tested for nitrogen. The neutral residue was now treated repeatedly with .15% KOH until the last extraction contained but about .02 per cent of proteid. The residue was made up to a definite volume and the following determinations then made,- total solids, total nitrogen, precipitate formed by acidifying with acetic acid or with HCl, precipitate formed by $ZnSO_4$, by Br, by Almen's tannin reagent, by phosphotungstic acid, and then the amount of nitrogen remaining in the solution.

The residue remaining from the extraction with KOH, was washed free from KOH, and then repeatedly treated with hot water, until nothing further went into solution. The extract from the hot water was made up to a definite volume when cold, and the following determinations made:- total solids, ash, total nitrogen, precipitate formed upon acidifying, precipitate formed by $ZnSO_4$, by Br, by Almen's tannin reagent, by phosphotungstic acid, and then the amount of nitrogen still in the solution.

The residue from the hot water extraction was dried, the fat extracted, and the nitrogen determined in the residue.

(2) The analysis of a second sample of lean, raw meat in duplicate. Each lot was extracted successively with the following solutions:- cold water, 10% NaCl, .15% HCl, .15% KOH, hot water, and the residue remaining was finally analysed and studied. Each filtrate obtained was analysed as before with the exception that the treatment with Almen's tannin reagent, and with phosphotungstic acid were omitted. A few other minor changes were made.

(3) The analysis of a sample of lean cooked meat in duplicate. The sample of meat used here was from the same cut as the second sample of raw meat analysed; the analysis of it was carried on simultaneously; and the treatment in both cases was made as much alike as possible.

(4) A special study of the proteids of flesh which are soluble in water, and also of those which are soluble in 10% NaCl solution.

PREPARATION OF THE SAMPLES FOR ANALYSIS.

Cuts of fresh, lean beef round from young animals were obtained. All bones, gristle, and visible fat were removed and the meat was then cut into strips, and run through a sausage mill two times. The piece that was cooked was subjected to the same treatment, after cooking. Samples from each lot were carefully air dried, and then analysed later on by ordinary methods of food analysis. The results of these analyses are shown in the following tables:-

Table no. 1.

Analysis of air-dried samples of the meats used.

Lab. no.	Kind of meat.	% air dried m.	per cent water.	% proteid.	% fat.	% ash.	Total.
1265	Raw beef	24.24	5.59	82.33	6.01	4.45	98.38
1266	" "	27.01	5.75	83.52	5.54	4.52	99.33
1267	Boiled "	44.68	6.75	84.39	6.83	2.00	99.97

Table no. 2.

Analyses calculated to fresh meats.

Lab. no.	Kind of meat.	% water.	% proteid.	% fat.	% ash.	Total.
1265	Raw beef round	77.12	19.96	1.46	1.08	99.62
1266	" " "	74.54	22.56	1.50	1.22	99.82
1267	Boiled " "	58.34	37.70	3.05	.89	99.98

Table no. 3.

Analyses calculated to water-free meats.

Lab. no.	Kind of meat.	% proteids.	% fat.	% ash.	Total.
1265	Raw beef round	88.72	6.48	4.80	100.00
1266	" " "	89.25	5.92	4.83	100.00
1267	Boiled " "	90.55	7.33	2.14	100.00

In the case of the first sample of raw meat, two lots of the freshly ground meat,- one of which weighed 100 grams, and the other 50 grams, were weighed out in one liter beakers. In the case of the second sample of the raw meat and of the sample of cooked meat, two lots of the fresh meat, each weighing 100 grams were weighed out.

Both samples of raw meat were from animals about two years

old. Nos. 1266 and 1267 were from the same cut. No. 1267 was cooked by plunging the piece of raw meat into boiling water, keeping it at that temperature for ten minutes, then gradually reducing the temperature to 80° to 85°, and thus continuing the cooking for 5 hours.

Extraction with cold water.

To each lot of the weighed meat, there was added about 500 c.c. of ice cold, nitrogen free water. The temperature was not allowed to rise above 10° C. at any time during the extraction. The mixture was thoroughly stirred about every fifteen minutes for about three hours. Liquid was then filtered. In the case of sample #1265 bolting cloth was used for this filtration. In the other cases it was found that a special grade of heavy parchment filter paper especially suited for this kind of work, gave the best results. The residue after the first extraction was again treated with water as before, and filtered a second time. This same process was repeated until the final extract showed that the extraction was complete or nearly so. This required from three to six extractions. The completeness of the extraction by means of cold water is shown in the following table no. 4.

Table no. 4.
Total solids in last extraction with cold water.

Lab. no.	Wt. sample	No. of extractions m.	Vol. of last extr.	Total sol. in last ex.	% total sol. in last ex.
1265	100	3	750	—	—
"	50	3	350	.0680	.13
1266	100	5	500	.0600	.06
"	100	5	500	.0500	.05
1267	100	7	500	.0280	.03
"	100	7	500	.0200	.02

To determine when the extraction was complete or nearly so, the entire filtrate from one extraction was measured,-after filtering if that was necessary, and a portion taken out for evaporation in a platinum or an aluminium dish. Both the total volume of the filtrate, and the amount taken out for the determination were carefully recorded. The succeeding extractions were similarily treated, and corrections made for all differences in the volumes of the different extractions. Thus if the filtrate from one extraction amounted to 500 c.c. and 25 c.c. were taken out for the determination of the total solids; and if the filtrate from the next extraction amounted to 650 c.c. then the total amount taken out was made proportional to that taken out in the first case:- 500 : 650 = 25 : x where x = 32.5 c.c. or the total amount taken out. All the extractions were similarily corrected for volume, and then thoroughly mixed. The total volume of all extractions from each lot was carefully noted, and used as the basis for subsequent calculations. The residue of the meat insoluble in cold water was carefully removed to the beaker, and treated with NaCl solution as will be described later on. The cold water extracts were analysed just as soon as practicable by the method which follows.

ANALYSIS OF THE COLD WATER EXTRACT.

Total solids.

The total solids were determined by evaporating in platinum dishes a definite amount,- 100 c.c. and in some cases 500 c.c. of the solution, to dryness upon the water bath, and then heating in the

water oven until nearly of constant weight.

Ash.

The dry residue obtained from the total solids determination was charred to a little below redness in the dishes. The residue still remaining was now extracted with hot water, filtered upon an ashless filter and washed with hot water. The filter paper and its contents were then returned to the dish, and burned until colorless or nearly so. The water filtrate was now returned to the proper dish, and evaporated to dryness upon the water bath, and finally ignited at a little below redness. The results of these two determinations are given the following table. #5.

Table no. 5.

Total solids and ash in cold water extract.

Lab. no.	Wt. of sample.	Tot. vol. extract.	c.c. ext. taken.	Wt. tot. solids.	% total solids.	Wt. tot. ash.	% total ash.
1265	100	2450	100	6.5256	6.53	1.0903	1.09
"	50	2250	100	3.1444	6.29	.5963	1.19
1266	100	2600	100	7.3502	7.35	1.1700	1.17
"	100	2500	100	7.3950	7.40	1.3200	1.32
1267	100	4500	500	3.4083	3.41	.8460	.85
"	100	4550	500	3.4534	3.45	.8509	.85

Total nitrogen in cold water extract.

A certain volume of the total water extract was measured out, and the amount of nitrogen in it determined by the Kjeldahl method. These results are shown in:-

Table no. 6.

Total nitrogen in the cold water extract.

Lab. no.	Wt. of sample.	Volume total solution.	c.c. solution used.	Wt. N in solution taken.	Wt. total N in water extract.
					.7350
1265	100	2450	50	.0150	.7351
"	50	2250	50	.0068	.3060
1266	100	2600	100	.0351	.9126
"	100	2500	100	.0369	.9225
1267	100	4500	250	.0203	.7654
"	100	4550	250	.0201	.7658

Determination of syntonin.

Portions of the solutions were measured off for the estimation of the syntonin. Delicate litmus paper was prepared especially for testing the acidity of the solution, but yet it was not possible to get satisfactory results with it. Phenolphthalein was then used as indicator. After carefully neutralizing, the solution was allowed to stand several hours, but in no case was there any syntonin precipitated.

Acidity of the solution.

All the cold water extracts were found to be distinctly acid to phenolphthalein. On account of the concentration of the solutions usually a certain amount of the solution was measured out and then diluted considerably with water before making the titration. Sol-

ution of NaOH containing .00539 gr. NaOH or .01214 grams of lactic acid per c.c. was used. It was found that upon boiling, and thus upon coagulating the albumin that the acidity of the solution kept increasing right along up to a certain point. When no further precipitate of albumin resulted then the solution remained neutral. In the adjoined table the total acidity of the solution is given. As an example of the increase in acidity the following case may be cited. The cold water extract from #1266 was titrated with the NaOH, 500 c.c. requiring 15.0 c.c. of NaOH. Solution was boiled down to small volume and filtered. A little more phenolphthalein was added to this filtrate, and the solution again titrated. It required 4.1 c.c. of the NaOH to neutralize it. Heated solution to boiling, filtered, washed precipitate with hot water, and titrated again. 2.1 c.c. of NaOH were used this time. Filtered, heated filtrate to small volume. This time no precipitate formed and the solution remained neutral. Thus it is shown that the acidity of the solution was increased by the boiling about one half. Similar results were obtained in all other cases. This work confirms the conclusions arrived at by Halliburton that the acidity of a solution increases with the coagulation of its proteids.

Table no. 7.

Lab. no.	Wt. of sample.	Total volume of extract.	c.c. NaOH required for each c.c.	Wt. total lactic acid in extract.	% lactic acid in meat.
1265	100	2450	.036	1.0707	1.07
"	50	2250	.0204	.5572	1.10
1266	100	2600	.0424	1.3583	1.34
"	100	2500	.0432	1.3111	1.31
1267	100	4500	.0206	1.1253	1.13
"	100	4550	.0206	1.1379	1.14
1267	984.56	1700	.3400	7.0169	.71
"	"	"	.3340	6.8930	.70

The acidity of the broth obtained by boiling #1267 is also shown in the table. It is seen by this that the total acidity found for the meat after cooking amounts to 1.84% computed to lactic acid while the total acidity of the cold water extract of #1266 amounts to only 1.34%. This would go to show that by boiling of meat additional acid is liberated.

Determination of albumins and globulins.

A portion of the extract was made slightly acid to phenolphthalein and then boiled and filtered. It was always found desirable to boil the solution down to as small a volume as possible. As already noted in speaking about the acidity of the solution it was always necessary to make solution nearly neutral again after each filtration and to boil down further. This process was repeated until no further precipitate resulted upon boiling and evaporating to small bulk.

Table no. 8.

Albumins and globulins in cold water extract.

Lab.no.	Wt. of sample.	Vol. total water extract.	c.c. sol. used in determination.	Wt. total N in extract.	Total wt. albumin & globulin.	% albumin & globulin in raw meat.
1265	100	2450	500	.3675	2.2962	2.30
"	50	2250	500	.1647	1.0325	2.06
1266	100	2600	500	.3890	2.4310	2.43
"	100	2500	500	.4190	2.6188	2.62
1267	100	4500	1000	.0077	.0477	.05
"	100	4550	1000	.0082	.0512	.05

Precipitate in cold water extract formed by adding H_2SO_4 to filtrate from albumin.

In the filtrate from the precipitation of albumin and globulin in the cold water extract of #1266, it was found that a considerable amount of proteid matter was precipitated upon the addition of H_2SO_4. This precipitate was filtered off, and the nitrogen in it determined. It is possible that this was some of the albumin whose acid compound is so much more soluble in than that found in neutral solutions. A similar precipitate was formed in other extracts which will be noted at the proper time.

Table no. 9.

Precipitate formed by H_2SO_4 in cold water extract

Lab. no.	Wt. of sample.	Vol. total water extract.	Vol. sol. taken.	Wt. N in total solution.	Wt. proteid in sol. taken.	% proteid ppted by H_2SO_4.
1266	100	2600	500	.1327	.2048	.20
"	100	2500	500	.0500	.3125	.31

Determination of proteoses and gelatin.

The filtrate from the determination of albumin and gelatin was evaporated to a small volume, and then made acid by the addition of two or three drops of 1 to 3 H_2SO_4. When no precipitate resulted at this stage, the solution was at once saturated with $ZnSO_4$. About 85 grams of $ZnSO_4$ are necessary for the saturation of 50 c.c. of the liquid at ordinary temperatures. Care was taken not to have the solution contain an excess of the salt as that would tend to cause bump-

ing in subsequent determinations of nitrogen. The precipitate was allowed to stand for several hours usually over night. The precipitate was never so voluminous but that it could be easily filtered and washed with a saturated solution of the $ZnSO_4$. The results obtained are given in table no. 10.

Table no. 10.

Proteoses and gelatin in cold water extract.

Lab. no.	Wt. of sample.	Vol. total water extract.	Vol. extract taken.	Wt. N in total extract.	Wt. proteid in total ex.	% proteose and gel. in raw meat.
1265	100	2450	500	.0189	.1225	.12
"	50	2250	500	.0031	.0194 .0217	.04 .05
1266	100	2600	500	.0338	.2113	.21
"	100	2500	500	.0435	.2719	.27
1267	100	4500	1000	.0198	.1218	.12
"	100	4550	1000	.0182	.1138 .2275	.11 .23

Determination of peptones in cold water extract.

The filtrate from the determination of the proteoses and gelatin was diluted with about an equal bulk of water, treated with about 3 c.c. of 10% HCl, and then saturated with bromine. The bromine was added little by little and the mixture well shaken after each addition. More bromine was added until finally a little of the bromine was left undissolved in the completely saturated liquid. The liquid was allowed to stand for 12 hours or more. The precipitate was then filtered off, and both it and the flask were thoroughly

washed with saturated bromine water. The filter paper and the precipitate were returned to the flask in which the precipitation had been made, and the nitrogen determined by the Kjeldahl method. Results are shown in table no. 11.

Table no. 11.

Peptones in the cold water extract.

Lab. no.	Wt. of sample.	Vol. total water extract.	Vol. extract taken.	Wt. N in total extract.	Wt. peptones in total ex.	% peptones in total ex.
1265	100	2450	500	.0044	.0276	.03
"	50	2250	500	.0020	.0133	.03
1266	100	2600	500	.0052	.0328	.03
"	100	2500	500	.0055	.0344	.03
1267	100	4500	1000	.0140	.0872	.09
"	100	4550	1000	.0173	.1081	.11

Precipitate by means of Almen's tannin reagent.

To the filtrate from the peptone precipitation there was added 10 c.c. of dilute acetic acid of the strength 1 to 4 and then a slight excess of Almen's tannin reagent which is supposed to precipitate all the proteids including peptones. It is prepared by dissolving 4 grams of tannic acid in 190 c.c. of 50% alcohol, and then adding 8 c.c. of 25% acetic acid. The reagent was tested for N, and found not to contain any. This test was not made but with the first sample of raw meat. The results obtained are given in table no. 12. The determinations were run in duplicate but only the average of the

duplicates in shown in the table.

Table no. 12.

Ppt by Almen's tannin reagent in the cold water extract.

Lab. no.	Wt. of sample.	Vol. total extract.	Vol. extract taken.	Wt. N in total extract.	Wt. proteids ppted by Almen's tan.	% proteids by Almen's tannin.
1265	100	2450	500	.0012	.0078	.01
"	50	2250	500	.0016	.0098	.2

Ppt formed by phosphotungstic acid in cold water extract.

To the filtrate from the tannin precipitation, was added phosphotungstic acid as long as a precipitate continued to form. The liquid was heated to about 95°, filtered while hot and the precipitate on the filter thoroughly washed with water having a temperature over 90°. It is very important to have the temperature of the water over 90° as otherwise some of the flesh bases are likely to be precipitated. This precipitation was made only with the first sample of raw meat. Results are given in table no. 13.

Table no. 13.

Ppt by phosphotungstic reagent in the cold water extract.

Lab. no.	Wt. of sample.	Volume of total water extract.	Volume of extract taken.	Wt. N in total extract.	Wt. proteids ppted by phos. photungstic.	% proteids ppted by phos. reagent.
1265	100	2450	500	.0008	.0047	.01
"	50	2250	500	.0036	.0225	.04

Nitrogen remaining unprecipitated.

The filtrate from the phosphotungstic acid was made up to a definite volume and the N determined in an eliquot part of the solution. The nitrogen found was calculated to flesh bases. The results obtained for sample #1265 are shown in table 14. In the case of samples nos. 1266 and 1267 the nitrogen remaining in the filtrate from the peptone determination, was determined. The results are shown in table no. 15. In all cases 4 separate determinations were made on each sample while the tables show only the average of the duplicates.

Table no. 14.

Nitrogen in filtrate from phosphotungstic reagent.

Sample no.	Wt. of sample.	Vol. total water extract.	Vol. extract taken.	Wt. N in total extract.	Wt. flesh bases in extract.	% flesh bases in meat.
1265	100	2450	500	.2934	.9155	.92
"	50	2250	500	.1823	.5690	1.14

Table no. 15.

Nitrogen in filtrate from peptone precipitation.

Sample no.	Wt. of sample.	Vol. total water extract.	Vol. extract used.	Wt. N in total extract.	Wt. flesh bases in filtrate.	% flesh bases in meat.
1266	100	2600	500	.3263	1.0180	1.02
"	100	2500	500	.3150	.9828	.98
1267	100	4500	500	.2914	.9092 .9592	.91 .96
"	100	4550	500	.2674	.8342 .8421	.83 .84

The above work nearly completed the study of the cold water

extract. It is evident from the last two tables that the amount of flesh bases found in the meat is larger than is commonly believed to be present. There is a possibility that some proteid bodies were not completely precipitated by the methods employed. Should such be the case it would indicate that these precipitants do not do their work as completely as is supposed.

A little further study was made with a portion of the water extract with the object of finding out the number of proteids in the solution, coagulable by heat, and also the temperatures at which they coagulate. Took 100c.c. of extract from #1266, and filtered it in order to get a perfectly clear solution. The solution had been standing for about 24 hours and in the meantime a slight precipitate had formed. In the above clear solution no precipitate was found to result upon boiling the solution either while distinctly acid or distinctly alkaline. Upon neutralization there occured a slight turbidity at 45°, a milkiness at 55°, and a heavy flocculent precipitate at 61° to 65°. At this stage the solution was filtered, and the clear filtrate heated again. A slight turbidity appeared again at about 45°, and a precipitate formed upon boiling. In one case the precipitate settled out at about 90°. It was usually found necessary to boil the solution very thoroughly, and down to a small volume before the liquid holding the precipitate became clear.

Lack of time at this stage of the work prevented the making of as thorough a study as would have been desired. The work shows at least that there is more than one proteid in the cold water extract from meat. This conclusion was reached also by further work done along a little different line, which will be described later on. It showed also how easily the precipitation of the proteids is influenced

by the condition of the solution.

Extraction with 10% NaCl solution.

The residue of the meat after complete extracti n with cold water, was extracted with a 10% solution of NaCl. The extraction was proceeded with as in the case of the cold water, but here it was found necessary to make a much larger number of extractions before dissolving out all or most of the proteids soluble in dilute NaCl. In #1265 the end of the extraction was determined somewhat unsatisfactorily by attempting to determine the total solids in a portion of a filtrate. This method would not work well on account of the large amount of salt present. The Biuret test, and the K_4FeCN_6 and acetic acid methods also failed to give satisfactory results, as they were not delicate enough. In the succeeding analyses a portion of a filtrate was tested for N by the Kjeldahl method, and this proved to be the most satisfactory way of arriving at the needed facts. The amount of proteid matter in the last extraction is shown in table no. 16. Parchment filter paper was used in making the filtration, and it was found to work very satisfactorily. After making all necessary volume corrections as under the cold water extract, all the different filtrates were mixed, and analysed by methods which will follow.

Table no. 16.

Proteid matter in last extraction with 10% NaCl.

Lab. no.	Wt. of sample.	No. of extractions made.	Vol. of last extraction.	Wt. N in last extraction.	Wt. proteid matter in last extraction.	% proteid matter in last extraction.
1265	100	5	1150	———	———	———
"	50	4	750	.0043	.0271	.05
1266	100	11	500	.0048	.0301	.03
"	100	11	500	.0019	.0120	.01
1267	100	8	500	.0019	.0120	.01
"	100	8	500	.0048	.0301	.03

The wash water after having completed the extraction with NaCl was all preserved and measured and the proteid matter which this treatment brought into solution was determined by means of the Kjeldahl method. The results so obtained are tabulated in:

Table no. 17.

Proteid matter dissolved out by wash water after extraction with NaCl.

Lab. no.	Wt. of sample.	Total vol of wash water.	Volume wash water analysed.	Wt. total N in wash water.	Wt. total proteid in wash water.	% total proteid in wash water.
1265	100	3500	100	.0118	.0737	.07
"	50	2900	100	———	———	———
1266	100	2750	500	.0071	.0447	.05
"	100	3000	500	.0075	.0470	.05
1267	100	2000	250	.0042	.0265	.03
"	100	2000	250	.0023	.0144	.01

Analysis of the 10% NaCl extract.

After thoroughly mixing all the different extractions with the NaCl, the analysis was carried on as rapidly as possible. In sample #1265 an attempt was made to determine the total solids but this was soon found to be impracticable on account of the large amount of salt present. On account of this fact this determination was abandoned. The other determinations made now follow.

Total nitrogen.

A measured quantity of the extract was placed in a Kjeldahl flask, and the total N determined by the usual method. On account of the large amount of salt it was found necessary to use, as a general thing a double quantity of H_2SO_4 for the digestion, and to proceed with the digestion very slowly especially at first. The mixture tends to foam a good deal. Results are given in the following table:-

Table no. 18.

Total N in 10% NaCl extract.

Lab. no.	Wt. of sample.	Vol. of extract.	Vol. extract taken.	Wt. total N in extract.	Wt. total proteid in extract.	% total proteid in extract.
1265	100	4350	100	.8449	5.2805	5.28
"	50	4250	100	.3062	1.9139	3.83
1266	100	6700	250	1.1015	6.8844	6.88
"	100	6400	250	1.1187	6.9919	6.99
1267	100	5400	250	.0907	.5669	.57
"	100	5000	250	.0864	.5400	.54

Determination of globulins by saturation with NaCl.

In the case of sample #1265 the globulins were estimated in one case by saturating a certain amount of the extract with NaCl. The precipitate formed in this way was filtered off, and the nitrogen in it determined. The filtering was found to be a very slow and tedious process, and upon the whole the method was found to give lower results than were obtained by coagulating the globulins by heat. By the latter method as the tables will show, the proteid found was .55% higher than by saturation with NaCl.

Table no. 19.

Globulins precipitated by saturation of NaCl extract with NaCl.

Lab. no.	Wt. of sample.	Vol. of extract.	Vol. of extract taken.	Wt. total N in ppt by NaCl.	Wt. total proteid ppted by NaCl.	% total proteid ppted by NaCl.
1265	100	4350	1000	.5224	3.2650	3.27
"	100	"	1000	.5420	3.3875	3.39
"	50	4250	500	.1853	1.1581	2.32

Globulins in NaCl extract by heat coagulation.

A fair sized volume of the extract was boiled down to a small bulk and then filtered. The precipitate was washed and the nitrogen in it determined.

Table no. 20.

Globulins precipitated by heat coagulation.

Lab. no.	Wt. of sample.	Vol. of total solution.	Vol. of extract taken.	Wt. total N in heat ppt.	Wt. total proteid in heat ppt.	% total proteid in heat ppt.
1265	100	4350	250	.6205	3.8776	3.88
"	50	4250	250	.1930	1.2059	2.41
1266	100	6700	500	.6888	4.3050	4.31
"	100	6400	500	.7142	4.4638	4.46
1267	100	5400	1000	.0075	.0469	.05
"	100	5000	1000	.0165	.1031	.10

Acidity of NaCl extracts.

To the filtrate from the heat coagulum was added in the case of the 100 gram lot in #1265, 1 c.c. of $\frac{N}{10}$ NaOH, and in the case of the 50 gram lot .5 c.c. of the NaOH. It had been found by previous titration of the original extract that that would be the amount necessary to neutralize the volume of the extract used. In neither case however was there a precipitate formed.

In samples #1266 and #1267 the original extract was neutralized before coagulating the globulins by heat. Boiled down to small volume, and then found solution acid again. Neutralized, filtered, heated again, and repeated this process until no further ppt formed. It was found that upon precipitation the solution became acid in reaction which further confirms the work of Halliburton.

Ppt by H_2SO_4 in filtrate from heat coagulum in NaCl extract.

The filtrate from the heat coagulation was treated with 3 to 4 drops of 1 - 3, H_2SO_4, and in all cases it was found that a con-

siderable ppt resulted. This was filtered off, washed and Kjeldahled. The ppt showed that there is either more than one globulin in meat which is soluble in 10% NaCl solution, or that the heating brings about a decomposition ////// should only one be originally present.

Table no. 21.

Ppt by H_2SO_4 in filtrate from heat coagulum.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. total N in H_2SO_4 ppt.	Wt. total proteid ppted by H_2SO_4	% total proteid ppted by H_2SO_4.
1265	100	4350	250	.1157	.7228	.72
"	50	4250	250	.0450	.2815	.56
1266	100	6700	500	.1983	1.2395	1.24
"	100	6400	500	.0730	.4563	.46
1267	100	5400	1000	.0124	.0775	.08
"	100	5000	1000	.0035	.0156	.02

Proteoses and gelatin in filtrate from H_2SO_4, precipitated by $ZnSO_4$.

The filtrate from the precipitate by H_2SO_4 was saturated with finely pulverized $ZnSO_4$, and allowed to stand for the usual time. The precipitate formed was filtered, washed, and the nitrogen in it determined.

Table no. 22.

Proteoses and gelatin in NaCl extract.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. extract taken.	Wt. total N as proteoses and gelatin.	Wt. total proteoses and gelatin.	% proteoses and gelatin in NaCl extract.
1265	100	4350	250	.0017	.0106	.01
"	50	4250	250	.0102	.0638	.12
1266	100	6700	500	.0134	.0837	.08
"	100	6400	500	.0243	.1519	.15
1267	100	5400	1000	.0070	.0438	.04 .06
"	100	5000	1000	.0155	.0969	.10

Peptones in NaCl extract from meat.

The filtrate from the $ZnSO_4$ precipitate was tested for peptones as described under the cold water extract. Results obtained are given in:

Table no. 23.

Peptones in NaCl extract precipitated by Br.

Lab. no.	Wt. of sample.	Vol. total extract.	Vol. extract taken.	Wt. N in NaCl ext. as peptones.	Wt. peptones in NaCl extract.	% peptones in NaCl.
1265	100	4350	250	.0105	.0653	.07
"	50	4250	250	.0085	.0532	.10
1266	100	6700	500	.0134	.0838	.08
"	100	6400	500	.0115	.0719	.07
1267	100	5400	1000	.0070	.0438	.04
"	100	5000	1000	.0065	.0406	.04

Treatment with Almen's tannin reagent, and with phosphotungstic acid.

The filtrate from the peptone precipitation in #1265 was treated successively with Almen's tannin reagent, and then with phosphotungstic acid but in neither case was a precipitate obtained which contained an appreciable amount of nitrogen. In the case of #1266 and of #1267 these tests were not made.

Nitrogen not precipitated by reagents used.

The filtrate from the phosphotungstic acid treatment in #1265 was made up to a definite volume, and the nitrogen determined in eliquot parts of it. In #1266 and #1267 the filtrate from the Er precipitate was tested for nitrogen. The results obtained indicate that the reagents employed failed to bring about a complete precipitation of all the proteid bodies. In all cases a relatively large amount of nitrogen remained unprecipitated as shown by:-

Table no. 24.

Nitrogen in NaCl extract not precipitated by reagents used.

Lab. no.	Wt. of sample.	Vol. total extract.	Vol. filtrate.	Wt. N in total filtrate.	Wt. total proteid not precipitated.	% total proteid not ppted.
1265	100	4350	350	.0426	.2662	.27 .30
"	50	4250	375	.0238	.1488	.29
1266	100	6700	1000	.2015	1.2563	1.26
"	100	6400	1000	.1536	.9600	.96
1267	100	5400	1000	.1188	.7425	.74
"	100	5000	1000	.1000	.6250	.63

Dialysis of the extract.

75 c.c. of the NaCl extract from #1265 were measured into a dialysing capsule. The capsule was attached to a ring of an iron stand so that it could be conveniently lowered into or raised out of the beaker in which the dialysis was to be carried on. The water in the beaker was changed eleven times. From 250 to 300 was used each time and each lot was carefully placed in a flask and at the end of the dialysis the entire amount was measured. Duplicate determinations were run on each lot. Nitrogen determinations were then made on the solutions used in dialysing but in no case was there a trace of nitrogen found. This showed that none of the proteid bodies had passed through the membrane, and also indicated that the bodies precipitated by bromine which we called peptones, must not have been peptones or they would have appeared in the solution dialysed. It is true however that by working with only 75 c.c. of the original solution, and in being able to take but from 1/8 to 1/12 of the dialysed solution which represents but six to nine c.c. of the original extract that such small quantities of peptones as were found, could get lost on the way.

The proteid separated in the capsule and began to decompose after about four days.

Extraction with .15% HCl.

The NaCl-free residue of meat from the extraction with NaCl was now treated with HCl, in the case of #1265, of the strength of about .15% and in the other cases very nearly of that strength. The extraction was carried out much as before. Parchment filter paper was used

for filtering. This treatment tended to swell the meat a good deal. The end of the extraction was determined in #1265 by finding total solids in different filtrates. The Biuret and K_4FeCN_6 tests were also used but without satisfactory results. In #1266 and #1267 the nitrogen was determined in parts of the filtrate and as usual was found to be the most rapid and most accurate method for arriving at the wanted facts. Corrections were made upon all the filtrates for the portions taken out in finding the end reaction, and then all those from the same lot of meat were very thoroughly mixed and analysed as will be described futher on. The completeness of the extraction is given in:-

Table no. 25.

Proteid matter in last extraction with dilute HCl.

Lab. no.	Wt. of sample.	No. of extractions made.	Vol. of last extraction.	Wt. N in last extraction.	Wt. proteid in last extraction.	% proteid mat. in last ext.
1265	100	6	2025	total solids=	.5670	.57
"	50	6	1195	" "	.3072	.61
1266	100	10	500	.0058	.0361	.04
"	100	10	500	.0058	.0361	.04
1267	100	13	450	.0078	.0487	.05
"	100	13	450	.0035	.0216	.02

After having made the last extraction with HCl, the residue of the meat was thoroughly washed with pure water until free from Cl. The filtrates thus obtained were all saved, and measured and the nitrogen determined in a portion. The table no. 26 shows the amount of proteid matter which this treatment dissolved out.

Table no. 26.

Proteid matter dissolved out by wash water after extraction with HCl.

Lab. no.	Wt. of sample.	Total volume of wash water.	Volume of wash water analysed.	Wt. total N in wash water.	Wt. total proteid in wash water.	% total proteid in wash water.
1265	not determined.					
1266	100	1700	200	.0073	.0460	.05
"	100	1500	200	.0065	.0406	.05
1267	100	2000	250	.0280	.1758	.18
"	100	2000	250	.0315	.1925	.19

Analysis of the HCl extract.

After having made all necessary volume corrections, and thoroughly mixed all the different portions from the same the analysis was carried on as rapidly as possible.

Total solids and ash and HCl extract.

A measured quantity of the extract was evaporated to dryness in platinum dishes on the water both and then heated to constant weight in the drying oven. The residue was saved and the ash in it determined by the method already described under the water extract. The ash was rather high in all cases, and was colored somewhat red by iron which probably came from the spatula used in removing the meat from the filter paper.

Table no. 28.

Total solids and ash in the HCl extract.

Lab. no.	Wt. of sample.	Total vol. of solution.	c.c. sol. taken.	Total wt. of resid-ue in extract.	% meat dissolved by HCl.	Total ash in HCl extract.	% ash in meat sol. in dil. HCl.
1265	100	6700	250	3.3835	3.38	.5052	.51
"	50	5000	250	2.0090	4.02	.6590	1.32
1266	100	6000	500	3.0960	3.10	.3648	.36
"	100	6100	500	3.2025	3.20	.3767	.34
1267	100	6600	500	3.1984	3.20	.4462	.45
"	100	6400	500	3.4317	3.43	.4749	.47

Table no. 29.

Total N in HCl extract from meat.

Lab. no.	Wt. of sample.	Total vol. of sol-ution.	Vol. of extract taken.	Wt. total N in ex-tract.	Wt. total proteid in extract.	% total proteid in extract.
1265	100	6700	250	.3766	2.3535	2.35
"	50	5000	250	.1720	1.0750	2.15
1266	100	6000	250	.3855	2.3250	2.33
"	100	6100	250	.3855	2.4094	2.41
1267	100	6600	500	.3472	2.1698	2.17
"	100	6400	250	.3866	2.4163	2.42

Precipitation of the proteids dissolved in HCl.

In #1265 a portion of the extract was exactly neutralized with N/10 NaOH, then boiled down to a small volume, and the precipitate formed filtered off. The filtrate was neutralized again, boiled and filtered. This was continued until no further precipitate re-

sulted. The filtrate was made up to a definite volume and the nitrogen determined in eliquot portions, and the results are given in table #31.

In nos. 1266 and 1267 the extracts were treated with N/10 NaOH until a turbidity began to appear. At this stage the solution was still distinctly acid which indicates that the acid salt of the proteid is less soluble than the neutral or alkaline compounds, as no precipitates occured with the solutions in those conditions. The solutions coagulated well when the proper quantity of acid was present. The precipitates were filtered off, and the filtrates treated again with more acid or with more alkali as necessary in order to effect further precipitation. The results are given in:-

Table no. 30.

Proteids in HCl extract precipitated in neutral or slightly acid solution.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. N in total extract.	Wt. total proteid in HCl ext.	% total prot. ppted by neut. or acid.
1265	100	6700	500	.2961	1.8506	1.85
"	50	5000	500	.1380	.8625	1.73
1266	100	6000	500	.2832	1.7700	1.77
"	100	6100	500	.3953	2.4706	2.47
1267	100	6600	500	.1993	1.2456	1.25
"	100	6400	500	.1690	1.0562	1.06

Table no. 31.

Proteids in HCl extract not precipitated by neutralization.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of tract taken.	Wt. N in total extract.	Wt. proteid in HCl extract.	% proteid not ppted by neut.
1265	100	6700	500	.0395	.2467	.25
"	50	5000	500	.0326	.2039	.40

Took the filtrate from the precipitation of the proteids by neutralization, or by making slightly acid, added 2 to 3 drops of 1-3 H_2SO_4 and then saturated with $ZnSO_4$. Treated the precipitate as usual. Results are given in:-

Table no. 32.

Precipitate formed by $ZnSO_4$ in the HCl extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. solution taken.	Wt. total N ppted by $ZuSO_4$.	Wt. total proteid ppted by $ZnSO_4$.	% total proteid ppted by $ZnSO_4$.
1265	100	6700	500	.0100	.0628	.06
"	50	5000	500	.0035	.0219	.04
1266	100	6000	500	.0708	.4425	.44
"	100	6100	500	.0586	.3663	.37
1267	100	6600	500	.1096	.6850	.69
"	100	6400	500	.1562	.9763	.98

The filtrate from the $ZnSO_4$ precipitation was tested for peptones by method which has already been described. The results are given in:-

Table no. 33.

Ppt formed by Br in HCl extract.

Lab. no.	Wt. of sample.	Vol. of total solution.	Vol. solution taken.	Wt. total N ppted by Br.	Wt. total prot. ppted by Br.	% total prot. ppted by Br.
1265	100	6700	500	.0107	.0669	.07
"	50	5000	500	———	———	———
1266	100	6000	500	.0108	.0675	.07
"	100	6100	500	.0177	.1106	.11
1267	100	6600	500	.0013	.0081	.01
"	100	6400	500	.0128	.0800	.08

In #1265 the filtrate from the Br precipitation was further studied with Almen's tannin reagent and phosphotungstic acid. In the other cases these treatments were omitted.

Table no. 34.

Ppt by Almen's tannin reagent in HCl extract.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. total N in Almen's ppt.	Wt. total prot. ppted by tannin.	% proteid ppted by tannin reagent.
1265	100	6700	500	.0020	.0125	.02
"	50	5000	500	.0020	.0125	.02

Table no. 34a.

Ppt by phosphotungstic acid in HCl extract.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. total N in phosphotungstic.	Wt. prot. in phosphotungstic.	% prot. in phosphotungstic ppt.
1265	100	6700	500	.0020	.0125	.01
"	50	5000	500	.0020	.0125	.02

In #1265 the filtrate from the precipitate by phosphotungstic acid was made up to a definite volume and the nitrogen was determined in eliquot parts. In the other samples the filtrate from the Br precipitate was so used. All of these results are given in the table which follows.

Table no. 35.

Proteids in HCl extract not ppted by the reagents used.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. total N not ppted.	Wt. total prot. not ppted.	% total prot. not ppted.
1265	100	6700	500	.0088	.0550	.06
"	50	5000	500	.0076	.0475	.09
1266	100	6000	500	____	____	____
"	100	6100	500	.0152	.1094	.11
1267	100	6600	500	.0132	.0825	.08
"	100	6400	500	.0320	.2000	.20

This practically completes the work which we did in studying the proteids soluble in .15% HCl.

Liebig[118] showed that when muscle is placed in dilute HCl - (.1%)

the greater part of the proteid matter is dissolved, to be reprecipitated when the solution is neutralized, as he claimed. As a result of our work we are inclined to believe that the amount of proteid matter soluble in HCl depends very largely upon the strength of the acid used. A slight increase in strength increases the amount soluble very considerably. With the strength of acid used in our experiments it was found that the percentage of proteid which went into solution decreased with each subsequent treatment, but yet with the number of extractions made we found a small amount continually going into solution.

The proteid dissolved by the HCl was found to be best precipitated in slightly acid solution as was the case with that dissolved by the KOH. We were however far from being able to obtain a complete precipitation by this means, as reference to the table would show. The above facts would either indicate the decomposition of the original proteid, or else the presence of more than one proteid soluble in HCl acid.

Extraction with .15% KOH.

The residue of the meat insoluble in .15% HCl was washed free from HCl and then treated with .15% KOH. The parchment filters gave the best results although even here the filtering was very slow. A sort of a sticky, gelatinous precipitate resulted which tended to clog up the filter. By frequently changing the filter, the filtration was finally all accomplished. The cooked meat swelled up very considerably by this treatment.

In #1265 the end of the extraction was determined by the

Biuret and the K_4FeCN_6 tests. Subsequent work showed these tests to be insufficiently delicate for very accurate work. In the other cases the N was determined in a portion of a filtrate and in that way very satisfactory results could be obtained. The completeness of the extraction is shown in the following table:-

Table no. 36.

Proteid matter in last extraction with KOH.

Lab. no.	Wt. of sample.	No. of extract-ions made.	Vol. of last ex-traction.	Wt. N in last ex-traction.	Wt. proteid in last ex-traction.	% prot. in last extraction.
1265	150	9	2000	not determined accurately.		
1266	100	16	400	.0123	.0771	.08
"	100	16	400	.0038	.0240	.02
1267	100	17	500	.0240	.1505	.15
"	100	17	500	.0394	.2468	.25

It is seen by reference to the above table that the proteid matter soluble in the last extract of cooked meat was rather high. At the end of the fourteenth extraction the nitrogen was determined in the extract and the proteid thus found amounted in one case to practically none and in the second case to about .04%. At this time our solution of KOH became used up and a new lot was prepared. This lot was just a trifle stronger than the other, and this fact accounts for the additional amount of proteid dissolved. This shows further how dependent the amount of soluble proteid is upon the strength of the solution used.

The wash water was all collected, and portions tested for nitrogen. In #1265 no nitrogen was found. In the other cases the

proceedure was considerably different. The meat in all the lots had swelled up so much that it would have been impossible to have washed it free of KOH before the mixture would spoil. Phenolpthalein was then added to the watery mass and HCl run in until neutral. This of course formed dilute KCl solution, but as the meat had once been extracted with salt solution, it was assumed that this could bring about but little further change. It was with the meat in this condition that the extraction with the hot water was carried on.

The various extractions were properly corrected for volume and then well mixed. The analysis was carried on as will now follow. An attempt was made to determine the total solids and the ash in the extract but this was soon abandoned on account of the alkali. Even such a dilute solution as that used would be found to bring about decompositions of the proteids when heated to 100. The alkali was neutralized with HCl, and then heated. It was found even after repeated treatments of this kind that the solution kept becoming alkaline again.

Table no. 37.

Total nitrogen in KOH extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. extract taken.	Wt. total N in extract.	Wt. total proteid in extract.	% total proteid in extract.
1265	150	16000	500	.8736	5.4600	3.64
"	150	16000	500	.8576	5.3600	3.57
1266	100	7100	500	.3493	2.1031	2.10
"	100	6600	500	.3524	2.2025	2.20
1267	100	115 0	500	.6785	4.2407	4.24
"	100	11200	500	.8692	5.4322	5.43

Precipitation of the proteids in the KOH extract.

The proteids in the KOH extract were precipitated by making the solution distinctly acid, with acetic or with HCl acid. It was found that no precipitate occured when the solution was either neutral, or acid beyond a certain point. Some experimenting is necessary in order to get the solution of just the proper strength. The results obtained are given in:-

Table no. 38.

Proteids in KOH extract precipitated in slightly acid solution.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. extract taken.	Wt. total N in extract.	Wt. total proteid in extract.	% proteid in extract.
1265	150	16000	1000	.3297	2.0606	1.37
"	"	16000	1000	.3808	2.3800	1.58
1266	100	7100	500	.3351	2.0945	2.10
"	100	6600	500	.3445	2.1533	2.15
1267	100	11500	500	.4968	3.1050	3.11
"	100	11200	500	.5869	3.6680	3.67

Table no. 39.

$ZnSO_4$ precipitate in KOH extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. extract used.	Wt. total N in $ZnSO_4$ ppt.	Wt. total proteid ppted by $ZnSO_4$.	% total proteid ppted by $ZnSO_4$
1265	150	16000	1000	.1776	1.1100	.74
"	150	"	1000	.1600	1.0000	.67
1266	100	7100	500	.0383	.2394	.24
"	100	6600	500	.0409	.2556	.26
1267	100	11500	500	.1012	.6325	.63
"	100	11200	500	.1770	1.1063	1.11

Table no. 40

Br precipitated in KOH extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. extract taken.	Wt. total N in extract.	Wt. total proteid in extract.	% proteid in extract.
1265	150	16000	1000	.1536	.9600	.64
"	"	16000	1000	.1296	.8100	.54
1266	100	7100	500	.0156	.0975	.10
"	100	6600	500	.0119	.0744	.07
1267	100	11500	500	.0138	.0863	.09
"	100	11200	500	.0113	.0706	.07

In #1265 the filtrate from the Br precipitate was treated with Almen's reagent and with phosphotungstic acid respectively. In the other cases this treatment was omitted, and the nitrogen remaining in solution in the Br filtrate was determined. In #1265 the nit-

rogen in the filtrate from the phosphotungstic acid was estimated.

Table no. 41.

Ppt by Almen's tannin reagent in the KOH extract.

Lab. no.	Wt. of sample.	Vol. total extract.	Vol. extract taken.	Wt. total N in ppt.	Wt. total prot. ppted by tannin.	% total proteid ppted by proteid.
1265	150	16000	1000	.0080	.0500	.03
"	"	16000	1000	.0064	.0400	.03

Table no. 42.

Ppt by phosphotungstic acid in KOH extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. solution taken.	Wt. total N ppted by phosphotungstic.	Wt. total prot. ppted by phos.	% total proteid ppted by phospho.
1265	150	16000	1000	.0048	.0300	.02
"	"	16000	1000	.0048	.0300	.02

Table no. 43.

Proteids in KOH extract not precipitated by reagents used.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. solution taken.	Wt. total N not ppted by reagents.	Wt. total proteid not ppted by reagents.	% total proteids not ppted by reagents.
1265	150	16000	1000	.1120	.7000	.47
"	150	16000	1000	.1080	.6750	.45
1266	100	7100	500	.0563	.3522	.35
"	100	6600	500	.0732	.4575	.46
1267	100	11500	500	.0886	.5440	.55
"	100	11200	500	.1617	1.0111	1.01

These results tend to show perhaps more clearly than any others, the fact that the methods employed did not effect the complete precipitation of the proteids dissolved in the KOH. In all cases a considerable amount of proteid matter remained in solution after all the different treatments. In some instances these amounts were relatively large representing about one fifth of the total proteid matter in solution.

Extraction with hot water.

The residue of the meat insoluble in all reagents used up to this stage was prepared for the extraction with hot water as has already been described. In #1265 the extraction was comparatively easy as more of the meat had been dissolved by other reagents. #1266 dissolved fairly well in the hot water but #1267 was so bulky as to be almost unmanageable. A considerable amount however went into solution. In all cases the residue insoluble in hot water was dried and the nitrogen in it estimated. The end of the extraction was determined by the usual method. In both raw meats the extraction was made practically complete, while with the cooked meat a large amount remained undissolved.

The usual volume corrections were made, and all filtrates were mixed as usual. The extracts were then analysed as rapidly as practicable. Along with the analyses of regular samples, there was made the analysis of the broth obtained by cooking #1267. The results of this analysis will be given along with the others. The analysis was made entirely with the clearly filtered broth.

Total solids and ash in hot water extract.

In the hot water extract from #1265 and in the clearly filtered broth from #1267 the total solids and the ash were determined by the usual methods. In the other cases these determinations were omitted on account of the method used in getting rid of the alkali. The KCl formed would have increased the ash content, and hence such an analysis would have had no value.

Table no. 44.

Total solids and ash in hot water extract.

Lab. no.	Wt. of sample.	Vol. of extract.	Wt. of total solids in ext.	% of total solids in	Wt. of ash in extract.	% of ash in extract.
1265	150	1500	.3798	.25	.2248	.15
"	"	1500	.3643	.24	.2170	.14
1267	984.56	1700	27.8256	2.83	6.8204	.69
"	"	1700	27.8513	2.83	6.7099	.68

Table no. 45.

Total N in hot water extract and in broth from #1267.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. of solution used.	Total N in hot water extract.	Wt. proteid in hot water extract.	% proteid in hot water extract.
1265	150	1550	200	.0310	.1938	.13
"	150	1550	200	.0310	.1938	.13
1266	100	5900	500	.1535	.9574	.97
"	100	4300	500	.1114	.6960	.70
1267	100	10400	500	.6323	3.9520	3.95
"	100	7200	500	1.3650	8.5184	8.52
1267Br.	984.56	1700	100	2.8339	———	———
"	984.56	1700	100	2.8526	———	———

Proteids in the hot water extract.

The hot water extracts were found to be neutral to phenolphthalein. Lots were measured out, treated with about 2 c.c. of N/10 acetic, boiled down to small volumes, filtered, washed and the nitrogen determined in the precipitate. The results thus obtained are shown in:-

Table no. 45.

Proteids in hot water extract precipitated in slightly acid solution.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract taken.	Wt. total N in hot water extract.	Wt. total proteid in hot water extract.	% total proteid in hot water extract.
1265	150	1550	100	.0124	.0775	.05
"	150	1550	100	.0124	.0775	.05
1266	100	5900	500	.0597	.3731	.37
"	100	4300	500	.0437	.2732	.27
1267	100	10400	500	.4181	2.6131	2.61
"	100	7200	500	1.0109	6.3181	6.32

Proteoses and gelatin in the hot water extract.

The filtrate from the proteid precipitate of the hot water extract and also a sample of the clearly filtered broth from #1267 were tested for proteoses and gelatin. As one would naturally expect a rather large quantity of these constituents were found to be present. The results are given in :-

Table no. 46.

$ZnSO_4$ ppted in hot water extracts and in the clearly filtered broth.

Lab. no.	Wt. of sample.	Vol. total extract.	Vol. of extract used.	Total N in $ZnSO_4$ ppt.	Total proteid ppted by $ZnSO_4$.	% proteid ppted by $ZnSO_4$.
1265	150	1550	100	———	———	———
"	150	1550	100	.0031	.0193	.01
1266	100	5900	500	.0414	.2588	.26
"	100	4300	500	.0189	.1181	.12
1267	100	10400	500	.1830	1.1438	1.14
"	100	7200	500	.3370	2.1063	2.11
Broth 1267	984.56	1700	100	.2924	1.8275	.18
"	984.56	1700	100	.2703	1.6894	.17

Table no. 47.

Br ppt in hot water extract, and in broth from #1267.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract used.	Total N in hot water extract ppted by Br.	Total prot. ppted by Br.	% total prot. ppted by Br.
1265	150	1550	250	.0019	.0019	.01
"	150	1550	250	.0019	.0019	.01
1266	100	5900	500	.0106	.0662	.07
"	100	4300	500	.0120	.0750	.08
1267	100	10400	500	.0291	.1819	.18
"	100	7200	500	.0245	.1531	.15
Broth 1267	984.56	1700	100	.0952	.5950	.06
"	984.56	1700	100	.0646	.4038	.04

Table no. 48.

Ppt by Almen's tannin reagent in hot water extract.

Lab. no.	Wt. of sample.	Vol. total solution.	Vol. of extract used.	Total N ppted by tannin reagent.	Total prot. ppted by tannin.	% total proteid ppted by tannin.
1265	150	1550	250	.0019	.0019	.01
"	150	1550	250	.0013	.0081	.01

In the #1265 there was no precipitate produced by phosphotungstic acid.

Table no. 49.

Broth analysis. Ppt formed in filtrate from Br ppt. After boiling off excess of Br and then allowing solution to stand.

Lab. no.	Wt. of sample.	Vol. of total solution.	Vol. of solution used.	Total N in ppt.	Total proteid in ppt.	% proteid ppted after Br.
1267	984.56	1700	100	.0323	.2019	.02
"	"	"	100	.0323	.2019	.02

The filtrate fro the above precipitate was made up to a definite volume and the nitrogen determined in eliquot parts. In 1265 the filtrate from the treatment with phosphotungstic acid wa likewise tested for nitrogen, while in #1266 and #1267 the filtrate from the precipitate by Br was used for this purpose. All of the results obtained in this way are given in the table which follows:-

Table no. 50.

Nitrogen remaining in solution in hot water extract and in the broth after treatments indicated.

Lab. no.	Wt. of sample.	Vol. of total extract.	Vol. of extract used.	Total N not precipitated.	Total prot not ppted.	% proteid not ppted.
1265	150	1550	250	.0087	.0543	.04
"	150	1550	"	.0078	.0485	.03
1266	100	5900	500	.0063	.0391	.04
"	100	4300	500	.0066	.0414	.04
1267	100	10400	500	.0220	.1375	.14
"	100	7200	500	.0104	.0650	.07
Broth 1267	984.56	1700	100	2.3928	_____	___
"	984.56	1700	100	2.2908	_____	____

Table no. 51.

Proteid in broth ppted by making the solution neutral or slightly alkaline.

Lab. no.	Wt. of sample.	Vol. total extract.	c.c. sol. taken.	Total N ppted in neut. or in alkaline sol.	Total prot. in broth ppted in neut.	% prot. ppted in neut. sol.
1267	984.56	1700	100	.0170	.1063	.02
"	"	1700	100	.0272	.1700	.02

Table no. 52.

Proteid ppted by H_2SO_4 in broth after filtering off the ppt formed in the neutral solution.

Lab. no.	Wt. of sample.	Vol. of total solution.	Vol. of solution used.	Total N in ppt by H_2SO_4.	Total prot. ppted by H_2SO_4.	% proteid ppted by H_2SO_4.
1267	984.56	1700	100	.0187	.1169	.02
"	"	"	"	.0170	.1063	.02

Analysis of the undissolved residues.

The residues from the different lots of meat which had up to this time remained undissolved, were now air dried on the water bath, and then in the case of the cooked meat in the water oven. The residues from #1265 was extracted with ether to remove the fat, and then the nitrogen in it wa determined. In #1266 the residue was Kjeldahled without previous extraction with ether. The residue from #1267 was so bulky that after drying the total amount of it was weighed and then a portion taken for the nitrogen determination. The table which follows shows the amount of the undissolved proteid matter.

Table no. 53.

Residues of proteids from meat insoluble in all the reagents used.

Lab. no.	Wt. of sample.	Wt. of air dried residue.	% of N in air dried residue.	Wt. N undissolved.	Wt. proteid undissolved.	% proteid in fresh meat undis.
1265	150	3.54	____	.1695	1.0591	.71
1266	100	____	____	.2135	1.3341	1.33
"	100	____	____	.2156	1.3473	1.35
1267	100	24.40	14.21	3.4672	21.6770	21.68
"	100	18.43	13.64	2.5039	15.7024	15.70

By comparing the results in table no. 53 with those in table no. 2 we find that the percentage of total proteids dissolved by the various treatments is as follows:- #1265 = 96.44%, #1266 = 94.06%, and #1267 = 50.42%. These figures indicate the relatively easier solubility of raw meats than of cooked meats and in a measure throw added light upon the differences in their digestibility.

In the following tables will give a summary of all the important results already recorded for purposes of more ready comparison. The first table contains the record of the total nitrogen found in all the different extracts and residues.

Table no. 54.

Total nitrogen in meats studied.

Portions containing nitrogen	100 grs. #1265	100 grs. #1266	100 grs. #1267
Total nitrogen in cold water extract	.7351	.9175	.3656
" " " 10% NaCl "	.7287	1.1101	.0886
" " " 15% HCl "	.3766	.3788	.3669
" " " 15% KOH "	.5771	.3509	.7739
" " " hot water "	.0203	.1325	.9987
" " " undissolved residue	.1130	.2171	2.9851
Total	2.6670	3.1069	5.5788
Total nitrogen found direct	3.1900	3.6100	6.0300

There is a very large and rather uniform discrepency between the total nitrogen found direct and that found by summing the various fractions. The difference is probably due to the fact that in arriving at the nitrogen in the total froctions, large factors had to be used. The total volume of all the extracts amounted to from 30 to 40 liters.

Table no. 54a.

Results of analysis of cold water extract.

	Per cent.		
	#1265	#1266	#1267
Proteids ppted in neutral or in slightly alk. sol.	2.18	2.57	.05
" " " H_2SO_4 acid solution.....	____	.26	____
" " by $ZnSO_4$.....	.09	.24	.18
" " " Br.....	.03	.03	.10
" " " Almen's tannin reagent...	.02	____	____
" " " phosphotungstic acid.....	.03		
Nitrogen record.....	Grams		
N ppted in neutral or in slightly alk. sol.	.3485	.4040	.0030
N " " " slightly acid solution.....	____	.0414	____
N " by $ZnSO_4$.....	.0126	.0387	.0190
N " " Br.....	.0043	.0053	.0107
N " " tannin and phospho.....	.0031	____	____
N " filt. from phos. or Br pptes.....	.3290	.3157	.2794
Total.....	.6975	.8051	.3161
Total nitrogen found direct.....	.7351	.9175	.3656

Table no. 55.

Summary of analysis of the HCl acid extract.

	Per cents		
	#1265	#1266	#1267
Proteids ppted in slightly acid solution	1.79	2.12	1.16
" " by $ZnSO_4$	.05	.41	.84
" " " Br	.04	.09	.05
" ppted by Almen's tannin reagent	.03	___	___
" " " phosphotungstic acid	.02	___	___
Total proteids precipitated	1.91	2.62	2.05
Nitrogen record	Grams		
N ppted in slightly acid solution	.2861	.3393	.1842
N " by $ZnSO_4$	.0085	.0647	.1329
N " " Br	.0107	.0143	.0072
N " " phosphotungstic acid	.0020	___	___
N " " Almen's tannin reagent	.0027	___	___
N in filt. from phosp. or Br ppt	.0082	.0152	.0226
Total	.3182	.4335	.3469
Total nitrogen found direct	.3766	.5788	.3669

Table no. 56.

Summary of analysis of NaCl extract.

	Per cent.		
	1265	1266	1267
Proteids ppted by heat coag. in neutral	3.15	4.39	.08
" " " acid	.64	.85	.05
" " " $ZnSO_4$	.06	.12	.08
" " " Br	.09	.08	.04
" remaining in solution	.28	1.11	.69
Nitrogen record	**Grams**		
N ppted by heat coag.	.5033	.7015	.0120
N " " H_2SO_4	.1029	.1355	.0075
N " " $ZnSO_4$	.0110	.0189	.0113
N " " Br	.0138	.0125	.0068
N not ppted by reagents used	.0451	.1776	.1094
Total	.6761	1.1460	.1468
Total nitrogen found direct	.7287	1.1101	.0886

Table no. 57.

Summary of analysis of KOH extract.

	Per cent		
	1265	1266	1267
Proteids ppted in acid solution	1.43	2.12	3.39
" " by $ZnSO_4$	.71	.25	.87
" " " Br	.59	.09	.08
" " Almen's reagent and phospho.	.05	___	___
" not ppted by reagents used	.46	.41	.73
Nitrogen record		Grams	
N precipitated in acid solution	.2368	.3398	.5419
N " by $ZnSO_4$	.1688	.0396	.1391
N " by Br	.0944	.0178	.0126
N in filt. from Br ppt	.0733	.0648	.1257
N ppted by Almen's and phosphotungstic	.0080	____	____
Total =	.5813	.4580	.8193
Total found direct	.5771	.3507	.7739

Table no. 58. hot water.

Summary of analysis of extract.

	Per cent		
	1265	1266	1267
Proteids ppted by heat and acid	.05	.32	4.47
" " " $ZnSO_4$	.01	.19	1.63
" " " Br	.01	.08	.17
" ppted by Almen's and phosphotungstic	.03	____	____
" not ppted by reagents used	.04	.04	.10
	Grams		
Nitrogen record			
N ppted by heat and acid	.0083	.0517	.7145
N " " $ZnSO_4$	.0021	.0302	.2600
N " " Br	.0013	.0113	.0268
N "by Almen's and phosphotungstic	.0010	____	____
N not ppted by reagents used	.0055	.0065	.0162
Total	.0182	.0997	1.0175
Total found direct	.0203	.1325	.9987

In the next table all the results given are calculated upon the basis of the amount of proteid which no. 1265 contained. For example #1265 contained 19.96% proteid while reference to table no. 2 shows these amounts to be 32.56% for #1266 and 37.70% for no. 1267.

In the table are grouped together all like precipitates produced in the different extracts. All the albumins and globulins, and acid and alkali albumins are grouped together under one heading.

Table no. 59.

Summary of the results figured upon basis of per cent of proteid in #1265.

	In 100 grs fresh meat #1265.	In 88.47 grs fresh meat #1266.	In 52.94 grams fresh meat #1267.
	grams	grams	grams
Albumins and globulins	8.60	10.12	4.80
Proteids ppted by H_2SO_4	.64	.98	.02
" " " $ZnSO_4$	1.50	1.72	1.02
" " " Br	.75	.25	.14
" undissolved by reagents	.71	1.18	0.89
Nitrogenous matters not ppted	1.89	2.36	1.38

Discussion of methods.

Enough has perhaps already been said in the way of pointing out the application of the methods used. A little further can however be added to good advantage. The extraction with cold water can be made complete if proper precautions are taken. There are so many things which influence the solubility of proteids in NaCl that this method demands considerable further study before it can be safely recommended. The extractions by means of HCl and KOH are necessarily unsatisfactory because the amount of proteid which such solutions can dissolve depends so much upon their strength.

The methods used for precipitating the proteids are influenced by so many factors which if not carefully controlled, give erroneous results.

Conclusions.

The amount of work done warrants us in drawing some conclusions which seem fairly well established.

(1) The acidity of muscle extract increases with the coagulation of its proteids, thus confirming the work of Halliburton and others.

(2) The methods in common use for precipitating proteids need a great deal of further study, for finding out the best conditions under which they can be used.

(3) The cooking of meat renders its proteids very insoluble in all ordinary reagents, as compared with raw meat. The solubility of boiled meat in 10% NaCl is almost nil. The treatment of the meat with dilute KOH seemed to partly gelatinize it so that considerable amounts of it became soluble in hot water. The amount of proteoses and gelatin in cooked meat is higher than in raw meat. These facts throw added light upon the increased digestibility of raw meat, as compared with that of cooked meat.

(4) The larger part of the proteids of meat are composed of albumins and globulins.

(5) The number of different proteids in flesh is probably considerably higher than is commonly supposed.

III.

THE PROTEIDS IN THE COLD WATER EXTRACT, AND IN THE NaCl EXTRACT FROM MEAT.

In this portion of the study an attempt was made to separate the different proteid bodies in the cold water extract and in the 10% NaCl extract from raw meat.

For this purpose about 700 grams of lean meat, freed from all bone and visible fat was run two times through the sausage mill and then mixed thoroughly with about 400 c.c. of ice cold water in a large dish. The mixture was then filtered in the filter press using 4 thicknesses of muslin and fitting the sack well in the press.

The extracts thus obtained were well mixed and studied as soon as possible. The volume amounted to 1000 c.c.

Treatment of water extract with saturated solution of $(NH_4)_2SO_4$.

(1) 500 c.c. of the cold water extract was treated with an equal volume of sat. $(NH_4)_2SO_4$ solution. A large ppt resulted which was filtered off after standing over night. This was called ppt 1.

(2) The 1000 c.c. filtrate from ppt 1 was treated with 1000 c.c. of sat. $(NH_4)_2SO_4$ solution. A ppt resulted about equal in bulk to ppt 1. It was filtered off after standing over night, and called ppt 2.

(3) The 2000 c.c. of filtrate from ppt 2 was treated with 2000 c.c. of $(NH_4)_2SO_4$ solution. A ppt nearly as large as that obtained under ppt 2 was gotten here. This ppt contained all the coloring matter of the extract as the filtrate from it was colorless.

Marked ppt 3.

(4) The 4000 c.c. of filtrate from ppt 3 was completely saturated with solid $(NH_4)_2SO_4$ but no precipitate resulted upon standing. The solution was doubly saturated by the use of $MgSO_4$ but again no precipitate resulted.

Treatment of ppt 1.

Ppt 1 was transfered to a beaker and treated with 500c.c. of 10% $(NH_4)_2SO_4$ solution, thoroughly stirred, allowed to stand over night in the refrigerator and filtered. The larger part of the ppt remained undissolved. Residue and filtrate were marked - residue 1a and filtrate 1b respectively.

Residue 1a

Residue 1a was treated with a second portion of 10% $(NH_4)_2SO_4$ solution, allowed to stand over night and filtered. Still a large amount of ppt remained undissolved. It was labelled residue 1c and filtrate 1d.

Residue 1c.

Residue 1c was again treated with 10% $(NH_4)_2SO_4$ and allowed to stand over night. Filtered, and residue washed with 10% $(NH_4)_2SO_4$. The residue was marked residue 1e and the filtrate 1f.

Residue 1e.

Upon saturating filtrate 1f with $(NH_4)_2SO_4$, no ppt resulted, and hence it was assumed that nothing further had gone into solution by the last treatment of residue 1c.

Experiments were made to test the properties of residue 1e. It did not dissolve readily in .73% HCl. It dissolved quite readily in .15% KOH. After about an hour with frequent stirring, giving a slightly yellow solution. A little slimy matter remained after filtering but this was probably due to material from the meat which had gotten into the extract.

The filtered solution was treated with phenolphthalein and N/10 acetic acid added until just neutral. No ppt. resulted. More acid was added and finally the solution became turbid, and then gave a heavy precipitate which settled in flakes upon the bottom of the beaker. Filtered, and washed with water. Swelled but did not dissolve in .15% KOH nor in .73% HCl, nor in $(NH_4)_2SO_4$. It was light, greyish yellow before filtering, but was sticky when collected upon the filter. It began to decompose after standing about 12 hours at room temperature. The filtrate was marked 1j.

Filtrate 1j.

Filtrate 1j was neutralized with KOH, and a portion of it treated with $(NH_4)_2SO_4$ until saturated. A ppt resulted which showed that the acetic acid had not completely precipitate the proteids. Ppt was insoluble in 10%$(NH_4)_2SO_4$. On account of small amount nothing further was done with it.

Filtrate 1b.

Filtrate 1b was treated with an equal bulk of sat. $(NH_4)_2SO_4$. Turbidity was at once produced, and upon standing a large white flocculent ppt resulted. Filtered. Ppt was called residue 1g and the filtrate, filtrate 1b.

Residue 1g.

Residue 1g was treated with 10% $(NH_4)_2SO_4$, stirred well and allowed to stand over night. Ppt no longer all soluble in 10% $(NH_4)_2SO_4$. Filtered. Residue 1g'and filtrate 1h.

Residue 1g'.

Residue 1g' was treated with about 500 c.c. more 10% $(NH_4)_2SO_4$. Filtered,washed and labelled residue 1g". Filtrate marked filtrate 1d.

Residue 1g".

Residue 1g" was dissolved in .15% KOH, neutralized with acetic acid and saturated with $(NH_4)_2SO_4$. Upon filtering and testing the filtrate further, it was found that the proteids had been very completely ppted. Nothing further was done with the ppt obtained here.

Filtrates 1d and 1f.

These filtrates were saturated with $(NH_4)_2SO_4$, and after standing over night only a trace of the ppt resulted.

Filtrate 1b'.

Filtrate 1b' was saturated with $(NH_4)_2SO_4$, and allowed to stand for some time. A small ppt, similar in appearance to others obtained was gotten here.

Filtrate 1h.

This filtrate was mixed with equal bulk of sat. $(NH_4)_2SO_4$. Upon standing a large ppt formed. It dissolved again almost completely in 10% $(NH_4)_2SO_4$, from which solution it was dialysed. .0295 grams

was recovered and the ash in it determined. Found to contain .0009 grams or 3.05% of ash. The ash was colored somewhat red.

Ppt 2.

Ppt 2 was removed from filter, and treated in beaker with about 500 c.c. of 10% $(NH_4)_2SO_4$, and allowed to stand over night. Residue was marked residue 2a, and the filtrate 2b.

Residue 2a.

Residue 2a was again placed in beaker and treated with the 10% $(NH_4)_2SO_4$. Much still undissolved, which after filtering was marked residue 2c, and the filtrate, filtrate 2d.

Residue 2c.

Filtrate 2d gave but slight ppt when treated with equal bulk of sat. $(NH_4)_2SO_4$. Residue 2c was then studied with the idea of finding some way of purifying it. Dissolved best in .15% KOH but not as well as residue 1c. A little slimy material remained undissolved. Attempts were made to reprecipitate the proteid but it was found to act differently than residue 1c had done. No ppt either when neutral to phenolphthalein or when acid to acetic acid. All the solution containing it was made alkaline with KOH and then saturated with $(NH_4)_2SO_4$, and allowed to stand over night. Filtered. Ppt 2d; filtrate 2e.

Ppt 2d.

The ppt was transferred to a beaker, treated with 10% $(NH_4)_2SO_4$, stirred, filtered washed. The filtrate was saturated with

$(NH_4)_2SO_4$, and little more than a turbidity resulted. On account of the small amount of residue little further was done than to test its solubility. It was quite readily soluble in .15% KOH, and somewhat in 10% $(NH_4)_2SO_4$.

Filtrate 2e.

Filtrate 2e was made acid with acetic acid, and saturated with $(NH_4)_2SO_4$. A small ppt resulted, but all proteids were not ppted. It is probable that if the solution had been neutral all of the proteid would have been reprecipitated.

Filtrate 2b.

Filtrate 2b was treated with an equal bulk of sat. $(NH_4)_2SO_4$, and found to give a large precipitate . Filtered. Filtrate found to be free from proteid. The ppt was dried, weighed and the ash in it determined. Wt. of ppt = .1268 grams; Wt. of ash =.0029 grams, % of ash = 2.29.

Precipitate 3.

Ppt 3 was removed from filter with knife, treated in beaker with about 500 c.c. of 10% $(NH_4)_2SO_4$. Filtered. Residue 3a; filtrate 3b.

Residue 3a.

Residue 3a was transferred to a beaker and treated with more 10% $(NH_4)_2SO_4$. Residue was filtered off and filtrate treated with equal volume of saturated $(NH_4)_2SO_4$. No ppt resulted after standing over night. Residue 3a was found to dissolve rapidly and completely in .15% KOH. The solution thus produced was bright red, showing that

coloring matter had all redissolved. Solution was first made neutral and then distinctly acid to phenolphthalein but in neither case did a ppt result. Both of the acid and of the alkaline solution were saturated with $(NH_4)_2SO_4$ and after standing over night similar ppts were found in both cases. Ppt arose to the top of the liquid. All solutions derived from this precipitate were mixed and found to be acid, then were saturated with $(NH_4)_2SO_4$. Ppt was transferred to a beaker. Found not to dissolve in 10% $(NH_4)_2SO_4$; again readily soluble in .15% KOH. All ppts were redissolved in .15% KOH and reprecipitated in sat. $(NH_4)_2SO_4$. Ppt was filtered after having stood over night, transferred to dialysing tube, and dialysed in running water. Ppt was dried and the ash in it determined. Wt. ppt = .0266 grams; wt. of ash = .0060 grams; % ash = 22.55%. The ash was red.

Filtrate 3b.

Filtrate 3b was treated with equal volume of saturated $(NH_4)_2SO_4$ and allowed to stand over night. Filtered. No proteid in filtrate. Ppt was transferred to a beaker and treated with 10% $(NH_4)_2SO_4$. It dissolved almost completely. Upon reprecipitation there was not enough product to make a further study possible.

Treatment with $MgSO_4$.

250 c.c. of the original solution was treated with $MgSO_4$ until saturated. The precipitate which resulted was filtered off, and the filtrate marked Mb. The filtrate was treated with 500 c.c. of 10% $(NH_4)_2SO_4$, stirred well and allowed to stand over night. Filtered. Residue Mc and filtrate Md.

Residue Mc.

Residue Mc was treated with more 10% $(NH_4)_2SO_4$, stirred and filtered. A good deal more material dissolved at this stage. This was shown by saturating the solution with $(NH_4)_2SO_4$. The insoluble matter decomposed after a few days.

Filtrates Mb and Md.

Filtrates Mb and Md were each treated with equal bulk of sat. $(NH_4)_2SO_4$ and allowed to stand for some time. In the case of Mb a slight reddish brown ppt resulted, while in the case of Md a white flocculent ppt came down. These were not studied further.

Extraction with NaCl.

The residue of the meat after almost complete extraction with cold water was treated with 10% NaCl using about 500 c.c. for the first extraction. The NaCl solution was allowed to act upon the meat for some time and then filtered in the press through four thicknesses of cloth. The process was repeated until the filtrate contained about 2000 c.c. No attempt was made to determine if all of the NaCl soluble material had been removed.

Equal bulk of saturated $(NH_4)_2SO_4$ was added to the extract, and very large ppt resulted. It was filtered off and the filtrate saturated with $(NH_4)_2SO_4$. Only a small ppt resulted in this case, and nothing further was done with it.

A study was made of the solubility of the above large ppt. 10% NaCl and 10% $(NH_4)_2SO_4$ dissolved it slowly and in about the same quantity. After standing several days some still remained in solution.

.15% KOH dissolved but very little of it, while .33% HCl seemed to dissolve a good deal more. Water did not dissolve any appreciable amount.

The entire ppt was treated with 10% NaCl in different lots so that finally about 8000 to 9000 c.c. had been used. Yet over one half of the original ppt remained undissolved.

The filtrate was saturated with $(NH_4)_2SO_4$ and a white flocculent ppt resulted. This was filtered off, and the ppt treated with 10% $(NH_4)_2SO_4$. Only part of it redissolved. The filtrate from the insoluble part was saturated with $(NH_4)_2SO_4$, the resulting ppt filtered, washed water until nearly free from SO_4, dried and the ash in it determined. Wt. of ppt = .3407 grams; wt. of ash in ppt = .0015 grams; % ash = .44%. This body was nearly transculent when dry while the ash was colored red.

The part which did not redissolve in 10% $(NH_4)_2SO_4$ was washed with water, transferred to a platinum dish and dried. It was black and gluten like when dry. Wt. of this residue = 1.0012 grams; wt. of ash in it = .0094 grams; per cent of ash = .94%.

Conclusions, continued.

(6) The proteids in the water extract of beef are at least three in number. These can be separated from one another by the method of fractional precipitation by means of $(NH_4)_2SO_4$.

(7) The prolonged action of salts renders these proteid bodies insoluble in media in which they once readily dissolved.

(8) The proteid or proteids soluble in 10% NaCl are rendered especially easily, insoluble in 10% NaCl solution after having been in contact with one another for any length of time. We are inclined to believe that not only one but several proteids are present in a NaCl

extract of muscle. We found a large ppt coagulated by heat, and another not coagulated by heat but ppted by dilute acid.

In closing, I wish to express my sincere and grateful thanks to Dr. H.S. Grindley for all the help and advice which I have received from him in carrying out this study.

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