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THE DETERIORATION AND COMMERCIAL PRESERVATION OF FLESH FOODS.

First Paper: General Introduction and Experiments on Frozen Beef.

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The increasing complexity of civilization works in such a way that a constantly increasing proportion of the population is removed from the production and immediate control of its food supply. Therefore, since foods are in their nature perishable, means and methods for maintaining them in a condition suitable for consumption for a longer or shorter time must be devised to meet this condition. The preservation of foods has had to meet much adverse criticism, which attacked not only faulty methods but the very principle of preservation itself. The duty of the scientist lies clearly in the way, not of attacking the principle of food preservation, but of devising better and better methods for carrying out that principle.

The problems connected with the preservation of meats and other animal food products are among the most important in the domain of food chemistry. Meat is the most perishable of foodstuffs, yet as in the case of other foods, its production is becoming more and more localized, thus necessitating longer storage, and transportation to greater distances. In all the world there is but one large area which produces great numbers of high-grade cattle and hogs. This area is the corn-producing region of the United States and includes the states of Indiana, Illinois, Iowa, Missouri, Kansas and Nebraska. It is within the province of the many, if they so desire, to cultivate vegetable gardens, while it would be difficult, if not impossible, for them to raise their own large meat-producing animals. It is worthy of note that in spite of its perish-

ability, meat is transported as far and in as good condition as other foodstuffs. England derives a large part of her meat supply from the valleys of the Mississippi and the La Plata. That this is so is due to improved methods of preservation in storage and in transit, and also to rapid transportation itself.

The term deterioration as applied to flesh foods may be defined as the change or series of changes which the food undergoes with the result of rendering it more or less unsuitable for consumption. In interpreting the meaning of the word, consideration must be given not only to actual changes which may have occurred in the food product, but also to the purpose for which the food is intended and the habits and customs of the consumers. For example, desiccated beef may be said to have deteriorated physically in the process of preparation; it is not as pleasing to the eye as fresh meat and, owing to the loss of moisture, its texture is leathery; but when it is understood that the loss of moisture means less weight for a given quantity of food, that no chemical or bacterial change has occurred which would affect the nutritive value of the product, that the product will keep indefinitely and that by proper preparation excellent soups, stews or boiled beef may be made from the product, its value as a ration for armies in case of siege or on a forced march, or as a store for an exploring expedition, is evident. For the purpose intended the beef has not deteriorated.

Probably the criteria which have been made use of to the largest extent in judging the suitability of meat for food are those which may be grouped together under the term sense-criteria. These are the color and general appearance of the sample, its texture, its odor and its flavor. Of these, the odor is more frequently applied than the others. Disparagement of these criteria would be ill-advised, especially since the chemistry of the subject has been so little investigated. They have been eminently useful in the past and probably will be for a long time to come. Without entering into a discussion of the various chemical substances which contribute to the odor of meat in its fresh or aged condition, it may be said that personal tastes differ widely in their judgments of what meat is suitable for food and what is not. Some demand meat in the freshest possible condition whether it be derived from a domestic animal, from fish or fowl, while others insist not only on a preliminary "ripening" (the enzymic action which sets in after rigor mortis and which is accompanied by acid production in the previously neutral or alkaline meat of the larger animals) preferably at a temperature near the freezing point, but also upon a typical putrefactive decomposition in order to bring out the so-called "gamey" flavor. This process would make the food entirely unpalatable to persons of the first class. The majority of people prefer fish and eggs in a perfectly fresh condition, but there are others,

notably certain islanders, who eat fish by preference in a condition of incipient decomposition and others (the Chinese) who allow eggs to undergo a peculiar putrefactive decomposition in order to prepare from them certain foods. There are others who allow fish roes to undergo a peculiar anaerobic fermentation, whereby they acquire the flavor, in part, of old cheese. In this connection the very general consumption of such cheeses as Camembert, Limburger and Roquefort may be cited to show the desire for "high" flavors in nitrogenous foods. The most important questions arise out of these differences in taste, inasmuch as the health officer and the food chemist are frequently called upon to decide as to the fitness or unfitness for food purposes of various lots of fish, meats and fowls. What shall be the grounds of their decision? To what extent may chemical change progress before a given sample becomes unfit for food? Again, are the changes in composition indicated by the development of a disagreeable odor necessarily deeply seated, or are they superficial? What compounds produce the odors and to what extent are they produced at different periods of storage and under different conditions? It is eminently desirable to have at hand the means of answering these various questions and others of like nature, if possible, by chemical determinations, the more so as meats which have caused severe symptoms of poisoning are described as normal or at most not noticeably decomposed or abnormal.[1] However, in the majority of cases of meat poisoning on record, the meat was derived from sick and dying animals; further, in the majority of cases the meat was observed to be abnormal in one or more respects.

A judgment of the suitability or unsuitability of various classes of foods for human consumption in general must be based on: (1) the results of general observation and experience; (2) the results of special scientific experiment. The subjects of nutrition and dietetics are ones which demand statistical treatment, as do all subjects which concern large numbers of individuals and in which undetermined influences and idiosyncrasy play large parts. It is difficult in dietary studies conducted along scientific lines to make the experiments sufficiently extensive and inclusive and to choose subjects sufficiently representative. There is, indeed, a serious doubt whether these difficulties can be satisfactorily and completely overcome and therefore the facts of general experience must be regarded as having a great value. This view of the question requires emphasis, as there appears to be a tendency in some investi-

[1] Ostertag-Wilcox, *Handbook of Meat Inspection*, pp. 715, 723; Thresh and Porter, *Preservatives in Food and Food Examination*, Chapter 25; Kolle and Wasserman, *Handbuch der Pathogenen Mikroörganismen II*, 669; Flügge, *Die Mikroörganismen II*, p. 239; König, *Nahrungs und Genussmittel II*, p. 439; Vaughn and Novy, *Cellular Toxins*, pp. 208–10).

gators to disregard the conclusions reached from long experience and to consider only the results of limited dietary experiments, whose data, if not inconclusive, are capable of various interpretations. On the other hand, experience, since it results from uncontrolled cases, should as far as possible be supplemented and confirmed by well-controlled experiments.

Alteration may be considered to occur in flesh foods in three ways: (1) physically; (2) chemically; (3) biochemically.

The physical changes are those which affect the appearance, the structure of the tissue, macroscopically and microscopically, and, in general, changes noticeable by the senses which do not affect the composition or nutritive value.

The chemical and biochemical changes are those which affect the composition of the food, hence possibly the nutritive value (advantageously or otherwise) and which may produce alterations in odor and flavor. The chemical agents of deterioration in the case of flesh foods are water and oxygen; in the absence of the former—that is in desiccated flesh foods—the changes which occur in the lean portions, even after long storage at ordinary temperatures are infinitesimal. In the absence of water and oxygen—for instance desiccated meat *in vacuo*—flesh foods will keep indefinitely without change. In desiccated flesh foods, if oxygen is present, rancidity usually occurs after a shorter or longer period of storage; this change is entirely chemical in nature and does not require the presence of microörganisms. Practically, the changes which occur in normal flesh foods are due to bacteria or molds; that is, these microörganisms are the exciting causes of the changes which occur, and in their absence, even though water and oxygen are abundantly present, the changes will be slow, uncertain and inconsiderable. The facts stated above are the basis of the preparation and preservation of nutrient media for bacterial culture, and are matters of commonplace observation in every bacteriological laboratory.

A tabular view of deterioration in the fat as well as in the lean of meat may be presented in the following form. In this scheme the formation of bacterial toxins is not considered and this topic will be discussed later.

In the decomposition of flesh by microörganisms, the tendency is chiefly toward the formation of simpler compounds by hydrolysis, and if the various changes are able to reach the limit, on account of a sufficiently abundant supply of water (which, among other functions, serves to dilate and render ineffective inhibitory substances), and of oxygen, the final products will be principally three: namely—water, ammonia and carbon dioxide. The sulphur compounds will remain as inorganic sulphates and the phosphorus compounds as phosphates. While the tendency of bacterial decomposition of flesh is principally in the direction indicated,

CHEMICAL AND BIOCHEMICAL DETERIORATION OF FLESH FOODS.

Three Principal Causes of Influences.

Chemical

(1) Water

(2) Oxygen of Air.

Biological

(3) Microörganisms.

- **Fat**
  - Hydrolysis—active agent water—products fatty acids and glycerol.
  - Oxidation (rancidity) active agents water and oxygen; products lower fatty acids, aldehydes, etc.
- **Lean**
  - **Hydrolysis**: In the absence of microörganisms this occurs but slowly, if at all, at ordinary temperatures—rapidly at high temperatures, with production of gelatin and albumoses (proteoses). At ordinary temperatures enzymes may act as accelerating agents.
  - **Oxidation**: Occurs slowly at ordinary and moderately high temperatures, in presence of oxygen of air, even in absence of microörganisms.
  - **Decomposition by microörganisms.** Hydrolysis chief chemical action. In presence of oxygen oxidation plays a part, the sulphur compounds being affected, and much CO_2 being evolved.
    - Putrefaction (anaerobic)
    - Decay (aerobic)
    - Moldering (aerobic by moulds).

Putrefaction produces reduction products of foul odors (NH_3, H_2S, amides, skatole acids). Decay produces products with scarcely any pronounced disagreeable odor (no H_2S). Moldering produces the typical odor of "moldiness," possibly due to acid amides.

at the same time the bacterial cells produce certain complex substances synthetically out of simpler materials. Thus bacteria can be cultivated upon nutrient media free of proteins and containing only minimal quantities of mineral salts, ammonia and carbon dioxide. Uschinsky grew various bacteria in solutions containing only glycerol, sodium chloride, calcium chloride, magnesium sulphate, potassium phosphate, ammonium lactate and sodium asparaginate. Voges and Fränkel[1] recommend a solution containing sodium chloride, sodium phosphate, ammonium lactate, and asparagine. Pasteur, Cohn, Nageli, Maasen and Proskauer and Beck have all proposed and used non-protein media.[2] Uschinsky grew the diphtheria bacillus with production of toxin upon his medium, and Proskauer and Beck cultivated *B. tuberculosis* upon theirs. This work indicates that toxins are synthetic substances. It indicates also

[1] *Hyg. Rundschau*, 1904, No. 17,769.

[2] Kolle und Wassermann, *Pathogenen Mikroorganismen*, *I*, p. 440.

that bacteria have the power to, and possibly do as a general rule, build up the entire protein cell contents and structure out of simple products. In other words, even in protein media it may be that analytic and degradation processes necessarily precede the upbuilding of the bacterial protoplasm and the products of its activity.

In the bacterial decomposition of flesh, besides the chemical changes noted, there will be parallel and progressive changes in the structure of the tissues, which will affect the texture and the macroscopic and microscopic appearance of it. The extent and rapidity of these changes, as well as the extent and rapidity of the chemical changes due to bacterial growth, will depend upon three factors: the structure of the meat, the nature of the meat, and the temperature. Bacterial growth proceeds easiest along surfaces. Colonies within solid media do not attain the size of surface colonies, except in those cases where liquefaction of the media proceeds somewhat rapidly, and this results in the production of larger surfaces. Because of this physical difficulty of growth, deterioration does not proceed rapidly in muscular tissue which is firm and continuous; the meats most liable to chemical change are those containing a bone, those much subdivided by connective tissue or those which have been comminuted by cutting, hashing or grinding. In each of these three cases there are bounding surfaces within the mass and these surfaces offer to microörganisms avenues of ingress and opportunity for rapid extension. The larger pieces of flesh of fairly uniform gross structure may begin to decay or mold on the surface rather quickly, if the temperature of storage is suitable, but the progress of interior decomposition is slow.

There are, at the present time, some differences of opinion as to how the different substances produced during bacterial growth and noxious to the human body when ingested with the food or injected into the circulation, should be viewed and classified. It is difficult to draw a clear distinction between the substances produced from the flesh itself and those elaborated within the living cell, although both are the result of bacterial growth, the more so as the soluble constituents of flesh may enter the bacterial cell and there be acted upon by insoluble endoenzymes.[1] The former result from a degradation process essentially analytic in character, consisting in the formation of relatively simple substances from more complex ones, while the formation of toxins is essentially synthetic.[2] Thus, as stated previously, toxins can be produced from simple compounds. Toxins appear to be sometimes present in foods in which de-

[1] Vinson, "*The Endo- and Ektoinvertase of the Date*," THIS JOURNAL, 30, 1013.

[2] Lehman and Neumann, *Bacteriology, Vol. II*, pp. 33–34; Muir and Ritchie, *Bacteriology*, pp. 177, 368; Kolle und Wassermann, *Pathogenen-Microörganismen*, pp. 344, *et seq.*

terioration is otherwise so slight as not to be noticeable, and on the other hand, deterioration may have progressed far without the formation of any substance definitely toxic. Further, in meat wherein bacterial decomposition is progressing uniformly, toxins may be absent in the beginning, present a little later and absent later still.[1] There seems to be, therefore, no necessary relationship between ordinary bacterial deterioration of flesh and toxicity.

The changes produced in flesh foods by bacteria, outside the bacterial cell, are according to the current view due to the action of enzymes secreted by the cell, and follow in greater or lesser degree the changes worked in foods by the digestive ferments secreted in the digestive tract. The bacterial enzymes, however, usually produce greater changes in proteins than do the digestive ferments. The enzyme, which commonly liquefies gelatin in cultures of bacteria, is not unlike, in its first action, the proteolytic enzyme of the pancreatic juice. In the first instance, both these enzymes when in contact with insoluble proteins (and water) convert the proteins into soluble and simpler compounds. If the action goes further still, as it does in the case of bacterial fermentations, simpler compounds are formed and finally acid amides, amino acids and amines. Now among these relatively simple basic bodies there are some which Selmi named the ptomains and of these, some are very poisonous, and cases of meat poisoning from eating putrid meat have probably been due to them. Some of them are not decomposed by boiling water but retain their toxicity even after boiling for some time, and this property distinguishes them from the bacterial toxins, none of which withstands a boiling temperature. The toxins proper (ectotoxins) appear to be elaborated within the bacterial cell and are excreted into the surrounding medium. Thus they are both soluble and diffusible. Contrasted with these are the so-called endotoxins, which are elaborated within the cell and remain therein. Thus, three classes of poisons, all of bacterial origin, must be considered in connection with meat poisoning.

The immense amount of work which has been carried out in the last ten or fifteen years upon the true toxins, has afforded means of defining and classifying them with a fair degree of precision, although their chemical nature is still unknown. They are characterized first by their extreme poisonousness and by their specificity, both as regards the disease symptoms produced and also the susceptible species or individuals; they are soluble in water, extremely labile, chemically and physically, and quite distinct both from ptomains and proteins; the bacterial toxins are synthetic products of the bacterial cells, and in the living body of

[1] Thresh and Porter, *Preservatives in Food*, pp. 308–312; Vaughn and Novy, *Cellular Toxins*, p. 206.

a susceptible individual they are able to produce specific antitoxins; in their poisonous properties they are closely related to the snake venoms, to ricin, abrin and crotin and to the poisons of the blood of the eels and muraenae. In a broader classification, they are linked by their chemical lability, their extreme activity catalytically, by the manner of their formation, by their ability to excite the production of antibodies, and by their inactivity at low temperatures and their destruction in the moist condition considerably below the boiling point of water, with the hydrolytic enzymes. Like these, too, in their resistance to higher temperatures in the dry condition, in the presence of certain salts and of amyl alcohol.

Although bacterial toxins are synthetic products, resulting from the activity of bacterial cells, this fact does not preclude the nature of the culture medium and the external physical conditions from having a great influence upon their production. The production of toxins at low temperatures has not been sufficiently investigated. The ordinary pathogenic varieties of bacteria whose toxins have been most studied have their optimum growth at or near 38°. Below 10° growth in the majority of cases stops, or at most is extremely slow. Therefore toxin production below 10°, by most pathogenic bacteria, must be a negligible quantity. The best known example of the class of pathogenic saprophytes of van Ermengem is the *B. botulinus* isolated by this investigator in 1895 from a ham which had caused botulism at Ellezelles. *Bacillus botulinus* is an obligate anaerobe which grows well at 18–25° but only sparingly at 35–37.5°. Six per cent. of common salt in the culture medium prevents growth. In cases of illness caused by this organism, the toxin appears to be formed previous to the ingestion of the food. The microbe develops but sparingly within the body. Presumably growth and toxin formation, if any, would proceed very slowly at the temperatures used for meat storage (below 2–4°).

In the literature there appear to be described three kinds of cases of meat poisoning, occasioned by bacteria or their products, in which the symptoms may be serious or fatal; first those due to specific bacteria which existed and occasioned disease in the animal before slaughter. Such an organism is the *B. enteritidis* of Gaertner. These cases have in times past occurred not infrequently in Germany (consult Ostertag) following cases of "emergency" slaughter of diseased and dying animals. It seems almost incredible that such meat should be eaten at all, even by a half starved population, and doubly incredible that it should be eaten without adequate preliminary cooking. Second, those due to bacteria which develop in the meat after slaughter and produce toxins therein and which are incapable of growth in the human body. Such an organism is the *B. botulinus* of van Ermengem previously mentioned, which belongs to the class named by him pathogenic saprophytes. Third,

there are those cases which in all probability are caused by the ordinary bacteria of putrefaction, not necessarily the Proteus varieties, but many kinds which under suitable conditions may develop in meat. These cases are probably not due to the action of specific organisms and may or may not be due to toxins, but in all likelihood are caused by protein degradation products, the toxicity of which may remain after they have been subjected to a boiling temperature. An effective system of ante-mortem and post-mortem meat inspection, such as obtains in large establishments in this country, obviates any danger from the first source—that is, from bacteria which have existed and caused disease in the animal before slaughter. The third source of danger can be entirely obviated by the careful buyer of meats; if decomposition has proceeded far enough to produce poisonous ptomains, the fact will be evident to the senses. The second variety of meat poisoning, which in the past has resulted only from consumption of cured hams and sausage, can be guarded against only by care in the handling, curing, transportation and storage of the meats up to the time they reach the consumer.

As stated, microörganisms and principally bacteria are the chief exciting cause in the deterioration of flesh foods; and it therefore follows that any means which destroys bacteria or lowers their vitality is, in degree, a means of preserving food and lessening deterioration. The various means which are used for this purpose follow:

(1) Low temperatures (freezing stops bacterial growth and activity entirely).

(2) Heat sterilization, absolute or partial (pasteurization).

(3) Desiccation.

(4) Application of antiseptics (including salt, saltpeter, sugar, vinegar, spices and wood smoke).

(5) Exclusion of air (may be practiced in connection with any of the foregoing).

There is no doubt that all these methods have been employed in some form since very ancient times, but in recent times they have been very greatly extended and improved because of the exigencies of commerce and the investigations of science. In particular, the methods of heat sterilization, vacuum desiccation and exclusion of air have been refined to a great degree. The only original contribution of modern science to the art of preserving foods, namely, the use of small quantities of non-condimental, antiseptics, falls under the second heading.

Among the majority of flesh-eating animals and birds a careful tendency is shown which is directed against the consumption of putrid flesh. The members of the vulture tribe are the only ones that by apparent preference eat meat in an advanced state of decomposition. The cat family (lions, tigers, cats, etc.) take their prey alive. So do owls,

hawks, and eagles. Thus among animals the discrimination between fresh and decomposed foodstuffs is marked. Primitive man must have been well aware of the liability of foods to decomposition and must have taken what means were available to him to prevent it. Very ancient peoples were acquainted with the alcoholic, lactic and putrefactive fermentations. Famine was an ever-present menace in ancient times, since agriculture over extensive areas was not practiced and since communication and transportation were difficult and slow. Any means by which alteration in foods might be obviated must have been eagerly seized upon. As a matter of fact, it is quite certain that all five methods enumerated above for preserving foods were observed and made use of to a considerable extent by ancient peoples. It would be out of place here to review the art of food conservation in ancient times, but a few examples of the methods used may be cited, if only to indicate that modern man has amplified, extended, particularized, specialized and differentiated old methods of food preservation rather than invented new ones. In the first place, the application of low temperatures to the preservation of flesh must have occurred of necessity in many instances to early man living in cold climates and the most superficial observation would have led to the voluntary application of it. Among primitive dwellers in the north at the present time, it is the ordinary method of food preservation. In many places, preservation of foods in cellars was commonly practiced. Preservation by heat was inaugurated with cooking and cooking is as old as the most ancient records of the race. Desiccation has been practiced from time immemorial for the preservation of fodder, tea, medicinal plants, grains, seeds, figs, dates, and even meat ("jerked" beef; smoked and dried hams). The application of antiseptics, too, is very old and exemplified in the use of gum resins, such as frankincense and myrrh, essential oils and various aromatic substances as preservatives. Smoking has long been applied in the preservation of fish and is in use at present among primitive tribes. Resins and other aromatic substances were used to preserve wine; salt, smoke and spices were used to preserve meat; various foods were pickled in vinegar. Ancient knowledge of the effects of antiseptics to arrest decomposition is further shown in the application of tannins in leather making and of gum resins and essential oils in the preservation of the dead as mummies. Even the exclusion of air was practiced, as in covering wine with olive oil to prevent acetification.

The original contribution of modern times to the art of preserving foods—the use of small quantities of non-condimental antiseptics—has had its application curtailed or prohibited in recent years, owing to the fear that these substances might adversely affect human health even if used in small quantities. A consensus of opinion on this point,

however, cannot be said to have been reached up to the present time.[1] More experiments on a large scale are desirable.

Since the ordinary deterioration of flesh foods is principally or wholly a bacterial process, it is logical to infer that it will follow in degree the course of the degradation of the proteins in general as accomplished by microörganisms. This process is characterized by the conversion of insoluble proteins into soluble ones, of coagulable proteins into non-coagulable ones by the continued hydrolytic splitting of large molecules into smaller ones with the production of amino-acids, acid amides, lower fatty acids and ammonia. During the progress of the changes indole and skatole and their compounds are set free as well as various benzene derivatives, mercaptans and hydrogen sulphide. Sometimes a change in reaction is observed. Organic sulphur and phosphorus are converted into inorganic. In the end, if the action proceeds to its conclusion, the substances concerned will have been converted for the most part into gases or vapors or inorganic salts. However, one of the peculiarities of bacterial decomposition is that the last stages of the process chemically considered, the formation of ammonium salts or derivatives and hydrogen sulphide, for instance, occur almost at the beginning of the decomposition. There seems to be little tendency to produce, first albumoses, then peptones, then amino compounds and so on, but all these substances are formed almost at the beginning, while the tendency and final end of the process is the formation of the simplest compounds. In the earlier stages, at least, the fermentations of the proteins bear a striking resemblance to protein hydrolysis as excited by hot water, digestive enzymes, acids, alkalies and salts. Whatever methods of analysis are adapted to follow changes such as these, are applicable to the work under consideration. The choice would depend upon the comparative simplicity, accuracy and rapidity of the methods considered. During the progress of the work about to be described, various methods were used at different times with different degrees of success, and the methods most used were those which proved most applicable, all things considered, to the work in hand.

The most useful determinations made may be grouped as follows:

(1) Moisture, ash, fat, total nitrogen, sulphur and phosphorus.

(2) (In the cold water extract) solids, ash, total nitrogen, coagulable nitrogen, nitrogen in albumoses and proteoses, nitrogen precipitable by tannic acid and salt, nitrogen in "meat bases," sulphur, inorganic and total phosphorus.

[1] Wiley, *Bulletin* 84 *Bureau of Chemistry, Liebreich's Treatises on the Effects of Borax and Boric Acid on the Human System*; Eccles, *Food Preservatives*; Thresh and Porter, *Preservatives in Food and Food Examination, Etc.*

(3) Sulphur volatile with steam, ammoniacal nitrogen, indole, acidity of extract, volatile acids, changes in phosphorus compounds, etc.

In general the methods of König,[1] of Grindley,[2] of Winton,[3] of Bigelow and Cook[4] were freely used and in some instances modified in the interest of simplicity, speed or accuracy. In particular the cold water extract method of Grindley was so modified that while complete extraction was obtained, the method was simplified and shortened. Grindley in determining moisture[5] enclosed the sample in a glass extraction tube covered with filter paper at one end. It would seem that the amount of surface exposed would be too small to give the best results. In the same paper, p. 660, the ash figures are on the whole lower than we have been able to obtain. The reason for this probably lies in the fact that the sample was incinerated without previous charring and extracting so that a loss might have resulted by the volatilization of volatile salts during the long heating necessary. The ash determination of the cold water extract[6] is open to the same criticism. It would also seem that the quantity of the sample used (10–15 g.) in making the cold water extract is too small to give a sufficient amount of substance to work upon for the various determinations (p. 1099). In Grindley's second paper, in making the cold water extract he makes use of 90 to 105 grams of meat and the combined extracts are diluted to 5000 cc. Portions of 100 cc. representing about two grams of meat were used for determining total solids, ash and total nitrogen. It would seem that these quantities could be increased with advantage. The equivalent of ten grams would not be too large a quantity, although our work was usually carried out upon the equivalent of five grams. In the first paper, pp. 1089 to 1094, nitrogen results are carried out to the fourth decimal place. This is manifestly too far, inasmuch as there is in several cases an "apparent loss of nitrogen" amounting to as much as 11.7 per cent. of the total nitrogen in the extract. In one instance this "apparent loss" in the several extracts and insoluble residues expressed in percentage of the total nitrogen in the meat is as high as 16.89 percent.

Considerable attention was given to the preparation of the sample for analysis. It was the aim to work on meat as lean and containing as much muscular fiber as possible and to this end, fatty matter, loose connective tissue, skin and other extraneous tissues were trimmed off, before the sample was ground. For grinding an "Enterprise" hasher was used and the meats were put through this apparatus several times.

[1] *Chemie der menschlichen Nahrungs und Genussmittel, Vol.* 3.
[2] THIS JOURNAL, 26, 1086 (1904); 27, 658 (1905); 28, 25 and 468 (1906).
[3] *Ibid.*, 29, 1499 (1907).
[4] *Ibid.*, 28, 1485 (1906).
[5] *Ibid.*, 27, 659.
[6] *Ibid.*, 26, 1088.

Any juice squeezed out during the grinding was carefully remixed with the ground meat.

In brief the analytical methods used were as follows:

Moisture.—Weigh out duplicates of 10 to 15 grams from a weighing bottle into porcelain dishes, spreading the meat over the bottom, and dry in an oven at 100 to 105° to constant weight.

Fat.—Grind the residue from the moisture determination in an agate mortar with sand and transfer to an extraction thimble and extract with petroleum ether for about 16 hours in a modified Wiley extraction apparatus.

Ash.—Weigh into a porcelain dish about 10 grams. Dry in the oven and then char the mass. Extract the charred mass with hot water and transfer to an ashless filter and wash. Transfer the filter and contents to a platinum dish and ignite until ashed. Transfer the soluble portion to the dish, evaporate to dryness, dry in the oven, cool in a desiccator and weigh.

Total Nitrogen.—Weigh out in duplicate from a weighing bottle about 3 to 4 grams of meat and proceed as in the Kjeldahl method, using potassium sulphate. When nitrates are present and chlorides absent, use the modified Gunning method. When chlorides are present, proceed as directed under cold-water-extract-total nitrogen.

"Ammoniacal Nitrogen," first method.—Weigh out in duplicate from a weighing bottle about 25 grams of meat into a 1 liter round bottom distilling flask, add 10 grams of magnesium oxide and 450 cc. of water and distil exactly 200 cc. into tenth normal acid, using 50 to 60 min. in the distillation. Comparable results are obtained in one distillation, but if sufficient water is added to make up to the original volume and distillation repeated, more ammonia distils over. After four or five distillations a practical limit is usually reached.

"Ammoniacal Nitrogen," second method.—Extract 100 grams of the sample three times, with 150 cc. of 60 per cent. alcohol, filter through cheese-cloth and wring tightly each time. Add 10 grams freshly calcined magnesium oxide and distil exactly 200 cc. into tenth normal acid, taking one-half hour for the distillation. Run a blank on the reagents and solvent. Results by this method are usually about one-third to one-half those obtained by the first method.

Cold Water Extract.—Duplicates of 100 grams are weighed out into 8 inch porcelain dishes and about 250 cc. of cold distilled water added. The mass is digested with occasional stirring for about twenty minutes in a room of low temperature (in summer 2–5°). It is then filtered into a liter volumetric flask through cheese-cloth and wrung out by hand. This process is repeated with lesser amounts of water five times until a liter of extract is obtained, or four portions of 250 cc. of distilled water

may be used. It was found in carrying out the extraction a second time in the manner described, that only negligible quantities of extractives were obtained. The extracts are filtered for all determinations.

Technic for Beef Juice.—In all cases the meat must first be frozen. The frozen meat is brought directly to the laboratory from the freezer and without thawing, chopped into blocks two to five centimeters on an edge. These pieces are then cut into pieces about one centimeter on an edge and the whole wrapped in cheese cloth or thin muslin and firmly tied. Pressure may be applied to the meat in a container with a perforated bottom, in a colendar by means of weights, or in a screw press. The length of time varies from a few hours to twenty-four hours. During the pressing the meat must be kept in a cool place. The juice is collected in such a way as to avoid evaporation of water. We find it advantageous to remove the meat after the juice has ceased to run freely, grind it in an Enterprise hasher and return to the press. We consider that at least twenty per cent. of juice should be obtained.

Total Solids.—Take 100 cc. in duplicate and evaporate to dryness in a platinum dish on the water bath. Dry in an oven at 100–105° for one hour, cool and weigh. Calculate to basis of original meat.

Ash.—Char residue from solids determination, extract with hot water and filter. Burn filter paper and residue in original dish and add soluble portion and evaporate to dryness, dry in oven and weigh.

Total Nitrogen.—50 cc. in duplicate are taken and the nitrogen determined by the Kjeldahl method, using potassium sulphate. When nitrates are present, these are first removed by adding 10 cc. of ferrous chloride and 5 cc. of strong hydrochloric acid and boiling a sufficient time (this on account of interference of chlorides with the modified Kjeldahl). Nitric nitrogen is determined by the Schloesing-Wagner method.[1]

Nitrogen in Coagulable Proteids.—50 cc. in duplicate are transferred to 100 cc. beakers and concentrated on the water bath to one-half the volume. Filter, wash and make the filtrate neutral to litmus with tenth normal alkali. If any precipitate forms upon further heating on the water bath, filter this off. The two residues with the filter papers are transferred to a Kjeldahl flask and the nitrogen determined in the usual manner, distilling into tenth normal acid.

Nitrogen in Albumoses and Proteoses.—To the filtrate from the coagulable determination add one cc. of 50 per cent. sulphuric acid. (The filtrate should measure about 30 cc.) Finely powdered zinc sulphate is next added until the solution is saturated. Place upon the water bath a few moments to clarify, then set aside to stand over night. In the morning the precipitate is filtered off and washed with saturated

[1] See THIS JOURNAL, 30, 421 (1908).

zinc sulphate solution acidified with sulphuric acid. Filter and precipitate are now transferred to a Kjeldahl flask and the nitrogen determined as usual, distilling into tenth normal acid.

Nitrogen in Meat Bases.—When nitrates are absent the filtrate from a second coagulable determination is taken direct and the usual Kjeldahl determination made. When nitrates are present they are removed after separating the coagulable matter, by adding 10 cc. of ferrous chloride solution and 5 cc. of concentrated hydrochloric acid, and boiling. The usual nitrogen determination is then made. To get the nitrogen present as "meat bases" the nitrogen as albumoses is subtracted from the figure obtained above.

Acidity of the Extract.—50 cc. are diluted with recently boiled and cooled distilled water and titrated against tenth normal caustic soda, using phenolphthalein. The acidity is expressed as lactic acid on basis of meat. A second determination is sometimes made on a separate 50 cc. portion after boiling one-half minute.

Nitrogen in Proteins Precipitated by Tannic Acid (Bigelow and Cook).—20 cc. of the cold water extract are placed in a 100 cc. volumetric flask, 50 cc. of a saturated salt solution added and then 30 cc. of a 24 per cent. tannic acid solution. The tannic acid must be of highest possible purity. The precipitation is made in a cold room not above 12° and the flask allowed to remain there over night. The next day the solution is filtered and the nitrogen determined in 50 cc. of the filtrate. The figure thus obtained is multiplied by two. A blank is run on 30 cc. of the tannic acid solution and the nitrogen found deducted from that found in the filtrate ($\times 2$). This figure, subtracted from the total nitrogen in the extract, gives the amount precipitated by the tannic acid. The tannic acid nitrogen minus the coagulable gives the albumoses and peptones.

Volatile Acids and Volatile Sulphur Compounds (sulphur dioxide, hydrogen sulphide, hydrosulphide, thio-ethers).—100 grams of the hashed sample are weighed into an evaporating dish containing 200 cc. distilled water and after stirring the mixture is poured into 1000 cc. distilling flask. The dish is rinsed with 100 cc. water. The procedure allows a thorough breaking up of the sample and obviates sticking. One cc. sirupy phosphoric acid is added and distillation carried on in a current of steam. The distillate is condensed by means of a Hopkins condenser and collected in a 500 cc. flask containing 10 cc. 0.5 *N* sodium hydroxide by means of a tube running to the bottom of the flask. The excess of alkali is titrated back with 0.5 *N* hydrochloric acid, using phenolphthalein as indicator. Bromine is then added to oxidize sulphur compounds, the solution taken to dryness in porcelain and the residue ignited until carbon is burned off. The residue is taken up with hydrochloric acid

and water and the sulphates determined by means of barium chloride in the usual way.

Total Sulphur (Dubois and LeClerc's modification of Osborne's method[1]). —Ten grams are weighed into a nickel crucible of 100 cc. capacity, 10 cc. of water added and 15 grams of sodium peroxide. The mixture is first evaporated on the water bath, then gently heated over an alcohol flame, great care being necessary to prevent ignition of the frothing mass, and as the mass approaches fusion, it is cooled, more sodium peroxide is added and the crucible reheated until the action is complete. The fused mass is dissolved in hot water in a beaker, hydrochloric acid added and the sulphates precipitated as usual with barium chloride.

Qualitative Test for H_2S in Meat.—Acidify 50 grams of finely chopped meat with dilute hydrochloric acid in a 450 cc. Erlenmeyer flask. Place a piece of filter paper saturated with lead acetate over the top and allow to stand in a warm place for ten minutes. The depth of color gives an indication of the amount present (Eber's test). Perfectly fresh meat responds to the test.

Qualitative Test for Indole.—To 10 cc. of the extract add 5 cc. of 10 per cent. sulphuric acid, then 2 cc. of a 0.05 per cent. sodium nitrite solution and warm to about 70°. A rose-red color indicates indole.

Total Phosphorus.—50 grams of the sample are weighed into a 250 cc. beaker and 50 cc. of a mixture of six parts nitric and one part hydrochloric acid are added. The resulting solution is taken nearly to dryness (10–15 cc.) on the hot plate, taken up with hot water and made up to volume after cooling, in a 250 cc. flask. The solution is filtered and portions of 100 cc. are taken in duplicate for the determination. This solution is precipitated with the ordinary acid ammonium molybdate solution, dissolved in ammonia and reprecipitated with nitric acid and a little of the molybdate solution. The precipitate is filtered, dissolved in ammonia and precipitated as usual with magnesia mixture and weighed in a Gooch crucible as magnesium pyrophosphate.

Inorganic Phosphorus.—100 grams of meat are extracted successively with four 250 cc. portions of boiling distilled water and filtered through a folded filter. The last portion is squeezed through cheese-cloth and the filter and contents washed with boiling water. Two portions of 500 cc. each are evaporated to about 100 cc. 30 cc. of nitric and 5 cc. of hydrochloric acid are added and the volume reduced on the hot plate to 10–15 cc. When oxidation is at an end hot water is added and the phosphorus determined as described under total phosphorus.

Each method was tested and proved before its adoption and none was used which did not give either absolute or at least comparable results.

[1] THIS JOURNAL, 24, 142 (1902); 36, 1108 (1904).

The methods of determining ammoniacal nitrogen by means of distillation with magnesium oxide, in particular, were carefully investigated. In most of the work one distillation only was carried out under the conditions specified (first method), and while in this way comparable results were obtained, it was thought better in some of the work to continue redistilling until a practical limit was reached. This usually required five or six repetitions of the distillations, the distilling flask each time being refilled to the 450 cc. mark.

TABLE I.—PART 1.

Ammoniacal nitrogen obtained by successive distillations of hashed frozen meat, the distillation flask being made up to volume between distillations.

No. of distillation.	Sample 1.				Sample 2.			
	Method 1. Per cent.		Method 2. Per cent.		Method 1. Per cent.		Method 2. Per cent.	
1	0.034	0.032	0.009	0.009	0.0034	0.0032	0.008	0.008
2	0.014	0.014	0.001	0.001	0.012	0.012	0.001	0.001
3	0.009	0.010	0.001	0.001	0.009	0.010	0.001	0.001
4	0.009	0.009	0.001	0.001	0.008	0.008	0.001	0.001
5	0.008	0.008	0.001	0.001	0.006	0.008	0.001	0.001
6	0.006	0.006	...	...	0.006	0.006	...	...
7	0.005	0.005	...	...	0.005	0.005	...	...
8	0.004	0.004	...	...	0.005	0.004	...	...
9	0.004	0.003	...	...	0.003	0.003	...	...
10	0.003	0.002	...	...	0.002	0.002	...	...
Sum	0.096	0.093	0.013	0.013	0.090	0.090	0.012	0.012

TABLE I.—PART 2.

Ammoniacal nitrogen obtained by successive distillations of hashed fresh meat, the flask being made up to volume between distillations.

No. of distillation.	Method 1.		Method 2.	
1	0.030	0.029	0.010	0.010
2	0.015	0.014	0.001	0.001
3	0.009	0.009	0.001	0.001
4	0.006	0.007	0.001	0.001
5	0.005	0.005	...	...
6	0.006	0.007	...	...
7	0.005	0.005	...	...
8	0.004	0.004	...	...
9	0.004	0.004	...	...
10	0.004	0.003	...	...
Sum	0.088	0.087	0.013	0.013

A comparison of the amounts of ammonia recovered during successive distillations is shown by the above figures (Table I). In order to obtain further data on the ammoniacal nitrogen determination with magnesium oxide, various amino compounds and acid amides were distilled by the methods given above, 1 g. of substance being used and 10 g. magnesium oxide, unless otherwise noted. Orthoaminobenzoic

acid, hippuric acid, diphenylamine, acetanilide, α-naphthylamine and glycocoll yielded no ammonia on the first distillation and they were therefore not distilled a second time. On the other hand asparagine, acetamide, uric acid, urea, aspartic acid, creatine, allantoin, leucine and Witte's peptone yielded the results given below, the figures representing percentages of nitrogen distilled over.

TABLE II.—PART 1.

Percentages of ammoniacal nitrogen obtained by distilling various substances with magnesium oxide and water (Method 1).

	Distillation No.									
Substance.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Creatine, 1 gram	0.048	0.048	0.048	0.048	0.042	0.048	0.048	0.043	0.054	0.052
Acetamide, 1 gram	0.336	0.182	0.182	0.186	0.212	0.200	0.210	0.182	0.182	...
Aspartic acid, 1 gram	0.357	0.030	0.014	0.012	0.00	...	...	...	...	...
Urea, 1 gram	2.80	3.00	3.05	3.00	3.08	2.40	2.60	...	...	...
Asparagine, 1 gram	0.510	0.532	0.546	0.546	0.525	0.504	0.500	0.400	0.475	...
Allantoin, 1 gram	0.633	1.03	1.31	1.36	1.66	1.60	1.50	1.70	...	...
Witte's peptone, 10 grams	0.105	0.063	0.035	0.037	0.027	0.024	0.021	...	...	...
Uric acid, 1 gram	0.262	0.049	0.035	0.000	...	...	...	...	...	...
Leucine, 1 gram	0.014	0.000	...	...	...	...	...	...	...	...
Sarcosine, 1 gram	0.070	0.035	0.014	0.014	0.00	...	...	...	...	...

TABLE II.—PART 2.

Ammoniacal nitrogen obtained by distilling various substances with magnesium oxide and 60 per cent. alcohol, the volume being restored between distillations (Method 2).

	Distillation No.								
Substance.	1.	2.	3.	4.	5.	6.	7.	8.	9.
Acetamide, 1 gram	0.385	0.018	0.018	0.018	0.028	0.042	0.042	0.042	0.042
Allantoin, 1 gram	0.035	0.063	0.077	0.120	0.217	0.261	0.273	0.336	0.0308
Asparagine, 1 gram	0.126	0.105	0.098	0.126	0.126	0.154	0.266	0.357	..
Aspartic acid, 1 gram	0.336	0.021	0.000	...	...	...	...	...	..
Urea, 1 gram	0.070	0.175	0.273	0.413	0.518	...	...	...	..
Creatine, 1 gram	0.035	0.049	0.014	0.014	0.000	...	...	...	..
Witte's peptone, 20 grams	0.038	0.011	0.007	0.006	0.003	0.003	...	...	..

TABLE II.—PART 3.

Same as Part 2, but 10 g. substance used.

Substance.	Distillation No. 1.	2.	3.	4.
Urea, 10 grams	0.075	0.182	0.256	0.552
Acetamide, 10 grams	0.490	0.052	0.018	0.030
Uric acid, 10 grams	0.076	0.103	0.028	0.023
Creatine, 10 grams	0.014	0.011	0.007	Foamed badly
Asparagine, 10 grams	0.018	0.058	0.082	0.191

In cases shown in Table II, wherein a negative result was obtained after one, two or three distillations, there was evidently present an impurity which yielded its ammonia rather quickly under the treatment, but we concluded that in those instances (aspartic acid, uric acid sarcosine and leucine) the pure substance yielded no ammonia. The determinations were run in duplicate and sometimes in quadruplicate, but in spite of this, complete regularity was not obtained in all cases. The irregularities were no doubt due to the impossibility of carrying out every determination under identical conditions and particularly on account of the small quantity of substance worked on in some instances.

During the past few years much work has been done on the determination of ammonia in animal and vegetable substances.[1] The difficulty of the determination lies in the fact that there are present also various derivatives of ammonia which yield ammonia by hydrolysis in the presence of water. To prevent this, various experimenters have made use of precipitating reagents, alcohol and low temperatures in the separation or distillation. Sodium chloride, alcohol, vacuum distillation and air aspiration at low temperature have been used in different combinations for the purpose. Besides the two methods adopted for our work and described above, we have experimented with other methods, either taken directly from the published records or modified in one way or another, and which we now briefly describe. The statement of the method experimented with appears on the left, the results obtained on the right.

AMMONIACAL NITROGEN DISTILLATION OF MEAT WITH MAGNESIUM OXIDE.

Method.	Results.	Per cent. nitrogen.
100 grams fresh beef knuckle 450 cc. of water 10 grams MgO One-half of volume distilled off	0.030 0.0141	(1st distillation) (2nd distillation)
100 grams fresh beef knuckle 100 cc. of water 100 cc. sat. salt solution 250 cc. ethyl alcohol 10 grams MgO	0.008 0.009	duplicates

[1] Marriott and Wolf, *Pr. Soc. exp. biol. med.*, March 20, 1907; Grafe, *Z. physiol. Chem.*, 48, 300 (1906); Moritz, *D. arch. klin. med.*, **83**, 567 (1905); Sherman and others, *J. Biol. Chem.*, **3**, 171; Trillat and Sauton, *Bull. soc. chim.*, **33**, 719 (1905).

AMMONIACAL NITROGEN DISTILLATION OF MEAT WITH MAGNESIUM OXIDE (*Continued*).

Method.	Results.	Per cent. nitrogen.
50 grams meat and above solvents	0.015 0.005	(1st distillation) (2nd distillation)
100 grams fresh beef knuckle 100 cc. water 100 cc. sat. salt solution 250 cc. ethyl alcohol 10 grams MgO Distilled under vacuum at 40° C.	0.007	
50 grams fresh beef knuckle 100 cc. water 100 cc. sat. salt solution 300 cc. ethyl alcohol 10 grams MgO Distilled at atmospheric pressure	0.010 0.009	
100 grams beef knuckle extracted with 3 portions of 150 cc. each of 60 per cent. alcohol. Extract distilled with 10 grams MgO in regular manner	0.013 0.013 0.004 0.004	(1st distillation) duplicates (2nd distillation) duplicates
100 grams frozen beef knuckle 435 days old, distilled as above	0.012	(average)
Same as above 455 days old	0.012 0.014	duplicates
Same as above 455 days old	0.012 0.013	duplicates
25 grams fresh meat 450 cc. water	0.025 0.027	duplicates
10 grams MgO 200 cc. distilled at atmospheric pressure (For comparison)	0.024 0.025	duplicates
25 grams frozen beef knuckle 455 days old 450 cc. water 10 grams MgO 200 cc. distilled at atmospheric pressure (For comparison)	0.021	
100 grams fresh beef knuckle 100 cc. sat. salt solution 300 cc. ethyl alcohol 10 grams MgO	0.0012 0.0016	(1st distillation)
Air aspirated through at room temperature (16° C.) for two hours	0.0006 0.0008	(2nd distillation) for ¾ hour
Vacuum distillation of same at 30° C.	0.0059	

DISTILLATIONS OF AMMONIUM CHLORIDE WITH MAGNESIUM OXIDE.

		First set.	Second set.
0.50 gram NH_4Cl in 50 cc. H_2O 100 cc. sat. salt solution 300 cc. ethyl alcohol 10 grams MgO Air aspirated for 1 hour at 12° C.	Duplicates	0.061 gram NH_4Cl 0.040 " "	0.027 gram NH_4Cl 0.036 " " (1 hour at 21° C.)
Above for 1 hour more at 30° C.		0.1699 " " 0.0909 " "	0.073 gram NH_4Cl 0.067 " "
Above for 1 hour more at 40° C. with several drops caustic soda solution added		0.1319 " " 0.0963 " "	0.077 gram (No caustic added) 0.069 gram (No caustic added)
Above for 1 hour more at 40 C. with 100 cc. water added		0.0749 " " 0.0776 " "	0.095 gram NH_4Cl 0.078 " "
The lack of agreement in the above four cases is very marked.			
0.50 gram NH_4Cl in 50 cc. water 100 cc. sat. salt solution 300 cc. ethyl alcohol 10 grams MgO Distilled at atmospheric pressure.		0.4943 " " 0.4939 " " (300 cc. distilled)	0.4851 " " 0.4851 " " (250 cc. distilled)
Same reagents as above distilled for 3 hours in vacuum at 30° C.		0.3612 gram NH_4Cl	0.3063 gram (2 1/2 hours ouly)
0.50 gram NH_4Cl in 50 cc. H_2O 400 cc. 60% ethyl alcohol 10 grams MgO	Duplicates	0.4895 " " 0.4977 " "	0.4949 gram NH_4Cl 0.4948 " "
0.50 gram NH_4Cl in 50 cc. H_2O 400 cc. water 10 grams MgO			0.4975 " " 0.4988 " "
0.05 gram NH_4Cl in 50 cc. H_2O 450 cc. 60% ethyl alcohol 10 grams MgO		0.0487 gram NH_4Cl 0.0481 " "	0.0497 " " 0.0497 " "
0.05 gram NH_4Cl in 50 cc. H_2O 400 cc. tap water 5 cc. caustic (50%).		0.0497 " " 0.0497 " "	0.0497 " " 0.0497 " "

A consideration of these methods and results convinces us that the two methods adopted for the determination of ammoniacal nitrogen are those most satisfactory for our purposes.

In an earlier paper the statement was made that the bacterial enzymes acting on meat followed in greater or lesser degree the action of the digestive ferments. Some experiments were made using various digestive ferments and determining the ammoniacal nitrogen produced by our method number 1. The results would indicate that papain acts more after the manner of bacteria than do pepsin and pancreatin, but that in general the action of bacteria on meats is more drastic and far-reaching than that of digestive ferments.

Experiment on Digestion of Beef Knuckle with Pepsin.

20 grams hashed beef digested at 38–40° C. with 0.0288 gram pepsin in 10 cc. water, 10 cc. dilute HCl (12 per cent. actual HCl) and 430 cc. water.

Hours	0	1	2	3	4	5	19	21
Ammoniacal nitrogen Method 1, per cent	0.030	0.030	0.038	0.036	0.039	0.040	0.042	0.043

Experiment Number 2 on Pepsin Digestion of Beef Knuckle.

20 grams hashed beef digested at 38–40° C. with 0.1 gram pepsin in 100 cc. water and 3 cc. dilute HCl. Just before distillation enough alcohol added to make 450 cc. of 60 per cent. alcohol (Method 2).

Hours	0	1	2	3	4	5	6	7
Ammoniacal nitrogen Method 2, per cent	0.017	0.019	0.020	0.021	0.022	0.021	0.021	0.021

Experiment on Pancreatin Digestion of Beef Knuckle.

20 grams hashed beef digested at 38–40° C., with 0.5 gram pancreatin, 2 grams $NaHCO_3$ and 450 cc. water.

Hours	0	1	2	3	4	5	6
Ammoniacal nitrogen Method 1, per cent	0.060	0.065	0.066	0.061	0.065	0.061	0.069

Experiment on Papain (Parke, Davis) Digestion of Beef Knuckle.

20 grams hashed beef knuckle digested at 38–40° C. with 1 gram papain and 450 cc. water.

Hours	0	1	2	3	4	5	6
Ammoniacal nitrogen Method 1, per cent	0.049	0.060	0.064	0.065	0.069	0.071	0.072

Experiment on Putrefaction of Beef Knuckle at 40° C.

20 grams meat digested at 40° C. with 100 cc. water after the addition of 2 cc. of a putrefying meat infusion; 350 cc. water added before distillation.

Hours	0	1	2	3	4	5	7
Ammoniacal nitrogen Method 1, per cent.	0.043	0.046	0.043	0.045	0.046	0.048	0.055

Experiment on Putrefaction of Beef Knuckle at Room Temperature (Summer) 34–33° C.

20 grams meat digested with 450 cc. water after addition of 1 cc. of putrefying infusion of meat.

Hours	0	5	21	29	45	93	117	165	309
Ammoniacal nitrogen Method 1, per cent	0.038	0.039	0.069	0.099	0.182	0.621	1.056	1.379	1.648

Experiment on Putrefaction of Beef Knuckle at Room Temperature 24–33° C.

20 grams meat digested with 100 cc. of water after addition of 1 cc. of putrefying meat infusion. Just before distillation enough alcohol was added to make 450 cc. (total of 60 per cent. alcohol, Method 2).

Hours	0	16	64	92	118	140	188	284
Ammoniacal nitrogen Method 2, per cent	0.012	0.018	0.191	0.353	0.607	0.814	1.015	1.045

The enzymes themselves yielded the following amounts of ammoniacal nitrogen by Method 1 (20 grams of the substance distilled with 10 g. magnesium oxide and 450 cc. water until 200 cc. distillate were collected): pepsin, 0.205 per cent.; pancreatin, 0.271 per cent.; papain, 0.243 per cent.

In the earlier series of experiments the tannin-salt method as developed by Bigelow and Cooke was used to precipitate such proteins as are brought down by it, but after a considerable experience the method was abandoned, as it proved unsatisfactory in the hands of several workers. Thereafter the zinc sulphate method was substituted for the tannin-salt method. Our results show that the "albumose" figures as determined by using the tannin-salt method are sometimes higher and sometimes lower than when the zinc sulphate method is followed, and that of the two, the latter is considerably easier of manipulation and gives more concordant and consistent results. The presence of nitrogen in the purest samples of tannic acid which we were able to obtain is a strong argument against the use of the method. In some cases more nitrogen is introduced into the determination with the reagent than is derived from the albumoses of the samples. However, the fact that the tannin-salt method produces a heavier precipitate from such a substance as Witte's peptone than does zinc sulphate must be given due consideration.

The technic used in the preserving, embedding, cutting and staining of beef, pork and chicken tissues was not different from the ordinary standard methods. Both the celloidin and paraffin methods were followed but the former was made use of to a greater extent than the latter. Alcohol and Zenker's fluid were used for hardening, the sections were cut about 8μ thick and they were stained with haematoxylin and eosine.

Experiments on Frozen Beef.

The opinions expressed in the literature on the subject of frozen beef and freezing as a method of preserving flesh foods are varied and lacking in agreement. One is impressed by the absence of careful experiments and observation on the subject. König (*Chemie der Menschlichen Nahrungs- und Genussmittel*, Vol. 2, "Fleischdauerwaaren") seems to have had but limited opportunities to investigate the subject, especially as it has developed since the application of modern refrigeration. H.

W. Wiley ("Foods and Their Adulteration," p. 34) says: "There is evidently also a limit to the length of time which meat should remain in cold storage, no matter how low the temperature may be, since the action of organisms which produce decay cannot be entirely overcome." This is a rather dogmatic assertion, especially as it is made without references to authorities or citations of experiments. The results of the present investigations were hardly needed to show that the statement has no foundation in fact. The consensus of bacteriological opinion is entirely against such a conclusion. The fact which many observers have made known, namely, that bacteria are able to withstand exposure to intense cold, ought not to arouse confusion, when placed beside the other fact equally well established, that a sufficiently low temperature does in fact absolutely prevent growth and reproduction of bacteria and stops all bacterial action. Here are fundamental points of difference. The same author in a later paragraph states that he has examined a quarter of beef which was kept frozen in a warehouse for more than eleven years, and which he has found to be wholly inedible. "It had an unpleasant and mummy-like odor, was light in fiber and color, having evidently lost a large part of its weight, and was of a character wholly unsuitable for consumption." Unless the history of this piece of meat was known throughout, it goes without saying that the observation counts for little, as a matter of scientific record. The lightness of fiber and color are easily accounted for by desiccation in storage, a physical effect quite without influence on the nutritive qualities of the meat, and easily prevented, if so desired. The odor may possibly have been absorbed from the atmosphere (as sometimes happens in badly managed warehouses) and may not have been produced in the meat. Again Dr. Wiley says: "It has been stated in semi-scientific publications that the flesh of a mammoth incrusted in polar ice and presumably thousands of years old has been found to be intact and edible. This story, lacking corroboration, is hardly in harmony with known facts." The author does not state what known facts do not harmonize with the story. The facts seem to be that the mammoth and rhinoceros remains when found are in a state of putrefaction (Nordenskiold, "Voyage of the Vega;" Herz, "Frozen Mammoth in Siberia"). It appears that the remains are uncovered by moderation in the weather of Northern Siberia (where intense sunshine occurs at times) which results in landslides and washouts, and are not discovered presumably for some time after their disclosure, when, however, the flesh furnishes food for wolves and birds of prey. The discoverers speak of mammoth "mummies" which term would indicate remains more or less desiccated. It is remarkable testimony in favor of preservation by freezing that animal flesh and tissue structures should be preserved at all after the lapse of the thousands of years since these animals

died. The marvel is that after so long a time any flesh should be left to putrefy.

There are no facts known at present which would militate against the possibility of flesh preservation for an indefinite length of time under proper conditions of storage. Ostertag-Wilcox, "Handbook of Meat Inspection," p. 824, says: "Cold is unquestionably the best method of preserving meat. It causes no alteration in the meat, either with regard to taste or nutritive value. On the other hand, it improves the quality of the meat considerably." Again, "it appears that bacteria, especially putrefactive bacteria, possess a quite unusual resisting power against low temperatures. This resistance does not in any way militate against the preservative effect of cold. While it is not possible to destroy putrefactive bacteria by cold, we may still prevent their multiplication by means of low temperatures and may keep them in a dormant condition and prevent the development of their proteolytic power." In a later passage, Ostertag recommends the construction of cold-storage chambers which are capable not only of producng cold but also dry air in order to induce superficial desiccation of the stored meats.

Mitchell ("Flesh Foods," p. 102) says: "This method of preservation by cold is perhaps more extensively employed than any other, especially in Russia, where the climate is favorable for its natural application. Preservation by means of artificial cold is also in general use, and enormous quantities of frozen meat are daily supplied to the markets of London and other large cities. * * * *Alterations in Frozen Flesh.*—Owing to the slow, continuous action of the sarcolactic acid, meat which has been frozen is often exceptionally tender. * * * But cold, though it does not destroy microörganisms, prevents their development or at least does so in the case of the putrefactive bacteria, which at low temperatures are unable to decompose the proteins of flesh. There are, however, certain non-proteolytic bacteria which are capable of developing in frozen meat, and especially in that which is kept at a temperature of 0°, instead of several degrees lower. To this cause Lafar ("Technical Mycology," p. 213) attributes the unpleasant flavor sometimes acquired by meat which has been kept in an ice-chamber for several days. This is confirmed by Popp (*Z. Fleisch u. Milch Hyg.*, **1898,** 33) who states that in cement-lined storage chambers the walls, when moist, swarm with bacteria which, when grown on beef gelatin, produce a moldy flavor, and he considers them to be the cause of the objectionable flavor frequently developed in stored meat." There is apparently some confusion in the mind of Mitchell, owing to the fact that he does not distinguish with sufficient precision between the application of temperatures above 0°, at 0° and below 0°. There is no such confusion in the passage of Lafar ("Technical Mycology," p. 213) referred to. Lafar clearly in-

dicates that meat laid upon ice, whereby it attains under favorable conditions a temperature not lower than 0°, is not frozen, whereas the passage quoted above from Mitchell is somewhat ambiguous on this point. In speaking of "frozen meat" the temperature of storage, if mentioned, would give a definiteness to the conception which is lacking without it. If the term "frozen" means anything in science it means the solid condition and as stated elsewhere, the temperature at which meat assumes the solid condition must be the cryohydric point of the solution which exists within the muscle fibers. At any temperature above this point, the meat can be only partially frozen, although it may be in a condition adapted to withstand invasion by bacteria and to prevent their growth and reproduction if artificially inoculated. Also in ordinary language it may be spoken of as "frozen." Mitchell's statement that "certain non-proteolytic bacteria are capable of developing in frozen meat" as it stands, is not true, even for partially frozen meat held at temperatures, say, below —10°. The statement in regard to sarcolactic acid is an unproved hypothesis.

The physical changes in frozen meat may be due to desiccation (from evaporation of water or ice) or to pressure produced by the expansion of the water contained in the meat, during the freezing process. The surface of water or a moist substance, when placed in a sufficiently low temperature, naturally freezes first. In this way a hard surface layer is produced and the interior when it freezes is subjected to considerable pressure, owing to the expansion of water when it assumes the solid state. In the case of meat, the ice crystals, as they form around adjacent cells, must press against the separating cell walls and such pressure exerted against a delicate membrane may possibly cause abrasions and ruptures although some facts would indicate that such is not the case. It is possible, also, that in the same way other tissue elements will be physically changed. And these physical changes resulting from ice pressure may possibly become somewhat greater with lapse of time. On the other hand, the evidence in the case of frozen animals being revived (recited below) would indicate the absence of general abrasions. There is data to show that during freezing or thawing of muscular tissues or both the sarcolemma becomes permeable to proteins, because these find their way into the spaces between the fibers and may there be hardened and stained, or pressed out in the juice. They appear to be absorbed again if the frozen tissues are thawed slowly, because they can not afterwards be pressed out. These phenomena could be explained by the hypothesis that at the time of freezing the sarcolemma (and possibly other tissue elements also) assumed a net-like structure with (chemically) coarse meshes, and became a permeable membrane and that its normal continuous, semi-permeable condition was not instantly resumed upon

thawing but only after the lapse of some time, and then only when the thawing was conducted slowly. Desiccation alone does not change the nutritive value of flesh foods, as has been demonstrated by various investigators. Physical abrasion due to ice pressure does not change the nutritive value as the analyses reported in this paper prove.

Several observers, and notably Franklin, have recorded the fact that fish, toads and frogs if frozen in ice and thawed gradually regain their vitality (Schafer, "Text Book of Physiology," I, p. 817). Pictet in 1893 (Verworm-Lee, "General Physiology," p. 290) found that fish frozen in ice and cooled to —15° regained vitality when thawed, while they were killed by a temperature of —20°, and that frogs frozen in ice withstood a temperature of —28°. These facts indicate that freezing of animal tissues does not necessarily result in abrasion and rupture of the cellular elements, for if these occurred generally death would certainly ensue. The determining influence in the case is probably the rapidity with which freezing and particularly thawing is conducted, although the strength and porosity of the cell walls would be important factors.

One other physical effect may take place in meat under conditions of storage—contraction of the insoluble tissue elements. Shrinkage appears to be characteristic of colloidal animal substances when kept for a long period. This occurs not only as an accompaniment to loss of moisture, but even when the moisture remains constant. Contraction may be observed in coagulated blood and milk, in dried albumen and blood, keratins such as hoof and horn, gelatin and caoutchouc. It is possible that alleged progressive tissue shrinkage observed in the histological examination of frozen meats (when not due to desiccation) may be due in some degree to this cause.

The effect of freezing upon the growth and reproduction of microörganisms is determined by the fact that in freezing the organisms become surrounded by a solid medium. In a solid rigid medium growth and reproduction of living things become impossible. They may not die, although the chances are that they will in time; but growth and reproduction, which imply increase in size, will be effectually resisted by the solidity of the surrounding mass. In the interpretation of the effect of low temperatures upon microörganisms, the fact should be emphasized that it is the solid state of the medium and not any specific temperature (for example 0°) which is the limiting condition for growth and reproduction, although retardation of growth ensues with lowering of temperature.

The question logically follows: At what temperature do flesh foods assume the solid condition? The answer to this question must take into consideration the fact that the water-soluble constituents of flesh food are, broadly considered, comprised in three groups: (1) soluble

proteins; (2) organic extractives, including the so-called meat bases; (3) mineral salts. In lean beef the total amount of dry solids soluble in pure cold water is about 6 per cent. Of this quantity about 1.20 per cent. are mineral salts (ash determination); 2.80 per cent. are proteins, leaving about 2.00 per cent. of "organic extractives." Lean beef contains about 76 per cent. water. On the other hand, expressed beef juice contains about 10.00 to 11.00 per cent. solids, of which 1.35 per cent. are mineral salts, 6.25 per cent. proteins and 2.50 per cent. "organic extractives."

It is somewhat surprising that cold water does not extract as much solid matter from beef as is contained in the juice when this is expressed. Assuming that beef contains 76 per cent. water, and the expressed beef juice contains 10.60 per cent. solids, we calculate that the beef contains 85.0 per cent. of this solution and 9.01 per cent. soluble solids, whereas cold water extracts on the average only about 6 per cent. We might conclude that some of the solids which exist in solution in beef juice are not soluble in pure water and therefore if beef juice is diluted with pure water some of the solids should separate out. A beef juice containing 10.52 per cent. was experimented on by weighing out 85 grams of it (which would correspond to the amount contained in 100 grams of the meat) and diluting to one liter, thus parallelling the procedure used in obtaining the cold water extract. Immediately the solution became turbid and a flocculent precipitate separated out and settled to the bottom. When 0.6 gram sodium chloride was added to 100 cc. of the suspension, a part but not all the flocculent matter went into solution. The results of quantitative experiments along the lines indicated above will now be described.

Experiment 1.—About five kilos of frozen beef knuckle were prepared for analysis in the usual way, a part was set aside for analysis and from the rest (4752 grams) the juice was expressed. The cold water extract was found to contain: total solids, 5.80 per cent.; ash, 1.26 per cent.; total nitrogen, 0.77 per cent. A yield of juice amounting to 1037 grams or 21.82 per cent. was obtained and this contained: total solids, 10.74 per cent.; ash, 1.36 per cent.; total nitrogen, 1.547 per cent. Calculated to the basis of the meat (on the assumption that the meat contains 85 per cent. juice of similar composition), these figures become: total solids, 9.13 per cent.; ash, 1.15 per cent.; total nitrogen, 1.314 per cent. Eighty-five grams of the juice were diluted to one liter, in order to have conditions comparable to those obtaining when the cold water extract method was used, and this solution, after the flocculent precipitate which formed was allowed to settle, contained: total solids, 8.94 per cent.; ash, 1.20 per cent.; total nitrogen, 1.298 per cent. These figures indicate the insolubility in dilute solution of a small quantity only of substance which was soluble in the original juice, whereas from the cold water extract figures, we might have expected a difference of something over 3 per cent. Why cold water under these circumstances did not extract 8.93 per cent. solids from the meat is not understood.

Experiment 2.—In this case a yield of juice amounting to 1129 grams or 24.8 per cent. was obtained from 4549 grams of hashed frozen meat.

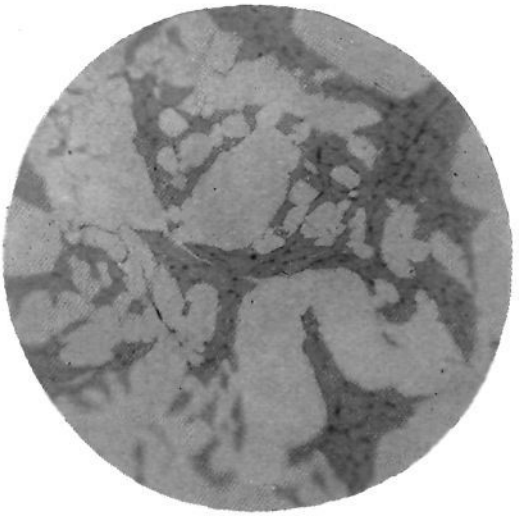

Fig. 1.

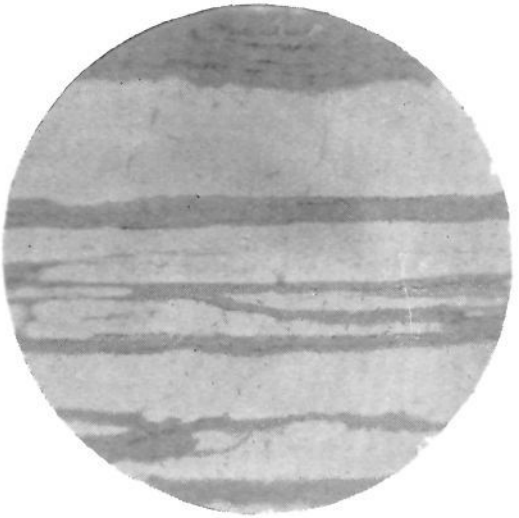

Fig. 2.

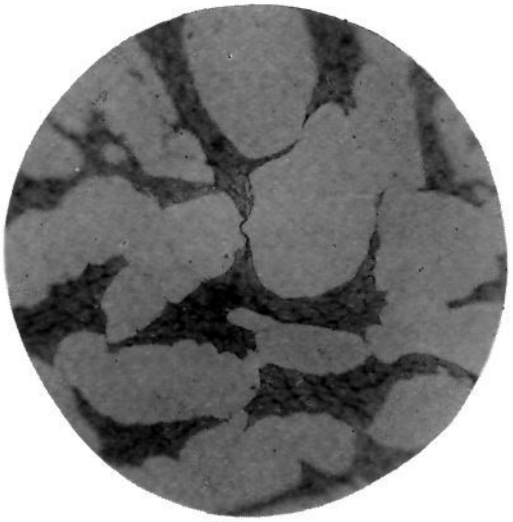

Fig. 3.

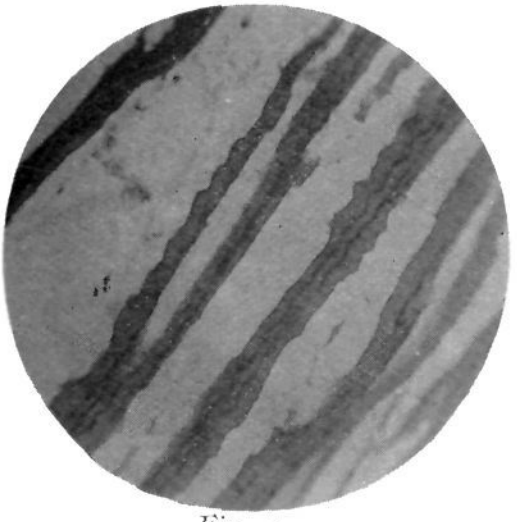

Fig. 4.

EXPLANATION: Fig. 1 is cross section, and Fig. 2 longitudinal section of muscular tissue from frozen beef knuckle hardened in the frozen condition. The light areas show the space occupied by ice. Fig. 3 is cross section, and Fig. 4 longitudinal section of muscular tissue from beef knuckle hardened while frozen.

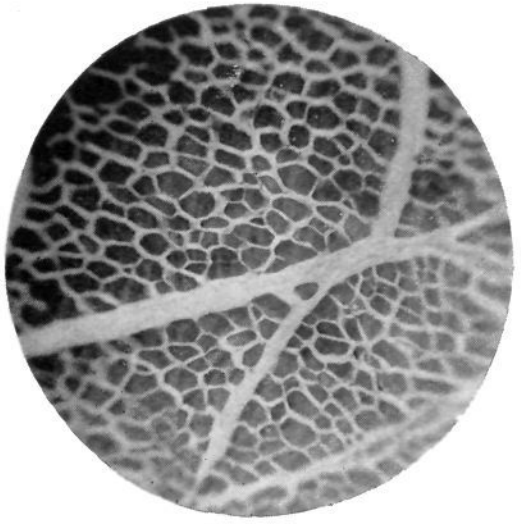

Fig. 5.

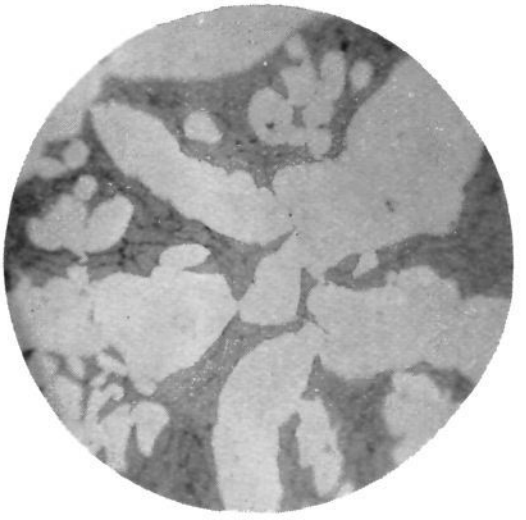

Fig. 6.

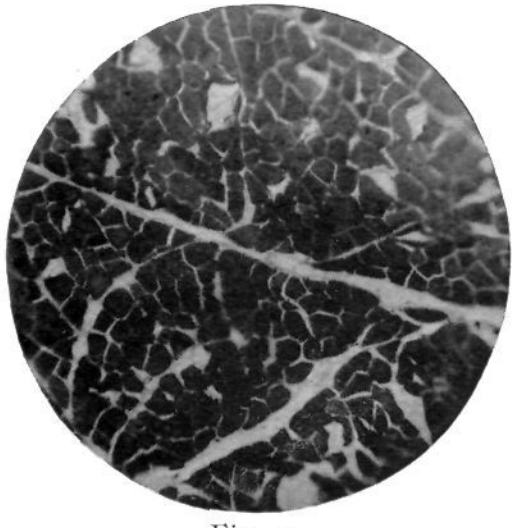

Fig. 7.

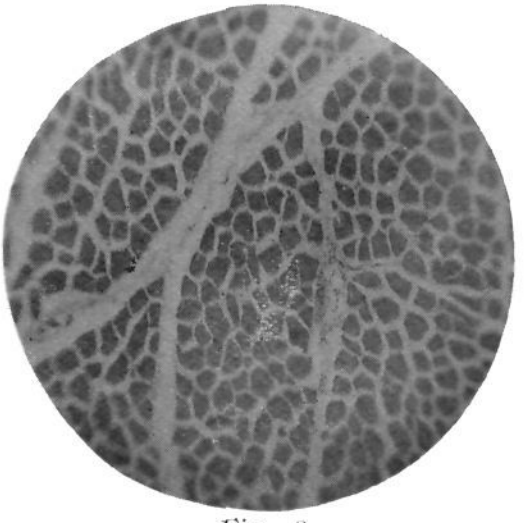

Fig. 8.

EXPLANATION: Fig. 5 is a section of normal beef knuckle. Fig. 6 shows the same piece hardened while frozen. Fig. 7 shows the effects of rapid thawing (the white spots show the remains of ice areas). Fig. 8 thawed slowly.

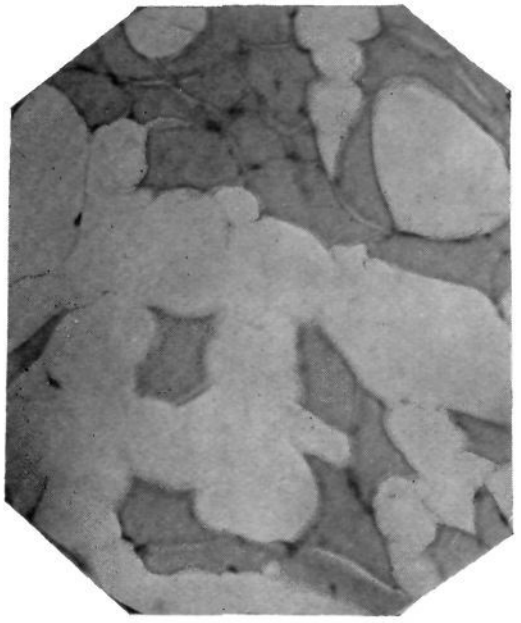

Fig. 9.

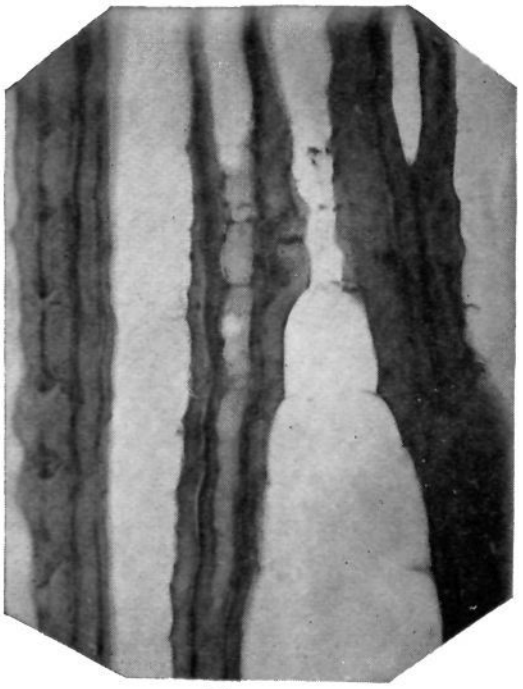

Fig. 10.

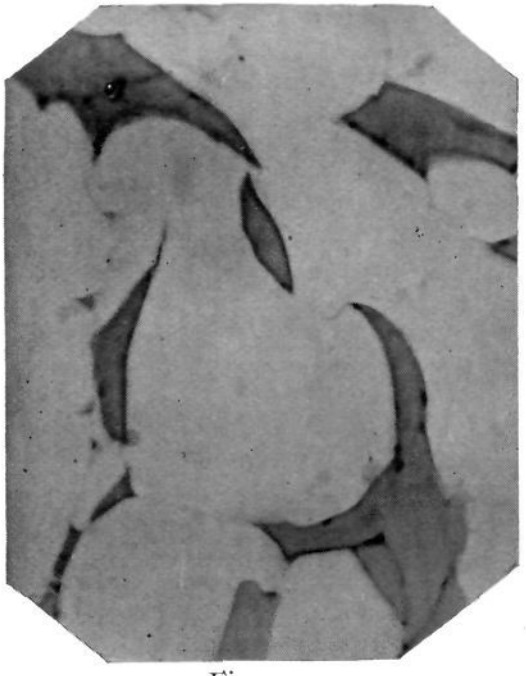

Fig. 11.

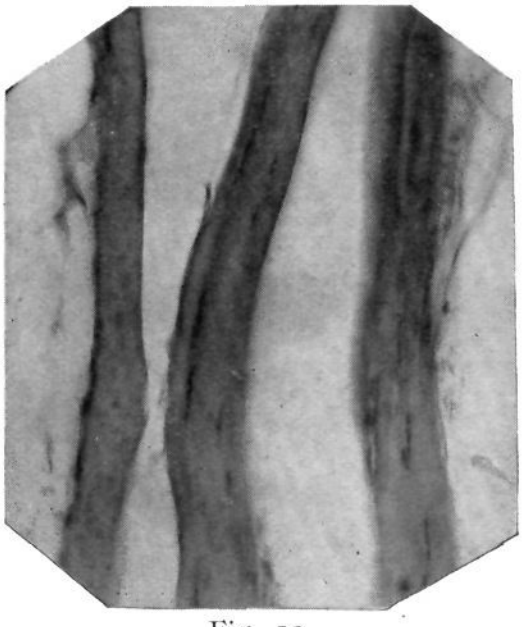

Fig. 12.

EXPLANATION: Fig. 9 is cross section, and Fig. 10 longitudinal section of beef knuckle hardened in frozen condition. Fig. 11 is a small area from frozen beef knuckle in cross section showing isolation of individual muscle fibers. Fig. 12 is a small area from frozen beef knuckle hardened while frozen, showing isolation of individual muscle fibers by ice (light areas).

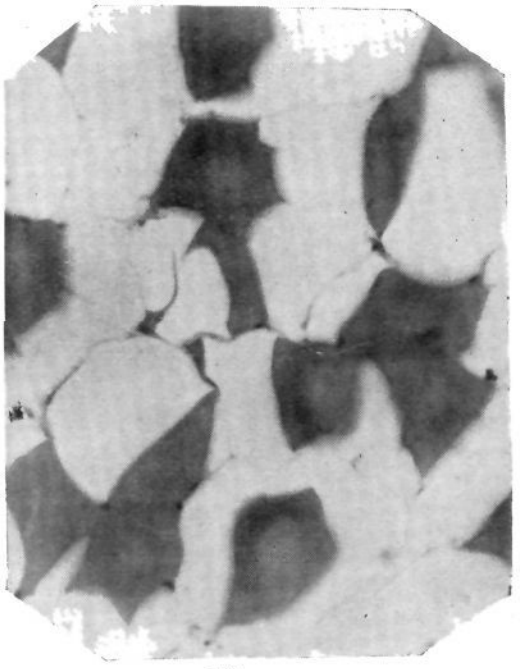

Fig. 13.

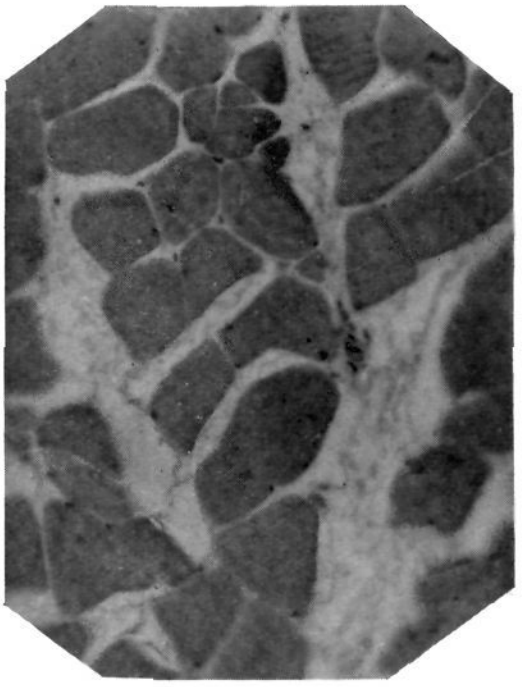

Fig. 14.

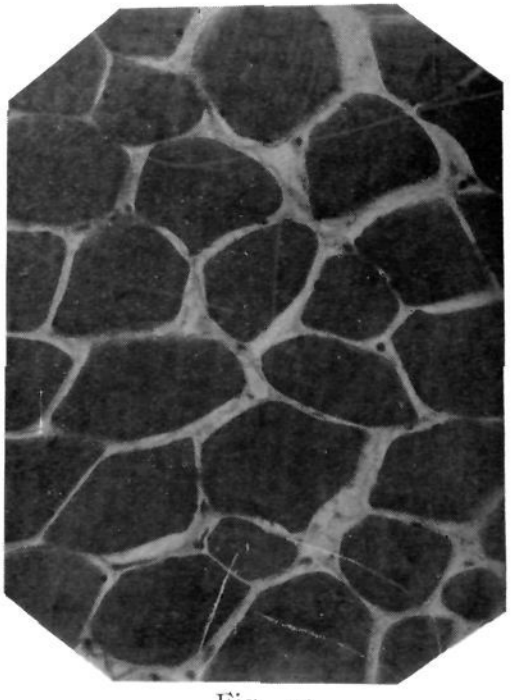

Fig. 15.

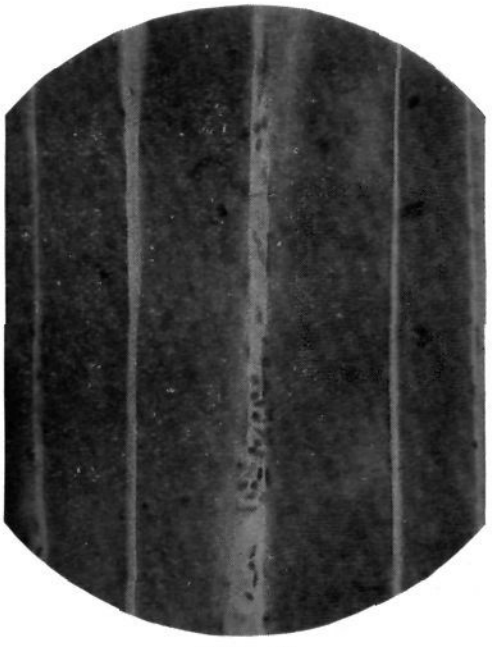

Fig. 16.

EXPLANATION: Fig. 13 (compare with two following plates): Beef knuckle in frozen condition. Fig. 14, same as Fig. 13 but thawed rapidly. The fibers approach the normal in appearance but the remains of the areas occupied by ice in the frozen condition are apparent. Fig. 15, same as Figs. 13 and 14, but thawed slowly; the muscular fibers have come back nearly or quite to the normal condition. Fig. 16, frozen beef knuckle 592 days old, thawed slowly.

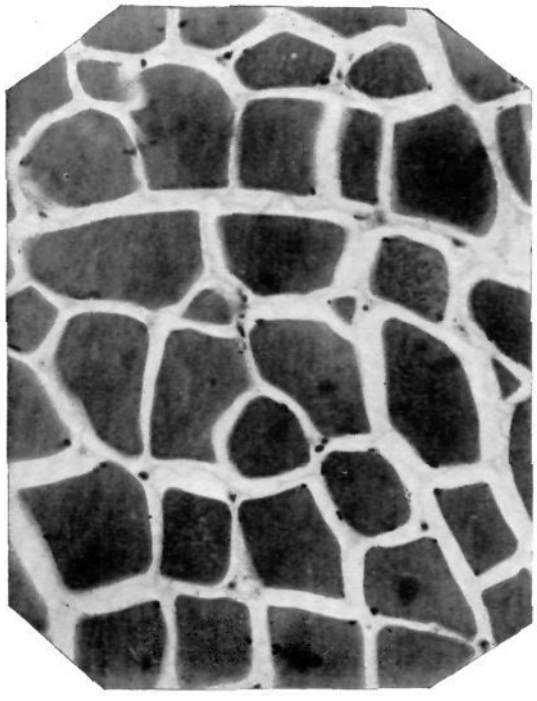

Fig. 17.

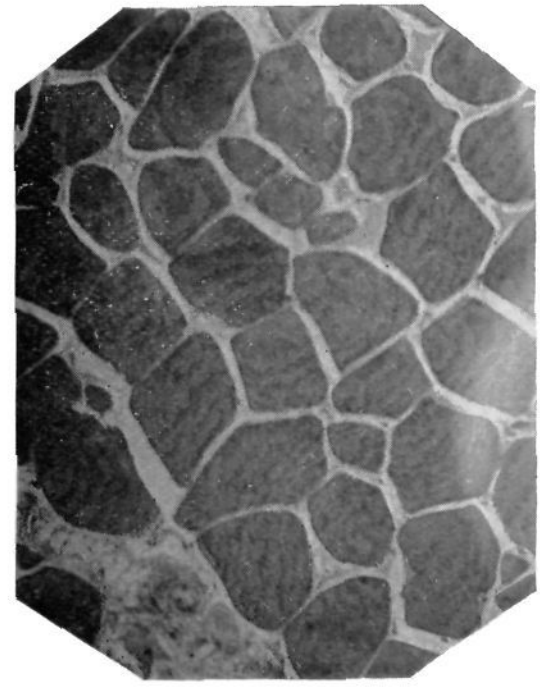

Fig. 18.

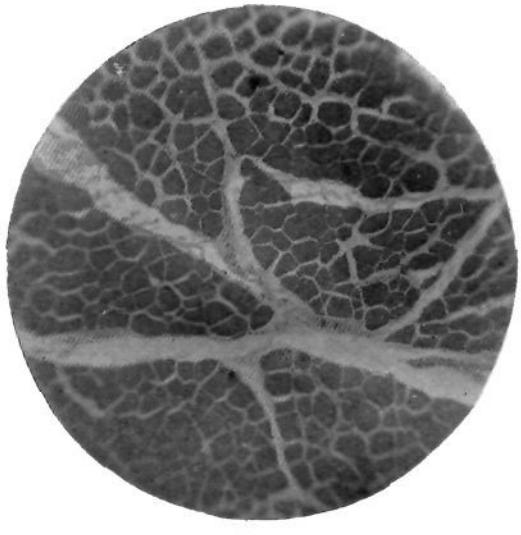

Fig. 19.

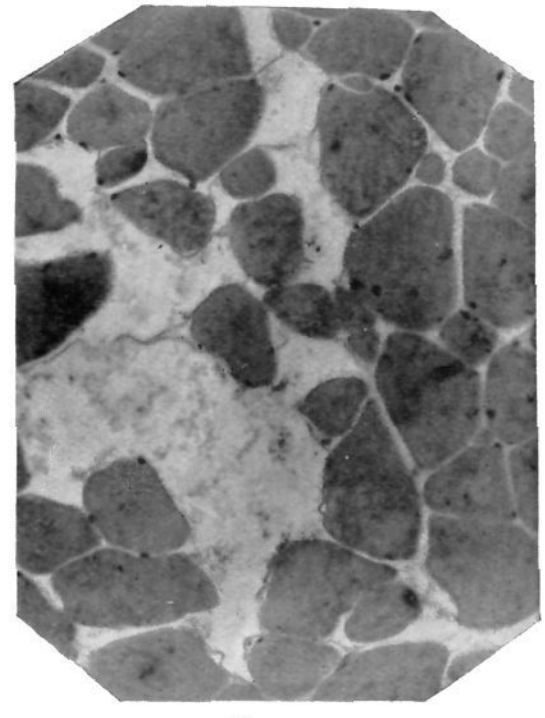

Fig. 20.

EXPLANATION: Figs. 17 and 18, specimens of frozen beef knuckle 554 days old, thawed slowly. Fig. 19, specimen of frozen beef knuckle 529 days old, thawed moderately rapidly. Fig. 20, a small area from frozen beef knuckle showing the effects of rapid thawing.

Analysis of Juice.

	Basis of juice.	Basis of meat.
Total solids	9.92 per cent.	8.43 per cent.
Ash	1.35 "	1.15 "
Total nitrogen	1.435 "	1.218 "

Analysis of Diluted Juice.

	On basis of meat.		Cold water extract from original meat.
	85 gms. to liter.	85 gms. to 2 liters.	
Total solids	8.16 per cent.	8.09 per cent.	5.52 per cent.
Ash	1.22 "	1.11 "	1.20 "
Total nitrogen	1.165 "	1.151 "	0.735 "

Pressed Residue after Removal of 24.8 Per Cent. as Juice.

Moisture	72.49 per cent.
Cold water extract of same:	
Total solids	4.64 "
Ash	1.16 "
Total nitrogen	0.581 "

In order to obtain more of the soluble matter of beef in solution, experiments were made, using as solvent common salt solutions of various concentrations and a solution of potassium phosphate (K_2HPO_4). The following table shows the results of this work.

TABLE III.—EXTRACTION OF BEEF KNUCKLES WITH WATER AND SOLUTIONS OF SALTS OF DIFFERENT CONCENTRATIONS.

100 grams meat extracted with the solvent and extract made up to one liter. 50 cc. (representing 5 grams of meat) taken for each determination. Two experiments (*a*) and (*b*).

Solvent.	Grams solids in 50 cc. solution	Grams ash in 50 cc. solution.	Per cent meat solids in solution.	Per cent. organic extractives.	Per cent. meat ash.	Per cent. total nitrogen.
Distilled water	(*a*)0.2824	(*a*)0.0597	(*a*)5.65	(*a*)4.46	(*a*)1.19	(*a*)0.770
	(*b*)0.2625	(*b*)0.0540	(*b*)5.25	(*b*)4.17	(*b*)1.08	(*b*)0.651
0.6 per cent. salt	(*a*)0.6097	(*a*)0.3441	(*a*)6.50.	(*a*)5.31	(*a*)1.19	(*a*)0.931
	(*b*)0.6412	(*b*)0.3227	(*b*)6.45	(*b*)5.37	(*b*)1.08	(*b*)0.858
2.0 per cent. salt	(*a*)1.2688	(*a*)0.9580	(*a*)7.40	(*a*)6.21	(*a*)1.19	(*a*)1.080
	(*b*)1.2075	(*b*) ..	(*b*) ..	(*b*) ..	(*b*)1.08	(*b*)0.913
5.0 per cent. salt	(*a*)2.6200	(*a*)2.2392	(*a*)8.81	(*a*)7.62	(*a*)1.19	(*a*)1.232
	(*b*)2.5252	(*b*)2.1517	(*b*)8.55	(*b*)7.47	(*b*)1.08	(*b*)1.158
10.0 per cent. salt	(*a*)4.8024	(*a*)4.4465	(*a*)8.30	(*a*)7.11	(*a*)1.19	(*a*)1.322
	(*b*)4.7867	(*b*)4.4216	(*b*)8.38	(*b*)7.30	(*b*)1.08	(*b*)1.274
20.0 per cent. salt	(*a*)9.1873	(*a*)8.8395	(*a*)8.14	(*a*)6.95	(*a*)1.19	(*a*)1.001
	(*b*) ..	(*b*) ..	(*b*) ..	(*b*) ..	(*b*) ..	(*b*) ...
1.1 per cent. K_2HPO_4	(*a*)0.8388	(*a*)0.5145	(*a*)7.67	(*a*)6.48	(*a*)1.19	(*a*)0.896
	(*b*)0.8194	(*b*) ..	(*b*) ..	(*b*) ..	(*b*) ..	(*b*)0.858

The 10 per cent. salt solution appears to extract the largest amount of nitrogenous substances, possibly not without alteration, whereas the 5 per cent. solution extracts more organic solids. However, on account of the large quantity of salt present, the ash figures are not to be considered as being absolutely accurate, although the determinations were

handled as carefully as possible. The nitrogen figures should be considered more accurate. In the case of the five and ten per cent. salt solutions, results in soluble meat solids and nitrogen are obtained of the same order as those representing the soluble solids and nitrogen in meat juice itself, calculated to the basis of the meat.

We have not explained in a satisfactory way why cold water does not extract from meat a larger percentage of soluble solids, especially since the same solids when pressed out in solution in meat juice, are not precipitated to a large extent when water is added.

Lean beef can then be considered as made up of insoluble protein tissues containing a solution of proteins and "organic extractives" in water. An analogous calculation would hold good for flesh foods of other kinds. There is no reason to suppose that this solution in freezing will behave differently from other solutions and therefore the phenomena connected therewith will be lowering of the freezing point of water by soluble solids and the formation of cryohydrates. The proteins, since they are colloids, have a negligible effect upon the depression of the freezing point. Now the course of the freezing process in the case of such a solution as that given above, would be as follows: At a temperature slightly below the freezing point of water (actually $-0.4°$) ice crystals would begin to separate and the solution would in consequence become more concentrated. As the temperature became lower, more ice would separate and a more concentrated solution would result. If at a still lower temperature the solution became supersaturated for any of the solutes, this one would separate out. Finally one or more of the soluble components would remain in solution in water and at a still lower temperature, possibly after a further separation of soluble components, or of ice, the entire solution would solidify. Below this temperature, the growth and reproduction of bacteria would be impossible; hydrolysis would become infinitesimal and enzyme action (which in flesh foods is but accelerated hydrolysis) would, in all probability, cease.

At a temperature above the point of complete solidification of meat juice, there would exist ice, deposited soluble compounds which had crystallized from the solution, and a concentrated solution of proteins, extractives and salts. Now in a solution of more than a definite concentration, or more than a definite osmotic pressure, microörganisms will not grow and reproduce. Their growth and development would also be affected adversely by the great viscosity of such a solution as the concentrated one produced by the freezing out of water from beef juice. Therefore bacterial development will cease at a point above the point of complete solidification. Again, the action of enzymes becomes progressively less as the temperature is lowered and it is possible, to say the least, that at the same point enzyme action would become a

negligible quantity. Thus, in the consideration of frozen flesh foods there are three temperatures of great interest: (1) the freezing point of water; (2) the point at which bacterial growth ceases, owing to the concentration of the meat extractives during the freezing process; (3) the point of complete solidification of all the constituents (that is, at the cryohydric point when only solid phases are present).

There are many interesting phenomena to be observed in the freezing of flesh foods. It is a fact well known in the physiology of plants, that when freezing ensues in tissues which contain the normal amount of moisture, and are not sufficiently prepared through desiccation to resist the harmful effects of freezing, that the water instead of freezing within the cell, freezes outside of it in a state of considerable purity. (Vines, "Text Book of Botany," p. 672; Sachs-Vines, "Text Book of Botany," 2nd Ed., p. 731; Pfeffer-Ewart, "Physiology of Plants," II, p. 235; Detmer-Moore, "Practical Plant Physiology," p. 125.) Such freezing does not necessarily kill the plant, especially if the subsequent thawing takes place slowly, but those plants which are unable to withstand desiccation are particularly likely to die if frozen, supposedly on account of the abstraction of water during the freezing. The complete withdrawal of water would probably not occur above —30° (cryohydric point of the plant juice?).[1] The current explanation of the freezing of water outside the plant cell is that a film of water surrounding the cell wall freezes first and continuously abstracts water from the cell to form ice as the temperature is lowered (*Ibid.*, p. 240).

Exactly the same thing occurs when animal tissues freeze, the water freezes outside the cells and between the muscle fibers in a fairly pure condition, leaving the muscle fibers more or less isolated and strangely distorted. Viewed in a cross or longitudinal section of lean beef at —12° to —14° the ice crystals cover larger areas than do the shrunken muscle fibers.

There are some difficulties in the way of making microscopic observations upon frozen meat. If the meat is removed to a warm place, it immediately begins to thaw, the muscle fibers re-absorb the water frozen outside of them, incompletely at a relatively high temperature and nearly completely at a relatively low one, and thus they tend to resume their normal shape. To make observations upon meat in the frozen state, microscope, microtome, and accessories must be carried into the freezer, and brought to the temperature of the freezer before observations are made. This was done by us in order to observe the true microscopic picture of beef in the frozen condition. The technic employed consisted in sawing out cubes of the frozen tissues to be ex-

[1] Pfeffer-Ewart, *Physiology of Plants*, 2nd Ed., II, p. 235.

amined, clamping these to the microtome and sectioning without further treatment. The sections were examined immediately and unstained.

In order to obviate the difficulties of working the freezer with microscope and accessories, it was thought that small cubes of the frozen beef to be examined could be dehydrated in alcohol cooled to the temperature of the freezer, and that with this technic the ice outside the muscle fibers would be rapidly dissolved by the alcohol and the fibers themselves would be hardened without losing their shape in the frozen condition. Bottles containing alcohol of the desired strength were usually left in the freezer over night and on the next day cubes of the meat to be examined were sawed or cut out, dropped into the alcohol and allowed to dehydrate at freezer temperature for twenty-four to forty-eight hours. After this the pieces were hardened, embedded, stained and mounted in the usual way. All the histological characters observed in the frozen sections when working in the freezer, were preserved in the stained sections prepared according to this method. When viewed either in cross or longitudinal section, the meat fibers appear to be surrounded by the transparent areas formerly occupied by the ice crystals. Adjacent fiber bundles are sometimes joined by extremely slender threads of connective tissue. This isolation of the fibers in the frozen condition has an important bearing upon the alleged penetration of bacteria into frozen meats, which has been reported from time to time. How can bacteria—assuming they are otherwise capable of growth and reproduction—in frozen meats in storage—pass from fiber to fiber across these solid barriers of ice? The only means of such progress would be along the threads of connective tissue mentioned above and these have been searched microscopically in vain for bacteria. It is almost an axiom of bacteriology that muscular tissue in healthy animals is free from bacteria. It has been shown that when fresh meat decomposes on account of bacterial action the bacteria begin their work on the surface of the meat, and that the interior of the muscular tissue decomposes last. Since these things are so, reports of bacteria obtained from the interior of frozen meat, otherwise properly handled, by cultural methods alone, must be looked upon with suspicion. Van Ermengem demonstrated *B. botulinus* in the tissues of the classical Ellezelles ham microscopically. It is an easy matter to demonstrate bacteria microscopically in meat which has spoiled and it should be equally easy to demonstrate them by the same means in the interior of stored frozen meats. All of our attempts at such demonstrations have resulted in failure.

Not only the alcohol technic is suitable for demonstrating the structure of frozen meat but other methods as well. Any hardening fluid which will not freeze at the temperature of the freezer can be used, if it is otherwise suitable. We have made use of Zenker's fluid, to which

common salt has been added to prevent freezing and five and ten per cent. formaldehyde with the same substance in proper amount. The cryohydric point of sodium chloride is —22°, which is lower than the temperature of the ordinary freezer, and therefore salt solutions with hardening substances added can be used, other things being equal, down to that temperature.

Nor is the microscope necessary to investigate, in general, the structure of frozen meat. If a frozen beef knuckle, for instance, is sawed across, the lines of ice forming a network all over the surface can be plainly seen. If a section three to six millimeters thick be sawed off and examined in the frozen condition by transmitted light the picture is still more striking, the ramifications of the ice areas showing brilliantly in contrast to the dark areas of tissue. Such sections can be hardened in the frozen condition by alcohol or by formaldehyde or Zenker's fluids to which a sufficient quantity of salt has been added, and then examined at leisure at ordinary temperatures. Specimens prepared in this way show open spaces which were formerly occupied by ice. If similar sections are allowed to desiccate in the open freezer or in specimen jars over calcium chloride, sulphuric acid or phosphoric anhydride, the same picture of the gross structure of frozen beef is preserved. It goes without saying that all other meats such as pork, mutton, chicken, turkey, etc., in the frozen condition, have a general structure very similar to that of frozen beef.

Wiley, Cook, Pennington and Stiles in a paper entitled "Cold Storage Results on Fowls and Eggs" read at the Chicago meeting of the American Chemical Society, Dec. 31st, 1907, to Jan. 3rd, 1908,[1] have reported that they find progressive histological changes in the breast muscle of chickens stored in the frozen condition at 13° F. and they assume that these changes are due to chemical or biochemical changes occurring in the muscles while in storage. After six months the muscle fibers are "scarcely recognizable." They mention particularly the shrinking or contraction of the muscle fibers, the apparent thinning or dissolving of portions of the same, and the changes in staining alleged to have been observed in the striae, the fibers and the capillaries. In the light of the present observations it would appear to be reasonable to ask to what extent the observations made by Wiley may be due to physical changes. After one hour in the frozen condition the muscle fibers of beef might be considered scarcely recognizable, if examined while frozen. Possibly many of the changes observed by Wiley and his collaborators can be explained as the result of physical changes. It may be true in a minor degree that the physical changes which occur in the interior of frozen meats increase with age, but unquestionably the principal changes occur

[1] *Abstract in Science*, **27**, 295 (1908).

during the period of freezing and after the meat is once frozen and has reached the temperature of the freezer, the after changes will amount to little. As stated elsewhere, the superficial changes, due to desiccation, cannot be considered to impair in any degree the nutritive qualities of the meat, and if desired, these changes, due to loss of moisture, can be easily guarded against. When frozen meat is thawed rapidly, the muscle fibers do not entirely reabsorb the water which has frozen outside of them, and they retain more or less of the distorted form they acquired in the freezing process. By rapid thawing some of the apparent histological changes observed by Wiley, Pennington and their co-workers can readily be reproduced, and all degrees of apparent histological change, also, according to the varying rapidity of thawing. On the other hand, the slower the pieces of frozen meat are thawed, the more nearly do the physically altered tissue elements resume their normal appearance. If frozen meat is thawed rapidly, it is impossible to avoid the loss of a quantity of meat juice; if it is thawed very slowly almost no juice escapes from the fibers. Since these things are so, the question forces itself: How large a part did rapidity in thawing play in the observation of supposed progressive alteration of the tissue elements, in the investigations of Wiley and his co-workers? Another point should be considered here. It is not pathological changes which are under observation in the histology of frozen flesh foods, and changes seen under the microscope which would be of immense importance in pathology may be of little moment or none at all, from the nutrition standpoint.

The histological method as applied in investigations like the present one, is open to serious question. The validity of the evidence which histology affords must depend upon a careful tracing of its relationship with chemical change; and if no chemical change appears, it would seem to be impossible to conclude that the nutritive value of the product was modified. While physical change in foods may in a slight degree modify the digestive process, it cannot be seriously thought that it is an important matter, in comparison with chemical change.

Two methods may be followed in examining objects for bacteria—the microscopic and the cultural. Either or both of these methods may be adopted for the purpose of demonstrating the presence of bacteria, saprophytic or pathogenic, in living or dead tissues. The cultural methods are open to the objection that on account of the universal distribution of microörganisms it is difficult to devise a technic which excludes absolutely, accidental contamination of the nutrient medium. In working on animal tissues and meat, this is particularly the case, as has been demonstrated by the contradictory results obtained by different investigators of the subjects of the distribution of bacteria in the living healthy animal, and in various meats, for example canned meats. In the last mentioned

case, cultures have been obtained in a minority (sometimes in a majority) of tests, from cans that were certainly sterile. T. J. Burrill, working on canned meats, with a most painstaking technic, obtained eleven cultures out of two thousand six hundred and one or 0.42 per cent. of the total. He thinks there is every reason to believe that these were contaminations in spite of the care taken to prevent them. He concludes that "cans which 'keep' are sterile" (*Science*, **27**, 215, Feb. 7, 1908), thus differing from other observers.

Thus the obtaining of cultures of bacteria does not necessarily prove the presence of bacteria in the thing examined. On the other hand, the microscopic method, if positive, furnishes conclusive evidence of the presence of bacteria which, however, may not be living. Unfortunately, bacteria must be present in considerable numbers—as in colonies—before the microscope affords certain evidence of their presence. If we accept the dictum that in the flesh of living healthy animals bacteria are absent[1] then we must conclude that bacteria, if they invade the tissues of meat intended for human consumption, must do so after the slaughter of the animal, starting from the surfaces exposed after death. Bacteria work with extreme slowness into firm muscular tissue, free from large blood or lymph vessels and sheaths of connective tissue. In our experiments they penetrate in a direction perpendicular to the muscle fibers only, about a centimeter at 2–4° in thirty days. And this invasion proceeds for the most part by the actual extension of the limits of the colony, by cell division. The mobility of some of the bacteria may aid the penetration when an avenue is offered along a moist surface, such as the surface of connective tissue or the interior wall of a blood vessel.

These facts concerning the invasion of meat by bacteria have an important bearing upon the validity of microscopic evidence of the presence of bacteria in flesh food. It seems all but certain that if the microscope fails to demonstrate bacteria in the interior of meat, from a healthy animal at any given point, that they are absent from that point; and in such cases, if cultural methods give positive results, the bacteriologist should look most carefully to his technic.

No very positive rule can be laid down with respect to the number of bacteria which must be present either in living or dead tissue to cause in the one case lesions or general symptoms of disease and in the other noticeable decomposition, but it seems to be true that the number of individuals has a determining influence on the effects produced, the necessary number varying noticeably with the kind and the environment. In all definitely known bacterial diseases, bacteria can be demonstrated microscopically in the body—in some cases, however, only with consid-

[1] This is the consensus of scientific opinion at the present day.

erable difficulty. In all fermentations, even at the commencement, microörganisms are easily demonstrated microscopically. This and the facts mentioned before would indicate that if bacteria cannot be demonstrated microscopically in the meat from healthy animals, that the meat is, in respect to bacteria, suitable for consumption.

We have examined a number of samples of frozen beef knuckles for bacteria microscopically, without finding them present. In the examination the pieces were cut across with knife or saw about one third the distance from the larger end. Scrapings from various parts of the section thus exposed were taken by means of a sterile scalpel (the surface scrapings were discarded and those from just below the surface retained), fixed on glass slides, stained and examined systematically, use being had of the two millimeter oil-immersion objective and mechanical stage. Frozen beef knuckles of all ages up to six hundred days were examined in this way, scrapings being taken at points varying from 1 centimeter below the surface to the center of the meat, without finding bacteria. In no case were indications of bacteria observed. In six hundred days, if bacteria have the ability to penetrate frozen beef it would seem that they ought to reach a depth of one centimeter.

Cultural methods were no more successful in demonstrating bacteria in frozen beef knuckles than were microscopic methods. A careful technic was followed in all cases. The knuckle was taken to the laboratory immediately from the freezer. A ring was seared around the piece about one-third the length from the larger end by means of a Bunsen flame. A meat saw which had been carefully cleaned with absolute alcohol and ether was used for sawing through the frozen sample, following the seared ring. After the piece was sawed across, the particular place from which it was desired to secure a piece was scorched by means of a hot branding iron, of circular form, one-fourth inch thick and two inches in diameter. Immediately after this one operator removed a piece of the meat from the seared spot (to a depth of one or two centimeters) by means of a scalpel heated just previously in a Bunsen flame and cooled. The piece was dropped into a bouillon flask whose plug had been burned and lip turned in the flame by a second operator. In all cases one flask was contaminated by exposure to the air or otherwise as a check on the culture medium and one flask was inoculated with meat taken from the unsterilized outer surface of the piece. These two flasks in all cases showed abundant growth at room temperature, usually in two days. If, after a sufficiently long period the other flasks (usually eight in number, four from one centimeter below the surface and four from nearer the center) failed to show growth, they were contaminated by exposure to the air in order to show the possibility of bac-

terial growth in the medium. In no case under these conditions was there a failure of growth.

In the cultural work on the penetration of bacteria into frozen meat anaerobic methods of cultivation were used less frequently than aerobic, inasmuch as we consider aerobic methods adequate for the purpose. If, as we assume, the muscular tissue of healthy animals is free from bacteria, then bacteria, if they gain access to the interior of meat, must do so from the outside, where of course they would find only aerobic conditions. According to this line of reasoning the bacteria which penetrate into meat must be aerobic or facultative. This, of course, opens up the question of the source of van Ermengem's *B. botulinus*. Such an organism might gain access symbiotically along with aerobies. The latter would then have the function of producing anaerobic conditions, in the presence of air, under which the obligate anaerobe could develop sufficiently to penetrate below the surface, where anaerobic conditions could easily be set up. But these facts would not invalidate the aerobic method of cultivation for our purposes, because facultatives would penetrate with the anaerobes and these would respond to aerobic methods of cultivation. In their presence in quiescent bouillon media, conditions would probably be sufficiently anaerobic also for the growth of anaerobes. In the bacterial work on frozen meats the 37° incubator was not largely used either, because that temperature is not favorable to the growth of bacteria which develop at low temperatures.

The cultural experiments are still in progress, although no demonstration of the presence of or penetration of bacteria into frozen beef knuckles has resulted up to the present time and details will be published in a later paper.

The chemical work on frozen meats was begun, following in general the methods previously described, with the object of determining whether or not there was progressive change in the chemical constituents of the meat, such as would result in an increase in the soluble constituents due to the auto-digestion (autolysis) of insoluble proteins, and also increase in the "ammoniacal nitrogen." In nearly all the work on beef, the muscular portion known as the "knuckle" to butchers, was made use of on account of its size, uniformity in structure and its freedom from fatty tissue. The knuckle is the group of muscles known as the Crural Triceps to anatomists and consists of the Rectus Femoris, Vastus Externus, Vastus Internus and Anterior Gracilis. It was desired to experiment primarily upon the lean portion of beef, and fatty matter and gristle was trimmed away as far as possible in the preparation of the samples for analysis. It was found that after long storage in the open freezer desiccation occurred in the surface layer of the pieces to a depth of two to four millimeters, and this desiccated layer also was trimmed away

TABLE IV.—PART 1.

ANALYSES FRESH BEEF KUNCKLES. ALL SAMPLES HELD AT 2–4° C. UNTIL ANALYZED.

AGE, 0–7 DAYS. ALL FIGURES ON BASIS OF ORIGINAL MEAT.

Source.	Lab. No.	Killed.	Analyzed.	Age, days.	Moisture.	Ash.	Fat pet. eth. ext.	Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Cold water extract. Total solids.	Organic extractives. Ash.	Organic extractives.	Total N.	Coag. N.	Albumose N.	Meat base N.	Acidity lactic.
Unknown	..	11/9 1907	11/9 1907	0.0	76.78	1.11	1.93	3.41	0.029	.....	6.24	0.97	5.27	0.807	0.444	0.026	0.332	0.63
Choice steer	3813	4/17 1908	4/22 1908	5.0	74.78	1.26	3.34	3.47	0.030	0.010	6.03	1.24	4.79	0.777	0.397	0.022	0.373	0.67
Old		4/17	4/22															
Bull	3814	1908 4/21	1908 4/22	5.0	76.20	1.31	1.08	3.65	0.033	0.011	6.09	1.27	4.82	0.849	0.448	0.027	0.385	0.68
Heifer	3815	1908	1908	1.0	75.26	1 23	2.30	3.58	0.029	0.010	6.02	1.17	4.85	0.795	0.413	0.021	0.363	0.67
Old cow		4/21	4/22															
Old cow	3816	1908 1/14	1908 1/18	1.0	77.27	1.23	0.95	3.46	0.027	0.011	5.55	1.13	4.42	0.742	0.358	0.022	0.360	0.69
Unknown	459	1908 1/14	1908 1/18	4.0	77.17	1.20	1.05	3.45	0.028	0.010	6.16	1.16	5.00	0.854	0.452	0.030	0.383	0.68
"	460	1908 1/26	1908 1/29	4.0	76.96	1.27	0.85	3.46	0.030	0.010	6.15	1.15	5.00	0.840	0.445	0.034	0.376	0.66
"	..	1908 4/2	1908 4/6	3.0	76.28	1.30	1.28	3.43	0.028	0.009	5.78	1.11	4.67	0.778	0.393	0.025	0.367	0.72
"	3263	1908 4/2	1908 4/6	4.0	76.30	1.28	0.78	3.56	0.033	0.011	6.02	1.22	4.90	0.837	0.409	0.024	0.398	0.82
"	3264	1908 10/12	1908 10/15	4.0	76.33	1.27	0.90	3.54	0.032	0.010	6.20	1.23	4.97	0.812	0.394	0.024	0.392	0.78
"	..	1907 3/2	1907 3/9	3.0	76.73	1.13	1.82	3.34	.....	.....	6.02	0.95	5.07	0.766	0.401	0.014	0.363	0.64
"	2326	1908 10/12	1908 10/19	7.0	77.04	1.28	1.06	3.43	0.022	0.010	5.94	1.25	4.69	0.840	0.423	0.019	0.378	0.65
"	..	1907	1907	7.0	75.56	1.10	1.25	3.59	.....	.....	5.89	1.06	4.83	0.787	0.393	0.033	0.355	0.58
Maximum				7.0	77.27	1.31	3.34	3.65	0.033	0.011	6.24	1.27	5.27	0.854	0.452	0.034	0.398	0.82
Minimum				0.0	75.26	1.11	0.78	3.34	0.022	0.009	5.55	0.95	4.42	0.742	0.358	0.014	0.360	0.63
Average				3.7	76.35	1.23	1.43	3.49	0.029	0.010	6.01	1.14	4.87	0.806	0.413	0.024	0.371	0.68

TABLE IV.—PART 2.

ANALYSES FRESH BEEF KNUCKLES. ALL SAMPLES HELD AT 2–4° C. UNTIL ANALYZED. AGE, 0–7 DAYS. FIGURES CALCULATED TO MOISTURE-, ASH- AND FAT-FREE BASIS.

Source.	Lab. No.	Killed.	Analyzed.	Age, days.	Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Organic extractives.	Cold water extract. Total N.	Coag. N.	Albumose N.	Meat base N.	Acidity lactic.
Unknown	....	11/9	11/9										
		1907	1907	0.0	16.90	0.144	.....	26.12	3.99	2.20	0.129	1.65	3.12
		4/17	4/22										
Choice steer	3813	1908	1908	5.0	16.83	0.145	0.048	23.23	3.77	1.92	0.107	1.81	3.25
		4/17	4/22										
Old bull	3814	1908	1908	5.0	17.05	0.154	0.051	22.51	3.96	2.09	0.126	1.80	3.17
		4/21	4/22										
Heifer	3815	1908	1908	1.0	16.88	0.137	0.047	22.87	3.75	1.95	0.099	1.71	3.16
		4/21	4/22										
Old cow	3816	1908	1908	1.0	16.83	0.131	0.053	21.51	3.61	1.74	0.107	1.75	3.36
		1/14	1/18										
Unknown	459	1908	1908	4.0	16.76	0.136	0.049	24.30	4.15	2.20	0.146	1.86	3.30
		1/14	1/18										
"	460	1908	1908	4.0	16.54	0.143	0.048	23.90	4.02	2.13	0.162	1.79	3.15
		1/26	1/29										
"	...	1908	1908	3.0	16.22	0.132	0.043	22.09	3.68	1.86	0.118	1.74	3.41
		4/2	4/6										
"	3263	1908	1908	4.0	16.45	0.152	0.051	22.64	3.87	1.89	0.111	1.84	3.79
		4/2	4/6										
"	3264	1908	1908	4.0	16.47	0.149	0.047	23.12	3.78	1.83	0.112	1.82	3.63
		10/12	10/15										
"	..	1907	1907	3.0	16.44	.....	.....	24.95	3.77	1.97	0.069	1.79	3.15
		3/2	3/9										
"	2326	1908	1908	7.0	16.63	0.107	0.048	22.75	4.07	2.05	0.092	1.83	3.15
		10/12	10/19										
"	..	1907	1907	7.0	16.25	.....	.....	21.87	3.56	1.78	0.149	1.61	2.63
Maximum				7.0	17.05	0.154	0.053	26.12	4.15	2.20	0.162	1.86	3.79
Minimum				0.0	16.22	0.107	0.043	21.51	3.56	1.74	0.069	1.61	2.63
Average				3.7	16.63	0.139	0.049	23.22	3.84	1.97	0.117	1.77	3.25

TABLE IV.—PART 3.

ANALYSES FRESH BEEF KNUCKLES. ALL SAMPLES HELD AT 2–4° C. UNTIL ANALYZED. AGE, 0–7 DAYS. NITROGEN FIGURES CALCULATED TO PER CENT. OF TOTAL NITROGEN.

Source.	Lab. No.	Killed.	Analyzed.	Age, days.	Amm. N. Method 1.	Amm. N. Method 2.	Cold water extract. Tot. N.	Cong. N.	Albumose N.	Heat base N.
		11/9	11/9		%	%	%	%	%	%
Unknown	..	1907	1907	0	0.850	...	23.66	13.02	0.763	9.73
		4/17	4/22							
Choice steer	3813	1908	1908	5	0.865	0.288	23.39	11.44	0.634	10.75
		4/17	4/22							
Old bull	3814	1908	1908	5	0.904	0.301	23.26	12.27	0.740	10.55
		4/21	4/22							
Heifer	3815	1908	1908	1	0.810	0.279	22.20	11.54	0.587	10.14
		4/21	4/22							
Old cow	3816	1908	1908	1	0.780	0.318	21.45	10.35	0.636	10.41
		1/14	1/18							
Unknown	459	1908	1908	4	0.812	0.290	24.75	13.10	0.869	11.10
		1/14	1/18							
"	460	1908	1908	4	0.867	0.289	24.28	12.86	0.983	10.87
		1/26	1/29							
"	..	1908	1908	3	0.816	0.262	22.68	11.46	0.729	10.70
		4/22	4/6							
"	3263	1908	1908	4	0.927	0.309	23.51	11.49	0.674	11.18
		4/2	4/6							
"	3264	1908	1908	4	0.904	0.282	22.94	11.13	0.678	11.07
		10/12	10/15							
"	..	1907	1907	3	...	...	22.96	12.01	0.419	10.87
		3/2	3/9							
"	2326	1908	1908	7	0.641	0.291	24.49	12.33	0.554	11.02
		10/12	10/19							
"	..	1907	1907	7	...	...	21.92	10.95	0.919	9.89
Maximum				7	0.927	0.318	24.75	13.10	0.983	11.18
Minimum				0	0.641	0.262	21.45	10.35	0.419	9.73
Average				3.7	0.834	0.291	23.11	11.83	0.707	10.64

in preparing samples for analysis. This was done, not in order to discard any portion of the sample but solely in the interests of uniformity and it was demonstrated in special analyses that this portion did not differ in composition from the interior, when analyses of both parts were calculated to the same moisture basis. In some of the work the pieces were enclosed in tinned pails in the storage chambers to prevent evaporation of moisture and in the preparation of these for analysis the surface layers were not trimmed away. Thawing of frozen pieces was accomplished at ordinary room temperature and no special precautions were taken in accomplishing it. The general results of the chemical work are shown in Tables IV and V. Table IV shows the results on fresh beef knuckles of ages from naught to seven days. Immediately after slaughter the beef from which these pieces were obtained was put

TABLE V.—PART 1.

ANALYSES OF FROZEN BEEF KNUCKLES. AGE, 33 TO 554 DAYS. TEMPERATURE OF FREEZER, 9–12°.

FIGURES ON BASIS OF ORIGINAL MEAT.

How stored.	Lab. No.	Killed.	Analyzed.	Age, days.	Moisture.	Ash.	Fat pet. eth. ext.	Amm. N. Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Cold water extract. Total solids.	Organic extractives. Ash.	Organic extractives. Total N.	Coag. N.	Albumose N.	Meat base N.	Acidity lactic.
		10/12	1/22														
Open freezer	..	1906	1907	102	77.02	1.20	1.80	3.64	0.030	.....	6.07	1.14	4.93	0.803	0.419	0.022	0.350	0.72
		10/12	1/13														
"	..	1906	1908	458	75.74	1.23	2.12	3.42	0.024	0.012	5.59	0.96	4.53	0.733	0.361	0.023	0.337	0.92
		10/12	1/13														
"	..	1906	1908	458	76.72	1.23	2.02	3.32	0.025	0.012	5.80	1.14	4.66	0.730	0.353	0.026	0.343	0.92
		10/12	3/27														
"	2925	1906	1908	529	76.59	1.24	1.73	3.50	.....	0.012	5.94	1.15	4.79	0.836	0.449	0.022	0.359	0.77
		10/12	3/27														
"	2926	1906	1908	529	75.32	1.29	2.13	3.61	0.029	0.013	6.27	1.09	5.18	0.861	0.468	0.021	0.360	0.74
		10/12	4/15														
"	3605	1906	1908	548	75.49	1.27	1.87	3.53	0.025	0.012	6.21	1.19	5.02	0.770	0.381	0.021	0.371	0.73
		10/12	4/15														
"	3606	1906	1908	548	76.83	1.25	1.05	3.47	0.024	0.012	5.98	1.18	4.80	0.828	0.458	0.025	0.379	0.66
		10/12	4/21														
"	3801	1906	1908	554	75.40	1.23	2.03	3.65	0.031	0.012	6.05	1.22	4.83	0.812	0.418	0.026	0.369	0.79
In hermetically sealed tinned		2/11	3/16														
pails	2546	1908	1908	33	76.76	1.20	1.17	3.31	0.026	0.009	5.56	1.16	4.40	0.766	0.373	0.023	0.359	0.72
In hermetically sealed tinned		2/11	4/14														
pails	3551	1908	1908	62	77.11	1.16	1.65	3.38	0.031	0.011	5.88	1.14	4.74	0.819	0.432	0.025	0.365	0.80
In closed glass		8/20	4/20														
jars	3735	1907	1908	212	76.66	1.22	1.31	3.65	0.030	0.010	5.98	1.09	4.89	0.795	0.420	0.021	0.350	0.88
In closed glass		8/20	4/20														
jars	3736	1907	1908	212	77.03	1.28	1.30	3.65	0.031	0.010	6.06	1.21	4.85	0.798	0.426	0.023	0.349	0.88
Maximum	..	.....	....	554	77.11	1.29	2.12	3.65	0.031	0.013	6.27	1.21	5.18	0.861	0.468	0.026	0.379	0.92
Minimum	..	.....	....	33	75.32	1.16	1.17	3.31	0.024	0.009	5.56	0.96	4.40	0.730	0.353	0.021	0.337	0.66
Average	..	.....	....	353	76.39	1.23	1.65	3.51	0.028	0.011	5.94	1.14	4.80	0.795	0.413	0.023	0.357	0.79

TABLE V.—PART 2.

ANALYSES FROZEN BEEF KNUCKLES. AGE, 33 TO 554 DAYS. TEMPERATURE OF FREEZER, 9–12°.
FIGURES CALCULATED TO MOISTURE-, ASH- AND FAT-FREE BASIS.

How stored.	Lab. No.	Killed.	Analyzed.	Age, days.	Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Organic extractives.	Cold water extract. Total N.	Coag. N.	Albumose N.	Meat base N.	Acidity lactic.
		10/12	1/22										
Open freezer	....	1906	1907	102	18.22	0.150	.....	24.67	4.02	2.10	0.110	1.75	3.60
		10/12	1/13										
"	....	1906	1908	458	16.36	0.115	0.057	21.67	3.51	1.73	0.110	1.61	4.40
		10/12	1/13										
"	....	1906	1908	458	16.58	0.125	0.060	23.27	3.64	1.76	0.130	1.71	4.59
		10/12	3/27										
"	2925	1906	1908	529	17.12	.....	0.059	23.43	4.09	2.19	0.108	1.76	3.77
		10/12	3/27										
"	2926	1906	1908	529	16.98	0.136	0.061	24.36	4.05	2.20	0.099	1.69	3.48
		10/12	4/15										
"	3605	1906	1908	548	16.52	0.117	0.056	23.49	3.60	1.78	0.098	1.74	3.41
		10/12	4/15										
"	3606	1906	1908	548	16.63	0.115	0.057	23.00	3.97	2.19	0.119	1.82	3.12
		10/12	4/21										
"	3801	1906	1908	554	17.10	0.145	0.056	22.63	3.80	1.96	0.121	1.73	3.70
In hermetically		2/11	3/16										
sealed tinned pails	2546	1908	1908	33	15.86	0.124	0.043	21.08	3.67	1.79	0.110	1.72	3.45
In hermetically		2/11	4/14										
sealed tinned pails	3551	1908	1908	62	16.83	0.154	0.055	23.60	4.08	2.15	0.124	1.82	3.98
In closed glass		8/20	4/20										
jars.	3735	1907	1908	212	17.54	0.144	0.041	23.50	3.82	2.02	0.101	1.68	4.22
In closed glass		8/20	4/20										
jars.	3736	1907	1908	212	17.90	0.152	0.049	23.78	3.91	2.09	0.112	1.71	4.31
Maximum	....	.....	....	554	18.22	0.154	0.061	24.67	4.09	2.20	0.130	1.82	4.59
Minimum	....	.....	....	33	15.86	0.115	0.041	21.08	3.51	1.73	0.098	1.61	3.12
Average	....	.....	....	353	16.97	0.133	0.049	23.20	3.93	1.99	0.111	1.73	3.83

TABLE V.—PART 3.

ANALYSES FROZEN BEEF KNUCKLES. AGE, 33 TO 554 DAYS. TEMPERATURE OF FREEZER, 9–12° C. NITROGEN FIGURES AS PERCENTAGES OF TOTAL NITROGEN.

How stored.	Lab. No.	Killed.	Analyzed.	Age, days.	Amm. N. Method 1. %	Amm. N. Method 2. %	Cold water extract. Total N. %	Coag. N. %	Albumose N. %	Meat base N. %
		10/12	1/22							
Open freezer.....	..	1906	1907	102	0.824	...	22.06	11.51	0.604	9.62
		10/12	1/13							
"	..	1906	1908	458	0.702	0.351	21.43	10.56	0.673	9.85
		10/12	1/13							
"	..	1906	1908	458	0.753	0.361	21.99	10.63	0.783	10.33
		10/12	3/27							
"	2925	1906	1908	529	...	0.343	23.89	12.83	0.628	10.26
		10/12	3/27							
"	2926	1906	1908	529	0.803	0.360	23.85	12.96	0.582	9.97
		10/12	4/15							
"	3605	1906	1908	548	0.708	0.340	21.81	10.79	0.595	10.51
		10/12	4/15							
"	3606	1906	1908	548	0.692	0.346	23.86	13.20	0.720	10.92
		10/12	4/21							
"	3801	1906	1908	554	0.849	0.329	22.25	11.45	0.712	10.11
		2/11	3/16							
In hermetically sealed tinned pails.....	2546	1908	1908	33	0.785	0.271	23.14	11.27	0.695	10.85
		2/11	4/14							
In hermetically sealed tinned pails.....	3551	1908	1908	62	0.917	0.325	24.23	12.78	0.739	10.80
		8/20	4/20							
In closed glass jars...	3735	1907	1908	212	0.822	0.274	21.78	11.51	0.575	9.59
		8/20	4/20							
In closed glass jars...	3736	1907	1908	212	0.849	0.274	21.86	11.67	0.630	9.56
Maximum				554	0.917	0.361	24.23	13.20	0.783	10.92
Minimum				33	0.702	0.271	21.43	10.56	0.575	9.56
Average				353	0.791	0.325	22.68	11.76	0.661	10.19

into ordinary coolers whose temperature varied from 2 to 4° and the meat was cut in the same temperature and held until analyzed. The cutting was usually done forty-eight hours after slaughter. Table V shows the results on the frozen samples, as far as they have been obtained, although the work will continue and analyses be made at much greater ages. In the case of frozen meats the treatment was the same as in the case previously described up to the time of cutting, when the pieces were hung in the freezer (temperature —9° to —12°) where they remained up to the time the analyses were made. The oldest knuckle analyzed was five hundred and fifty-four days or somewhat more than a year and a half old and considerably older than meat is usually held in the frozen condition. The average age is a little less than one year. Table VI shows a summary and comparison of the maximum and minimum fig-

TABLE VI.

SUMMARY OF RESULTS OF ANALYSES OF FRESH BEEF KNUCKLES AND FROZEN BEEF KNUCKLES.

PART 1. ON BASIS OF ORIGINAL MEAT.

	No. samples.	Age, days.	Moisture.	Ash.	Fat.	Total N.	Amm. N. Method I.	Amm. N. Method II.	Cold water extract. Total solids.	Ash.	Organic extractives.	Total N.	Coag. N.	Albumose N.	Meat base N.	Acid as lactic.
Fresh—Maximum	13	7	77.27	1.31	3.34	3.65	0.033	0.011	6.24	1.27	5.27	0.854	0.452	0.034	0.398	0.82
Minimum		0	75.26	1.11	0.78	3.34	0.022	0.009	5.55	0.95	4.42	0.742	0.358	0.014	0.360	0.63
Average		3.7	76.35	1.23	1.43	3.49	0.029	0.010	6.01	1.14	4.87	0.806	0.413	0.024	0.355	0.68
Frozen—Maximum	12	554	77.11	1.29	2.12	3.65	0.031	0.013	6.27	1.21	5.18	0.861	0.468	0.026	0.379	0.92
Minimum		33	75.32	1.16	1.17	3.31	0.024	0.009	5.56	0.96	4.40	0.730	0.353	0.021	0.337	0.66
Average		353	76.39	1.23	1.65	3.51	0.028	0.011	5.94	1.14	4.80	0.795	0.413	0.023	0.357	0.79

PART 2. ON MOISTURE-, ASH- AND FAT-FREE BASIS.

	No. samples.	Age, days.	Total N.	Amm. N. Method 1.	Amm. N. Method 2.	Cold water extract. Organic extractives.	Total N.	Coag. N.	Albumose N.	Meat base N.	Acid as lactic.
Fresh—Maximum	13	7	17.05	0.154	0.053	26.12	4.15	2.20	0.162	1.86	3.79
Minimum		0	16.22	0.107	0.043	21.51	3.56	1.74	0.069	1.61	2.63
Average		3.7	16.63	0.139	0.049	23.22	3.84	1.99	0.117	1.77	3.25
Frozen—Maximum	12	554	18.22	0.154	0.061	24.67	4.09	2.20	0.130	1.82	4.59
Minimum		33	15.86	0.115	0.041	21.08	3.51	1.73	0.098	1.61	3.12
Average		353	16.93	0.133	0.049	23.20	3.93	1.99	0.111	1.73	3.83

PART 3. NITROGEN FIGURES AS PERCENTAGES OF THE TOTAL NITROGEN.

	No. samples.	Age, days.	Amm. N. Method 1.	Amm. N. Method 2.	Cold water extract. Total N.	Coag. N.	Albumose N.	Meat base N.
Fresh—Maximum	13	7	0.927	0.318	24.75	13.10	0.983	11.18
Minimum		0	0.641	0.262	21.45	10.35	0.419	9.73
Average		3.7	0.834	0.291	23.11	11.83	0.707	10.64
Frozen—Maximum	12	554	0.917	0.361	24.23	13.20	0.783	10.92
Minimum		33	0.702	0.271	21.43	10.56	0.575	9.56
Average		353	0.791	0.325	22.68	11.76	0.661	10.19

ures and averages of the results obtained on both fresh and frozen knuckles.

A general inspection of the tables shows very little difference on the whole between the analyses of fresh and frozen samples. The variations in the individual fresh samples are hardly greater than would be expected in material of this kind into whose composition so many factors enter, and the differences between maxima and minima in fresh and frozen samples are of the same order. In general, the variations do not tend definitely in one direction in the frozen samples, the ammoniacal nitrogen, the coagulable nitrogen and the albumose nitrogen figures do not show a progressive tendency to increase or decrease. The largest difference between the analyses of fresh and frozen samples occurs in the acidity figures which are larger on the average by a small amount in the case of the frozen samples; but even here no progressive tendency is shown, since the oldest samples do not show the highest acidity, nor the youngest the lowest. Nor is there any indication of the acidity reaching a maximum at a certain age and then falling off. It is possible that the analysis of a larger number of samples would produce agreeing averages in the case of acidity. It should also be mentioned that on account of the nature of the acidity determination and the lack of sharpness in the end point, even when using phenolphthalein, it is a questionable analytical procedure to magnify the minute differences as shown in the original determinations by calculating to the moisture-, ash- and fat-free basis. It is, in general, a doubtful procedure, to magnify the necessary errors of analytical methods. We attach no importance to the slight differences in the acid figures.

A detailed discussion of the results follows:

Age of the Samples.—The fresh samples varied in age from 0 to 7 days and from the time of slaughter were stored in a temperature which varied from two to four degrees centigrade. We had shown in another series of experiments that meat seven days old and even much older stored under these conditions was entirely wholesome and fit for consumption, judged as meat usually is, by appearance, consistency, smell and taste. The frozen samples varied from thirty-three to five hundred and fifty-four days in age and were stored while frozen in a room whose temperature varied from minus nine to minus twelve degrees centigrade. The temperature of this room was taken at regular intervals during the day and was under good control.

Moisture.—The maximum amount of moisture found in the fresh knuckles was 77.27 per cent., minimum 75.26 per cent. and average 76.35 per cent. In the case of the frozen samples the figures were: maximum 77.11, minimum 75.32 and average 76.39 per cent. The exterior dried to a depth of from two to four millimeters in the course of a year in the open freezer, but thereafter the progress of desiccation was ex-

tremely slow. The moisture content of the desiccated portion was found to be about 30 per cent. The line of demarcation between the desiccated exterior and the interior was always sharply defined. Holding the samples in glass jars or tinned pails, with reasonably close-fitting covers or hermetically sealed, effectually prevented surface desiccation. The general agreement in the moisture figures requires no special comment. It indicates no loss or gain of moisture in the stored samples.

Ash.—The ash figures for fresh beef knuckles are: maximum 1.31 per cent., minimum 1.11 per cent., average 1.23 per cent.; for the frozen samples: maximum 1.29 per cent., minimum 1.16 per cent., average 1.23 per cent. We believe that, with the method used and on account of the care taken in making the determinations, not only are the results comparable but they are quite accurate. Potassium phosphate and probably secondary potassium phosphate is the chief component of the ash. This, when heated to a red heat loses 5.46 per cent. water and is transformed into potassium pyrophosphate. However, in our determinations a full red heat was not applied and the soluble ash was finally dried at a temperature of 105°. No change would be expected in the ash of meats even under more drastic conditions than cold storage at temperatures below the freezing point, and the agreeing results bear this out.

Fat.—The fat, as would be expected, varies more than any other constituent of meats. Even after trimming away all the larger growths of fatty tissue, as was done in preparing samples for analysis, the amounts found closely interwoven with the muscular tissue, varied considerably. The figures for fresh meat are: maximum 3.34, minimum 0.78, average 1.43 per cent.; for frozen meat: maximum 2.12, minimum 1.17 and average 1.65 per cent. The sample containing the maximum amount came from a choice steer. It is needless to say that the variation in the fat figures has no bearing upon the present research and the figures are used merely as a basis in calculating.

Total Nitrogen.—Other things being equal, the nitrogen figures will vary inversely as the sum of the moisture, ash and fat figures. For fresh meat the maximum amount found was 3.65 per cent., minimum 3.34 and average 3.49 per cent.; for frozen samples the maximum was 3.65 per cent., minimum 3.31 and average 3.51 per cent. We have not followed the prevalent practice of multiplying by a fixed quantity to convert the nitrogen figures into proteins, since for our purpose the figures found are as useful as the others would be in making comparisons. We have used the total nitrogen figures mainly as a basis of calculation in determining the ratios of other nitrogen figures to this one.

Ammoniacal Nitrogen.—We attach considerable importance to the ammoniacal nitrogen determination by both the methods which we have used, although these methods are comparative and not absolute. We

convinced ourselves in the beginning that whenever decomposition of meat occurs to any noticeable extent, there occurs a formation of volatile basic substances (principally ammonia) which increases with the degree of decomposition. Hashed meats and whole pieces were stored under different conditions and the ammoniacal nitrogen determined by our methods at different intervals, and in all cases, above the freezing point, a progressive increase (with age) in the amount of ammoniacal nitrogen was recorded. These results will be published in a later paper, which will discuss the deterioration of flesh foods at temperatures above the freezing point of water. It would be incorrect to say that all the nitrogen reported under the head of "ammoniacal" is derived from ammonia, but in all probability the bulk of it is so derived from and exists in the decomposed meats as ammonium salts. In the distillate from meat which has undergone noticeable decomposition, the odor of acetamide is marked.

As the method of determining ammoniacal nitrogen is refined to eliminate other basic substances and to prevent hydrolysis, as by adding alcohol and salt and distilling *in vacuo*, the quantity found becomes progressively less. We conclude from our experiments that the amount of nitrogen in ammonium salts in fresh lean beef is less than one part in ten thousand and is possibly none at all. Perfectly fresh hashed beef extracted with boiling ammonia-free water shows immediately an intense yellow color which quickly fades into a dirty green precipitate when tested with Nessler's reagent. Ammonia, if the above test indicates ammonia, may have been produced during extraction by hydrolysis. The maximum amount of ammoniacal nitrogen found by our first method in fresh meat was 0.033 per cent., minimum 0.022 per cent., average 0.029 per cent. In the frozen samples we found maximum 0.031, minimum 0.024, average 0.028 per cent. The figures found by the second method are between one-half and one-third of these and preserve nearly the same ratios and are in as good agreement. We conclude that no change in composition is shown by the ammoniacal nitrogen figures in the samples analyzed and hence that no bacterial decomposition occurred. This work will be continued for a number of years, and we may have occasion to change our opinion later, but from the data obtained and the absence of progressive change in these figures, we see no reason to expect an increase or decrease in the ammoniacal nitrogen figures within any reasonable length of time.

Solids in the Cold Water Extract.—While on account of our work on the composition of beef juice, a part of which has been previously described, we cannot believe that the cold water extract method yields absolute results in the sense that it extracts all the substances which exist in solution in the meat, nevertheless we believe that when deter-

minations are carried out under strictly comparable conditions, comparative results may be obtained. For example, determinations on duplicate extractions which we carried out side by side (or on succeeding days) always agreed extremely well. The significance of the amount of solids in the cold water extract is that these increase, although by no means with great regularity, with the age of decomposing meat (unpublished results). The maximum amount found by our method in the case of fresh beef knuckles was 6.24, minimum 5.55 and average 6.01 per cent. From the frozen samples cold water extracted as a maximum 6.27, minimum 5.56, average 5.94 per cent. These figures are in close agreement. It is likely that the acidity of the meat has a definite effect upon the percentage of extractives soluble in cold water, but it is not the only factor affecting the determination and we have not been able to show a very definite relationship in the direction indicated.

Ash in Cold Water Extract.—Ash determinations in the extracts from fresh and frozen samples are in good agreement and the average figures are identical. There is about one-tenth per cent. difference, on the average, between the ash in the original meat and in the extract.

Organic Extractives in the Cold Water Extract.—This figure is obtained indirectly by subtracting the ash figures from total solids figures and indicates nothing which is not indicated by the figures obtained directly.

Total Nitrogen in the Cold Water Extract.—In meat undergoing bacterial decomposition the total nitrogen in the cold water extract increases with the age of the sample, and also increases more or less proportionally with the total solids in normal samples. In the fresh meat analyses our figures show a maximum total nitrogen in the extract of 0.854 per cent., a minimum of 0.742 per cent., and an average of 0.806 per cent. In the frozen samples the maximum is 0.861 per cent., minimum 0.730 per cent., average 0.795 per cent. These figures agree very well and require no special comment.

Coagulable Nitrogen in the Cold Water Extract.—With advancing decomposition of meat there is an increase in the coagulable nitrogen content of the cold water extract, although this increase would hardly be expected. It would appear that proteins previously insoluble in water were brought into solution (through bacterial action) in coagulable condition. The fact is interesting, but considering our insufficient knowledge of meat proteins and of the effects of change of reaction on their solubility, too great stress should not be laid upon it. We found in the fresh samples a maximum of 0.452 per cent., a minimum of 0.358 per cent., and an average of 0.413 per cent. coagulable nitrogen in the cold water extract. In the frozen samples the figures are maximum 0.468 per cent., minimum 0.353 per cent. and average 0.413 per cent.

Albumose Nitrogen in the Cold Water Extract.—The proteolytic en-

zymes of bacteria have the power of rendering soluble such substances as fibrin, gelatin and coagulated albumen, and hence it is to be expected that in decomposing meat an increase in albumose nitrogen will occur at some period of the decomposition. Bacterial action, however, does not rest with the formation of albumoses, but these compounds are decomposed further into simpler substances, principally amino acids. During decomposition of meat the albumose nitrogen represents the balance between coagulable proteins and the mixed group which we have termed "meat bases." So far as we have followed the changes worked in meat by bacterial action there is a small but definite and progressive increase in the amount of albumoses precipitable by zinc sulphate. Other things being equal, this increase taken together with the increase in ammoniacal nitrogen and total nitrogen in the cold water extract is a measure of the extent of decomposition. Our figures for fresh beef knuckles show a maximum of 0.034 per cent., a minimum of 0.014 per cent., and an average of 0.024 per cent. The figures for the frozen samples are: maximum 0.026, minimum 0.021, average 0.023 per cent. For the fresh samples the maximum is higher and the minimum is lower than, but the average nearly the same as, the corresponding figures for the frozen samples.

Meat Base Nitrogen.—The "meat base" nitrogen in our tables represents nitrogen in soluble compounds which are not coagulated by heat nor precipitated in a saturated solution of zinc sulphate. In the fresh samples we found a maximum of 0.398, a minimum of 0.360 and an average of 0.355 per cent. In the frozen samples the maximum was 0.379, the minimum 0.337 and the average 0.357 per cent.

Acidity (calculated as lactic acid).—We calculate the acidity of meat determined using phenolphthalein as indicator, to lactic acid, as a matter of convenience and for the sake of comparison with other investigators. Whether the entire acidity of the cold water extract of meat is due to sarcolactic acid or not we do not know at this time. It has been suggested that a part of the acidity is due to phosphoric acid, but it is probable that the principal salt of phosphoric acid present is the secondary potassium compound, which is neutral to phenolphthalein. Practically all the viscera of the food animals at the time of slaughter are neutral to litmus; that is, if touched on the surface or interior with moist neutral litmus paper this does not change color. At the same time the meat is acid to phenolphthalein and if an extract be promptly made of it and titrated, using this indicator, it is found to be of about the same acidity as when the meat is older. An important change in the reaction of meat juice toward litmus occurs after it has been stored at refrigerator temperature (2–4°) for some time, since it becomes acid in reaction toward this indicator.

In order to determine whether or not there was any change in flavor

in frozen beef after storage which would distinguish it from fresh beef, a frozen beef knuckle six hundred and ten days old was boiled without seasoning and eaten. The flavor was identical with that of fresh beef nor was it possible for one not knowing the age or source of the meat to distinguish it from fresh meat. We may say that similar tests of frozen poultry have resulted similarly.

On the whole the results of the various lines of work reported in this paper, chemical, histological and bacteriological, indicate that cold storage, at temperatures below —0.9° C. at least, is an adequate and satisfactory method for the preservation of beef for a period of five hundred and fifty-four days and probably for a much longer time.

LABORATORY OF SWIFT AND COMPANY,
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[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE.]

THE INVERSION OF CANE SUGAR BY INVERTASE, II.[1]

BY C. S. HUDSON.

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CONTENTS.

1. *Summary of Previous Work on the Laws of Action of Invertase.*— In most plants and animals there occurs a substance that changes cane sugar to invert sugar, which is a mixture of equal parts of dextrorotatory *d*-glucose and levorotatory *d*-fructose.[2]

This inverting substance, which plays such an important part in plant and animal life, has never been obtained in a pure condition because it does not crystallize from its solutions. Although there is some evidence indicating it to be an albuminoid its exact composition is unknown. Its synthesis takes place exclusively in the tissues of living organisms. Its presence in plants and animals is known only from the fact that cer-

[1] Published by permission of the Secretary of Agriculture. Read at the New Haven meeting of the American Chemical Society, June 30–July 2, 1908.

[2] For proof of its very wide if not universal distribution in the plant world see Kastle and Clark, *Amer Chem. J.*, 30, 422–427 (1903). It occurs in the intestinal walls of all mammalian animals that have been examined. For its occurrence in bees, locusts, butterflies, spiders and many other insects (excluding, however, the silk worm) see Axenfeld, *Zentralblatt für Physiologie*, **17**, 268 (1903). O'Sullivan and Thompson state that "probably all organisms which have the power of assimilating cane sugar contain invertase."