Had not the first reduction been complete, the results would not have agreed so well.

The mean atomic value from the hexachloride is 184.04, that from the oxidation of metal 184.065, or the average of the two independent series is 184.05, which probably approximates the truth very closely and may be safely regarded as the atomic weight of tungsten.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

THE NITROGENOUS CONSTITUENTS OF FLESH.[1]

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(PRELIMINARY PAPER.)

THE results reported in this paper form a part of an extended investigation which has for its object a study of the chemistry of the nitrogenous constituents of the flesh of meats. The present knowledge of both the proteïd and the non-proteïd substances occurring in the animal body is very incomplete. The past researches upon the proteïds of animal substances have been almost, if not entirely, confined on the one hand to the proteïds of blood, and on the other hand to the proteïds of muscle freed from blood. However, flesh as sold for food always contains more or less blood. From the standpoint of physiological chemistry, in the study of the chemistry of the digestion of meats, and also in the study of the nutritive value of foods, it is highly desirable that the present very limited knowledge of the nitrogenous principles of flesh, as they exist in meat as used for food, be increased.

As it was impossible to find any definite data regarding the extent of the solubility of the different nitrogenous constituents of flesh in cold or hot water or in dilute solutions of acids, alkalies or salts, it was deemed necessary for the future work of this investigation to study somewhat carefully this question. In the first place this was done by extracting flesh successively with the following reagents: Cold water, 10 per cent. sodium chloride solution, 0.15 per cent. hydrochloric acid solution, 0.15 per cent. potassium hydroxide solution and, lastly, with hot water.

[1] The expenses in connection with this research have been in part defrayed out of a grant from the Elizabeth Thompson Science Fund.

DESCRIPTION AND PREPARATION OF THE SAMPLES OF MEATS.

Two samples of lean beef round from animals about two years old and one sample of cooked meat were taken. The first sample of raw meat was given laboratory No. 1265, the second sample was labeled No. 1266 and the sample of cooked meat was given laboratory No. 1267. The two last samples were from the same cut. The sample No. 1267 was cooked by plunging the piece of raw meat into boiling water, keeping it at that temperature for ten minutes, then gradually reducing the temperature to 85° C. and finally cooking it at this temperature for five hours. In preparing the samples for the investigation all bone, gristle and fat were removed and the meat was cut into strips and run through a sausage mill three times. A portion of each sample was air-dried and completely analyzed by the ordinary methods. The results of the analyses are given in the following table:

TABLE I.—CHEMICAL COMPOSITION OF THE ORIGINAL MATERIAL OF MEATS.

Laboratory No.	Kind of meat.	Water. Per cent.	Proteid. N×6.25. Per cent.	Fat. Per cent.	Ash. Per cent.	Total. Per cent.	Total nitrogen. Per cent.
1265	Beef, raw	77.12	19.96	1.46	1.08	99.62	3.190
1266	do	74.54	22.56	1.50	1.22	99.82	3.610
1267	Beef, boiled	58.34	37.70	3.05	0.89	99.98	6.030

EXTRACTION WITH COLD WATER.

Weighed portions in duplicate of the three samples of meat were extracted with ice-cold, nitrogen-free water. The temperature of the water was not allowed to rise above 10° C. at any time during the extraction. The mixture was thoroughly stirred every fifteen minutes during the extraction and the undissolved residue was removed by filtration. The residue, after the first extraction, was again treated with water as before and filtered a second time. The extraction with cold water was continued until the final extract showed that practically nothing further went into solution.

METHODS OF ANALYSIS OF THE COLD WATER EXTRACTS.

The cold water extracts were diluted to a definite volume and then used for the following determinations. The total quantity of solid matter dissolved from the meat was determined by evaporating measured volumes of the aqueous solution to dryness upon the water-bath and then drying to constant weight in the water-oven. The quantities of organic matter and ash in the extracts

were estimated by igniting the residues from the above determinations. The total nitrogen was determined by the ordinary Kjeldahl method.

The proteïds coagulated by heat were estimated by heating measured volumes of the aqueous extracts upon the water-bath until the coagulum settled completely. The evaporation was usually continued until the volume of the original solution was reduced about one-half. The supernatant liquid was then carefully neutralized with N/10 sodium hydroxide, using litmus paper as an indicator. The solution was again warmed upon the water-bath for a few minutes, filtered and thoroughly washed with hot water. The nitrogen in the coagulated residue was determined by the Kjeldahl method.

The albumoses were determined by evaporating the filtrate from the above determination to a volume of about 30 cc. and precipitating the slightly acidified solution by the addition of zinc sulphate to complete saturation. The saturated solution was warmed upon the water-bath until it became perfectly clear, allowed to stand twelve hours, filtered and washed with a saturated solution of zinc sulphate. The resulting precipitate was Kjeldahled.

The peptones were determined by diluting the filtrate from the zinc sulphate precipitate with an equal volume of water and then precipitating with bromine. For the time being it has been considered that the sum of the coagulated proteïds, albumoses and peptones, as determined by the methods briefly described above, represent the total proteïd matter. The results of the analyses are given in the following table:

TABLE II.—RESULTS OF ANALYSES OF COLD WATER EXTRACTS OF MEAT.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Coagulable proteïds	2.18	2.79	0.05
Albumoses	0.08	0.24	0.12
Peptones	0.03	0.03	0.10
Total proteïd matter	2.29	3.06	0.27
Flesh bases	1.05	1.00	0.87
Acid (calculated as lactic)	1.09	1.33	1.14
Ash	1.14	1.25	0.85
Total solids by summation	5.57	6.64	3.13
Total solids by direct determination	6.41	7.38	3.42
Other substances by difference	0.84	0.74	0.29

TABLE III.—NITROGEN RECORD OF COLD WATER EXTRACTS OF MEAT.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Nitrogen as coagulated proteïd....	0.3485	0.4454	0.0080
Nitrogen as albumose	0.0126	0.0387	0.0190
Nitrogen as peptones.....	0.0043	0.0053	0.0160
Total proteïd nitrogen.....	0.3654	0.4894	0.0430
Nitrogen as flesh bases (direct determination).....	0.3365	0.3205	0.2788
Total nitrogen by summation	0.7019	0.8099	0.3218
Total nitrogen by direct determination	0.7351	0.9175	0.3656
Apparent loss of nitrogen during analysis.....	0.0331	0.1076	0.0438
Nitrogen as flesh bases (by difference)	0.3696	0.4281	0.3226

EXTRACTION WITH A 10 PER CENT. SODIUM CHLORIDE SOLUTION.

The residues from the three samples of flesh, laboratory Nos. 1265, 1266 and 1267, after complete extraction with cold water, were extracted with a 10 per cent. solution of sodium chloride. The details of the extraction were the same as used above in the preparation of the cold water extracts, but it was found necessary to make a greater number of extractions before dissolving out all or most of the proteïds soluble in the sodium chloride solution.

At first an attempt was made to determine the end of the extraction by evaporating a portion of one of the last filtrates to dryness. This method would not work well on account of the large amount of salt present. The biuret test, and the potassium ferrocyanide and acetic acid test also failed to give satisfactory results, as they were not sufficiently delicate. Finally, a portion of the last filtrates were tested for nitrogen by the Kjeldahl method, and this proved to be the most satisfactory method of determining the completion of the extraction with sodium chloride solution. When the extraction was complete, the residues remaining insoluble in the salt solution were thoroughly washed with water until the wash-water was free from sodium chloride. The wash-liquid thus obtained was added to the sodium chloride extract and the whole diluted to definite volume.

ANALYSIS OF THE 10 PER CENT. SODIUM CHLORIDE EXTRACTS OF MEAT.

The total nitrogen proteïds precipitated by heat coagulation, proteïds precipitated by acidifying the filtrate from the heat co-

agulation with sulphuric acid, albumoses precipitated by saturating the filtrate, from the precipitate produced by sulphuric acid, with zinc sulphate, proteïds precipitated by bromine in the filtrates from the zinc sulphate precipitates and the nitrogen not precipitated by the above reagents were determined in the sodium chloride extracts. A condensed summary of the analytical results is given in Tables IV and V.

TABLE IV.—RESULTS OF THE ANALYSES OF 10 PER CENT. SODIUM CHLORIDE EXTRACTS OF MEAT.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Proteïds coagulated by heat in neutral solutions	3.88	4.39	0.08
Proteïds precipitated by acids in filtrate from above	0.64	0.85	0.05
Proteïds precipitated by zinc sulphate	0.12	0.12	0.07
Proteïds precipitated by bromine	0.09	0.08	0.04
Proteïds not precipitated by the above reagents	0.29	0.96	0.69

TABLE V.—NITROGEN RECORD OF 10 PER CENT. SODIUM CHLORIDE EXTRACTS OF MEAT.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Nitrogen as coagulated proteïd	0.6205	0.7015	0.0120
Nitrogen as proteïd precipitated by acids	0.1029	0.1355	0.0075
Nitrogen as proteïd precipitated by zinc sulphate	0.0192	0.0189	0.0113
Nitrogen as proteïd precipitated by bromine	0.0138	0.0125	0.0068
Nitrogen as proteïd not precipitated by the above reagents	0.0451	0.1536	0.1104
Total nitrogen by summation	0.8015	1.0220	0.1480
Total nitrogen by direct determination	0.8449	1.1101	0.0886
Apparent loss of nitrogen during analysis	0.0434	0.0881	0.0594

EXTRACTION WITH 0.15 PER CENT. HYDROCHLORIC ACID SOLUTION.

The thoroughly washed residues from the samples of flesh, laboratory Nos. 1265, 1266 and 1267 remaining insoluble in a 10 per cent. solution of sodium chloride, were extracted with a 0.15 per cent. hydrochloric acid solution. The details of the ex-

traction were the same as used above in the preparation of the cold water and sodium chloride extracts. The acid extracts were diluted to definite volume and portions of the acid solution were used for the following determinations: Total solids, ash, total nitrogen, coagulable proteïds, albumoses, peptones and nitrogen not precipitated by the above reagents. A condensed summary of the analytical results is given in Tables VI and VII.

TABLE VI.—RESULTS OF ANALYSES OF 0.15 PER CENT. HYDROCHLORIC ACID EXTRACTS OF MEATS.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Proteïds coagulated by heat in slightly acid solutions	1.79	2.12	1.16
Proteïds precipitated by zinc sulphate	0.05	0 41	0.84
Proteïds precipitated by bromine	0.07	0.09	0.05
Proteïds not precipitated by above reagents	0.11	0.11	0.14
Total proteïd by summation	2.02	2.73	2.19
Ash	0.51	0.35	0.46
Total solids by summation	3.53	3.08	2.65
Total solids by direct determination	3.70	3.15	3.32
Other substances by difference	0.17	0.07	0.67
Total proteïd, direct determination (total N $\times$ 6.25)	2.20	2.37	2.30

TABLE VII.—NITROGEN RECORDS OF 0.15 PER CENT. HYDROCHLORIC ACID EXTRACTS OF MEATS.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Nitrogen as coagulated proteïd	0.2861	0.3393	0.1842
Nitrogen as proteïd precipitated by zinc sulphate	0.0085	0.0647	0.1329
Nitrogen as proteïd precipitated by bromine	0.0107	0.0143	0.0071
Nitrogen not precipitated by above reagents	0.0112	0.0152	0.0226
Total nitrogen by summation	0.3165	0.4335	0.3468
Total nitrogen by direct determination	0.3603	0.3855	0.3669
Apparent loss (—) or gain (+) of N during analysis	—0.0438	+0.0480	—0.0201

EXTRACTION WITH 0.15 PER CENT. POTASSIUM HYDROXIDE SOLUTION.

The thoroughly washed meat residues not dissolved by treating with 0.15 per cent. hydrochloric acid were now extracted with a 0.15 per cent. solution of potassium hydroxide. The details of the extraction were the same as followed in the above extractions. The alkaline extract was diluted to a definite volume and portions used for the following determinations: Total nitrogen, proteïds precipitated in slightly acid solution, albumoses, peptones and nitrogen not precipitated by the reagents used in the above determinations. A summary of the results is tabulated below in Tables VIII and IX.

TABLE VIII.—RESULTS OF ANALYSES OF 0.15 PER CENT. POTASSIUM HYDROXIDE EXTRACTS OF MEAT.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Proteïds precipitated in slightly acid solutions	1.48	2.13	3.39
Proteïds precipitated by zinc sulphate	0.71	0.25	0.87
Proteïds precipitated by bromine	0.59	0.09	0.08
Proteïds not precipitated by above reagents	0.51	0.41	0.78
Total proteïd by summation	3.29	2.88	5.12
Total proteïd, direct determination (total N × 6.25)	3.61	2.15	4.84

TABLE IX.—NITROGEN RECORDS OF 0.5 PER CENT. POTASSIUM HYDROXIDE EXTRACTS OF MEATS.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Nitrogen as proteïds precipitated in very slightly acid solution	0.2370	0.3398	0.5419
Nitrogen as proteïd precipitated by zinc sulphate	0.1125	0.0396	0.1391
Nitrogen as proteïds precipitated by bromine	0.0944	0.0138	0.0126
Nitrogen not precipitated by above reagents	0.0773	0.0648	0.1252
Total nitrogen by summation	0.5212	0.4570	0.8188
Total nitrogen by direct determination	0.5771	0.3509	0.7739
Apparent loss (−) or gain (+) of N during analysis	−0.0359	+0.1061	+0.0449

EXTRACTION WITH BOILING WATER.

In sample laboratory No. 1265 the residue insoluble in 0.15 per cent. potassium hydroxide solution was washed thoroughly until all potassium hydroxide was removed. The wash-liquid thus

obtained contained no nitrogen. In the other two cases, laboratory Nos. 1266 and 1267, the residues were suspended in water, phenolphthaleïn added and then dilute hydrochloric acid was added until the mixture was exactly neutral. This, of course, formed a dilute solution of potassium chloride, but as the meat had once been extracted with a 10 per cent. sodium chloride solution it was assumed that this could bring about but little further change. It was with the meat in this condition that the extraction with hot water was carried on. The details of the procedure in the extraction with boiling water were similar to those used above in the case of the cold water extraction. The cold water extracts were diluted to definite volume and portions of this solution were used to determine the total nitrogen, proteïds precipitated in slightly acid solution, albumoses, peptones, and nitrogen not precipitated by the reagents used in the above determinations. A summary of the analytical results is given in Tables X and XI.

TABLE X.—RESULTS OF ANALYSES OF THE HOT WATER EXTRACTS OF MEATS.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Proteïds coagulated in slightly acid solutions	0.05	0.32	4.47
Proteïds precipitated by zinc sulphate	0.01	0.19	1.63
Proteïds precipitated by bromine	0.01	0.08	0.17
Proteïds not precipitated by above reagents	0.05	0.04	0.11
Total proteïd by summation	0.12	0.63	6.38
Total proteïd, direct determination (total N × 6.25)	0.13	0.84	6.24

TABLE XI.—NITROGEN RECORDS OF HOT WATER EXTRACTS OF MEATS.

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Nitrogen as proteïds precipitated in very slightly acid solution	0.0083	0.0517	0.7145
Nitrogen as proteïds precipitated by zinc sulphate	0.0020	0.0302	0.2600
Nitrogen as proteïds precipitated by bromine	0.0019	0.0113	0.0268
Nitrogen not precipitated by above reagents	0.0066	0.0065	0.0162
Total nitrogen by summation	0.0188	0.0997	1.0175
Total nitrogen by direct determination	0.0206	0.1325	0.9987
Apparent loss (−) or gain (+) of N during analysis.	−0.0018	−0.0328	+0.0188

ANALYSIS OF THE RESIDUES INSOLUBLE IN ABOVE REAGENTS.

The residues of the meats which were not dissolved by the solvents used above were air-dried and the nitrogen determined in the same. The nitrogen in No. 1265 = 0.1130 per cent., in No. 1266 = 0.2171 per cent., in No. 1267 = 2.9856 per cent. of the original weight of the meat. Calculating from these figures the quantity of proteïds in the residues, the following results were obtained. Residue No. 1265 contained 0.71 per cent., No. 1266 contained 1.34 per cent. and No. 1267 contained 18.69 per cent. of proteïds.

The following table gives, in a condensed form, the percentages of nitrogen contained in the several extracts and insoluble residues:

TABLE XII.—NITROGEN RECORDS OF MEATS.

(Expressed in percentage of fresh substance.)

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Total nitrogen in cold water extract	0.7351	0.9175	0.3656
Total nitrogen in 10 per cent. sodium chloride solution	0.8449	1.1101	0.0886
Total nitrogen in 0.15 per cent. hydrochloric acid solution	0.3603	0.3855	0.3669
Total nitrogen in 0.15 per cent. potassium hydroxide solution	0.5771	0.3509	0.7739
Total nitrogen in hot water solution	0.0206	0.1325	0.9987
Total nitrogen in undissolved residue	0.1130	0.2171	2.9856
Total nitrogen by summation	2.6510	3.1136	5.5793
Total nitrogen by direct determination	3.1900	3.6100	6.0300
Apparent loss of nitrogen	0.5390	0.4964	0.4507

The nitrogen in the several extracts and insoluble residues was calculated to the form of percentages of the entire amount of nitrogen occurring in the fresh meat and this data is given in the following table:

TABLE XIII.—NITROGEN RECORDS OF MEATS.

(Expressed in percentage of the total nitrogen in meats.)

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Total nitrogen in meat by direct determination	100.00	100.00	100.00
Total nitrogen in cold water extract	23.05	25.42	6.07

TABLE XIII.—(*Continued*).

	Raw beef. No. 1265. Per cent.	Raw beef. No. 1266. Per cent.	Boiled beef. No. 1267. Per cent.
Total nitrogen in 10 per cent. sodium chloride solution.....	26.49	30.76	1.47
Total nitrogen in 0.15 per cent. hydrochloric acid solution....	11.29	10.68	6.09
Total nitrogen in 0.15 per cent. potassium hydroxide solution...	18.09	9.69	12.83
Total nitrogen in hot water extract	0.65	3.67	16.56
Total nitrogen in undissolved residue.....	3.54	6.02	49.51
Total nitrogen by summation	83.11	86.24	92.53
Apparent loss of nitrogen...	16.89	13.76	7.47

DISCUSSION OF RESULTS.

(1) In studying the data presented in Table II, it can clearly be seen that a considerable proportion of raw flesh is soluble in cold water. There was extracted from the raw flesh of beef round No. 1266, 3.06 per cent. of proteïd, 1 per cent. of flesh bases, 1.33 per cent. of organic acids, 1.25 per cent. of ash, and 0.74 per cent. of other substances, making a total of 7.38 per cent. of soluble matter. The work upon the sample of meat cooked by boiling in hot water, however, shows quite different results. In the first place it will be observed that the amount of total proteïds and also the amount of coagulated proteïds are much less in the case of the cooked meat, laboratory No. 1267, than in the raw sample, laboratory No. 1266, both samples being from the same cut and from the same animal. The total proteïds in the water extracts of the raw samples of meat were equal to 3.06 per cent., while in the cooked meat they were only 0.27 per cent. The proteïds coagulated by heat in the water extract of raw meat amounted to 2.79 per cent., while they amounted to only 0.05 per cent. in cooked meat. The data here given also shows that the amount of flesh bases, acid, ash, and other substances are present in smaller proportions in the cooked meat than in the raw meat.

Referring to Table XIII it will be seen that in one sample of the raw meat 23.05 per cent. of the total nitrogen existing in the flesh was soluble in cold water. In the other sample of raw meat 25.42 per cent. of the nitrogen present in the raw flesh was soluble in water, while only 6.07 per cent. of the total nitrogen in the cooked meat was soluble under the same conditions.

From the figures given in Tables I and II it will be observed that boiled meat is much less soluble in cold water than raw meat. In the boiled meat the total nutrients dissolved by cold water formed only 8.21 per cent. of the total nutrients existing in the fresh substance of the cooked meat, while in the raw meats the soluble nutrients formed 29.20 per cent. of the total nutrients contained in the fresh substance.

The cold water extracts were found to be distinctly acid to phenolphthaleïn. It was found that upon boiling, and thus upon coagulating the albumin, that the acidity of the solution kept increasing. When no further precipitation of coagulated proteïd resulted, then the solution remained neutral. This work confirms the conclusion reached by Halliburton, namely, that the acidity of a solution of muscle increases upon the coagulation of its proteïds. The total acidity of the broth resulting from the cooking of the meat, laboratory No. 1267, was determined and the total acidity of the cooked meat was also determined. The sum of the acidity of the broth and of the cooked meat was equal to 1.84 per cent. of the original weight of the fresh meat. The acidity of the raw meat was only equal to 1.34 per cent. of the weight of the original meat. This goes to show that in the boiling of meat in water, acid is produced.

A portion of the water extract of one of the samples of raw meat was studied with the object of noting the influence of increased temperature upon the same. The perfectly clear solution, if it was either distinctly acid or distinctly alkaline, gave no coagulum even upon boiling. Upon warming the neutral solution of the cold water extract there results a slight turbidity at 45°, a milkiness at 55°, and a heavy flocculent precipitate at 61° to 65°. At this stage the solution was filtered and the clear filtrate heated again. A slight turbidity appeared again at about 45° and a precipitate formed upon boiling. It was always found necessary to boil the solution very thoroughly and to evaporate it to a small volume before the liquid holding the precipitate became clear and before all proteïd matter, removable by coagulation, was separated.

The apparent loss of nitrogen here and in the other extracts is undoubtedly due, in part, if not entirely, to the use of bromine as a precipitant for peptones. It is a well known fact that bromine acts upon flesh bases and similar nitrogenous compounds liberating

free nitrogen. Van Slyke[1] has shown that the water extracts of cheese in some cases lose 44 per cent. of their nitrogen by this treatment with bromine.

(2) A 10 per cent. solution of sodium chloride extracts from raw meats have been freed from all water-soluble constituents, a considerable quantity of proteid matter. The average quantity of proteïd matter thus dissolved amounts to 6.10 per cent. of the entire weight of the flesh. In other words, almost one-third of the proteïd of the meat is dissolved by this solution from the water-extracted raw meats. In the case of the boiled meat only about 0.5 per cent. of proteïd matter is dissolved by the salt solution, that is equivalent to only about one-seventieth of the total proteïd in the boiled meat. Referring to Table XIII. it will be seen that in one sample of raw meat 26.49 per cent. of the total nitrogen existing in the flesh was soluble in the 10 per cent. sodium chloride solution. In the other sample of raw meat 30.76 per cent. of the nitrogen present in the raw flesh was soluble in the 10 per cent. sodium chloride solution, while only 1.47 per cent. of the total nitrogen in the cooked meat was soluble under the same conditions.

The results of the analysis of the sodium chloride extracts indicate plainly that there is present in this extract more than one proteïd compound. Further work is now being done in this laboratory which has for its object the separation and purification of these bodies.

(3) Liebig maintained that when muscle is placed in dilute hydrochloric acid (0.1 per cent.) the greater part of the proteïd matter is dissolved, to be reprecipitated when the solution is neutralized. Our work indicates clearly that the amount of proteïd matter of meats, soluble in hydrochloric acid, depends very much upon the strength of the acid. A slight increase in the strength of the acid increases very much the quantity of proteïd soluble. The proteïd, dissolved by hydrochloric acid, was found to be precipitated best in slightly acid solution. However, all of the proteïd matter was not precipitated by this means. This fact seems to indicate the decomposition of the original proteïds or else the presence of more than one proteïd soluble in hydrochloric acid.

The boiled meat, which gave but little soluble matter to the cold water and 10 per cent. sodium chloride solution, was much more

[1] *Chem News*. 88, 92.

soluble in the 0.15 per cent. hydrochloric acid solution. In fact, the boiled meat is as soluble in this solvent as the raw meat.

(4) The proteïds in the potassium hydroxide extract were precipitated by making the solution distinctly acid with acetic acid or with hydrochloric acid. It was found that no precipitate occurred when the solution was either neutral or acid beyond a certain point.

These results show clearly that the reagents used did not affect the complete precipitation of the proteïds dissolved in the potassium hydroxide solution. A considerable amount of proteïd matter remained in the solution after all the different treatments.

The data here given indicate that the proteïds of boiled meat, while much less soluble in water and 10 per cent. sodium chloride solution, are more soluble in 0.15 per cent. potassium hydroxide solution than the proteïds of raw meat.

(5) Hot water dissolves only a very small amount of matter from the raw meats after extraction with the above reagents, but it dissolved a large quantity from boiled meat which had been treated in a similar manner. The amount of the proteïd dissolved by hot water and coagulated by heat in slightly acid solution is large and the amount of nitrogenous substances precipitated by zinc sulphate indicates the presence of a considerable quantity of albumoses.

(6) By referring to Table XII, it will be observed that the percentage of total nitrogen dissolved by the various treatments, is as follows: No. 1265 = 96.46 per cent., No. 1266 = 93.98 per cent., and No. 1267 = 50.49 per cent. These figures indicate the relatively easier solubility of raw meats than of cooked meats and, in a measure, throw added light upon the differences in their digestibility.

A STUDY OF THE WATER EXTRACTS OF FLESH.

As the above preliminary experiments proved conclusively that at least one-fourth of the nitrogenous constituents of flesh is soluble in cold water, it seemed desirable in the next place to study, somewhat in detail, the nature of the water-soluble nitrogenous bodies. For this purpose the cold water extracts were prepared by treating, repeatedly, 10- to 15-gram portions of the finely ground flesh with small quantities of cold water. The water and meat were constantly stirred. The extracts were filtered. The ex-

traction was continued until the filtrate from the above quantity of flesh measured about 500 cc. A number of tests, which were made in order to determine if the extraction was complete under these conditions, proved conclusively that practically all soluble substances were removed by this treatment. A number of these extracts were prepared and then diluted to a definite volume for use in the determination of the total quantity of solid matter, the ash, the total nitrogen, the proteïds coagulated by heat, the albumoses precipitated by zinc sulphate and the peptones by bromine, by the methods briefly indicated at the beginning of this paper.

However, in order to get, if possible, a further insight into the nature of the nitrogenous constituents present in cold water extracts of flesh and also to study and compare methods for determining these bodies in animal substances, the following determinations were made:

(*a*) Nitrogenous compounds precipitated by bromine directly in the cold water extracts. These substances were determined by completely saturating measured portions of the extracts with bromine after slightly acidifying with dilute hydrochloric acid. After allowing to stand for twelve hours the precipitate was removed by filtration and thoroughly washed with water, saturated with bromine. The nitrogen was determined in the residue.

(*b*) Nitrogenous constituents precipitated by tannin and sodium chloride. The amount of such bodies were estimated by treating known volumes of the aqueous extracts with 1 gram of sodium chloride and a slight excess of a solution of tannin in water. The solutions were diluted to definite volume, allowed to stand twelve hours, filtered through dry filters and the nitrogen determined in the filtrates.

(*c*) Nitrogen compounds precipitated by phosphotungstic acid in the cold. In order to determine these substances, measured portions of the liquid obtained in treating meats with cold water were acidified with 5 cc. of 50 per cent. sulphuric acid and then a solution of phosphotungstic acid was added in slight excess. The solutions were diluted to definite volume, allowed to stand ten to twelve hours, filtered through dry filters and the nitrogen was determined in measured fractions of the filtrates.

(*d*) Nitrogenous compounds separated by a hot solution of phosphotungstic acid. For this determination portions of the solution

TABLE XIV.—CONSTITUENTS OF MEATS, SOLUBLE AND INSOLUBLE IN COLD WATER.

(Results expressed in percentage of original material.)

Laboratory No.	Kind of meat.	Water.	Proteïd.	Flesh bases.	Fat.	Ash.	Other substances.	Total.	Total nitrogen.
		(*a*) Total nutrients in original meat.							
1649	Beef, sirloin, raw, lean	75.46	18.93	1.16	3.08	1.02	1.48	25.67	3.401
1685	Beef, sirloin, raw, fat	60.39	16.07	0.84	20.89	1.11	1.39	40.30	2.840
1677	Beef, round, raw, lean	74.01	18.71	1.21	4.63	1.05	3.13	28.74	3.382
1693	Beef, round, raw, lean	76.22	18.46	1.23	2.36	1.08	1.71	24.84	3.348
	Average,	71.52	18.04	1.11	7.74	1.07	1.93	29.89	3.243
1656	Veal, leg, raw, lean	75.97	19.17	1.26	0.96	1.15	1.94	24.47	3.470
1662	Veal, leg, raw, lean	75.53	18.02	1.08	3.99	1.10	1.37	25.57	3.230
	Average,	75.75	18.60	1.17	2.48	1.13	1.66	25.02	3.350
		(*b*) Nutrients soluble in water.							
1649	Beef, sirloin, raw, lean	....	2.31	1.16	none	0.91	1.48	5.86	0.742
1685	Beef, sirloin, raw, fat	....	1.81	0.84	none	0.77	1.39	4.81	0.559
1677	Beef, round, raw, lean	....	2.44	1.21	none	0.84	3.13	7.63	0.779
1693	Beef, round, raw, lean	....	2.21	1.23	none	0.82	1.71	5.97	0.748
	Average,	....	2.19	1.11	none	0.84	1.93	6.07	0.707
1656	Veal, leg, raw, lean	....	2.83	1.26	none	1.00	1.94	7.02	0.855
1662	Veal, leg, raw, lean	....	1.90	1.08	none	0.91	1.37	5.26	0.650
	Average,	....	2.37	1.17	none	0.96	1.66	6.14	0.753
		(*c*) Nutrients insoluble in water.							
1649	Beef, sirloin, raw, lean	....	16.62	none	3.08	0.11	none	19.81	2.659
1685	Beef, sirloin, raw, fat	....	14.26	none	20.89	0.34	none	35.49	2.281
1677	Beef, round, raw, lean	....	16.27	none	4.63	0.21	none	21.11	2.603
1693	Beef, round, raw, lean	....	16.25	none	2.36	0.26	none	18.87	2.600
	Average,	....	15.85	none	7.74	0.23	none	23.82	2.536
1656	Veal, leg, raw, lean	....	16.31	none	0.96	0.15	none	17.45	2.615
1662	Veal, leg, raw, lean	....	16.12	none	3.99	0.19	none	20.30	2.580

were acidified, as above, with 50 per cent. sulphuric acid, heated to boiling and precipitated by the addition of a very slight excess of phosphotungstic acid. The solutions were maintained at the boiling-point for from three to five minutes and immediately rapidly filtered. The precipitates were thoroughly washed with boiling water and the nitrogen which they contained determined by the Kjeldahl method.

(*e*) The amount of nitrogen as ammonia or its compounds was determined by distilling portions of the water extracts with magnesium oxide or barium carbonate.

In addition to the above determinations upon the water extracts a complete analysis of the meats was made, including the determination of moisture, fat, total nitrogen and ash. These determinations were made directly upon the fresh substance of the meats without first preparing an air-dried sample, which is the usual practice.

The results of this study of the proteids of flesh, soluble in cold water, are given in Tables XIV, XV, XVI and XVII, which are sufficiently clear to explain themselves.

TABLE XV.—NITROGEN RECORDS OF THE COLD WATER EXTRACTS OF RAW MEATS.

(Results expressed in percentage of meat taken.)

Nitrogen.	Beef, sirloin. No. 1649.	Beef, sirloin. No. 1685.	Beef, round. No. 1677.	Beef, round. No. 1693.	Beef average.	Veal leg. No. 1656.	Veal leg. No. 1662.	Veal leg average.
In meat.....	3.400	2.840	3.382	3.348	3.245	3.470	3.230	3.350
In water extract....	0.742	0.559	0.779	0.748	0.707	0.855	0.650	0.753
Coagulated by heat.	0.291	0.271	0.360	0.328	0.313	0.396	0.264	0.330
Precipitated by $ZnSO_4$ (albumoses)	0.059	0.015	0.027	0.022	0.031	0.044	0.024	0.034
Sum of coagulated and albumose.....	0.350	0.286	0.387	0.350	0.343	0.440	0.288	0.364
Precipitated by Br in filtrate from $ZnSO_4$ precipitate.....	0.019	0.004	0.004	0.003	0.005	0.012	0.015	0.014
As proteid in water extract.....	0.369	0.290	0.391	0.353	0.351	0.452	0.303	0.378
As non-proteid in water extract.....	0.373	0.269	0.388	0.395	0.356	0.403	0.347	0.375

TABLE XV.—(*Continued.*)

Nitrogen.	Beef, sirloin. No. 1649.	Beef, sirloin. No. 1685.	Beef, round. No. 1677.	Beef, round. No. 1693.	Beef average.	Veal leg. No. 1656.	Veal leg. No. 1662.	Veal leg average.
Precipitated by Br directly	0.292	0.247	0.245	0.306	0.273	0.370	0.226	0.298
Precipitated by phosphotungstic acid (hot)	0.177	0.266	0.358	0.327	0.282	0.389	0.235	0.312
Precipitated by tannin and salt	0.376	0.256	0.383	0.361	0.344	0.442	0.290	0.366
Precipitated by phosphotungstic acid (cold)	0.372	0.270	0.381	0.384	0.352	0.419	0.253	0.336
As free ammonia	....	0.006	0.021	0.011	0.013	0.013	0.020	0.017

TABLE XVI.—NITROGEN RECORDS OF THE COLD WATER EXTRACTS OF RAW MEATS.

(Results expressed in percentage of total nitrogen of meats taken.)

Nitrogen.	Beef, sirloin. No. 1649.	Beef, sirloin. No. 1685.	Beef, round. No. 1667.	Beef, round. No. 1693.	Beef average.	Veal leg. No. 1656.	Veal leg. No 1662.	Veal leg average.
In meat	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
In water extract	21.80	19.66	23.04	22.34	21.71	24.64	20.13	22.39
Coagulated by heat	8.57	9.53	10.63	9.80	9.63	11.42	8.18	9.80
Precipitated by $ZnSO_4$ (albumoses)	1.74	0.52	0.79	0.65	0.93	1.26	0.74	1.00
Sum of coagulated and albumose	10.31	10.05	11.42	10.45	10.56	12.68	8.92	10.80
Precipitated by Br in filtrate from $ZnSO_4$ precipitate	0.57	0.13	0.11	0.10	0.23	0.35	0.46	0.41
As proteid in water extract	10.88	10.18	11.53	10.55	10.79	13.03	9.38	11.21
As non-proteid in water extract	10.92	9.48	11.51	11.79	10.92	11.61	10.75	11.18
Precipitated by Br directly	8.59	8.67	7.24	9.13	8.41	10.67	6.99	8.83
Precipitated by phosphotungstic acid (hot)	5.20	9.37	10.59	9.77	8.73	11.20	7.28	9.24
Precipitated by tannin and salt	11.06	9.02	11.32	10.79	10.55	12.74	8.97	10.86
Precipitated by phosphotungstic acid (cold)	10.93	9.49	11.27	11.47	10.79	12.10	7.84	9.97
As free ammonia	...	0.22	0.62	0.34	0.39	0.39	0.61	0.50

TABLE XVII.—NITROGEN RECORDS OF THE COLD WATER EXTRACTS OF RAW MEATS.

(Results expressed in percentages of total nitrogen of cold water extracts.)

Nitrogen.	Beef, sirloin. No. 1649.	Beef, sirloin. No. 1685.	Beef, round. No. 1677.	Beef, round. No. 1693.	Beef average.	Veal leg. No. 1656.	Veal leg. No. 1662.	Veal leg average.
In water extract ...	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Coagulated by heat.	39.30	48.48	46.14	43.88	44.45	46.36	40.66	43.51
Precipitated by $ZnSO_4$ (albumoses)	8.00	2.63	3.44	2.91	4.25	5.11	3.66	4.39
Sum of coagulated and albumose	47.30	51.11	49.58	46.79	48.70	51.47	44.32	47.90
Precipitated by Br in filtrate from $ZnSO_4$ precipitate	2.60	0.68	0.46	0.44	1.05	1.40	2.29	1.85
As proteid in water extract	49.90	51.79	50.04	47.23	49.74	52.87	46.61	49.74
As non-proteid in water extract.....	50.10	48.21	49.96	52.77	50.26	47.13	53.39	50.26
Precipitated by Br directly.....	39.37	44.13	31.41	40.87	38.95	43.33	34.71	39.02
Precipitated by phosphotungstic acid (hot)	23.82	47.64	45.98	43.74	40.30	45.48	36.14	40.81
Precipitated by tannin and salt.....	50.73	45.85	49.12	48.27	48.50	51.68	44.55	48.12
Precipitated by phosphotungstic acid (cold)	50.17	48.27	48.94	51.35	49.68	49.03	38.97	44.00
As free ammonia ...	....	1.11	2.67	1.53	1.77	1.57	3.06	2.32

DISCUSSION OF RESULTS.

In studying the data presented in Table XIV it can clearly be seen that a considerable proportion of raw flesh is soluble in cold water. There was extracted by cold water from the four samples of beef flesh an average of 2.19 per cent. of proteïd, 1.11 per cent. of flesh bases, 0.84 per cent. of ash and 1.93 per cent. of other substances, making a total of 6.07 per cent. of soluble matter. In the case of the two samples of veal there was extracted by water 2.37 per cent. of proteïd, 1.17 per cent. of flesh bases, 0.96 per cent. of ash and 1.66 per cent. of other substances, giving a total of 6.14 per cent. of soluble material.

If we consider the data regarding each individual nutrient, it will be observed that from 11.26 to 13.39 per cent. of the total proteïd in the beef was soluble in water. The average for the four samples of raw beef shows that 12.14 per cent. of the total proteïd was soluble. The average result for the two samples of veal shows that there was a somewhat greater proportion of the proteïds soluble in water in this meat than in beef. All of the flesh bases and also all of the "other substances" are soluble in cold water, while none of the fat of the flesh enters into solution. The greater part of the ash is soluble in water, the average per cent. soluble in the case of beef flesh being 78.50, while the average for veal flesh is 84.96.

The total nutrients soluble in cold water, expressed in per cent. of total nutrients in original meat, varies from 11.93 in laboratory No. 1685 to 26.55 in the case of laboratory No. 1677, the average for beef being 20.31 and for veal 24.54. In other words, these experiments indicate that one-fifth to one-fourth of the total nutrients of the raw flesh of beef and veal are soluble in cold water.

The average results of the experiments here reported indicate that the solid matter obtained by treating beef flesh with cold water contains 11.65 per cent. of nitrogen, 36.08 per cent. of proteïd, 18.45 per cent. of flesh bases, 13.84 per cent. of ash, 31.47 per cent. of other substances and no fat. Judging from the examination of two samples, the cold water extract of veal contains 12.27 per cent. of nitrogen, 38.60 per cent. of proteïd, 20.36 per cent of flesh bases, 15.63 per cent. of ash, 25.73 per cent. of other substances and no fat.

Table XV gives the nitrogen data of the cold water extracts of the raw meats expressed in percentage of the fresh meat. The total nitrogen in the fresh meats varies from 2.840 per cent. in No. 1685, which was a fat sample of beef, to 3.470 per cent. in No. 1656, which was a lean sample of veal, the average amount of nitrogen in the beef flesh being 3.245 per cent. and in the veal flesh 3.350 per cent.

The total nitrogen soluble in water formed 0.559 to 0.855 per cent. of the weight of the meat. The lowest result was again in No. 1685, and the highest was, as before, in No. 1655. Upon studying the results more closely it will be observed that the water-

soluble nitrogen bears a direct relation to the total nitrogen in the meat. In other words, the greater the nitrogen in the meat the more nitrogen there will be dissolved by cold water. The average nitrogen soluble in water amounted to 0.707 per cent. in the samples of beef and to 0.753 per cent. in the samples of veal.

The nitrogen in the form of proteïds coagulated by heat varies from 0.264 per cent. in laboratory No. 1662 to 0.396 per cent. in laboratory No. 1656, the average for beef being 0.313 per cent. and for veal 0.330 per cent. The albumose nitrogen precipitated by zinc sulphate was less than one-tenth, as a rule, of the nitrogen coagulated by heat. The average albumose nitrogen in the samples of beef is equal to 0.031 per cent., while in the samples of veal it is 0.034 per cent. The nitrogen precipitated by bromine in the filtrate from the zinc sulphate is, in all cases, very small in amount.

The total proteïd nitrogen varies from 0.290 to 0.452 per cent. of the weight of the meats studied, the average for the flesh of beef being 0.351 per cent. and for the flesh of veal 0.378 per cent. The non-proteïd nitrogen is quite similar in amount to the proteïd nitrogen and seems to vary as the proteïd nitrogen, but it is in most cases somewhat less in quantity.

It will be noticed that the nitrogen precipitated by bromine directly, or by phosphotungstic acid in the hot solution, is, in no case, equal to the total proteïd nitrogen. This is especially true of the bromine, notwithstanding the fact that it has been so generally used lately as a precipitant for proteïds. The results here given show clearly that bromine does not precipitate proteïds completely.

Finally, the data given in this table indicate that there is a small amount of nitrogen as ammonia or ammonium salts invariably present in cold water extracts of raw flesh. This has been found to be true even where barium carbonate has been used to remove the ammonia by distillation.

Table XVI gives the nitrogen data of the cold water extracts of the raw meats, expressed in percentages of total nitrogen in the meats taken. The data show that from 19.66 to 24.64 per cent. of the total nitrogen in raw meats is soluble in cold water, the average in the case of the beef being 21.71 per cent. and in the veal 22.39 per cent. The nitrogen in the form of proteïd in the

cold water extracts of raw meat forms from 9.38 to 13.03 per cent. of the total nitrogen in the fresh material of the raw meats. The non-proteïd nitrogen in the water-soluble portion forms from 9.48 to 11.79 per cent. of the total nitrogen in the raw flesh. From 0.22 to 0.62 per cent. of the nitrogen in the fresh substance of the meats enters the water solution in the form of ammonium salts.

In Table XVII the nitrogen data of the cold water extracts of raw flesh are expressed in percentages of the total nitrogen in the cold water extract. The data show that from 39.30 to 48.48 per cent. of the soluble nitrogen exists in the form of compounds coagulated by heat. The average of the four samples of beef shows that 44.45 per cent. of soluble nitrogen was precipitated by coagulation, while in the case of the veal the average was 43.41 per cent. From 2.63 to 8 per cent. of the soluble nitrogen was precipitated by saturating the filtrate from the coagulated proteïd with zinc sulphate. By this reagent it is supposed that the albumoses are precipitated. Bromine in the filtrate from the zinc sulphate precipitate separates 0.46 to 2.60 per cent. of the soluble nitrogen. This reagent, as thus used, is supposed to precipitate peptones.

It is evident from the data here presented that the nitrogen existing in the cold water extracts of raw meats is quite equally divided between the proteïd and the non-proteïd substances. The average of the six samples of flesh shows that 49.74 per cent. of the soluble nitrogen exists as proteïds and 50.26 per cent. as non-proteïd material.

CONCLUSIONS.

(1) It is evident that a considerable proportion of raw flesh is soluble in cold water.

(2) The data show that 12.14 per cent. of the total proteïd of raw flesh was soluble in cold water.

(3) Of the total nitrogen in raw meats 22 per cent. was soluble in water.

(4) The nitrogen existing in cold water extracts of raw meat is equally divided between proteid and non-proteid substances.

(5) The acidity of a solution of flesh increases upon the coagulation of its proteïds.

(6) The proteids of cooked meat are much less soluble thar

those of raw flesh in cold water and 10 per cent. sodium chloride solution.

(7) Cold water extracted 3.06 per cent. of nitrogenous matter from raw meats and only 0.27 per cent. from boiled meat.

(8) A 10 per cent. solution of sodium chloride extracted from raw meats 6.10 per cent. of proteïd matter and only 0.5 per cent. from boiled meat.

(9) A 0.15 per cent. solution of hydrochloric acid dissolved from raw meat 2.28 per cent. of proteïd and from boiled meat 2.30 per cent.

(10) A 0.15 per cent. solution of potassium hydroxide extracted from raw meats 2.88 per cent. and from boiled meat 4.84 per cent. of proteid.

(11) Hot water removed from raw meats 0.49 per cent. and from boiled meats 6.24 per cent. proteïd matter.

(12) Of the total proteïd existing in the original raw meats 95.22 per cent. was dissolved by extracting successively with the following reagents: Cold water, 10 per cent. sodium chloride solution, 0.15 per cent. hydrochloric acid solution, 0.15 per cent. potassium hydroxide solution and hot water, while only 50.59 per cent. of the total proteïd of the boiled meat was thus made soluble.

Much work has been done in this laboratory in endeavoring to separate and purify the several proteïds of flesh. Encouraging results have been obtained and it is hoped that in the near future they will be put in shape for publication. In addition the several fractions of nitrogenous substances obtained from flesh, as above described by the successive treatment with cold water, 10 per cent. sodium chloride solution, 0.15 per cent. hydrochloric acid solution, 0.15 per cent. potassium hydroxide solution and hot water, are each being subjected to a thorough chemical investigation with a view of throwing some light upon their nature.

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