two methyl groups. If we consider, further, the formulas of aminolauronic and of camphoric acids, the two possible formulas for laurolene are 1,2-dimethyl-Δ^1-cyclohexene, $\begin{matrix} CH_2—CH_2—C—CH_3 \\ | \qquad\qquad \| \\ CH_2—CH_2—C—CH_3 \end{matrix}$, and 1,2,3-trimethyl-$\Delta^1$-cyclopentene, $\begin{matrix} CH(CH_3)—C—CH_3 \\ | \qquad\quad \| \\ CH_2—CH_2—C—CH_3 \end{matrix}$, as proposed by Eijkman. The first of these and also the 2,6-octanedione, which it would give by oxidation, are necessarily inactive as neither contains an asymmetric carbon atom. As both laurolene and the diketone are optically active, Eijkman's formula seems to be altogether probable.

On heating, the diketone appears to condense with loss of water, probably to form a cyclic ketone, either 3,4-dimethyl-Δ^2-cyclohexenone, $\begin{matrix} CH_3—C=CH—CO \\ | \qquad\quad \diagdown \\ CH_3—CH—CH_2—CH_2 \end{matrix}$, 3,6-dimethyl-$\Delta^2$-cyclohexenone, or 1,3-acetyl-methyl-Δ^2-cyclopentene. It seems quite possible that this condensation product is identical with laurenone of Tiemann and Tigges.[1] The work will be continued and a discussion of the mechanism of the rearrangements which give laurolene is reserved for a later communication.

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FACTORS WHICH INFLUENCE THE CREATININE DETERMINATION.[2]

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During the past five years, since the Folin[3] colorimetric method for the estimation of creatinine has been available, a great amount of work involving this method has been performed. The determination of creatinine now holds an important place in Nitrogen Metabolism experiments, and in all investigations on the chemistry of meat products. The author applied this method to beef extracts and similar products during the winter of 1905, and obtained fairly satisfactory results for total creatinine. The determination of the original creatinine in meat products has not appeared to be entirely satisfactory, and the factors which influence this determination have not been studied.

The object of this investigation was to obtain some additional information on the various factors which influence the determination of crea-

[1] *Ber.*, **33**, 2950.

[2] Published by permission of the Secretary of Agriculture.

[3] *Z. physiol. Chem.*, **41**, 223 (1904).

tine and creatinine as well as to compare several of the methods which have been used in various laboratories for their determination, and to select the method which gave the most satisfactory results. After the errors in these determinations due to dilution were shown, a correction factor for this error was worked out.

The factors studied which have more or less influence on the creatinine colorimetric determination are: the amount of picric acid added, the amount of alkali added, the influence of dilution, of standing, of coagulable protein and finally of proteoses and peptones. The methods compared were: the method as outlined by Grindley and Woods,[1] the Benedict and Myers autoclave method,[2] the method of heating for four hours on a boiling water bath,[3] and finally the method as formerly employed in the laboratory of Armour and Company.[3]

Historical.

The color reaction which takes place when a creatinine solution is treated with picric acid and an alkali was described by Jaffé[4] in 1886. Creatinine from different sources was investigated by Schmidt[5] who found that the creatinine in wine, in meat, synthetic creatinine, and the creatinine prepared from creatine of urine were identical, all melting at 212° and forming identical salts. A quantitative colorimetric method based on the Jaffé reaction was described by Folin[6] in 1904, and its application to the estimation of this substance in urine[7] was made soon after.

Previous to 1904 the Neubauer[8] method of precipitation with zinc chloride was used for determining creatinine, it being isolated as the zinc salt and weighed as such.

Methods for the application of Folin's colorimetric determination to meat products have been described by Grindley and Woods,[1] Bauer and Barschall,[9] and Bigelow and Cook.[10]

In a recent article Hehner[11] claims that an excess of alkali diminishes the color of the creatinine picrate ($C_4H_7N_3O$–$C_6H_3N_3O_7$). He further states that 15 cc. of picric acid contain too little reagent and the amount of creatinine in meat extracts is accordingly underestimated. On using 25 cc. of picric acid Hehner found 10–11 per cent. of creatinine, while

[1] *J. Biol. Chem.*, 2, 309 (1907).
[2] *Am. J. Physiol.*, 18, 397 (1907).
[3] *Bur. Chem., Bull.* 116, p. 47.
[4] *Z. physiol. Chem.*, 10, 391 (1886).
[5] *Arch. Pharm.*, 234, 380 (1896).
[6] *Z. physiol. Chem.*, 41, 223 (1904).
[7] *Am. J. Physiol.*, 13, 45 (1905).
[8] *Ann. Chem. and Pharm.*, 119, 33 (1861).
[9] *Arb. Kais. Gesundheitzamte*, 24, 552 (1906).
[10] *Bur. Chem., Bull.* 114.
[11] *Pharm. J.*, 78, 683 (1907).

but 6–7 per cent. was found on using 15 cc. picric acid. This work prompted Emmet and Grindley[1] to make a study of the application of Folin's method to meats and meat extracts, and they investigated the effects of various amounts of alkali and picric acid on this reaction, and concluded that increasing the amount of picric acid according to Hehner's suggestion makes no difference in determining the original creatinine, but does make a difference when creatine is present. They advise the use of 30 cc. of picric acid in the latter case. In regard to the amount of alkali the use of 5 cc. is recommended in determining the original creatinine, while 10 cc. should be used when creatine is determined as creatinine. Benedict and Myers[2] describe a method for the conversion of creatinine to creatinine by means of the autoclave. On heating a creatine solution in the autoclave for 15 minutes at 117° theoretical yields of creatinine were obtained. Some results[3] obtained by the author as associate referee on meat proteins of the Association of Official Agricultural Chemists show that the Folin method for creatinine in meat extracts gives fairly satisfactory results in the hands of different analysts.

Mellanby[4] has recently published an excellent paper on the subject of creatine and creatinine. Folin's method was tested for its accuracy. Various substances that occur in urine—namely, urea, dextrose, sodium chloride, calcium chloride, disodium phosphate, and monosodium phosphate—were added to pure creatinine solutions, both singly and together, and were found to be without influence on this test when present in reasonable quantities. Mellanby suggests that the temperature of the reagents be kept constant, and he places great reliance on the colorimetric method as long as the conditions are kept rigidly constant.

Chapman[5] has recently stated that the color which is the basis of this determination does not depend on the formation of creatinine picrate, but in fact that creatinine, which is a powerful reducing agent, reduces the picric acid in alkaline solutions to picramic acid, and diaminonitro phenol, the alkali salts of which are deeply colored and serve under definitely defined conditions for the colorimetric estimation of creatinine.

Experimental.

In his original paper, Folin recommended in this determination the employment of a 1.2 per cent. solution of picric acid and 5 cc. of a 10 per cent. solution of sodium hydroxide. The volume of sample used was 10 cc. The volume of solution was consequently always constant (10 cc.) before adding reagents, and his results were worked out on this

[1] *J. Biol. Chem.*, **3**, 491 (1907).
[2] *Am. J. Physiol.*, **18**, 397 (1907).
[3] *Bur. Chem., Bull.* 116.
[4] *J. Physiol.*, **36**, 447 (1908).
[5] *Brit. Med. J.*, Dec. 12, 1908.

concentration. A tenth-normal bichromate solution (24.54 grams per liter) was used as the standard color.

In testing the influence of the various factors that might affect the creatinine test, solutions of pure creatine and creatinine and meat extract were employed. The creatine was changed to creatinine by the addition of 10 cc. of normal hydrochloric acid and the volumes made to 500 cc. before taking the readings on a Duboscq colorimeter.

(*a*) *Picric Acid Influence.*—The methods employed were either the boiling water bath method or the autoclave method. In using the latter method it was found necessary to heat in the autoclave for fifteen minutes under fifteen pounds pressure.

In these experiments 25 cc. of the creatine solution were treated with 10 cc. of normal hydrochloric acid in a small Erlenmeyer flask, and heated on the boiling water bath for four hours under a reflux condenser. The samples were cooled and 10 cc. of normal sodium hydroxide added to neutralize the 10 cc. of normal hydrochloric acid previously added to dehydrate the creatine—15 or 30 cc. picric acid were added and 10 cc. of 10 per cent. sodium hydroxide. The flask was well shaken and stood for five minutes in order to allow the red color to develop. The contents were transferred to a 500 cc. graduate flask, diluted to volume and well mixed. The color was compared with that of a tenth-normal bichromate solution (24.54 grams per liter), the scale of the Duboscq colorimeter being set at 8 mm. The average of five readings was taken as the final figure. The results, using a commercial sample of creatine, are recorded in Table I.

TABLE I.—INFLUENCE OF PICRIC ACID ON CREATININE.

Creatine solution taken. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
25	15	10	12.3	6.59
25	15	10	10.0	8.10
25	30	10	9.0	9.00
25	30	10	9.2	8.80
25	15	10	11.5	7.04
25	30	10	9.7	8.35
25	15	10	7.3	11.09
25	30	10	6.9	11.74
25	15	10	8.0	10.13
25	15	10	8.0	10.13
25	30	10	7.3	11.09
25	30	10	7.3	11.09

The creatine solution was the same in the case of the first six samples, while the last six samples were taken from another solution. The results are somewhat higher in the cases where 30 cc. of picric acid (1.2 per cent.) were added than where 15 cc. were used.

TABLE II.—INFLUENCE OF PICRIC ACID ON CREATININE IN MEAT EXTRACT.

Method.	Solution of meat extract taken. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Autoclave.....	10	15	10	7.1	11.41
"	10	15	10	7.2	11.25
"	10	25	10	7.6	10.66
"	10	25	10	7.1	11.41
"	10	35	10	7.1	11.41
"	10	35	10	7.1	11.41
Boiling water bath, 4 hours.	10	15	10	6.9	11.74
" " 4 " ..	10	15	10	7.0	11.57
" " 4 " ..	10	25	10	7.0	11.57
" " 4 " ..	10	25	10	6.9	11.74
" " 4 " ..	10	35	10	7.1	11.41
" " 4 " ..	10	35	10	7.2	11.25

In Table II results showing the effects of varying amounts of picric acid on the total creatinine of a beef extract solution are given. The solution was made so that 10 cc. were equivalent to 0.23 gram of beef extract. The results as recorded for this sample show that it makes no difference whether 15 or 30 cc. of picric acid are added, while in the case of some samples not recorded here, a slightly higher figure for creatinine has been indicated where 30 cc. of picric acid were used. It is evident that in the case of meat extracts practically no difference in the creatinine value is indicated whether 15 or 30 cc. of picric acid are used. In the case of commercial creatine the presence of 30 cc. of picric acid gives slightly higher results than where but 15 cc. were used. This is in agreement with the findings of Grindley and Emmett.[1]

(*b*) *Alkali Influence.*—The next point studied was the influence of alkali on the creatinine test. Both the autoclave and the boiling water bath methods were used. 25 cc. of picric acid were added in all cases, but the amount of alkali added was varied. In all these experiments each series was performed on the same solution of creatine or beef extract. The results using commercial creatine are recorded in Table III and using beef extract in Table IV.

These results show a tendency for 15 cc. of alkali to give lower results than obtained with either the 5 or 10 cc. portions of alkali where pure creatine was used. In the case of the samples treated with 5 or 10 cc. portions of alkali little difference was noted except in the case of the beef extract where very low results were obtained when 5 cc. of alkali were employed. In the beef extract solution little difference was observed in the results where 10 and 15 cc. of alkali were used. Other results with pure creatine show that 15 cc. gave lower results than the

[1] *Loc. cit.*

5 or 10 cc. portions. The most satisfactory amount for all cases appears to be 10 cc.

TABLE III.—INFLUENCE OF ALKALI ON CREATININE TEST.

Method.	Creatine solution taken. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Water bath, 4 hours.....	20	25	5	6.90	11.74
" 4 "	20	25	5	7.05	11.48
" 4 "	20	25	5	6.85	11.82
" 4 "	20	25	10	6.95	11.65
" 4 "	20	25	10	7.05	11.33
" 4 "	20	25	10	6.90	11.74
" 4 "	20	25	15	7.3	11.09
" 4 "	20	25	15	7.7	10.52
Autoclave.....	20	25	5	7.2	11.25
"	20	25	5	7.2	11.25
"	20	25	5	7.2	11.25
"	20	25	10	7.1	11.41
"	20	25	10	7.5	10.80
"	20	25	10	7.3	11.09
"	20	25	15	7.7	10.52
"	20	25	15	7.6	10.66
Water bath, 4 hours.....	20	25	5	10.4	7.79
" 4 "	20	25	5	13.0	6.23
" 4 "	20	25	10	12.0	6.75
" 4 "	20	25	10	13.5	6.00
" 4 "	20	25	15	14.0	5.78

TABLE IV.—INFLUENCE OF ALKALI ON CREATININE TEST—BEEF EXTRACT.

Method.	Solution of beef extract taken. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Autoclave.....	20	25	5	22.0	3.68
"	20	25	5	22.0	3.68
"	20	25	10	4.1	19.76
"	20	25	10	4.1	19.76
"	20	25	15	4.5	18.00
"	20	25	15	4.4	18.41
Boiling water bath, 4 hours.	20	25	5	22.0	3.68
" " 4 "	20	25	5	22.0	3.68
" " 4 "	20	25	10	4.5	18.0
" " 4 "	20	25	10	4.1	19.76
" " 4 "	20	25	15	4.2	19.29
" " 4 "	20	25	15	4.1	19.76

(*c*) *Influence of Dilution.*—As stated above, Folin originally applied this method to 10 cc. of solution, and later used 10 cc. of urine and based his calculations on this concentration. In determining the amount of creatinine in various meat products, especially where the original creatinine is determined, the volume is often considerably increased above the 10 cc. originally prescribed by Folin. Some experiments are recorded below,

which show the effect of dilution on the creatinine readings. The two volumes compared were 50 and 200 cc., respectively. Ten cc. of 10 per cent. alkali were added in each case and the boiling water bath method was employed.

TABLE V.—INFLUENCE OF DILUTION ON CREATININE DETERMINATION.

Creatine solution used. cc.	Picric acid added. cc.	10% NaOH added. cc.	Volume of solution. cc.	Colorimetric reading.	Mgs. creatinine found.
25	15	10	50	8.15	9.94
25	15	10	50	8.35	9.70
25	15	10	150	9.10	8.90
25	15	10	150	8.55	9.47
25	30	10	50	7.50	10.80
25	30	10	200	7.45	10.88
25	15	10	50	7.3	11.09
25	15	10	200	9.00	9.00
25	30	10	50	6.9	11.73
25	30	10	200	7.7	10.52
25	15	10	50	10.8	7.50
25	15	10	50	10.8	7.50
25	15	10	200	20.0	4.05
25	15	10	200	20.0	4.05
Beef extract solution used.					
10	25	10	50	7.9	10.25
10	25	10	50	8.0	10.12
10	25	10	200	14.0	5.78
10	25	10	200	13.0	6.23

The results recorded above were all performed on 25 cc. of a solution of commercial creatine; while 25 cc. were always used, the solution varied in strength somewhat from time to time. Ten cc. of the meat extract solution used represented 0.23 gram of beef extract. The figures show what a decided influence the volume of the solution has upon the colorimeter reading. The solutions in the case of the 50 cc. volumes were not diluted, the reagents being added directly to the 25 cc. of creatine solution. In the cases of the 200 cc. volume, 150 cc. of H_2O were added to the 25 cc. of dehydrated creatine solution and the 15 cc. of picric acid, and 10 cc. of 10 per cent. alkali then added.

After studying the error in the creatinine determination due to dilution an attempt was made to ascertain if this error was relatively constant and if it was possible to obtain a factor which would correct the error in question. A solution of a commercial sample of creatinine was prepared as noted above, 10 cc. of which would give a reading, approximately, of 6 mm. on the scale of the Duboscq instrument. Five portions of 10 cc. each of this creatinine solution were placed in 500 cc. flasks, and varying amounts of water added to each. No water was added to number 1 in each case. When the 25 cc. portions of picric acid and 10 cc. of 10%

TABLE VI.—ERROR DUE TO VOLUME—PURE CREATININE.

No. of experiment.	Solution of creatinine taken. cc.	Water added. cc.	Volume before adding reagents. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Creatinine found. Gram.	Difference creatinine. Gram.	Creatinine error. Per cent.	Difference creatinine per 10 cc. dilution. Gram.
1	10	0	10	25	10	5.35	0.01514	.....	...	.....
2	10	30	40	25	10	5.66	0.01431	0.00083	5.5	0.00028
3	10	60	70	25	10	5.91	0.01370	0.00061	9.5	0.00020
4	10	90	100	25	10	6.20	0.01307	0.00063	13.7	0.00021
5	10	150	160	25	10	6.95	0.01166	0.00141	23.0	0.00024
									Average,	0.00023
1	15	0	15	25	10	5.40	0.0150	....	...	.....
2	15	30	45	25	10	5.53	0.0146	0.0004	2.6	0.00013
3	15	60	75	25	10	5.80	0.0140	0.0006	6.6	0.00020
4	15	90	105	25	10	6.00	0.0135	0.0005	10.0	0.00017
									Average,	0.00017
1	10	0	10	25	10	5.30	0.01528	.....	...	.....
2	10	30	40	25	10	5.55	0.01460	0.00068	4.4	0.000227
3	10	60	70	25	10	5.82	0.01392	0.00068	8.9	0.000227
4	10	90	100	25	10	6.11	0.01326	0.00066	13.2	0.000220
									Average,	0.000225
1	10	0	10	25	10	5.6	0.01446	.....	...	.....
2	10	90	100	25	10	6.2	0.01307	0.00139	9.6	0.00015
3	10	130	140	25	10	6.35	0.01277	0.00030	11.7	0.00008
4	10	160	170	25	10	6.6	0.01227	0.00050	15.2	0.00018
									Average,	0.00015
1	10	0	10	25	10	5.90	0.01373	.....	...	.....
2	10	40	50	25	10	6.21	0.01304	0.00069	5.0	0.00017
3	10	90	100	25	10	6.62	0.01224	0.00080	10.9	0.00016
4	10	140	150	25	10	7.20	0.01125	0.00101	18.0	0.00020
5	10	190	200	25	10	7.80	0.01038	0.00087	24.4	0.00017
									Average,	0.000175
1	10	0	10	25	10	5.6	0.01446	.....	...	.....
2	10	40	50	25	10	lost	.....	.....	...	.....
3	10	90	100	25	10	6.3	0.01286	0.00160	11.1	0.00018
4	10	140	150	25	10	6.75	0.01200	0.00086	17.0	0.00017
5	10	190	200	25	10	7.25	0.01117	0.00083	22.8	0.00017
									Average,	0.000173
1	10	0	10	25	10	5.55	0.01460	.....	...	.....
2	10	40	50	25	10	5.95	0.01361	0.00099	6.8	0.00025
3	10	90	100	25	10	6.50	0.01246	0.00115	14.7	0.00023
4	10	140	150	25	10	7.0	0.01157	0.00089	20.8	0.00018
5	10	190	200	25	10	7.8	0.01039	0.00118	28.9	0.00024
									Average,	0.000225

sodium hydroxide were added the flasks were well shaken and allowed to stand for five minutes. They were then made to volume and the colors compared with the half-normal potassium bichromate solution in the usual manner. In these experiments the amount of creatinine found using the 10 cc. of undiluted solution was taken as a basis for the calculations. The various amounts of creatinine found under the different dilutions are recorded in Table VI above. Likewise the variations in the amount due to each successive dilution. In the next column the percentage of error in the creatinine estimation is given and in the last column the average amount of creatinine error in grams per 10 cc. dilution is recorded.

It is seen from Table VI that the error is a remarkably constant one, increasing with an increase in the dilution. The average error in the different series varied per 10 cc. of dilution from 0.00015 gram to 0.00023 gram which, considering the colorimetric method, was fairly constant. The general average error per 10 cc. dilution was 0.00019 gram creatinine. Attention is called to the real error which exists when the correction factor for dilution is not applied. At 100 cc. volume the error varied from 9.6% to 14.7%. With 150 cc. volume the error varied from 17.0% to 23.0%, and with a 200 cc. volume the errors varied from 18.8% to 28.9%. This method was next extended to a study of the creatine of meat. The meat was washed free of creatine by the following method: Place 25 grams of meat (finely divided beef) in a 450 cc. Erlenmeyer flask, add 200 cc. of water and shake for one hour; decant into an evaporating dish holding 500 cc. and add 200 cc. of water to the Erlenmeyer flask and shake for the second hour; decant and wash the meat with 50 cc. of water. Evaporate the entire volume of liquid to a volume of 75 or 100 cc., filter, wash, and evaporate the filtrate to a 50 cc. volume, and place in a small Erlenmeyer flask, add 25 cc. of normal hydrochloric acid and convert the creatine to creatinine by the autoclave method of Benedict and Myers.[1] The solution is evaporated to 50 cc. after neutralizing and 10 cc. are used for the colorimetric determination of creatinine. The 10 cc. portion used represents 5 grams of beef. Four samples of beef treated by this method read 5.0, 4.9, 4.9 and 5.0 respectively on the colorimeter. The water extract of meat was subjected to the same treatment as the commercial creatinine samples above described. The results were not so uniform as those obtained where pure creatinine was used. The average error per 10 cc. of dilution was 0.00019 gram or exactly the same figure that was obtained using the pure creatinine. The error when the volume was 100 cc. was 6.9, 16.2, 10.1 and 11.1 per cent., the average showing 11.1 per cent. loss of creatinine. The error when a 150 cc. volume was used was as follows: 18.3, 21.6, 17.4 and 26.8, averaging 21 per cent.

[1] *Loc cit.*

For correction of error in Table VII, p. 682, column 7, see "Errata," p. 728.

When the volume was diluted to 200 cc. the errors were 22.1, 26.2, 29.6, 31.8 or an average loss of 27.4 per cent. of the total creatinine actually present.

TABLE VII.—ERROR DUE TO VOLUME—CREATININE IN COLD WATER EXTRACT OF BEEF.

No. of experiment.	Cold water extract of beef used. cc.	Water added. cc.	Volume before adding reagents. cc.	Picric acid added. cc.	10% NaOH added cc.	Colorimetric reading.	Creatinine. Gram.	Difference creatinine. Gram.	Creatinine error. Per cent.	Difference creatinine per 10 cc. dilution. Gram.
1	10	0	10	25	10	0.70	0.01209	.....	...	.....
2	10	40	50	25	10	0.95	0.01166	0.00043	3.5	0.00011
3	10	90	100	25	10	0.20	0.01125	0.00041	6.9	0.00008
4	10	140	150	25	10	0.20	0.00988	0.00137	18.3	0.00027
5	10	190	200	25	10	0.60	0.00942	0.00046	22.1	0.00009
									Average,	0.00014
1	10	0	10	25	10	6.20	0.01307	.....	...	.....
2	10	40	50	25	10	7.05	0.01149	0.00158	12.1	0.000375
3	10	90	100	25	10	7.40	0.01095	0.00054	16.2	0.000110
4	10	140	150	25	10	7.90	0.01025	0.00070	21.6	0.000140
5	10	190	200	25	10	8.40	0.00964	0.00061	26.2	0.000120
									Average,	0.000186
1	10	0	10	25	10	5.70	0.01421	.....	...	.....
2	10	40	50	25	10	6.15	0.01317	0.00104	7.3	0.00026
3	10	90	100	25	10	6.34	0.01277	0.00040	10.1	0.00008
4	10	140	150	25	10	6.90	0.01174	0.00103	17.4	0.00021
5	10	190	200	25	10	8.10	0.01000	0.00174	29.6	0.00035
									Average,	0.000225
1	10	0	10	25	10	6.0	0.01350	.....	...	.....
2	10	40	50	25	10	6.3	0.01286	0.00064	4.7	0.00016
3	10	90	100	25	10	6.75	0.01200	0.00086	11.1	0.00017
4	10	140	150	25	10	8.2	0.00988	0.00212	26.8	0.00042
5	10	190	200	25	10	8.8	0.00920	0.00068	31.8	0.00014
									Average,	0.00022

Grindley and Woods,[1] in an article on the chemistry of flesh, recommended the use of 200 cc. of water extract of meat in determining the creatinine content and an exactly similar procedure in the case of meat extracts. The experiments here recorded show the error to average 27.4 per cent. in cases where the volume is 200 cc. In Table VIII given below the same procedure was carried out on a sample of beef extract to which commercial creatinine was added in quantity sufficient to obtain a reading

[1] *J. Biol. Chem.*, 1906–7, 2, 309.

of approximately 6 mm. on the Duboscq scale, using 10 cc. of the extract solution.

TABLE VIII.—ERROR DUE TO VOLUME CREATININE IN BEEF EXTRACT (CONTAINING ADDED CREATININE.)

No. of experiment.	Beef extract solution used cc.	Water added. cc.	Volume before adding reagents. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Creatinine found. Gram.	Difference creatinine. Gram.	Error creatinine. Per cent.	Difference creatinine per 10 cc. dilution. Gram.
1	10	0	10	25	10	6.25	0.01296	.....	...	.....
2	10	40	50	25	10	6.78	0.01194	0.00102	7.9	0.00025
3	10	90	100	25	10	7.30	0.01109	0.00085	14.4	0.00017
4	10	140	150	25	10	7.82	0.01036	0.00073	20.1	0.00015
5	10	190	200	25	10	8.50	0.00953	0.00083	26.5	0.00017
									Average,	0.00021
1	10	0	10	25	10	6.1	0.01238	.....	...	.....
2	10	40	50	25	10	6.35	0.01278	0.00050	3.8	0.00012
3	10	90	100	25	10	6.85	0.01182	0.00096	11.0	0.00019
4	10	140	150	25	10	7.68	0.01055	0.00127	20.6	0.00025
5	10	190	200	25	10	8.45	0.00595	0.00096	27.8	0.00019
									Average,	0.00019

The average error per 10 cc. of dilution here shows 0.0002 gram of creatinine which is practically the same, *viz.*, 0.00019 gram found in the case of pure creatinine, and also obtained when a water extract of beef was used. The error per 10 cc. of dilution, using the commercial solution of creatinine, has been shown to be 0.00019 gram creatinine and the same holds true of water extracts of beef and of beef extract. It is therefore proposed to use this figure 0.00019 as a factor in correcting the creatinine error due to dilution.

The table below, IX, shows the error due to dilution in the case of beef and beef extracts, both the loss of creatinine in grams and the percentage loss of creatinine. In addition, the corrected values both in grams of creatinine and in the per cent. error based on the corrected values are given. The values using 10 cc. of the original solution are taken as the standard creatinine values. The uncorrected error for 100 cc. dilution averages 11.63 per cent.; the corrected error is reduced to 1.19 per cent. With a 150 cc. volume the uncorrected error averages 20.8 per cent., the corrected error 0.55; using 200 cc. volumes the uncorrected error averages 27.3 per cent., the corrected error 1.18 per cent. It is evident from the above figures that the correction factor tends to give very nearly the correct values for creatinine. The common dilutions used in determining creatinine in meat extracts are 100,

TABLE IX.—INFLUENCE OF DILUTION ON CREATININE IN BEEF AND BEEF EXTRACTS.

(Corrected and uncorrected results showing errors due to dilution.)

Substance.	Creatinine per 10 cc. volume. Gram.	Creatinine per 100 cc. volume.				Creatinine per 150 cc. volume.				Creatinine per 200 cc. volume.			
		Obtained value. Gram.	Difference 10 and 100 volumes. Gram.	Corrected value. Gram.	Difference 10 cc. and 100 cc. corrected. Gram.	Obtained value. Gram.	Difference 10 and 150 cc. volumes. Gram.	Corrected value. Gram.	Difference 10 cc. and 150 cc. corrected. Gram.	Obtained value. Gram.	Difference 10 and 200 cc. volumes. Gram.	Corrected value. Gram.	Difference 10 and 200 cc. corrected. Gram.
Creatinine beef	0.01307	0.01095	0.00212	0.01266	0.00041	0.01025	0.00282	0.01291	0.00016	0.00964	0.00342	0.03125	0.00018
			—16.2%		—3.14%		—21.6%		—1.22%		—26.2%		+1.38%
Creatinine beef	0.01421	0.01277	0.00144	0.01448	0.00027	0.01174	0.00247	0.01440	0.00019	0.01000	0.00421	0.01361	0.00060
			—10.1%		+1.90%		—17.2%		+1.34%		—29.6%		4.24%
Creatinine beef	0.01350	0.0120	0.00150	0.01371	0.00021	0.00988	0.00362	0.01254	0.00096	0.00920	0.00430	0.01382	0.00068
			—11.1%		+1.6%		—26.8%		—7.1 %		—31.8%		—5.04%
Creatinine beef	0.01209	0.01125	0.00084	0.01283	0.00074	0.00988	0.00221	0.01254	0.00045	0.00942	0.000267	0.01303	0.00094
			—6.95%		+6.12%		—18.3%		+3.72%		—22.1%		+7.77%
Creatinine beef extract.....	0.01296	0.01109	0.00187	0.01280	0.00016	0.01036	0.00260	0.01302	0.00006	0.00953	0.00343	0.01214	0.00082
			—14.4%		—1.23%		—20.0%		+0.47%		—26.5%		—6.33%
Creatinine beef extract.....	0.01328	0.01182	0.00146	0.01353	0.00025	0.01055	0.00273	0.01321	0.00007	0.00959	0.003692	0.01320	0.00008
			—11.0%		+1.9%		—20.6%		—0.53%		—27.8%		—0.60%
	Average errors,		—11.63%		+1.19%		—20.8%		—0.55%		—27.3%		—1.18%

150 and 200 cc. volumes. It is proposed in the case of 100 cc. volumes to add 0.00019 × 9 or 0.00171 gram of creatinine to the creatinine value obtained; when the volume is 150 cc., to add 0.00019 × 14 or 0.00266 gram creatinine and in the case of 200 cc. volume add 0.00019 × 19 or 0.00361 gram creatinine to the obtained values.

(*d*) *Influence of Standing.*—The length of time usually recommended for the creatinine solution to develop its orange color with the alkali and picric acid reagents is from five to ten minutes. The destruction of creatinine and its change to creatine on standing has been noted by several investigators. It was deemed advisable to investigate the influence of standing on the determination of creatinine by the colorimetric method. The solutions were all made to 500 cc. volume and stood fifteen hours. The results given below in Table X were performed on commercial samples of creatine, using the boiling water bath method.

TABLE X.—EFFECT OF STANDING ON THE CREATININE DETERMINATION.

Creatine solution taken. cc.	Picric acid added. cc.	10% NaOH added. cc.	Period of standing. Hrs.	Colorimetric reading.	Mgs. creatinine found.
25	30	10	..	7.1	11.41
25	30	10	..	7.1	11.41
25	30	01	15	7.7	10.52
25	30	10	15	7.6	10.66
25	30	10	..	7.3	11.09
25	30	10	..	7.3	11.09
25	30	10	15	7.9	10.25
25	30	10	15	7.8	10.38
25	30	10	..	8.0	10.13
25	30	10	..	7.9	10.25
25	30	10	15	8.0	10.13
25	30	10	15	8.0	10.13
25	30	10	..	7.1	11.41
25	30	10	..	6.8	11.91
25	30	10	15	8.0	10.13
25	30	10	15	8.0	10.13
25	15	10	..	8.0	10.13
25	15	10	..	7.9	10.25
25	15	10	15	9.1	8.90
25	15	10	15	8.9	9.10
25	15	10	..	8.5	9.54
25	15	10	..	8.4	9.64
25	15	10	..	8.5	9.54
25	15	10	15	8.6	9.42
25	15	10	15	8.7	9.31
25	15	10	15	8.6	9.42

These results show that on standing fifteen hours a somewhat higher reading was obtained on the colorimeter, indicating that a portion of the creatinine compound had been destroyed.

TABLE XI.—EFFECT OF STANDING ON THE CREATININE DETERMINATION.

Weight creatine taken. Gram.	Picric acid used. cc.	10% NaOH added. cc.	Time of heating. hrs.	Period of standing. hrs.	First reading.	Mgs. creatinine found.	Period of standing. min.	Second reading.	Mgs. creatinine found.
0.02	25	10	4	..	5.70	14.21	30	5.80	13.97
0.02	25	10	4	..	6.00	13.20	30	6.20	13.06
0.02	25	10	6	..	5.70	14.21	30	6.35	12.76
0.02	25	10	6	..	5.70	14.21	30	5.95	13.61
Sol. creatine used. cc.									
25	15	10	4	..	8.0	10.13	30	9.0	9.0
25	15	10	4	..	8.0	10.13	30	9.0	9.0
25	30	10	4	..	7.3	11.09	30	7.8	10.38
25	30	10	4	..	7.3	11.09	30	8.0	10.13
25	15	10	4	15	8.0	10.13	..	...	...
25	15	10	4	15	8.0	10.13	..	...	...
25	30	10	4	15	7.7	10.52	..	...	...
25	30	10	4	15	7.9	10.25	..	...	...

In Table XI, results are recorded which show the effect of standing but a short period of time (30 minutes) on the creatinine test. The last four samples stood fifteen hours. These are the same samples that gave an initial reading of 8.0–8.0, 7.3–7.3 and a reading thirty minutes later of 9.0–9.0, 7.8–8.0, respectively. We have an opportunity here to observe the action of standing, both for a short and long period of time, on the same sample. The first two samples of this series read 8 on the first reading, then 9, thirty minutes later, and after fifteen hours, dropped to 8, the original reading. The second series treated with 30 cc. of picric acid read 7.3 at first reading, thirty minutes later read 7.8 and 8.0, and fifteen hours later read 7.7 and 7.9. This indicates that considerable change had taken place in these samples in the first thirty minutes. The first four samples in Table XI show that there is an increased reading on standing thirty minutes.

(*e*) *Influence of Coagulable Protein.*—A solution of egg albumin in water was prepared and used for the coagulable protein sample in this test. Ten cc. of this egg albumin solution contain 0.027 gram of nitrogen, or 0.169 gram of protein, which is practically all coagulable. Where no egg albumin was added, 10 cc. of water were added in order to keep the volumes uniform. Both the autoclave and the boiling water bath methods were used. The largest amount of creatine indicated by this method was 82 per cent. in the first sample as shown in Table XII below.

It is evident from the figures in Table XII that the presence of 0.169 gram of coagulable egg albumin has a noticeable influence in increasing the reading of the colorimeter in the determination of creatinine. The effect was equally noticeable whether meat extract or a commercial sample of creatine was used.

TABLE XII.—INFLUENCE OF COAGULABLE PROTEIN ON CREATININE TEST.

Method used.	Wt. creatine taken. Gram.	Egg albumin added. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Boiling water bath, 4 hrs.	0.02	..	25	10	5.7	14.21
" " "	0.02	..	25	10	6.0	13.50
" " "	0.02	10	25	10	5.8	13.97
" " "	0.02	10	25	10	6.0	13.50
" " "	0.016	..	25	10	7.9	10.25
" " "	0.016	..	25	10	7.8	10.38
" " "	0.016	10	25	10	8.2	9.88
" " "	0.016	10	25	10	8.3	9.76
	Meat extract solution used. cc.					
Autoclave	15	..	25	10	9.0	9.00
"	15	..	25	10	9.2	8.80
"	15	10	25	10	9.5	8.53
"	15	10	25	10	9.7	8.35
"	15	10	25	10	9.3	8.71
"	20	..	25	10	5.7	14.21
"	20	..	25	10	5.8	13.97
"	20	10	25	10	6.0	13.50
"	20	10	25	10	6.2	13.07
"	20	10	25	10	6.1	13.28

In all cases 25 cc. of picric acid were added. It appears as if the coagulable albumin combined with a portion of the picric acid, leaving an amount too small to give the deepest color in the creatinine reaction. The precipitate formed appeared to be entirely dissolved by the alkali but nevertheless may *per se* have caused the lower creatinine figures.

(*f*) *Influence of Peptones.*—A solution of somatose, a powder consisting of albumoses and peptones, was prepared, 10 cc. of which contained 0.0547 gram of nitrogen, which represents 0.342 gram of proteoses and peptones. As in the previous experiment both a commercial creatine sample and a commercial meat extract solution were used. Ten cc. of 10 per cent. alkali were added in all cases. Where no proteoses and peptones were added, 10 cc. of water were introduced to keep the volumes constant. The results are given in Table XIII below.

Just as in Table XII the coagulable protein showed a tendency to increase the reading of the colorimeter likewise in Table XIII where a proteose and peptone solution was added a higher reading was obtained. It appears from the table to make no difference whether 15 or 25 cc. of picric acid are present. In both cases the readings are increased by the presence of the proteins. The average sample of commercial beef extract does not contain a sufficient amount of either coagulable or lower protein bodies to materially influence this test. An occasional sample is found, however, which contains considerable amounts of higher proteins which

by their presence may cause a notable error in the creatinine estimation. A series of results is recorded below, using a commercial sample of creatinine.

TABLE XIII.—INFLUENCE OF PROTEOSES AND PEPTONES ON CREATININE TEST.

Method.	Creatine solution taken. cc.	Peptone solution added. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Boiling water bath, 4 hrs.	25	..	15	10	8.5	9.53
" " "	25	..	15	10	8.5	9.53
" " "	25	10	15	10	8.7	9.31
" " "	25	10	15	10	8.8	9.21
	Weight creatine. Gram.					
" " "	0.02	..	25	10	5.70	14.21
" " "	0.02	..	25	10	6.00	13.50
" " "	0.02	10	25	10	5.95	13.61
" " "	0.02	10	25	10	6.15	13.17
" " "	0.016	..	25	10	7.9	10.25
" " "	0.016	..	25	10	7.8	10.38
" " "	0.016	10	25	10	8.1	10.00
" " "	0.016	10	25	10	8.4	9.64
	Meat extract solution used. cc.					
Autoclave.....	15	..	25	10	9.0	9.00
"	15	..	25	10	9.2	8.80
"	15	10	25	10	9.1	8.90
"	15	10	25	10	9.0	9.00
"	15	10	25	10	9.5	6.53
"	20	..	25	10	5.8	13.98
"	20	..	25	10	5.7	14.21
"	20	10	25	10	6.0	13.50
"	20	10	25	10	5.85	13.84
"	20	10	25	10	6.20	13.07

(g) *Influence of Alkali and Picric Acid on the Original Creatinine.*—The results recorded in Table XIV below show the effect of varying the amount of alkali from 5 to 15 cc. and also the influence of both 15 and 25 cc. portions of a 1.2 per cent. solution of picric acid. Two series of experiments are recorded in Table XIV, the first eleven results being comparable and the last twelve results comparable.

As in the case of the results shown in Table III with the total creatinine, so here in the cases where 15 cc. of alkali were used, lower results were obtained than where 5 or 10 cc. were used. There is an indication that the results using 10 cc. of alkali run lower than where 5 cc. were used. This helps to explain the lower results where 15 cc. of alkali are present, showing the solvent effect of the alkali on the creatinine compound. In regard to the amount of picric acid to be added, there seems to be very little difference in the results whether 15 or 25 cc. are added; if there is any difference the higher results are indicated where 25 cc. are present.

TABLE XIV.—INFLUENCE OF ALKALI AND PICRIC ACID ON ORIGINAL CREATININE.

Creatinine solution taken. cc.	Water added. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
10	20	15	5	7.9	10.25
10	20	15	5	7.85	10.32
10	15	15	10	8.1	10.00
10	15	15	10	8.2	9.88
10	10	15	15	8.6	9.42
10	10	15	15	8.5	9.53
10	10	25	5	7.8	10.38
10	10	25	5	7.9	10.25
10	5	25	10	7.8	10.38
10	5	25	10	7.8	10.38
10	0	25	15	9.1	8.90
10	20	15	5	11.30	7.17
10	20	15	5	11.55	7.01
10	15	15	10	11.4	7.11
10	15	15	10	11.7	6.92
10	10	15	15	12.0	6.75
10	10	15	15	12.1	6.69
10	10	25	5	10.8	7.50
10	10	25	5	11.2	7.23
10	5	25	10	11.75	5.89
10	5	25	10	11.45	7.07
10	0	25	15	12.1	6.69
10	0	25	15	12.2	6.63

(*h*) *Influence of Dilution on Original Creatinine.*—The few experiments recorded below seem to check up the results shown in Table V, dealing with the total creatinine. As in the experiments recorded in Table V, the dilutions were made previous to adding the reagents. The results show that dilution has a marked action in lowering the creatinine results.

TABLE XV.—INFLUENCE OF DILUTION ON ORIGINAL CREATININE.

Creatinine solution taken. cc.	Volume diluted. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
10	10	25	10	7.7	10.52
10	10	25	10	7.6	10.59
10	150	25	10	8.3	9.76
10	150	25	10	8.5	9.53

(*i*) *Influence of Standing on Original Creatinine.*—Here as in (*g*) and (*h*), mentioned above, 10 cc. of a commercial creatinine solution were used. Four samples were taken, two of which stood for fifteen hours in the presence of the reagents, but without being made to volume. The other two samples were made to volume, and the creatinine determined immediately; then again after two hours, and finally after fifteen hours standing. The results are recorded in Table XVI below.

TABLE XVI.—INFLUENCE OF STANDING ON ORIGINAL CREATININE.

Creatinine solution taken. cc.	Period of standing. hrs.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
10	0	25	10	7.8	10.38
10	0	25	10	7.85	10.32
10	2	25	10	8.7	9.31
10	2	25	10	8.7	9.31
10	15	25	10	11.0	7.36
10	15	25	10	11.3	7.17
10	15[1]	25	10	22.0	3.68
10	15[1]	25	10	19.0	4.26

The readings show a steady increase on standing indicating that the amount of creatinine present gradually decreases, depending on the length of time that has elapsed before taking the readings. The readings after fifteen hours were 11.0 and 11.3 in the cases where the solutions were made to volume, but where the solutions stood 15 hours before being made to volume, the readings were 19.0 and 22.0. This shows the destructive action of the alkali on the creatinine compound.

(*j*). *The Influence of Coagulable Protein and of Proteoses and Peptones on Creatinine.*—These experiments were of the same general nature as those recorded earlier in this article. Ten cc. of a commercial creatinine solution were used.

TABLE XVII.—INFLUENCE OF COAGULABLE PROTEIN AND OF PROTEOSES AND PEPTONES ON ORIGINAL CREATININE.

Creatinine solution taken. cc.	Substances added. 10 cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
10	Water	25	10	7.65	10.59
10	"	25	10	7.7	10.52
10	Peptone	25	10	8.3	9.76
10	"	25	10	8.0	10.13
10	"	25	10	7.9	10.25
10	Egg albumin	25	10	8.0	10.13
10	"	25	10	8.2	9.88
10	"	25	10	8.1	10.0

The results recorded in Table XVII show that both the solution of egg albumin and the proteose and peptone solution have a distinct action in increasing the creatinine readings, that is, they give lower figures for the amount of creatinine present. These figures coincide with the results in Tables XII and XIII, and indicate that the presence of protein in any considerable amount tends to lower the creatinine values whether for the original creatinine or the total creatinine.

The commercial samples of creatine and creatinine are of varying degrees of purity and only in an exceptional case was over 85 per cent. of creatine or creatinine found in a commercial sample.

[1] Stood without being made to 500 cc. vol.

(*k*) *Comparison of Methods.*—Four methods for the determination of creatinine in meat products have been used in this country, namely, the autoclave method of Benedict and Myers;[1] the method as described by Emmet and Grindley;[1] the method as formerly employed in the chemical laboratory of Armour and Company;[1] and the boiling water bath method.[1] In all of the experiments recorded in Table XVIII below, 25 cc. of picric acid and 10 cc. of 10 per cent. alkali were used.

TABLE XVIII.—COMPARISON OF CREATININE METHODS.

Method employed.	Meat extract solution used. cc.	Volume diluted. cc.	Aliquot used. cc.	Equivalent to original solution. cc.	Picric acid added. cc.	10% NaOH added. cc.	Colorimetric reading.	Mgs. creatinine found.
Autoclave	50	100	20	10	25	10	10.5	7.71
	50	100	20	10	25	10	11.0	7.36
Boiling water bath, 4 hours	50	100	20	10	25	10	12.5	6.48
	50	100	20	10	25	10	12.3	6.59
Grindley and Woods	50	100	20	10	25	10	11.6	6.98
	50	100	20	10	25	10	13.0	6.23
Armour	10	...	..	10	25	10	10.9	7.42
	10	...	..	10	25	10	10.9	7.42
Autoclave	50	100	30	15	25	10	8.4	9.64
	50	100	30	15	25	10	8.4	9.64
Boiling water bath, 4 hours	50	100	30	15	25	10	9.0	9.0
	50	100	30	15	25	10	9.0	9.0
Grindley and Woods	50	100	30	15	25	10	9.9	8.18
	50	100	30	15	25	10	8.3	9.76
Autoclave	50	100	20	10	25	10	10.8	7.50
	50	100	20	10	25	10	11.0	7.36
Boiling water bath, 4 hours	50	100	20	10	25	10	11.50	7.04
	50	100	20	10	25	10	12.00	6.75
Grindley and Woods	50	100	20	10	25	10	10.8	7.50
	50	100	20	10	25	10	11.0	7.36
Armour method	10	...	all	10	25	10	11.4	7.11
	10	...	all	10	25	10	10.6	7.64
Autoclave	50	100	30	15	25	10	6.9	11.74
Boiling water bath, 4 hours	50	100	30	15	25	10	7.6	10.66
Grindley and Woods	50	100	30	15	25	10	7.0	11.57

After dehydration, the solutions in all cases with the exception of the Armour method, were made to a volume of 100 cc. and an aliquot of either 20 or 30 cc. used. Four series of results are recorded in Table XVIII.

The autoclave method appears to give the highest average results and the duplicates are very close. The boiling water bath method is quite satisfactory, but gives lower results than obtained by the autoclave

[1] *Loc. cit.*

method. Moreover, it is a time-consuming method. The other two methods give poorer duplicates and both being evaporation methods, depend for their accuracy on a uniform evaporation, which is often difficult to obtain. For practical work the autoclave method seems to be the best at hand to-day. Fifteen minutes are sufficient for the conversion of all the creatine to creatinine; longer heating gives no higher results. In this laboratory the method was performed as follows: Fifty cc. of beef extract solution representing 1.15 grams of extract were used. Fifty cc. of normal hydrochloric acid were added and the solution well mixed. The mixture was heated for fifteen minutes in the autoclave under fifteen pounds pressure. The solution was made to 100 cc. and 20 cc. aliquots used for the colorimetric estimation of creatinine. In order to keep the volume to Folin's original figure, 10 cc., the author recommends evaporating the solution after neutralizing to 50 cc. and using a 10 cc. aliquot. The aliquot is treated with 25 cc. of picric acid and 10 cc. of 10 per cent. sodium hydroxide in a 500 cc. graduate flask, well shaken, allowed to stand five minutes, diluted to volume and the readings taken.

All of the experiments recorded above were carried out on the dehydrated sample, that is, the total creatinine was estimated in each case.

Conclusions.

1. Using commercial samples of creatine and creatinine slightly higher results were obtained when 30 cc. of picric acid were added than when 15 cc. were added. In the case of meat extracts it appears to have made little difference whether 15 or 30 cc. of picric acid were used. It is accordingly advisable to have 25 or 30 cc. of picric acid present in all cases as it has been shown that an excess of picric acid does not alter the results.

2. Practically the same results were obtained on using 5 and 10 cc. portions of 10 per cent. sodium hydroxide in the case of both creatine and creatinine. Fifteen cc. of sodium hydroxide gave lower results; evidently the excess of alkali tended to destroy the creatinine compound. With beef extract 10 and 15 cc. portions of alkali gave practically identical results, the results where 5 cc. of alkali were used being lower. The combination of the alkali with the protein of the meat extract may account for this difference. Ten cc. of alkali gave the most universally satisfactory results.

3. The values for creatine and creatinine both alone and in meat and meat extracts were lowered upon dilution. The error due to dilution appears to be a fairly constant one, and averages 0.00019 gram creatinine per 10 cc. of dilution. It is proposed to correct for the dilution error by adding 0.00019×9 or 0.00171 gram to the creatinine value obtained where the dilution is 100 cc. In the case of 150 cc. dilution add 0.00019×14 or 0.00266 gram creatinine and where the volume is 200 cc. add 0.00019×19

or 0.00361 gram to the creatinine value obtained. If it is impossible to keep the volume of the solution at 10 cc. it is advisable to use the correction factor.

4. The period of standing appeared to influence the creatinine values. The longer the solution stood the lower were the results. This point was worked out on commercial creatine and creatinine samples. It is advisable to allow the solution to stand five minutes after adding the reagents and read at once.

5. The presence of coagulable protein lowered the results in the case of solutions containing only creatine and creatinine, and on adding egg albumin to a beef extract solution the creatinine results were also lowered.

6. The presence of proteoses and peptones lowered the values for solutions of creatinine. This was also the case with commercial samples of creatine and on adding a proteose and peptone solution to a sample of meat extract the creatinine values were slightly lowered. The ordinary meat extract does not contain a sufficient quantity of protein bodies to seriously affect the creatinine results. In an exceptional case it is necessary to remove the protein bodies before applying this test.

7. The autoclave method of Benedict and Myers, modified so as to apply to meat products, gave the most satisfactory results in determining creatine.

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CHEMISTRY OF ANIMAL FECES.

SECOND PAPER.[1]—THE DETERMINATION OF FATTY MATTER IN ANIMAL FECES BY ETHER AND CARBON TETRACHLORIDE.

By A. D. Emmett.

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In the first paper upon this subject, relating to the possibility of analyzing the fresh dung from cattle and swine without previously air-drying the samples, the determination of the fatty matter was reported as having been made with both anhydrous ether and carbon tetrachloride.

The use of the last-mentioned solvent was recommended by Bryant[2] as a substitute for ether in extracting fat from foods and feeding-stuffs, because of the great rapidity of the extraction, the decrease of danger from fire and the reduction of the expense. Lately, Herty, Stem, and Orr[3] have made use of carbon tetrachloride in determining the percentage of oil in cotton-seed products. They have found that this reagent, when compared with gasoline, removes quantitatively the same amount

[1] A. D. Emmett and H. S. Grindley, This Journal, **31**, 569 (1909).

[2] This Journal, **26**, 568 (1904).

[3] *J. Ind. Eng. Chem.*, **1**, 76 (1909).