



THE PREPARATION OF CREATININE FROM CREATINE

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THE PREPARATION OF CREATININE FROM CREATINE

I. Introduction.

Creatine and creatinine are found distributed very extensively in the animal kingdom. Creatine is a constituent of all vertebrate muscles but apparently is absent from invertebrate muscle. The creatine content for the muscles of a given species is very constant, varying among the different species from about 0.36 % or less to 0.52 %. Creatinine on the other hand is almost or entirely absent from muscles but is a normal constituent of mammalian urine the percentage varying. Creatinine also occurs in wheat, rye, clover and many other crops and hence occurs in cultivated soil.

Thus creatine and creatinine are of importance to the biological chemist. There is a demand here for creatine and creatinine for numerous experiments with the aim of discovering the biological significance of these two substances.

Just recently a tentative method is being tried for diagnosing nephritis by administering creatinine and then analysing the output in the urine. (R. H. Major, Jr., J. Am. Med. Assoc., Feb. 1923)

Both creatine and creatinine were expensive chemicals a few years ago. At present creatine is very readily obtained from a by-product of a commercial meat-juice plant.



(J. Ind. Eng. Chem. <u>14</u>, 984). The by-product is about 50 % creatine from which it is readily obtained by several recrystallizations. This has given a relatively cheap supply of creatine yet the price of creatinine is still high even tho it is theoretically very easy to convert the one into the other.

II. Historical.

(a). Creatinine from Urine.

Creatinine has been obtained directly from urine by several methods the two best known and most satisfactory being the method of Folin and Blanck (J. Biol. Chem. <u>13</u> (1910) 395-97) and the improved one of Benedict (J. Biol. Chem. <u>18</u> (1914) 183-190).

1. In the method of Folin and Blanck a hot alcoholic solution of picric acid is added to undecomposed urine in the proportion of 125 g. of picric acid to 8 liters of urine. After settling over night the precipitate is collected on a Buchner funnel, washed thoroughly with dilute picric acid and then with cold water. The picrate, mixed with 400 cc. of water, is decomposed by 60 g. of potassium bicarbonate, keeping the temperature at 45-50° C. until evolution of CO_2 ceases, care being taken to prevent loss by foaming over.

After standing in cold water over night the precipitate is filtered, washed and then 50 % acetic acid added to the filtrate until there is an acid reaction again watching the tendency to foam. Finally alcoholic zinc chloride is added

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producing the double chloride of zinc and creatinine.

This double chloride is dissolved in 10 % warm sulfuric acid when a compound having the formula (creatinine)₂SO₄, $2nSO_4$, $6H_2O$ is formed, which is almost quantitatively precipitated on addition of acetone or alcohol and ether. This precipitate is purified by boiling with bone black, filtering and again precipitating with organic solvents.

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Creatinine is obtained from this salt by adding to its solution the theoretical amount of barium acetate required to precipitate the sulfuric acid and then passing hydrogen sulfide into the hot mixture to precipitate the zinc. After filtering, the solution is evaporated to dryness in a vacuum at about 50° C. The remaining acetic acid being removed by washing with alcohol.

2. In Benedicts method the creatinine picrate is obtained in the same manner as in the preceding method. However instead of decomposing it with potassium bicarbonate concentrated hydrochloric acid is used, about 60 cc. being required for 100 g. of creatinine picrate. The creatinine hydrochloride solution which forms is filtered from the insoluble picric acid with suction and then neutralized with solid magnesium oxide keeping the flask cooled by running water. The mixture is again filtered with suction, washed and then the filtrate is strongly acidified with acetic acid and diluted with about four volumes of alcohol. The slight



precipitate which forms is removed by filtering and the filtrate treated with 30 % zinc chloride, using about 4 cc. for each liter of urine used. After standing over night the creatinine zinc chloride is filtered, washed with water and then with alcohol.

The product should be a nearly white crystalline powder. With care a 90-95 % yield should be obtained.

The zinc salt is purified by dissolving it in water, 10 g. to 100 CC., adding 60 CC. N H₂SO₄ and heating to boiling. About 4 g. of animal charcoal are added and boiling continued for one minute after which the material is filtered, pouring the filtrate thru until clear. The filter is then washed with hot water and filtrate treated with strong zinc chloride solution after which a solution containg 7 g. of potassium acetate is added. The solution is next diluted with an equal volume of alcohol when creatinine zinc chloride crystallizes out on standing in a cold place. It is filterdoff and any potassium sulfate removed by stirring it up with its own weight of water, filtering and washing with alcohol.

Creatinine is best obtained by adding a volume of concentrated aqueous ammonia equal to seven times the weight of the recrystallized creatinine zinc chlowide and warming the mixture just enough to affect solution. The solution is then placed in an ice-box to allow the creatinine to crystalize out. The yield should be 60-80 %. In case of a yellow product it may be purified from alcohol or ammonia.

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(b) Creatinine from Creatine.

Creatinine is obtained directly from creatine when the latter is heated under pressure due to the catalytic action of the water of crystallization. This is the autoclave process of Folin and Denis. (J. Biol. Chem. <u>13</u> (1910) 399-400). They place creatine which contains water of crystallization in a stoppered bottle, enclosing this in an ordinary preserving jar that fastens with a clamp. The jar is then placed in water in an autoclave and heat applied until a pressure of 4.5 kilos per sq. cm. is developed maintaining the same for three hours. Upon cooling the contents should consist entirely of crystalline creatinine. The product is usually less white than the original and small amounts of ammonia may form. It may be purified by means of alcohcl. About a 90 % yield is obtained analysing 99-100 %.

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Anhydrous creatine will not convert without the addition of a small amount of water. The ammonia generated does not interfere. Creatinine may be present originally in the creatine, otherwise it must be pure.

(c) Discussion of Former Methods.

One can readily see that the methods of obtaining creatinine from urine are difficult in technique, require much labor and give a low yield. Benedict says the method of Folin is unsatisfactory and gives a low and uncertain yield. (J.Biol. Chem. <u>18</u> (1914) 184). The method of Bene-



dict is also a laborious process giving a large loss in passing from creatinine zinc chloride to creatinine. Thus it would give a low yield if creatine was used as the starting point.

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The autoclave process of Polin and Denis apparently fails in giving satisfactory results when larger quantities of material are handled.

Because of the various reasons mentioned heretofore a more satisfactory method was sought for obtaining creatinine.

III. Methods of Investigation.
The investigation was conducted along two main lines.
(a) Direct conversion of creatine to creatinine
by catalytic agents.

1. Since creatinine is formed from creatine by the loss of a molecule of water, dry gaseous amnonia was passed thru a tube of dry creatine heated to 150° C. Dry ammonia is a good dehydrating agent and thus it was thought that it might bring about this conversion.

2. Glacial acetic acid vapor was substituted for the ammonia. Acids are known to convert creatine to creatinine but where a strong acid is used a salt is formed, as creatinine hydrochloride with hydrochloric acid. With this weak acid it was thought that perhaps conversion may take place and at the same time leave the creatinine free.



3. It is known that steamunder pressure and slightly superheated, as in the autoclave process, will bring about the conversion of creatine to creatinine. If this occurs under several atmospheres pressure the same reaction might take place at a higher temperature and under atmospheric pressure. Therefore, steam heated to 150° C. was passed thru creatine.

(b) Conversion with intermediate creatinine hydrochloride.

Since, as shown below, none of the above methods proved satisfactory, a scheme based on the following known facts was adopted.

First, it is known that creatine is readily and quantitatively converted into creatinine hydrochloride in presence of an excess of hydrochloric acid. Secondly, creatinine is a weak base even weaker than ammonia and thus by adding ammonia to a solution of creatinine hydrochloride ammonium chloride should form leaving uncombined creatinine. Thirdly, creatinine is very much less scluble in a cold concontrated ammonium hydroxide solution than is ammonium chloride and thus it should be possible to separate the two very readily.

IV. Experimental.

(a) Materials.

For these experiments commercial creatine was used.

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This was a white crystalline material containing a few per cent of creatinine and a small amount of muscle extractives that gave the material a slightly disagreeable odor. The yields given are based upon the total creatinine content of the material upon conversion with hydrochloric acid. In a few cases, as noted, the commercial creatine used was purified by several recrystallizations.

- (b) Experimental Methods.
- 1. Method of analysis.

All analyses given in this paper were made with a Bausch and Lomb Dubosc Colorimeter with 100 mm. prisms by the Folin method using creatinine or one of its salts as a standard. For most of the analyses pure creatinine, made by H.J. Bean of Boston, was used as a standard. The standard solution was made approximately N/10 acid by hydrochloric acid. This standard checked with a creatinine picrate standard. (For a discussion of standards see paper by Graham Edgar, J. Biol. Chem., May 1923).

Since the Folin method has been variously modified the procedure adopted in this investigation will be given in some detail. The standard contained approximately 1 mg. per cubic centimeter and the material to be analysed was made into a solution containing as near the same amount of creatinine as could be judged. At least if 10 mg. of creatinine was used as a standard the sample should contain not less than 5 mg. mor more than 15 mg. of creatinine.

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For an analysis 10 cc. of the standard and 10 cc. of the sample, measured in a precision pipette, were placed in separate graduated 500 cc. flasks. 25 cc. of a saturated solution of picric acid was added to each flask and then 10 cc. of a 10 % solution of sodium hydroxide. The contents in each flask were mixed by shaking and allowed to stand for exactly seven minutes after which the flasks were filled to the mark with water. The solutions were made uniform by thorough shaking and then compared in the colorimeter. Readings were taken at approximately 30 mm. To insure correct readings the sample and standard were reversed and new readings taken. The mean of these two sets gave the data which is given in the tables.

Like all colorimetric methods this is only accurate to one or two per cent.

2. Apparatus and Procedure for the Direct Hethods. The apparatus and procedure was essentially as follows:

The creatine was placed in a small U-tube fitted with stop-cocks. Free passage of vapor was assured by placing alternate thin layers of creatine and glass-wool followed by a large plug of glass-wool to prevent any of the powdery creatine from being carried from the tube. One end of the U-tube connected to a small tube thru which either dry air or the different gases could be conducted into $it_{\Lambda}^{\text{and}}$ from which they passed into a condensing bottle <u>or into-bottles-con</u>

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densing bottle.or, when ammonia was used, into bottles containing water for absorbing it.

The U-tube containing the creatine was immersed in an oil-bath heated by means of a Bunsen burner. The temperature was kept at about 150° C. by means of hand control, not varying more than a few degrees from this at any time.

Each time, before admitting the gas or vapor, dry air was conducted slowly thru the tube while heated to 150° C. to remove all moisture from the sample. This was continued for one-half hour when the tube was removed from the bath, the hot oil carefully removed by means of clean towels and the tube allowed to cool in a desiccator, containing calcium chloride as the drying agent, before weighing. The same procedure was followed when the gas or vapor was cut off at the close of an experiment.

If the creatine would completely convert into creatinine there would be a 13.7 % loss in weight. Thus with a three gram sample, which is approximately the amount used, even a slight conversion could be detected by weighings provided there were no other reactions.

(1). With Gaseous ammonia.

The ammonia was supplied from a cylinder of compressed gas. No change in color or form could be detected.

The data given in the following table were obtained when using ammonia:


Wt. of :Wt.of tupe: creatine:creatine &: used :glass-wool:	Hrs.NH ₃ was passed	Wt.of tube,etc., after passing NH ₃	Loss in wt. -sign means gain in wt.
J.2860 g: 33.0873 g.	11/2	33.0876 g.	-0.0003g.
2.6531 :33.2150	7	33.2144	0.0006
2.6531 :33.2150	61/2	33.2157	-0.0007
2.6531 :33.2194	21/2	33.2123	0.0001

The slight gains and losses are well within the experimental error of the method used in removing oil from the U-tubes and thus there is no evidence of conversion.

(2). With Acetic Acid Vapor.

A. Heated to 150° C.

In using acetic acid vapor in place of ammonia a few changes were made in the apparatus. A glass-tube coil, immersed in the oil-bath, was inserted just before the U-tube to insure the acid being in the form of vapor before coming into contact with the creatine. In this case the vapor was drawn thru the apparatus by slight suction, dry air being allowed to bubble thru the flask containing acetic acid which was kept at about 100° C. The results are given in the following table.

Wt. of	WL.of tube,	Hrs. acetic	Nt.of tube,	Lossin wt.
creatine	creatine &	acid vapor	etc.,after	
2.6531 g.	33.2±09 g.	21/2	33.1374 g.	0.0735 g.
51/2 hrs.	longer)	· 7	.32.8388	· 0.3721
3.2795	:33.2573	· 11/2		• 0.2673

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In this case there was both decomposition and conversion. Almost as soon as the acid vapors came into contact with the creatine a slight dark brown coloring was noticed which increaded as the vapor continued to pass thru the material. Besides this product there was a lighter colored, slightly volatile and tarry substance formed much of which was carried across with the acid making a brownish colored solution in the condensing bottle. When a few drops of water were added to this solution a precipitate was formed and continued to form until about one-fourth as much water as there originally was solution had been added. On standing over-night a light brown powdery substance settled to the bottom and also a colorless substance crystallized out in fine needles. No investigation of these substances was undertaken.

Both of the tubes containing creatine lost more than the theoretical weight necessary for complete conversion. Analyses were made giving the following data:

From the first tube.

Wt. of sample for analysis.	creatinine before conversion.	Creatinine after con- version with HCl.
29.01 mg.	20.17 mg.	22.56 mg.

Thus about 30.4 % of the sample was creatinine and creatine the remainder being decomposition products and permaps a few very small particles of glass-wool that escaped detection. Of this 80.4 %, 89.4 % had been converted to creatinine.

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From the second tube.

Wt. of sample for analysis.	Creatinine before conversion.	Creatinine after con- version with HC1.
37.84 mg.	29.45 mg.	30.20 mg.

About 79.3 % of this comple was creatinine the remainder being decomposition products. Thus no creatine remained unconverted.

In both cases the material removed for analysis was from the part which had apparently suffered least decomposition. No analysis was made of the part that appeared to be almost entirely decomposed. There was no regularity to the rate of decomposition as the second tube showed the greatest amount with the shortest time. This was most probably due to better contact with acid vapors.

B. With Acetic Acid Wapor Heated to 1250 C.

Another tube was prepared containing 1.1331 g. of creatine and the same procedure carried out at 125° C. to see if it was possible to get conversion without the accompanying decomposition. The loss in weight after 1 $\frac{1}{2}$ hours was 0.0065 g. but apparently decomposition, as shown by the darkening of the creatine, was as great at this temperature as for the same length of time at the higher temperature while the loss in weight, and hence conversion, was less.

(3). With Superheated Steam.When superheated steam was used as the catalytic

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agent the same form of apparatus and procedure was used as described for acetic acid. The water gave off sufficient vapor when heated to 90-95° C., the temperature being increased as it passed thru the coil immersed in the oil-bath. No apparent decomposition occurred.

The following data were obtained:

Wt. of creatine	:Wt.of tube,: creatine & : glass-wool :	Hrs. steam was passed	Wt. of tube, : etc.,after : passing steam:	Loss in wt.
3.9510 g.	:46.5995 g.	51/6	:46.5925 g.	0.0070 g.
ų.	:46.5925	11/2	:46.5892	0.0033
\$1	46.5892	3	46.5864	0.0028
ŧr	:46.5864	2	:46.5837	0.0027
11	:46.5837	2	:46.5774	0.0063

There is evidently a slow conversion with superheated steam at atmospheric pressure but according to the loss in weight only 4.3 % was converted to creatinine after passing superheated steam for nearly 14 hours.

3. Procedure for Method Involving Intermediate Creatinine Hydrochloride.

(1). Preliminary Experiments.

Eight samples of creatine of 10 g. each were taken. To each sample 20 cc. of 6 N hydrochloric acid was added. The first four samples and the last two were allowed to evaporate to dryness on the water-bath, about 15-20 hours being



required. Samples 5 and 6 were evaporated almost to dryness on a hot-plate, just below boiling, and then to dryness on the water-bath. This required only about one-third the time the others did and yielded a less colored product.

Samples 1 to 6 were from commercial creatine and samples 7 and 8 from almost pure material, the creatine having been recrystallized several times.

After conversion to creatinine hydrochloride the samples were treated as follows:

10 cc. of concentrated ammonium hydroxide was added to samples 1 and 2, the creatinine hydrochloride being broken up in the mixture by means of a rubber-tipped class rod. The beakers were then placed in an ice-water bath for 1 to 2 hours after which the creatinine was filtered out, by suction in a Buchner funnel, washed with 10 cc. ice-cold, concentrated ammonium hydroxide and then with 15-20 cc. of alcohol. It was then dried for about an hour in an oven heated to 100-105° C., cooled and then weighed. The material was non-crystalline and hardened on drying but could be readily made fine in a mortar.

Samples 3 and 4 were first dissolved in 10 cc. of water by warming, saturated with gaseous ammonia while kept in an ice-water bath and then allowed to remain in same for an hour or more after which they were treated as samples 1 and 2. A finely crystalline material was obtained colored slightly brown by impurities.

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Samples 5 and 6 were broken up in 10 cc. of concentrated ammonium hydroxide and then, while kept in an ice-water bath, were saturated with ammonia after which they received the usual treatment. The product resembled that from samples 1 and 2.

Samples 7 and 8 received the same treatement as samples 3 and 4. The creatinine obtained was crystalline and from one sample almost pure white, the other one being slightly yellow colored due, for the most part, to slight oxidation during evaporation of the acid solution, also the creatine used was not absolutely pure.

Tests for chlorine were made with silver mitrate in a solution acidified with nitric acid. The chlorine could not be found quantitatively due to reaction between silver nitrate and the creatinine after all chlorine was precipitated.

A quantitative yield of creatinine from the commercial material would be 7.650 g. for each 10 g. sample as given by several analyses, due to the creatinine as an impurity in the creatine, and 7.5630 g. for pure samples.

The following table gives yields and purity of samples:



Sample: No.:	Yield in : grams :	% Yield	Purity of the second se	<u>ne_creatinine_formed</u> Chlorine (qualitative)
1 :	7.3730 g.:	96.4 %	92.0%	Heavy precip.with AgN63
2	6.8240	89.3	97.5	Chlorine present
3	6.9130	90.3	100.4	No chlorine present
4	6.9200	90.4	Not det.	0 - B - B
5	7.3650	96.3	1 1 1	Chlorine present
6	7.4150	96.9	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	n u
7	6.7680	89.4	100.0	No chlorine present
8	6.7390	89.0	Not det.	0 0 9

Samples 4 and 8 while not analysed were undoubtedly 100 % creatinine. Both were crystalline and free from chlorine, No. 8 being pure white.

Whenever the creatinine hydrochloride was not dissolved in water before adding the ammonia chlorine was present, sometimes in considerable quantity. The presence of chlorine was most likely due to some creatinine hydrochloride in hard particles with which the ammonia failed to come into contact and also to the difficulty of washing all the ammonium chloride from the pasty, non-crystalline product.

(2)Investigation on A Larger Scale. To further investigate this procedure a 25 g. sample of creatinine hydrochloride was finely powdered in a mortar, run thru a 40 mesh sieve and then treated with 25 cc. concentrated ammonium hydroxide as in the preliminary samples 1 and 2.

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The washing with alcohol was very thorough. The yield was 90 % but chlorine was still present tho in a less quantity, perhaps, than in samples 1 and 2, whereas in the case of the four crystalline samples not a trace of chlorine was found.

The proportion of 10 cc. of water to 10 g. of creatinine hydrochloride is slightly in excess of the minimum amount of solution necessary to hold the resulting ammonium chloride in solution, especially since there is a slight increase in volume with saturation by ammonia. 50 g. of creatinine hydrochloride in 40 cc. of water should the ammonium chloride which forms in solution. Accordingly this was tried and found to give a yield of about 92.5 % using a 50 g. sample of creatinine hydrochloride, the treatment after saturation with ammonia being the same as with previous samples. The creatinine was free from chlorine and in a crystalline condition.

That this method is applicable to large quantities of material is shown by obtaining a 92 % yield from 1 kilogram of creatine which was converted to creatinine hydrochloride and then treated as described in the preceding paragraph.

Creatinine made by any of the methods described in this paper can be further purified by the ordinary methods of purifying creatinine when the product is not pure enough for a particular use. When the crystalline material still contains a trace of chlorine, due to insufficient washing, it may be removed by stirring the creatinine in a small amount of ice-cold concentrated ammonium hydroxide then filtering and washing as above.

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(3). Substitute for the Method with Gaseous Ammonia.

To use the preceding method of obtaining creatinine from creatinine hydrochloride in the absence of gaseous ammonia the following procedure was found to give a satisfactory crystalline product tho the yield was less:

50.000 g. of creatinine hydrochloride was dissolved, by warming, in 35 cc. of water and then 35 cc. of concentrated ammonium hydroxide added and the mixture quickly cooled in an ice-water bath. It was allowed to stand in the bath for an hour or two with frequent stirring then filtered and treated the same as previous samples.

The following data were obtained by this procedure:

Sample No.	Wt. of sample	Yield in grams	% yield
10	50.00 g.	31.45	83.5 %
11	50.00	31.91	84.7

Both samples yielded creatinine free from chlorine. The smaller yield is largely due to the larger volume of solution from which the creatinine crystallized. Also in the preliminary experiments apparently the ones less saturated with ammonia, like sample 2, gave a smaller yield than samples 5 and 6 which were saturated after the ammonium hydroxide solution had reacted with the creatinine hydrochloride.

(4). Preparation of Purer Creatinine Hydrochloride from Commercial Creatine.



All of the samples indicated that the purity of color of the creatinine depends largely upon the color of the creatinine hydrochloride. When the latter was white or very light colored the creatinine obtained was white. Therefore a method of obtaining pure creatinine hydrochloride from commercial creatine was sought.

In the preparation of the former samples it was noticed that there was less undesirable color produced the shorter the time of heating with the hydrochloric acid and also that the upper surface of the residue was more colored than that underneath showing that oxidation from prolonged contact with the air caused some of the brown color.

A. By Rapid Conversion with Concentrated Hydrochloric Acid.

To determine the effect of a shorter time of heating in converting the creatine, 50 cc. of concentrated hydrochloric acid was added to 50 g. of creatine and the mixture slowly boiled to apparent dryness, being completely dried in an oven at about 105° C. The mixture boiled dry in 35-40 minutes. Analysis however showed that only 97 % had been converted but the product was very light colored. Increasing sufficiently the time of boiling to dryness would no doubt affect complete conversion or a somewhat greater quanity of acid would do the same and still leave a light product.

B. Without Evaporating Acid Solution to Dryness.

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Rather light colored creatinine hydrochloride was obtained by heating the commercial creatine with hydrochloric acid in the proportion of 100 g. of creatine to 125 cc. of concentrated acid in a flask on the water-bath until conversion has taken place, with partial evaporation, and then allowing the creatinine hydrochloride to crystallize out when the flask is kept in an ice-water bath. The yield of creatinine hydrochloride, however, is not quantitative as in the case of evaporation to dryness. The creatinine hydrochloride remaining in solution may be recovered by evaporation to dryness and working up the colored residue as described in the next paragraph.

C. By Decolorizing with Animal Charcoal.

Animal charcoal has been used to purify creatine and creatinine and so it was tried on solutions of creatinine hydrochloride. A clear solution was obtained by the use of about 10 g. of powdered "activated charcoal" for a solution containing 25 g. of creatinine hydrochloride. The volume was 35-40 cc. and after addition of charcoal the solution was kept at about 40-60° for about 15 minutes then filtered with suction, washing the residue with hot water. The filtrate was concentrated until it contained 20 cc., and then saturated with gaseous ammonia as in former cases.

The following data were obtained:

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No.	Wt. of sample	Yield in grams	% Yield	Purity
14	25.042 g.	15.530 g.	81.3 %	100 %
15	26.621	16.510	81.2	

This gave a loss of about 10 % over samples treated by the same procedure but omitting the clarification by charcoal.

D. By the Use of Gaseous Hydrogen Chloride.

It is known that dry gaseous hydrogen chloride will form creatinine hydrochloride with creatine. Preliminary experiments showed that if dry hydrogen chloride was passed into a beaker containing dry creatine for about a day with frequent stirring it would entirely convert the matereal into creatinine hydrochloride with very slight formation of colored decomposition products even from the commercial creatine used. Following this 75-100 g. of dried creatine were placed in an Erlenmeyer flask. This was closed by a two-hole rubber stapper. A tube leading into the flask thru one hole was connected to a Kipp type of hydrogen chloride generator and conducted the gas to the bottom of the flask, and thus beneath most of the creatine, and a short tube from the other hole connected with a U-tube containing mercury to prevent the escape of the gas and yet allow for some variation in pressure. The hydrogen chloride was only generated at the same rate it was absorbed and thus there was no waste. After two orthree days, depending somewhat on quantity used, conversion was complete the only

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attention required being to break up the more or less hard mass which was formed two or three times during conversion. The creatinine hydrochloride was white or very light colored and analysed 100.2% in the colorimeter.

E. Suspending Dry Creatine in an Inert Solvent and Converting with Dry Hydrogen Chloride.

As another possible means of obtaining better creatinine hydrochloride dry creatine was suspended in toluene at room temperature and dry hydrogen chloride passed into the mixture. Apparently a loose crystalline compound was rapidly formed which, perhaps, was creatine hydrochloride. After passing the gas into it for an hour or more the toluene was filtered off and the residue allowed to dry at room temperature. It was thought that by heating it creatinine hydrochloride might form. Analysis with silver nitrate showed that the substance contained hydrochloric acid. Accordingly, it was placed in an oven at 105-110° C. The first sample lost all of its hydrogen chloride with no conversion. The second sample gave about 36 % creatinine hydrochloride after 7-8 hours of heating.

Next dry creatine was suspended in xylene, since this could be readily heated to 100° C. on a water-bath giving a temperature at which rapid conversion is usually obtained. Hydrogen chloride was passed into this mixture while on a water-bath for about four hours. Apparently less



than 5 % was converted as shown by an attempted analysis.

F. Converting in Hydrochloric Acid Solution out of Contact with Air.

Since apparently the greatest amount of undesirable colored products formed where the material was exposed to the air for the greatest length of time, conversion was made in a stoppered flask with only the theoretical amount or a slight excess of acid present and a solution of the correct volume for saturation with ammonia after the conversion. This method gave a very light colored product, even starting with commercial creatine, and analysed 99.6 % pure, a figure within the limits of error for a pure sample when only one analysis was made.

When creatine is heated in hydrochloric acid solution many of the impurities from the muscle extractives found in commercial creatine become insoluble in the acid in a short time forming small brown, flocculent masses and thus could be largely removed by filtering, while hot, just before saturating the solution with ammonia. This method with filtering would give a very good grade of creatinine from commercial creatine.

By this method or any mentioned before, creatinine containing creatinine could be used as well as creatine free from creatinine and without loss of the latter.

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V. Conclusions.

(a). The action of gaseous ammonia, glacial acetic acid vapor and superheated steam on dry creatine, at atmospheric pressure and a temperature of 1500 C., are unsatisfactory as a means of obtaining creatinine.

Gascous armonia will not bring about conversion under these circumstances.

Acetic acid vapor caused conversion but could not be used as a practical method because of the large amount of decomposition that takes place.

Superheated steam apparently converts the creatine to creatinine without decomposition, so far as could be detected, but has no practical application because of its extreme slowness compared with other methods.

(b). The method of obtaining creatinine from creatine, with creatinine hydrochloride as an intermedicte product, gave very satisfactory results under some conditions.

The most satisfactory methods of obtaining creatinine hydrochloride, both from the standpoint of labor involved and purity of product, are:

1. The method of converting dry creatine by means of dry paseous hydrogen chloride as described on page 22.

2. Converting the creatine in hydrochloric acid solution in a closed flask to prevent oxidation. Since the creatinine will later be precipitated from this solution the

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proportions should be 50 g. of creatine to 40 cc. of acid solution. The quantity of acid should be the theoretical amount or only slightly in excess of this, at most, in order to prevent an excess of amionium chloride formation when the solution is later saturated with ammonia to obtain the creatinine.

From the standpoint of labor and materials the second method of obtaining creatinine hydrochloride will perhaps be the most satisfactory. If the insoluble impurities which form when using commercial creatine are filtered off before saturating the solution with anmonia, as described below, a very satisfactory product should be obtained.

For obtaining creatinine from the creatinine hydrochloride the following method gave the maximum yield, over 92 %, consistent with a sufficiently pure product.

50 g. of creatinine hydrochloride are dissolved in 40 cc. of water, warming being necessary, and this solution saturated with gaseous armonia, the saturation being completed at 0° C. The mixture is then kept at this temperature for about an hour after which it is filtered with suction, washed with about 25 cc. of ice-cold concentrated anmonium hydroxide and then with about 50 cc. of 95 % alcohol. The creatinine obtained is dried in an oven at 100° C. It should be finely crystalline, free from chlorides and test 100 % even when using creatinine hydrochloride obtained from commercial creatine.

The volume obtained here for saturation with ammonia is the same as that resulting from the conversion of creatine to creatinine hydrochloride in a closed flask as described previously.

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All emounts mentioned are merely relative. A kilogram of material has been worked up by this method at one time.

In the absence of gaseous ammonia creatinine may be obtained from creatinine hydrochloride as follows:

50 g. of creatinine hydrochloride are dissolved in 30-35 cc. cf water, 35 cc. of concentrated ammonium hydroxide added and the mixture allowed to stand at 0° C. for at least an hour, after which it is treated as described in the preceding method. This gives a smaller yield due to the increased volume cf solution and, perhaps to a small extent, from the lesser concentration of ammonia. The product is equally as satisfactory as in the former case.

VI. Summery.

(a). A small emount of conversion was obtained by the action of glacial acetic acid vapor and by superheated steam on dry creatine at 150° C. and atmospheric pressurc. No conversion was obtained with gaseous amaonia.

(b). A method of obtaining creatinine from creatine, involving the intermediate formation of creatinine hydrochloride, is described and a satisfactory technique developed for its application on a commercial scale.

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