

THE PREPARATION OF CREATININE FROM CREATINE.

By GRAHAM EDGAR AND W. S. HINEGARDNER.

(*From the Cobb Chemical Laboratory of the University of Virginia, University.*)

(Received for publication, May 4, 1923.)

INTRODUCTION.

The usual methods for the preparation of creatinine have involved the use of urine as the original source. This has made its preparation rather laborious and its price almost prohibitive for any other purpose than small scale research. In view of the fact that creatine is now available in large quantity and at much reduced prices it has seemed desirable to consider methods for the preparation of creatinine from creatine, with the object of discovering a simple method, suitable for large or small scale operation, and involving relatively little time or expense.

The two chief methods already described for the preparation of creatinine from creatine are those of Folin and Denis (1), and of Benedict (2). The former involves the heating of solid creatine in an autoclave; the latter involves the intermediate preparation of creatinine zinc chloride by one of several methods, and the subsequent decomposition of this salt with ammonia. These methods do not seem as simple or as satisfactory for the purpose outlined above as the one described below.

General Method.

The principle upon which the method depends is extremely simple, involving (a) the conversion of creatine into creatinine hydrochloride by treatment with hydrochloric acid, and (b) the formation of creatinine by treatment of the creatinine hydrochloride with ammonia, taking advantage of the facts that ammonia will liberate creatinine from solutions of its salts; that creatinine is only moderately soluble in cold concentrated ammonia; that ammonium chloride is readily soluble in cold ammonia

solutions; and that ammonia has very little tendency to bring about the conversion of creatinine into creatine.

The experimental technique necessary to obtain a good yield is described below. A number of variations in procedure are possible, and the choice of them may be determined somewhat by the scale of operation, the facilities of the particular laboratory, and the quality of the creatinine which may be desired.

EXPERIMENTAL TECHNIQUE.

The materials used were largely commercial creatine, containing 1 molecule of water of crystallization, together with small amounts of creatinine and traces of meat extractives. Pure recrystallized creatine (hydroxide) was also employed in certain cases.

Since the process divides clearly into two distinct steps, (1) the preparation of creatinine hydrochloride, and (2) the conversion of creatinine hydrochloride into creatinine, it seems best to consider these separately, particularly as the two may be combined in a number of different ways.

Preparation of Creatinine Hydrochloride.

(a). Creatine may be converted quantitatively into creatinine hydrochloride by evaporation to dryness upon a steam bath with an excess of hydrochloric acid, or by boiling such a solution slowly to dryness over a small flame. This is essentially the method employed by Benedict (3), who worked, however, only with relatively small quantities of creatine. We have found that complete conversion takes place when solid creatine is treated with twice its weight of 6 N hydrochloric acid, and the solution evaporated as indicated above. The process is rather a slow one when large quantities of creatine are employed, as a crust of creatinine hydrochloride forms when the solution becomes concentrated, and this must be broken up frequently. Furthermore, when crude creatine is thus treated considerable color develops, which increases somewhat with the time of heating. Material prepared by this process will be known as "creatinine hydrochloride (a)."

(b). Creatine may be converted into creatinine hydrochloride by treatment with *gaseous* HCl at room temperature. The

reaction is a slow one, but the process is practically automatic and has certain other advantages. In our experiments 100 gm. of creatine, previously dried at 100°C. to remove water of crystallization, were placed in a closed flask connected to an automatic HCl generator (*e.g.*, a Kipp generator employing fused NH_4Cl and H_2SO_4). The HCl is absorbed rapidly at first, and in the course of a few hours the mixture has caked somewhat. Two or three times a day the flask is disconnected and the cakes are broken up with a glass rod. By the end of 48 hours the reaction is complete, as is evidenced by failure to absorb more HCl. The mixture will have become moist at the end of the reaction, because of the liberation of water in the reaction. The moist mass is dried for a short time at 100°C. It will be known as "creatinine hydrochloride (*b*)."

(*c*). Creatinine may be converted into creatinine hydrochloride *solution* by adding to it a very slight excess of hydrochloric acid and heating the mixture on the steam bath in a closed flask for a sufficient length of time. In our experiments 150 gm. of creatine (hydroxide), 85 cc. of concentrated HCl (sp.gr. 1.19), and 25 cc. of water were heated for 24 hours or more on the steam bath, when conversion was found to be complete. This solution will be known as "creatinine hydrochloride solution (*c*)."

Conversion of Creatinine Hydrochloride into Creatinine.

The conversion of creatinine hydrochloride into creatinine may also be accomplished in several ways.

(*d*). Solid creatinine hydrochloride is added to a volume of cold concentrated ammonia (sp.gr. 0.90) equal to the weight of creatinine hydrochloride used. (It may be noted that this is almost exactly equal to the weight of creatine hydroxide originally taken.) The mixture is stirred, lumps are broken up as thoroughly as possible, and after standing at 0°C. for an hour or so the creatinine is filtered off (with suction), washed with a little ice-cold concentrated ammonia, and finally with alcohol. It is dried at 100°C.

(*e*). Solid creatinine hydrochloride is dissolved in 0.8 of its weight of water, warming to effect solution, and ammonia gas is rapidly passed into the solution, the mixture being cooled in ice

at the same time. When the ice-cold mixture gives a *strong* odor of ammonia the current of gas is stopped and after standing an hour or so the creatinine is filtered off, washed, and dried as above.

(f). Solid creatinine hydrochloride is dissolved in 0.6 of its weight of water, heating to effect solution, and an equal volume of cold concentrated ammonia is added, with constant stirring. The mixture is cooled as rapidly as possible, and is allowed to stand at 0°C. for an hour or so, and is then filtered and treated as above.

(g). Concentrated creatinine hydrochloride solution (c) is treated with ammonia gas exactly as described in (f).

Results.

1. The *yield* of creatinine by the above methods may be summarized as follows. Combining (a) or (b) with (d) or (e) gives very uniformly a yield of about 90 per cent of the theory (69 to 70 per cent of the weight of creatine hydroxide used; theory 76 per cent). Combining (c) and (g) also gives about 90 per cent of the theory. Combining (a) or (b) with (f) gives 83 to 85 per cent of the theory, the lower yield being due to the larger volume employed.

2. The *purity* of the creatinine depends partly upon the purity of the original creatine and partly upon the process employed. Process (d) gives a product nearly always containing a small amount of chlorine, and while very convenient, is not recommended where a good grade of creatinine is desired. Process (e) gives a product which is practically 100 per cent pure, when tested by the Folin method of analysis against pure creatinine picrate (4) or against pure creatinine.¹ If *pure* creatine was used as a starting point the creatinine will be perfectly white; commercial creatine gives a faintly yellow product, the color depending upon the process employed for preparing the creatinine hydrochloride. Process (b) gives a whiter product than (a). The creatinine should give no test for chlorine with silver nitrate, or at most only a faint trace. Process (f) gives a product similar in every respect to that produced in (e). Process (g) gives a

¹ We are indebted to Mr. H. J. Bean for a sample of pure creatinine prepared especially for standardization purposes.

product similar in purity to that obtained by (e) or (f), but usually of lighter color than that produced by combining (a) with (e) or (f).

Attempts to remove the color entirely by decolorizing the creatinine hydrochloride solutions with charcoal were successful, but the yield was materially lowered thereby (to 80 per cent) as the charcoal adsorbs considerable creatinine from the very concentrated solution.

DISCUSSION.

Considerable quantities of creatinine have been prepared by the methods outlined above, as much as 1 kilo of creatine having been handled at one time, although most of the experiments employed 10, 25, 50, or 100 gm. All the methods seem suitable for large or small scale operation. It is believed that the small amount of attention required, the low cost of chemicals, and the high yield of creatinine obtained make these methods superior to any others hitherto proposed for the preparation of creatinine.

It should be noted that the methods are equally applicable to treatment of mixtures of creatine and creatinine, or to the re-purification of creatinine.

The creatinine thus prepared should be of sufficient purity for any ordinary purpose (including Major's test for renal function (5)). It is not intended as an analytical standard, but it may be noted that in our experience it gives results undistinguishable from those obtained with samples prepared especially for this purpose.

Recrystallization of Creatinine.

The purification of creatinine by crystallization presents certain difficulties. If recrystallized from water at high temperatures or from aqueous alcohol, there is always the danger that some creatine may be formed, and this is almost impossible to separate by recrystallization, as it is much less soluble in all ordinary solvents than creatinine, and has a similar temperature coefficient of solubility. Crystallization from pure alcohol is satisfactory for a small sample, but the solubility is too small for convenient handling of large quantities of creatinine.

We have found the following procedure fairly satisfactory. 1 part by weight of creatinine is dissolved in 5 parts of water, previously heated to 65°C., as rapidly as possible. 2 volumes of acetone are added at once, and the mixture is cooled in ice. After standing a few hours the creatinine is filtered off, washed with acetone, and dried. About 65 per cent of the original creatinine is recovered. The solubility of creatinine in aqueous acetone is less than in aqueous alcohol of the same volume percentage, and the purity of the recrystallized material seems higher with acetone than with alcohol.

SUMMARY.

1. Simple methods have been described for the preparation of creatinine from creatine.
2. The yield and purity of the creatinine by these methods are most satisfactory.

BIBLIOGRAPHY.

1. Folin, O., and Denis, W., *J. Biol. Chem.*, 1910, viii, 399.
2. Benedict, S. R., *J. Biol. Chem.*, 1914, xviii, 183.
3. Benedict, S. R., *J. Biol. Chem.*, 1914, xviii, 191.
4. Edgar, G., *J. Biol. Chem.*, 1923, lvi, 1.
5. Major, R. H., *J. Am. Med. Assn.*, 1923, lxxx, 384.

THE PREPARATION OF CREATININE FROM CREATINE

Graham Edgar and W. S. Hinegardner

J. Biol. Chem. 1923, 56:881-886.

Access the most updated version of this article at
<http://www.jbc.org/content/56/3/881.citation>

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
<http://www.jbc.org/content/56/3/881.citation.full.html#ref-list-1>