

1937

# Some Reactions of Creatinine with Certain Aliphatic Halogen Derivatives

Howard Edwards Wright Jr

Follow this and additional works at: <http://scholarship.richmond.edu/masters-theses>



Part of the [Organic Chemistry Commons](#)

---

## Recommended Citation

Wright, Howard Edwards Jr, "Some Reactions of Creatinine with Certain Aliphatic Halogen Derivatives" (1937). *Master's Theses*. 1270.  
<http://scholarship.richmond.edu/masters-theses/1270>

This Thesis is brought to you for free and open access by the Student Research at UR Scholarship Repository. It has been accepted for inclusion in Master's Theses by an authorized administrator of UR Scholarship Repository. For more information, please contact [scholarshiprepository@richmond.edu](mailto:scholarshiprepository@richmond.edu).

SOME REACTIONS OF CREATININE WITH CERTAIN ALIPHATIC  
HALOGEN DERIVATIVES

-----

THESIS

Presented in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in the Graduate  
Department of the University of Richmond

by

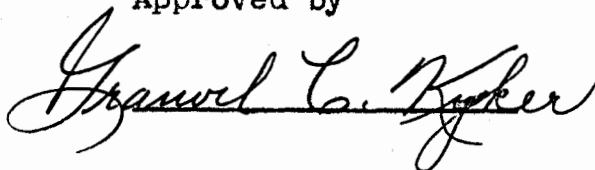
Howard Edwards Wright, Jr., B. S.

-----

The University of Richmond

1937

Approved by

A handwritten signature in cursive script, reading "Samuel C. Byker". The signature is written in dark ink and is positioned below the "Approved by" text.

LIBRARY  
UNIVERSITY OF RICHMOND  
VIRGINIA

## TABLE OF CONTENTS

	Page
1. Introduction.....	3
2. History.....	7
3. General Chemistry and Derivatives.....	11
4. Experimental Part.....	32
5. Discussion of Results.....	50
6. Summary.....	64
7. Acknowledgement.....	66
8. Autobiography.....	67

## INTRODUCTION

In view of the work that has been done on creatine and creatinine, especially in recent years, it was thought it would be interesting to attempt to condense creatinine with certain aliphatic halogen derivatives and to study carefully the reactions involved. The results of the work of previous investigators indicate the imino and imido groups of the creatinine molecule to be the positions in which substitution occurs, the imido group being the most susceptible.

Udransky and Baumann<sup>1</sup> prepared benzoylcreatine by treating creatine solutions with benzoyl chloride and sodium hydroxide.

Korndörfer<sup>2</sup>, Schmidt<sup>3</sup>, and Kunze<sup>4</sup> obtained methylcreatine by heating creatinine, methyl iodide, and methyl alcohol in a sealed tube at 100°. The free base

---

(1) Hunter: Creatine and Creatinine. London: Longmans, Green and Co., Ltd. (1928)

Udransky and Baumann, Ber., 21, 2938-41 (1888)

(2) Hunter: Ibid. Korndörfer, Arch. Pharm., 242, 641-48 (1904)

(3) Hunter: Ibid. Schmidt, Arch. Pharm., 248, 568-78, (1910)

(4) Hunter: Ibid. Kunze, Arch. Pharm., 248, 578-93 (1910)

was prepared through its hydriodide or hydrochloride. Kunze<sup>4</sup> and Greenwald<sup>5</sup> have prepared the hydriodide of dimethylcreatinine by treating methylcreatinine with methyl iodide. They did not describe the free base. Kunze<sup>4</sup> and Schmidt<sup>3</sup>, by converting the mother liquor from the preparation of dimethylcreatinine hydriodide into the hydrochloride and subsequent treatment with platonic chloride, were able to isolate trimethylcreatinine platinichloride. However, the position which the last methyl group occupied was not shown.

Neubauer<sup>6</sup> and, later, Henzerling<sup>7</sup> prepared ethylcreatinine through its hydriodide or hydrochloride. They obtained the hydriodide by heating creatinine, ethyl iodide, and absolute alcohol in a sealed tube at 100°. Henzerling<sup>7</sup> has also described diethylcreatinine platinichloride and methylethylcreatinine platinichloride, obtained by further treatment with ethyl iodide and methyl iodide respectively.

Jaffé<sup>8</sup>, in treating dimethylolcreatinine with

- 
- (5) Hunter: Ibid. Greenwald, J. Am. Chem. Soc., 47, 1443-47 (1925)
- (6) Hunter: Ibid. Neubauer, Ann. Chem. Pharm., 119, 27-52 (1861); Ibid, 120, 257-68 (1861)
- (7) Hunter: Ibid. Henzerling, Arch. Pharm., 248, 594-608 (1910)
- (8) Hunter: Ibid. Jaffé, Ber., 35, 2896-2901 (1902)

benzoyl chloride by a modified Baumann-Schotten method, isolated a dibenzoyl derivative.

Hennig<sup>9</sup> obtained benzylcreatinine through its hydrochloride by the action of benzyl chloride on creatinine in a sealed tube. Greenwald<sup>5</sup> prepared benzoylcreatinine by carrying out the reaction of creatinine and benzoyl chloride in pyridine. Tribenzoylcreatinine was obtained by treating creatinine in pyridine with an excess of benzoyl chloride.

Nicolet and Campbell<sup>10</sup> methylated 5-benzalcreatinine with methyl iodide and alkali and obtained N<sup>8</sup>-methyl-5-benzalcreatinine, evidenced by the fact that on hydrolysis it was converted into 1-methyl-5-benzalhydantoin and methylamine. Nicolet and Campbell prepared methylcreatinine by the same method used by Korndörfer<sup>2</sup> and Kunze<sup>4</sup> to be used in an attempt to show the position of the methyl group.

Ing<sup>11</sup> prepared 3-methyl-5-benzalcreatinine by treating the potassium salt of N<sup>8</sup>-acetyl-5-benzalcreatinine with methyl iodide. This product on acid hydrolysis yielded 1,3-dimethyl-5-benzalhydantoin. Ing also prepared benzoyl-

---

(9) Hunter: *Ibid.* Hennig, *Arch. Pharm.*, 251, 396-400 (1913)

(10) Nicolet and Campbell, *J. Am. Chem. Soc.*, 50, 1155 (1928)

(11) Ing, *J. Chem. Soc.*, 1932, 2647

creatinine from benzoyl chloride and diphenoxyphosphoryl creatinine from diphenoxyphosphoryl chloride, obtaining a second product, creatinine diphenoxyphosphate, in the case of the latter derivative if experimental conditions were not anhydrous.

The object of this investigation is to prepare some derivatives of creatinine from certain aliphatic halogen compounds with emphasis on the nature of the reactions through which condensation takes place.

## HISTORY

In a report to the French Academy of Science in 1832 Chevreul<sup>12</sup> described an organic substance extracted from meat and which he called creatine. He did not determine its constitution and proposed that it might be the ammonium salt of an organic acid.

Many unsuccessful attempts to confirm this discovery of Chevreul were made within the next fifteen years. In 1847 Liebig<sup>13</sup> described the general distribution of creatine and established its empirical formula as  $C_4H_9O_3N_3 + H_2O$ , by which it is known to-day. He discovered that by heating it with mineral acids it yielded a base whose empirical formula he established as  $C_4H_7ON_3$  and to which he gave the name creatinine. Liebig hydrolysed creatine and obtained urea and sarcosine. He assumed it to be a substituted urea. Weltzien<sup>14</sup>, on this assumption, presumed it to be ethyl-biuret,  $NH_2.CO.NH.CO.NH.C_2H_5$ ; but experimental evidence was lacking for this hypothesis

(12) Hunter: Ibid. Chevreul, J. pharm., 21, 231-42 (1835)

J. prakt. Chem., 6, 120-30  
(1835)

(13) Hunter: Ibid. Liebig, C. R. Acad. Sci., 24, 69-73 (1847)

Ann. Chem. Pharm., 62, 257-369 (1847)

(14) Hunter: Ibid. Weltzien, Ann. Chem. Pharm., 100, 191-98  
(1856)



and it was discarded.

Dessaignes<sup>15</sup>, on heating creatine in aqueous solution with mercuric oxide, obtained carbon dioxide, oxalic acid, and a strong base having the empirical formula  $C_3H_7N_3$  which he named "methyluramine". He thought creatine might be a condensation product of sarcosine and urea, and, analogously, "methyluramine" might be a product of urea and methylamine. He considered sarcosine to be a combination of glycollic acid with methylamine, and creatine an analogous combination of glycollic acid with "methyluramine".

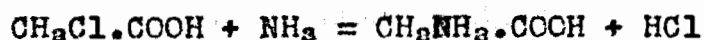
In 1861 Strecker<sup>16</sup> discovered guanidine among the oxidation products of guanine. He considered Dessaignes' "methyluramine" to be methylguanidine and proposed creatine to be a combination of sarcosine (methylglycocoll) and cyanamide. From this he synthesized glycocyamine from aqueous solutions of glycocoll and cyanamide to which a few drops of ammonia were added and established its empirical formula as  $C_3H_7O_2N_3$ . He treated glycocyamine with HCl and obtained glycocyamidine,  $C_3H_5ON_3$ . It was now clear that glycocyamidine was related to glycocyamine similarly as creatinine was to creatine, and that creatine was

(15) Hunter: Ibid. Dessaignes, C. R. Acad. Sci., 38, 839-43 (1854)

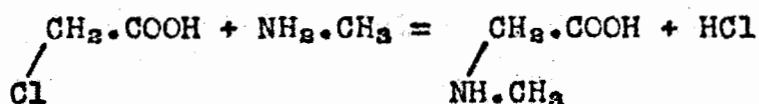
(16) Hunter: Ibid. Strecker, C. R. Acad. Sci., 52, 1210-13 (1861)

methylglycocyanine, glycocyanine being a lower homologue.

In 1858 Cahours<sup>17</sup> and Perkin and Duppa<sup>18</sup> established the constitution of glycoll as aminoacetic acid by its synthesis from monochloro- or monobromo-acetic acid and ammonia:



In 1862 Volhard<sup>19</sup> synthesized sarcosine from methylamine and acetic acid:



This synthesis established sarcosine as methylaminoacetic acid. In 1868 Volhard<sup>20</sup> synthesized creatine from sarcosine and cyanamide, the reaction being carried out in an alcoholic solution at 100° for several hours.

In 1867 Strecker<sup>21</sup> and, later, Erlenmeyer<sup>22</sup> first correctly considered creatine as methylguanidine-acetic acid

(17) Hunter: Ibid. Cahours, Ann. Chem. Pharm., 107, 147-51 (1858)

(18) Hunter: Ibid. Perkin and Duppa, Ann. Chem. Pharm., 108, 106-13 (1858)

(19) Hunter: Ibid. Volhard, Ann. Chem. Pharm., 123, 261-65 (1862)

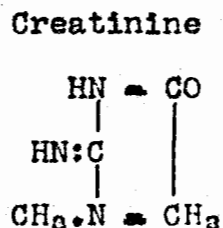
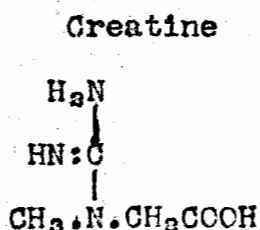
(20) Hunter: Ibid. Volhard, Sitzungsber. Königl. Bayer. Akad. Wissensch., 1868, II, 472-79

(21) Hunter: Ibid. Strecker, Lehrbuch der organischen Chemie, 5te Aufl., pp. 586-88

(22) Hunter: Ibid. Erlenmeyer, Ann. Chem. Pharm., 146, 259-60 (1868)

and creatinine as its internal anhydride (lactam).

Erlenmeyer proposed their structural formulae as they are written to-day:



Methylguanidine-acetic acid      Glycolyl-methylguanidine

However, this interpretation of Strecker and Erlenmeyer was not universally accepted. In 1885 Horbaczewski<sup>23</sup> removed all doubt when he prepared creatinine from guanidine carbonate and sarcosine and ultimate treatment with HCl. This synthesis was the final confirmation that creatine is methylguanidine-acetic acid, and creatinine its internal anhydride or lactam.

---

(23) Hunter: Ibid. Horbaczewski, Wiener med. Jahrbücher, 1885, 459-62

## GENERAL CHEMISTRY AND DERIVATIVES

The interconvertibility of creatine and creatinine and their many similar properties seems to justify a detailed consideration of each with its respective derivatives. The discussion will be outlined as follows:

## A Creatinine

## I General Properties

## II Salts

## III Halide Derivatives

## IV Aldehyde Derivatives

## V Miscellaneous Derivatives

## B Creatine

## I General Properties

## II Salts

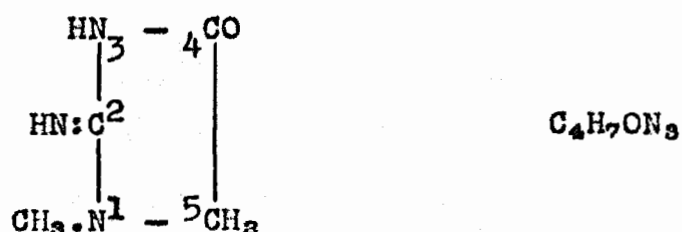
## III Miscellaneous Derivatives

## A Creatinine

## I General Properties

Creatinine (methylglycocyanidine or 1-methyl-2-imino-4-keto-tetrahydro-imidazole) is the internal anhydride of creatine, decomposing at about 270° without melting. (Note: The system of numbering is the same as that used in Chemical Abstracts. This is done to avoid confusion since other systems have been used in the literature). The chemical nature of the substance is

somewhat self-explanatory from the structural formula of the molecule:



Crystallographically, creatinine belongs to the monoclinic system. It is slightly soluble in water, much less soluble in alcohol, and practically insoluble in ether. Aqueous solutions of creatinine are slightly alkaline to litmus<sup>24,25</sup>. According to Eadie and Hunter<sup>26</sup> it is about 66 times stronger as a base than creatine. Creatinine is partly transformed by all hydrolysing agents into creatine, except acid media. Neubauer<sup>27</sup> and Poulsson<sup>28</sup>, on heating it with Ba(OH)<sub>2</sub> in a closed tube, obtained methylhydantoin and ammonia. However, the work of Gaebler<sup>29</sup> indicates methylhydantoin to

---

(24) Hunter: Ibid. Salkowski, Z. Physiol. Chem., 4, 133-36 (1880)

(25) Hunter: Ibid. Salkowski, Z. Physiol. Chem., 12, 211-15 (1888)

(26) Hunter: Ibid. Eadie and Hunter, J. Biol. Chem., 67, 237-44 (1926)

(27) Hunter: Ibid. Neubauer, Ann. Chem. Pharm., 137, 288-98 (1866)

(28) Hunter: Ibid. Poulsson, Arch. exp. Path. Pharm., 51, 227-38 (1904)

(29) Hunter: Ibid. Gaebler, J. Biol. Chem., 69, 613-24 (1926)

be the product of secondary reactions and that alkalies primarily convert creatinine into sarcosine and urea. The same hydrolytic decomposition products are obtained by the action of syrupy phosphoric acid<sup>30</sup>.

When acted on by oxidising agents such as mercuric oxide, lead peroxide, hydrogen peroxide, or mercuric acetate, creatinine yields methylguanidine, glyoxylic acid, oxalic acid, carbon dioxide, formaldehyde, and formic acid (probably by further oxidation of formaldehyde)<sup>15,31,32,33,34</sup>.

Creatinine reduces alkaline solutions of copper<sup>35</sup>, Nessler's solution and alkaline solutions of mercuric chloride<sup>36</sup>, salts of gold<sup>37</sup>, and alkaline potassium

---

(30) Hunter: Ibid. Schöndorff, Arch. ges. Physiol., 62, 1-57 (1895)

(31) Hunter: Ibid. Dakin, J. Biol. Chem., 1, 271-78 (1906)

(32) Hunter: Ibid. Baumann and Igvaldsen, J. Biol. Chem., 35, 277-80 (1918)

(33) Hunter: Ibid. Dessaignes, C. R. Acad. Sci., 41, 1258-61 (1855)

(34) Hunter: Ibid. Schmidt, Arch. Pharm., 256, 308-12 (1918)

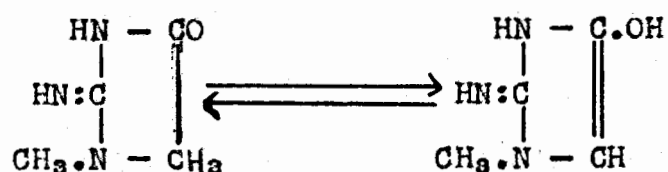
(35) Hunter: Ibid. Winogradoff, Arch. path. Anat. Physiol., 27, 533-73 (1863)

(36) Hunter: Ibid. Johnson, Proc. Roy. Soc., 42, 365-70 (1887); Ibid, 43, 493-534 (1888)

(37) Hunter: Ibid. Greenwald, J. Biol. Chem., 59, 329-37 (1924)

ferricyanide<sup>38</sup>.

Of the several color reactions for creatinine, the Jaffe<sup>39</sup> test is the best, though a non-specific. If a creatinine solution containing aqueous picric acid is made alkaline with sodium or potassium hydroxide a reddish-orange to deep blood-red coloration is produced, the color varying with the concentration of creatinine. Greenwald<sup>5</sup> has shown that for this to occur there must exist a keto-enol tautomerism as follows:



The picric acid adds on to the imido group of the creatinine molecule. The color test is only given by those derivatives of creatinine which can form salts and which retain the  $-\text{CH}_2-\text{CO}-$  group.

According to Engeland<sup>40</sup> dry distillation of creatinine yields ammonia, dimethylamine, and probably pyrrole and hydrogen cyanide.

(38) Hunter: Ibid. Holmes, E. G. and Holmes, B. E.,  
Biochem. J., 20, 595-97 (1926)

(39) Hunter: Ibid. Jaffé, Z. Physiol. Chem., 10, 391-400  
(1886)

(40) Hunter: Ibid. Engeland, Z. Physiol. Chem., 57, 65-66  
(1908)

The ease with which creatine and creatinine are transformed into one another is a characteristic common to both. The results of the work of Hahn and Barkan<sup>41</sup> proved the presumption to be correct that in neutral and alkaline media the creatine-creatinine transformation is a reversible reaction and that both creatine and creatinine in alkaline media undergo a progressive decomposition. In the presence of sufficient concentration of mineral acids the conversion of creatine to creatinine is irreversible.

## II Salts

Creatinine Hydrochloride<sup>13,42,43</sup>  
 $C_4H_7ON_3.HCl$

Creatinine Hydrochloride-Methyl Alcohol<sup>44</sup>  
 $C_5H_{11}O_3N_3.HCl$ ; m. p. 139-40°

Creatinine Hydrochloride-Ethyl Alcohol<sup>44,45</sup>  
 $C_6H_{13}O_3N_3.HCl$ ; m. p. 163°

Creatinine Hydrochloride-n-Butyl Alcohol<sup>44,45</sup>  
 $C_8H_{17}O_3N_3.HCl$ ; m. p. 138°

- 
- (41) Hunter: Ibid. Hahn and Barkan, Z. Biol., 72, 25-36 (1920)  
 (42) Hunter: Ibid. Edgar and Hinegardner, J. Biol. Chem., 56, 881-86 (1923)  
 (43) Hunter: Ibid. Benedict, J. Biol. Chem., 18, 191-94 (1914)  
 (44) Hunter: Ibid. Dox and Yoder, J. Biol. Chem., 54, 671-73 (1922)  
 (45) Hunter: Ibid. Kapfhammer, Biochem. Z., 156, 182-89 (1925)



Creatinine Hydr iodide<sup>2,4,7,46</sup>

$C_4H_7ON_3 \cdot HI$ ; m. p. 195°

Creatinine Sulfate<sup>13</sup>

$(C_4H_7ON_3)_2 \cdot H_2SO_4$

Creatinine Platinichloride<sup>13,47,48</sup>

$(C_4H_7ON_3 \cdot HCl)_2 PtCl_4$ ; m. p. 220-25° (Anhydrous),  
210° (Hydrated)

Creatinine Aurichloride<sup>36,47,48,49,50,51</sup>

$C_4H_7ON_3 \cdot HCl \cdot AuCl_3$

Creatinine Phosphomolybdate<sup>52</sup>

Creatinine Phosphotungstate<sup>52,53,54,55</sup>

$(C_4H_7ON_3)_8 \cdot H_3PO_4 \cdot 12 WO_3$

Creatinine Tartrate<sup>28</sup>

$(C_4H_7ON_3)_2 \cdot C_4H_6O_6$ ; m. p. 207-9°

Creatinine Oxalate<sup>28</sup>

$(C_4H_7ON_3)_2 \cdot C_2H_2O_4$

(46) Hunter: Ibid. Neubauer, Ann. Chem. Pharm., 120, 257-68  
(1861)

(47) Hunter: Ibid. Wörner, Z. physiol. Chem., 27, 1-13 (1899)

(48) Hunter: Ibid. Toppelius and Pommerehne, Arch. Pharm.,  
234, 380-97 (1896)

(49) Hunter: Ibid. Reuter, Z. Biol., 72, 129-40 (1920)

(50) Hunter: Ibid. Korndörfer, Arch. Pharm., 242, 373-79 (1904)

(51) Hunter: Ibid. Engeland, Z. physiol. Chem., 57, 49-64  
(1908)

(52) Hunter: Ibid. Kerner, Arch. ges. Physiol., 2, 200-43  
(1869)

(53) Hunter: Ibid. Demjanowski, Z. physiol. Chem., 80,  
212-17 (1912)

(54) Hunter: Ibid. Drummond, Biochem. J., 12, 5-24 (1918)

(55) Hunter: Ibid. Wechsler, Z. physiol. Chem., 73, 138-43  
(1911)

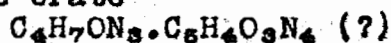
Creatinine Acid Oxalate<sup>56</sup>



Creatinine Kynurenate<sup>39</sup>



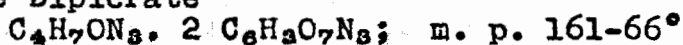
Creatinine Urate<sup>57</sup>



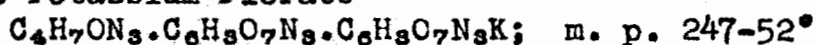
Creatinine Picrate<sup>39,47,48,50,58</sup>



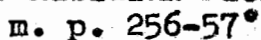
Creatinine Dipicrate<sup>59</sup>



Creatinine Potassium Picrate<sup>39,60</sup>



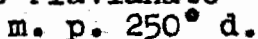
Creatinine Rubidium Picrate<sup>61</sup>



Creatinine Caesium Picrate<sup>61</sup>



Creatinine Flavianate<sup>62</sup>



(56) Hunter: Ibid. Thesen, Z. physiol. Chem., 24, 1-17 (1898)

(57) Hunter: Ibid. Klemperer, Fortschritte d. Medizin, 19, 328-29 (1901)

(58) Hunter: Ibid. Folin, J. Biol. Chem., 17, 463-68 (1914)

(59) Hunter: Ibid. Mayerhofer, Wien. Klin. Wochenschr., 22, 90-92 (1909)

(60) Hunter: Ibid. Greenwald and Gross, J. Biol. Chem., 59, 601-12 (1924)

(61) Hunter: Ibid. Greenwald and Gross, J. Biol. Chem., 59, 613-14 (1924)

(62) Hunter: Ibid. Kossel and Gross, Z. physiol. Chem., 135, 167-74 (1924)

Creatinine Zinc Chloride<sup>6,13,63,64,65</sup>  
 $(C_4H_7ON_3)_2 \cdot ZnCl_2$

Creatinine Zinc Chloride Hydrochloride<sup>46,66,67</sup>  
 $(C_4H_7ON_3)_2 \cdot ZnCl_2 \cdot 2 HCl$

Double Sulfate of Creatinine and Zinc<sup>68</sup>  
 $(C_4H_7ON_3)_2 \cdot H_2SO_4 \cdot ZnSO_4 \cdot 8 H_2O$

Creatinine Cadmium Chloride<sup>6</sup>  
 $(C_4H_7ON_3)_2 \cdot CdCl_2$

Creatinine Mercuric Nitrate<sup>6</sup>  
 $(C_4H_7ON_3)_2 \cdot HgO \cdot Hg(NO_3)_2$

Mercuric Chloride Compounds of Creatinine<sup>13,36</sup>  
 $4 (C_4H_7ON_3 \cdot HCl \cdot HgO) \cdot 3 HgCl_2$   
 or  
 $4 (C_4H_7ON_3 Hg \cdot HCl) \cdot 3 HgCl_2 \cdot 2 H_2O$

Double Salts of Creatinine and Copper<sup>13</sup>

Creatinine-Cuprous Oxide<sup>69</sup>

Creatinine-Silver Nitrate<sup>6,13,70</sup>  
 $C_4H_7ON_3 \cdot AgNO_3$ ; m. p. 188-91°

(63) Hunter: Ibid. Heintz, Poggendorff's Ann. Phys. Chem., 70, 466-80 (1847)

(64) Hunter: Ibid. Shorey, J. Am. Chem. Soc., 34, 99-107 (1912)

(65) Hunter: Ibid. Edgar, J. Biol. Chem., 56, 1-6 (1923)

(66) Hunter: Ibid. Dessaignes, J. pharm. chim., 32, 37-50 (1857)

(67) Hunter: Ibid. Benedict, J. Biol. Chem., 18, 183-90 (1914)

(68) Hunter: Ibid. Folin and Blanck, J. Biol. Chem., 8, 395-97 (1910)

(69) Hunter: Ibid. Maschke, Z. anal. Chem., 17, 134-41 (1878)

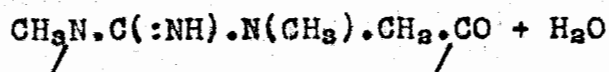
(70) Hunter: Ibid. Smorodinzew, J. Russ. Phys.-chem. Soc., 47, 1275-79 (1915)

Creatinine-Silver<sup>3,71</sup>  
 $C_4H_7ON_3Ag$

The compounds listed above are all double salts. The most useful ones for the identification or isolation of creatinine are the picrate, the double picrate with potassium, and the compound with zinc chloride.

III Halide Derivatives

Methylcreatinine<sup>2,3,4,5,10</sup>; m. p. 79-81°



Hydriodide; m. p. 210-12°

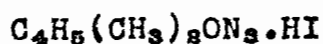
Hydrochloride; m. p. 233-37°

Aurichloride; m. p. 170-76°

Platinichloride; m. p. 227-29°

Picrate; m. p. 183°

Dimethylcreatinine hydriodide<sup>2,4,5</sup>; m. p. 179-80°



Aurichloride; m. p. 128-29°

Platinichloride; m. p. 177-79°

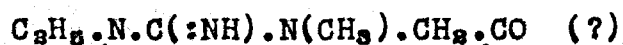
Platinochloride; m. p. 244°

Trimethylcreatinine platinichloride<sup>3,4</sup>; m. p. 205° d.



Aurichloride; m. p. 137-38°

Ethylcreatinine<sup>3,6,7</sup>; m. p. Slowly decomposes above 100°



Hydriodide; m. p. 217-19°

Hydrochloride

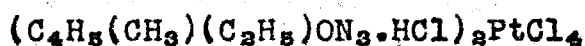
Platinichloride; m. p. 197-211° d.

Aurichloride; m. p. 151-52°

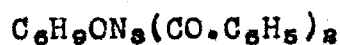
Diethylcreatinine platinichloride<sup>7</sup>; m. p. 201-02°



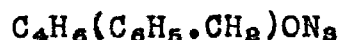
Methylethylcreatinine platinichloride<sup>7</sup>; m. p. 181-82°



Dibenzoyl derivative of dimethylolcreatinine<sup>8</sup>; m. p. 265-66°



N<sup>2</sup>(or<sup>3</sup>)-Benzylcreatinine<sup>9</sup>; m. p. 225°



Hydrochloride; m. p. Carbonizes above 230°

Aurichloride; m. p. 158°

Platinichloride; m. p. 177-78°

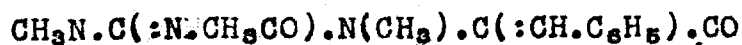
Picrate; m. p. 174-75°

5-Benzylcreatinine picrate<sup>5</sup>; m. p. 206-08°

Benzoylcreatinine<sup>5,11</sup>; m. p. 190°

Tribenzoylcreatinine<sup>5,11</sup>; m. p. 238-40°

N<sup>2</sup>-Acetyl-3-methyl-5-benzalcreatinine<sup>11</sup>; m. p. 129-30°

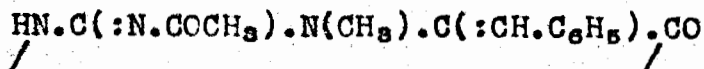


N<sup>2</sup>-Methyl-5-benzalcreatinine<sup>11</sup>; m. p. 129°



## IV Aldehyde Derivatives

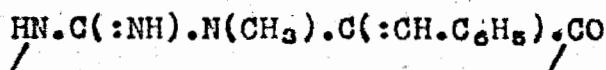
N<sup>2</sup>-Acetyl-5-benzalcreatinine<sup>5,10,11,72</sup>; m. p. 213°



Picrate; m. p. 250°

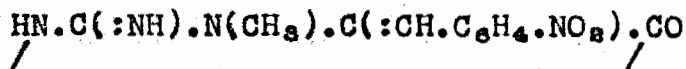
Potassium salt

5-Benzalcreatinine<sup>5,10,11,73</sup>; m. p. 247°

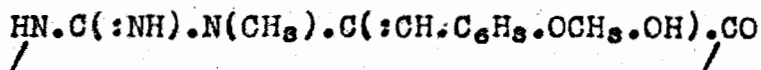


Picrate; m. p. 260°

5-m-Nitrobenzalcreatinine<sup>73</sup>; m. p. 288°



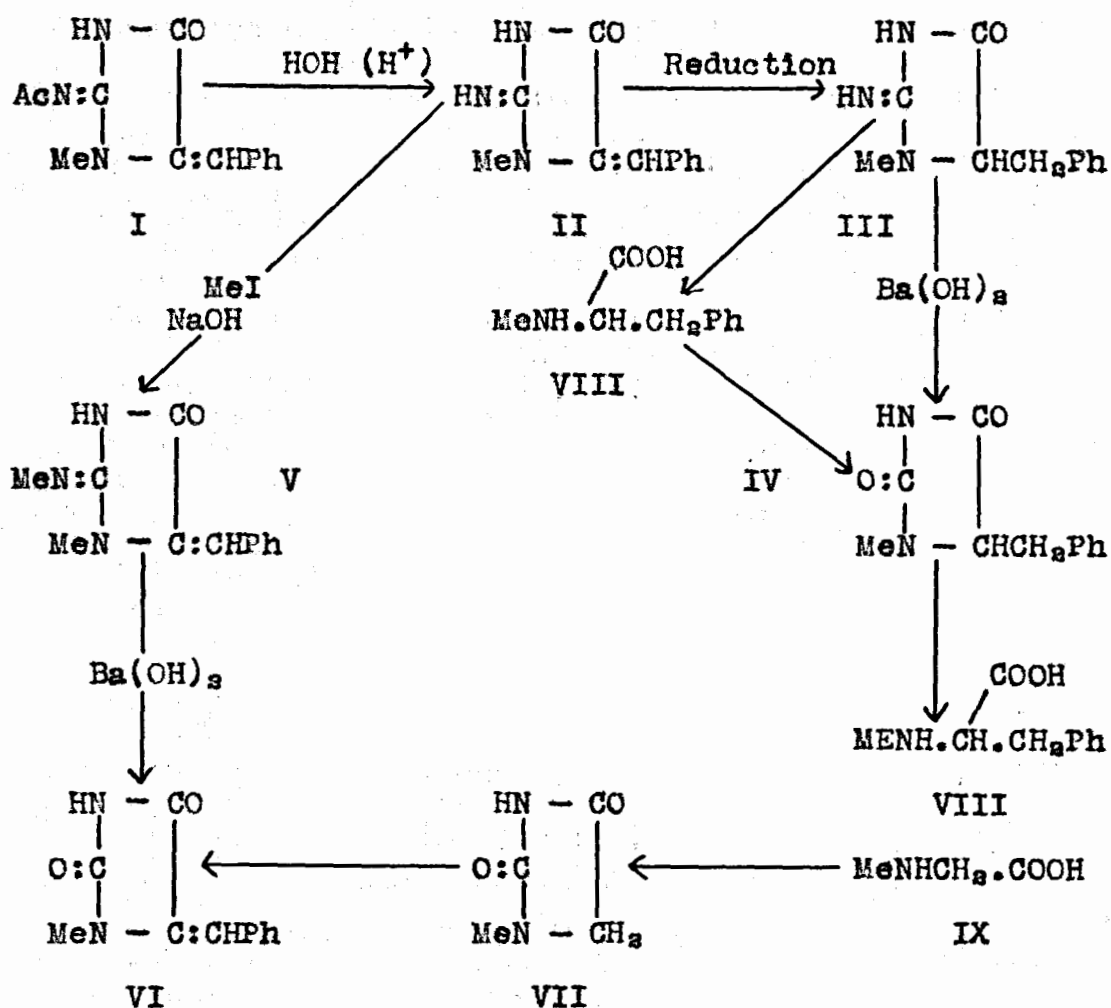
5-(m-Methoxy-p-hydroxybenzal)-creatinine<sup>73</sup>; m. p. 267° d.



Nicolet and Campbell<sup>10</sup>, in an attempt to establish the position of the methyl group in methylcreatinine and to determine the possibility of obtaining alpha-methylamino acids through the aldehyde condensation products of creatinine, carried out the following series of reactions with benzalcreatinine:

(72) Hunter: Ibid. Erlenmeyer, Ann. Chem., 284, 49-52 (1895)

(73) Richard, Welch, and Calvert, J. Am. Chem. Soc., 51, 3075, (1929)



## Legend:

I Acetyl-5-benzalcreatinine; m. p. 208-09°

II 5-Benzalcreatinine; m. p. 244°

III 5-Benzylcreatinine; m. p. 282° d.

IV 1-Methyl-5-benzylhydantoin; m. p. 106°

V N<sup>s</sup>-Methyl-5-benzalcreatinine; m. p. 129°

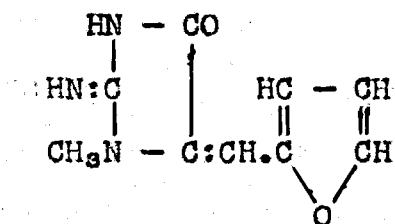
VI 1-Methyl-5-benzalhydantoin; m. p. 193-94°

VII 1-Methylhydantoin

VIII N-Methylphenylalanine

IX Sarcosine

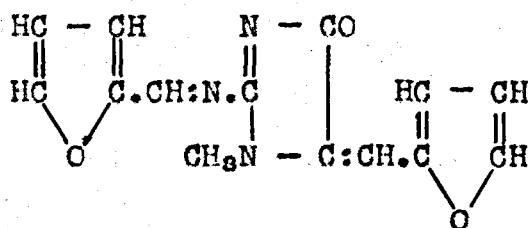
5-Furfuralcreatinine<sup>74</sup>; m. p. 273° d.



Picrate; m. p. 244° d.

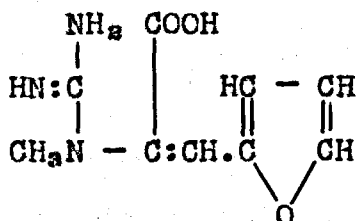
Hydrochloride; m. p. 257° d.

Difurfuralcreatinine<sup>74</sup>; m. p. 243° (Proposed formula)

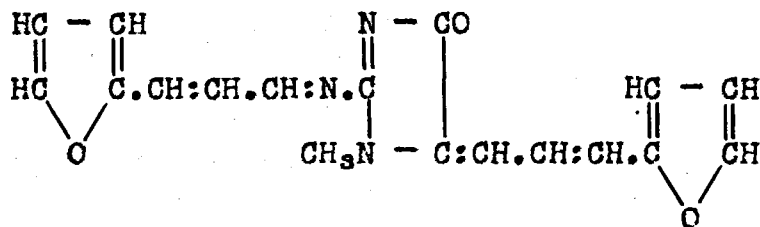


Picrate; m. p. 205° d.

Furfuralcreatine<sup>74</sup>; m. p. 254° d.



Di-(furfural-acrolein)-creatinine<sup>74</sup>; m. p. 268° (Proposed formula)



Picrate; m. p. 200° d. (Without melting)

---

(74) Cornthwaite and Jordan, J. Am. Chem. Soc., 56, 2733 (1934)



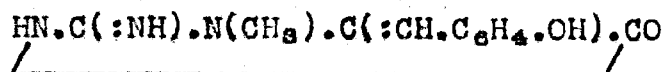
5-Furfural-methylcreatinine<sup>74</sup>; m. p. 134°

Picrate; m. p. 235° d.

Difurfural-methylcreatinine<sup>74</sup>; m. p. 137°

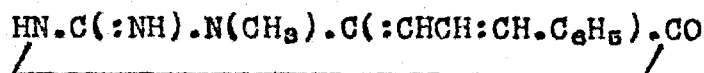
Picrate; m. p. 205° d.

5-Salicylcreatinine<sup>74</sup>; m. p. 232° d.



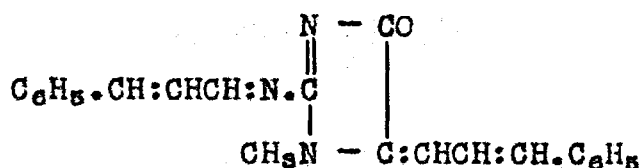
Picrate; m. p. 269° d.

5-Cinnamylcreatinine<sup>74</sup>; m. p. 280° d.



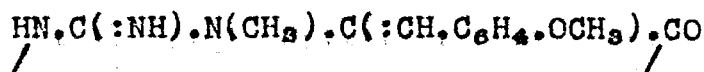
Picrate; m. p. 261° d.

Dicinnamylcreatinine<sup>74</sup>; m. p. 220° d. (Proposed formula)



Picrate; m. p. 193° d.

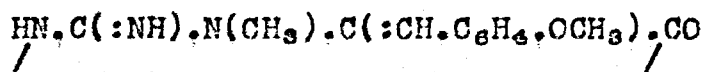
5-p-Methoxybenzalcreatinine<sup>75</sup>; m. p. 248-49° d.



Picrate; m. p. 244° d.

Hydrochloride; m. p. 247-48° d.

5-o-Methoxybenzalcreatinine<sup>75</sup>; m. p. 241°



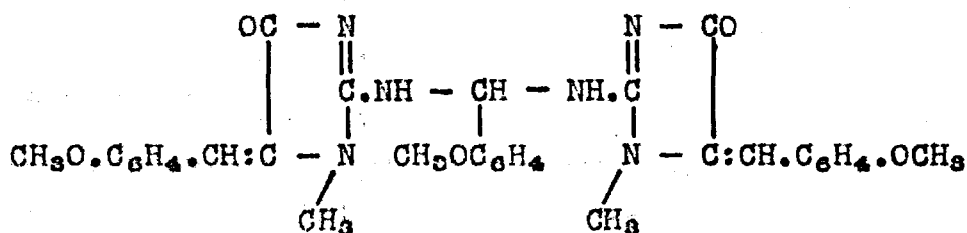

---

(75) Cornthwaite, Lazarus, Snellings, and Denoon,  
J. Am. Chem. Soc., 58, 628-29 (1936)

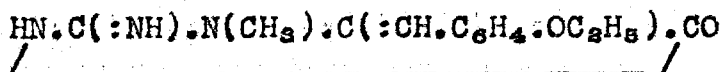
Picrate; m. p. 258-59° d.

Hydrochloride; m. p. 255-70° d.

Tri-o-Methoxybenzal-di-creatinine<sup>75</sup>; m. p. 292° (Proposed formula)



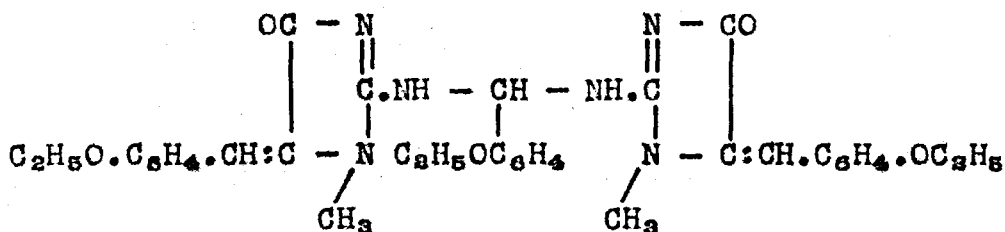
5-o-Ethoxybenzalcreatinine<sup>75</sup>; m. p. 236° d.



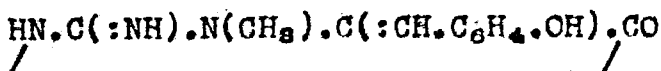
Picrate; m. p. 244-46° d.

Hydrochloride; m. p. 214° d.

Tri-o-Ethoxybenzal-di-creatinine<sup>75</sup>; m. p. 297° (Proposed formula)

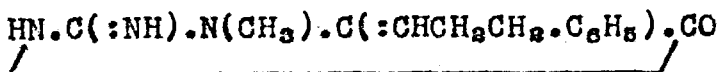


5-p-Hydroxybenzalcreatinine<sup>75</sup>; m. p. 289°



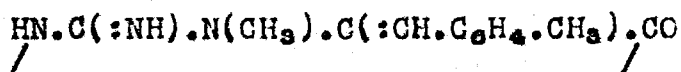
Picrate; m. p. 252-57° d.

5-Hydrocinnamylcreatinine<sup>75</sup>; m. p. 225-30°



Picrate; m. p. 221°

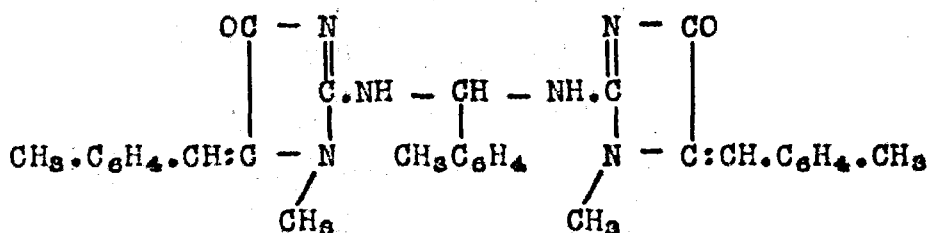
5-p-Methylbenzalcreatinine<sup>75</sup>; m. p. 285° d.



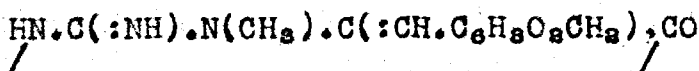
Picrate; m. p. 256° d.

Hydrochloride; m. p. 256° d.

Tri-p-Methylbenzal-di-creatinine<sup>75</sup>; m. p. 309° (Proposed formula)

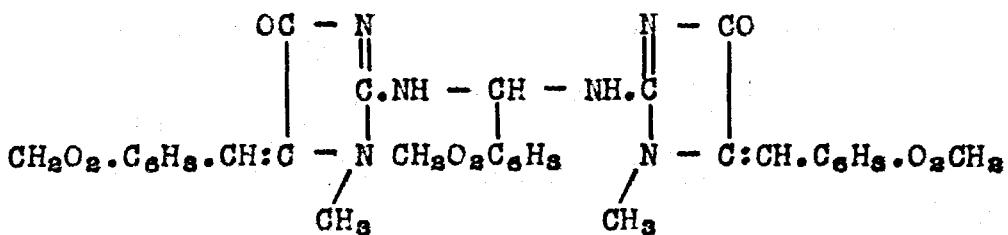


5-Piperonalcreatinine<sup>75</sup>; m. p. 274° d.

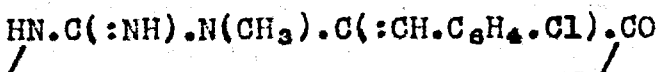


Picrate; m. p. 255°

Tri-Piperonal-di-creatinine<sup>75</sup>; m. p. 327° (Proposed formula)



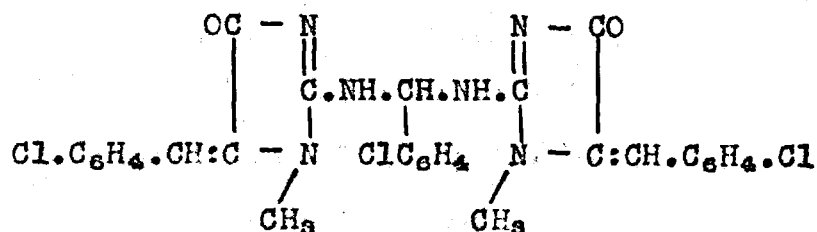
5-o-Chlorobenzalcreatinine<sup>75</sup>; m. p. 242° d.



Picrate; m. p. 260°

Hydrochloride; m. p. 241°

Tri-o-Chlorobenzal-di-creatinine<sup>75</sup>; m. p. 270° (Proposed formula)



#### V Miscellaneous Derivatives

N<sup>2</sup>-Acetylcreatinine<sup>11</sup>; m. p. 124-25°

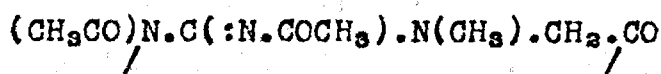
Picrate; m. p. 170-72° d.

Hydrochloride; m. p. 185-86° d.

Potassium salt

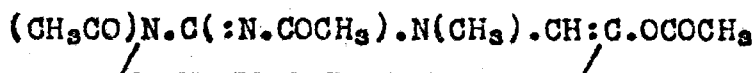
Diacetylcreatine<sup>11</sup>; m. p. 177-78°

Diacetylcreatinine<sup>11</sup>; m. p. 164-65°



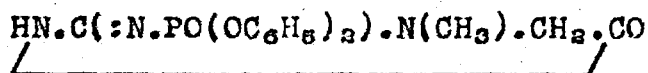
Picrate; m. p. 139-40°

Triacetylcreatine<sup>11</sup>; m. p. 63-65° (Proposed formula)



Benzoylcreatinine<sup>11,76</sup>; m. p. 187°

Diphenoxyphosphorylcreatinine<sup>11</sup>; m. p. 127-28°

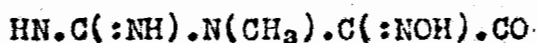


Sodium salt

Creatinine diphenoxyphosphate<sup>11</sup>; m. p. 158-59°

(76) Hunter: Ibid. Urano, Beitr. chem. Physiol. Pathol., 9, 183-84 (1907)

Creatinine-Oxime<sup>77,78</sup>; m. p. 220° d. (Without melting)



Hydrochloride; m. p. 200-05° d.

Nitrato; m. p. 123-25° d.

Platinichloride

Aurichlorides;

$\text{C}_4\text{H}_8\text{O}_2\text{N}_4.\text{HCl}.\text{AuCl}_3$ ; m. p. 187°

$(\text{C}_4\text{H}_8\text{O}_2\text{N}_4.\text{HCl})_2.\text{AuCl}_3$ ;

m. p. 194-96°

Silver-creatinine-oxime; Dehydrates at 125°

Diacetylcreatinine-oxime; m. p. 210°

Dimethylolcreatinine<sup>8</sup>; Decomposes above 250° without melting.

Creatinine trinitro-m-cresol<sup>5</sup>; m. p. 218°

Dicreatinine phthalate<sup>79</sup>.

## B Creatine

### I General Properties

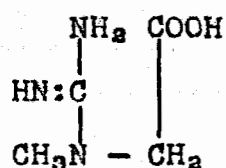
Creatine (methylguanidine-acetic acid or methyl-glycocyanine) is an amino acid decomposing with

(77) Hunter: Ibid. Kramm, Centr. med. Wissensch., 35, 785-87 (1897)

(78) Hunter: Ibid. Hennig, Arch. Pharm., 250, 370-81 (1912)

(79) Ing, J. Chem. Soc., 1932, 2198

effervescence at about 291°. Crystallographically,



creatine belongs to the monoclinic system. It is very soluble in boiling water, slightly soluble in cold water (but considerably less than creatinine), less soluble in alcohol, and insoluble in ether.

Creatine is an amphoteric substance. It is a very weak base and a still weaker acid. According to Hahn and Fasold<sup>80</sup>. It is about 1000 times weaker as an acid than as a base. The hydrolytic, oxidative, and reductive products of creatine are exactly the same as those of creatinine. Creatine is converted wholly or partly to creatinine when heated with water, dilute acids or alkalies. This fact has already been mentioned.

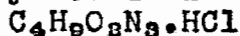
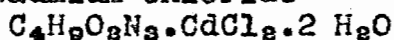
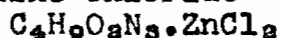
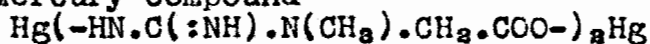
One of the best color reactions to distinguish creatine from creatinine is the diacetyl test<sup>81,82</sup>. When creatine is treated with fresh 1% diacetyl in a slightly alkaline solution a pink coloration is produced. Creatinine does not give this test.

(80) Hunter: Ibid. Hahn and Fasold, Z. Biol., 82, 473-84 (1925)

(81) Hunter: Ibid. Harden and Norris, J. Physiol., 42, 332-36 (1911)

(82) Hunter: Ibid. Walpole, J. Physiol., 42, 301-08 (1911)

## II Salts

Creatine Nitrate<sup>15</sup>Creatine Sulfate<sup>15</sup>Creatine Hydrochloride<sup>15</sup>Creatine Phosphomolybdate<sup>52</sup>Creatine Cadmium Chloride<sup>83</sup>Creatine Zinc Chloride<sup>83,84</sup>Creatine Copper Chloride<sup>83,84</sup>Creatine Mercuric Nitrate<sup>83,84</sup>Creatine Mercury Compound<sup>85</sup>

Creatine Picrate<sup>86</sup>; m. p. 260°. It is possible that this substance was creatinine picrate.

## III Miscellaneous Derivatives

Diacetylcreatine<sup>11,72</sup>; m. p. 177-78°Benzoylcreatine<sup>1</sup>

The salts and derivatives of creatine possess no

(83) Hunter: Ibid. Neubauer, Ann. Chem. Pharm., 137, 298-301 (1866)

(84) Hunter: Ibid. Neubauer, Z. anal. Chem., 2, 22-34 (1863)

(85) Hunter: Ibid. Engel, C. R. Acad. Sci., 80, 885-88 (1875)

(86) Hunter: Ibid. Riesser, Abderhalden's Handbuch der biologischen Arbeitsmethoden, Abt. I., Teil 7, 859-900, Berlin (1923)

distinctive characteristics such as would make it useful for purposes of identification. The recognition of creatine depends solely on its analysis as such or on its conversion to creatinine or some derivative of creatinine.



## EXPERIMENTAL PART

The creatinine used in this investigation was prepared from commercial creatine. The method of conversion was a slight modification of that of Edgar and Hinegardner<sup>42</sup>. 150 g. of creatine were added to a mixture of 85 cc. of concentrated HCl, sp. gr. 1.19, and 25 cc. of water. A small quantity of decolorizing charcoal was added and the mixture heated on the water bath at 100° for several minutes. The reaction mixture was filtered and the residue on the filter paper washed with a small quantity of concentrated HCl. The filtrate and washings were put in a sealed flask along with about 1 g. of decolorizing charcoal and heated at 100° for about 24 hours. The reaction mixture was then filtered and the residue on the filter paper washed with a few cc. of concentrated HCl. The filtrate and washings were concentrated under reduced pressure to the original volume. The creatinine hydrochloride solution was cooled to 0° and treated with an equal volume of ice cold  $\text{NH}_4\text{OH}$ , sp. gr. .9, with constant stirring. The solution was kept in an ice bath for about one hour. The precipitated creatinine was filtered, washed thoroughly with ice cold concentrated  $\text{NH}_4\text{OH}$  and finally with cold 95% ethyl alcohol. The product was dried in the oven at 105° for about one hour. The creatinine obtained was

perfectly white and crystalline. This method gave a 60% yield of the theoretical. The product was free of chlorides and creatine and melted with decomposition at 270-72°, and was not further purified.

#### Reaction of Creatinine with n-Butyl Bromide

The reaction with n-butyl bromide on creatinine was considered first since its possibilities of complex reactions were much less than with some of the reagents to be used.

#### Experiment I

2.26 g. (.02 mole) of creatinine, 2.74 g. (.02 mole) of n-butyl bromide, and 4 cc. of n-butyl alcohol were intimately mixed and refluxed at 117° for 23 hours, during which time the reaction mixture became yellow. A solid phase existed throughout the reaction. The appearance of the solid was distinctly different from that of creatinine. At the end of the refluxing the mixture was cooled to 0° and filtered. The solid product was yellow and gave a positive halide test. It melted with decomposition at 192-99°. The product was dried at 105° and analyzed for n-butylcreatine hydrobromide.

Anal. Calcd. for  $C_8H_{15}ON_3.HBr$ : N, 16.8

Found: 24.13, 23.88

The above product was dissolved in hot absolute

alcohol and the solution stratified with ether until precipitation seemed almost complete. This treatment removed some coloration. The recrystallized product gave a positive halide test and melted with decomposition at  $201^{\circ}$ . The product was infinitely soluble in water, very soluble in alcohol, and insoluble in acetone, ether, and  $\text{CCl}_4$ . The product was analyzed for nitrogen. Found, 23.71%, 23.97%.

Since the product contains ionizable bromide, it was thought to be a mixture of hydrobromide and free base. The nitrogen analysis after evaporation with concentrated HBr should indicate this. The recrystallized product was evaporated with 48% HBr, giving a brownish orange solid, which melted with decomposition at  $233-36^{\circ}$ . It was infinitely soluble in water, slightly soluble in alcohol, and insoluble in acetone and ether. It gave a positive halide test. This product was analyzed for nitrogen. Found, 21.31%.

#### Experiment II

The experiment was repeated using the same procedure. The solid matter which separated from the reaction mixture was filtered and washed several times with absolute alcohol to remove n-butyl alcohol and any unreacted n-butyl bromide. The solid product was evaporated with about 3 cc. of 48% HBr almost to dryness. Water was added at the boiling

point until all solid had barely dissolved. The solution was cooled and filtered. The product obtained was yellow, and distinctly crystalline. It decomposed with partial melting at 225-35°. It was infinitely soluble in water, slightly soluble in absolute alcohol, and insoluble in acetone and ether. The product was dried at 105° and analyzed for n-butylcreatinine hydrobromide.

Anal. Calcd. for  $C_8H_{15}ON_3 \cdot HBr$ : N, 16.8

Found: 21.30, 21.35

The above product was dissolved in absolute alcohol. No crystallization took place when cooled. On standing overnight only a few crystals appeared. The alcoholic solution was stratified with ether until precipitation was almost complete. The white flocculent product was filtered with suction and dried for about one hour in the oven at 105°. The solid decomposed at 225-38° without melting. It was infinitely soluble in water, slightly soluble in absolute alcohol, and insoluble in acetone and ether. The product was analyzed for nitrogen. Found, 21.21%, 21.29%.

The mother liquor from the reaction mixture was stratified with ether until two layers separated. A solid appeared at the interface; this was filtered off. It was very soluble in water, very slightly soluble in absolute alcohol, and insoluble in acetone and ether. It decomposed

without melting at 232-40°. The product was analyzed for nitrogen. Found, 21.34%, 21.32%.

The liquid portion of the reaction product from Experiment I and all washings from Experiment II were combined and evaporated on the steam bath to a very thick and tarry looking substance. The substance was cooled and about 20 cc. of acetone added, resulting in complete solution. A small quantity of ether was added. A black oil separated. This oil was washed and worked up several times with ether until the ether was no longer discolored. A semi-crystalline tarry material resulted. To this was added 5 cc. of 48% HBr and 10 cc. of water. This was done in an effort to isolate a salt from the portion which refused to crystallize. The solution was heated to boiling and charcoaled for about 5 minutes, and then filtered. The charcoal treatment gave an amber colored solution. This solution was evaporated. No crystalline solid appeared even after evaporation was almost complete, but the residue remained resinous. When the substance was cooled it became very viscous but remained clear and gummy. After long stirring a crystal from the original reaction product was added. This caused turbidity and further stirring gave a light yellow pasty solid. This solid was treated with acetone and ether. The solvents were decanted, leaving a gummy, semi-crystalline substance. The entire treatment was

repeated. All efforts to separate a crystalline substance failed.

### Experiment III

4.52 g. (.04 mole) of creatinine, 5.48 g. (.04 mole) of n-butyl bromide, and 8 cc. of n-butyl alcohol were refluxed over an open flame for 8 hours. The reaction mixture at the end of the time of refluxing consisted of a yellow white solid and a dark liquid layer. The solid matter was filtered with suction. The solid was washed with 95% alcohol repeatedly, then with a 50% solution of alcohol, next with water, next with absolute alcohol, and finally with acetone. The product suffered a loss during all these washings, but it was hoped to remove any hydrobromide salt formed. The product after washing gave only a very slight halide test. It was slightly soluble in water, less soluble in absolute alcohol, and insoluble in acetone and ether. It melted with decomposition at 241-53°. Yield, 1.4 g. Product A. The product was dried at 105° and analyzed for butylcreatinine.

Anal. Calcd. for  $C_8H_{15}ON_3$ : N, 24.85

Found: 36.75, 36.63

The mother liquor from A was treated with ether. An oily mass separated. The washings from A and the ethereal solution of the mother liquor were combined. At the junction of the two layers there appeared a solid

substance which was filtered off. It gave a negative halide test and had the same solubilities as A. The solid, product B, decomposed without melting at 254-62°. B was dried at 105° and analyzed for nitrogen. Found, 36.86%, 36.65%.

The mother liquor from B was put in a separatory funnel and the oily substance separated. This substance was treated with acetone, yielding a yellowish white precipitate which was destroyed. The ethereal solution left in the separatory funnel was evaporated to dryness, giving a reddish orange product. This solid matter was dissolved in the least amount of absolute alcohol, a brownish green solid separating on cooling. This substance was filtered off and discarded. On stratifying the alcoholic filtrate with ether an orange yellow solid separated. It was filtered off and dried in the oven at 105°. This product was very soluble in water, slightly soluble in absolute alcohol, and insoluble in acetone and ether. It gave a negative halide test and melted with decomposition at 230-38°. Yield, .3 g. The product was analyzed for nitrogen. Found, 33.45%, 33.68%.

#### Creatinine Hydrobromide

A sample of creatinine was evaporated to dryness twice with an excess of 42% HBr. The product was recrystallized from absolute alcohol. Approximately a 50%

recovery was made. The recrystallized product was filtered with suction and dried in the oven at  $105^{\circ}$  for about 30 minutes. The product was white with a rhombic crystalline structure. It was extremely soluble in water, slightly soluble in absolute alcohol, and insoluble in acetone and ether. The salt melted with decomposition at  $235-43^{\circ}$ . It was analyzed for nitrogen and bromide.

Anal. Calcd. for  $C_4H_7ON_3 \cdot HBr$ : N, 21.65; Br, 41.23

Found: N, 21.37, 21.43, 21.40, 21.40

Br, 41.26, 41.27

### Reaction of Creatinine with Ethylene Chlorohydrin

#### Experiment I

2.26 g. (.02 mole) of creatinine and 1.6 g. (.02 mole) of ethylene chlorohydrin were mixed and heated at  $116^{\circ}$  for 2 hours. Only slight signs of a reaction were noticeable. The temperature was raised to  $142^{\circ}$  and kept there for about 15 minutes. The solid dissolved. The mixture was then heated over a bare flame for 2 or 3 minutes during which period some  $NH_3$  was evolved. The material became a very viscous brown mass. When boiled with alcohol a white solid separated. This solid was filtered and dried in the oven at  $105^{\circ}$ . It gave a positive halide test, and was very soluble in water and insoluble in absolute alcohol, acetone, and ether. It darkened and gradually decomposed without melting above  $265^{\circ}$ . The



product was analyzed for ethanolcreatinine hydrochloride.

Anal. Calc. for  $C_6H_{11}O_3N_3 \cdot HCl$ : N, 21.71

Found: 25.07, 25.93

### Experiment II

The experiment was repeated using the same quantity of creatinine with just a slight excess of ethylene chlorohydrin. The mixture was heated at  $140^\circ$  for about 1 hour. All solid material went into complete solution which became a gummy mass on cooling. This mass was treated with a few cc. of 10%  $K_2CO_3$  and the resulting solution stratified with a little acetone. A white solid precipitated. This solid was filtered and dried. It gave a positive halide test. The compound proved to be KCl.

### Experiment III

The experiment was repeated, using the same quantities of reagents. After heating at  $140-50^\circ$  for about 45 minutes all solid matter went into complete solution. 1.5 cc. of ethylene chlorohydrin were added and the temperature raised to  $160^\circ$ , and held there for 45 minutes. A copious flocculent precipitate separated during the latter part of the heating. The mother liquor was reddish orange in color. The solid matter was filtered off. It was dissolved in hot absolute alcohol and the solution was covered with a layer of ether. A white solid

precipitated. This solid was evaporated to dryness with HCl, yielding a rather hygroscopic substance which partially melted with complete decomposition at 324-27°. The product proved to be  $\text{NH}_4\text{Cl}$ . The calculated percentage of nitrogen in  $\text{NH}_4\text{Cl}$  is 26.18. Found, 25.47.

The mother liquor from the above product was treated with about twice its volume of absolute alcohol. On stratifying this alcoholic solution with ether an oily liquid separated. The oil was taken back into solution with more absolute alcohol. The solvents were evaporated, leaving a dark reddish orange viscous substance with an odor of putrefaction. The gummy syrup was treated with a few cc. of concentrated HCl. To this acid solution concentrated  $\text{NH}_4\text{Cl}$  was added drop by drop. The white precipitate which formed was filtered, washed with acetone, and dried in the oven at 105°. The product gave a positive halide test. It darkened slightly at 265° and gradually decomposed without melting above 320°. The product proved to be  $\text{NH}_4\text{Cl}$ . The percentage composition of  $\text{NH}_4\text{Cl}$  is N, 26.18; Cl, 66.02. Found, N, 25.98, 26.04; Cl, 66.03.

#### Experiment IV

The condensation was attempted again, but carried out in the presence of pyridine. 1.13 g. (.01 mole) of creatinine, .8 g. (.01 mole) of ethylene chlorohydrin, and .8 g. (.01 mole) of pyridine were mixed and heated in a

sealed tube at 120-30° for 7 hours. The temperature was raised to 140-50° for 30 minutes and then to 150-60° for another 30 minutes. The reaction mixture had the appearance of a dark gummy mass. The contents of the tube were worked up with  $\text{CH}_3\text{OH}$ . A white crystalline substance separated. This product was filtered, washed with  $\text{CH}_3\text{OH}$ , and dried in the oven at 105° for about 45 minutes. The product gave a negative halide test. It was slightly soluble in water, less soluble in 95% alcohol, and insoluble in acetone and ether. A melting point determination showed the solid to gradually decompose at 258° and then melt with further decomposition at 285°. The product proved to be creatinine. The calculated percentage of nitrogen in creatinine is 37.16. Found, 36.47, 36.34.

The mother liquor from the reaction product and the alcoholic washings were combined and evaporated to a gummy mass which was put in a vacuum desiccator for about one hour. The orange yellow gummy mass was then treated with 5 cc. of 95% ethyl alcohol. When stratified with acetone, this solution gave a white precipitate. The precipitate was filtered and dried in the oven at 105° for 30 minutes. This solid gave a negative halide test and had the same solubilities as the first solid isolated from the reaction mixture of this experiment. It began to decompose slowly at 248° and melted with further decomposition at 276°.

This product proved to be creatinine. Percentage nitrogen found, 35.98, 36.13.

The mother liquor from the latter product was evaporated down as far as possible to a dark oily gum on the water bath and then put in a vacuum desiccator for several days. All attempts to induce crystallization failed.

#### Experiment V

The latter experiment was repeated, using a sealed tube and identical experimental conditions, but in the absence of pyridine. The reaction mass was removed from the tube by solvation with  $\text{CH}_3\text{OH}$ . The solvent was evaporated off and left an oily, viscous substance, with an odor of putrefaction. The gummy mass was put in a vacuum desiccator for several days but crystallization could not be induced. Ether, absolute alcohol,  $\text{CCl}_4$ , concentrated and dilute  $\text{HCl}$ , and various mixtures of these solvents failed to start crystallization.

#### Experiment VI

2.26 g. (.02 mole) of creatinine and a slight excess of ethylene chlorohydrin were mixed and heated in an open tube at  $150-60^\circ$ . Complete solution of all solid matter resulted after one hour of heating. On heating at the same temperature for another hour a solid precipitated. The reaction mixture, on cooling, became a viscous gummy mass. This mass was dissolved in hot absolute alcohol to

which an equal volume of acetone was added. A tan solid precipitated and was filtered off and dried in the oven at 105°. This product gave a positive halide test. It was very soluble in water, slightly soluble in alcohol, and insoluble in acetone and ether. It decomposed gradually without melting above 267°. Percentage nitrogen found, 26.12, 25.87.

The mother liquor from this product was destroyed and so the experiment was repeated, using the same quantities of reagents and identical experimental conditions. A white product was isolated from the reaction mixture by the same method as employed in the previous experiment. This solid had the same solubilities as the other compound, but darkened at 280° and decomposed without melting above 326°. It gave a positive halide test. As in previous cases, this product and the preceding one were  $\text{NH}_4\text{Cl}$ . Percentage nitrogen found, 25.75, 25.86.

The mother liquor from the latter product was evaporated over a water bath to a gummy substance. It had an odor of putrefaction and was reddish orange in color. All attempts to get crystallization by methods previously described failed.

#### Reaction of Creatinine with Trimethylene Bromohydrin

##### Experiment I

11.3 g. (.1 mole) of creatinine and 13.9 g. (.11 mole)

of trimethylene bromohydrin were mixed and heated at  $115^{\circ}$  for 2.5 hours. The reaction mixture was very viscous and orange yellow in color. 10 cc. of 48% HBr were added to the mixture and the gummy substance went into complete solution. The solution was poured into a beaker and allowed to cool. On cooling crystals separated. The crystals were filtered and washed with acetone. This washing removed any unreacted reagent and left a pure white product. The solid was dried as well as possible at the pump and then further dried in the oven at  $105^{\circ}$  for about 30 minutes. It gave a positive halide test, and was extremely soluble in water and insoluble in acetone and ether. The product decomposed slowly above  $240^{\circ}$  without melting. It was analyzed for propanolcreatinine hydrobromide.

Anal. Calcd. for  $C_7H_{13}O_2N_3 \cdot HBr$ : N, 16.7

Found: 14.98, 14.86.

The impure product was recrystallized from 95% alcohol in which it was only slightly soluble. The recrystallized solid decomposed without melting at  $268-300^{\circ}$ . The product was analyzed for nitrogen. Found, 14.32%.

The mother liquor from the original reaction product was cooled to  $0^{\circ}$  and 4 cc. of cold concentrated  $NH_4OH$  was added with stirring, precipitating a flocculent solid. The solution and precipitate were cooled in ice for about an hour. The solid matter was then filtered and

washed with cold 95% ethyl alcohol. This washing removed all coloration, leaving a white substance which was dried as well as possible at the pump and then further dried in the oven at 105° for one hour. However, the product gave a halide test. It was very soluble in water and only slightly soluble in alcohol. It decomposed at 285-301° without melting. The product was analyzed for propanolcreatinine hydrobromide.

Anal. Calcd. for  $C_7H_{13}O_2N_3 \cdot HBr$ : N, 16.7; Br, 31.74

Found: N, 14.49, 14.58; Br, 82.57, 82.47.

#### Experiment II

Another attempt to condense trimethylene bromohydrin with creatinine was made, this time using a pyridine medium. 2.26 g. (.02 mole) of creatinine, 5.56 g. (.04 mole) of trimethylene bromohydrin, and 3.2 g. (.04 mole) of pyridine were heated at 114-17° for 5 to 6 hours. After allowing the reaction mixture to cool a white solid separated. The product was filtered off. It was only slightly soluble in water, and insoluble in alcohol and acetone. 10 cc. of cold 95% alcohol were added to the product and then 10 cc. of cold acetone. The solid matter was worked up in an ice bath for about 30 minutes. The supernatant liquid was poured off and the residue treated with 5 cc. of cold water. On standing white crystals separated. The crystals were filtered, washed with cold 95% alcohol, and dried in the oven at 105°. The product

gave a negative halide test, and decomposed at 235° without melting. The solid proved to be creatinine. Percentage nitrogen found, 38.48%, 34.86%.

The mother liquor from the reaction product and all the washings were combined but time did not permit investigation.

### Experiment III

Another attempt to condense trimethylene bromohydrin with creatinine was made. 4.52 g. (.04 mole) of creatinine and 5.56 g. (.04 mole) of trimethylene bromohydrin were heated in an open tube at 114-17° for 5 to 6 hours. After cooling to room temperature the reaction mixture became a pale yellow gummy mass. The mass was cooled to 0° and 1.5 cc. of absolute alcohol and 5 cc. of acetone were added. The solid matter was filtered and washed with cold absolute alcohol, and then dried in the oven at 105°. The product gave a negative halide test, and was only slightly soluble in absolute alcohol and insoluble in acetone. It was a white powdery substance decomposing without melting at 217-40°. The product proved to be creatinine. Percentage nitrogen found, 36.47%. Time did not permit investigation of the mother liquor.

### Reaction of Creatinine with Ethyl Chlorocarbonate

#### Experiment I

1.13 g. (.01 mole) of creatinine, 1.1 g. (.01 mole) of ethyl chlorocarbonate, and an excess of pyridine were



heated for a few minutes at 100°. All solid material went into total solution. The reaction mixture was cooled to 0° and filtered. The white product was washed with absolute alcohol. It was very soluble in water, and insoluble in absolute alcohol, acetone, and ether. It gave a positive halide test. The solid melted with decomposition at 238°, although gradual decomposition had set in at 215°. The compound was dried in the oven at 105° and analyzed for ethyl creatinyl formate.

Anal. Calcd. for  $C_7H_{11}O_3N_3$ : N, 22.58

Found: 29.96.

#### Experiment II

The condensation was attempted again. 1.13 g. (.01 mole) of creatinine and an excess of ethyl chlorocarbonate were heated in a sealed tube at 100° for 4 hours. The reaction product was removed from the tube and washed thoroughly with acetone and then dried in the oven at 105°. The solid was almost white, having a slight brownish yellow tint. It gave a positive halide test and melted with decomposition at 240-45°. It was very soluble in water, slightly soluble in absolute alcohol, and insoluble in acetone and ether. The compound was analyzed for nitrogen and chloride. Found, N, 27.61%, 27.62%; Cl, 21.85%.

Reaction of Creatinine with 2,4-Dinitrochlorobenzene

1.13 g. (.01 mole) of creatinine, 2.02 g. (.01 mole) of 2,4-dinitrochlorobenzene, and 30 cc. of 95% alcohol were heated over a water bath under a reflux condenser for 16 hours. The solution became orange yellow in color. On cooling light yellow crystals separated. The crystals were recrystallized from hot ethyl alcohol after which they became pale yellow in color. The crystals appeared different from those of creatinine. The product was slightly soluble in water, less soluble in alcohol, and insoluble in acetone and ether. It softened at 190° and decomposed gradually from 202° to 267° at which temperature it decomposed. The product was dried in the oven at 105° and analyzed for creatinine 2,4-dinitrochlorobenzene.

Anal. Calcd. for  $C_4H_7ON_3 \cdot C_6H_3O_4N_2Cl$ : N, 22.22

Found: 36.40, 36.45.

The mother liquor from the above product was diluted with water and allowed to stand overnight. Crystals separated. However, all solid matter immediately became a brown oily liquid upon the slightest application of heat, showing the crystals to be 2,4-dinitrochlorobenzene.

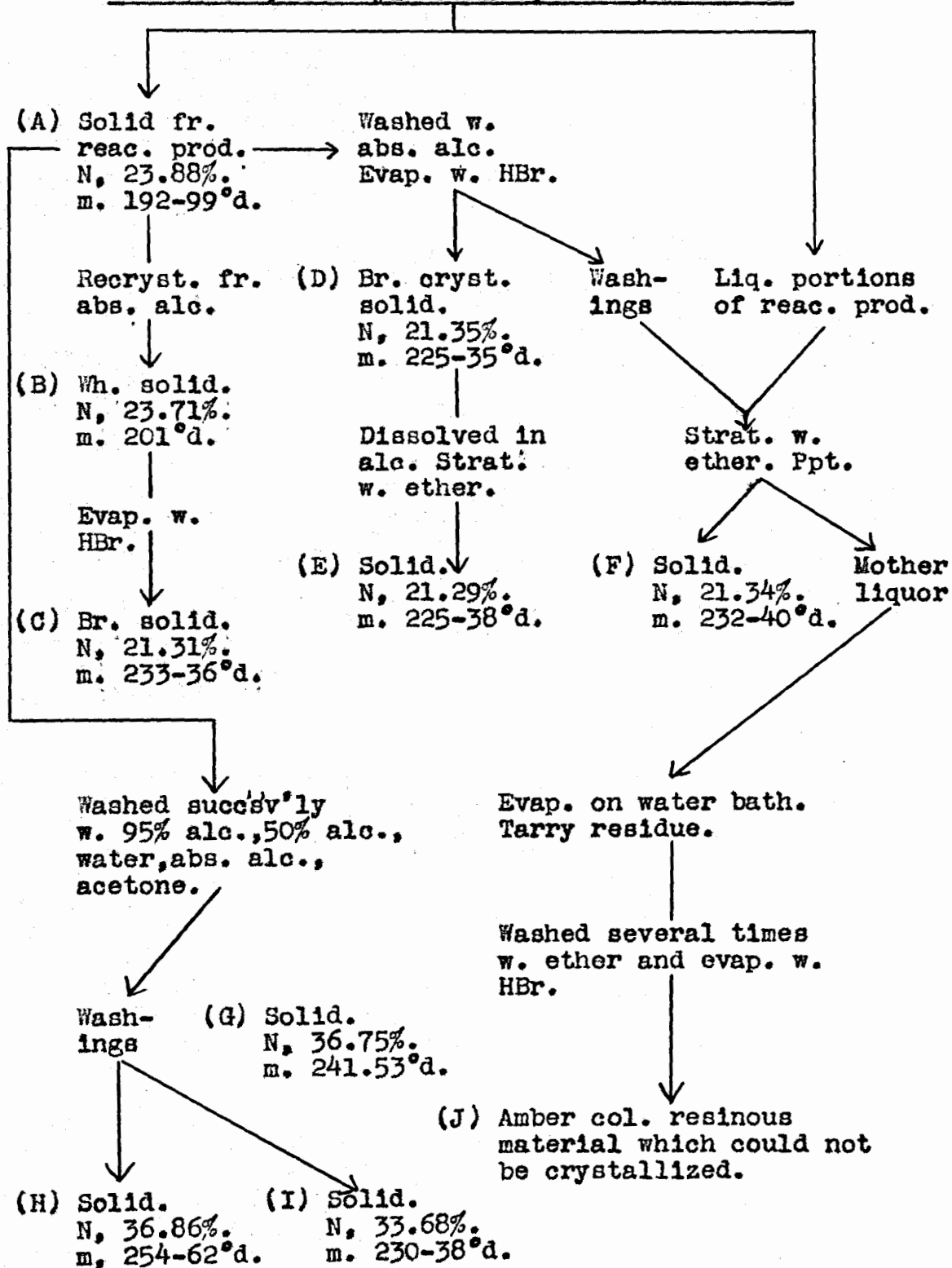
## DISCUSSION OF RESULTS

All attempts to condense n-butyl bromide with creatinine were unsuccessful. The halogen in n-butyl bromide did not seem to be particularly active towards the basic nitrogen atoms of the creatinine molecule. In every instance there was evidence of some reaction. Some of the products isolated gave positive halide tests. The source of the halogen must be the alkyl halide. For this to happen it seems that the butyl radical must substitute either the imido or imino hydrogen.

In some attempted condensations about 50% of unreacted creatinine was recovered. Most of the products isolated were mixtures of creatinine hydrobromide, unreacted creatinine, and possibly some butylcreatinine hydrobromide. No method of separation of a butyl derivative could be found because of the similar solubilities.

All the mother liquors, when evaporated to almost dryness, yielded oily or gummy masses which could not be crystallized. Absolute alcohol, ordinary alcohol, acetone, ether, and various mixtures of these solvents were tried. It is possible that the desired product of the reaction was contained in this gummy residue, since all solid matter isolated consisted of mixtures. These gummy residues had the odor of putrefaction, indicating decomposition. This odor was noticed in every attempt to condense n-butyl bromide with creatinine.

Creatinine, n-Butyl Bromide, n-Butyl Alcohol



Products (A) and (B) must be a mixture of creatinine hydrobromide and creatinine since (C) was later proved by synthesis to be pure creatinine hydrobromide. The other products giving a similar percentage of nitrogen are also creatinine hydrobromide. Products (G) and (H) are creatinine. Product (I) being from ionizable bromide and giving too low a percentage of nitrogen could be a mixture of creatinine and n-butylcreatinine. No separation could be made. The desired derivative, n-butylcreatinine, must be in product (J) because some kind of a reaction does take place with creatinine and n-butyl bromide as evidenced by the odor, color, and the fact that creatinine hydrobromide was isolated from the reaction product.

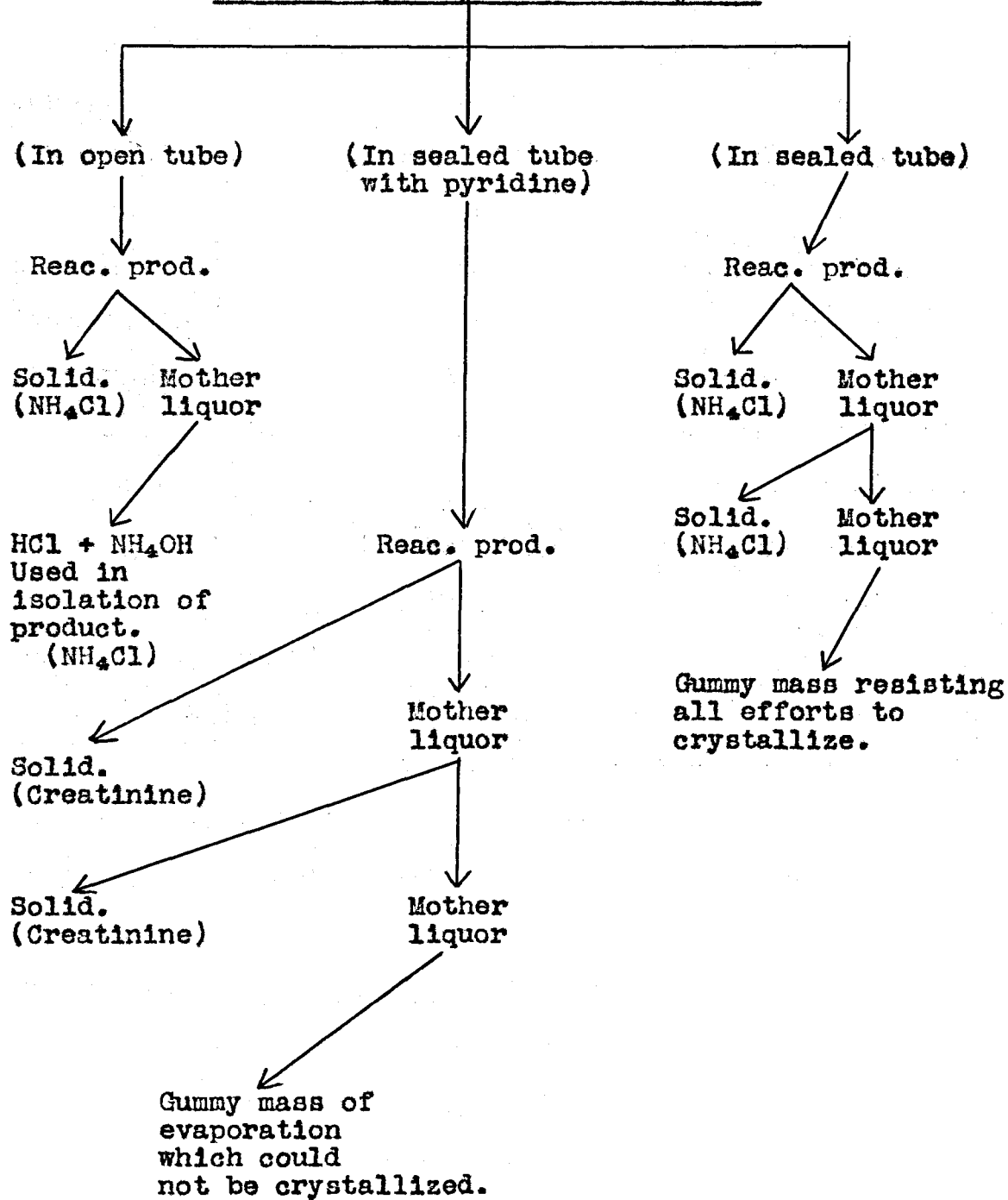
Creatinine hydrobromide does not appear in the literature; it was prepared in order to become acquainted with its solubilities and other properties since product (C) on page 51 was believed to be creatinine hydrobromide. As was expected, the product proved to be identical in every respect.

There was evidence of a positive reaction of creatinine with ethylene chlorohydrin, though no condensation product was isolated. In all reactions, except those in the presence of pyridine, any solid material isolated gave a positive halide test; indicating that a reaction must have occurred. The products analyzed to give a nitrogen content which could be interpreted as

possible mixtures of the desired compound, ethanolcreatinine, creatinine hydrochloride, and unreacted creatinine. On further investigation part of these proved to be ammonium chloride. It must be remembered that there was always a gummy, viscous residue resulting from evaporation of the mother liquors of the reaction mixtures, and all attempts to induce crystallization failed. It is justifiable to believe that had the gummy mass crystallized a condensation product would have been isolated. When the reaction was carried out in the presence of pyridine there was a darkening in color but only unreacted creatinine could be isolated. Pyridine seemed to inhibit reaction. The coloration could have been due to the reaction between pyridine and ethylene chlorohydrin.

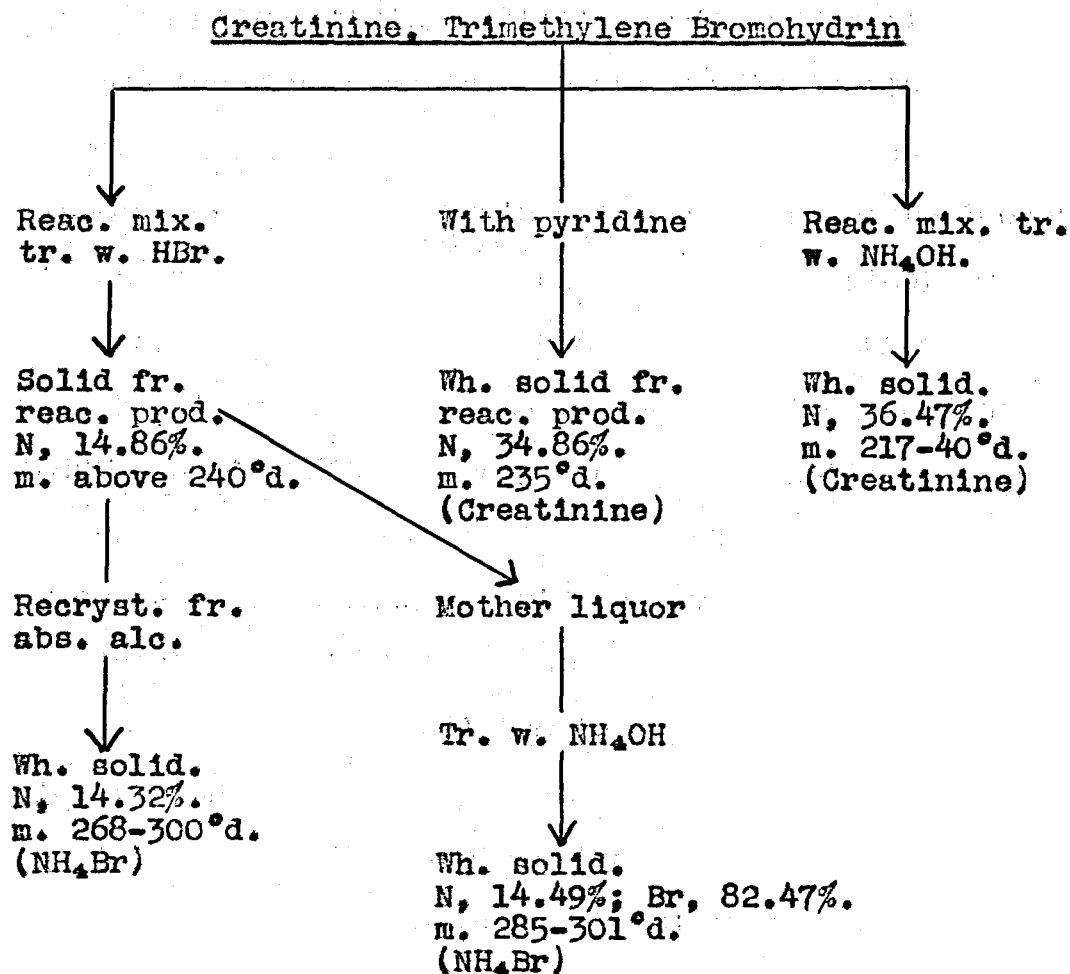
The criteria for saying that the various products were ammonium chloride were its decomposition point, its nitrogen and chloride analysis, and the fact that ammonia was copiously evolved in the cold with sodium hydroxide. The explanation for the formation of this product is not proposed since from the general chemistry of creatinine one does not expect a decomposition at the temperature used unless in the presence of alkali. Products in different cases gave halide tests and in the preliminary experiment with this reagent ammonia was evolved. These observations show that the elements of ammonium chloride are available from the reactants.

Creatinine, Ethylene Chlorohydrin

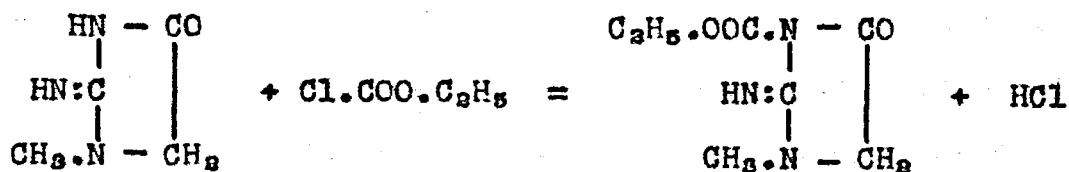


A reaction was obtained with creatinine and trimethylene bromohydrin. In the first attempted condensation a product was obtained which, according to nitrogen analyses, was evidently a mixture of the mono and di compounds of propanolcreatinine hydrobromide. The mono derivative has a calculated nitrogen content of 16.70% and the di derivative 13.50%. The analyzed product gave 14.98% and 14.86% nitrogen. Recrystallization gave a product analyzing 14.32% nitrogen. No method of separation of the possible mixture could be advanced. However, it was later shown to be probably ammonium bromide since it evolved ammonia with cold sodium hydroxide. When the mother liquor from this product was treated with  $\text{NH}_4\text{OH}$  the precipitate was beyond a doubt ammonium bromide. The percentage composition of  $\text{NH}_4\text{Br}$  is; N, 14.31%; Br, 81.80%. These figures tally very closely with those given under experimental results, i. e., N, 14.58%, 14.47%; Br, 82.57%, 82.47%. When the condensation was attempted in the presence of pyridine there was very little reaction, the isolated product being creatinine. This fact was noticeable in all reactions carried out in a pyridine medium, such as with ethylene chlorohydrin and ethyl chlorocarbonate.





Since according to the literature acetyl and benzoyl creatinine have been prepared from the acyl halides, with and without the use of pyridine, it was thought that ethyl chlorocarbonate would react in the same way to give the ethyl ester of creatinyl formic acid;



A condensation of ethyl chlorocarbonate with creatinine was tried and was unsuccessful. The first attempt, carried out in an open tube, gave a chloride test even when the reaction was carried out in the presence of pyridine. One might interpret by the nitrogen analyses that the product contained a considerable part of creatinine hydrochloride along with the expected product, ethyl creatinyl formate. These supposed constituents could not be separated. In the reaction carried out in the sealed tube, there was evidence of a reaction. The reactants became a solid mass at the end of the period of heating. Lack of time prohibited further investigation of this product.

Since the halogen in 2,4-dinitrochlorobenzene is so very active it was thought that an addition or substitution compound of creatinine could be prepared which could be applied as a means of identification of creatinine and its derivatives. The preparation of such a compound was unsuccessful. Even after such a long period of refluxing, nitrogen analyses indicated the product to be creatinine. From the physical appearance of the solid and its peculiar melting point it was concluded that the crystals consisted of creatinine covered with a very thin film of 2,4-dinitrochlorobenzene. Further evidence in support of this was offered by examining the mother liquor. By dilution of the mother liquor with water and allowing to stand

overnight unreacted 2,4-dinitrochlorobenzene was recovered.

A reaction was obtained with glycerol dichlorohydrin, glycerol dibromohydrin, and trimethylene bromide, but the products were not investigated. Reagents containing the di-halide presented too many possibilities and in view of this the investigation consisted of carrying out reactions with mono-halide compounds. A reaction was also obtained with benzene sulfonyl chloride, using a procedure similar to that of Greenwald<sup>5</sup> in benzoylating creatinine. However, no solid product could be isolated and the reaction was not further investigated.

The fact that condensation products of creatinine with halogen derivatives were not obtained is not surprising in view of previous derivatives similarly prepared. Only the methyl and ethyl, and benzyl derivatives are recorded as prepared directly from the halides. The methyl and ethyl derivatives were prepared through their iodides in a sealed tube at 100°. The benzyl derivative was made from benzyl chloride at 140° for 6 hours in a closed tube<sup>9</sup>. These previously used halogen compounds are more reactive than those assigned for this work. The formation of acyl derivatives, such as the benzyl<sup>o</sup> and acetyl, take place only with difficulty and in the presence of pyridine.

The original assignment of this work by Dr. Wm. R. Cornthwaite consisted of a proposed study of the reaction

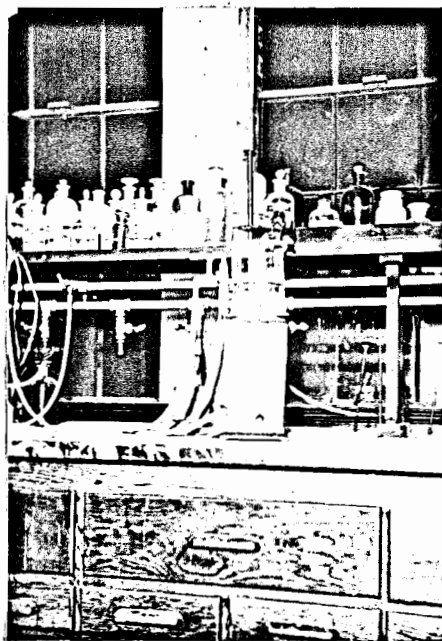
of creatinine with ethylene dibromide, ethylene chlorohydrin, trimethylene bromide, and glycerol dichlorohydrin. Early in the work it was concluded that the di-halogen compounds afforded more possible ways of reaction than the mono-halogen derivatives. The probable number of products would be greatly increased also. For this reason, some other reagents were considered.

Since the experiments recorded show negative results, they were confirmed by the results of various repetitions of work.

## APPARATUS

During the pursuance of this investigation small quantities of reagents were used in all reactions carried out. If a condensation should occur, the experiment was to be repeated using, of course, large quantities of the reactants. In order to carry out the reactions using small samples, it became necessary to construct special apparatus to facilitate reaction and to conserve time. Professor G. C. Kyker, directing the investigation, very obligingly and ingeniously, designed and constructed the pieces of apparatus described and pictured below.

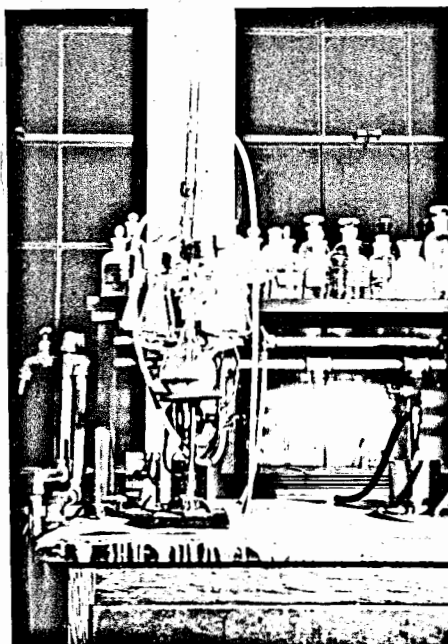
1 - Constant level water bath



This apparatus was designed to maintain a constant water

level and temperature for any desired period of time. It was made of Pyrex glass and consisted of a liter beaker to which control gauges were attached. A protected flame was set beneath it. This apparatus was used principally for the conversion of creatine to creatinine.

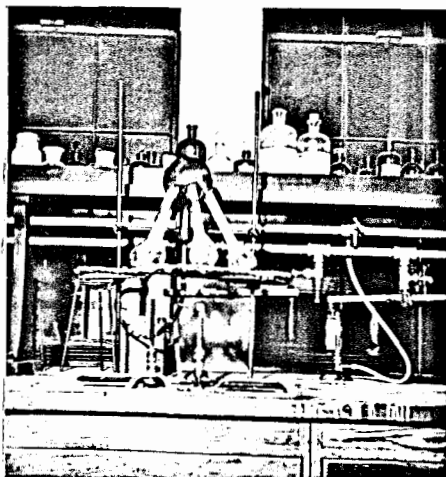
2 - Constant temperature heating apparatus



This apparatus was designed with a capacity to carry out at least four reactions, if desired, simultaneously. The reactants could be heated to any desired constant temperature for any period of time. It consisted of a central flask, which contained the solution with the desired boiling point, to which four Erlenmeyer flasks were attached by tubes leading from their bottoms. Each Erlenmeyer flask was

connected, by a tube leading from its side near the top, to a central bulb over which a reflux condenser was attached. The central bulb was connected by a perpendicular tube to the flask containing the constant boiling solution. In order to operate the apparatus the reaction mixtures were put in large ignition tubes and inserted in the Erlenmeyer flasks through cork stoppers. The boiling mixture would circulate its vapors through the entire system and return via the reflux condenser to its starting point, thus maintaining a constant temperature for any desired length of time. The entire apparatus was made of Pyrex glass. All reactions in this investigation were carried out with this apparatus unless otherwise specified.

### 3 - Digestion hood



This digestion hood was used in the semi-micro Kjeldahl nitrogen analyses. It was made from a large distilling

flask from which the side arm had been removed. The neck of the flask was drawn out and was attached to an aspirator by means of a length of rubber tubing. Five holes were blown through the bulb of the flask through which, upon inverting the flask, the necks of the Kjeldahl flasks were inserted. On applying suction the obnoxious fumes which evolved during the digesting process were automatically removed.



## SUMMARY

1. No condensation product of creatinine and n-butyl bromide was isolated. Some kind of a reaction was obtained as evidenced by the color, peculiar odor, and the isolation and identification of creatinine hydrobromide.
2. Creatinine hydrobromide was prepared and its properties described.
3. No condensation product of creatinine and ethylene chlorohydrin was isolated. The color, odor, and isolation of ammonium chloride indicated a reaction or decomposition of the reactants; the change occurring is not described.
4. There was a reaction between creatinine and trimethylene bromohydrin as indicated by color, odor, and isolation of a product which was shown to be ammonium bromide. The mechanism for the formation of the ammonium salt is not proposed.
5. All reactions tried with creatinine and halogen compounds gave a solid phase which proved to be either unreacted creatinine or its hydrohalide, or an ammonium halide, and a liquid phase which could be obtained as a yellow resinous residue which resisted all attempts toward crystallization. The reaction product and the resinous residue in every case was accompanied by an odor of putrefaction.
6. Creatinine does not give a condensation product with the

very active halogen in 2,4-dinitrochlorobenzene after refluxing for 16 hours.

7. Creatinine and ethyl chlorocarbonate gave a reaction when heated in a sealed tube. The product according to the nitrogen analyses and the expected reaction is probably a mixture of ethyl creatinyl formate and unreacted creatinine. No separation of a pure product could be found in the time that remained.

## ACKNOWLEDGEMENT

I am indebted to Dr. Wm. R. Cornthwaite who originally outlined the problem to be pursued, but who did not return to the University of Richmond to direct the investigation.

I wish to express my appreciation to Professor G. C. Kyker for his unceasing efforts to stimulate and maintain my enthusiasm and interest during the entire investigation, for his many timely suggestions, and for the aid he rendered in designing and constructing special apparatus.

I thank the Valentine Meat Juice Company, Richmond, Virginia, for its generous contribution of creatine without which this investigation could not have been undertaken.

## AUTOBIOGRAPHY

I, Howard Edwards Wright, Jr., was born June 29, 1913, at Petersburg, Virginia. My early education was obtained at the D. M. Brown Grammar School and the Bolling Junior High School in Petersburg. In June, 1931, I graduated from the Petersburg High School. The following September (1931) I matriculated at Hampden-Sydney College, and received the Bachelor of Science degree in June, 1935. In September, 1935, I entered the University of Richmond, having been granted a Service Scholarship, and have held the appointment to the present time.