THE NINHYDRIN REACTION WITH AMINES AND AMIDES.

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In the preceding paper Harding and Warneford have examined and discussed the interaction of amino-acids and ammonium salts with triketohydrindene hydrate (ninhydrin). They showed that the ninhydrin reaction with amino-acids could be explained by adopting the hypothesis of Dakin and Dudley which supposes the decomposition of the amino-acid into ammonia and the corresponding glyoxal,

\[ \text{RCHNH}_2 \cdot \text{COOH} \rightarrow \text{RCO} \cdot \text{CHO} + \text{NH}_3 \]

the glyoxal acting as the reducing agent. They showed that the ninhydrin reaction with ammonium salts, first pointed out as a general reaction by Neuberg, only took place in the presence of hydroxyl ions, and that these had been observed by Ruhemann to hydrolyze triketohydrindene hydrate to phenyglyoxal-o-carboxylic acid.\(^5\)

\(^5\) In this and the preceding paper triketohydrindene hydrate has sometimes been written as the triketone for the sake of simplicity in explaining the decompositions. Its true formula, as pointed out by Ruhemann is,

\[ \text{C}_4\text{H}_4\left(\text{CO}\right)_2\text{C(OH)}_2 \]

and it will be found that we have used this where the reactions could be more clearly shown by its use.

337

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Ninhydrin Reaction

\[
C_2H_4\left(\begin{array}{c}CO \\
CO
\end{array}\right) + H_2O = C_2H_4\left(\begin{array}{c}\cdot CH_2 \\
COOH
\end{array}\right)
\]

In this way the requisite glyoxal was produced and the two series of facts were brought into agreement.

There still remains, however, the second criticism brought against the ninhydrin reaction by Neuberg, who noticed that a large number of organic bases give a positive test with triketohydrindene hydrate. This is in contradiction to the statements of Abderhalden and Schmidt.

In our experimental method we have followed the same procedure as that adopted in the previous paper. The bases examined were tested in varying concentration, with and without the presence of pyridine, and the general results followed those obtained with ammonium salts.

### Table I

<table>
<thead>
<tr>
<th>Base</th>
<th>1 per cent solution</th>
<th>Solution: 1 cc. = 0.05 mg. N.</th>
<th>N decomposed in 1 per cent ninhydrin</th>
<th>2 per cent ninhydrin and pyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>Methylamine</td>
<td>Strong reaction</td>
<td>No reaction</td>
<td>0.041</td>
<td>0.045</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>0.037</td>
<td>0.044</td>
</tr>
<tr>
<td>Allylamine</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>0.021</td>
<td>0.022</td>
</tr>
<tr>
<td>Pentamethylenediamine</td>
<td>Reddish shades</td>
<td>&quot; &quot;</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>Strong reaction</td>
<td>&quot; &quot;</td>
<td>0.043</td>
<td>0.046</td>
</tr>
<tr>
<td>Ethyl glycoll</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>0.036</td>
<td>0.037</td>
</tr>
<tr>
<td>Phenocoll</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>0.021</td>
<td>0.021</td>
</tr>
<tr>
<td>Isopropylamine</td>
<td>No</td>
<td>&quot; &quot;</td>
<td>No reaction</td>
<td></td>
</tr>
<tr>
<td>Aniline</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>Diethylamine</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>Diisopropylamine</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>(\omega)-Aminocetophenone</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
<td></td>
</tr>
</tbody>
</table>

It is seen from an inspection of Table I that those bases which react with triketohydrindene hydrate do so in relatively high con-

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centration only. When in very dilute solution (1 cc. = 0.05 mg. amino nitrogen), no reaction is obtained. In the presence of pyridine, however, those bases which react in the higher concentration are found to react even in the very dilute solution. In this respect the ninhydrin reaction with amines follows that with ammonium salts. It is to be noted that if a base does not react with triketohydrindene hydrate alone when in the higher concentration neither does it do so when in the lower concentration and in the presence of pyridine. Furthermore, the amount of coloring matter (ammonium salt of diketohydrindylidene-diketohydrindamine) formed by the reactive bases in dilute solution in presence of pyridine is not constant, as it is in the case of the ammonium salts. The amount of color formed is distinctly and clearly dependent on the constitution of the amine from which it is formed, a point which we will discuss later. The identity of the coloring matter was proved by the three tests mentioned in the previous paper. The constitution given to this compound,

\[
\text{C}_6\text{H}_5\begin{array}{c}
\text{CO} \\
\text{C} - \text{N}^* \\
\text{C (ONH)}
\end{array} \text{C}_6\text{H}_4
\]

and its formation by the interaction of certain bases with triketohydrindene hydrate, mean a complete decomposition of the amines, in order to supply the nitrogen atom marked with the asterisk. The salt need not necessarily be an ammonium salt, as a salt with an organic base would fit the facts equally well, but the substitution of any other group for the nitrogen atom marked with the asterisk, or an alteration in the linkages, would cause a marked alteration in the absorption spectrum. In explaining the mechanism of the ninhydrin reaction with amino-acids, the nitrogen atom was supplied by the decomposition of the acid into the corresponding glyoxal and ammonia, the former causing a reduction of the triketone to 1,3-diketohydrindol,

\[
\text{C}_6\text{H}_5\begin{array}{c}
\text{CO} \\
\text{CO}
\end{array} \rightarrow \text{C}_6\text{H}_4\begin{array}{c}
\text{CO} \\
\text{CHOH}
\end{array}
\]

which condensed with loss of water with the ammonia.
Ninhydrin Reaction

\[
\text{C}_6\text{H}_4\text{CO} \text{CHOH} + \text{NH}_3 = \text{C}_6\text{H}_4\text{CHNH}_2 + \text{H}_2\text{O}
\]

A second molecule of triketohydridene hydrate then condensed with the 1, 3-diketohydridamine

\[
\text{C}_6\text{H}_4\text{CO} \text{CHNH}_2 + \text{OC} \text{CO} \text{C}_2\text{H}_4 =
\]

\[
\text{H}_2\text{O} + \text{C}_6\text{H}_4\text{CO} \text{CH} - \text{N} = \text{C} \text{CO} \text{C}_2\text{H}_4
\]

to give diketohydridylidene-diketohydridamine. In the reaction with the ammonium salts the glyoxal was supplied by the hydrolysis of part of the triketone, the ammonia necessary to supply the nitrogen atom coming from the ammonium salt. The production of ammonia, however, from such organic amines as methylamine and ethylamine is a matter of difficulty. Such bases do not hydrolyze readily into the corresponding alcohol and ammonia. Any dissociation theory, such as that of methylamine into ammonia and methylene,\(^7\)

\[
\text{CH}_3\text{NH}_2 \rightleftharpoons \text{CH}_2: + \text{NH}_3
\]

has not sufficient facts in its favor to warrant its assumption. Moreover, such a dissociation theory would fail to explain the negative reaction with isopropylamine.

As the ninhydrin reaction with amines is clearly dependent on the constitution of the base, it becomes important to see if some insight into its mechanism cannot be gained by a classification of the bases, according to their action towards triketohydridene hydrate and to their chemical constitution. This has been done and the results are given in the following tables.

In tabulating our own results we have always taken into consideration the concentration of the base, and a negative reaction given by us means that the base gives no blue coloring matter when 1 cc. of a 1 per cent solution is heated with 1 cc. of 1 per cent ninhydrin solution in a boiling water bath for a period of 20 minutes.

The results given by this type of amine are clear and unequivocal. The only two exceptions previously known, i.e., allylamine and ethyl glycocholl, on examination are shown to be errors. The finding with ethyl glycocholl (H₂NCH₂CO₂C₂H₅) and phenocoll (C₅H₇OCH₄NHCOCH₃NH₂) is of great interest as it finally dis-
poses of the claim of Ruhemann and Abderhalden and Schmidt that it is necessary for both the carboxyl and amino group of an amino-acid to be unsubstituted in order to obtain a positive result. The carboxyl group may be substituted, but substitution on the amino group certainly prevents the reaction (hippuric acid).

TABLE III.

<table>
<thead>
<tr>
<th>Base</th>
<th>Observer</th>
<th>Reaction</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropylamine</td>
<td>Harding and MacLean</td>
<td>-</td>
<td>Reddish</td>
</tr>
<tr>
<td>Camphylamine</td>
<td>Neuberg</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abderhalden and Schmidt</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>Neuberg</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harding and MacLean</td>
<td>+</td>
<td>Strong reaction</td>
</tr>
</tbody>
</table>

The very small number of bases of this type which have been examined makes it difficult to draw definite conclusions. It would appear, however, that simple bases of this type do not react with triketohydrindene hydrate (isopropylamine and camphylamine). In the case of glucosamine, where we found an undoubted reaction, we have a negative aldehyde group on the carbon atom adjacent to the amino group, a fact which is well known to increase the reactivity of the neighboring groups, and the explanation of the reactivity of glucosamine may be sought in this direction. Moreover, it must not be forgotten that the amino-acids which are obtained by the hydrolysis of proteins and which show the greatest reactivity, belong to this group (except glycine). A number of di- and tri-peptides, quoted by Abderhalden and Schmidt as giving a positive reaction, are also to be classed in this category, so that the position occupied by glucosamine is not an isolated one. As a consequence we are brought to the conclusion that the grouping $R_2\text{CHNH}_2$, when one of the radicles is negative in character, reacts with ninhydrin, otherwise it is unreactive.

With the exception of the two tertiary amino-acids examined by Ruhemann, bases of this type do not give any reaction with ninhydrin. The two exceptions mentioned probably give a posi-

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Table IV.

<table>
<thead>
<tr>
<th>Base</th>
<th>Observer</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Aminoisobutyric acid</td>
<td>Ruhe mann</td>
<td>+</td>
</tr>
<tr>
<td>α-Aminoethylbutyric acid</td>
<td>“</td>
<td>+</td>
</tr>
<tr>
<td>β-Aminocrotonic ester</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Adenine</td>
<td>Abderhalden and Schmidt</td>
<td>-</td>
</tr>
<tr>
<td>Guanine</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
<tr>
<td>Aniline</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Benzidine</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
<tr>
<td>p-Aminoacetophenone</td>
<td>“</td>
<td>-</td>
</tr>
</tbody>
</table>

tive reaction on account of the ease with which tertiary amino-acids lose ammonia to form unsaturated acids. Indeed, Ruhe-
mann himself makes the observation that these two amino-acids required boiling with the reagent in order to give the reaction, whereas the other amino-acids only required warming.

Table V.

Types: R₃NH and R₂N.

<table>
<thead>
<tr>
<th>Base</th>
<th>Observer</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylamine</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Diisopropylamine</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
<tr>
<td>Diisobutylamine</td>
<td>“</td>
<td>-</td>
</tr>
<tr>
<td>Piperidine</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>Harding and MacLean</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>Neuberg</td>
<td>+</td>
</tr>
<tr>
<td>Skatole</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>“</td>
<td>-</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Pyridine</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
<tr>
<td>Quinoline</td>
<td>Neuberg</td>
<td>-</td>
</tr>
<tr>
<td>Isoquinoline</td>
<td>Harding and MacLean</td>
<td>-</td>
</tr>
</tbody>
</table>

* Red shades.
With the exception of adrenaline secondary and tertiary bases are unreactive towards triketohydrindene hydrate. The instability of solutions of adrenaline is well known and this exception can well be explained in this way.

It is evident, then, that there are only two general classes of organic bases which give the ninhydrin reaction, $RCH_2NH_2$ and $R_2CHNH_2$ where one radicle is strongly negative in character.

In attempting to explain the ninhydrin reaction with these classes of organic bases, two facts must be borne in mind: (1) The reaction does not take place when the amine is in very dilute solution, unless in the presence of pyridine; i.e., it parallels the reaction with ammonium salts. (2) The extent of the reaction depends on the constitution of the amine, the simpler amines being the more reactive unless a negative group is present (Table I).

In the previous paper we have shown how the analogy between triketohydrindene hydrate and alloxan enabled Ruhemann to arrive at the constitution of the blue color produced by the interaction of triketohydrindene hydrate and amino-acids. The analogy, however, between the formation of murexide from alloxan and its derivatives and the formation of the ammonium salt of diketohydrindylidene-diketohydrindamine in the ninhydrin reaction is much closer than has previously been recognized. In Tables VI and VII we have collected the results of the different investigators in the two fields. In Table VI we have collected the work of Ruhemann. The analogies, which were first drawn by him, are very clear and striking and extend not only to the methods of preparation of the compounds but to many of their physical and chemical properties. This is particularly striking in the case of the formation of chrome salts. It also shows the formation of 7-ethyluramil by the action of ethylamine on alloxantin, an observation which is as yet unknown in the hydrindene series. Table VII shows the relationships in the formation of murexide and the ammonium salt of diketohydrindylidene-diketohydrindamine. It shows that murexide can be formed by the action of ammonia, alanine (amino-acids), or ethylamine upon alloxan or alloxantin. If the analogy is a true one it is not surprising, then, to find that ammonia and ammonium salts, and organic bases of the type of ethylamine, in addition to amino-acids react with triketohydrindene hydrate to give the ninhydrin reaction.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Preparation</th>
<th>Formula</th>
<th>Compound</th>
<th>Preparation</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan</td>
<td></td>
<td>NH - CO</td>
<td>Triketohydrindene</td>
<td></td>
<td>C₆H₅CO C(OH)₂</td>
</tr>
<tr>
<td>Violuric acid</td>
<td>Hydroxylamine on alloxan.</td>
<td>CO C( OH)₂</td>
<td>Oximo 1, 3-diketohydrindene</td>
<td></td>
<td>C₆H₅CO C = NOH</td>
</tr>
<tr>
<td></td>
<td>Nitrous acid on barbituric acid.</td>
<td>NH - CO</td>
<td>Hydroxylamine on triketohydrindene hydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialuric acid</td>
<td>Reduction of alloxan by hydroiodic acid.</td>
<td>CO CHOH</td>
<td>Nitrous acid on 1,3-diketohydrindene.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH - CO</td>
<td>1,3-Diketohydrindol. *</td>
<td></td>
<td>C₆H₅CO CHOH</td>
</tr>
<tr>
<td>Uramil</td>
<td>Reduction of violuric acid. Action of ammonium chloride on hydrindantin.</td>
<td>NH - CO CO CHNH₂</td>
<td>1,3-Diketoethylhydrindamine.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH - CO</td>
<td>Reduction of oximo 1,3-diketoethylhydrindene.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxantin</td>
<td>Reduction of alloxan by HzS.</td>
<td>NH - CO CO - NH</td>
<td>Hydrindantin.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO CH - O - C(OH)CO</td>
<td>Reduction of triketohydrindene hydrate by HzS.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Ethyl-uramil</td>
<td>Action of ethylamine on alloxantin.</td>
<td>NH - CO CO CHNH₂</td>
<td>1,3-Diketoethylhydrindamine.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH - CO</td>
<td>Unknown.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE VII.  
The Relationship of Murexide and the Ammonium Salt of Diketohydrindylidene-diketohydrindamine.

<table>
<thead>
<tr>
<th>Preparation from</th>
<th>By the action of</th>
<th>Preparation from</th>
<th>By the action of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uramil.</td>
<td>I₂ or HgO.</td>
<td>1,3-Diketohydrindamine.</td>
<td>Air.</td>
</tr>
<tr>
<td></td>
<td>Alloxan in presence of ammonia.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Ethyluramil.</td>
<td>Alloxan.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The papers of Piloty and Finckh,⁹ Slimmer and Stieglitz,¹⁰ Möhlau,¹¹ and Möhlau and Litter¹² supply us with the clue to the ninhydrin reaction with amines. The similarity of the reaction with amines to that with ammonium salts in requiring the presence of large amounts of base or the presence of pyridine, again suggests that the production of phenylglyoxal-o-carboxylic acid represents the first stage in the mechanism

\[ \text{C}_6\text{H}_4\text{COCHO} + \text{H}_2\text{O} = \text{C}_6\text{H}_4\text{CO} + \text{COOH} \]

and that this acts as a reducing agent giving 1, 3-diketohydrindol or hydrindantin.

\[ C_8H_4(\overset{CO}{\overset{CH}{\overset{CO}{\overset{CHOH}{\text{ }}\text{)}}}} \text{C}_6H_4 \]

The action of ethylamine on alloxantin, as observed by Piloty and Finckh, Simmer and Stieglitz, and Mohlau and Litter, gives 7-ethyluramil, and there is no reason to suppose that the action of ethylamine on 1, 3-diketohydrindol or on hydrindantin would not produce 1, 3-diketoethylhydrindamine.

\[ C_8H_4(\overset{CO}{\overset{CH}{\overset{CO}{\overset{CHNHCH_3H_6}{\text{}}}}}) \text{C}_6H_4 \]

Just as 7-ethyluramil and alloxan in the presence of ammonia give the ammonium salt of purpuric acid (murexide) and ethyl alcohol

\[
\begin{align*}
\text{NH} - \text{CO} & \quad \text{CO} - \text{NH} \\
\text{CO} & + \text{CHNHCH}_3H_6 + C(OH)_2 \text{CO} = \text{H}_2O + C_2H_6\text{OH} + \\
\text{NH} - \text{CO} & \quad \text{CO} - \text{NH} \\
\text{NH} - \text{CO} & \quad \text{CO} - \text{NH} \\
\text{CO} & + \text{CH} - \text{N} = \text{C} \quad \text{CO} \rightarrow \text{CO} \\
\text{NH} - \text{CO} & \quad \text{CO} - \text{NH} \\
\text{NH} - \text{C(OH)}_2 \text{CO} & = \text{H}_2O + C_2H_6\text{OH} + \\
\text{NH} - \text{C(OH)}_2 \text{CO} & = \text{H}_2O + C_2H_6\text{OH} \\
\text{NH} - \text{C(OH)}_2 \text{CO} & = \text{H}_2O + C_2H_6\text{OH} \\
\end{align*}
\]

Purpuric acid. Murexide.

so it would be expected that 1, 3-diketoethylhydrindamine and triketohydrindene hydrate would undergo a similar condensation to give diketohydrindylidene-diketohydrindamine

\[
\begin{align*}
\text{C}_8H_4 & + \text{CHNHCH}_3H_6 + C(OH)_2 \text{C}_6H_4 = \text{H}_2O + C_2H_6\text{OH} \\
\text{C}_8H_4 & + \text{CH} - \text{N} = \text{C} \quad \text{C}_6H_4
\end{align*}
\]
which would give a blue salt with either an excess of ethylamine or pyridine. It is this latter reaction, *i.e.*, the removal of ethyl alcohol by condensation, which probably represents the constitutive part of the ninhydrin reaction with bases, as it is well known that condensations depending on the removal of an alcohol become more and more difficult as the molecular weight of the alcohol increases.

It must not be forgotten, however, that amino-acids themselves may also react in the presence of pyridine according to this mechanism, as well as by the mechanism expressed in the previous paper. Indeed, Piloty and Finckh claim to have isolated from the interaction of alloxan and glycine a compound possessing the constitution of uramiloacetic acid,

\[ \text{NH - CO} \]

\[ \text{CO} \quad \text{CHNHCH}_2\text{COOH} \]

\[ \text{NH - CO} \]

though Hurtley and Wooton\(^7\) take an entirely different view of the reaction.

This view of the mechanism of the ninhydrin reaction with amines also is not entirely satisfactory, as it does not explain why bases of the type of isopropylamine are unreactive, and there is still room for investigation on this part of the ninhydrin reaction. Lack of material at present forces us to postpone such an inquiry.

We are, however, strongly of the opinion that the production of ammonia from amino-acids and the production of the nitrogen atom marked with an asterisk (page 339) from amines is not brought about by hydrolysis, and are confirmed in this belief by the behavior of amides towards ninhydrin. This class of compounds are generally readily hydrolyzed to give ammonia, and yet they give only the faintest of ninhydrin reactions in the presence of pyridine. Alone, in a 1 per cent solution, only two of them showed any reaction, urea and allantoin, and these, though definite, are weak. The results are tabulated in Table VIII. Substituted amides, such as hippuric acid and glycocholic acid, cyclic imides, such as the various cyclic ureides, and the imino compounds, such as guanidine, creatine, and creatinine, do not give any reaction, either with or without the presence of pyridine.
The non-reactivity of the guanidine grouping is important from our standpoint as it means that the quantitative ninhydrin reaction worked out by us is confined to the α-amino group of arginine. The slight reactivity of pentamethylenediamine also leads us to the conclusion that the amino group in lysine will not introduce any serious error into our method.

**TABLE VIII.**

<table>
<thead>
<tr>
<th>Amide</th>
<th>1 per cent solution, without pyridine</th>
<th>1 cc. amide solution (0.05 mg. N). 1 cc. pyridine (10 per cent). 1 cc. ninhydrin (1 per cent).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formamide*</td>
<td>No reaction.</td>
<td>Very faint reaction.</td>
</tr>
<tr>
<td>Acetamide</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Oxamide</td>
<td>&quot; &quot;</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Gallamide trimethyl ether</td>
<td>&quot; &quot;</td>
<td>No reaction.</td>
</tr>
<tr>
<td>Urea**</td>
<td>Very faint reaction.</td>
<td>Reaction.</td>
</tr>
<tr>
<td>Biuret</td>
<td>No reaction.</td>
<td>Very faint reaction.</td>
</tr>
<tr>
<td>Urethane</td>
<td>&quot; &quot;</td>
<td>No reaction.</td>
</tr>
<tr>
<td>Allantoin*</td>
<td>Distinct reaction.</td>
<td>Reaction (0.003 mg. N).</td>
</tr>
<tr>
<td>Asparagine</td>
<td>&quot; &quot;</td>
<td>No reaction (α-amino only).</td>
</tr>
</tbody>
</table>

* Neuberg.⁸  
** Abderhalden and Schmidt.⁶

**CONCLUSIONS.**

It is evident from the results of this and the foregoing paper that the ninhydrin reaction is open to serious objections. The appearance of a positive blue color, even if proved spectroscopically to be the ammonium salt of diketohydrindylidene-diketohydrindamine, does not show definitely the presence of amino-acids. Thus, its application to saliva, urine, serum, etc., unless precautions are taken to remove ammonium salts and certain amines, can lead to totally erroneous conclusions. Of the three classes of substances which give a positive test, amino-acids are the most reactive, as they give a strong reaction when in a concentration of 1 cc. = 0.05 mg. nitrogen, whereas the two other classes fail to react in a concentration of 1 cc. = 0.1 mg. nitrogen, so that dilution of the liquid to be tested until its concentration of nitrogen is 0.1 mg. per cc., and the subsequent heating of 1 cc. of such a
liquid with 1 cc. of 1 per cent ninhydrin solution in a boiling water bath for a period of 20 minutes, can be used as means of overcoming these difficulties. Even here, however, the solution must be free from large amounts of phosphates as these would tend to act like pyridine and, giving a few hydroxyl ions, cause a positive reaction with ammonium salts and certain amines.

These same criticisms apply with equal force to the quantitative reaction discovered by us. It is only in the absence of large amounts of ammonium salts and bases that the method will yield accurate determinations of amino-acid α-nitrogen, conditions which are fulfilled in the hydrolysis of proteins by pancreatic enzymes.

The removal of ammonium salts and reactive amines, however, does not present insuperable difficulties, and it is hoped shortly to present a method of determining amino-acid α-nitrogen in physiological fluids.

A large part of the experimental work described in this paper was carried out in the Biochemical Laboratory of Cornell University, Ithaca, N. Y., during the summer of 1915, and we wish to thank Professor S. Simpson and Dr. J. B. Sumner for the facilities they extended and for the many kindnesses received at their hands. Thanks are also due to Messrs. Parke, Davis and Company for the specimen of pure adrenaline.

SUMMARY.

1. The ninhydrin reaction is given by organic bases of the type RCH₂NH₂, and R₂CHNH₂ where one radicle is negative in character.
2. Other bases which readily yield ammonia or are readily oxidized give the ninhydrin reaction.
3. With the fatty amines and in the presence of pyridine the strongest reaction is given by the simplest members.
4. Amides give no reaction with ninhydrin.
5. Guanidine and its derivatives give a negative test.

Harding and MacLean, J. Biol. Chem., 1916, xxiv, 503.
THE NINHYDRIN REACTION WITH AMINES AND AMIDES
Victor John Harding and Reginald M. MacLean


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