A COMPARATIVE STUDY OF PIPERAZINO-ACETIC ACID
AND ANALOGOUS COMPOUNDS, ESPECIALLY OF THEIR BEHAVIOUR ON OXIDATION.

A Thesis presented by Freda G. Clubb for the Ph.D. degree of the University of London,
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A COMPARATIVE STUDY OF PIPERAZINO-ACETIC ACID AND ANALAGOUS COMPOUNDS; ESPECIALLY OF THEIR BEHAVIOUR ON OXIDATION.

ABSTRACT OF THESIS

The view has been expressed that the copper salt of piperazino-acetic acid could exist in two isomeric forms, a blue and a green; the aim of the present investigation was to determine the nature of the isomerism.

A successful method for preparing the pure blue copper salt has been worked out and the physical and chemical properties of this and of the green solid have been studied; determinations of molecular weight and attempts to ascertain relative copper ion concentrations revealed no essential difference between the two substances in these respects.

Copper salts of related acids have been prepared in an attempt to determine the part of the molecule essential to the change.

The effect of modification of conditions of preparation of the green solid from the blue salt has been investigated. Attempts to prepare a green salt of constant composition failed, and attempts to determine its nature by treatment with p-toluene-sulphonyl chloride, phenyl isothiocyanate, picric acid etc., gave few positive results.

The general indication of the above work was that oxidation was taking place during the colour change; accordingly the action of various oxidising agents on piperazino-acetic acid was studied. Cold aqueous hydrogen peroxide was found to give a definite product which was isolated and investigated.
The action of oxidising agents on amino-acids and amines is discussed; it is shown that by the action of hydrogen peroxide on piperazino-acetic acid an amine-oxide is produced which can be converted into a secondary hydroxylamine, a change involving transference of oxygen within the molecule. An explanation is given of this change and of the part played by alcohol in effecting it.

Finally, it has been shown that the colour change in the copper salt is due to aerial oxidation and that the green solid contains the same secondary hydroxylamine.
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Abbreviations used in the Thesis

Biochem. Z. .... Biochemische Zeitschrift.
Compt. rend. ... Comptes rendus hebdomadaires des Séances de l'Académie des Sciences.
J.A.C.S. ....... Journal of the American Chemical Society.
J. Biol. Chem. .... Journal of Biological Chemistry.
J.C.S. ......... Journal of the Chemical Society.
The very dark blue copper salt of piperazino-acetic acid gives on dehydration a green salt which does not revert to the blue under any conditions tried, and in both the blue and the green solution some of the normal reactions for copper are absent" [Moore, Boyle and Thorn, J.C.S., 1929, p44.]

The anomalous behaviour of the copper salt of piperazino-acetic acid noted in this paper suggested itself as a problem to be investigated in detail. The salt had been prepared by Miss Thorn by treatment of the acid with copper hydroxide and subsequent evaporation; on dehydration either by warming the salt with alcohol or heating it alone at 110°C., a green gummy solid was obtained, which dissolved in water to give a green solution. The reverse change from the green to the blue salt could not be effected.

The analyses were not perfectly definite, but the percentage of copper found in the blue salt was approximately that required for the formula
and in the green salt for a formula with three molecules of water. Neither of the two solutions gave the normal reactions for the cupric ion; for example, they did not liberate iodine from potassium iodide, and when reduced with sulphur dioxide, and treated with ammonium thiocyanate, they gave no precipitate of cuprous thiocyanate. Moreover, with dilute acid curious colour changes took place, the blue solution becoming practically colourless and the green a reddish-brown colour. The molecular weights of the salts were found by depression of the freezing point, and though they were not accurate owing to the small quantities of material available, they were sufficient indication that the difference was not due to polymerisation. The fact that the salts retain their individual colour in solution, and react differently towards acid seemed to exclude the view that the change was merely one of hydration. It was suggested that the two salts were different stereo-isomeric forms, both showing internal complex formation, as they do not give the normal reactions; various possibilities of configuration were discussed.

Two main questions presented themselves, whether the isomerism was due to the presence of the -NH group in the piperazine ring, and whether the length of the acid chain
was an important factor; in short, which part of the mole-
cule was essential to the change. Accordingly it was
proposed to prepare the copper salts of piperidino-acetic
acid, benzoyl- and ethyl-piperazino-acetic acid, which
should determine the first point, and of piperazino-propionic
and butyric acids, which should determine the second. With
certain facts thus established, it should be possible to
gain more information as to the configuration by a detailed
study of the composition, the molecular weights, and the
chemical reactions of the two isomeric forms; determinations
of the concentrations of copper ions, by electromotive force
methods, and measurements of conductivity and transport
numbers were to follow, and it was thought that an explana-
tion of the isomeric change should then be possible. Finally,
it was considered that it would be of interest to know whether
other metallic salts of piperazino-acetic acid, such as the
iron, cobalt, nickel and zinc salts, exhibit the same property.

The first step was an attempt to prepare pure specimens
of the two salts which could be analysed completely. Here,
unexpected difficulties were confronted, which will be des-
cribed in detail in the next chapter, and the problem with
which the work was begun assumed, quite early in the inves-
tigation, a different aspect; the first indication that the
change was not a simple case of isomerism lay in the discovery
of the extreme susceptibility of the acid to oxidation by
metallic salts, and this fact opened up a new problem. The steps by which the investigation progressed from an attempt to define an apparently new type of isomerism to a study of the oxidation of piperazino-acetic acid and related compounds will be described in the following pages.

*** *** ***
The preparation and properties of the blue copper salt of piperazino-acetic acid.

(a) In general, there are three possible methods for preparing a soluble copper salt; namely, treatment of an aqueous solution of the acid with copper hydroxide, either in the cold, or with heating, treatment of a boiling solution of the acid with copper carbonate, and the addition of equivalent quantities of copper sulphate and baryta to a solution of the acid.

Each of these was used in the preliminary attempts to prepare the copper salt of piperazino-acetic acid; small experiments were carried out to compare the efficiency of the different methods, but it was found that in none of the concentrated solutions could crystallisation be induced, and when an analysis was carried out of the solids obtained by allowing the liquids to evaporate to dryness in the desiccator, it was found that in all the copper content was too low. The highest obtained was about 11% for the anhydrous salt, whilst a salt containing as much as eight molecules of water has 12.8% copper.

Part of the difficulty in the preparation of a pure copper salt is due to the fact that both the acid and the
salt are extremely soluble in water, and practically insoluble in every other solvent. Although very little of either dissolves in absolute alcohol, both are very soluble in 50% alcohol, and even 80 to 90% dissolves them to a considerable extent. This means that the method of preparation of the copper salt must be one in which the reaction can be made to go to completion, for separation from a large excess of acid is impossible. The solution has to be concentrated to a gummy consistency before crystallisation of the salt can begin, owing to the high solubility, and in this state it is difficult completely to separate the crystals from the mother liquor, and thus from the excess of acid remaining in solution. Moreover, attempts to precipitate the salt from the solution by adding alcohol must fail if there is much excess acid, since this is slightly less soluble in aqueous alcohol than the copper salt itself.

A closer study of the action of copper hydroxide with piperazino-acetic acid, and of the conditions with which a maximum copper content can be obtained, was therefore imperative. An experiment was carried out in which an aqueous solution of the acid was first shaken in the cold with freshly precipitated copper hydroxide, and finally heated for about an hour, but the salt obtained from this contained only 8% copper and was so deliquescent as to be practically impossible to deal with. An attempt was then made to observe
the progress of the reaction in the solution, by analysing portions, at hourly intervals, for copper and acid; the solution was boiled until the copper content was constant, at a value which did not correspond to that required for the normal salt. Again evaporation gave a gummy solid which could not be made to crystallise and was only hardened by repeated treatment with absolute alcohol. This experiment suggested that hydrolysis of the salt was taking place, in which case a solution containing copper and acid in the right proportions could never be obtained.

In order to determine whether or not this apparent hydrolysis was due to traces of alkali in the copper hydroxide or to impurities in the glass surface of the vessel which might inhibit the reaction, similar experiments were performed in a silica flask, with copper hydroxide prepared with special precautions. The results were, however, just the same, syrupy liquids, which would not crystallise, being obtained.

After a series of experiments in which the temperature of the solution in contact with the hydroxide was varied, it was concluded that heat was definitely unfavourable to the reaction; further, when the temperature was allowed to rise above 50°-60°C., the formation of cuprous oxide was noted and at the same time the solution assumed a bluish-green colour. Such solutions invariably became gummy on evaporation and
would not crystallise. It was found, too, that whatever the temperature the maximum copper content was not equal to that required for the salt. The value found in most cases agreed, within the limits of experimental error, with that found in the first experiment of this kind.

It was then shown that when a solution of piperazino-acetic acid is boiled with copper oxide, with consequent reduction, carbon dioxide is evolved and piperazine is found in the solution. This accounts for the failure of any method in which heat was applied to the solution, for the adverse effect of piperazine is two-fold; not only does it, as a base, prevent the solution from attaining the maximum copper content, but being impossible to remove accounts also for the deliquescent nature of the solids obtained. It does not account for the green colour of some of the solution, for mixtures of piperazine and acid with copper hydroxide never give a green solution.

In order to avoid the long heating and consequent oxidation in the copper oxide experiments, attempts were made to prepare the salt by mixing equivalent quantities of copper sulphate, baryta and piperazino-acetic acid in the cold. The solution after filtration of the barium sulphate was tested for \( \text{Ba}^{+2} \) and \( \text{SO}_4^{2-} \) and as none could be detected was evaporated in the cold. Crystallisation began spontaneously and the crystals were separated as well as possible
from the mother liquor. After drying to constant weight in a desiccator, the copper percentage was found to be only 10.3%, a much lower value than that of the anhydrous salt (18.1%). Parts of the same solution were heated and treated with alcohol respectively, and in neither case were crystalline products obtained. With alcohol, gums could be precipitated which hardened in air, but no real separation from the mother liquor was effected and the copper percentages varied considerably.

A repetition of the experiment, in which the whole of the solution was allowed to evaporate in the cold, gave the same crystalline salt which was found to contain 8.6% copper in the air-dried state, and 10.1% in the anhydrous state, and subsequently this salt was prepared many times. No attempts at seeding the solutions with specimens already obtained have been successful, but crystallisation has always occurred spontaneously.

The precipitate of barium sulphate which was formed in these experiments was always associated with a slight blue precipitate, presumably copper hydroxide from the hydrolysed salt. An estimation of the copper in this showed that the amount of hydrolysis corresponded to about one tenth of the acid used which would be left as excess in the solution, the copper percentage thus becoming approximately the same as the maximum found in the oxide preparations.
But as the salt which crystallised out contained a yet lower percentage of copper, the mother liquor must have had more copper in proportion to the acid than is required for the normal salt. That such was the case was eventually shown by a deposit of an insoluble copper salt from all the residues; analysis showed it to be a basic salt of the formula CuAg₂Cu(OH)₂ [where A represents the molecule of piperazino-acetic acid.]

The composition of the blue soluble salt showed that it must be an acid salt of the formula CuAg₂.2HA with six molecules of water in the air-dried state, which it loses over phosphoric oxide, or at 100°C. The preparation has been repeated many times and a salt of the same composition obtained, so that it must be a definite compound, though why it should crystallise out, in preference to the normal salt, is not at present known.

It was observed that a slow evaporation of the solution favoured the formation of the basic salt, but when that was formed before crystallisation of the acid salt had begun, the mixture was warmed and the basic salt dissolved and did not reappear on cooling. The fact that it was formed in dilute solution on standing suggested that this might be the precipitate associated with the barium sulphate. It seemed that it might be avoided if the baryta were added to a hot solution of copper sulphate and piperazino-
acetic acid, a method which would have the additional advantage that the sulphate precipitate would be filterable immediately, thus avoiding the long standing. A trial showed, however, that the precipitate was not the basic salt but copper hydroxide, and the effect of heat was merely to increase the amount of hydrolysis.

The results of the experiments with baryta and copper sulphate may be epitomized thus:—by this method, an acid salt of definite composition can be obtained by cold evaporation; further purification of this is impossible, any attempt at recrystallisation leading to the formation of insoluble salts and gummy solids. An insoluble basic salt is obtained from the mother liquors after standing. The normal salt cannot be obtained.

It was thought that since the acid salt is a definite compound it should crystallise from a solution prepared by shaking the acid with copper hydroxide, provided that it was not heated. For it is clear from the above experiments that the effect of heat is to prevent the formation of a crystalline salt. A solution of the acid was therefore shaken with copper hydroxide in the cold for two days, and the filtered solution evaporated in the cold. Crystallisation began, and when a reasonable quantity of salt had come out it was separated, dried and analysed. It was found to contain 12.8% copper and was thus apparently the same as
that originally prepared by Miss Thorn. In similar experiments, attempts were made to start the crystallisation by seeding, by cooling in ice, and to effect a purification by recrystallisation, or precipitation with alcohol. These attempts resulted in products containing less and less copper, and it soon became clear that any disturbance of the equilibrium led to salt and acid coming out together. The only way to prepare the salt is to leave the solution undisturbed until crystallisation has begun; while the liquid is still thin enough to be filterable, the crystals are separated by suction and dried carefully on porous plate. Even purification by washing is inadvisable, and as the salt has a definite composition it has been assumed to be pure.

When dried at 100°C. the salt loses water rapidly until the percentage of copper is 15.8%, corresponding to \( \text{CuAg}_2.3\text{H}_2\text{O} \), but further heating leads to a very slow loss in weight accompanied by a change to the green solid, and finally by decomposition.

The action of dilute acids on the aqueous solution of the blue salt has been studied in some detail; dilute hydrochloric acid causes the deep blue colour of the solution to disappear leaving a practically colourless liquid. Two equivalents of acid appear to be necessary for this change, and no further change is apparent when more is
added. When the colourless solution is treated with alcohol, a light green crystalline compound is obtained which dissolves in water to give a deep blue solution; concentration of the acid solution leads to a deposit of yellow crystals.

These facts can be explained on the assumption that by the addition of hydrochloric acid copper chloride and free piperazino-acetic acid are formed, and in the presence of excess acid the only colour in the solution is that of the cupric chloride, which is slight. Alcohol precipitates a mixture of copper chloride crystals and piperazino-acetic acid, and this with water gives a deep blue solution. The explanation was confirmed by dissolving equivalent quantities of copper chloride and the acid in water, producing a deep blue solution. Another equivalent of hydrochloric acid decolourised this, and alcohol precipitated the same light green crystals. Further, it was shown that concentration of copper chloride solution with excess hydrochloric acid gives yellow crystals.

With sulphuric and oxalic acids, similar changes are observed which can be explained in the same way. Caustic soda precipitates most of the copper as hydroxide, but ammonia produces no precipitate and no colour change. With other reagents, too, the blue salt behaves abnormally; iodine is not liberated from potassium iodide in neutral solution, although in acid solution the normal reaction
takes place; with potassium cyanide no precipitate is formed, but the solution becomes colourless; with potassium sulphocyanide and potassium ferricyanide no precipitate is formed.

Thus in its reactions in neutral solution, the blue copper salt gives evidence of very little free copper ion; the complex must, however, be broken up both by acid and by alkali.

It has already been mentioned that the effect of heat or warm alcohol on the blue salt is to change it to a green gummy substance; this hardens in a desiccator but is extremely deliquescent in air; it is very soluble in water, and insoluble in all other solvents. In this it resembles the blue salt, being only slightly less soluble in aqueous alcohol. With acids, the deep green solution changes to a reddish-brown colour which does not change further with more acid; the green colour is restored by alkali, but with excess very little precipitate of the hydroxide is formed, and the solution changes to a light straw colour. If the slight precipitate which is formed is filtered off, acid no longer restores the green colour to the solution, but changes it directly to the reddish-brown originally obtained with acid.

With other reagents, the green salt resembles the blue in its inactivity, but it appears to be a yet more
stable complex, since it does not give the reactions even in acid solution.

An attempt was made to determine the molecular weights of the blue and the green salts by depression of the freezing point of water. This could not be done with any accuracy owing to the rate at which both substances were hydrolysed in solution; the deposition of copper hydroxide introduces a large error into the weight on which the molecular weight is to be calculated, as well as the error due to the presence of free piperazino-acetic acid. Allowances for its effect were made in the calculation, and the results do indicate that the green substance is not a polymer of the blue. Both solutions gave a depression corresponding to molecular weights of the same order, ranging from 300 to 400.

In order to gain some information as to the copper ion concentration in the two solutions, attempts were made to determine the potentials with respect to a copper electrode. Unexpected difficulties were confronted at the outset, the chief being that of preparing a copper electrode which was stable to the solution and gave constant values for the electrode potential. It was found from the literature that satisfactory results had not been obtained for the potential of copper in copper salt solutions, some writers advocating a smooth adherent deposit, and others a spongy non-adherent deposit. The results have been summarised by
Getman [J. Phys. Chem., 1930, p. 1454] who concluded that a spongy deposit was the most reliable, while G. N. Lewis, and Riley have used a smooth deposit. In the present case it was found by trial that the most satisfactory electrode was prepared by a combination of the two, a smooth deposit being produced first and a more porous layer deposited on that, thus reducing the surface strains. The electrode was allowed to stand for some time in contact with a copper sulphate solution of the same strength as that eventually to be used in the electrode vessel, the solution itself was shaken with pure electrolytically deposited copper to ensure equilibrium between cuprous and cupric ions, and the electrode vessel was filled with nitrogen; in this way, a fairly constant potential was obtained. Fresh difficulties occurred, however, when copper piperazino-acetate was used in the electrode vessel; the hydrolysis of the salt caused a deposition of copper hydroxide on the electrode and therefore variations in the readings were found. Attempts were made to avoid this by adding to the solution small known quantities of the free acid, but even then hydrolysis occurred on standing and no satisfactory readings could be taken. From those which were obtained it could be inferred that in this respect there was no essential difference between the blue and green solutions. Thus the only information so far gained as to the nature of the blue and green salts, was that the green was a slightly more stable complex.
(b) Experimental details.

Piperazino-acetic acid was prepared by the method described in the paper already referred to [J.C.S., 1929, p.48]. The copper hydroxide used in the preliminary experiments was prepared by adding the equivalent of caustic soda to an approximately 0.5N solution of copper sulphate and the precipitate filtered and washed until free from alkali. In subsequent experiments, the caustic soda solution (approximately N.) was added to the sulphate slowly, with vigorous stirring; the precipitate was filtered, suspended in water and again subjected to vigorous stirring; this process was repeated five or six times until the precipitate gave no alkaline reaction to litmus, even when boiled with a little water.

To determine which method should be employed for the determination of copper in the salts, a series of comparative experiments was carried out in which different methods were used with the same specimen of salt; the methods were estimation as cuprous thiocyanate, as cuprous sulphide, as cupric oxide, and volumetric determination by thiosulphate of the iodine liberated from potassium iodide. In the last case, 0.1N thiosulphate was used and the conditions had to be modified accordingly; that is, the standard solution was stabilised by the addition of sodium carbonate,
and the titration carried out in an atmosphere of nitrogen. [See Moore and Young, J.C.S., 1932, p.2694].

Results.

Specimen I.

Copper estimated as Cu₂(CNS)₂ = 3.93%
" " Cu₂S = 3.95%
" " by iodide method = 3.66%

According to these results, there is not much difference between the thiocyanate and sulphide estimations, which were not in agreement with the determination by the iodide method. Later work did not confirm the first conclusion.

Specimen II.

Copper estimated as Cu₂(CNS)₂ = 14.7%
" " Cu₂S = 15.0%
" " CuO = 15.2%
" " by iodide method = 4.07%

Specimen III.

Copper estimated as Cu₂(CNS)₂ = 7.65%
" " Cu₂S = 15.1%
" " CuO = 15.1%
From this work the conclusion was drawn that estimation as oxide or sulphide were the only reliable methods, the other two being inaccurate owing to the complex nature of the salt, and as the ignition to oxide is a quicker method than the sulphide method, it was adopted. It is known to be a very accurate method of estimation, as long as no volatile compound of copper with nitrogen is formed, and in the case of copper piperazino-acetate, none could be detected.

Results of preliminary experiments on the preparation of the blue copper salt.

(1) Prepared from Cu(OH)₂, desiccator-dried,
    Copper = 11.7%.
(2) "   " CuCO₃, desiccator-dried,
    Copper = 6.24%.
(3) "   " CuSO₄ + Ba(OH)₂, desiccator-dried,
    Copper = 11.2%.

Results of analysis of the solution during the reaction.

Two equal volumes were pipetted from the solution: one was evaporated to dryness on the water bath, and ignited to copper oxide; the second was acidified with dilute hydrochloric acid, the copper precipitated as sulphide and the filtrate evaporated to dryness on the water bath, and the residue dried in a desiccator. It was found by trial that
there was a large source of error in this method as the hydrochloride loses some of its hydrochloric acid as well as moisture when dried in the desiccator. On the other hand, if a neutral solution is used, the copper is not completely precipitated. The estimation can therefore only be regarded as an approximate one.

(1) Wt. of CuO = 0.0338 gm. " acid hydrochloride = 0.3569 gm.
This represents an anhydrous salt containing 10.1% Cu.

(2) One hour later
Wt. of CuO = 0.0477 gm. " acid hydrochloride = 0.3565 gm.
13.8% Cu.

(3) One hour later
Wt. of CuO = 0.0605 gm. " acid hydrochloride = 0.4560 gm.
13.8% Cu.

Preparation of salt by baryta method
Equivalent quantities of copper sulphate and piperazino-acetic acid were dissolved in a little water, and the requisite
volume of standard baryta was added slowly with stirring. The precipitate of barium sulphate was allowed to settle, and then filtered off; the solution was treated in various ways the results of which are shown in the following table.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Evaporated on water-bath</th>
<th>Evaporated in cold, pptd. with alcohol</th>
<th>Evaporated on water-bath, pptd. with alcohol</th>
<th>Evaporated in the cold.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue gummy solid</td>
<td>Blue gummy solid</td>
<td>Blue gummy solid, hardened in air</td>
<td>Blue crystalline solid</td>
<td></td>
</tr>
<tr>
<td>(10.2% Cu)</td>
<td>(Not analysed)</td>
<td>(10.2% Cu)</td>
<td>(8.6% Cu).</td>
<td></td>
</tr>
<tr>
<td>No separation from mother liquor</td>
<td></td>
<td></td>
<td>[Acid salt.]</td>
<td></td>
</tr>
<tr>
<td>Blue solution</td>
<td></td>
<td></td>
<td>Blue insoluble salt</td>
<td></td>
</tr>
<tr>
<td>Evaporated in cold</td>
<td></td>
<td></td>
<td>23.3% Cu (air-dried)</td>
<td></td>
</tr>
<tr>
<td>Blue gummy solid</td>
<td></td>
<td></td>
<td>[Basic salt]</td>
<td></td>
</tr>
<tr>
<td>(dried in desiccator)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water added</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaporated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results of analysis of acid salt from baryta experiment.

<table>
<thead>
<tr>
<th>Wt. of air-dried salt</th>
<th>= 0.2211 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; CuO</td>
<td>= 0.0238 gm.</td>
</tr>
</tbody>
</table>

\[\text{Cu} = 8.60\%\]

\[\text{Loss of water at } 100^\circ\text{C.} = 15.8\%\]

\[\text{In anhydrous salt, Cu} = 10.1\%\]

\[\text{In salt dried over } \text{P}_2\text{O}_5, \text{Cu} = 9.95\%\]

\[\text{Calculated for } (\text{NH}_2\text{C}_4\text{H}_6\cdot\text{N} \cdot \text{CH}_2 \cdot \text{COO})_2\text{Cu} \cdot 2(\text{NH}_2\text{C}_4\text{H}_6\cdot \text{N} \cdot \text{CH}_2 \cdot \text{COOH}) + 6\text{H}_2\text{O} = 8.58\% \text{ Cu.}\]

Results of analysis of basic insoluble salt.

<table>
<thead>
<tr>
<th>Wt. of air-dried salt</th>
<th>= 0.1593</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; CuO</td>
<td>= 0.0464</td>
</tr>
</tbody>
</table>

\[\text{Cu} = 23.3\%\]

\[\text{In salt dried at } 100^\circ\text{C, Cu} = 28.0\%\]

| Wt. of salt dried at 100^\circ\text{C.} | = 0.1250 |

\[\text{Wt. of } \text{CO}_2 = 0.1472\]

| " H\text{H}_2\text{O} | = 0.0593 |

\[\text{C} = 32.1\%\]

\[\text{H} = 5.27\%\]

\[\text{Calculated for } \text{CuA}_2 \cdot \text{Cu(OH)}_2 \cdot 5\text{H}_2\text{O} \]

\[\text{Cu} = 28.4\%\]

\[\text{C} = 32.2\%\]

\[\text{H} = 5.56\%\]

\[\text{Cu} = 23.6\%\]
Preparation of pure blue salt.

The salt was prepared by shaking a solution of about 15 gms. of piperazino-acetic acid in 40-50 c.c.s water with freshly precipitated copper hydroxide for two days. The solution was filtered and evaporated in the cold over sulphuric acid until crystallisation occurred. The crystals were filtered from the syrupy mother liquor by suction and carefully dried on porous plate.

Wt. of air-dried salt = 0.1493 gm.
" CuO = 0.0239 gm.
Cu = 12.8%
Calculated for Cu(NH₂C₄H₈N.CH₂.COO)₂·6H₂O = 12.8%.
First rapid loss of water at 100°C. = 19.5%
In this compound, Cu = 15.9%
Calculated for loss of 5H₂O = 18.2%
" Cu(NH₂C₄H₈N.CH₂.COO)₂·3H₂O = 15.8%.

After this, loss in weight is very slow and is accompanied by a change to the green solid and finally by decomposition.
Results of molecular weight determinations

Blue salt

(1) Wt. of salt = 0.2453
   "  " H_2O = 19.3958
   Depression = 0.056  M.Wt. = 419.6

Corrected for hydrolysis  M.Wt. = 297.3

(2) Wt. of salt = 0.5296
   "  " H_2O = 26.8739
   Depression = 0.108  M.Wt. = 339.0

Green salt

(1) Wt. of salt = 0.6056
   "  " H_2O = 21.7748
   Depression = 0.122  M.Wt. = 423.8

(2) Wt. of salt = 0.1970
   "  " H_2O = 21.7748
   Depression = 0.042  M.Wt. = 400.3
**E.M.F. experiments.**

**Preparation of copper electrodes.**

A current of 0.5 amp./100 sq.cm. was passed through a solution of the composition

- 30 gm. CuSO$_4$·5H$_2$O
- 10 gm. H$_2$SO$_4$
- 50 gm. alcohol
- 200 gm. H$_2$O

to give the first smooth deposit.

A current of 0.5 amp./10 sq.cm. was passed through a concentrated solution of copper sulphate ($\approx 1.4N$) to give a spongy deposit.

**Electrode vessel.**

The vessel used was of the hydrogen electrode type and a stream of nitrogen was passed through when the solution was introduced into the vessel. The exclusion of air prevented to some extent the deterioration of the copper electrode.

**The copper salt solutions.**

The solutions of the blue and the green salts were shaken with electrolytically-deposited copper in order to establish equilibrium between the cuprous and cupric ions. As it was found that in the electrode vessels hydrolysis occurred causing deposition of copper hydroxide on the
electrode, small quantities of piperazino-acetic acid were added to the solutions. After standing the deposition and consequent variation of potential still occurred and no satisfactory results were obtained. The readings found indicated that there was no essential difference between the blue and the green solutions in the respect of their copper ion concentrations but no calculation could be made owing to the uncertain composition of the solutions.
CHAPTER 3.

The copper salts of related acids and other metallic salts of piperazino-acetic acid.

(a) A study of the behaviour of the copper salts of other acids under the action of heat seemed desirable; if it could be definitely established whether or not these salts assume another form under the conditions necessary for the transformation of the original salt, such knowledge would help to elucidate the nature of the change. A colour change could not be assumed to prove that the same molecular rearrangement was taking place, nor could its absence be considered as conclusive evidence that no change had occurred, but when an observation of the effect of the treatment on the colour of the salt was combined with a study of the effect on chemical properties, an opinion could be formed as to which cases were parallel to the copper salt of piperazino-acetic acid.

With the experiments described in the last chapter as a guide, heat was as far as possible avoided in the preparation of the salts. Piperazino-propionic acid had already been prepared [J.C.S., 1929, p.49] and an aqueous solution of this was treated with copper hydroxide in the cold; during evaporation, however, extensive hydrolysis took place, and as this acid has the same high solubility
as the lower homologue a pure salt could not be obtained. This result is in accordance with observations recorded by Ley [Ber., 1909, p.354.] that β-amino-acids do not easily form copper salts owing to extensive hydrolysis.

The impure gummy solid obtained by evaporation was converted into a green solid by heat or by warm alcohol; the green solution obtained from this showed the same colour changes with acid and alkali which have been found with copper piperazino-acetate, and was similar in its behaviour towards other reagents; thus it may be assumed that the corresponding change takes place, that the longer acid chain makes no difference.

The next salt to be prepared was the copper salt of piperidino-acetic acid; the acid was made by a method described by Kraut [Ann., 157, p.66] and the salt obtained by treatment with copper hydroxide. An analysis of the crystalline compound obtained showed it to be the same as that prepared by Ley [Ber., 1909, p.354]; its reactions were similar to the blue salt of piperazino-acetic acid, showing complex formation, but no corresponding change either of colour or properties was brought about by heating alone or with alcohol, implying that the second N atom in the ring is in some way concerned in the transformation.

This conclusion was borne out by the fact that the salts of acids of the formula X.N.C₄H₆.N.CH₂.CO₂H could
not be transformed, implying that not only the second nitrogen, but a free -NH group was essential to the change. For example, the copper salt of carbethoxy-piperazino-acetic acid, \([\text{COOEt.N.C}_4\text{H}_5.N.\text{CH}_2.\text{COO}]_2\text{Cu}\), a specimen of which had been prepared by Dr. Boyle, was stable under all conditions tried; on being heated, it did indeed assume a lighter purple shade, but this was due merely to dehydration, as with water the same blue solution was obtained, with the same reactions.

Again, the salt of p-toluene-sulphonyl-piperazino-acetic acid, prepared from the acid by adding an equivalent quantity of copper sulphate and caustic soda, was found to be perfectly stable to heat and alcohol.

It was realised, however, that the size and nature of the group attached to the second nitrogen atom might be a very important factor. A group such as the p-toluene-sulphonyl- is bound to change the character of the product considerably: that it does so is shown by the fact that both the acid and salt are practically insoluble in cold water. A smaller group such as the ethyl- might be expected to have less effect in this way, giving a compound which, while retaining certain characteristics of the original acid would yet have no -NH group. The preparation of the ethyl-piperazino-acetic acid presented great difficulty owing partly to the insolubility of the piperazino-acetic acid in
alcohol, which excluded methods for the direct ethylation of the acid by ethyl bromide or iodide, and partly to the difficulty of extracting ethyl-piperazine from aqueous solution, a disadvantage if the method chosen is to put the acetic acid group into the base. It was eventually prepared from ethyl-piperazine dihydrochloride without isolation of the base itself, an operation which entails considerable loss of material, by neutralising with sodium carbonate in a little water, evaporating the mixture to dryness over potash, and treating the residue, which consists of sodium chloride and ethyl-piperazine, with chloroacetic ester and sodium carbonate. An alternative method in which sodium alcoholate was substituted for sodium carbonate in the neutralisation was also used, but in each case poor yields were obtained at every stage of the process. The preparation of the copper salt offered no difficulty; copper hydroxide dissolved more readily than in the original acid, and the salt crystallised out at a greater dilution.

When the copper salt was heated alone, a curious change was observed; mixed with particles of the blue salt, particles of a brown substance could be distinguished, and these increased on further heating until the whole solid appeared brown. This gave a brown solution, while at the intermediate stage a green solution was obtained. With
ethyl alcohol, the blue salt formed a solution which changed colour very slowly, the alcohol evaporating before much change had occurred; with a higher boiling-point alcohol, such as butyl alcohol, the solution changed, through green, to brown.

It appeared from these preliminary observations that the transformation from blue to green, in the case of the ethyl derivative proceeds further than it does with copper piperazino-acetate; in the latter it seems that the change does not involve the whole of the blue salt, some of which is present in the green solid. That this is so was also suggested by its behaviour towards acid and alkali: alkali gives a slight precipitate of copper hydroxide, and acid restores the green colour, but if the small precipitate is removed, the green colour is not re-formed, indicating that the precipitate corresponds to the unchanged blue solid, and that the new product is not green but brown in solution [See Chap.2, p. 16.]. Before this could be stated as fact it was necessary to gain more information as to the conversion of the blue to the green salt, and the effect of modifying the conditions; it seemed possible that by some alteration in treatment, the transformation might be made to proceed further. These attempts to ascertain the composition of the green solid will be described in the next chapter.

The attempt to prepare salts of other metals with
piperazino-acetic acid met with little success; many metals were tried, but these experiments will not be described in detail, since few positive results accrued.

From two metals, namely nickel and mercury, some information was obtained. A pure nickel salt could not be prepared, mixtures of acid and salt invariably crystallising out or being precipitated together; this agrees with Ley's view that nickel salts of amino-acids are more difficult to prepare, because they are more easily hydrolysed than the copper salts [Ber., 1909, p.354]. The solid obtained was heated alone, and warmed with alcohol, but no change was apparent, and its reactions in solution were unaltered; when the nickel was precipitated as sulphide in the presence of dilute acetic acid, pure piperazino-acetic acid was obtained.

In the attempt to prepare the mercury salt, a solution of the acid was shaken with mercuric oxide; some mercury went into solution, but during evaporation mercury was deposited and both mercurous and mercuric ions were present in solution. This made a separation of a pure salt impossible, but it was of interest in connection with the work on the oxidation of piperazino-acetic acid which followed.
(b) Experimental details

Preparation of copper piperazino-propionate.

Copper hydroxide was prepared as before [p. 17] and was shaken with a solution of piperazino-propionic acid in the cold for 3-4 hours. The solution after filtration was evaporated over sulphuric acid, a gummy solid being obtained which could not be made to crystallise.

Copper in solid dried over $\text{H}_2\text{SO}_4 = 3.95\%$

Preparation of copper piperidino-acetate.

Piperidino-acetic acid was prepared by a method described by Kraut [Ann. 157, p.66]. A solution of the acid was boiled with copper hydroxide for an hour, the solution filtered and evaporated on the water bath. Portions were cooled from time to time to see if crystallisation occurred; when this was so, the solution was cooled and the crystals formed were filtered and recrystallised from water.
Results of analysis of copper piperidino-acetate.

Wt. of air-dried salt = 0.1339 gm.
" " Cu₂S = 0.0251 gm.

" Cu = 15.1%
Loss of water at 100°C. = 17.2%
In anhydrous salt, Cu = 18.2%

Calculated for \((\text{C}_5\text{H}_{10}\text{N.C}_2\text{H}_4\text{COO})_2\text{Cu.4H}_2\text{O}\) [Prepared by Ley]

Cu = 15.2%
Loss of 4H₂O = 17.2%
In anhydrous salt, Cu = 18.3%.

Preparation of p-toluene-sulphonyl-piperazino-acetic acid.

(1) An ethereal solution of p-toluene-sulphonyl chloride was added to an aqueous solution of piperazino-acetic acid; a slight excess of normal caustic soda was added, and the ether and aqueous solutions shaken together for about 24 hours. The solid formed was then filtered off, washed several times with ether to remove traces of sulphonyl chloride, and recrystallised from hot water. If more than a slight excess of alkali is used, the acid
has to be precipitated from the aqueous solution by acetic acid.

M.pt. of compound = 183°C.

(2) Mono-toluene-sulphonyl-piperazine was prepared [J.C.S., 1929, p.46], and boiled on the water bath, under reflux, with equivalent quantities of chloracetic ester and anhydrous sodium carbonate. The alcohol was evaporated, after evolution of carbon dioxide had ceased, and the solid crystalline mass was separated into sodium chloride and the ester C7H7SO2.N.C4H9.N.CH2.COOEt, by treatment with hot absolute alcohol. The ester was only sparingly soluble in ether; it melted at 110°C. [A mixed melting point of this with mono-toluene-sulphonyl-piperazine, m.p. 110-112°C., was 80°C.]. The ester was boiled under reflux with alcoholic potash for three or four hours and the alcohol evaporated; the residue was dissolved in water and on acidification with acetic acid, p-toluene-sulphonyl-piperazino-acetic acid was precipitated. Melting point = 183°C.

Results of analysis.

\[
\begin{align*}
\text{Wt. of anhydrous compound} & = 0.1633 \text{ gm.} \\
\text{Vol. of nitrogen} & = 13.1 \text{ c.c. at 16.5°C. and 751 mm.} \\
\% N_2 & = 9.2\% \\
\text{Calculated} & = 9.4\%
\end{align*}
\]
Results of analysis of copper salt.

Wt. of salt = 0.4109 gm.
" " Cu₂S = 0.0494 gm.
Cu = 9.6%

Calculated for \((\text{C}_7\text{H}_7\text{SO}_2\cdot\text{N}_4\text{H}_3\cdot\text{N}_6\text{CH}_2\cdot\text{COO})_2\cdot\text{Cu}\) = 9.67%.

Preparation of ethyl-piperazino-acetic acid.

(1) Ethyl-piperazine dihydrochloride was dissolved in a little water, and the equivalent of sodium carbonate added; when the evolution of carbon dioxide had ceased, the solution was evaporated in a vacuum desiccator, over potash. After two days, the residue, being a mixture of sodium chloride and ethyl piperazine, was treated with chloracetic ester and sodium carbonate in absolute alcohol, and boiled on the water bath until the action was finished. The alcohol was evaporated, the residue treated with ether, and the ether solution was dried over sodium sulphate and then distilled. The oil thus obtained was boiled under reflux with concentrated hydrochloric acid and the hydrochloride precipitated with alcohol. The analysis of the hydrochloride corresponded to \(\text{C}_2\text{H}_5\cdot\text{N}_4\text{H}_3\cdot\text{N}_6\text{CH}_2\cdot\text{COOH}.2\text{HCl}\). It was dehalogenised with silver carbonate, and after precipitation of silver, the solution was evaporated to a syrupy liquid which eventually crystallised. This was purified by
dissolving in alcohol and precipitating with acetone.
Melting point = 113.4°C. Yield = 25%.

Results of analysis

(1) Hydrochloride

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of salt</td>
<td>0.1905</td>
</tr>
<tr>
<td>AgCl</td>
<td>0.2197</td>
</tr>
</tbody>
</table>

\[ \text{Cl} = 28.6\% \]

Calculated for C$_2$H$_5$.N.C$_4$H$_8$.N.CH$_2$.COOH,2HCl

\[ \text{Cl} = 29.0\% \]

(2) Acid

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of acid</td>
<td>0.2378</td>
</tr>
<tr>
<td>Vol. of N$_2$</td>
<td>33.1 c.c. at 16.3°C and 766.6 mm.</td>
</tr>
</tbody>
</table>

\[ \text{N}_2 = 16.3\% \]

Calculated for C$_2$H$_5$.N.C$_4$H$_8$.N.CH$_2$.COOH

\[ \text{Nitrogen} = 16.3\% \]

(3) Copper salt

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of salt</td>
<td>0.2457</td>
</tr>
<tr>
<td>CuO</td>
<td>0.0364</td>
</tr>
</tbody>
</table>

\[ \text{Cu} = 11.8\% \]

Dried at 100°C, Cu = 15.4%

Calculated for (C$_2$H$_5$.N.C$_4$H$_8$.N.CH$_2$.COO)$_2$Cu.7H$_2$O

\[ \text{Cu} = 11.9\% \]

In anhydrous salt, Cu = 15.7%. 
(2) In another preparation, the dihydrochloride was treated with its equivalent of dry sodium alcoholate. Equivalent amounts of chloracetic ester and sodium carbonate were added, and the mixture boiled under reflux on the water bath until no more carbon dioxide was evolved. The oil obtained after evaporating the alcohol and extracting with ether was found to contain a large proportion of chloracetic ester which had not reacted, and a yield of about 10% of the ethyl ester was obtained, which was converted into the hydrochloride and thence into the acid.

(3) Later, a modification of the first method was used; equivalent amounts of the dihydrochloride of ethyl piperazine and sodium carbonate were mixed and 96% alcohol added. The moisture present was sufficient to cause reaction, and the alcohol solution of ethyl piperazine was dried over sodium sulphate, and treated with chloracetic ester and sodium carbonate as before. The yield was, however, no greater.
CHAPTER 4

The investigation of the green solid.

(a) The methods by which the blue copper salt of piperazino-acetic acid is transformed into the green deliquescent solid already described should themselves throw some light on the nature of the change. Hitherto the effect of varying the conditions had not been observed so that little could be deduced as to the mechanism. The present chapter describes the differences produced by variations of solvent and temperature and the attempts to ascertain the nature of the green solid formed.

A series of comparative experiments was carried out, using cold alcohol of different strengths; the blue copper salt was finely powdered and shaken with the various specimens of alcohol, and it was found that the presence of a little water made an extraordinary difference to the time taken for the colour change to occur; with absolute alcohol and the dried salt, three or four hours were sufficient, while with 96% alcohol there was only an indication of green colour after two days.

This does indeed suggest that dehydration is an essential part of the change, though from the properties of the green solution already described it is clear that it does
not constitute the whole of the change. That there is something beyond dehydration occurring is confirmed also by the fact that other dehydrating solvents do not promote the colour change, although they give a lighter, less hydrated, blue salt. Ethers, ketones, esters, benzene and chloroform are quite without effect; the only class of compounds which causes the transformation being that of the alcohols. Very little of the dried salt dissolves, but both the solution and the undissolved solid gradually assume a green appearance; the higher alcohols, butyl, amyl, isocamyl, dissolve the salt to a somewhat greater extent, but in the course of the change, some decomposition occurs, insoluble tarry products being formed. The process is always accelerated by heat, whatever solvent is used.

Remarkable results were obtained with benzyl alcohol and acetaldehyde. In both these, the blue salt dissolved to a large extent, and a colour change was produced, the solution becoming first green and then brown; in acetaldehyde solution the change took place in the cold. Attempts to use these methods for producing a pure specimen of the brown compound failed; a slight deposit of an insoluble brown substance was formed, even when the conditions were made as mild as possible, showing that decomposition had taken place. Just as with ethyl alcohol, the presence of a little water inhibited the change.
It was, of course, possible that both benzyl alcohol and acetaldehyde coupled up with the molecule in some way and that the colour change thus produced did not correspond to that produced by heat or by ethyl alcohol; but it was regarded as significant that with every solvent used, when a change from blue to green was observed in the copper salts of piperazino-acetic and propionic acids, no change occurred in the salts of piperidino-acetic and p-toluene-sulphonyl-piperazino-acetic acids [that is, in those which are not affected by heat or ethyl alcohol], although these dissolve easily in benzyl alcohol and acetaldehyde. Another possibility is that the initial change is the same and that the product couples up with the solvent, for it must be regarded as unlikely that such active agents as aldehydes and benzyl alcohol should remain unaffected by an -NH group. Experiments showed that a coupling did occur between piperazino-acetic acid and benzyl alcohol, or acetaldehyde, but the loose complex was broken up by dissolving in water and reprecipitating with alcohol.

Many other attempts were made to prepare a green salt of constant composition by the action of heat alone, or by warming with alcohol. The loss in weight found when the blue salt is heated at 110°C. does not correspond to the total dehydration of the salt, so that the solid obtained cannot be regarded as the pure anhydrous salt. If some
chemical change were taking place, there would be no reason to assume that the solid contained nothing but the new product. Therefore a method of purification had to be adopted, and this consisted of dissolving the gummy product in a little water, precipitating with alcohol, and repeating this process several times. Many specimens were obtained but the copper percentages were never in agreement, varying from 10% to 15%. The solids obtained by the alcohol method were no better, the only positive fact which emerged from the analytical results being that, on the whole, the green solid is less hydrated than the blue.

In addition, it was found that all the green solutions gave, on standing in the cold, or more rapidly with heat, a deposit of cuprous oxide. These facts indicated that the green gummy solid was not a pure salt, but a mixture which might contain cuprous or cupric salts of an oxidation product or of the original acid itself.

The formation of cuprous oxide on boiling was investigated more closely; each of the green solutions gave this deposit showing that oxidation had taken place, but whether the colour change was due to the oxidation or to some other cause was not certain. One fact which supported the theory that oxidation was the cause, was that only those salts change colour, the acids of which are oxidised by simple metallic oxidising agents. It was found that no carbon dioxide was
evolved during the change to green, and the solution did not contain piperazine, so that some other oxidation product was the result of the reduction of the copper in the salt to cuprous oxide. The amount of cuprous oxide was determined in two specimens of the solid; an aqueous solution was boiled until no further deposit was obtained, and it was found that approximately one third of the total copper was present as cuprous; if this represents the full extent of the oxidation, that is, if all the cuprous copper were precipitated by boiling, only about one twelfth of the acid used could be oxidised. That this was so was proved by the use of an indicator described by Kolthoff [J.A.C.S., 1930, p.2222]. p-Dimethyl-amine-benzylidene-rhodamine provides a sensitive test for the cuprous ion and it was found that while none of the blue copper salts contained cuprous copper, all the green solutions did, but after precipitation by boiling, no further formation was indicated.

If the copper is precipitated from the green solution by sulphuretted hydrogen, and the filtrate evaporated a brown gummy solid is obtained which hardens in a desiccator but immediately deliquesces on exposure to air. It must therefore contain something besides the original acid, and as the attempts to obtain a pure specimen of the green salt had failed, it was thought that an investigation of this product obtained from it should be carried out.
This product, which will be referred to as A, did not become entirely crystalline even on long standing with alcohol; an aqueous solution still gave a green solution with copper hydroxide; repeated treatment with water and alcohol, alternate dissolving and precipitation, gave a precipitate which became more and more like the original acid, and a solution of the gummy part, until finally a pure specimen of the original acid was obtained, giving a blue solution with copper, and a gummy solid, still containing some original acid, which gave a deep green solution with copper. These facts, together with the formation of cuprous oxide, prove that the colour change from blue to green is not a simple case of isomerism, but that some new compound, presumably an oxidation product, is formed and in the following experiments attempts were made to identify it.

Owing to the extremely deliquescent nature of A, the only possible line of attack was to prepare derivatives. Accordingly, it was treated with p-toluene-sulphonyl chloride under the usual conditions; a little solid was formed which was washed with ether, and recrystallised from hot water. A melting point and mixed melting point determination proved that it was the derivative of the original acid 

\[ \text{C}_7\text{H}_7\text{.SO}_2\text{.N.C}_4\text{H}_8\text{.N.CH}_2\text{.COOH, m.pt. 183}^\circ\text{C.}] \]. The solution was shaken with ether to remove traces of the sulphonyl chloride and then acidified and evaporated; a little more
of the acid was obtained and traces of di-toluene-sulphonyl-
piperazine (M.pt. 296°C.) but no other compound could be
isolated. Several specimens of A were treated in this way,
but in most cases the solid which separated contained only
the two known compounds mentioned above; in one case a
sodium salt was obtained from the alkaline solution which
was soluble in chloroform, and therefore a larger experiment
was performed with the view of preparing a larger quantity
of the sodium salt. In this case A was prepared by precipi-
titating the copper in neutral solution, boiling the fil-
trate just to expel the HgS, and the solution was treated
with the acid chloride without evaporation. A little
di-toluene-sulphonyl-piperazine separated and then a mixture
of sodium salts was obtained by evaporation to dryness.
This was treated successively with chloroform, cold and hot
alcohol and the result is shown in the following Table.
Mixture of sodium salts

- Chloroform solution evaporated
  - Solid
    - Chloroform
      - Slight residue of NaCl
      - Gummy substance which hardens
        - alcohol
          - Solution
            - NaCl
              - Na salt (10.95% Na)
            - Residue NaCl

- Cold alcohol solution evaporated
  - Solid
    - Chloroform
      - Gummy substance

- Hot alcohol
  - Solid
    - Chloroform
      - Insoluble part II

Insoluble in the three solvents (NaCl)

The solids I and II from the cold and hot alcohol solutions were dissolved in dilute hydrochloric.

Solution

Evaporated alcohol added

NaCl

Solution

Na-toluene-sulphonate + a little p-toluene-sulphonic acid.
The sodium salt which was analysed and found to contain 10.95% sodium had the same solubilities as the small specimen obtained in a previous experiment, being soluble in chloroform and alcohol and insoluble in acetone. With acid, however, it was not decomposed and it was found by comparison that sodium toluene sulphonate, which contains 10.8% sodium, behaves in this way also. Therefore it was concluded that the salt obtained was sodium toluene sulphonate.

Small experiments were performed on the copper salts themselves; the blue salt gave a purple salt, insoluble in water and other common solvents, which was identical with the copper salt obtained from p-toluene-sulphonyl-piperazine-acetic acid; the green salt gave a precipitate which appeared to be a mixture of the purple salt, copper hydroxide, and di-toluene-sulphonyl-piperazine. Nothing could be extracted from the residue which retained its green colour, indicating that the compound which is the essential part of the green salt was unaffected by the acid chloride.

Another reagent used was phenyl isothiocyanate; this has an advantage over the isocyanate in that it is not so easily decomposed by water, and can therefore be used in aqueous solution without fear of complications in the form of decomposition products; in the present case this is a necessary property; as one of the reactants (A) is a gum,
an exact equivalent of the isothiocyanate cannot be added, and as A is only soluble in water, an aqueous solution has to be used.

The phenyl thioureas of the original acid and of piperazine were prepared for comparative purposes; the most convenient method was to add a few drops of the isothiocyanate to an approximately equivalent weight of acid in water, and then to add alcohol, insufficient to precipitate the acid, but sufficient to dissolve some of the isothiocyanate. The mixture was warmed on the water bath until the precipitate appeared.

Specimens of A were treated in this way, and compounds obtained which were shown to be the derivative of the original acid. Evaporation and cooling led to further small quantities of the same compound and in no case was any new derivative obtained.

As these efforts to prepare derivatives were fruitless, it was decided to attempt a separation by forming insoluble salts, and picric acid was the first acid used. A disadvantage is that it is so sparingly soluble in water, and yet an aqueous solution has to be used since the compounds involved are insoluble in everything else; the result is, of course, that the solutions become inconveniently dilute. An insoluble picrate of the original acid was prepared, however, although the yield was less than theoretical by 20-30%. The product A
gave a precipitate which appeared to consist largely of the piperazino-acetic acid picrate, and no separation into constituent parts could be effected. A picrate was obtained from A by first treating it exhaustively with phenyl isothiocyanate, that is until no more precipitate of a phenyl thio urea was obtained, and then, after washing the residue with ether and alcohol, a solution of picric acid was added; a golden-brown precipitate of a picrate was obtained which was carefully recrystallised from water, to remove piperazine picrate, and then from alcohol. A micro-analysis showed that this was impure, and it was thought that it might have contained a little sulphur from the decomposition of the isothiocyanate; no conclusions could be drawn, therefore, from the analytical results, as to the formula of the product.

Although the experiments which have been described with p-toluene-sulphonyl chloride, phenyl isothiocyanate, and picric acid, yielded no information as to the composition of the product contained in the green salt, certain conclusions can be drawn as to its nature. One possibility is that the new product is present in very small quantity, the bulk of A being the original acid, and that this small quantity cannot form N-derivatives; an alternative is that the product is originally present in considerable quantity, but that it is an unstable one which easily reverts to piperazino-acetic acid. In either case it must be concluded that it cannot
form N-derivatives, that is, has no -NH group. The fact that 
cuprous oxide is formed shows that some oxidation product must 
be present and though it had not been proved at this stage 
that the colour change is due to the oxidation, it seemed 
reasonable to suppose that the gummy part of the product A 
was the oxidation product.

It was thought that as this new compound appeared to 
be so unstable, some decomposition product might be obtained 
from it by distillation, which would throw light on its struc­
ture. Accordingly a solution of the green solid was dis­
tilled with dilute sulphuric acid, and the distillate col­
lected; it was found to be faintly acid to litmus, strongly 
reducing with ammoniacal silver nitrate solution, and it 
gave an indication of a phenyl hydrazone, although there was 
not sufficient to collect and examine. These properties 
indicated the presence of an aldehydic acid, which must be 
volatile in steam, and glyoxylic acid seemed a possible one; 
the characteristic reaction of glyoxylic acid, the precipita­
tion of the calcium salt with excess of lime water, was not 
obtained, but it was thought that the solution might be too 
dilute. The residue was found to contain a little piper­
azine, but subsequent experiments showed that the actual 
amount of piperazine was not equivalent to the reducing sub­
stance distilled. The latter distilled over very slowly 
and an end-point was never attained, the reducing action being
still present when as much as a litre of distillate had been collected; it was found to give the colour reactions for formaldehyde, and none of the glyoxylic acid reactions. The quantities obtained were too small and the solutions too dilute to be of much practical use, but the experiments were considered to have established the fact that an oxidation product was present, and attention was therefore turned to the action of various other oxidising agents in the hope of finding the nature of the product.
(b) Experimental details.

Conversion of blue salt to green by:

(1) **cold alcohol.**

Some of the salt was dried over $\text{P}_2\text{O}_5$.

**Strength of alcohol**

<table>
<thead>
<tr>
<th>Dried powdered blue salt with 100% alcohol.</th>
<th>Time taken for change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrated salt with 100% &quot; 100% &quot; (3 c.c.) + 1 drop H$_2$O</td>
<td>3 to 4 hours</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 96% alcohol.</td>
<td>24 hours</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 90% &quot;</td>
<td>Indication of green after 48 hours.</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 80% &quot;</td>
<td>Indication of green after 60 hours.</td>
</tr>
</tbody>
</table>

(2) **warm alcohol.**

The dried, powdered blue salt was treated with alcohol in the cold, and filtered immediately; the filtrate and residue were then treated as shown in the following table:
(3) heat.

The blue salt was heated alone at 110°C.; after the first loss of water, the change proceeded very slowly, and the total loss of weight did not correspond to the anhydrous...
salt. The solid was dissolved in water and the solution was treated as shown in the table:-

Solution

- bluish-green solution
  - evaporated in cold
    - bluish-green solid
      - heat
        - green solid
          - water, then alcohol
            - green gummy solid
              - treatment with water and alcohol repeated six times
                - green gummy solid (15% Cu)

- green gummy solid (12% Cu)

- green solution containing excess acid.

- green solid
  - water, then alcohol
    - green gummy solid (15% Cu)
Results of analysis of green salts.

(1) Solid obtained by warm alcohol on blue salt

<table>
<thead>
<tr>
<th>Wt. of salt</th>
<th>CuO</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4991 gm.</td>
<td>0.0910 gm.</td>
<td>14.6%</td>
</tr>
</tbody>
</table>

(2) Solid obtained by heat.

<table>
<thead>
<tr>
<th>Wt. of salt</th>
<th>CuO</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3636 gm.</td>
<td>0.0691 gm.</td>
<td>15.2%</td>
</tr>
</tbody>
</table>

(3) Solid obtained by heat.

<table>
<thead>
<tr>
<th>Wt. of salt</th>
<th>CuO</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1036 gm.</td>
<td>0.0157 gm.</td>
<td>12.1%</td>
</tr>
</tbody>
</table>

Determination of Cu₂O in green salts.

(1) Wt. of CuO from cuprous copper = 0.0062 gm.

<table>
<thead>
<tr>
<th>Wt. of CuO</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0062 gm.</td>
<td>0.0224 gm.</td>
</tr>
</tbody>
</table>

\[ \cdot \cdot \cdot \% \text{ of total as cuprous} = 36.2\% \]

(2) Wt. of CuO from cuprous copper = 0.0077 gm.

<table>
<thead>
<tr>
<th>Wt. of CuO</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0077 gm.</td>
<td>0.0262 gm.</td>
</tr>
</tbody>
</table>

\[ \cdot \cdot \cdot \% \text{ of total as cuprous} = 34.0\% \]
Results of analysis of sodium salt in experiment with p-toluene-sulphonyl chloride. [p. 49.]

Wt. of salt = 0.1884 gm.
" " Na₂SO₄ = 0.0637 gm.
.. Na = 10.9%

Calculated for sodium toluene-sulphonate
= 10.8%

Preparation of phenyl thioureas.

An approximately equivalent weight of phenyl isothiocyanate was added to an aqueous solution of piperazine-acetic acid; alcohol to about 40% was added and the mixture warmed on the water bath until a precipitate appeared. This was filtered, recrystallised from hot water and analysed.

Wt. of acid = 0.1573 gm.
Vol. of N₂ = 20.1 c.c. at 11.9°C. and 756.7 mm.
.. N₂ = 15.0%

Calculated for C₆H₅.NH.CS.N.C₄H₈.N.CH₂.COOH
= 15.1%.

M.pt. of C₆H₅.NH.CS.N.C₄H₈.N.CH₂.COOH = 223°C.
The piperazine compound was prepared in the same way but could not be recrystallised as it is insoluble in all the common solvents. It was purified by boiling with water.

M.Pt. of \( \text{C}_6\text{H}_5\cdot\text{NH.CG.SN.C}_4\text{H}_6\cdot\text{N.CG.SN.C}_6\text{H}_5 \) = 274°C.

Preparation of picrates.

A saturated aqueous solution of picric acid was added to an aqueous solution of piperazino-acetic acid. A precipitate was formed which was filtered and recrystallised from water and then from alcohol.

M.Pt. = 232°C.

An alcohol solution of picric acid was added to an alcohol solution of piperazine. The precipitate formed was easily soluble in water, soluble in hot alcohol and insoluble in cold alcohol. This compound had been prepared before [Sieber. Ber., 1890, p.326].

Results of micro-analysis of picrate of A after treatment with phenyl isothiocyanate.

(1) Wt. of salt = 3.694 mgm.

" " \( \text{CO}_2 \) = 5.320 mgm.

" " \( \text{H}_2\text{O} \) = 1.474 mgm.

" " ash = 0.018 mgm.
(2) Wt. of salt = 2.362 mgm.
Vol. of N₂ = 0.403 c.c. at 20°C. and 759 mm.

\[ \text{Ratio is } \frac{C}{N} = 2.36, \quad \frac{H}{O} = 3.14, \quad \frac{O}{H} = 1.65 \]

No satisfactory formula could be obtained from these results.

Tests used for formaldehyde.

(1) A solution of phenyl hydrazine hydrochloride and a freshly prepared solution of potassium ferricyanide added to a solution containing formaldehyde give on acidification with concentrated hydrochloric acid a deep magenta colour.

(2) A solution of phenyl hydrazine hydrochloride and of sodium nitroprusside with a solution containing formaldehyde give on addition of caustic soda a prussian blue colour.

Test for glyoxylic acid

With excess of lime water, a precipitate is formed which, on warming, immediately decomposes into calcium glycollate and calcium oxalate and the latter is insoluble in acetic acid, and can be detected.
The result of the work described in the last chapter was the conclusion that a study of the oxidation of the acid would assist in the solution of the problem under investigation; it was hoped to find some agent which would be comparable in its action to the copper in the copper salt transformation, and with this end in view, a series of oxidising agents were used and their action examined.

Quite early in the work it had been discovered, accidentally in the first instance, that piperazino-acetic acid was remarkably susceptible to oxidation by metallic compounds. It was known that many amino-acids form double compounds with mercuric chloride, which are often insoluble in water; during the experimental work on the preparation of the copper salt, it was thought that if an insoluble compound of the acid could be made, this might afford a means of removing the excess acid from the solution. Accordingly, mercuric chloride was added to a solution of the acid, and a white precipitate was obtained; it was found to be soluble in hot water, but during the boiling an insoluble deposit was formed which proved to be calomel,
showing that some oxidation of the acid must have occurred. Preliminary experiments were then carried out with related compounds, and it was found that although precipitates were formed in every case only those with the -CH₂COO chain in the molecule gave deposits of calomel; thus while the compounds of piperazine and derivatives such as mono- and ditoluene-sulphonyl-, and mono-carbethoxy-piperazine were unaltered by boiling, those of piperazino-propionic acid and carbethoxy-piperazino-acetic ester were extensively oxidised.

When it became clear that oxidation was taking place in the copper salt change, these experiments were repeated on a larger scale and attempts were made to isolate the product; these have not been successful, owing probably to the inconvenient solubilities of the substances involved and the instability of the product itself.

During the boiling of the double salt with water, no carbon dioxide was evolved, and as far as could be tested, none during the subsequent evaporation; the formation of calomel became very much slower after the first ten to fifteen minutes, although the weight did not then correspond to the oxidation of all the acid; it was thought that this might be due to the hydrochloric acid liberated being unfavourable to the reaction. When the oxidation appeared to be over, an attempt was made to isolate the product by adding
more mercuric chloride to precipitate the double salts; after removing the mercury from these, and dehalogenising, only original acid could be detected. The calomel contained a little organic matter, but when this was suspended in boiling dilute hydrochloric acid, and H₂S passed in, a little of the original acid was obtained and nothing else could be isolated. In a subsequent experiment, a trace of a new compound melting at 127-8°C. was found, but it could not be prepared in quantities large enough for further examination; in fact it was not always obtained.

Similar experiments were performed with carbethoxy-piperazino-acetic ester, and after precipitation of mercury, a deliquescent hydrochloride was obtained which was analysed for chlorine and nitrogen, but the results showed that it was not pure; in another experiment, the solution was dehalogenised and evaporated in the cold, giving a gummy substance which was hardened by repeated treatment with alcohol and ether, but was extremely deliquescent in air. An attempt to prepare the same hydrochloride from this failed, and this indicated that the product is affected by evaporating with acid and some decomposition product formed; in all subsequent experiments, therefore, long heating either in acid or neutral solution was avoided, but no more conclusive results were obtained.

The chlorine and nitrogen percentages in the hydrochloride
suggested that the product might be the compound \( \text{NH}_4\text{C}_4\text{H}_8\text{N.CO.COOCH}_2\text{H}_5\text{HCl} \), contaminated with a little of the original carbethoxy ester. \([\text{Found Cl 16.2\%}, \text{N 11.5\%}]. \text{Calculated Cl 16.0\%, N 12.6\%}]\). It was recognised however that it was extremely unlikely that the ester group would remain when the carbethoxy group had been lost, as it is known that the latter is more difficult to hydrolyse with acid; such a compound should easily decompose into piperazine and oxalic acid, but in no case could oxalic acid be found.

It was clear that no positive result was forthcoming from the experiments with mercuric chloride; other mercuric salts were substituted, but although oxidation again took place, no product could be isolated. Mercuric oxide was found to behave differently; a rapid oxidation occurred, carbon dioxide being evolved and piperazine formed. This is in accordance with the fact recorded in Chapter 3, that a mercuric salt could not be prepared owing to extensive reduction to mercurous oxide even in the cold. It was apparent that this was a case of an oxidation of the side chain; no intermediate product could be isolated, even when the conditions of oxidation were modified, and the reaction seemed analogous to that of the formation of cuprous oxide, carbon dioxide and piperazine in the attempts to prepare the copper salt of piperazino-acetic acid by
boiling with copper oxide. In this connection it may be noted that in the preparation of the original acid, a little piperazine was formed by oxidation by silver oxide; the amount was minimised by carrying out the dehalogenisation at as low a temperature as possible.

The progress made in these experiments as regards the nature of the oxidation product was small, but they indicated that a further study of the oxidation was important; the facts established as to the oxidation or non-oxidation of related compounds made it not improbable that the colour change of the copper salt was connected with the oxidation of the acid; if not directly due to that, then due to the same property which makes oxidation possible. It should be borne in mind that the primary aim in the experiments which followed was to find an agent which, besides being similar in action to the copper in the transformation of the blue salt, should allow of isolation of the products. Accordingly, when this proved impossible, the agent was discarded even though the nature of the action had not been established. The means chosen for investigating the latter was in general that of distilling the solution with acid, in order to determine the volatile products present and to compare them with those obtained from the distillation of the green salt. [See pp. 52-53.]

Preliminary experiments were carried out by Professor T.S. Moore, on the action of permanganate and bromine water
on the acid. He found that while piperazino-acetic acid undergoes rapid oxidation with both neutral and acid permanganate, the toluene-sulphonyl derivative and piperazine itself are oxidised only slowly, and piperidino-acetic acid requires more than twenty-four hours for the reduction of one equivalent. It was found that after oxidation, the solution of the acid had a strong reducing action with ammoniacal silver nitrate, and that this reducing substance distilled over on heating with acid, and the residue contained very little piperazine. The action of bromine water appeared to be quite different; in acid solution the action was much slower, and distillation yielded a volatile reducing substance and a good test for piperazine in the residue. The evaporation of the distillates gave minute residues, but the desiccators smelt strongly of formaldehyde.

A small experiment was also performed with lead tetracetate; a reducing substance was again obtained by distillation, and the solution was found to contain very little piperazine.

Further experiments were then carried out with the various agents, in a roughly quantitative way. One equivalent of the agent was used in each case, and both distillate and residual solution were examined with a view to finding the proportions in which the various products were present. No essential difference was observed in the nature of the distillates, all of which gave the formaldehyde reactions,
but in the case of the bromine oxidation, the reducing substance came over more rapidly, a definite end point being reached; all were slightly acidic, all gave on evaporation with ammonia, a white solid which formed an insoluble lead salt, and all thus appeared to be identical with the distillate from the green salt. When they were neutralised with alkali and distilled again, formaldehyde came over, and the residue could not be identified owing to the large quantities of sodium salts present. The residual solutions were different; in the bromine oxidation, treatment with benzoyl chloride gave a quantitative yield of dibenzoyl piperazine showing that the oxidation is a straightforward one of the side chain; only a trace of piperazine was found in the permanganate and lead tetracetate experiments.

The view was still held that the acid portion of the distillate might be glyoxylic acid, and therefore several modifications of the experimental methods were made with the sole end of establishing this point. A practical difficulty hitherto had been the inevitable dilution of the solution both with bromine water and permanganate; a solution of bromine in chloroform was used, of much higher concentration than the aqueous solution, but even so no tests for either glyoxylic acid, or its decomposition product, oxalic acid, could be obtained from the solution.
Another attempt consisted of neutralising the solution with lime water immediately after oxidation, and distilling; methyl alcohol came over, formic acid was found in the residue, showing that formaldehyde, which in this case underwent a Cannizzaro reaction, must be one product of oxidation; no test for glyoxylic or oxalic acids could be obtained, and it was therefore concluded that glyoxylic acid was not an intermediate product in the oxidation of piperazino-acetic acid.

In the permanganate experiments a great practical difficulty in the isolation of the products is the presence of potassium salts, and it was therefore suggested that the substitution of barium permanganate would be an advantage; if the manganese were precipitated by baryta, and the barium by sulphuric acid, a solution containing only the products would be obtained. It was found, however, that the large bulk of the solution arising from the necessity of adding an excess of baryta to precipitate the manganese completely made this method useless.

In the oxidation by lead tetracetate in concentrated solution, formaldehyde was evolved immediately; the lead was precipitated in the presence of a stream of nitrogen, and on evaporation a syrupy liquid which could not be hardened or made to crystallise, and no derivatives could be obtained.

It was then thought that, from the point of view of
isolating a product, hydrogen peroxide would be preferable to many other agents, for an aqueous solution containing only the products would be thus obtained. In the early experiments, an aqueous solution of piperazino-acetic acid was warmed for a short time with hydrogen peroxide; the liquid became straw-coloured and was found to give a deep green solution with copper hydroxide. In another experiment on a larger scale the solution was evaporated to dryness and a gummy solid was obtained which could not be made to crystallise; a little dibenzoyl piperazine was formed with benzoyl chloride, but the amount did not correspond to the total solid; this was found to have aldehydic properties, reducing ammoniacal silver nitrate, giving a pink colour with Schiff's reagent and an opaqueness with phenyl hydrazine, indicating the formation of a phenyl hydrazone. The next attempt was to carry out the oxidation as quantitatively as possible, and accordingly the hydrogen peroxide solution was standardised and titrated into the acid, the end point being determined by a starch-iodide solution as an external indicator. When the reaction in the cold appeared to be over (after only a few c.c.s had been added) the solution was warmed and a little ferrous sulphate (0.01 gm.) was added to catalyse the reaction; a definite end point was not reached but the point at which the slowing-down was very marked was taken as an indication that the first step was
complete; very little piperazine was found in the solution, which was strongly reducing, and no glyoxylic or oxalic acids could be detected. Nothing could be isolated from the solid, after evaporation, except piperazine and the original acid.

In another experiment equivalent quantities of hydrogen peroxide and piperazino-acetic acid were mixed in the cold and allowed to stand. Tests were made from time to time and it was found that at the end of two days all the hydrogen peroxide had disappeared; part of the solution was then treated with phenyl isothiocyanate and a new phenyl thiourea \( \text{m.pt.} = 186^\circ \text{C.}, \text{with decomposition} \) was precipitated and none of the thiourea of the original acid obtained. The solution was then evaporated in the cold, and a solid product obtained by precipitation with alcohol \( \text{m.pt.} = 135-6^\circ \text{C.} \). Analyses of this product and of the thiourea showed it to contain one atom of oxygen more than the original acid.

The solution was found to have a very slight reducing action, and when distilled the merest trace of a reducing substance came over; distillation with acid yielded appreciably more of the reducing product, but still insufficient to give a phenyl hydrazone. No carbon dioxide was evolved during the experiment, and only a trace of piperazine was found in solution. With copper hydroxide
a deep blue solution was obtained. When the product was warmed with acid, either hydrochloric, sulphuric or acetic acids, for some time, the solution darkened in colour, and when neutralised gave a deep green colour with copper hydroxide; the green solution underwent the same colour changes with acid and alkali as that obtained from copper piperazino-acetate. It seemed therefore that some change had occurred in the nature of the oxidation product, which might possibly correspond to the change in the copper salt; accordingly experiments were performed to find the mildest conditions under which this change could be effected. As a result it was found that by heating the product at 100°C., warming with alcohol, or hot evaporation in concentrated aqueous solution, a brown gummy solid was obtained which in solution gave a green colour with copper hydroxide; long heating in dilute aqueous solution did not have the same effect. In other words, the conditions for the change correspond exactly to those necessary for the conversion of the blue salt to the green solid.

A comparison of the action of various oxidising agents on piperidino-acetic acid and p-toluene-sulphonyl-piperazino-acetic acid is of some interest when it is remembered that the copper salts of these do not undergo the change with heat. It has already been mentioned that the oxidation of piperidino-acetic acid with potassium
permanganate is very slow; after oxidation and precipitation of the manganese by alkali, the solution was benzoylated and a compound obtained, showing that either the acid group must have been removed or the ring opened. Analysis of the hydrochloride obtained by hydrolysing the benzoyl compound indicated that the ring was opened, and that two extra atoms of oxygen had entered. [Found Cl 29.2%, Calculated for C$_7$H$_{12}$NO$_4$ is 29.1% Cl]. One possibility was

\[
\begin{align*}
&\text{CH}_2 \\
&\text{CH}_2 \\
&\text{CH}_2 \\
&\text{COOH} \\
&\text{NH} \\
&\text{CH}_2\text{COOH}
\end{align*}
\]

but whatever the formula assigned to it, it must have an -NH group to give the benzoyl compound, and in this it is different from the oxidation product of the original acid with permanganate. The p-toluene-sulphonyl- derivative appears to resemble the original, very little mono-p-toluene-sulphonyl piperazine being formed, just as very little piperazine could be detected in the other case; the main product could not be isolated or identified. A complete oxidation of all the piperazine derivatives can be effected by boiling with excess of permanganate, ammonia and carbon dioxide being among the products.

With hydrogen peroxide, quite different results are found; neither the piperidino-acetic acid, nor the p-toluene-
sulphonyl- acid were oxidised by standing in the cold with hydrogen peroxide. Modifications such as the addition of acid or alkali produced no effect, and finally experiments were carried out with the solutions brought to the same $p_H$ value as the original acid, but still no oxidation occurred. With ethyl-piperazino-acetic acid, the oxidation seemed to proceed very slowly, hydrogen peroxide disappearing at less than half the rate found with the original acid; from the solution nothing could be isolated, decomposition apparently occurring very easily. When the solution was evaporated in the cold and then warmed with alcohol tarry products were formed and these were found to contain acetaldehyde and piperazine, suggesting the oxidation of the ethyl group. When the solution after oxidation was boiled with acid, formaldehyde was obtained; attempts to isolate ethyl piperazine from the residue by making alkaline failed, but a basic smell was noted which might have been that of ethyl piperazine. From these experiments therefore it would appear that the oxidation product of ethyl-piperazino-acetic acid can break down by oxidation at either end of the molecule.

Piperazine and carbethoxy-piperazine, in aqueous solution were also treated with hydrogen peroxide; the peroxide disappeared on standing but the solution gave the original derivatives, dibenzoyl piperazine, and the phenyl thiourea of carbethoxy-piperazine, almost quantitatively.
It was then found that the solutions after the disappearance of hydrogen peroxide had a strong reducing action; it appeared therefore that some oxidation had taken place giving a small quantity of a strongly reducing substance. The experiment was repeated with alcoholic solutions of piperazine and carbethoxy-piperazine and the equivalent volume of aqueous hydrogen peroxide. The latter appeared to react more quickly in the presence of the alcohol but a similar result was obtained, a solution which had a reducing action and which gave a considerable quantity of the derivatives of the original amine was obtained in each case.

The p-toluene-sulphonyl derivative of the oxidation product [B] of piperazino-acetic acid was prepared by the method used for the acid itself. [p.36]. This compound and the phenyl thiourea of B were both heated with alcohol under the same conditions as those effecting the change of B to the gummy solid but no change was observed in either of them, the melting points being the same after the treatment as before.

In the light of this work it seemed extremely likely that a knowledge of the nature of the oxidation product B, and of the gummy solid derived from it, might provide the solution of the copper salt anomaly, besides being of great interest in itself. The process of identification of both these substances will be the subject of the following chapter.
Experimental details.

The oxidation of piperazino-acetic acid by mercuric chloride.

(1) 5 gm. of acid, and 17 gm. of mercuric chloride, in aqueous solution were mixed, and boiled. 8 gm. calomel (containing a little organic matter) were collected; the theoretical yield, if all the acid were oxidised, is 14.8 gm. The calomel precipitate was suspended in dilute hydrochloric acid, and HgS passed in to the boiling liquid to precipitate the mercury; a trace of the hydrochloride of the original acid was obtained. 6.5 gm. of the original mercuric chloride compound was obtained from the solution, and nothing more could be isolated.

(2) 4.2 gm. acid and 13 gm. HgCl₂ gave 7 gm. of calomel. The theoretical yield is 11.3 gm. Nothing was isolated from the solution except the original acid and a little piperazine.

Results of analysis of the hydrochloride from the oxidation of carbethoxy-acetic ester of piperazine.

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of salt</td>
<td>0.1662 gm.</td>
</tr>
<tr>
<td>AgCl</td>
<td>0.3374 gm.</td>
</tr>
<tr>
<td>Cl</td>
<td>16.2%</td>
</tr>
</tbody>
</table>
Wt. of salt = 0.2173 gm.
Vol. of \(N_2\) = 21.6 c.c. at 16\( ^\circ \)C. and 757 mm.
\[
\therefore \ N_2 = 11.5\%
\]

Calculated for \(\text{NH}_4\text{C}_4\text{H}_8\text{N.CO.COOC}_2\text{H}_5\text{HCl}\)
\[
\begin{align*}
\text{N}_2 & = 12.6\% \\
\text{Cl} & = 16.0\%
\end{align*}
\]

This analysis does not prove that the hydrochloride obtained was the compound \(\text{NH}_4\text{C}_4\text{H}_8\text{N.CO.COOC}_2\text{H}_5\text{HCl}\), but if it had been contaminated with a little of the original carbethoxy-piperazine-acetic ester the percentages might have been altered to those found. Nevertheless, it was regarded as unlikely that the ester group would have remained when the carbethoxy group was removed. Nothing more conclusive was obtained from the experiments with mercuric chloride.
The oxidation of piperazino-acetic acid by:-

(1) KMnO₄.

<table>
<thead>
<tr>
<th>2 gms. acid</th>
<th>230 c.c. 0.1092N.KMnO₄</th>
<th>55 c.c. N.H₂SO₄</th>
<th>[40 c.c. N. acid in excess]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume of solution = 295 c.c.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

30 c.c. were treated with C₆H₅.COCl

30 c.c. were left for 24 hours and then treated with C₆H₅.COCl

30 c.c. were neutralised, left for 24 hours and treated with C₆H₅.COCl

205 c.c. were distilled (1.4 gm. acid)

<table>
<thead>
<tr>
<th>Residue Vol. = 65 c.c.</th>
<th>distillate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Made alkaline and distilled</td>
<td></td>
</tr>
</tbody>
</table>

formaldehyde

0.039 gm. [≈ 0.2 gm. acid]

13 c.c. (0.28 gm.) were treated with C₆H₅.COCl

0.02 gm. dibenzoyl piperazine.

13 c.c. were left for 24 hours and treated with C₆H₅.COCl

0.02 gm. "

13 c.c. were neutralised and left for 24 hours and treated with C₆H₅.COCl

0.02 gm. "

13 c.c. were treated with phenyl hydrazine.

0.01 gm. of a hydrazone.

3 c.c. were tested for oxalate.

None.
(2) **Bromine water.**

2 gms. acid
236 c.c. Br₂ water [1 atom of Br per molecule]
15 c.c. N.HCl [Acidity being the same as in KMnO₄ expt.]

Total volume = 295 c.c.

30 c.c. were treated with C₆H₅.COCl 0.15 gm.
30 c.c. were treated as before 0.2 gm.
30 c.c.

205 c.c. were distilled.

Residue
Vol. = 80 c.c.

Distillate
Made alkaline and distilled

Formaldehyde
0.048 gm. [= 0.26 gm. acid]

Residue

16 c.c. (0.28 gm.) were treated with C₆H₅.COCl 0.35 gm.
16 c.c. were treated as before. 0.35 gm.
16 c.c.

16 c.c. were treated with phenyl hydrazine <0.01 gm.

3 c.c. were tested for oxalate None.
(3) Lead tetracacetate.

0.5 gm. acid.
1.2 gm. lead tetracetate
10 c.c. dilute acetic acid.

Total volume = 80 c.c.

8 c.c. (0.05 gm.) were treated with C₆H₅COCl 0.02 gm.
8 c.c. were left for 24 hours and treated with C₆H₅COCl.

24 c.c. (0.15 gm.) were treated with H₂S, and solution evaporated → A gummy residue containing a little piperazine from which nothing could be isolated.

40 c.c. (0.25 gm.) were distilled

↓ Residue Vol. = 50 c.c. Distillate
↓ Made alkaline and distilled

Formaldehyde Residue
0.02 gm. [≈ 0.11 gm. acid]

10 c.c. (0.05 gm.) were treated with C₆H₅.COCl 0.02 gm.
10 c.c. were left for 24 hours and treated with C₆H₅.COCl
10 c.c. were treated with phenyl hydrazine No compound.
3 c.c. were tested for oxalate. None.
Distillation of the green salt — comparative experiment.

4 gms. of green solid
45 c.c. N. H₂SO₄.

Total volume = 100 c.c.

10 c.c. (0.4 gm.) were treated with C₆H₅.COCl

Trace

10 c.c. were treated as before.

" "

10 c.c. were allowed to stand and solid filtered.

Cuprous oxide.

60 c.c. (2.4 gm.) were distilled.

Residue Vol. = 50 c.c.

Distillate

Made alkaline and distilled

Formaldehyde Residue

0.03 gm. [≥ 0.165 gm. acid]

10 c.c. (0.5 gm.) were treated with C₆H₅.COCl

0.03 gm.

10 c.c. were treated as before.

0.03 gm.

10 c.c. were treated with phenyl hydrazine

Precipitate containing copper.

3 c.c. were tested for oxalate.

None.
Comparison of amounts of piperazine (estimated as dibenzoyl-piperazine) and formaldehyde in the above experiments.

<table>
<thead>
<tr>
<th></th>
<th>Acid equiv. to HCHO formed</th>
<th>Acid equiv. to pip. formed</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMnO₄</td>
<td>1.4 gm. acid</td>
<td>0.2 gm.</td>
<td>0.05 gm.</td>
</tr>
<tr>
<td>Br₂</td>
<td>1.4 &quot;</td>
<td>0.26 &quot;</td>
<td>1.2 &quot;</td>
</tr>
<tr>
<td>PbAc₄</td>
<td>0.25 &quot;</td>
<td>0.11 &quot;</td>
<td>0.05 &quot;</td>
</tr>
<tr>
<td>Green solid</td>
<td>2.5 &quot;</td>
<td>0.16 &quot;</td>
<td>0.1 &quot;</td>
</tr>
</tbody>
</table>

Thus, except in the case of the bromine oxidation the piperazine formed did not correspond to the total acid used, nor were the amounts equivalent to the formaldehyde produced. In the bromine experiment, the formaldehyde was less than was expected, for the amount of piperazine and in the other cases more. This disagreement is explained by the fact that piperazine is stable to bromine water at least within the time limits of the experiment, and formaldehyde is oxidised to formic acid; thus the formaldehyde found is less than that corresponding to the piperazine obtained.

In the case of the permanganate and lead tetracetate oxidations, the reverse is true; formaldehyde is stable to these agents while piperazine is attacked and broken down, so that the formaldehyde is more than the equivalent of the piperazine found. The discrepancy in the case of the green solid cannot be explained at this stage.
Preparation of the oxidation product of piperazino-acetic acid with hydrogen peroxide.

12-15 gms. piperazino-acetic acid were dissolved in about 20 c.c.s of water and the equivalent of 3% hydrogen peroxide was added. The mixture was allowed to stand in a loosely corked flask for about 48 hours and it was then evaporated in the cold over sulphuric acid. When a solid began to appear, alcohol was added to precipitate the product which was then filtered and dried.

The solution was tested for hydrogen peroxide by means of a solution of titanous sulphate which gives a yellow colour with hydrogen peroxide. [Sensitive to 1 part in 180,000].

The solution after oxidation was treated with phenyl isothiocyanate in a little alcohol and warmed until a precipitate appeared. This was filtered, dried, and analysed.

The p-toluene-sulphonyl compound of the oxidation product was also made by shaking an alkaline solution of the product with an ethereal solution of p-toluene-sulphonyl chloride, and precipitating by acidification with acetic acid.

The analyses of these three compounds were in agreement with the formula C_{6}H_{12}N_{2}O_{3} for the oxidation product, that is the original acid with an additional atom of oxygen per molecule.
Results of analysis

(1) Phenyl thiourea of oxidation product.

(a) Specimen I.
Wt. of product = 0.1248 gm.
Vol. of N₂ = 15.8 c.c.s at 16.2°C and 756.2 mm.
:. N₂ = 14.6%
Calculated for phenyl thiourea
of C₆H₁₂N₂O₃ = 14.4%

(b) Specimen II prepared from different specimen of oxidation product.

(i) Wt. of product = 0.1311 gm.
Vol. of N₂ = 16.4 c.c.s at 14°C and 757.4 mm.
:. N₂ = 14.6%

(ii) Wt. of product = 0.2036 gm.
" " BaSO₄ obtained = 0.1604 gm.
:. S = 10.8%

(iii) Wt. of product = 0.2268 gm.
" " CO₂ = 0.4407 gm.
" " H₂O = 0.1075 gm.
:. C = 53.0%
H = 5.27%

:. Found C = 53%, N = 14.6%, H = 5.27%, S = 10.8%
Calculated for phenyl thiourea of C₆H₁₂N₂O₃
= C = 53.1%, N = 14.4%, H = 5.22%, S = 10.8%.
(2) **Oxidation product**

Wt. of product = 0.1986 gm.

Vol. of N\textsubscript{2} = 30.1 ccs. at 17.6\textdegree C. and 760 mm.

\[ \therefore \text{N}_2 = 17.4\% \]

Calculated for C\textsubscript{6}H\textsubscript{12}N\textsubscript{2}O\textsubscript{3} = 17.5\%

(3) **p-toluene-sulphonyl derivative of oxidation product.**

Wt. of product = 0.2257 gm.

Vol. of N\textsubscript{2} = 17.45 ccs. at 20.2\textdegree C. and 760 mm.

\[ \therefore \text{N}_2 = 8.9\% \]

Calculated for p-toluene sulphonyl derivative of C\textsubscript{6}H\textsubscript{12}N\textsubscript{2}O\textsubscript{3} = 8.92\%

*** *** ***

Preparation of the gum C from oxidation product.

A quantity of the product B (3-4 gms.) was warmed with about 20 ccs. of 75-80\% alcohol. The aqueous alcohol was allowed to evaporate and when the solution had been evaporated to dryness a gummy solid was obtained which contained some of the product B unchanged. The process was repeated two or three times, and finally a gummy solid was obtained which gave no phenyl thiourea with phenyl isothiocyanate and therefore contained neither B nor piperazine.

It was extremely deliquescent, rapidly becoming a syrupy liquid on exposure to the atmosphere. In this state,
piperazine is formed by decomposition, some of the diphenyl thiourea of piperazine being obtained in less than half an hour after preparation.

*** *** ***

Oxidation of piperazine by hydrogen peroxide.

12 gms. piperazine hexahydrate were dissolved in water and divided into four equal parts of 18 ccs. Two parts were treated with hydrogen peroxide in the proportion one molecule $\text{H}_2\text{O}_2 :$ one molecule of piperazine; the other two were kept blank for comparative purposes.

After about 30 hours, the hydrogen peroxide had disappeared; one of the oxidised portions and one blank were treated with benzoyl chloride and caustic soda. Approximately equal weights of product were obtained, the amount being almost the theoretical weight for dibenzoyl piperazine. The mother liquors retained no reducing power. The product from the oxidised portion had a low melting point and appeared to be a mixture of dibenzoyl piperazine and some other benzoyl compound. With silver nitrate, slow reduction took place, and after hydrolysing with hydrochloric acid and removing benzoic acid, a trace of a hydrochloride was obtained which reduced ammoniacal silver nitrate in the cold, and Fehling's solution on warming. The second product, from the blank solution, treated in the same way gave no reducing product.
Thus the oxidised portion appeared to contain $\text{HN.C}_4\text{H}_8\text{.N-OH}$ which with benzoyl chloride gives $\text{C}_6\text{H}_5\text{.CO.N.C}_4\text{H}_8\text{.N.O.CO.C}_6\text{H}_5$.

The remaining two portions of the solution were treated with phenyl isothiocyanate and similar results were obtained. The phenyl thiourea from the oxidised portion contained some reducing substance and had a low melting point.

Thus, some oxidation of piperazine by hydrogen peroxide takes place in aqueous solution.
CHAPTER 6

The oxidation of amino-acids and amines, and the nature of the product of oxidation of piperazine-acetic acid with hydrogen peroxide

(a) The action of various oxidising agents on amino-acids is of interest in relation to the problem now under discussion, namely the nature of the oxidation products obtained from piperazine-acetic acid. It has long been known that primary aliphatic amino-acids give on complete oxidation with potassium permanganate ammonia and carbon dioxide; Denis [J. Biol. Chem. 9, p.365] stated that from glycine, ammonia and carbon dioxide, with traces of oxalic and nitric acids, were obtained; if insufficient permanganate were used, glyoxylic and formic acids could be detected. A comparison of the results of oxidation by hydrogen peroxide, and electrolytically, showed that in both cases ammonia and carbon dioxide were the main products. [Fichter and Kuhn, Helv. Chim. Acta, 1924, p.167].

The first suggestion as to the mechanism of the process came from Wieland and Bergel [Ann. 439 (1924), p.196] who put forward the theory that an imino-acid was formed intermediately; they studied the oxidation of glycine and alanine by oxygen in the presence of a catalyst, and found that ammonia and carbon dioxide were produced in the ratio
1:1 and in from 6 to 40% yield, according to the catalyst and concentration used; an aldehyde with one less carbon atom was also formed and the following mechanism was therefore suggested.

\[
\begin{align*}
R\cdot CH\cdot COOH & \quad R\cdot CO\cdot COOH & \quad R\cdot CHO + CO_2 \\
\text{NH}_2 & \quad \text{NH} & \quad \text{NH}_3 \\
\text{or} & \quad \text{NH} & \quad \text{NH}_3
\end{align*}
\]

In support of this theory, it was found that the compound \( \text{Me}_2\cdot C\cdot (\text{NH}_2)\cdot \text{COOH} \), which contains no hydrogen on the carbon atom and could not therefore undergo dehydrogenation, was scarcely oxidised under the same conditions.

Two years later Goldschmidt and Beuschel [Ann. 447 (1926), p.197] confirmed this view by isolating the imino-compound in several cases:-

\[
\begin{align*}
\text{Ph}_2\cdot \text{CH}\cdot \text{NH}_2 + \text{KMnO}_4 & \quad \rightarrow \quad \text{Ph}_2\text{C}=\text{NH} \quad \text{(isolated)} \\
& \quad \rightarrow \quad \text{Ph}_2\text{C}=\text{O} + \text{NH}_3 \\
\text{Me}\cdot \text{CH}\cdot (\text{NH}_2)\cdot \text{COOEt} & \quad \rightarrow \quad \text{MeC}=\text{NH}\cdot \text{COOEt} \\
& \quad \rightarrow \quad \text{NH}_3 + \text{Me}\cdot \text{CO}\cdot \text{COOEt}
\end{align*}
\]

The formation of aldehydes by the oxidation of amino-compounds has frequently been recorded; for example Suto [Biochem. Z. 1915, p.169] found that by the action of hydrogen peroxide in the presence of iron ethylamine and benzylamine gave acetaldehyde and benzaldehyde, and amino-ethyl alcohol gave a mixture of glycollaldehyde and glyoxal. Again,
Neuberg [Biochem. Zeit. 1909 (20), p.531] found that aldehydes are obtained by the oxidation of amino-acids by hydrogen peroxide. For example, amino-acetaldehyde is formed from isoserine, and glycollaldehyde from serine. Similar results were obtained by Dakin [J. Biol. Chem. 1908 (4), p.63, and 1909 (5), p.409] who studied the action of hydrogen peroxide on such acids as glutamic and aspartic acids. From glutamic acid he obtained finally ammonia, carbon dioxide and succinic acid, the latter being formed from the semi-aldehyde of succinic acid which is the first product of oxidation.

\[
\begin{align*}
\text{CH}_2\text{CHO} & \rightarrow \text{CH}_2\text{COOH} + \text{NH}_3 + \text{CO}_2 \\
\text{CH}_2\text{CHO} & \rightarrow \text{CH}_2\text{COOH} \\
\text{CH}_2\text{COOH} & \rightarrow \text{CH}_2\text{CDOH}.
\end{align*}
\]

Aspartic acid undergoes a similar reaction but the results are complicated by the fact that the semi-aldehyde of malonic acid which is first formed decomposes into acetaldehyde and carbon dioxide.

\[
\begin{align*}
\text{CH(NH}_2\text{)}\text{COOH} & \rightarrow \text{CH}_2\text{CHO} \\
\text{CH}_2\text{COOH} & \rightarrow \text{CH}_2\text{COOH} \rightarrow \text{CH}_3\text{CHO} + \text{CO}_2
\end{align*}
\]

It is to be noted that in all the cases described the amino-group was a primary one; in piperazino-acetic acid, the group corresponding to the one which becomes an imino-group in Wieland and Bergel's acids is a tertiary one and cannot therefore undergo dehydrogenation. Further neither
ammonia nor carbon dioxide have ever been detected during the oxidation of this acid with hydrogen peroxide in the cold, and the particular product of oxidation, described in the last chapter, has no aldehyde properties. It is clear therefore that the same mechanism cannot be applied.

When attention is turned to the oxidation of secondary and tertiary amines by hydrogen peroxide some interesting results are found. A great deal of work has been done on this subject by Wolffenstein and his collaborators, and one feature of significance in the present connection is that the conditions of oxidation were similar to those described on p. 82. Wolffenstein first treated piperidine with an excess of hydrogen peroxide and obtained glutarimide, glutaric acid and ammonia; a gentler oxidation with one equivalent gave a compound which he first characterised as amino-valeraldehyde, as it had all the reducing properties of aldehydes and gave on heating tetrahydropyridine

\[
\begin{align*}
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{N} & \\
\end{align*}
\]  [Ber. 1892, p.2777]

Wernick and Wolffenstein [Ber. 1898, p.1553] then found that when N-alkyl piperidines were oxidised under the same conditions a series of compounds with quite different properties from amino-valeraldehyde was obtained. These substances were not reducing, they contained one extra atom of oxygen per molecule but no primary or secondary nitrogen, and they
were monomolecular. They liberated iodine from potassium iodide, giving the original alkyl piperidines again. It was therefore concluded that they were amine-oxides

\[ \text{Alk} \quad \overset{\text{O}}{\equiv} \]

If the same mechanism is applied to piperidine itself two possibilities for the formula of the product arise:

\[ \text{NH} \quad \rightarrow \quad \left[ \begin{array}{c} \text{NH} \\ \overset{\text{O}}{\equiv} \end{array} \right] \quad \rightarrow \quad \text{CHO} \quad \text{or} \quad \text{N} \quad \overset{\text{OH}}{\equiv} \]

The latter form, the secondary hydroxylamine, would have the same reducing properties as the aldehyde, but a derivative such as a benzoyl compound would no longer be reducing, whereas the same derivative of the aldehyde would retain its reducing properties. Haase and Wolffenstein [Ber. 1904, p. 3228] prepared derivatives and found that they were not reducing, and therefore the oxidation product must be assumed to be the secondary hydroxylamine.

When acyl-substituted piperidines were treated in the same way, different results were obtained; Auerbach and Wolffenstein [Ber. 1899, p. 3507] found that acetyl and benzoyl piperidines could not be oxidised by hydrogen peroxide, the acidic group apparently preventing the nitrogen from becoming pentavalent.
Amine-oxides are easily reduced back to the original amines; Wolffenstein found that in some cases the oxygen is lost by warming or by allowing to stand with acid. Bamberger and Tschirner [Ber. 1899, p. 342] stated that dimethylaniline-oxide can be reduced by such agents as zinc dust, sulphuretted hydrogen or sulphur dioxide. The same agents will reduce secondary hydroxylamines, which are on the whole even more easily decomposed than the amine-oxides. A number were prepared by Mamlock and Wolffenstein [Ber. 1900, p. 159] by the action of hydrogen peroxide on a secondary amine; they reduce Fehling's solution, ammoniacal silver nitrate, copper acetate, they give a pink coloration with Schiff's reagent, and form compounds with sulphur dioxide. Such compounds are acidic, having the formula $\text{Alk}_2\text{N}.\text{SO}_3\text{H}$ or $\text{Alk}_2\text{NH}.\text{SO}_3^-$; that is, of a dialkyl sulphamic acid. They can be prepared either directly by passing sulphur dioxide into the solution, or by adding a saturated solution of sodium bisulphite and then acidifying with hydrochloric acid [Ber. 1900, p. 160]. With carbon bisulphide, a diethyl-dithio-carbamic acid is obtained, which is also formed directly from diethylamine; the oxygen splits off from the hydroxylamine and oxidises the carbon bisulphide to sulphur.

These two classes of compounds have also been studied by Dunstan and Goulding [J.C.S. 1899, pp. 792 & 1004], who prepared them by the action of hydrogen peroxide on the amines, and by the treatment of hydroxylamine with methyl-
or ethyl-iodides. Similar properties were found except that trimethylamine oxide did not liberate iodine from potassium iodide. The secondary hydroxylamines would not give double salts with gold or platinum chlorides, the latter being immediately reduced to the metal. The oxalates and sulphates were prepared but no derivatives such as benzoyl- or $p$-toluene-sulphonyl compounds, or phenyl ureas are described. An interesting point in this work is the consideration of the possibility that the "amine-oxide" is really tri-alkyl hydroxylamine, $\text{Alk}_2\text{NOAlk}$. The fact that formaldehyde and dimethylamine were obtained from the trimethyl compound gave support to this view, but against it were the facts that with hydrogen iodide no methyl iodide was formed, and that it was easily reduced with zinc dust. The discovery that formaldehyde could be obtained from the compound $\text{CH}_3\text{NH}_2\text{OH}$, where the methyl group is attached to the nitrogen and not to the oxygen, removed the objection to the amine-oxide form.

Amine-oxides have also been prepared by the action of ozone on tertiary amines; under similar conditions, primary amines were oxidised to ammonia and formaldehyde, and secondary amines gave ammonia, formaldehyde, acetaldehyde, formic and acetic acids [Strecker and Thiemann. Ber. 1920, p.2112].

The results described above may be briefly summarised thus: by the action of hydrogen peroxide on a tertiary amine a loosely-linked oxygen is added, giving an amine-oxide, and under the same conditions the hydrogen of a secondary amine
is converted into an -OH group, forming a secondary hydroxylamine.

The compound formed by oxidation of piperazino-acetic acid with hydrogen peroxide contains one additional atom of oxygen [Chap.5, p. 70] and it has only a very slight reducing action; in addition, it contains an imino-group capable of giving derivatives with phenyl isothiocyanate and p-toluene-sulphonyl chloride [Chap.5]. The instability of this product [B] and the readiness with which it loses the oxygen atom was shown by a further study of its reactions. It was found that it is readily and quantitatively split up by hot alkali into piperazine and sodium carbonate; towards cold acid it is stable but distillation with dilute acid gives a trace of formaldehyde and piperazine and some other compound with no free -NH group. The physical properties of this substance and its reducing action towards silver nitrate suggested the possibility of its being the same as the sticky solid [C] obtained by the action of heat or warm alcohol [p. 84]. The product B is reduced by zinc dust in neutral or dilute acetic acid solution to the original piperazino-acetic acid, which was identified as the phenyl thiourea; no other thiourea was obtained which showed that none of the oxidation product remained unreduced, and no piperazine was formed. B also liberates iodine from potassium iodide in the presence of a little dilute acetic acid, giving first piperazino-acetic acid; this however reacts with iodine to give piperazine.
This somewhat surprising result was confirmed by allowing a solution of piperazino-acetic acid to stand with an iodine solution; it was found that the iodine disappeared rapidly giving piperazine, and it appears therefore that iodine has a similar action to bromine [See p. 66.]. This can be explained if it is assumed that the iodine first gives

\[
\text{N} \quad \text{N-I} \\
\text{CH}_2\text{COOH}
\]

which with water would be in equilibrium with

\[
\text{N} \quad \text{NH} + \text{HIO}.
\]

The hypoidous acid can then oxidise the acid chain to give piperazine. Some evidence of the formation of such a halogen compound was found in the oxidation of piperazino-acetic acid by bromine in chloroform [p. 67.]. It will be remembered that this oxidation in concentrated solution was carried out for the specific purpose of determining the first product of oxidation of the side chain; during the oxidation a white crystalline compound was momentarily formed which was thought to be a derivative with the halogen attached to the nitrogen atom. The instability of this compound was such that it could not be investigated, as it disappeared very rapidly, but in the light of the result recorded above of the action of iodine on the acid, it seems not unlikely that such halogen derivatives are formed, to be immediately split up by water in the manner indicated.

A result of fundamental importance in a consideration of the constitution of B was afforded by an experiment with
acetic anhydride, in which a quantitative yield of diacetyl piperazine was obtained from B. This not only shows the readiness with which the product parts with its third atom of oxygen, but also that the product itself contains an unbroken piperazine ring, and therefore that the oxygen must have attacked some other part of the molecule. Two alternatives are then possible for the process of oxidation; either the oxygen must attack the acetic acid chain or become loosely linked to one of the two nitrogen atoms. In the former case, glyoxylic or oxalic acids would almost certainly be formed during the decomposition of the product by acid or alkali, but neither of these were ever detected; an attempt to find the effect of a further oxidation of the product B gave a result which added to the improbability of the first oxidation attacking the acid chain. It was found that when two equivalents of hydrogen peroxide were added to piperazino-acetic acid, only one oxygen was effective under the normal conditions, the phenyl thiourea of B being obtained with phenyl isothiocyanate. Accordingly 0.01 gm. of ferrous sulphate was added to catalyse the second oxidation, and as a result great heat was developed and a large quantity of piperazine was formed in the solution. In a second experiment the ferrous sulphate was added to a solution of B and a second equivalent of hydrogen peroxide was added slowly with cooling. From the solution a new phenyl thiourea was obtained [melting point = 156°C.] a nitrogen estimation of
which indicated that it might be the diphenyl thiourea of an open chain dicarboxylic acid

\[
\text{C}_6\text{H}_5\text{NHCSNCH}_2\text{CH}_2\text{NCSNH.C}_6\text{H}_5
\]

[Found \(N = 12.8\%\), Calcd. 12.5\%]

The yield was small and insufficient for a complete analysis so that a definite formula could not be assigned to the product; the indication was however that the only substance with a free iminic group formed by the oxidation of \(B\) was one, containing one atom of oxygen per molecule more than \(B\), in which the piperazine ring was opened giving two nitrogen groups capable of forming derivatives, and which still contained the acetic acid chain. If in the first oxidation of piperazino-acetic acid the acid chain had become \(-\text{CO.COOH}\) or \(-\text{CHOH.COOH}\), it is unlikely that further oxidation would attack a \(-\text{CH}_2\) group in the ring in preference to the side chain; the experiment just described is therefore a further indication that the oxygen atom acquired by oxidation of piperazino-acetic acid with hydrogen peroxide is loosely attached to the nitrogen and is capable under different conditions of oxidising different parts of the molecule.

Although the product of the oxidation of \(B\) had not been identified by the above experiment no further attempt to do so was made; the facts which had been brought to light indicated that a complete investigation would be of no
assistance in elucidating the problem of the nature of B.

It has been said that certain reactions of B indicate that the oxygen is loosely linked to one of the two nitrogen atoms in the molecule; if it were to the tertiary nitrogen, an amine-oxide would be formed, and if to the secondary, a secondary hydroxylamine. The properties of B which have already been described are in complete agreement with an amine-oxide formula

\[
\begin{align*}
\text{NH} & \text{H} \\
N\text{CH}_2\text{COOH},
\end{align*}
\]

and they do not agree with the hydroxylamine form

\[
\begin{align*}
\text{OH} & \text{H} \\
N\text{CH}_2\text{COOH}.
\end{align*}
\]

The latter would be strongly reducing towards silver and copper, and the product B has no reducing action. Furthermore the reaction of B with phenyl isothiocyanate shows that an \(-\text{NH}\) group is present in the molecule. The fact that B is more acid in solution than piperazino-acetic acid, the \(p_H\) value being about 4 compared with 7.5, is also in accordance with the amine-oxide formula; in the grouping

\[
0 \leftarrow \text{NCH}_2\text{C}^\text{OH},
\]

the effect of the oxygen, transmitted through the chain, is to increase the tendency to ionise, that is, to make a stronger acid.

A brief recapitulation of the ways in which piperazino-
acetic acid might be oxidised and of the properties of the product found will make it quite clear that the product is an amine-oxide. The experiment with acetic anhydride excluded the possibility of oxidation in the ring either with formation of a \(-\text{C}=\text{O}\) group or an open-chain aldehyde; the latter was again proved impossible by the absence of reducing properties in the product B. Oxidation of the side chain with formation of \(-\text{CO}\cdot\text{COOH}\) or \(-\text{CHOH}\cdot\text{COOH}\) appeared unlikely from the fact that neither glyoxylic acid nor oxalic acid could ever be detected among its decomposition products, and the attempt to oxidise B further gave additional evidence against this view. Finally, B has all the properties attributed to amine-oxides by Wolffenstein and the analysis agrees with the formula \(\text{C}_6\text{H}_{12}\text{N}_2\text{O}_3\).

The discussion of the identity of B cannot be concluded without a consideration of the nature of the substance C obtained from it by the action of warm alcohol. The properties of C are quite different from those of B, except the solubility in water and insolubility in organic solvents which was found with B and with piperazino-acetic acid; when first prepared C is slightly more alkaline in solution than piperazino-acetic acid \([\text{pH} = 8, \text{compared with} \ 7.5]\), but on standing it becomes more acid. It reduces Fehling's solution, ammoniacal silver nitrate and copper acetate. It contains no piperazine when first prepared, but a trace can be detected within a few minutes, and the amount increases on standing in
aqueous solution, or in the sticky condition which is its natural state in air; the decomposition is accelerated both by acid and alkali. Distillation with acid gives formaldehyde and piperazine, while with alkali sodium carbonate and piperazine are the chief products. Like the amine-oxide, it liberates iodine from potassium iodide in acetic acid solution, and is reduced by zinc dust to give piperazino-acetic acid; but unlike B, no derivative can be made from it with such reagents as p-toluene-sulphonyl chloride or phenyl isothiocyanate.

The product itself is so deliquescent and so unstable that an analysis of it as such is impossible; the hydrochloride could not be prepared as evaporation with hydrochloric acid led to decomposition. Accordingly some other means of characterisation of C had to be found. A specimen was treated with acetic anhydride in the same way that B had been, and a quantitative yield of diacetyl piperazine was obtained which proved that the piperazine ring was still intact in C. It was clear from the inability to form derivatives that the secondary nitrogen group must be in some way involved in the transformation and it seemed therefore that if the change could be effected in the presence of another base a new compound might be obtained which should still have an -NH group. B was therefore heated with a little piperidine and a brown gummy solid was quickly formed which yielded by treatment with alcohol a pure specimen of
piperazine-acetic acid. No other definite compound could be isolated and it seemed that the piperidine must have caused the reduction of B. The experiment therefore failed in its purpose but was of value in that it showed the readiness with which B loses an atom of oxygen.

Another attempt was then made to prepare a compound of G which could be analysed; experiments with a number of acids showed that only the picrate could be prepared as a dry solid. On addition of an aqueous solution of picric acid to a solution of G, a precipitate was obtained which was found to be very sparingly soluble in hot water and to decompose explosively at 255-60°C. It was therefore different from the picrates of both the original acid and the product B which dissolve easily in hot water and decompose at 230°C. and 180°C. respectively. The new picrate was boiled with water to remove traces of these and of piperazine picrate, and then dried and analysed, but the results indicated that it was impure. Moreover as all attempts to estimate the amount of picric acid in the salt failed, no conclusions could be drawn as to the formula of G; the usual method of dissolving the salt in hydrochloric acid and extracting the picric acid with benzene or chloroform was tried, but it was found that only a small proportion of the picric acid was extracted. Even evaporation to dryness with hydrochloric acid did not entirely decompose it and the portion insoluble in alcohol was a mixture of a hydrochloride
and undecomposed picrate.

The solution from which the picrate had been precipitated was evaporated in the presence of hydrochloric acid and the picric acid extracted by treating with alcohol. The residue was found to contain both piperazine and piperazino-acetic acid; the former might have been formed by decomposition of C in the presence of acid but it was shown that the latter was not due merely to the heating with hydrochloric acid. A comparative experiment in which the conditions were the same except that picric acid was not present resulted only in piperazine. It was concluded therefore that the effect of standing and heating with picric acid had been to cause a splitting off of oxygen from C similar to the decomposition of B found in the piperidine experiment, showing that the oxygen in C is loosely attached.

This fact was also shown by a number of experiments with p-toluene-sulphonyl chloride and the substance C; only the derivatives of piperazine and piperazino-acetic acid could be obtained.

The new picrate described above was again prepared but this time no attempt was made to purify it by boiling with water as the intervening experimental work had made it clear that C was an unstable compound. Accordingly a specimen of C was prepared by warming B with 75% alcohol and evaporating to dryness; it was found that C could only be prepared free from the amine-oxide by evaporating the alcohol,
adding fresh alcohol and again evaporating. The substance was then immediately treated with picric acid and a precipitate obtained which decomposed between 260 and 270°C. A second specimen of C was prepared and treated with phenyl isothiocyanate to remove the piperazine present; the thio-urea was filtered off, the excess of the reagent extracted and the solution treated with picric acid. A substance was obtained with the same decomposition point. A nitrogen estimation was carried out with each of these and the agreement between them showed that the specimen obtained by treating the solution of C with picric acid was as pure as that obtained after the removal of piperazine, which was therefore an unnecessary procedure. This experiment was important in that it showed that apart from the decomposition into piperazine, the change from B to C appears unaccompanied by side reactions. The first specimen referred to was analysed for carbon, hydrogen and nitrogen, and picric acid content; the latter was estimated by heating and evaporating with 80% sulphuric acid, shaking the sulphuric acid solution with benzene and then chloroform, and evaporating these solutions to dryness. The sulphuric acid solution was then quite colourless and on diluting with water there was still no trace of yellow colour, so that the picric acid appeared to be completely extracted by this method. The residue of picric acid was dissolved in a little water and the amount estimated by the potassium iodide-potassium iodate method.
The results showed that the proportion of picric acid to base was 2:1, the base having a formula $C_6H_{11}N_2O_3$; that is, the additional atom of oxygen in B is retained in C.

Molecular weight determinations by the freezing point depressions offered some difficulty owing to the formation of piperazine in C both during the drying and in solution. A specimen of C was dried to constant weight and a freezing point determination of a solution made; the solution was then immediately treated with benzoyl chloride and the amount of dibenzoyl piperazine formed was found. In another experiment the freezing point and the amount of piperazine were determined simultaneously with two portions of the same specimen of C. It appeared that very little more piperazine was formed during the time taken for the freezing point determination. The estimation of the amount of piperazine was checked by precipitating the picrate of the new base from the solution, for it was known to be very sparingly soluble, and the two results were in agreement. Allowance was made in the calculation of the molecular weights for the free piperazine present, and the values 162, 161, and 148 were obtained for the molecular weight of the product C. The experimental error in these experiments was great and the results could not be expected to be in perfect agreement; the values obtained were considered satisfactory proof that the product is monomolecular, there being no reason to suppose that the amphoteric nature of the amino-acids would give an
abnormal value for the molecular weight, as glycine is known to give the true value by the freezing point method. [J.A.C.S. 1912, p.383].

Bearing in mind the fact that the product C contains no secondary nitrogen several possibilities for its constitution suggest themselves:

![Chemical Structures]

It soon becomes clear however that the properties known to be possessed by C are not in accordance with most of these formulae; with the exception of IV the compounds formulated above should not reduce silver nitrate or Fehling's solution, and VI, being a form of a per-acid should be an oxidising agent. Again it would seem that I, II, V, and VI should be perfectly stable in aqueous solution, and V should also be stable towards acid and alkali. II could not revert to
piperazino-acetic acid by a simple splitting off of oxygen, but only by the action of a reducing agent. III seems stereochemically unlikely, but cannot be said to be impossible; the strains in a 4-ring containing two nitrogen atoms would probably be less than in a 4-carbon ring as the angles between the three valencies of a tertiary nitrogen should be larger than those between the four carbon valencies. It is however difficult to explain why III should give formaldehyde on hydrolysis with acid. A compound such as VII, with a double molecule, is ruled out by the molecular weight determinations, and I, II, V and VI by the results of analysis of the picrate which showed that the product C still retains the additional atom of oxygen. A compound of the formula IV should be strongly reducing, and should be easily reduced by zinc to piperazino-acetic acid; it should part with its oxygen easily and should be unstable to acids and alkalis, giving piperazine either by inter- or intra-molecular oxidation of the side chain.

These properties are those already described as belonging to the product C, and as the formula IV agrees with the analysis and molecular weight determinations, there is a great deal of evidence in favour of the secondary hydroxylamine form for C. It appears therefore that by the action of hydrogen peroxide on piperazino-acetic acid an amine-oxide is produced which is converted by the action of heat or alcohol into a secondary hydroxylamine, the oxygen migrating to the secondary
nitrogen atom.

The migration of the oxygen of an amine-oxide to another nitrogen in the molecule is not unknown; Bamberger and Tschirner [Ber. 1899, p.342] prepared dimethylaniline oxide, converted this into $p$-nitroso-dimethylaniline oxide and found that this was readily transformed into $p$-nitro-dimethylaniline.

Another point of interest in the present connection is found in some work of Meisenheimer [Ber. 1922, p.513]. This is the formation of a hydroxylamine from an amine-oxide due in this case to the migration, not of the oxygen but of an alkyl group.

A possibility for C which must be considered further is formula III above; namely

$$\begin{align*}
\text{C}_6\text{H}_5 & \quad \text{N}--\text{O} & \quad \text{C}_6\text{H}_5 \quad \text{N}--\text{O}--\text{C}_3\text{H}_5 \\
\text{C}_3\text{H}_5 & \quad \text{N}--\text{O} & \quad \text{C}_3\text{H}_5 \quad \text{N}--\text{O}--\text{C}_3\text{H}_5 \\
\end{align*}$$

Provided that such a molecule would exist in that form and not in the dehydrated state.
the analysis and molecular weight determination are in agreement with this, the molecular formula being the same as that of the secondary hydroxylamine. If such were the case, it must be assumed that the ionised hydroxyl group can take up a position on either nitrogen giving either the secondary hydroxylamine or the amine-oxide reactions. In the majority of its reactions apparently the hydroxylamine predominates.

The basic assumption, that the substance exists in its hydrated form is not unjustifiable; Stoltzenberg \[Z. Physiol. Chem. 92, p. 445\] states that anhydrous betaines of which this compound is a type quickly take up a molecule of water from the air, and Pfeiffer \[Ber. 1922, p. 1769\] records that trans-p-aminocinnamic acid trimethyl betaine only loses half its molecule of water over phosphoric oxide, during some weeks, and is very deliquescent in air. Further it is noted that slight impurities or decomposition products cause betaines to become very hygroscopic. There is here nothing contradictory to the known properties of the product C. Again there is the analogy of the acridinium salts; the compound
when treated with soda gives the pseudo-base

![Chemical Structure]

It is thought that the true base is first formed but this passes over into the pseudo-base. Spectroscopic examination by Tinkler showed that the absorption spectra of the base resembled the open ring compound, dihydroacridine, and not acridine methiodide. Here therefore we have an example of a compound containing a quaternary nitrogen undergoing a change in which a hydroxyl group migrates and the nitrogen becomes tertiary; this process is the same as that which would take place if

\[
\begin{array}{c}
\text{CH}_2-\text{N}-\text{CH}_2 \\
\text{CH}_2-\text{N}-\text{CH}_2 \\
\text{OH}
\end{array}
\]

passed into the secondary hydroxylamine

\[
\begin{array}{c}
\text{OH} \\
\text{CH}_2-\text{N}-\text{CH}_2 \\
\text{CH}_2-\text{N}-\text{CH}_2 \\
\text{CH}_2-\text{COOH}
\end{array}
\]

When the properties of the product C are compared with those of secondary hydroxylamines recorded by Wolffenstein and by Dunstan and Goulding, several discrepancies are apparent. The most important is the liberation of iodine from potassium iodide by C \( \text{p. 100.} \); in previous work on secondary
hydroxylamines no mention was made of this property. It is true that in the attempts to obtain methyl iodide from them by treatment with hydriodic acid the original amine was obtained but this was a more drastic treatment than the addition of potassium iodide to C in the presence of acetic acid. It has been shown by Prof. Moore that the solutions produced by the oxidation of diethylamine and piperidine by hydrogen peroxide liberate iodine in the presence of sulphuric acid but not in the presence of acetic acid; this reaction has been proved to be due to the presence of nitrous acid, the acetates of the unchanged diethylamine and the amine-oxide having sufficient buffering action to prevent the liberation of iodine. In the case of C, however, iodine was liberated in the presence of acetic acid, and the probability is therefore that it was due not to nitrous acid but to the secondary hydroxylamine itself. This property is merely an indication of the readiness of the compound to part with its oxygen and is in keeping with experiments already described showing the unusually high oxidising power of C. Further, the oxygen in the amine-oxide $\text{NH}_2\text{C}_4\text{H}_9\text{N}\cdot\text{CH}_2\cdot\text{COOH}$ appears to be more active as an oxidiser than the oxygen in $(\text{C}_2\text{H}_5)_3\text{N} \rightarrow \text{O}$; for example, in the former case the oxygen is split off merely by warming with piperidine, oxidising the latter, and under certain conditions oxidises a part of the molecule, namely, the side chain. It is
reasonable, therefore, that the secondary hydroxylamine \( \text{HO-N.C}_4\text{H}_6\text{.N.CH}_2\text{.COOH} \) should be more active as an oxidising agent than \( (\text{C}_2\text{H}_5)_2\text{N-OH} \).

It will be remembered that in Wolffenstein's description of the properties of secondary hydroxylamines mention was made of the reaction with carbon bisulphide and of compounds with sulphur dioxide \([p. 92\,].\) Experiments have been performed with a view to studying the behaviour of C towards the same reagents. With carbon bisulphide the action was comparable; no solid compound could be isolated but it was found that C had reverted to the original piperazino-acetic acid, which was identified as the phenyl thiourea, and sulphur had been formed. With diethyl hydroxylamine, the diethyl-dithiocarbamic acid was obtained, a compound which is formed directly from diethylamine and carbon bisulphide, and sulphur was formed by oxidation. It is obvious that the same action occurred with C, but in this case the reduced product, piperazino-acetic acid, does not form a compound with carbon bisulphide. With sulphur dioxide a sulphamic acid could not be obtained; the experimental method was varied, sulphur dioxide being passed into an aqueous solution, into an alcohol suspension, and a concentrated solution of sodium bisulphite being added to an acid solution, but in no case could a precipitate be obtained. Eventually it was found that after sulphur dioxide had been passed into the aqueous solution, the phenyl thiourea of
piperazino-acetic acid was obtained showing that reduction of the hydroxylamine had taken place; the solution also gave a precipitate of barium sulphate. The failure to prepare a sulphamic acid can therefore be explained by the ease of reduction of C; the sulphurous acid causes the reversion to the original acid being at the same time oxidised to sulphuric acid. It is possible that under different conditions, in alcohol solution for example, the compounds with sulphur dioxide are more stable than in aqueous solution, but in the present case only an aqueous solution can be used; it may be too that the compound of C with sulphur dioxide is formed but is soluble in water and decomposed by it while in other cases it would be precipitated from the solution.

Thus although in certain particulars the compound C does not resemble the secondary hydroxylamines previously described, the apparent anomaly is explained by the instability and high oxidising power of C, combined with the remarkable solubility of the substances concerned.

If, however, the secondary hydroxylamine is capable of existence, it appears strange that it should not be formed to some extent in the action of hydrogen peroxide on piperazino-acetic acid; it seems that the product B should be a mixture of the amine-oxide and the secondary hydroxylamine, whereas it is known to be a homogeneous product, the amine-oxide. It must be remembered however, that the >NH group has a strong tendency to coordinate and this can operate in
two ways to prevent the free $\text{NH}$ group remaining in aqueous solution; it can either coordinate a molecule of water or become positively charged in an internal salt formation. For example,

\[
\begin{align*}
\text{N} \rightarrow \text{H.OH} & \quad \text{or} \quad \text{NH}_2^+ \\
\text{N.CH}_2\text{.COOH} & \quad \text{N.CH}_2\text{.COO}^-
\end{align*}
\]

The hydrogen in the one case and the water molecule in the other will of course go to the nitrogen with the highest coordinating power, and there is much experimental work available to determine which this will be. The evidence falls into three sections, the first being the properties of amino-acids in general in relation to Bjerrum's theory of internal salt formation, the second being the relative hydration of primary, secondary and tertiary amines, and the third being the particular properties of the piperazine compounds. Bjerrum [Z.physikal.Chem. 104 (1923), p.147] put forward the theory that in aqueous solutions of aliphatic amino-acids about 95% of the neutral molecules present are in the form of an internal salt or "zwitterion", e.g. $\text{NH}_3\text{.CH}_2\text{.COO}^-$. He showed that the apparent dissociation constants, $k_a$ and $k_b$, which had been calculated from conductivity measurements were in reality hydrolytic constants; the presence of hydrogen and hydroxyl ions indicates not the acidity or basicity, but the amount of hydrolysis of the salt. That is, the amounts of hydrogen and hydroxyl ions are determined not by the equilibria represented by
\[
\begin{align*}
\text{NH}_2\cdot\text{CH}_2\cdot\text{COOH} & \rightleftharpoons \text{NH}_2\cdot\text{CH}_2\cdot\text{COO}^- + \text{H}^+ \\
\text{and} \quad \text{NH}_2\cdot\text{CH}_2\cdot\text{COOH} + \text{H}_2\text{O} & \rightleftharpoons \text{NH}_3\cdot\text{CH}_2\cdot\text{COOH} + \text{OH}^- \\
\text{but by} \quad +\text{NH}_3\cdot\text{CH}_2\cdot\text{COO}^- + \text{H}_2\text{O} & \rightleftharpoons +\text{NH}_3\cdot\text{CH}_2\cdot\text{COOH} + \text{OH}^- \\
\text{and} \quad +\text{NH}_3\cdot\text{CH}_2\cdot\text{COOH} & \rightleftharpoons +\text{NH}_3\cdot\text{CH}_2\cdot\text{COO}^- + \text{H}^+ + \text{H}_2\text{O}.
\end{align*}
\]

The true dissociation constants determined by the equilibria
\[
+\text{NH}_3\cdot\text{CH}_2\cdot\text{COOH} \rightleftharpoons +\text{NH}_3\cdot\text{CH}_2\cdot\text{COO}^- + \text{H}^+
\]
and
\[
\text{NH}_2\cdot\text{CH}_2\cdot\text{COO}^- \rightleftharpoons +\text{NH}_3\cdot\text{CH}_2\cdot\text{COO}^- + \text{OH}^-.
\]
are connected with the hydrolytic constants in the relation
\[
K_A = \frac{K\text{H}_2\text{O}}{K_B} \quad \text{and} \quad K_B = \frac{K\text{H}_2\text{O}}{K_A}
\]
where \(K_A\) and \(K_B\) are the true dissociation constants and \(K\text{H}_2\text{O}\) the ionic product of water. The values of \(K_A\) and \(K_B\) thus found, expressing the strength of the amino-acids, their power to neutralise acidic and basic groups, are in accordance with the structural formulae of the acids; that is the values which can be attributed to \(K_A\) and \(K_B\) from the nature of the acidic and basic groups agree with those calculated from the formulae above.

Various physical properties of amino-acids are in accordance with their salt-like form; they are soluble in water and insoluble in organic solvents, and they have high melting points. In the present instance, the high solubility of piperazino-acetic acid in water, and its insolubility in all other solvents may be cited as evidence of its salt-like form.

In the case of piperazino-acetic acid there are two nitrogen atoms available but it has been shown by Moore and Winmill that a secondary amine has a greater coordinating
power than a tertiary [J.C.S. 1912, p.1635]. The hydration constant and true ionisation constant of a number of bases were calculated from the apparent dissociation constants and partition coefficients at three different temperatures. The equilibria concerned are represented by the equations:

$$NX_3 + H_2O \rightleftharpoons NX_3\cdot H\cdot OH \rightleftharpoons NX_3H^+ + OH^-$$

It was found that secondary amines had very large hydration constants so that the true and apparent ionisation constants are the same. The two tertiary amines examined both had very small hydration constants. Thus while diethylamine exists largely as diethyl ammonium hydroxide, in aqueous solution, triethylamine exists largely in the unhydrated form.

The same conclusion, as to the greater coordinating power of the secondary nitrogen, can be reached on theoretical grounds; the proximity of the acid group to the tertiary nitrogen, the presence of a -CH2-COOH in the place of a -H would tend to make the tertiary nitrogen more positive than the secondary so that the latter would have a greater tendency to act as donor.

There are a number of facts in connection with the piperazine derivatives which can only be explained by the assumption of a high coordinating power in the molecule. It has been observed [J.C.S. 1929, p.44] that piperazino-acetic acid retains a molecule of water with great tenacity; it does not become anhydrous over phosphoric oxide, losing water very slowly, and has to be heated at 100°C. in order to drive
off the water completely. This can only be explained on the assumption that a molecule of water is attached by a coordinate link to the nitrogen. Again, with the exception of monotoluene sulphonyl piperazine, all the mono-derivatives are extremely soluble in water and like piperazine itself cannot be extracted from aqueous solution by ether; for example, ethyl-piperazine, although completely miscible with ether, is not extracted by ether from water, carbethoxy-piperazine is only extracted when the solution is saturated with potassium carbonate. Ethyl carbethoxy piperazine, Et-N.C₄H₉-N.COOC₂H₅, on the other hand is extracted by ether, as is carbethoxy-piperazino-acetic ester COOC₂H₅.N.C₄H₉.N.CH₂.COOC₂H₅, and other di-derivatives of piperazine. These properties are evidence of a tendency for the >NH group to coordinate with water. Another example of its effect is in the preparation of carbethoxy-piperazino-acetic ester from carbethoxy piperazine and of ethyl-piperazino-acetic ester from ethyl piperazine; in both cases the reaction does not take place unless anhydrous materials are used [See p. 37 and J.C.S. 1929, pp.45-48]. The readiness of the >NH group to coordinate is also shown by the violent reaction of carbethoxy-piperazine with methyl or ethyl iodide in cold alcohol solution to give the quaternary iodides, with very little, or none, of the mono-alkyl compound. [J.C.S. 1929, p.46 and Thesis presented by V.M. Thorn for M.Sc. in the University of London, 1928, p.6].

It seems therefore that the secondary nitrogen group
will have a very much greater tendency to coordinate than the tertiary nitrogen, and that in an aqueous solution of piperazino-acetic acid there can be very little free $>\text{NH}$; the oxygen from the hydrogen peroxide cannot then attack the secondary nitrogen which is already pentavalent but it forms an amine-oxide by attaching itself to the tertiary nitrogen.

The fact already stated [p. 73.] that piperazine and carbethoxy piperazine are oxidised to some extent in aqueous solution, though more slowly than piperazino-acetic acid shows that it cannot be coordination of a molecule of water which renders the $>\text{NH}$ group inactive to hydrogen peroxide, but the internal salt formation

$$\text{NH}_2^+ \quad \text{N} \cdot \text{CH}_2 \cdot \text{COO}^-.$$

If the coordinating power of the $>\text{NH}$ group exhibited itself in a link with a molecule of water which prevented attack by hydrogen peroxide, then piperazine and mono-carbethoxy-piperazine should not be oxidised at all in aqueous solution; for in piperazine both the nitrogen groups would be blocked thus, and in carbethoxy-piperazine the secondary nitrogen would be blocked and the tertiary nitrogen, being attached to an acyl group would be prevented from becoming pentavalent [p. 91. Wolffenstein and Auerbach, Ber. 1899, p.3507]. It appears therefore that piperazino-acetic acid, in aqueous solution, exists chiefly as the "zwitterion".
Goldschmidt has found [Z. physikal. Chem. 1921, (99), p. 116, and 1925 (117), p. 312] that a number of weak acids have very much smaller dissociation constants in alcohol than in water. Among the acids which were investigated were the fatty acid series from acetic to valeric acid, glycollic acid, picric acid, benzoic, nitrobenzoic and salicylic acids. He gave the equations \( \frac{K_{H_2O}}{K_{MeOH}} = 10^4 \) and \( \frac{K_{MeOH}}{K_{EtOH}} = 10^4 \), so that the dissociation constant in water is \( 10^8 \times \) that in ethyl alcohol.

On this view it is possible to explain the transformation of the \( \text{N-amine-oxide} \) into the \( \text{N'-secondary hydroxylamine} \). In alcohol or aqueous alcohol below a certain proportion of water, the acid becomes weaker, existing not as

\[
\begin{align*}
\text{N-CH}_2\text{COO}^- & \quad \text{but as} \quad \text{N-CH}_2\text{COOH}. \\
\text{C}_4\text{H}_8 & \\
\end{align*}
\]

It is likely that there will be an equilibrium between the normal form and the internal salt, which is necessarily influenced by the amount of water present. The equation

\[
\begin{align*}
\text{N-CH}_2\text{COOH} + \text{water} & \rightleftharpoons \text{NH}_2^+ \text{N-CH}_2\text{COO}^- \\
\end{align*}
\]

is a rough representation of the equilibrium, an excess of water leading to a greater proportion of the internal salt.
With the water content below a certain limit, therefore, some of the oxide has a free $>\text{NH}$ group and a transfer of oxygen can take place giving the secondary hydroxylamine.

\[
\text{NH}_2^+ \quad \rightarrow \quad [\text{NH} \quad \rightarrow \quad \text{OH}]
\]

\[
\text{N.CH}_2\cdot\text{COO}^- \quad \rightarrow \quad [\text{N.CH}_2\cdot\text{COOH} \quad \rightarrow \quad \text{N.CH}_2\cdot\text{COOH}]
\]

In cold alcohol, very little of the amine-oxide dissolves so that the freeing of the $>\text{NH}$ group and the transference of oxygen cannot proceed very far; addition of water to dissolve the amine-oxide of course prevents the formation of the free $>\text{NH}$. If however the alcohol or aqueous alcohol solution is warmed on the water bath the change is accelerated as the moisture already present is removed with the alcohol. When the alcohol has evaporated, the addition of fresh alcohol and a repetition of the process makes the change a continuous one and the amine-oxide is completely transformed into the secondary hydroxylamine. The reason for the completion of the change, for the fact that none of the amine-oxide remains, is probably the same as that for the initial blocking up of the $>\text{NH}$ group, namely the greater coordinating power of the secondary nitrogen which will continue to co-ordinate an atom of oxygen until none of the N-oxide remains.

The fact that the p-toluene-sulphonyl derivative of the amine-oxide, and the phenyl thiourea do not undergo the corresponding changes with alcohol [p. 74.] can now be
explained; the proximity of the acyl group was observed by Wolffenstein to prevent the nitrogen from becoming pentavalent \[pp. 91 \] and therefore the transfer of oxygen from N to N' cannot take place.

The non-oxidisability of piperidino-acetic acid and of p-toluene-sulphonyl-piperazino-acetic acid by hydrogen peroxide can be explained on the same lines. In the former case, there is only one nitrogen in the molecule and in aqueous solution this becomes pentavalent in the internal salt formation; it cannot therefore be attacked by the oxygen of the hydrogen peroxide to give an amine-oxide. In the case of the p-toluene-sulphonyl derivative another factor enters, the effect of the acyl group referred to in the last paragraph; the N' is therefore preventing from forming an internal salt, also from forming an amine-oxide by oxidation. The N is then in the same state as that in the piperidino-acetic acid, and no oxidation can take place.

The behaviour of the oxidation product of the ethyl-piperazino-acetic acid \[p. 73. \] can also be explained; the ethyl group is likely to make the coordinating power of the N' greater than that of N, \( \text{C}_2\text{H}_5^- \) being more strongly electron-repelling than \( \text{H}^- \), and the reaction with hydrogen peroxide is therefore similar to that of piperazino-acetic acid, giving the N-amine-oxide. This compound can split up into ethyl-piperazine and formaldehyde just as the amine-oxide B gives piperazine and formaldehyde. The effect of alcohol,
too, is similar, the internal salt passing into the normal form, and the transfer of oxygen taking place giving the N'-amine-oxide; this is apparently unstable and readily splits up into piperazine and acetaldehyde.

If the view is held that the product C consists of

\[
\begin{align*}
\text{CH}_2-N-\text{CH}_2 \\
\text{CH}_2-N^+\text{CH}_2 \\
\text{CH}_2\cdot\text{COOH}
\end{align*}
\]

the action of alcohol in promoting the change is clearly one of dehydration, the conversion of the hydrated form of the amine-oxide

\[
\begin{align*}
\text{NH} \\
\text{OH} \\
\text{CH}_2\cdot\text{COOH}
\end{align*}
\]

into the ring compound involving the loss of a molecule of water. Again, the p-toluene-sulphonyl derivative could not undergo the change since there is no hydrogen available for loss of water. So far, then, the ring formula explains the facts as well as the secondary hydroxylamine formula. But this is not the case when the behaviour of the amine-oxide of ethyl-piperazino-acetic acid is considered; this acid, like the p-toluene-sulphonyl derivative, has no hydrogen available for loss of water and should undergo no change if the ring formula is the true explanation. It is known, however, that alcohol has some effect, and the formation of acetaldehyde is a clear indication that the oxygen has been in some way transferred to the N'; it is difficult to explain
this in any other way than that given above. It should be remembered however that very little is known about the oxidation of the ethyl acid; the experimental difficulty in preparing the acid, the small yields obtained, as well as the ready decomposition of the oxidation product and the practical impossibility of isolating the products, have prevented a detailed investigation. It can however be said that as far as the present investigation has gone the facts as to the oxidation of derivatives of piperazino-acetic acid can best be explained by the view described above, namely the formation of an amine-oxide which is capable, under certain conditions and when the nature of the molecule allows, of undergoing a change in which an atom of oxygen is transferred from the N to the N'.

It appears strange that the >NH groups which are inactive to hydrogen peroxide in water yet react easily with p-toluene-sulphonyl chloride and phenyl isothiocyanate; for example, the >NH in piperazino-acetic acid. It would seem that the >NH is normal in its reactions, except in that with hydrogen peroxide, and it may be that this is the only one for which a free >NH is essential. It is possible that the other reactions can take place whether the molecule is in the internal salt form or not, since it is through the hydrogen of the >NH that action takes place, and not by coordination of nitrogen and oxygen as in the oxidation. Thus the reaction might be represented:—
\[
R >^\dagger \text{NH}_2 + \text{C}_7\text{H}_7\text{SO}_2\text{Cl} \rightarrow \left[ R >^\dagger \text{NH-SO}_2\cdot\text{C}_7\text{H}_7 \right] \rightarrow H.R >^\dagger \text{N-SO}_2\cdot\text{C}_7\text{H}_7
\]

In other words, the reaction with hydrogen peroxide is essentially a coordination and cannot take place if the coordinating power of the \( >\text{NH} \) is already engaged, whereas the reaction with other reagents involves the hydrogen atom in the \( >\text{NH} \), that is the third valency bond and not the coordinate link. An alternative expression of this view is that the reactions may be represented by an equilibrium

\[
R >^\dagger \text{NH}_2 + \text{Reagent} \rightleftharpoons \left[ H.R >^\dagger \text{NH} \right] \downarrow \text{Reagent}
\]

and the complex then \( \rightarrow H.R >^\dagger \text{N-X} \). Then with hydrogen peroxide the equilibrium is in favour of the zwitterion and with other reagents in favour of the complex with the reagent.

The reason for the equilibrium being so altered in the reaction with \( p \)-toluene sulphonyl chloride is that an alkaline solution is used which prevents the formation of the zwitterion; in the reaction with phenyl isothiocyanate a possible reason is the presence of alcohol which is necessary to the reaction, but it should be remembered that the necessity may be due to its effect in dissolving some of the reagent, which is insoluble in water, and quite unconnected with its effect of preventing the zwitterion formation.

The position with regard to the oxidation of piperazine derivatives by hydrogen peroxide may now be briefly summarised thus; in cases which in aqueous solution contain a tertiary nitrogen free to coordinate an atom of oxygen, an amine-oxide...
is formed; among these compounds, those which contain a second nitrogen capable of becoming pentavalent pass over, under the influence of an agent such as alcohol which prevents the formation of internal salts, into a secondary hydroxylamine or an amine-oxide according to whether the second nitrogen is secondary or tertiary. Those which have another tertiary nitrogen, attached to such groups that it cannot become pentavalent, are unchanged by the action of alcohol. Substances which contain no carboxy-group, and which do not therefore form internal salts, and which contain secondary nitrogen groups are oxidised by hydrogen peroxide to give secondary hydroxylamines.

Some points of special interest in the work on the oxidation of piperazino-acetic acid may here be recapitulated. The amine-oxide described is of a new type, being the oxide of an amino-acid; those previously described were oxides of tertiary amines containing alkyl- or aryl groups with no acid chain. Thus although the method of oxidation by hydrogen peroxide to give amine-oxides is not new, it having been extensively applied by Wolffenstein and others, this is the first case of application to amino-acids; earlier work on this subject was concerned with more violent oxidations with such agents as potassium permanganate and hydrogen peroxide catalysed by ferrous sulphate, resulting in the breakdown of the amino-acid into aldehydes and ammonia. It is true that piperazino-acetic acid is of a different type from those
previously investigated which consisted chiefly of primary amino-acids; but it has been shown that with stronger oxidising agents such as bromine water piperazine and formaldehyde are formed from the acid, a reaction parallel to the formation of ammonia and aldehydes from the primary acids. It would seem therefore that the method of oxidation by hydrogen peroxide might be applied to other amino-acids with similar results, provided they contained a secondary or tertiary nitrogen group; a secondary hydroxylamine or an amine-oxide should then be formed. A full investigation of the secondary hydroxylamine formed from piperazino-acetic acid, through the amine-oxide, is difficult owing to the instability and high solubility of the compound; it would be of interest therefore to investigate the properties of secondary hydroxylamines from other amino-acids with different solubility relations and to compare them with the dialkyl hydroxylamines which have already been described.

It appears at first strange that the amine-oxide should be transformed into the secondary hydroxylamine by transfer of an atom of oxygen without any oxidation of the acid chain taking place, while the oxygen in the secondary hydroxylamine does oxidise the acid chain very easily. Other properties of the secondary hydroxylamine, showing the great instability and great mobility of the oxygen have been described. The amine-oxide appears to be fairly stable except in its change to the secondary hydroxylamine which is
certainly easily effected. But it should be remembered that the primary force effecting it is the greater coordinating power of the \( >\text{NH} \) group, resulting in a shift of a coordinate link from one nitrogen to the other, when the internal salt passes into the normal form in alcohol. When that has occurred, the oxygen oxidises the \( >\text{NH} \) to \( >\text{N-OH} \), thus forming an extremely reactive oxidising group.

In connection with the breakdown of the molecule by oxidation of the acid chain, the much greater stability of the \( >\text{CH}_2 \) group in the piperazine ring compared with that in the chain should be noted. In all decompositions of the amine-oxide and of the secondary hydroxylamine, the ring remains intact while the acid chain is split off. Again oxidation by permanganate and by bromine water causes first the breakdown of the acid chain leaving the piperazine ring unattacked.

The properties of the two compounds, the amine-oxide and the secondary hydroxylamine, here described afford further evidence that in piperazine derivatives the groups attached to one nitrogen atom have some influence on the other end of the ring, that there is some action through the ring not expressed by the ordinary formula. Anomalies previously noted include the extraordinary difference in solubility between the mono- and di-carbethoxy compounds. [J.C.S. 1929, p.45]. It is found, in the preparation of the former, that the di-carbethoxy piperazine is extracted
from an aqueous solution by ether, whereas the mono-carb-
ethoxy piperazine is only extracted when the solution is
saturated with potassium carbonate. Another point of
interest in the same connection is found in the properties
of the benzoyl derivatives of piperazine; mono-benzoyl
piperazine is so unstable that it is hydrolysed by cold
aqueous alkali on standing, and very rapidly by cold alco-
holic potash, while dibenzoyl piperazine is only decomposed
after boiling for some hours with alcoholic potash. [V.M.
Thorn's thesis, p. 8]. In the course of the present work,
too, many attempts were made to prepare the benzoyl deriva-
tive of piperazino-acetic acid; an outstanding difficulty
in the way is the solubility in water, but the general in-
dication of the experimental work was that this was not the
only reason for the failure to prepare it, but that it was
very easily hydrolysed, giving benzoic acid and piperazino-
acetic acid. An indication of similar forces acting through
the ring is given by the high oxidising power and consequent
instability of the secondary hydroxylamine compared with
other secondary hydroxylamines.

The explanation given above, based on the "zwitterion"
formation, of the oxidation of piperazino-acetic acid and
its derivatives breaks down at one point; although the non-
oxidation of piperidino-acetic and p-toluene-sulphonyl
piperazino-acetic acids is explained by the theory, the same
theory requires that the sodium salts in aqueous solution
should be oxidised. The experimental facts are that very little, if any, oxidation takes place under the same conditions as those used for piperazino-acetic acid, or in double the time, the original acid being recovered from the solution. This anomaly can only be explained by the assumption of an influence of the groups at one end of the ring on the other end.

In order wholly to account for the experimental facts therefore, the theory of "zwitterion" formation must be amplified by the theory of action through the ring. Looking at the results from this point of view, the conclusion is reached that in the molecule

![Chemical Structure]

oxidation can only take place at N when R carries a positive charge. When R is not positive, the negative charge of the carboxyl group inhibits oxidation at N. Considering the lower part of the molecule, it is seen that oxidation, with the formation of an amine-oxide, leads to

![Chemical Structure]

The oxygen of the amine-oxide would not carry a whole unit charge but dipole moments of amine-oxides show that the charge is large. Therefore, just as the negative charge produced by the first ionisation of a dibasic acid offers a great resistance to the appearance of a second negative charge, so the effect of the negative charge of the carboxyl group is
to oppose the oxidation resulting in the appearance of another negative charge. Now when R is positive, the carboxyl group will be attracted towards R and away from N, the positive and negative charges tending to approach near to each other; thus the inhibiting effect on oxidation is diminished or altogether removed. This is the case with piperazino-acetic acid and ethyl-piperazino-acetic acid; in water they are represented by

\[
\begin{align*}
\text{NH}_2 & \quad \text{O}^- \\
\text{N} & \quad \text{CO} \\
\text{CH}_2 & \\
\end{align*}
\quad \text{and} \quad 
\begin{align*}
\text{C}_2\text{H}_5 & \quad \text{O}^- \\
\text{NH}^+ & \quad \text{CO} \\
\text{CH}_2 & \\
\end{align*}
\]

and oxidation can take place. Further, when the amine-oxide is formed, the effect of the charge on the nitrogen is to make the acid stronger than piperazino-acetic acid, as described on p. 98. With the p-toluene-sulphonyl derivative and piperidino-acetic acid, R is not positive and the inhibiting effect on oxidation is therefore great. In alkali, too, the carboxyl charge would inhibit the formation of an amine-oxide, the salts being represented by the formulae:

\[
\begin{align*}
\text{C}_7\text{H}_7\text{SO}_2 & \\
\text{N} & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{O} & \quad \text{O} \\
\end{align*}
\quad \text{and} \quad 
\begin{align*}
\text{N} & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

On the same view the instability of the amine-oxides to alkali can be explained. The oxide of piperazino-acetic acid in alkali can be represented by
and the carboxyl charge renders the oxygen less stable. It is then liberated and oxidises the $-\text{CH}_2$ of the acid chain, giving piperazine and sodium carbonate.

It would seem, therefore, from this work, that a further investigation should be made of the influence of substituents in the piperazine ring, of the very large effect that the introduction of a second group has on the solubility and stability of the compounds and of products, in particular oxidation products obtained from them.

The result of the investigation of the oxidation of piperazino-acetic acid and related compounds by hydrogen peroxide may therefore be briefly stated thus:— the experimental facts as to the oxidation or non-oxidation of the compounds concerned, and the properties of the oxidation products obtained can be explained on the view that in aqueous solution, the carboxylic derivatives of piperazine exist chiefly as the zwitterion

\[
\text{or } \quad \begin{array}{c}
\text{R} \\
\text{NH}^+ \\
\text{N} \cdot \text{CH}_2 \cdot \text{COO}^- \\
\end{array}
\]

modifications must however be made on account of the influence of substituent groups through the piperazine ring, about which very little can at present be stated with certainty.
Properties of the amine-oxide B.

(1) A weighed quantity of the product was boiled for about ten minutes with an excess of normal caustic soda. After being cooled, the solution was treated with benzoyl chloride, and the precipitate of dibenzoyl piperazine was collected, dried, and weighed. It was found that an almost quantitative yield was obtained.

0.4 gm. B gave 0.58 gm. dibenzoyl piperazine, m.pt. 196°C.

Theoretical yield = 0.65 gm.

(2) A weighed quantity of B was dissolved in water and warmed on the water-bath with zinc dust. After half an hour, the solution was filtered from the zinc and treated with phenyl isothiocyanate under the usual conditions [p. 58]. The phenyl thiourea obtained was shown to be that of piperazine-acetic acid [M.pt. and mixed M.pt. = 223°C.] No other product was obtained.

0.2 gm. B gave 0.24 gm. phenyl thiourea.

Theoretical yield = 0.35 gm.
(3) A small quantity of B was dissolved in water and dilute sulphuric acid added; the mixture was distilled. The distillate was found to contain a trace of formaldehyde, identified by the reduction of ammoniacal silver nitrate and the colour tests [p. 60.]; the residue was made alkaline and treated with benzoyl chloride. A trace of dibenzoyl piperazine was obtained. The main product was not isolated and no derivatives could be obtained.

(4) A small quantity of B was dissolved in water and a little acetic acid added; to this was added some potassium iodide solution. The colour deepened and by testing with starch solution, it was shown that iodine had been liberated. The iodine quickly disappeared and when the solution was treated with phenyl isothiocyanate, only the diphenyl thiourea of piperazine was obtained, melting point = 274°C.

(5) A weighed quantity of B was boiled under reflux with acetic anhydride for one hour. The excess of anhydride was then distilled off, and the remainder decomposed by pouring the mixture into a little water. Evaporation of this solution to dryness, followed by treatment with alcohol gave a substance soluble in alcohol, and recrystallised from benzene which proved to be diacetyl piperazine.

M.pt. of product = 135°C.
M.pt. of diacetyl piperazine = 138°C.
Mixed M.pt. = 136°C.
Composition of product, C = 57.3%, H = 8.4%, N = 15.3%,
O = 19.0%.

Calculated for diacetyl piperazine, C₈H₁₄N₂O₂,
C = 56.5%, H = 8.3%, N = 16.4%,
O = 18.8%.

Yield. 0.4 gm. B gave 0.38 gm. acetyl compound.
Theoretical (for diacetyl piperazine) = 0.42 gm.

***    ***    ***

Oxidation of piperazino-acetic acid with two equivalents of hydrogen peroxide.

A weighed quantity of piperazino-acetic acid was dissolved in water and two equivalents of hydrogen peroxide were added. The mixture was allowed to stand for forty-eight hours. At the end of that time, the solution still contained hydrogen peroxide, and this amount did not appear appreciably to decrease during further standing. Evaporation of the solution and precipitation with alcohol gave a substance which proved to be the amine-oxide B, giving the same phenyl thiourea [M.pt. 186°C].

The experiment was repeated and at the end of two days, 0.01 gm. of ferrous sulphate was added to the solution, to catalyse the second oxidation. Great heat was immediately developed, and when the solution was tested with phenyl isothiocyanate, it was found that a quantity of piperazine
had been formed. Dibenzoylpiperazine [M.pt. 196°C.] was also obtained from the solution with benzoyl chloride.

The solution of the amine-oxide B, prepared by treating piperazino-acetic acid with one equivalent of hydrogen peroxide, was cooled in ice, and 0.01 gm. of ferrous sulphate added. An equivalent of hydrogen peroxide was then added slowly with stirring and cooling. The solution was evaporated and a gummy solid was obtained; treatment with phenyl isothiocyanate gave a small quantity of a phenyl thiourea which had not previously been obtained [M.pt. 156°C.]. A nitrogen estimation was carried out and the result indicated that it might be the diphenyl thiourea of an open chain dicarboxylic acid.

\[
\begin{align*}
\text{C}_6\text{H}_5\text{NH-CS-NCH}_2\text{CH}_2\text{CH}_2\text{N-CS-NH-C}_6\text{H}_5 \\
\text{CH}_2\text{COOH} \\
\text{CH}_2\text{COOH}
\end{align*}
\]

Found \( N = 12.8\% \). Calculated for \( \text{C}_{20}\text{H}_{22}\text{N}_8\text{S}_2\text{O}_4 \) \( N = 12.5\% \).

No other derivative could be obtained from the residue.

Properties of the substance \( C \).

(1) A little of the freshly-prepared gummy solid \( C \) was dissolved in water and a drop of a universal indicator added. The colour indicated that the \( p_H \) value was approximately 8, showing that \( C \) is slightly more alkaline than either the amine-oxide \( B \) \( [p_H \approx 4] \) or piperazino-acetic acid \( [p_H \approx 7.5] \). After an hour's standing in solution, the \( p_H \) value was found to have changed to 5-6.
A solution of freshly-prepared C was treated with phenyl isothiocyanate. No trace of the diphenyl thiourea of piperazine was found.

A similar solution was allowed to stand for about half an hour, and at the end of that time, a further test was made and some diphenyl thiourea of piperazine was precipitated.

A solution of C in alkalı decomposed more rapidly than the aqueous solution; a precipitate of dibenzoyl piperazine was obtained by treatment with benzoyl chloride after a few minutes.

A solution of C in acid also decomposed rapidly, piperazine again being obtained. Some of the solution was distilled, and the distillate was shown to contain a trace of formaldehyde. [See tests on p. 60.]

A solution of C was found to be very strongly reducing. A part was added to an ammoniacal silver nitrate solution and the mixture warmed on the water-bath. Rapid reduction took place.

A second part was treated with Fehling’s solution and warmed; a copious precipitate of cuprous oxide was formed.

A third part was treated with copper acetate and warmed; a precipitate of cuprous oxide was rapidly formed.

A solution of C was warmed on the water bath with zinc dust for about an hour. The solution was then filtered
and tested with phenyl isothiocyanate; a precipitate was obtained which consisted of the phenyl thiourea of piperazino-acetic acid, and of the diphenyl thiourea of piperazine.

This experiment was not done quantitatively as it was difficult to weigh the gummy solid C with any accuracy without long drying; the result indicated that about half the substance underwent decomposition into piperazine while the rest was reduced by the zinc to the original acid.

\[
\text{Wt. of } \text{C}_6\text{H}_5\text{NH.CS.N.C}_4\text{H}_8\text{.N.CH}_2\text{.COOHN} = 0.2 \text{ gm.} \\
\text{Wt. of } \text{C}_6\text{H}_5\text{NH.CS.N.C}_4\text{H}_8\text{.N.CS.NH.C}_6\text{H}_5 = 0.25 \text{ gm.}
\]

(5) A solution of C was acidified with acetic acid solution and potassium iodide added. A trace of iodine was momentarily formed, but it rapidly disappeared, the action presumably being comparable with that in the case of the amine-oxide \( \text{pp. 95-96} \).

(6) A weighed quantity of C was boiled under reflux with acetic anhydride. The residue after the removal of the excess anhydride was treated as before \( \text{p. 132.} \) and a quantitative yield of diacetyl piperazine was obtained.

Yield from 0.3 gm. of C = 0.3 gm.

Theoretical yield = 0.31 gm.
Preparation and analysis of picrate of the substance C.

A specimen of C was prepared by warming the amine-oxide with 75% alcohol on the water bath, and then evaporating to dryness; more alcohol was added and again evaporated. The gummy solid was then immediately treated with an aqueous solution of picric acid and a precipitate obtained which was filtered and dried over sulphuric acid. It was found to be very sparingly soluble in hot water and hot alcohol and was therefore different from the picrates of piperazine, piperazino-acetic acid, and the amine-oxide B. It decomposed explosively between 260 and 270°C.

A nitrogen estimation was carried out with the following result:

Vol. of nitrogen from 0.0814 gms. picrate, at 19.8°C.
and 757 mm. = 12.95 c.c.s.

\[ \text{N}_2 = 18.1\% \]

A second specimen of C which had been allowed to stand for an hour after being prepared and so contained some piperazine, was treated with phenyl isothiocyanate under the usual conditions and the diphenyl thiourea of piperazine was removed. The excess of the reagent was removed by treating the residue with alcohol, and the gummy solid was treated with an aqueous solution of picric acid. A precipitate was obtained, which showed the same insolubility and the same high decomposition
point (260-70°C).

A nitrogen estimation was carried out:

Vol. of nitrogen from 0.0825 gm. picrate at 21°C. and 767 mm. = 13.5 ccs.

\[ \therefore N_2 = 18.3\% \]

The agreement between these two results showed that the removal of piperazine was an unnecessary procedure.

A third specimen of the picrate was prepared by the first method, i.e. directly from C, and a nitrogen estimation was carried out:

Vol. of nitrogen from 0.0833 gm. at 20.1°C. and 767 mm. = 13 ccs.

\[ \therefore N_2 = 18.0\% \]

The first specimen referred to was then analysed for carbon and hydrogen.

0.0697 gm. picrate yielded 0.0912 gm. CO₂ and 0.0173 gm. H₂O.

\[ \therefore C = 35.7\% \]
\[ H = 2.7\% \]

A weighed quantity of the same specimen was heated on the water-bath with 80% sulphuric acid, and the solution evaporated; the sulphuric acid solution was shaken with benzene, and then with chloroform to extract the picric acid. The benzene and chloroform solutions were evaporated to dryness and the residue dissolved in a little water and the
amount estimated by the potassium iodide - potassium iodate method. The sulphuric acid solution was quite colourless, and on diluting with water there was still no trace of yellow colour, showing that the picric acid had all been extracted and the estimation therefore reliable.

0.1034 gm. picrate yielded 0.0767 gm. picric acid.

**Discussion of results.**

Owing to the explosive nature of the picrate, the combustions had to be carried out with very small quantities of the material well mixed with powdered copper oxide, and the heating was done very slowly. The experimental error, necessarily greater than in an ordinary combustion, was greater in the carbon and hydrogen estimation than in the nitrogen as the copper oxide was not spread out so extensively, and the increases in weight were very small. The nitrogen values therefore, being in agreement, have been taken as more reliable.

Taking the nitrogen content 18.1% as a basis, it was calculated that the picrate consisted of two molecules of picric acid to one of a base of molecular weight 160.

\[
\text{[Picric acid in 0.1034 gm., calculated on this basis]} = 0.078 \text{ gm.} \\
\text{Found} = 0.0767 \text{ gm.}
\]
Composition found was $C = 35.7\%$
$H = 2.7\%$
$N = 16.1\%$
$O = 43.5\%$

giving as the formula of the base

$$C_{18}H_{16}N_{8}O_{17} \cdot 2 \times \text{mol. picric acid}$$

$$= C_{6}H_{10}N_{2}O_{3}$$

Formula of secondary hydroxylamine $\quad \text{HO-N} \quad \text{CH}_{2}\cdot\text{CH}_{2} \quad \text{CH}_{2}\cdot\text{CH}_{2} \quad \text{N-CH}_{2} \quad \text{COOH}$

$$= C_{6}H_{12}N_{2}O_{3}$$

and calculated percentages for the picrate $C_{18}H_{16}N_{8}O_{17}$

are $C = 35.0\%$
$H = 2.9\%$
$N = 16.1\%$
$O = 44.0\%$

These results indicate that the base contains one atom of oxygen more than piperazino-acetic acid.

*** *** ***

Determination of the molecular weight of the base $C$ by the depression of the freezing point.

A specimen of the base $C$ was introduced into a weighing bottle and dried to constant weight over sulphuric acid.
A solution of the solid was made and the freezing point found; the solution was then immediately treated with benzoyl chloride, and the dibenzoyl piperazine formed was filtered, dried and weighed.

In a second experiment, two portions of the same specimen of G were dried to constant weight and the amount of piperazine was found in one, simultaneously with the freezing point of a solution of the other. The amount of piperazine in the solution was also found and it appeared that very little more was formed during the time taken for the freezing point determination.

In the third experiment similar to the first, a portion of the same specimen of G was treated with picric acid at the same time as the piperazine was estimated in the solution. The picrate was filtered and weighed. It appeared to be the picrate of the base C [Decomposition point = 260-70°C.] and the amount found was in agreement with the amount of piperazine found.

Results

(1) Specimen I

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of solid</td>
<td>= 0.2557 gm.</td>
</tr>
<tr>
<td>&quot; water</td>
<td>= 17.75 gm.</td>
</tr>
<tr>
<td>Depression</td>
<td>= 0.18°C.</td>
</tr>
<tr>
<td>Wt. of piperazine</td>
<td>= 0.0179 gm.</td>
</tr>
<tr>
<td>&quot; C</td>
<td>= 0.2378 gm.</td>
</tr>
<tr>
<td>Depression due to C</td>
<td>= 0.154°C.</td>
</tr>
<tr>
<td>.&quot; Molecular Weight</td>
<td>= 162</td>
</tr>
</tbody>
</table>
(2) Specimen II.

Wt. of solid = 0.3046 gm.
" " water = 21.75 gm.
Depression = 0.192°C.

Wt. of piperazine in this wt. of solid, found simultaneously = 0.0207 gm.
Wt. found after the freezing-point determination = 0.0213 gm.
Mean = 0.021 gm.
Wt. of C = 0.2836 gm.
Depression due to C = 0.171°C.
∴ Molecular Weight = 148

(3) Specimen III.

Wt. of solid = 0.2991 gm.
" " water = 19.15 gm.
Depression = 0.20°C.
Wt. of piperazine = 0.037 gm.
" " C = 0.2621 gm.
Depression due to C = 0.1582°C.
∴ Molecular Weight = 161

Wt. of picrate obtained from 0.526 gm. of same specimen = 1.7745 gm.
∴ Wt. of C = 0.4595 gm.

Calculated from amount of piperazine found above,
Wt. of C = 0.4609 gm.

***   ***   ***
Experiments with carbon bisulphide and sulphur dioxide.

(1) A little of the gummy solid G was treated with carbon bisulphide; as the solid was stirred and broken up a cloudiness appeared in the liquid indicating the formation of a compound. After standing for a few minutes the cloudiness was resolved into a precipitate which was filtered from the excess carbon bisulphide. On being exposed to the atmosphere, it immediately deliquesced and when the sticky solid was dissolved in water a suspension of sulphur was found; the solution was then treated with phenyl isothiocyanate under the usual conditions and a precipitate of the phenyl thiourea of piperazino-acetic acid was obtained [M.pt. 223°C.]

(2) Sulphur dioxide was passed into an aqueous solution of the solid G. No precipitate was formed, and evaporation and treatment with alcohol gave a precipitate of the original piperazino-acetic acid [M.pt. 276°C.]

A concentrated solution of sodium bisulphite was then added to an aqueous solution of G; the mixture was then acidified with hydrochloric acid, but no sulphur compound was precipitated.

A solution which had been saturated with sulphur dioxide was then divided into two parts; one part was treated with phenyl isothiocyanate and the precipitate obtained was the phenyl thiourea of piperazino-acetic acid [M.pt. 223°C.]
The other part was acidified with hydrochloric acid and barium chloride added; a white precipitate proved the presence of sulphuric acid, indicating that the substance had oxidised the sulphurous to sulphuric acid, being at the same time reduced to piperazino-acetic acid.
The nature of the green solid obtained from copper piperazino-acetate

(a) The problem of the nature of the green solid, with which this investigation began, was reconsidered in the light of the theoretical and practical work described in the last chapter. It was thought to be not unlikely that the change which the amine-oxide undergoes with alcohol was connected with the change which the blue copper salt of piperazino-acetic acid undergoes under similar conditions. It seemed therefore that a purer specimen of the green salt should be obtained from the oxidation product itself; a solution of the amine-oxide was then shaken with copper hydroxide in the cold, and the deep blue solution evaporated, also in the cold. The solution remained blue until it was of a thick gummy consistency and then as the edges began to dry a greenish colour appeared; when it was completely dry it was quite green in colour and gave a greenish-blue solution. In a later experiment it was found that the beginning of the colour change was simultaneous with the formation of cuprous ions in solution, detected by p-dimethyl-amino-benzylidene-rhodamine. There was no means of testing whether the cuprous salt was present in the dry solid or only formed in
solution. When the green solid was heated or warmed with alcohol a brown solid was formed which gave an immediate precipitate of cuprous oxide with water, and a greenish-brown aqueous solution. This solution resembled in its reactions the green solution obtained directly from the blue copper salt of piperazino-acetic acid. With phenyl isothiocyanate, a trace of the diphenyl thiourea of piperazine was formed but no other compound could be isolated, so that apparently there was a product with no free >NH group. Distillation with acid yielded some formaldehyde and in the residue piperazine was found approximately equivalent to the formaldehyde. [Found 0.04 gm. piperazine, 0.01 gm. formaldehyde. Calculated equivalent of 0.04 gm. piperazine is 0.014 gm. formaldehyde]. Distillation with alkali yielded piperazine in the distillate; only sodium carbonate could be found in the residue although tests were made for oxalate, formate and glyoxylate.

It was thought that if the brown salt contained the product C, the sparingly soluble, high melting-point picrate should be obtained from the brown solution; in order to keep the copper in solution, hydrochloric acid had to be added, and this would keep some of the picrate of C in solution. It is however known to be only sparingly soluble in dilute hydrochloric acid [p. 15] and it was thought that some might be precipitated. A precipitate was in fact obtained by adding picric acid to an aqueous solution of the
brown salt acidified with hydrochloric acid. This was very sparingly soluble in water and decomposed at 260-270°C. with explosive violence, and thus appeared to be identical with the picrate of C. It was clear therefore that the change which takes place in the product B, takes place also in the copper salt of B. The formation of cuprous oxide is now explained by reduction of the cupric salt by the hydroxylamine group.

An attempt had been made to prepare the brown salt directly from a solution of C but it was unsuccessful; very little copper appeared to go into solution. This failure can now be explained; the presence of the copper causes decomposition of the hydroxylamine to piperazine and this prevents the copper hydroxide from dissolving and causes decomposition of some of the copper salt already formed.

The similarity in reaction between the brown salt and the green solid obtained directly led to the idea that the same product might be present in the latter. It became of importance therefore first to establish definitely whether oxygen was essential to the colour change, or whether oxidation occurred at the expense of the alcohol, and secondly to ascertain whether the amount of oxygen absorbed was of molecular proportion. Oxidations by oxygen catalysed by copper have been described before; I.S. McLean [Biochem.J. 1929, p.593] found that copper salts were more active catalysts than ferrous salts in the oxidation of fatty acids, and
that they gave reproducible results. She suggested that the oxygen atom attaches itself loosely to the copper to give a cupric peroxide which then splits up again in oxidising the fatty acid. Traube and Schönewald [Ber. 1909, p.179] found that amines were oxidised in air, in the presence of copper powder as catalyst, to aldehydes and ammonia.

Comparative experiments were performed in which suspensions of the blue copper piperazino-acetate in alcohol were shaken in an atmosphere of oxygen, nitrogen and carbon dioxide, respectively. A remarkable difference was observed between the first of these experiments and the two last; in the presence of oxygen the solid began to turn green in less than an hour, while in the presence of nitrogen and carbon dioxide, the colour was unchanged after several days.

It was thought that in the conversion of the blue salt to the green substance in the presence of alcohol, the oxygen might first react with either the alcohol or the water present to give acetaldehyde or hydrogen peroxide which would then oxidise the copper salt. Accordingly experiments were carried out to ascertain whether either of these compounds were formed intermediately. For this purpose an alcoholic suspension of the blue copper salt was heated in an atmosphere of oxygen, and the distillate was tested for acetaldehyde by the usual tests, namely ammoniacal silver nitrate, Schiff's reagent, and the sodium nitroprusside test. No trace of aldehyde was obtained. The test used for hydrogen
peroxide was a very sensitive one, the details of which were kindly supplied by Dr. Gulland of the Lister Institute; it depends on the combination of hydrogen peroxide with cerous hydroxide to give an insoluble ceric hydro-peroxide which is yellow-orange in colour, and it is capable of detecting quantities of the order of $10^{-5}$. It is particularly useful in cases where the hydrogen peroxide is formed intermediately as the yellow hydroperoxide is formed and the hydrogen peroxide is no longer free to undergo its usual reaction. In the present case, the alcoholic suspension of the blue salt was shaken with a little precipitated cerous hydroxide in an atmosphere of oxygen. No trace of yellow colour was observed in any of a number of experiments and it was therefore concluded that the oxidation of the copper salt was a direct attack by the oxygen.

Fresh light was thrown on the colour change of the copper salt by an experiment performed to determine whether the amount of oxygen absorbed was of molecular proportions. A flask containing the alcohol suspension of the solid was connected with a Lunge nitrometer filled with mercury, and with a source of oxygen. Oxygen was drawn into the vessel and the volume noted, and the apparatus was left for several hours, the flask being shaken from time to time. A definite absorption of oxygen took place, corresponding to about one third of the theoretical volume; after about ten hours no further change of volume occurred. Control experiments, in
which alcohol was substituted for the suspension of the salt, showed that the absorption found was not due to absorption by the alcohol or small leakages in the apparatus, as there was no change of volume during the same time. In another experiment with the copper salt, a little aqueous potash was introduced into the nitrometer after the absorption of oxygen. There was no change in volume, showing that no carbon dioxide had been evolved during the oxidation.

In two similar experiments, in which the volumes of oxygen absorbed were different, the greenish-blue solids obtained by the oxidation were dissolved in water and the solution boiled until there was no further deposition of cuprous oxide; in both cases the ratio of cuprous oxide to the oxygen absorbed was approximately Cu₂O : 0. This fact indicates that an oxidation product is formed which reduces the cupric ions to cuprous, giving a precipitate of cuprous oxide.

The results of the two experiments mentioned are given below:

| I. | Wt. of copper salt | = 0.5 gm. |
|    | Vol. of oxygen absorbed | = 9 ccs. |
| Theoretical volume for one O : one molecule of acid | = 23 ccs. |
| Wt. of Cu₂O obtained on boiling | = 0.1 gm. |
| Calculated Wt. of Cu₂O equivalent to 9 ccs. O₂ in ratio Cu₂O : O | = 0.11 gm. |
II. Wt. of copper salt = 1.5 gm.
Vol. of oxygen absorbed = 20 ccs.
Theoretical volume = 69 ccs.
Wt. of Cu₂O = 0.23 gm.
Calculated in ratio Cu₂O : O = 0.26 gm.

The significance of the above experiments was enhanced by the fact that no other copper salt in the series studied absorbed oxygen under the same conditions; copper piperidino-acetate and the copper salts of ethyl-, p-toluene-sulphonyl-, and carbethoxy-piperazino-acetic acids were suspended in alcohol and connected with a measured volume of oxygen in the same way as in the experiments described above, but in no case was there the slightest change of volume. With the exception of the copper salt of the ethyl-acid, none of these salts undergo any change by warming with alcohol, or alone at 110°C., and therefore the fact that they do not absorb oxygen is of great importance in indicating that the colour change of copper piperazino-acetate is directly due to oxidation. Finally from two specimens of green solid, one obtained from the oxygen absorption experiment, and the other by heating the blue salt with alcohol, traces of picrates were obtained resembling the picrate of the secondary hydroxylamine C; the picrate was precipitated in the presence of hydrochloric acid for the reasons given on p. 146, and consequently only a small yield was obtained, but it was found to be very sparingly soluble in water and like the
picrate of G, decomposed explosively between 260-270°C.

It seemed clear, therefore, that the same product was present in the green solid as was obtained by the treatment of the oxidation product of the acid with alcohol. It was then thought that the first result of the oxygen absorption might be the formation of the amine-oxide which then underwent transformation to the hydroxylamine; accordingly a number of specimens of the greenish-blue solid obtained in the absorption experiments were treated with phenyl isothiocyanate under the usual conditions but no trace of the phenyl thiourea of the amine-oxide could be found.

It would appear from this, and from the non-absorption of oxygen by the copper salt of the ethyl-acid, that an amine-oxide is not easily formed by aerial oxidation, the secondary hydroxylamine being formed preferentially in this case. In the case of the ethyl-derivative, since the copper salt was not changed under the conditions used in the absorption experiments, it can be said that the colour change noted earlier in the work [p. 32.] is not parallel to the change from blue to green of copper piperazino-acetate; it is possibly due to a decomposition of the acid. For reasons given above [p. 131.] the investigation of the ethyl derivative has not been carried further.

The composition of the green deliquescent solid cannot be definitely established, but it is clear that it contains both the unchanged blue salt and the secondary hydroxylamine.
The amount of the latter cannot at present be stated. It would appear from the amount of oxygen absorbed that about one third of the salt is oxidised, but this cannot be confirmed by the amount of the picrate formed for two reasons. First, owing to the fact that the picrate has to be precipitated in the presence of hydrochloric acid [p. 146] some remains in solution; secondly, the immediate formation of cuprous oxide in aqueous solution shows that some decomposition of the hydroxylamine takes place. The green solid therefore probably contains some decomposition product of the hydroxylamine, in addition, formed by oxidation by cupric ions. Owing to the solubilities of the compounds concerned and to the difficulty in obtaining derivatives, especially in the presence of copper this point could not be decided experimentally. But some light on the process of oxidation is thrown by the reduction of copper salts by other hydroxylamines. For example dialkyl hydroxylamines with copper sulphate give a variety of products among which the corresponding dialkylamines, ammonia, aldehydes and acids have been identified. The presence of the dialkylamine indicates that the first step in the decomposition is a self-oxidation by the -OH group, catalysed perhaps by the copper salt, giving aldehydes and ammonia, in addition to the amine. The aldehydes may then be oxidised to the corresponding acid by the cupric salt, which is of course reduced to the cuprous salt. If a similar process occurred
in the case of the hydroxylamine C, piperazine, formaldehyde and carbon dioxide are the most likely products of self-oxidation, formaldehyde being oxidised further to formic acid or carbon dioxide by the copper present. The whole change of the blue salt would then be represented thus:

\[
\begin{align*}
&\text{Blue copper salt of piperazino-acetic acid} \\
&\text{by oxidation} \\
&\text{Green solid containing original blue salt} \\
&\text{and Cu salt of secondary hydroxylamine C} \\
&\text{by self oxidation} \\
&\text{Cu(OH)}_2 + \text{piperazine} + \text{CH}_2\text{O} + \text{CO}_2 \\
&\text{Cu}_2\text{O} + \text{piperazine} + \text{H.COOH} + \text{CO}_2 \\
&\text{or H}_2\text{O} + 2\text{CO}_2
\end{align*}
\]

Some piperazine is in fact found in the green solutions after decomposition; this does not, however, prove that the oxidation proceeds according to the above table as some piperazine is always formed by the direct decomposition of the secondary hydroxylamine in the absence of copper. The proportions of cuprous oxide and absorbed oxygen found agree with the above scheme, if the oxidation of formaldehyde only proceeds as far as formic acid. It must be noted however that formic acid has not been found in the green solutions. Further, the piperazine obtained does not correspond to the amount required by the scheme given. Small quantities
have been found, but the amounts indicated that some other product was formed by the decomposition of the secondary hydroxylamine, which still contained no free $\text{>NH}$ group. It was said above that owing to the solubilities of the substances concerned and the difficulties in obtaining derivatives, the investigation of this other product has not been possible. It has therefore not been possible definitely to establish the result of the reduction of cupric to cuprous ions by the secondary hydroxylamine.

It would seem from the experimental facts that the only product of oxidation of piperazino-acetic acid by air catalysed by copper is the secondary hydroxylamine. When the oxidation is carried out under the mildest conditions, as in the quantitative absorption experiments described, apparently only about a third is oxidised, the rest remaining unchanged; from the mixture the picrate of C is obtained. The view that the oxidation is of this simple character, resulting in the one product, is supported by the fact that the copper salts of related acids in which that simple change cannot take place are not oxidised at all. It was shown that the application of heat to the green solid obtained in the absorption experiments, or treatment with water led immediately to the breakdown of the hydroxylamine to piperazine and some other product or products at present unknown. In the early experiments with the green solution these decomposition products must have been present, as the
green solid was always prepared by heating the blue salt, either alone or with alcohol. The reactions described therefore were complicated by the presence of other substances and not due entirely to the unchanged blue salt and the secondary hydroxylamine. The reactions with potassium iodide, potassium ferricyanide, etc. were not very different from those of the blue salt solution; neither gave the normal reactions, and the chief difference between the green and the blue lay in colour changes. These cannot be regarded as of great importance, since the mere physical mixing of the deep blue solution with other coloured solutions produces changes, unconnected with chemical reactions. The most important property of the green solution, exhibiting a difference from the blue, is that in neutral solution the copper is not entirely precipitated by sulphuretted hydrogen. This indicated that some stable complex of copper was present and support for this view was found in the reaction with caustic soda [p. 16]. It will be remembered that some copper hydroxide was precipitated, leaving the solution of a greenish-brown colour still containing copper.

The nature of the green solid obtained from the blue copper salt has not therefore been determined, but experimental work has shown that the cause of the colour change is an aerial oxidation catalysed by the copper resulting in the formation of a secondary hydroxylamine. The ready
decomposition of this, spontaneously and by oxidation by the copper, has prevented the preparation of the pure salt of the hydroxylamine; it has also prevented a study of its reactions. The peculiar properties of this class of compounds have made it impossible to determine the nature of the decomposition products. Copper salts of related acids, which contain no $>\text{NH}$ group, are not oxidised by air and undergo no colour change corresponding to that of the blue salt.

The difficulty experienced in preparing the pure blue copper salt of piperazino-acetic acid [Chap.2] can now be explained. It will be remembered that in every experiment in which heat was applied a gummy solid was obtained which would not crystallise and in many cases the colour changed to a greenish blue. In the light of the more recent work it is clear that under these conditions of heating with copper oxide, or heating the concentrated aqueous solution, some oxidation had taken place, and the oxidation product had decomposed. When once these impurities have been introduced, there seem no means of removing them and obtaining the pure blue salt.

In the attempts to prepare derivatives from the green solid with $p$-toluene-sulphonyl chloride, it will be remembered that the corresponding derivative of the piperazino-acetic acid was frequently obtained from the solution after the precipitation of copper when the copper salt apparently
contained none. Apparently the sulphuretted hydrogen not only precipitated the copper but reduced the oxidation product, either at the same time or during the subsequent boiling and evaporation [Chap.4].

The conditions necessary for the oxidation of the blue copper salt of piperazino-acetic acid, for the colour change from blue to green, are so similar to those required for the conversion of the amine-oxide to the secondary hydroxylamine, that the conclusion is reached that there must be a close parallelism between the mechanism in the two cases; the identification of the oxidation product from the copper salt as the same secondary hydroxylamine as that from the amine-oxide lends support to this view. In the oxidation of organic substances by oxygen catalysed by charcoal, it is assumed that the oxygen and the substance to be oxidised are adsorbed on the same surface; nothing is known as to the mechanism of aerial oxidations catalysed by copper, but it seems reasonable to suppose that the oxygen and the substance to be oxidised (in this case, the molecule of piperazino-acetic acid) are attached to the same copper atom by coordination. Now since oxidation takes place at the >NH of piperazino-acetic acid, it would seem that the molecule is attached to the copper at this end. It has already been shown [p157] that in aqueous solution there is very little free >NH; therefore in aqueous solution the acid could not become attached to the copper atom through the >NH group.
In alcohol, or aqueous alcohol of certain concentrations, the >NH end of the molecule is freed and could be coordinated to the copper and thus oxidised. This is seen to be parallel to the amine-oxide change, in which alcohol plays the part of preventing internal salt formation [p. 118].

It is known that copper has a strong tendency to coordinate; further the properties of the blue copper salt of piperazino-acetic acid show that it exists in some complex form, many of the normal reactions for copper ions being absent. It is possible that the coordination valency of copper is used up, except in so far as it is already exerted in the formation of the salt, wholly on molecules of water, when more than a certain amount of water is present. On this view oxidation, involving coordination of an acid molecule and of an atom of oxygen could not take place until the water molecules had been removed, and this could not happen until a certain proportion of alcohol was present.

It is clear, therefore, that no one theory will suffice to explain the conversion of the amine-oxide into the secondary hydroxylamine, and of the blue copper salt to the green solid, by the influence of alcohol. The explanations have this in common, however, that the part played by the alcohol is essentially that of dehydration, resulting in the freeing of the >NH group in the molecule, making it available for oxidation.
Several possibilities for the configuration of the blue copper salt suggest themselves; one is the formula

\[
\text{HO} \quad \text{NH} \cdot \text{C}_4\text{H}_8 \cdot \text{N} \cdot \text{CH}_2 \cdot \text{COOH} \quad \text{Cu} \quad \text{HO} \quad \text{NH} \cdot \text{C}_4\text{H}_8 \cdot \text{N} \cdot \text{CH}_2 \cdot \text{COOH}
\]

This however seems unlikely from the fact that piperazinopropionic and butyric acids do not easily form copper salts, and on this view they, and piperazine itself, should form them just as readily as the acetic acid derivative. Another possibility is

![Structure 1]

This again is very unlikely, as, with valency angles everywhere of 110°, the structure

![Structure 2]

is practically unstrained, so that the above formula would involve a considerable amount of strain or a puckered ring.

A third possibility is the formula

![Structure 3]

or its trans-isomer.

In alcohol, or in aqueous alcohol of more than a certain concentration of alcohol, some or all of the water
molecules will be removed; the copper can then coordinate with a molecule of acid with a free $>\text{NH}$. The complex might then become

\[
\begin{align*}
\text{Pip} & \quad \text{Pip} \\
\text{CH}_2-N & \quad \text{H}_2\text{O} \\
\text{CO-O} & \quad \text{Cu} \\
\text{NH} & \quad \text{O-CO} \\
\text{C}_4\text{H}_8 & \\
\text{CH}_2-N & \quad \text{Cu} \\
\text{CO-O} & \quad \text{etc.}
\end{align*}
\]

Further removal of water would enable the copper to coordinate an atom of oxygen and oxidation could then take place. It is not necessary to assume that such a complex would be stable; the formation of a very small proportion would enable oxidation to take place at the $>\text{NH}$, and then these oxidised molecules would be replaced by unoxidised molecules. The existence of the acid salt of piperazino-acetic acid [p. 12] lends support to this view; the normal and acid salts are $\text{CuA}_2.8\text{H}_2\text{O}$ and $\text{CuA}_2.2\text{HA}.6\text{H}_2\text{O}$.

The existence of these may be determined solely by geometrical requirements of crystallisation, but it may be that in the acid salt, the $>\text{NH}$ groups of the two molecules of acid replace two molecules of water in the normal salt.

A fourth formula can be suggested which is analogous to ammonia-copper hydroxide complexes; this is
It is not suggested that this represents the blue salt as a whole for reasons given above [p. 160], but there are indications that hydrolysis of the blue salt occurs and therefore all aqueous solutions, or solutions containing a certain proportion of water, contain such a complex. In alcohol, this might be partially or entirely dehydrated, enabling coordination with oxygen to take place. The disadvantage of this view is that oxidation of ammonia attached to copper hydroxide takes place in aqueous solution, so that it would seem that the removal of the water molecules should not be essential.

It is apparent therefore that the nature of the change undergone by the blue copper salt is not by any means fully known, nor can the mechanism of the part played by alcohol in facilitating oxidation be established. Of the views described above, that based on the formula

\[
Pip.\quad Pip. \\
\begin{array}{c}
\text{CH}_2-N-Cu-N-\text{CH}_2 \\
\text{CO--O} \\
\text{H}_2\text{O}
\end{array}
\]

is the most satisfactory, but at best it is little more than a theory unsupported by experimental evidence. To the conclusions reached at the end of the last chapter, however, can be added the fact that the copper salt change with which
this work began has been definitely linked up with the oxidation of the acid of the salt by hydrogen peroxide; there is a close similarity in that the copper salt change is primarily an oxidation, by the oxygen of the air catalysed by copper, resulting in the secondary hydroxylamine which was also obtained indirectly by the oxidation of the acid. The close connection between the two reactions is further demonstrated by the similarity of the physical conditions promoting them; these are essentially a continuous dehydration of the substance concerned, enabling the high coordinating power of the \textgreater NH group, always in aqueous solution satisfied by water molecules, to be directed towards oxygen thus forming a highly reactive and unstable compound, the secondary hydroxylamine of piperazino-acetic acid.