580. *Chemical Actions of Ionising Radiations in Solutions.*
Part X. The Action of X-Rays on Ammonia in Aqueous Solution.*

By Tyson Rigg, George Scholes, and Joseph Weiss.

It has been found that ammonia in dilute aqueous solutions is oxidised to nitrite at room temperature by the action of the radicals produced by irradiation with *X*-rays, provided that molecular oxygen is present. The influence of pH, concentration, and other factors on this process has been investigated. Hydroxylamine is apparently not involved as an intermediate. Attention has been drawn to the related biological process.

Ionising radiations can deaminate various amino-compounds in aqueous solution, leading to the formation of ammonia. These compounds include amino-acids (Stein and Weiss, *J.*, 1949, 3256; Dale, Davies, and Gilbert, *Biochem. J.*, 1949, **45**, 93), and nucleic acids (Scholes, Stein, and Weiss, *Nature*, 1949, **164**, 709; Scholes and Weiss, *ibid.*, 1950, **166**, 640). The amino-group can also be liberated to a smaller extent in the form of hydroxylamine, the yield of which depends on the nature of the substrate (Scholes and Weiss, *ibid.*, 1951, **167**, 693).

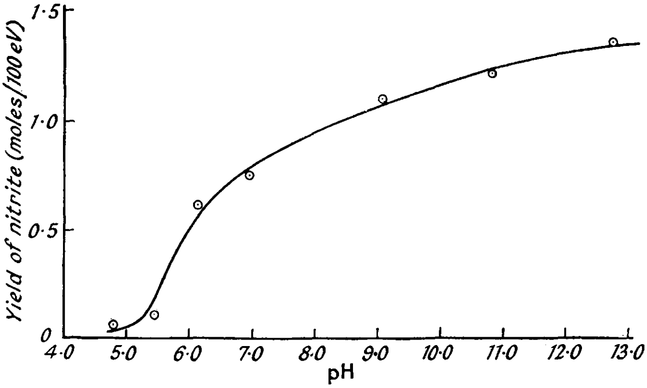

Fig. 1.

pH-*Dependence of the formation of nitrite from* 0·1M-$(NH_4)_2SO_4$ *solutions, in the presence of air* (*constant dose of* $1{\cdot}6 \times 10^4$ *rep*).

On the other hand, irradiation of ammonia solutions with *X*-rays in the presence of air results in an oxidation to nitrite but in this case no hydroxylamine is formed. The yield of nitrite was found to depend markedly on the pH (Fig. 1) and it can be shown that it is essentially dependent on the concentration of the free ammonia, according to the equilibrium, $NH_3 + H^+ \rightleftharpoons NH_4^+$. This is confirmed by the results presented in Fig. 2, showing that the production of nitrite in a dilute solution of ammonium sulphate (5×10^{-4}M) at pH 9·62 follows practically the same course as in a more concentrated solution (10^{-1}M) at pH 6·90, in which the concentration of the free NH_3 molecules is approximately the same.

There was no difference in the behaviour of air- or oxygen-saturated solutions (Fig. 3, curve *a*). In experiments with evacuated solutions, however, no oxidation could be detected—the solutions were tested for nitrite, hydroxylamine, and hydrazine. From these observations it is concluded that the presence of oxygen is essential in the radiation-induced oxidation of ammonia. The ionic yield for the formation of nitrite is rather low, and the curve for air shown in Fig. 3 exhibits no break which would correspond to exhaustion of atmospheric oxygen (Stein and Weiss, *J.*, 1949, 3245; Rigg, Stein, and Weiss, *Proc. Roy. Soc.*, 1952, *A*, **211**, 375). The similarity between the results with air and oxygen can be explained on the grounds that here, even in air-saturated solutions, sufficient oxygen is always available under the conditions of the experiments represented by Fig. 3.

The yield–dose plots for the formation of nitrite display a marked curvature. This

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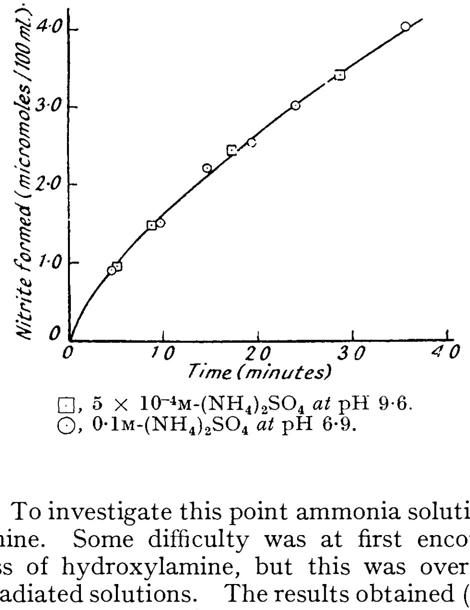
* Part IX, *J.*, 1951, 1247.

is presumably a consequence of further oxidation of the nitrite since addition of the latter before irradiation results in a much lower rate of nitrite production, as shown by the initial slopes of curves *b* and *a* in Fig. 3. This view is further supported by the results, presented in Fig. 4, of experiments in more concentrated ammonia solutions, in which the initial steep slope is maintained up to a greater total dose. From this it appears that ammonia (NH_3) in sufficiently high concentrations can exert a " protective " effect on the nitrite. Further oxidation of the nitrite presumably leads to formation of nitrate (cf. Fricke and Hart, *J. Chem. Phys.*, 1935, **3**, 365).

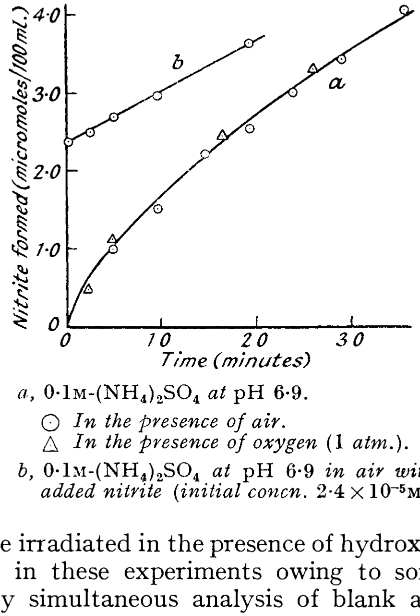
The absence of detectable amounts of hydroxylamine in any of these experiments indicates that, if this substance is an intermediate in the oxidation of ammonia, it must be present in a stationary concentration of the order of *less* than 10^{-6} mole/l. and should therefore be itself oxidised very rapidly; for instance, in the oxidation of a 10^{-1}M-solution of ammonia any hydroxylamine, acting as an intermediate, would, therefore, have to be oxidised about 10^6 times faster than the ammonia.

FIG. 2. *Dependence of the formation of nitrite on the time of irradiation, in the presence of air (dose rate* 3200 *rep/min.).*



☐, 5 × 10^{-4}M-$(NH_4)_2SO_4$ *at* pH 9·6.
○, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9.

FIG. 3. *Dependence of nitrite formation on the time of irradiation (dose rate* 3200 *rep/min.).*

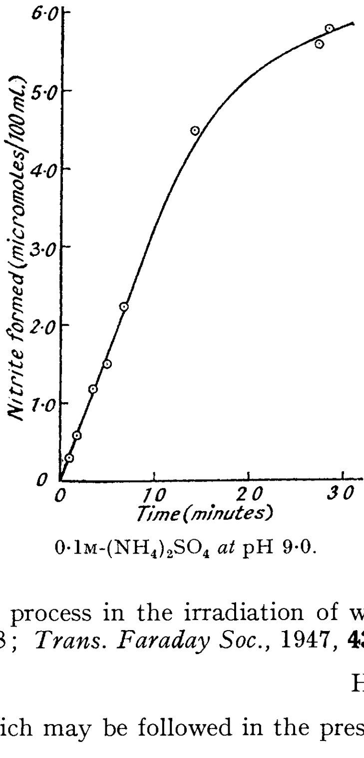


a, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9.
○ *In the presence of air.*
△ *In the presence of oxygen* (1 *atm.*).
b, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9 *in air with added nitrite* (*initial concn.* 2·4 × 10^{-5}M).

To investigate this point ammonia solutions were irradiated in the presence of hydroxylamine. Some difficulty was at first encountered in these experiments owing to some loss of hydroxylamine, but this was overcome by simultaneous analysis of blank and irradiated solutions. The results obtained (Fig. 5) show that the hydroxylamine is oxidised only about 5 × 10^3 times faster than ammonia under similar conditions. This can be deduced, *e.g.*, from curve *b'* (Fig. 5) which shows that hydroxylamine (initial concentration of about 1 × 10^{-5} mole/l.) after 5 minutes of irradiation decreases by 0·55 × 10^{-6} mole/100 ml., whereas the nitrite formed from the ammonia (at a concentration of about 10^{-1} mole/l.), during the same time interval, is of the order of 1 × 10^{-6} mole/100 ml. Therefore, it may be concluded that hydroxylamine is not formed here as an intermediate in the oxidation process. Although some of the added hydroxylamine is oxidised there is no effect on the overall nitrite yield (Fig. 5, curve *a*), which suggests that, under these conditions, ammonia and hydroxylamine are oxidised independently to nitrite in about the same yield.

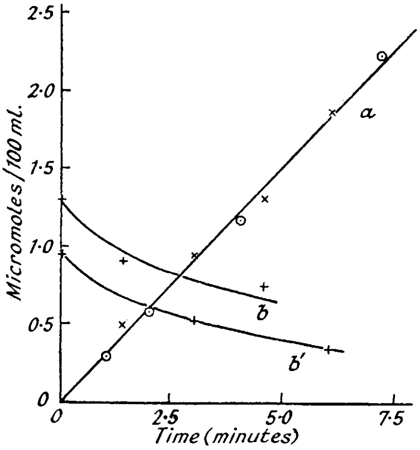
Discussion.—It is well known that ammonia in the gaseous phase in the presence of oxygen is relatively easily converted into oxides of nitrogen by means of a suitable catalyst. However, in aqueous systems, ammonia is relatively inert towards ordinary oxidising agents (cf. Mellor, " Treatise on Inorganic and Theoretical Chemistry," Vol. VIII, p. 205). In biological systems under aërobic conditions on the other hand, it is easily oxidised to nitrous acid. It is, therefore, of interest that ammonia in aqueous systems can be likewise oxidised to nitrite, at room temperature, in the presence of oxygen, if the reaction is initiated by the free radicals produced by the irradiation of the aqueous solution with *X*-rays.

FIG. 4. *Dependence of the formation of nitrite on the time of irradiation, in the presence of air (dose rate* 3200 *rep/min.).*



0·1M-$(NH_4)_2SO_4$ *at* pH 9·0.

FIG. 5. *Dependence of the formation of nitrite on the time of irradiation in the presence and absence of added hydroxylamine, in air.*



a, ⊙ *Nitrite formed from* 0·1M-$(NH_4)_2SO_4$ *at* pH 9.
× *As above but with added* NH_2·OH (1 × 10^{-5}M).
b *and* b′ *show the decrease of the added hydroxylamine* :
b *for initial concn. of* 1·3 × 10^{-5}M-NH_2·OH.
b′ „ „ 0·95 × 10^{-5}M-NH_2·OH.

One of the most interesting features of the oxidation under the influence of *X*-rays is the important rôle of oxygen, without which no reaction appears to take place. Recently, other evidence has indicated that the presence of oxygen during irradiation may lead to marked changes in the nature of the end-products. For example, in the presence of oxygen, pyruvic acid is formed on irradiation of alanine solutions (Johnson, Scholes, and Weiss, *Science*, 1951, **114**, 112) and *o*-benzoquinone is obtained from phenol solutions under suitable conditions (Stein and Weiss, *J.*, 1951, 3265). It is known that the primary *net* process in the irradiation of water by ionising radiations (Weiss, *Nature*, 1944, **153**, 748; *Trans. Faraday Soc.*, 1947, **43**, 314) is represented by

$$H_2O \rightsquigarrow H + OH \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

which may be followed in the presence of molecular oxygen by the very fast reaction :

$$H + O_2 \longrightarrow HO_2 \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (2)$$

One function of the oxygen, therefore, may be to inhibit the recombination of the radicals produced according to reaction (1) and it may, on the other hand, also react with some other intermediate radicals produced in the system. In general, the presence of molecular oxygen should enhance oxidative processes and in certain systems the participation of oxygen may be essential. The oxidation of ammonia falls into the latter category.

The nature of the intermediate steps in the oxidation of ammonia is, as yet, a matter of some conjecture, but it follows from the experimental results discussed above that hydroxylamine is very unlikely to be an intermediate.

Neither hydrazine nor hydroxylamine could be detected in the experiments carried out *in vacuo*. However, under the conditions prevailing in these experiments recombination of the radicals primarily formed according to reaction (1), or back-reactions such as

$NH_2 + H \longrightarrow NH_3$, may account for the absence of any observable oxidation. Such back-reactions may be inhibited by oxygen and a plausible mechanism, involving NH_2 radicals, may be represented as follows :

$$NH_3 + OH \longrightarrow NH_2 + H_2O \quad \ldots \quad (3)$$

$$NH_2 + O_2 \longrightarrow NH_2O_2 \quad \ldots \quad (4a)$$

where it is assumed that reaction (4*a*) is very fast and is followed by a dehydrogenation reaction, *e.g.* :

$$NH_2O_2 + OH \longrightarrow HNO_2 + H_2O \quad \ldots \quad (4b)$$

Alternatively, the NH_2 radicals formed by reaction (3) could undergo disproportionation, leading to the formation of ammonia and the imine radical (NH), *viz.* :

$$2NH_2 \longrightarrow NH_3 + NH \quad \ldots \quad (5)$$

It is known that the imine radical is rather unstable and decomposes to ammonia and nitrogen and possibly also hydrogen (cf. Sidgwick, " The Chemical Elements and their Compounds," Oxford Univ. Press, 1950, p. 711). This could also explain the lack of formation of any oxidation products in *vacuo*, whereas in the presence of oxygen one could have the reaction :

$$NH + O_2 \longrightarrow HNO_2 \quad \ldots \quad (6)$$

leading directly to nitrous acid.

The yield of nitrite reaches a maximum of $G \sim 1{\cdot}3$ (cf. Fig. 1) where $G =$ moles/100 ev. This leads to a yield per radical of $\gamma \sim 0{\cdot}26$, obtained from the relation $\gamma = WG/100$, where W is the energy required to form one radical pair in water (W for liquid water has been taken to be 19·4 ev, Rigg, Stein, and Weiss, *loc. cit.*). This yield is compatible with the above mechanism.

Some preliminary experiments on the effects of *X*-rays on aqueous solutions of primary aliphatic amines show that these substances are oxidised with the formation of hydroxylamine and small amounts of nitrite. The presence of an alkyl group therefore must modify the course of the reaction. On the other hand, the presence of a hydroxyl group (as in $NH_2{\cdot}OH$) does not apparently change the nature of the products, although hydroxylamine is more readily attacked than ammonia (Fig. 5).

In view of these results it seems likely that certain other oxidations of ammonia may involve radical intermediates. For instance, nitrite is formed when alkaline ammonia solutions are treated with copper in the presence of oxygen (Müller, *Z. Elektrochem.*, 1926, **32**, 109). It is also of interest that although perbenzoic acid reacts with ammonia and aliphatic amines it apparently does not react with their salts (Botvinnik, *J. Gen. Chem. U.S.S.R.*, 1946, **16**, 863).

As is well known, the formation of nitrite from ammonium salts is of great importance in the biochemistry of the nitrogen cycle. Virtanen (*Suomen Kem.*, 1945, **18**, *B*, 48) has presented evidence that hydroxylamine is apparently an intermediate; other authors, however (*e.g.*, Pethica, Roberts, and Winter, *Nature*, 1949, **163**, 408), have disputed this. The biological process has now been simulated by radicals produced by *X*-rays in the presence of oxygen and apparently without the intermediate formation of hydroxylamine.

EXPERIMENTAL

Solutions of ammonium sulphate (100 ml.) were irradiated with *X*-rays from a Victor Maximar set operating at 200 kv and 15 mA, the experimental arrangement being essentially that employed by Farmer, Stein, and Weiss (*J.*, 1949, 3241). Doses were determined by the ferrous sulphate actinometric method (Miller, *J. Chem. Phys.*, 1951, **18**, 79); the mean dose rate was found to be about 3200 rep/minute.

Triply distilled water was used throughout for the solutions, ordinary distilled water being redistilled from permanganate and then from dilute orthophosphoric acid. Buffer solutions were used to ensure that the pH decrease during irradiation did not exceed 0·2 pH unit. For the range of pH 5—8, Sørensen phosphate buffer was used and, for pH 8—10, borate–sodium hydroxide mixtures were found to be quite satisfactory; for higher pH values sodium hydroxide alone was added.

Experiments were performed (*a*) in air, (*b*) with solutions saturated with oxygen at 1 atm. (oxygen being bubbled through the solution for about 30 minutes), and (*c*) *in vacuo*. Evacuation of solutions was carried out as follows: the vessel containing the solution to be irradiated was de-aërated as fully as possible by means of a Hyvac oil-pump and (oxygen-free) nitrogen was then admitted to ordinary pressure; this process was repeated once more. After this procedure it was found (on a gas-analysis apparatus) that less than 0·01 ml. of non-condensable gas remained. Alkaline ammonia solutions cannot, however, be subjected to this treatment on account of the volatility of ammonia and, for this reason, unbuffered solutions were used and the necessary amount of sodium hydroxide was placed in a side-arm of the irradiation vessel until evacuation was completed. The hydroxide was then tipped into the ammonium sulphate solution, the pH being measured after irradiation.

Commercial " oxygen-free " nitrogen was further purified by passage through a solution of 10% sodium hydrosulphite (dithionite) in 10% sodium hydroxide solution containing 1% of sodium anthraquinone-2-sulphonate (Fieser, *J. Amer. Chem. Soc.*, 1924, **46**, 2639), through a copper sulphate solution, then through a calcium chloride and soda-lime scrubber, and finally through a tube containing copper gauze at ~380°. The gas so purified was found to contain less than one part of oxygen per million when tested with a trypaflavine–silica gel (Kautsky and Hirsch, *Z. anorg. Chem.*, 1935, **222**, 126; Franck and Pringsheim, *J. Chem. Phys.*, 1943, **11**, 21).

Determination of Reaction Products.—Nitrite and hydroxylamine. Endres and Kaufman's colorimetric method (*Annalen*, 1937, **530**, 184) was adopted; when applied in conjunction with a " Spekker " colorimeter it gave accurate results with nitrite or hydroxylamine concentrations down to 1×10^{-6}M.

For determination of nitrite, 10 ml. of sample (neutral) were treated with 2 ml. of sulphanilic acid solution and 2 ml. of α-naphthylamine reagent, the mixture was set aside for 15 minutes until the colour (red) was fully developed, and the whole finally diluted to 50 ml. The optical density was then measured, a green filter (Ilford 604) being used. The colour was found to be stable for several hours.

Hydroxylamine was determined as nitrite after oxidation with iodine. To 10 ml. of sample (neutral) were added 2 ml. of sulphanilic acid solution and 0·2 ml. of iodine solution (0·65 g. in 100 ml. of glacial acetic acid). After 15 minutes, the excess of iodine was removed with the minimum amount of thiosulphate (2% w/v) and the solution immediately treated with the α-naphthylamine reagent as above.

By a combination of these two methods nitrite and hydroxylamine, even if present together, could be accurately determined.

The sulphanilic acid reagent consisted of a solution of 5·25 g. in 400 ml. of water to which, after the solid had dissolved, 100 ml. of glacial acetic acid were added. The α-naphthylamine reagent was prepared as follows: 3·0 g. of α-naphthylamine were boiled in 500 ml. of water for several minutes, the solution filtered hot, and the filtrate acidified with 25 ml. of glacial acetic acid. The latter reagent is comparatively unstable and was used only if the reagent blank values were very low.

Hydrazine. In the vacuum experiments tests for hydrazine were carried out by Pesez and Petit's method (*Bull. Soc. chim.*, 1947, 122), with a *p*-dimethylaminobenzaldehyde reagent. This reagent gives a yellow-red colour with hydrazine and is capable of detecting one micromole per 100 ml. Hydroxylamine does not interfere but nitrite gives a similar colour to hydrazine.

Throughout this investigation " AnalaR " chemicals were used.

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