

Chemical Action of Ionising Radiations in Solution. Part XIV. The Action of X-Rays (200 kv) on Some Aliphatic Amines in Aqueous Solution with Particular Reference to the Formation of Oximes.*

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Oximes are produced by irradiation with X-rays (200 kv) of primary and secondary aliphatic amines in alkaline solutions provided that molecular oxygen is present. Quantitative studies have been carried out with ethylamine and diethylamine, and the effects of pH and other factors examined. The process involves radical attack at the free (uncharged) amino-group. Some light has been thrown on the mechanism of this reaction by the observation that, in addition to oximes, nitroparaffins are produced in the irradiation of the primary aliphatic amines; it is suggested that organic peroxy-radicals act as intermediates.

In a preliminary publication (Scholes and Weiss, *Nature*, 1951, **167**, 693) it was reported that hydroxylamine was detected in the aqueous solutions of the following amino-compounds which had been irradiated with X-rays (200 kv): amino-acids (*e.g.*, alanine, lysine), amino-purines (*e.g.*, adenine), and amines (*e.g.*, ethylamine, propylamine, histamine). In a large number of the systems, however, particularly at pH ~ 7 , the yields of hydroxylamine were quite small. On the other hand, more significant quantities could be detected on irradiation of alkaline solutions of the primary aliphatic amines which, therefore, appeared suitable for further studies of the nature of this process.

In general, chemical transformations induced by ionising radiations in dilute aqueous systems are due, in the first instance, to the production of free radicals from the water, according to the net process (Weiss, *Nature*, 1944, **153**, 748; *Brit. J. Radiol.*, 1947, Suppl. 1, 56):



In the presence of dissolved molecular oxygen this can be followed by the reaction



The radicals thus formed may then react with the solutes. Recently it has been shown that, in addition to reaction (1), decomposition of water takes place with the production of molecular hydrogen and hydrogen peroxide (Allen, *Ann. Rev. Phys. Chem.*, 1952, **3**, 57), *viz.*,



With hard X-rays, the extent of reaction (3) is small compared to that of reaction (1), but must, nevertheless, be considered in any quantitative studies of the action of these radiations.

Preliminary Experiments.—In confirmation of previous observations, hydroxylamine could be readily detected in irradiated alkaline solutions of several primary aliphatic amines by Endres and Kaufman's method (*Annalen*, 1937, **530**, 184), involving oxidation to nitrite by iodine at room temperature, a process which is normally complete within about 15 minutes, the nitrite thus formed being estimated by the Griess-Ilosvay reagent.

Aerated solutions of the amines (0.1M) at pH ~ 11 were irradiated with 200 kv X-rays, total doses varying between 2 and 6×10^{-6} ev/N per ml. being used. However, oxidation was incomplete after 15 min. at room temperature, additional quantities of nitrite being slowly produced. Further, the corresponding aldehydes could be isolated from the irradiated solutions as their 2:4-dinitrophenylhydrazones. These observations suggested that oximes and not necessarily free hydroxylamine were present after irradiation. Total hydroxylamine content was, therefore, determined by the "hot oxidation" procedure for oximes as first suggested by Endres (*Annalen*, 1935, **518**, 109).

* Part XIII, *J.*, 1954, 3248.

Table 1 shows some results obtained by irradiating methyl-, ethyl-, and propyl-amine. It can be seen that additional amounts of nitrite are produced by the iodine oxidation at higher temperatures; this is characteristic of solutions containing oximes (*e.g.*, formaldoxime) but unlike those of pure hydroxylamine.

Because of the equilibrium between aldehyde, hydroxylamine, and oxime in aqueous systems, it is not possible on the basis of these experiments alone to decide whether oxime or hydroxylamine is the primary product of irradiation; this question is discussed more fully below. The yields of free nitrite in the irradiated solutions (*cf.* Table 1) were determined by direct addition of the Griess-Ilosvay reagent. However, nitrite was present only in solutions which had received relatively high doses.

TABLE 1. *Irradiation of aqueous solutions of some primary aliphatic amines with X-rays (200 kv). Comparison of yields of nitrite by "cold" and "hot" oxidation with iodine.*

Solution	Dose [10^{-8} (ev/N) per ml.]	Free nitrite (10^{-8} mole per ml.)	Nitrite after cold oxidation (10^{-8} mole/ml.)	Nitrite after hot oxidation (10^{-8} mole/ml.)
NH ₂ Me, 0.09M; pH 12.1...	2.08	0.00	0.91	1.27
	2.59	0.08	1.29	1.73
NH ₂ Et, 0.13M; pH 10.3...	1.73	0.00	0.52	1.00
	3.46	0.10	1.10	2.00
NH ₂ Pr ⁿ , 0.10M; pH 12.0	6.06	0.50	1.30	1.60
Hydroxylamine	—	—	1.95	1.90
Acetaldoxime	—	—	1.08	1.81

The possibility that *N*-substituted hydroxylamines were formed by irradiation and then oxidised to nitrite by the above method was examined in tests with *N*-methylhydroxylamine solutions. With a solution containing 2.5×10^{-7} mole/ml. (*i.e.*, of the order of the concentrations which may be expected to be produced in the irradiated solutions) no nitrite was formed on oxidation by iodine at room temperature, and the "hot oxidation" procedure converted only about 1% of the *N*-methylhydroxylamine into nitrite. Hence it was concluded that the nitrite-forming substances present in the irradiated solutions were oximes. These observations do not, of course, exclude the possibility that substituted hydroxylamines are also formed.

Further light was thrown on the mechanism by the observation that the corresponding nitroparaffins were also produced in the irradiation of primary amines in aqueous solutions containing oxygen. This has been investigated particularly in the case of ethylamine; the nitroethane formed was detected by Türber and Uhlin's method (*Angew. Chem.*, 1949, 61, 74). Analytically, the method is not particularly suitable for this system, there being some interference in colour development. However, by blank tests (see Experimental) estimates of the yields have been obtained from which it would appear that the amounts of nitroparaffin formed are roughly equivalent to those of the oxime.

Irradiations of alkaline solutions of diethylamine and triethylamine were carried out in a similar manner. In the case of the secondary amine, oxime (acetaldoxime) was again present after irradiation. However, only aldehyde (acetaldehyde) could be detected in the irradiated tertiary amine solutions. Thus, oxime formation can occur only if the amine nitrogen carries a hydrogen atom.

Since all these experiments were in the presence of molecular oxygen, the formation of hydrogen peroxide was expected and was detected by means of the titanium sulphate reagent. In each instance, the formation of ammonia was established by Nessler's reagent.

When, however, the primary and secondary amine solutions were irradiated in the absence of dissolved molecular oxygen, no oxime (or nitroparaffin) could be detected. It seemed evident therefore, that oxygen plays an important part in the mechanism of these processes.

Quantitative Experiments.—Ethyl-, diethyl-, and triethyl-amine were selected as representative of the primary, secondary, and tertiary aliphatic amines. Total hydroxylamine (oxime) was determined by the "hot iodine oxidation" method, hydrogen peroxide with titanium sulphate, and acetaldehyde as its 2 : 4-dinitrophenylhydrazone. (This method was shown to determine total aldehyde content, *i.e.*, including that available in the form of oxime).

(a) *Ethylamine*. Fig. 1 shows some typical yield-dose plots obtained from the irradiation of an aqueous ethylamine solution (0.068M; pH = 10.75). Experiments were carried out with oxygen-saturated and with air-equilibrated solutions. The initial yields were identical under both these conditions, that of total hydroxylamine (oxime) corresponding to a G -value (molecules/100 ev of energy absorption) of ~ 0.6 , and that of total measurable aldehyde of $G \sim 1.4$. Small quantities of nitrite were also detected but only after relatively high doses had been applied to the solutions (cf. Fig. 1) and, therefore, they appear to be the result of secondary oxidation of the primary products of irradiation.

FIG. 1. Irradiation of aqueous solutions of ethylamine (0.068M, pH = 10.75) with X-rays (200 kv). (Temp. 20°.)

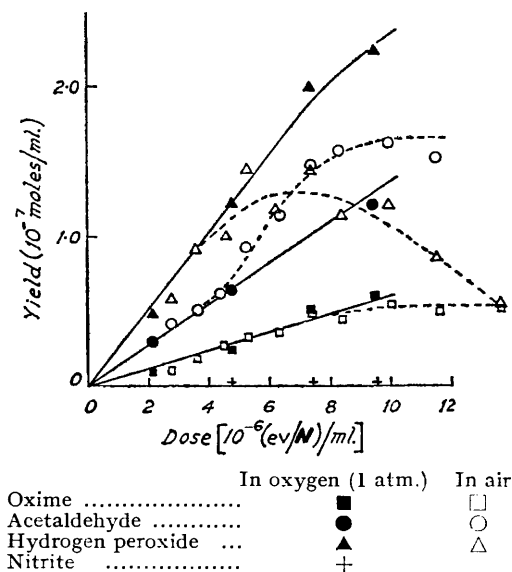
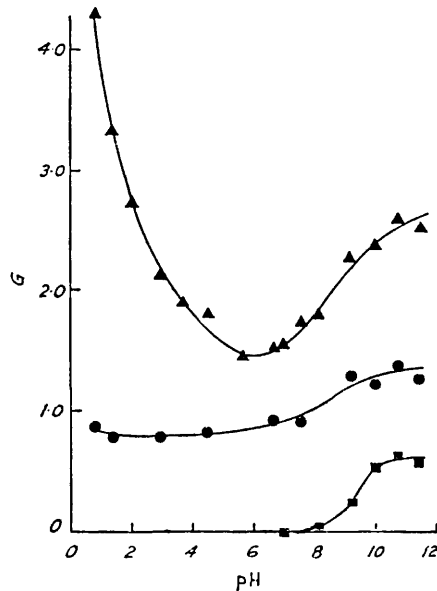


FIG. 2. pH-Dependence of the initial yields of oxime (■), acetaldehyde (●), and hydrogen peroxide (▲) in irradiation of aqueous solutions of ethylamine (0.068M) with X-rays (200 kv) in the presence of oxygen (1 atm.). (Temp. 20°.)



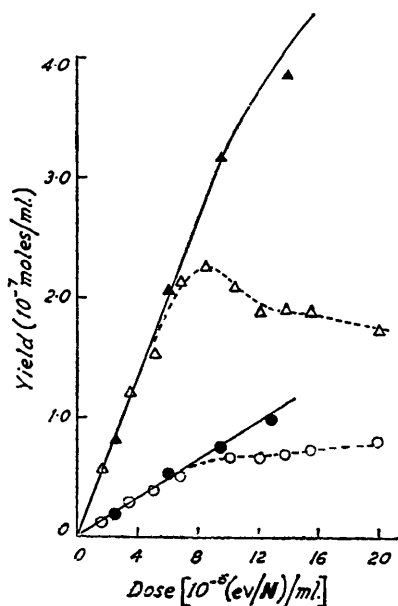
The pH-dependence of the initial yields in oxygen-saturated solutions was obtained from such yield-dose plots, as shown in Fig. 2. The initial yields of all the measured products vary with pH; oxime formation occurs only in alkaline solutions and increases with increasing alkalinity at $\text{pH} > \sim 8$, giving eventually a yield of $G \sim 0.6$ at $\text{pH} \sim 11$; the yields of acetaldehyde also increase over the same pH range, and it is noteworthy that this increase is of the same order as that of oxime. Since dissociation of the ethylamine cation takes place in this range according to $\text{Et}\cdot\text{NH}_3^+ \rightleftharpoons \text{Et}\cdot\text{NH}_2 + \text{H}^+$, these observations suggest that oxime can only result from radical attack on the free uncharged amino-group.

In solutions saturated with air (Fig. 1) oxime formation more or less ceases after a total dose of the order of 7.5×10^{-6} ev/N per ml. Secondary decomposition of hydrogen peroxide and an enhanced formation of aldehyde begin even before this dose is reached. These effects can be attributed to the utilisation and eventual exhaustion of the dissolved molecular oxygen. At least one-third (Fig. 1) of the available oxygen is not converted into measurable hydrogen peroxide. If no peroxide decomposition had occurred before the "break point" (at $\sim 7.5 \times 10^{-6}$ ev/N per ml.) the maximum yield expected after this dose would have been 2×10^{-7} mole/ml., *i.e.*, corresponding to that in solutions saturated with oxygen; included in this value is the hydrogen peroxide produced by reaction (3), which, up to this point amounts to 0.38×10^{-7} mole/ml. ($G = 0.50$). Thus the peroxide

formed by radical processes is 1.62×10^{-7} mole/ml. Under these conditions the total available oxygen present in the solutions saturated with air was $\sim 2.9 \times 10^{-7}$ mole/ml. (at 20°). Therefore, $\sim 1.3 \times 10^{-7}$ mole/ml. of oxygen (corresponding to $G \sim 1.7$) have entered into reactions involving the solute molecules and are eventually incorporated in the final products. That these reactions are essentially those leading to oxime is suggested by the fact that "oxygen loss" in acid solution, where no oxime is formed, is much less ($G \sim 0.5$, Fig. 3).

(b) *Diethylamine and triethylamine.* Fig. 4 gives the yield-dose plots obtained from the irradiation of an alkaline solution of diethylamine in which the concentration of the

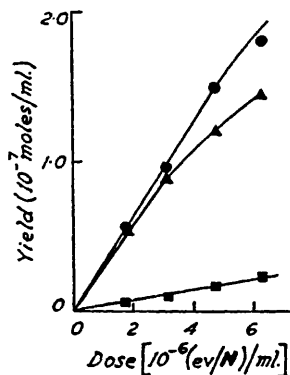
FIG. 3. Irradiation of aqueous solution of ethylamine (0.068M, pH = 1.35) with X-rays (200 kv). (Temp. 25°.)



In oxygen (1 atm.) In air

Acetaldehyde ● ○
 Hydrogen peroxide ... ▲ △

FIG. 4. Irradiation of aqueous solutions of diethylamine (0.0655M, pH = 11.2) with X-rays (200 kv) in the presence of oxygen (1 atm.). (Temp. 20°.)



■ Oxime
 ● Acetaldehyde
 ▲ Hydrogen peroxide.

secondary amino-group was identical with that of the free primary amino-group in the above ethylamine solutions. Table 2, in addition, summarises some G values obtained from di- and tri-ethylamine solutions irradiated at different pH values. The yields of aldehyde from these compounds in alkaline solution were much greater than those obtained

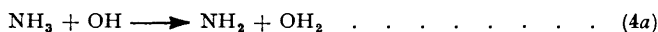
TABLE 2. Irradiation of aqueous solutions of di- and tri-ethylamine with X-rays (200 kv) at different pH.

Solution	pH	Yield (G)		
		Hydrogen peroxide	Acetaldehyde	Oxime
NH ₂ Et ₂ (0.065M)	11.16	3.16	3.16	0.36
	2.82	2.58	0.77	0.00
NEt ₃ (0.059M)	11.95	2.95	3.20	0.00
	2.35	2.70	0.76	0.00

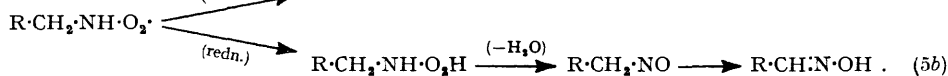
from ethylamine at similar pH's; in acid solution, however, all three amines yielded about the same amount of aldehyde ($G = 0.7-0.8$).

It seems, therefore, that three conditions are necessary for the production of oximes when aqueous amine solutions are subjected to ionising radiations: (i) The presence of

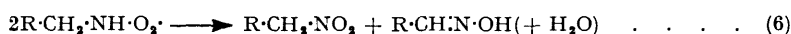
a hydrogen atom on the nitrogen of the amino-group. (ii) The primary or secondary amino-group must be present in the free (uncharged) state. (iii) Molecular oxygen must be present in solution. These conditions are closely similar to those required for the production of nitrite by irradiation of ammonium salt solutions (Rigg, Scholes, and Weiss, *J.*, 1952, 3034), where the presence of molecular oxygen was essential, and the yield was dependent on the concentration of free ammonia molecules. The mechanism then proposed for ammonia oxidation involved the following initial steps:



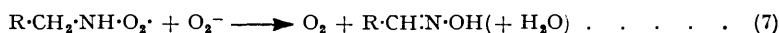
Nitrite can then readily result from further oxidation of the $\text{NH}_2\cdot\text{O}_2\cdot$ radical. In the case of a primary amine the organic peroxy-radical ($\text{R}\cdot\text{CH}_2\cdot\text{NH}\cdot\text{O}_2\cdot$) produced in an identical fashion could on oxidation lead to a nitroparaffin, or, on reduction directly to an oxime, *i.e.*:



Simultaneous formation of oxime and nitroparaffin, moreover, can result from the simple dismutation process:



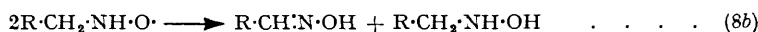
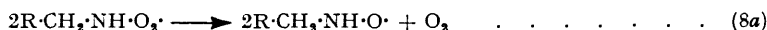
In alkaline solution reduction may also take place according to



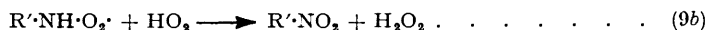
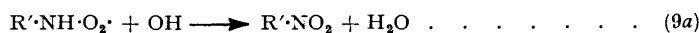
where O_2^- arises from the dissociation of any primarily formed hydroperoxy-radical ($\text{HO}_2 \rightleftharpoons \text{H}^+ + \text{O}_2^-$). The mere fact that nitroparaffins are formed lends considerable support to the mechanism outlined above, since it is difficult to see how these compounds can be produced except by a process involving molecular oxygen.

With regard to the dismutation process (reaction 6), it may be of some significance that in the case of ethylamine, at least, oxime and nitroethane are formed in about equal amounts, and also that the increase in aldehyde over the pH range 8—11 is equal to that of oxime over this region (Fig. 2).

However, dismutation according to the scheme



i.e., oxime production accompanied by formation of a substituted hydroxylamine, cannot be entirely ruled out, since it is not known whether the latter substance is produced. Nitroparaffin could then only arise from an oxidation involving a hydroxyl (or HO_2) radical, *viz.*,



The almost constant yield of aldehyde at pH < 8 is presumably the result of dehydrogenation of the amine at the α -carbon atom, and there is no reason to believe that such an attack does not also take place in alkaline solution. Indeed, the excess of aldehyde over total oxime and also the formation of ammonia in alkaline solutions suggest the occurrence of such processes.

With secondary amines, *e.g.*, diethylamine, oxime formation is linearly dependent upon dose (Fig. 4); the compound must therefore be a primary product of the irradiation. On a basis similar to that outlined above, one would expect oxime production to involve, as intermediates, peroxy-radicals of the structure $\text{R}''_2\cdot\text{NO}_2\cdot$. The higher yields of aldehyde

from the secondary and tertiary amines are not unexpected, since radical attack at the nitrogen atom can presumably lead to oxidation of more than one alkyl group.

The yields of total hydroxylamine produced on irradiation of some amino-compounds of biological importance are shown in Table 3; in these experiments the "hot oxidation"

TABLE 3. "Total hydroxylamine" by irradiation with X-rays (200 kv) of aqueous solutions of organic amino-compounds of biological interest.

Solution irradiated	pH	Yield of "total hydroxylamine"	
		10 ⁻⁸ moles/ml.	G
Dose = 5.4 × 10 ⁻⁶ ev/N per ml.			
L-Lysine (2.3 × 10 ⁻³ M)	11.0	0.61	0.11
D-Arginine (2.3 × 10 ⁻³ M)	11.5	0.63	0.12
Histamine (2.7 × 10 ⁻³ M)	10.3	0.41	0.08
Albumin * (0.075%)	10.8	0.22	0.03
Glycine (0.028M)	12.1	2.79	0.51
Glycylglycine (0.028M)	11.85	1.96	0.36
Diglycylglycine (0.028M)	11.90	1.11	0.20

* Dose = 9.2 × 10⁻⁶ ev/N per ml.

method was employed. The yields were in all cases higher than those which had been obtained previously by using the cold oxidation procedure (Scholes and Weiss, *loc. cit.*; Scholes, Ph.D. Thesis, University of Durham, 1952) and also indicate the production of oximes. Concentrations of the order of 10⁻⁵M (total) hydroxylamine can be produced by doses of about 5 × 10⁴ r (Table 3). It is known that hydroxylamine is a strong cell poison and enzyme inhibitor (cf. Kaplan, *J. Biol. Chem.*, 1954, **211**, 419); it is not impossible therefore that the production of this substance from amino-compounds may be of some significance in the biological action of ionising radiations; this may be particularly accentuated in local regions of relatively high pH.

EXPERIMENTAL.

Irradiations.—The amine solutions (200 ml.) were irradiated with X-rays generated from a Victor Maximar set, operating at 200 kv and 15 mA, the irradiation arrangements being essentially those employed by Farmer, Stein, and Weiss (*J.*, 1949, 3241). The dose rate was determined by measuring the oxidation of 10⁻³M-ferrous sulphate in 0.1N-sulphuric acid and was 1.72 × 10⁻⁷ ev/N per ml. per min., $G_{(Fe^{2+})}$ being taken as 15.5 (Farmer, Rigg, and Weiss, *J.*, 1955, 582).

Triply distilled water was used for all irradiation experiments, obtained by redistilling ordinary distilled water from alkaline potassium permanganate and then from dilute orthophosphoric acid. "AnalaR" sulphuric acid was used when pH's other than the natural alkaline pH's of the amine solutions were required.

All amines were purified before irradiation *via* their hydrochlorides, the free amines being then regenerated by distillation from alkali into triply distilled water.

Irradiations were carried out with air-equilibrated, oxygen-saturated, and evacuated solutions. Saturation with oxygen was effected by passing the gas through the solutions for approximately 15 min. For evacuation, first a Hyvac oil pump and finally a mercury-diffusion pump were employed. To avoid losses of amine it was necessary to evacuate, in the first instance, acidic solutions (in sulphuric acid), which were then made alkaline by tipping into the solution the required amounts of sodium hydroxide stored in a side arm of the radiation vessel.

The pH's of the solutions were checked after irradiation.

Detection and Determination of the Irradiation Products.—*Ammonia.* Owing to interference from the amines it was only possible to carry out qualitative tests. An exact amount of Nessler's reagent was added to 5 ml. of irradiated solution and also to 5 ml. of the blank. Both gave a precipitate (due to the amine), but the yellower colour of the irradiated samples was quite distinct in all the cases examined.

Nitroethane. This was detected by the red-brown coloration on addition of diazotized sulphamic acid (Türber and Uhlin, *loc. cit.*). The relative quantities of reagents given by these authors were adhered to; the volumes, however, were increased so as to allow measurement on a "Spekker" photoelectric absorptiometer, a 4-cm. cell and a blue-green filter (Ilford 603) being used. Although the amine present gave a yellow-green colour with the reagents; it was

possible in test solutions to detect quantities down to $10^{-6}M$. From a quantitative point of view, difficulty was encountered owing to interference from other irradiation products. However, estimates of the yields could be obtained by comparison with standard nitroethane solutions, containing ethylamine and also the other known radiation products, in such quantities as to simulate the irradiated solutions.

Hydrogen peroxide. This was determined by means of titanium sulphate (Eisenberg, *Ind. Eng. Chem. Anal.*, 1943, 15, 327). Aliquot parts were slightly acidified, treated with 5 ml. of titanium reagent, and then made up to 50 ml. The extinctions were measured on the "Spekker" instrument by use of an Ilford 601 (blue-violet) filter.

Oxime, hydroxylamine, and nitrite. The methods described by Endres and Kaufman (*loc. cit.*) were used for these compounds.

To determine nitrite, 10-ml. aliquot parts were treated with 2 ml. of sulphanilic acid solution (5.25 g. in 100 ml. of 20% v/v acetic acid) and 2 ml. of α -naphthylamine reagent (0.6 g. in 100 ml. of 5% acetic acid). The mixture was set aside for 15 min. It was then diluted to 50 ml., and the extinction measured by means of a large (4 cm.) cell and a green (Ilford 604) filter. Hydroxylamine was determined as nitrite after oxidation with iodine. To 10-ml. aliquot portions, 2 ml. of sulphanilic acid solution and 0.2 ml. of iodine reagent (0.6 g. in 100 ml. of glacial acetic acid) were added. After 15 min. at room temperature, the excess of iodine was removed with a few drops of aqueous sodium thiosulphate (2% w/v), and the solution immediately treated with α -naphthylamine reagent as above.

Oxime was also determined as nitrite: 0.2 ml. of iodine reagent was added to 10 ml. of the aliquot portion. The solution was brought quickly to the boil and cooled at once in running water; 2 ml. of sulphanilic acid and a further 0.1 ml. of iodine reagent were added. The analysis was then continued as described above for hydroxylamine. This method was accurate to within $\pm 5\%$, there being invariably some very slight losses owing to further oxidation of nitrite to nitrate.

The α -naphthylamine reagent is somewhat unstable and was only used when controls gave very low extinctions.

Aldehydes. These were identified as their 2:4-dinitrophenylhydrazones. 100 ml. of irradiated amine solution were acidified and subsequently treated with 50 ml. of 2:4-dinitrophenylhydrazine (0.25% w/v in 30% perchloric acid). The mixture was set aside for $\frac{1}{2}$ hr., then extracted with carbon tetrachloride. The solvent was removed by vacuum-distillation, and the residue chromatographed on paper by using a light petroleum-methanol solvent (Meigh, *Nature*, 1952, 170, 579).

The quantitative determination of acetaldehyde in irradiated amine solutions was carried out by Johnson and Scholes's method (*Analyst*, 1954, 79, 217): 10 ml. of sample were slightly acidified with sulphuric acid, 5 ml. of the 2:4-dinitrophenylhydrazine reagent were added, and the mixture set aside for 15 min. It was then extracted for 2-min. and for $\frac{1}{2}$ -min. periods with 20 ml. and 5 ml. of carbon tetrachloride, respectively. The extract was diluted with ethanol, 2 ml. of 0.1N-ethanolic sodium hydroxide were added, and after thorough mixing the solution was made up to 50 ml. with ethanol. Extinctions were measured in a 4-cm. cell with a blue-green (Ilford 603) filter. By this method the total amount of acetaldehyde, including that combined as oxime, in solution was measured.

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