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RADIOLYSIS OF LIQUID N-ETHYLACETAMIDE

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RADIOLYSIS OF LIQUID N-ETHYLACETAMIDE\*

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September 1970

ABSTRACT

The  $\gamma$ -radiolysis of liquid N-ethylacetamide leads to formation of acetaldehyde and ethyl amine as major decomposition products. The mechanism of formation of these products is examined through use of added electron and radical scavengers such as RCl, RSH and Fe III. The results are consistent with a reaction scheme in which the radiation-induced steps involve the stoichiometries:  $\text{RCONHCHR}_2 \xrightarrow{\gamma} \text{RCONH}\dot{\text{C}}\text{R}_2 + \text{H}^+ + \text{e}^-$  with  $G = 3.2$ ,  $\text{RCONHCHR}_2 \xrightarrow{\gamma} \text{RCON} = \text{CR}_2 + \text{H}_2$  with  $G = 0.8$ , and  $\text{RCONHCHR}_2 \xrightarrow{\gamma} \dot{\text{R}} + \text{CO} + \dot{\text{N}}\text{HCHR}_2$  with  $G = 0.5$ . A detailed reaction scheme for the intermediate processes is presented.

\* This work was performed under the auspices of the U. S. Atomic Energy Commission.

## INTRODUCTION

The radiation chemistry of simple organic amides is of considerable intrinsic interest and at the same time has potential application in the development of our understanding of the actions of ionizing radiations on more complex biochemical systems. Studies of the radiation chemistry of liquid formamide<sup>(1,2)</sup>, of liquid N,N-dimethyl formamide<sup>(3)</sup> and of acetamide<sup>(4)</sup> in both liquid and solid states have recently been published. However, none of the simple amides studied contain the characteristic peptide function,  $RCONHCHR_2$ , which is of particular interest from the radiation biochemical viewpoint. In the present work we consider the radiation chemistry of one of the simplest peptide derivatives, viz N-ethylacetamide.

## EXPERIMENTAL

The N-ethylacetamide (Eastman White Label) was fractionally redistilled under nitrogen at reduced pressure (50 microns). A middle-third fraction was collected and stored under dry nitrogen at atmospheric pressure. The product showed a conductivity of  $< 1 \times 10^{-7} \text{ ohm}^{-1} \text{ cm}^{-1}$ . Samples were transferred to glass-stoppered cells (10 ml) under dry nitrogen in a glove box. In some instances the irradiation cells were attached to the vacuum line, evacuated by the freeze-thaw technique and then sealed off. Data obtained under the two conditions of sample preparation were indistinguishable.

All other chemicals were of reagent grade and were used without further purification.

A 5 kc  $^{60}\text{Co}$  "Gamma Cell 200" was used in the irradiations. Two dose rates were employed:  $3.5 \times 10^{16} \text{ ev/gm-min}$ ,  $1 \times 10^{18} \text{ ev/gm-min}$ .  $[\text{G}(\text{Fe}^{+3}) = 15.5, t_{305} = 2200 \text{ at } 25^{\circ}\text{C}]$ .

The irradiated N-ethylacetamide was passed through a 10 cm Dowex-50 column (acid form) to remove amine and any ammonia. After washing with water the column was stripped with 6N hydrochloric acid and the eluate was taken to dryness, redissolved in distilled water and analyzed. Ethylamine was identified by vapor-phase chromatography<sup>(5)</sup> (Aerograph 600C) and was assayed colorimetrically as the picrate<sup>(6)</sup>. Assay of the amine by the ninhydrin method<sup>(7)</sup> gave identical yield values; hence free ammonia is produced in negligible yield since ninhydrin reagent detects both amine and ammonia with essentially the same sensitivity.

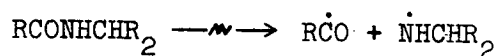
Carbonyl products were identified through filter-paper chromatography of the 2,4-dinitrophenyl hydrazones<sup>(8)</sup>. Assay was by the method of Johnson and Scholes<sup>(9)</sup>. Chloride ion was determined by the method of Hayon and Allen<sup>(10)</sup>.

Gaseous products were identified and determined by mass spectrometrically (Consolidated 120). Appropriate control runs established the specificity of the above procedures in their present applications.

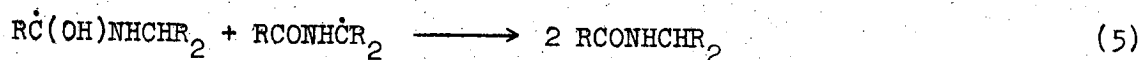
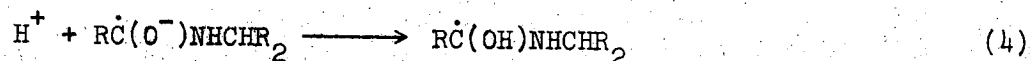
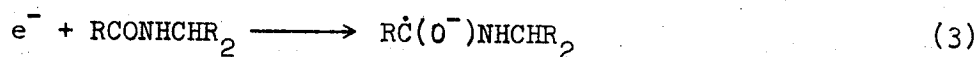
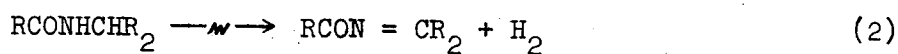
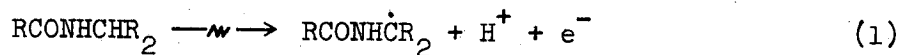
## RESULTS AND DISCUSSION

The 100 ev yields (G-values) of degradation products formed in the  $\gamma$ -radiolysis of liquid N-ethylacetamide are summarized in Table I. Yields are essentially independent of dose-rate over the range  $3.5 \times 10^{16}$  ev/gm-min to  $1 \times 10^{18}$  ev/gm-min and independent of total dose up to values of approximately  $5 \times 10^{19}$  ev/gm.

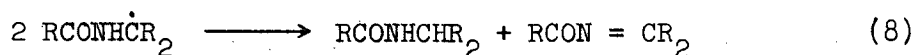
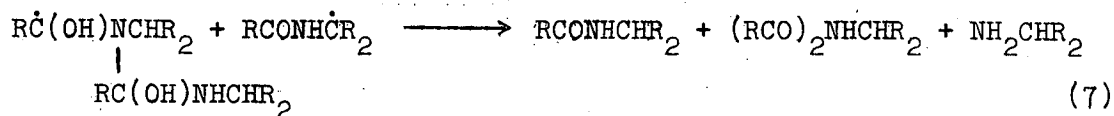
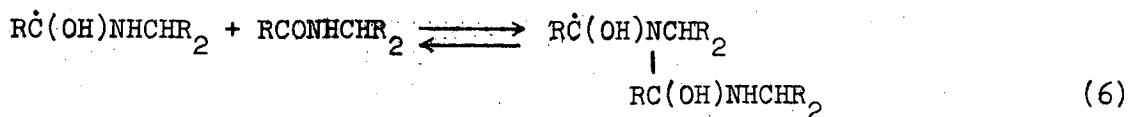
We assume that chemistry arising from the homolytic cleavage



is of no more than minor importance in the production of acetaldehyde and ethylamine since caging effects in the condensed phase would lead to preferential recombination of the heavy-radical pair,  $\dot{\text{R}}\text{C}\text{O} + \dot{\text{N}}\text{HCHR}_2$ . Rather, the evidence is that heterolytic processes are of prime importance in the radiolytic degradation of the peptide bond to yield the aldehyde and amine functions. As discussed in following sections, the scheme of reactions given in equations 1-9 are consistent with the major experimental requirements of the present system.



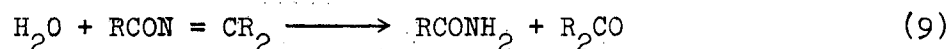




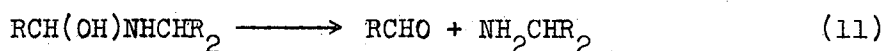
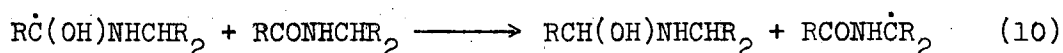
The ionization act as formulated in equation 1 incorporates the concept that the parent positive ion in a polar medium\* is converted to the corresponding radical species through reaction of the type<sup>(12)</sup>  $\text{RH}^+ + \text{RH} \rightarrow \text{R} + \text{RH}_2^+$ . Localization of the radical site at the carbon position  $\alpha$  to the nitrogen function is consistent with esr studies of  $\gamma$ -irradiated amides of the type  $\text{RCNHCHR}_2$ <sup>(13,14)</sup>. The stoichiometry of reaction 2 accounts for the production of molecular hydrogen with  $G \approx 0.8$ . Scavenger studies described below indicate that electrons formed in reaction 1 escape the parent positive charge and add to the carbonyl function of the peptide bond as formulated in reaction 3. Reaction 4 represents the process of charge neutralization. Interaction of the radicals  $\text{RC}(\text{OH})\text{NHCHR}_2$  and  $\text{RCNH}\dot{\text{C}}\text{HR}_2$  in step 5 leads to reconstitution of the parent peptide. Since the reduced radical  $\text{RC}(\text{OH})\text{NHCHR}_2$  is an amine species we assume that it is involved in the equilibrium reaction 6 which yields an adduct radical. Reaction of the latter with  $\text{RCNH}\dot{\text{C}}\text{HR}_2$  leads to liberation of free amine with degradation of the peptide chain as formulated in equation 7. Analogues of reactions 6 and 7 have been invoked in interpretations of the radiation chemistry of peptides in concentrated aqueous solution<sup>(16)</sup>. The

\*The dielectric constant of N-ethylacetamide at 25°C corresponds to  $\epsilon = 130$  (Ref. 15).

dehydropeptide,  $\text{RCON} = \text{CR}_2$ , formed in reaction 8 is labile and yields carbonyl through hydrolysis during the sequence of analytical manipulations



We note that the scheme of reactions 1-9 requires that the production of acetaldehyde from N-ethyl acetamide in the absence of added scavengers occurs exclusively through the formation of the dehydropeptide as formulated in reactions 8 and 9. Net reduction of the acetyl group to yield acetaldehyde through reactions 3 and 4 followed by



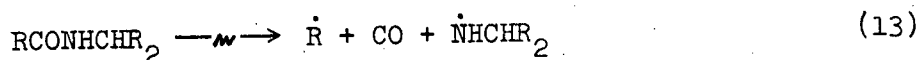
is not invoked. We cannot, of course, rule out reactions 10 and 11 just on the basis of the N-ethyl acetamide results since with this compound acetaldehyde is a product of both reactions 8, 9, and 10, 11. Actually the main argument against reactions 10 and 11 is derived from work with N-propyl acetamide. This derivative shows no acetaldehyde on radiolysis; the carbonyl product is exclusively propionaldehyde in accord with the idea that carbonyl production in these systems arises through the dehydropeptide.

Electrons produced in the radiation-induced step 1 can be detected through use of electron scavengers such as organic chloro compounds,  $\text{RCl}$ , which species have been shown to be effective in trapping electrons produced in the radiolysis of polar media<sup>(17,18)</sup>.

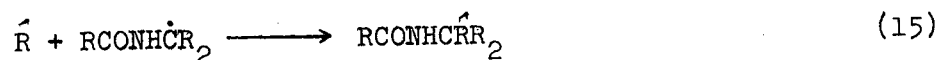
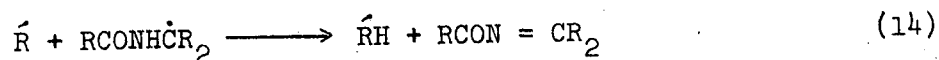


The production of chloride ion in the  $\gamma$ -radiolysis of liquid N-ethyl acetamide containing increasing concentrations of ethylene dichloride is shown in Fig. 1; a reciprocal yield plot is shown in the insert. These results provide a measurement of the yield of reaction 1 v.z.,  $G_e \approx 3.2^*$ .

The effect of ethylene dichloride on the yields of ethyl amine and acetaldehyde is summarized in Table IIA. In the presence of RCl, reaction 12 occurs in competition with reaction 3 and this leads to a decrease in the amine yield since reactions 3, 4, and 7 are blocked. The fact that the amine yield levels off at a limiting value of  $G(\text{RNH}_2) \approx 0.5$  at the higher RCl concentrations is interpreted as evidence that part of the amine production arises from a "high-energy" reaction, e.g.



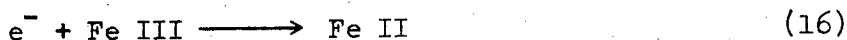
where the radical products of reaction 13 have excess energy and abstract H from  $\text{RCONHCHR}_2$  to yield  $\text{RCONH}\dot{\text{C}}\text{R}_2$ . Such reaction also provides the source of the small amounts of methane and carbon monoxide reported in Table I. The acetaldehyde yield increases in the presence of ethylene dichloride as seen in Table IIA. At sufficiently high concentrations of RCl, reaction 1 is followed preferentially by reaction 12. Subsequent removal of  $\text{RCONH}\dot{\text{C}}\text{R}_2$  involves step 8 and



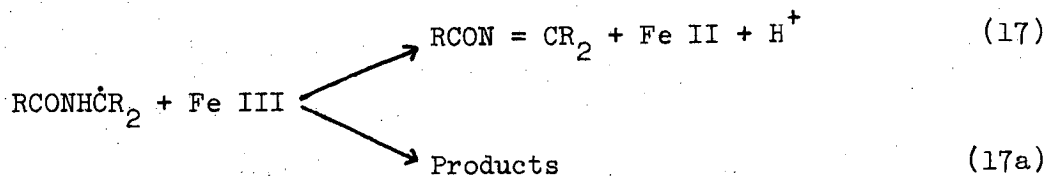
Reactions 2, 8, and 14 followed by the hydrolysis step 9 yield acetaldehyde with  $G \approx 2$ .

\* Through use of  $\text{N}_2\text{O}$  as electron scavenger ( $\text{N}_2\text{O} + e^- \rightarrow \text{N}_2 + \text{O}^-$ ), Head and Walker (Ref. 2) find  $G_e = 3.3$  for liquid formamide.

Oxidizing solutes such as Fe III scavenge both electrons and radicals. Effects of increasing Fe III concentrations on the yield of aldehyde and amine are shown in Table IIB. At sufficiently high concentrations of Fe III, the electrons produced in reaction 1 are preferentially scavenged via



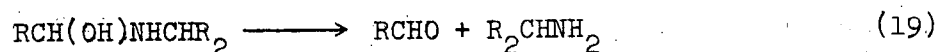
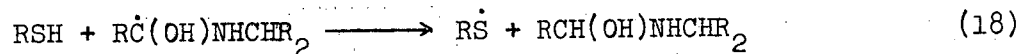
Here again the scavenging of  $e^-$  prevents reaction 3 and as a result the amine yield decreases to the limiting value of  $G(\text{RNH}_2) = 0.5$ . The  $\alpha$ -carbon radicals are scavenged by Fe III



We find at the higher Fe III concentrations that  $G(\text{RCHO}) = 3.9$ . The magnitude of this value indicates that a major fraction of the  $\text{RCONH}\dot{\text{C}}\text{R}_2$  radicals are removed via step 17.

Consider now the effects of a labile H-atom donor, RSH, in the radiolysis of liquid N-ethylacetamide. Table IIC shows the effects of increasing concentrations of mercaptoethanol on the yields of acetaldehyde and ethylamine. Yields of both products increase markedly with increasing RSH concentration and then level off at  $G(\text{RCHO}) \simeq 3.9$ ,  $G(\text{RNH}_2) \simeq 2.7$  at RSH concentrations above  $\sim 10^{-3}\text{M}$ . These results are consistent with the concept that  $e^-$  formed in the radiation-induced step 1 is quantitatively removed through addition to the carbonyl group of the peptide function as formulated in reactions 3 and 4. The

reduced peptide radical,  $\text{RC}(\text{OH})\text{NHCHR}_2$ , in the presence of appropriate concentrations of RSH is preferentially removed via\*



while the  $\alpha$ -carbon radical formed in step 1 are removed through



In accord with this formulation we find that the aldehyde produced in the radiolysis of liquid N-propylamide containing  $10^{-3}\text{M}$  RSH is predominantly acetaldehyde.

The limiting value  $G(\text{RCHO}) \approx 3.9$  obtained with N-ethylacetamide at the higher RSH concentrations represents the yield for reduction of the peptide bond via steps 3, 4, and 18 plus the yield of reaction 2 which as proposed above gives aldehyde with  $G = 0.8$  (via the dehydropeptide intermediate). Hence the RSH data give  $G_e^- = 3.9 - 0.8 = 3.1$  in good agreement with the direct measurement of  $G_e^-$  by the RCl method.

We are indebted to M. E. Jayko for the mass spectrometric analysis and to B. M. Weeks, M. E. Jayko, and H. A. Sokol for assistance during the early stages of this study.

\* An analogous radiation-induced reduction of the amide function in aqueous systems containing RSH has been observed (Ref. 19).

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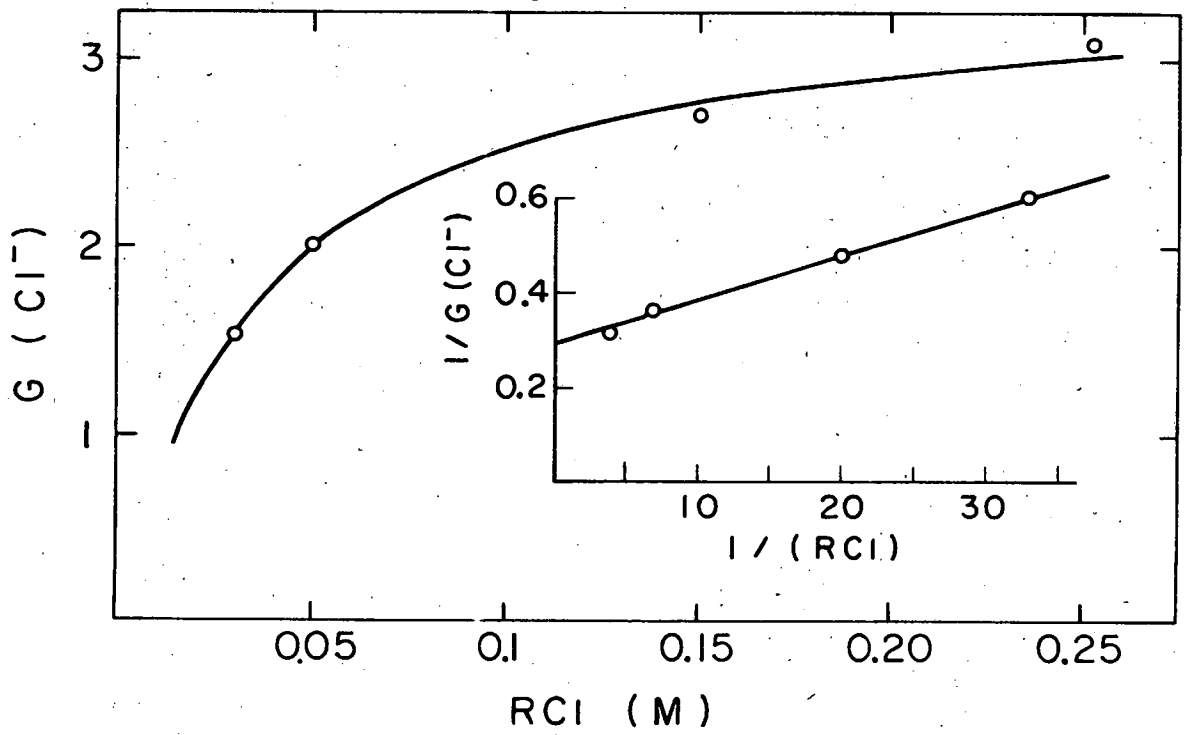
Table I. Product Yields in the  $\gamma$ -Radiolysis of N-ethylacetamide

Product	G
Acetaldehyde	1.1
Ethylamine	1.2
Ammonia	< 0.1
Hydrogen	0.8
Carbon Monoxide	0.4
Methane	< 0.3
Ethane	

Table II. Effect of Added Scavengers on Acetaldehyde and Ethylamine Yields in the  $\gamma$ -Radiolysis of N-ethylacetamide

	G(RCHO)	G(RNH <sub>2</sub> )
A. Ethylene dichloride (M)		
none	1.10±.05	1.21±0.1
0.05	1.61	0.66
0.16	1.82	0.76
0.25	1.89	0.47
0.32	2.03	—
0.39	2.06	0.61
B. Ferric ion (mM)		
none	1.10	1.21
0.13	1.36	—
1.32	2.79	—
5.48	—	0.68
13.2	3.92	—
132	3.96	0.50
C. Mercapto ethanol (mM)		
none	1.10	1.21
.43	1.52	2.04
1.48	3.90	2.72
82.0	3.90	2.73





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Fig. 1

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