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RADIATION CHEMISTRY OF ORGANO-NITROGEN COMPOUNDS

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RADIATION CHEMISTRY OF ORGANO-NITROGEN COMPOUNDS

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1. Introduction

The radiation chemistry of the organic compounds of nitrogen in their various ionic forms is of considerable intrinsic interest from the strictly physico-chemical standpoint, and also has important applications in numerous other areas of radiation research. Among these, for example, are the radiation-chemical synthesis and modification of nitrogenous chemicals and fibers, the radiation preservation and sterilization of foods and drugs, and, of course, the elucidation of the basic and elementary processes of radiobiology.

This paper treats some of the more recent investigations of reaction mechanism in the radiolysis of certain bio-organic derivatives of nitrogen. Included are studies of amino acids, amines, peptides, polypeptides, pyrimidines, and purines. The emphasis here is primarily on reactions in irradiated aqueous solution which are initiated by the radiation-induced step

$$H_2O \longrightarrow\!\!\!\!\!\sim\!\!\!\!\!\rightarrow H_2O_2, H_2, OH, H, e^-_{aq}, H^+ \qquad (1)$$

where e^-_{aq} represents the hydrated electron[1]. In the closing section we also consider a few solid-state systems for which specific and detailed reaction mechanisms have been outlined.

[1] (BARR and ALLEN, 1959; BAXENDALE and HUGHES, 1958; BOAG and HART, 1963; BOAG, 1963; CZAPSKI and SCHWARZ, 1962; HAYON and WEISS, 1959; HART and BOAG, 1962; KEENE, 1963; MAGEE, 1961; PLATZMAN, 1953; STEIN, 1952; WEISS, 1960).

2. Deamination

2.1. DEAMINATION REACTIONS

That the radiolytic deamination of the simpler α-amino acids in aqueous solutions arises as a consequence of the attack of labile species formed in water radiolysis was established in the quantitative studies of STEIN and WEISS [1949] and DALE, DAVIES, and GILBERT [1949]. The work of MAXWELL et al. [1954, 1955] and of SHARPLESS et al. [1955a, 1955b] provided the first identification of the major reaction stoichiometries involved in the radiation-induced degradation of glycine and alanine in evacuated and in oxygenated solutions. WEEKS and GARRISON [1956a, 1956b, 1958] identified the higher molecular weight products and offered a detailed mechanism that accounted both qualitatively and quantitatively for the formation of major and minor products. At the time, it was quite generally assumed that the initial reducing species formed in water radiolysis was the H atom. As we now know, this is not the case and only recently has the role of e^-_{aq} in the chemistry of these systems been clarified [GARRISON 1964; WEEKS, COLE, and GARRISON 1965].

The principal actions of ionizing radiation on the simpler α-amino acids such as glycine and alanine in oxygen-free aqueous solution leads to both oxidative and reductive deamination with formation of the corresponding keto acid and fatty acid as major degradation products. Smaller amounts of acetaldehyde, carbon dioxide, hydrogen, and higher molecular weight products are also observed [SHARPLESS et al. 1955a, 1955b; WEEKS and GARRISON 1958]. The yields of these products are strongly dependent on the amino acid concentration, which must be in the decimolar range to ensure the quantitative scavenging of the oxidizing and reducing species derived from water as shown in fig. 1. Major products

yields from 1 M glycine and 1 M alanine in oxygen-free solution under γ rays[1] are summarized in table 1.

The magnitude of the observed $G(NH_3)$ values and the fact that both keto acid and fatty acid are produced as major products indicate that deamination by both e^-_{aq} and OH occurs. To separately evaluate these processes chemically, it is convenient to add a scavenger which is preferentially reactive to OH (and H) but relatively unreactive towards e^-_{aq}. Formate is such a scavenger in that the rate constants for the reactions

$$OH + HCOO^- \longrightarrow H_2O + COO^- \qquad (2)$$

$$H + HCOO^- \longrightarrow H_2 + COO^- \qquad (3)$$

are $\sim 3\times10^8$ M^{-1} sec^{-1} and $\sim 1\times10^9$ M^{-1} sec^{-1} respectively whereas the rate of

$$e^-_{aq} + HCOO^- \longrightarrow HCOO^{-2} \qquad (4)$$

is $< 10^6$ M^{-1} sec^{-1} [HART, 1964]. The effect of added formate on $G(NH_3)$ from

[1] The relatively small but apparently very real discrepancies in the reported 100-eV yields of the oxidizing and reducing species formed in the γ radiolysis of water have been discussed by ALLEN [1964]. More recent measurements include those of HAYON [1965], MAHLMAN [1966], and HOCHANADEL and CASEY [1965]. The latter authors give $G_{OH} = 2.59$, $G_{e^-_{aq}} = 2.58$, $G_H = 0.55$, $G_{H_2} = 0.45$, and $G_{H_2O_2} = 0.72$.

oxygen-free solutions of glycine and alanine, 1 M, pH 7, is shown in fig. 2. It is seen that $G(NH_3)$ in both cases drops rapidly with increasing formate concentration and then levels off and becomes essentially independent of the concentration of the radical scavenger. The fatty acid yields, however, are wholly unaffected by formate ion even at the highest concentrations; whereas, the keto acid yields drop essentially to zero with the drop in $G(NH_3)$. Typical data for alanine are shown in fig. 3. The conclusion, then, is that the hydrated electron reacts with these α-amino acids according to the stoichiometry [GARRISON, 1964; WEEKS, COLE, and GARRISON, 1965]

$$e^-_{aq} + NH_3^+CH(R)COO^- \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (5)$$

$$\longrightarrow NH_2 + CH_2(R)COO^-. \qquad (5a)$$

You will notice in table 1 that $G(H_2)$ from 1-M glycine is appreciably greater than it is from 1-M alanine and we interpret this in terms of the branching reaction

$$e^-_{aq} + NH_3^+CH(R)COO^- \longrightarrow H + NH_2CH(R)COO^-. \qquad (5b)$$

That is, the glycine zwitterion acts in part simply as a proton donor, whereas alanine scavenges e^-_{aq} quantitatively via reaction (5,5a). Reaction (5b) is analogous to the conversion of e^-_{aq} to H by NH_4^+ as observed by JORTNER et al. [1962]. The fact that the keto acid products from these amino acids are wholly quenched by the added formate is consistent with the view that OH attacks these simpler amino acids preferentially at the α-carbon position

$$OH + NH_3^+CH(R)COO^- \longrightarrow H_2O + NH_3^+\dot{C}(R)COO^-. \qquad (6)$$

Reactions (5) and (6) are then followed by

$$NH_3^+CH(R)COO^- + \dot{C}H(R)COO^- \longrightarrow NH_3^+\dot{C}(R)COO^- + CH_2(R)COO^- \quad (7)$$

$$NH_3^+\dot{C}(R)COO^- + \dot{C}H(R)COO^- \longrightarrow NH_2^+=C(R)COO^- + CH_2(R)COO^- \quad (7a)$$

$$\longrightarrow \text{dimer} \quad (7b)$$

$$2NH_3^+\dot{C}(R)COO^- \longrightarrow NH_2^+=C(R)COO^- + NH_3^+CH(R)COO^- \quad (8)$$

$$\longrightarrow \text{dimer} \quad (8a)$$

$$NH_2^+=CH(R)COO^- + H_2O \longrightarrow NH_4^+ + RCOCOO^- \quad (9)$$

where reactions (7b) and (8a) occur in relatively low yield and account for the higher molecular weight products. The predominant path for removal of the α-carbon radical $NH_3^+\dot{C}(R)COO^-$ is through the disproportionation reactions (7a,8). The reaction sequence (1,5 to 9) accounts both qualitatively and quantitatively for the radiation chemistry of the glycine and alanine zwitterions at pH 7 [WEEKS and GARRISON, 1958; WEEKS, COLE, and GARRISON, 1965].

Although the zwitterions of glycine and alanine undergo reductive deamination on reaction with e^-_{aq}, this does not necessarily mean that the cation (protonated) forms also undergo reductive cleavage of the N-C bond. It is conceivable that the cation form, $NH_3^+CH(R)COOH$, reacts simply as an organic acid in which case the chemistry would be confined to the carboxyl group

$$NH_3^+CH(R)COOH + e^-_{aq} \longrightarrow NH_3^+CH(R)COO^- + H \quad (10)$$

$$\longrightarrow NH_3^+CH(R)CO + OH^- \quad (10a)$$

as observed by THOMAS [1964] with acetic acid. We find experimentally,

however, that reactions (10,10a) do not occur to any appreciable extent, at least with glycine and alanine. The only effect of protonation is to increase the velocity constant of the reductive deamination reaction [WILLIX and GARRISON, 1965a, 1967]. These effects of ionic form on reaction rates are described more fully in a following section. Of course, as the pH is decreased, the conversion reaction

$$e^-_{aq} + H_3O^+ \longrightarrow H + H_2O \qquad (11)$$

becomes of increasing importance, and, with these simplest amino acids, both H and OH are removed at the α-carbon position

$$H + NH_3^+CH(R)COOH \longrightarrow NH_3^+\dot{C}(R)COOH + H_2. \qquad (12)$$

Hence in strongly acidic solutions the fatty acid yield approaches zero and ammonia and keto acid appear as the only major products in accord with the reaction scheme given by equations (6,12,8) [WEEKS, COLE, and GARRISON, 1965].

2.2. EFFECTS OF HEAVY METAL IONS

The effects of heavy metal ions such as Cu^{+2} and Fe^{+3} on the radiation chemistry of the α-amino acids is of interest from both the chemical and biochemical standpoint. Such ions are effectively chelated by the amino acids according to the pH dependent equilibria

$$[Cu(NH_2CH_2CO_2^-)]^+ + NH_3^+CH_2CO_2^- \rightleftharpoons [Cu(NH_2CH_2CO_2^-)_2] + H^+ \qquad (13)$$

$$Cu^{+2} + NH_3^+CH_2CO_2^- \rightleftharpoons [Cu(NH_2CH_2CO_2^-)]^+ + H^+ \qquad (14)$$

where the equilibrium constants are $K_{12} = 10^{-1.4}$, $K_{13} = 10^{-2.9}$ at 20°C. Since the free Cu^{+2} ion has been shown to react rapidly with e^-_{aq}

$$e^-_{aq} + Cu^{+2} \longrightarrow Cu^{+1} \qquad (15)$$

with $k_{15} = 3 \times 10^{10}$ M^{-1} sec^{-1} [BAXENDALE, FIELDEN, and KEENE, 1963], there arises the question of whether the glycine-Cu(II) chelates react with e^-_{aq} through simple capture analogous to reaction (15) or by a path that leads to chemical degradation of the ligand through reaction akin to that given in equation (5), i.e.,

$$e^-_{aq} + [Cu(NH_2CH_2CO_2^-)_2] + H_2O \rightarrow [Cu(NH_3)(NH_2CH_2CO_2^-)]^+ + CH_2CO_2^- + OH^- . \qquad (16)$$

The effects of Cu^{+2} on the radiation chemistry of glycine in oxygen-free solution over the pH range ~2.5 to 9 have recently been studied [WILLIX and GARRISON, 1965a]. The presence of Cu^{+2} leads to a considerable simplification in the radiation chemistry at pH values below 6 as shown in fig. 4 and table 2. Under these conditions the bulk of the cupric ion is present as Cu^{+2} or $[Cu(NH_2CH_2CO_2^-)]^+$ with the appropriate number of water molecules of hydration. The stoichiometry of the over-all radiation-induced reaction is given by

$$2Cu(II) + NH_2CH_2CO_2H + H_2O \rightarrow 2Cu(I) + 2H^+ + CHOCO_2H(CH_2O + CO_2) + NH_3 \qquad (17)$$

with $G(NH_3) \simeq G(RCHO) \simeq 2.2$. A consistent explanation is that the reducing species in the form of e^-_{aq} or H is preferentially scavenged by Cu^{+2} or $[Cu(NH_2CH_2COO^-)]^+$ at pH values below 6 to give cuprous ion without net chemical effect in glycine. Glycine degradation is ascribed to OH attack via

reaction (6) followed by the stoichiometry

$$Cu(II) + NH_3^+\dot{C}HCOO^- + H_2O \rightarrow Cu(I) + NH_4^+ + CHOCOO^- + H^+ \qquad (18)$$

with some contribution from[1]

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH + OH^-. \qquad (19)$$

As the pH of the glycine-Cu(II) system is increased above ~pH 6, the concentration of the bis(glycinato)Cu(II) chelate increases sharply and at pH 8.5 essentially all of the Cu^{+2} is so bound. The carbonyl yield $G(CHOCOOH) + G(CH_2O)$ remains essentially constant with increasing alkalinity, indicating that oxidation by OH via steps (6) and (18) retains the stoichiometry of equation (17). The abrupt increase in $G(NH_3)$ and $G(CO_2)$ over the range pH 6 to 9 is associated with the onset of a competing reaction of e^-_{aq} that leads to glycine deamination. Solutions of preformed bis(glycinato)Cu(II) at pH 8 also give $G(NH_3) \simeq 5.0$ (and maximal yields of the other products). It is concluded therefore that bis(glycinato)Cu(II) scavenges e^-_{aq} as indicated by reaction (16). The addition of formate as a competing scavenger of OH radicals reduces $G(NH_3)$ from ~5.0 to a limiting value of ~3.3 (fig. 5). This value is somewhat greater than would be expected on the basis of the

[1] Evidence that the "free" OH may not be produced in the Fenton-type reaction $M^{+n} + H_2O_2 \rightarrow M^{+n+1} + OH^- + OH$ has been reported by PIETTE, BULOW, and LOEFFLER [1964] and by SHIGA [1965].

published values of $G_{e^-_{aq}}$. Apparently, the Cu^{+1} in reaction (19) is present in the chelate form $[Cu(NH_2CH_2COO^-)]^+$ and as a result the OH radical is liberated in close proximity to a glycine molecule and is not then available for scavenging by moderate concentrations of formate in the bulk. The product stoichiometries require that the carboxymethylene radical, $\dot{C}H_2COOH$, formed in reaction (16) in the presence of Cu(II) be removed by the equivalent of

$$CH_2CO_2^- + Cu(II) + H_2O \rightarrow Cu(I) + CH_3OH + CO_2. \qquad (20)$$

ANBAR, MUNOZARD, and RONA [1963] have studied the effect of cupric ion on the radiolysis of a number of amino compounds that form stable complexes with heavy metal ions and find no evidence for reductive deamination in dilute oxygen-free solution. For example the Cu(II) complex of ethylene diamine $[Cu(NH_2(CH_2)_2NH_2)]^{+2}$ reacts with e^-_{aq} through simple capture to yield Cu(I)

$$e^-_{aq} + [Cu(NH_2(CH_2)_2NH_2)]^{+2} \rightarrow [Cu(NH_2(CH_2)NH_2)]^{+1} \qquad (21)$$

as does the glycine complex $[Cu(NH_2CH_2CO_2^-)]^{+1}$, as we have noted above. Apparently the difference between these complex ions and the neutral bis(glycinato)Cu(II) chelate, $[Cu(NH_2CH_2CO_2^-)_2]$, is that, in the chelates, the reactive orbitals of the metal moiety are adequately shielded and deamination occurs preferentially as shown in equation (16).

2.3. EFFECTS OF OXYGEN

The introduction of molecular oxygen at a sufficiently high relative concentration results in a quenching of the reductive deamination reaction since the reducing species e^-_{aq} and H are preferentially scavenged

$$e^-_{aq}(H) + O_2 \rightarrow O_2^-(HO_2) \qquad (22)$$

where O_2^- and HO_2 are related by the equilibrium

$$HO_2 \rightleftharpoons H^+ + O_2^-. \qquad (23)$$

Reactions of OH are not inhibited by molecular oxygen and in the case of glycine and alanine the α-carbon radicals so formed are removed by O_2 to form the peroxy radical

$$NH_3^+\dot{C}(R)COO^- + O_2 \rightarrow NH_3^+C(\dot{O}_2)(R)COO^- \qquad (24)$$

which either dissociates to form the labile imino acid

$$NH_3^+C(\dot{O}_2)(R)COO^- \rightarrow NH_2^+=C(R)COO^- + HO_2 \qquad (25)$$

$$H_2O + NH_2^+=C(R)COO^- \rightarrow NH_3 + (R)C\dot{O}COOH \qquad (26)$$

$$\rightarrow NH_3 + (R)CHO + CO_2 \qquad (26a)$$

or reacts to form the unstable hydroperoxide

$$HO_2 + NH_3^+C(\dot{O}_2)(R)COO^- \rightarrow NH_3^+C(OOH)(R)COO^- + O_2 \qquad (27)$$

$$H_2O + NH_3^+C(OOH)(R)COO^- \rightarrow NH_3 + (R)COCOOH + H_2O_2 \qquad (28)$$

$$\rightarrow NH_3 + (R)CHO + CO_2 + H_2O_2 \qquad (28a).$$

In any case, the major product stoichiometry in dilute oxygenated solutions of glycine and alanine is given by

$$G(NH_3) \simeq G(R_2CO) \simeq G_{OH}$$

[BARRON, AMBROSE, and JOHNSON, 1955; MAXWELL, PETERSON, and WHITE, 1955; WEEKS and GARRISON, 1956b, 1958].

2.4. EFFECTS OF SUBSTITUTION

Although the reactions of e^-_{aq}, H, and OH are localized at the α-carbon position of glycine, it is clear that, with the more complex α-amino acids, other competing loci become available for reaction.

For example, increasing the length of the aliphatic side chain increases the number of C-H bonds susceptible to OH attack. Hence the relative importance of oxidative deamination at the α-carbon position would be expected to decrease. This effect is shown in fig. 6 where $G(NH_3)$ for a number of aliphatic amino acids is plotted as a function of the total number of C-H bonds in the amino acid residue [HOLIAN and GARRISON, 1967a]. The solutions at pH 5 to 6 were oxygen-flushed and the amino acid concentrations are such that it may be assumed on the basis of known rate constants[1] that e^-_{aq} is quantitatively removed by O_2 and that OH is quantitatively scavenged by the amino acid. Since in oxygenated solution one molecule of ammonia is liberated per OH radical removed at the α-carbon position as formulated in reactions (6,22 to 28), it would appear that there is an approximate linear relationship between $G(NH_3)$ and the number of

[1] (ADAMS et al., 1965; BAXENDALE et al., 1964; BRAAMS, 1965, 1966; DAVIES, EBERT, and SWALLOW, 1965; HART, 1964; SCHOLES et al., 1965).

C-H bonds of a particular residue. This result is somewhat unexpected and indeed may involve certain fortuitous factors to be discussed in sections 5.2 and 5.3. KOPOLDOVA, LIEBSTER and BABICKÝ[1961,1962,1963a,1963b] have made detailed studies of the products formed in the γ radiolysis of a number of aliphatic amino acids ranging in molecular weight from α-amino butyric acid to leucine, in both evacuated and oxygenated solution. They find higher molecular weight products resulting from the dimerization of radicals formed through hydrogen abstraction at β, γ, , etc. positions of the aliphatic side. For example, with oxygen-free 0.05 M solutions of α-amino butyric acid they find diamino suberic acid

$$COOH - CH(NH_2) - (CH_2)_4 - CH(NH_2) - COOH$$

as the principal dimer product together with lesser amounts of diaminomethyl pinelic acid

$$COOH - CH(NH_2) - (CH_2)_3 - CH(CH_3) - CH(NH_2)COOH.$$

It is somewhat surprising to see that the yield of the former is almost 10 times that of the latter which would suggest that radical attack occurs almost exclusively at the terminal methyl group. It is even more surprising to find that the initial product yields correspond to -G(aminobutyric acid) $\simeq$ 8, G(diaminosuberic acid) $\simeq$ 3.0, $G(NH_3) \simeq 4$, together with $G \simeq 3$ for the combined yield of lesser products. It is very difficult to explain the magnitude of these product yields in terms of accepted G values for formation of e^-_{aq}, H, and OH in the radiation decomposition of water. The fact that high dimer yields are also observed in the oxygenated system must be attributed to an early depletion of dissolved oxygen during the irradiation period.

Deamination at the α-carbon position is a relatively minor process in the radiolysis of the aromatic amino acids. Phenylalanine, tyrosine, and tryptophane give $G(NH_3)<0.5$ in both evacuated and oxygenated solutions; the carbon-carbon double bond appears to represent the major locus of reaction in these systems[1]. However, the possibility of parallel OH attack at the β carbon position does not seem to be completely excluded.

The recent work of EL SAMAHY, WHITE, and TRUMBORE [1964] and of ARMSTRONG and WILKENING [1964] has established that e^-_{aq} reacts directly with the SH group of cysteine and other simple thiols

$$RSH + e^-_{aq} \rightarrow R + HS^- \qquad (29)$$

where $k_{29} \sim 10^{10}\ M^{-1}\ sec^{-1}$. The radiation chemistry of cysteine[2] in dilute oxygen-free solution may be interpreted in terms of reaction (29) and the

[1] (ALEXANDER and ROSEN, 1961; FLETCHER and OKADA, 1961; JAYSON, SCHOLES, and WEISS, 1954; KORGAONKAR and DONDE, 1962; NOSWORTHY and ALLSOPP, 1956; PETER and RAJEWSKY, 1963; ROWBOTTOM, 1955).

[2] (ARMSTRONG and WILKENING, 1964; DALE and DAVIES, 1951; IBRAGIMOV, TULYAGANOV, and TUICHIEV, 1962; KOCH and FRÄNZ, 1960; LITTMAN, CARR, and BRADY, 1957; MARKAKIS and TAPPEL, 1960; PACKER, 1963; RIESZ and BURR, 1962; EL SAMAHY, WHITE, and TRUMBORE, 1964; SHAPIRO and ELDJARN, 1955; SWALLOW, 1952; WHITCHER, ROTHERHAM and TODD, 1953).

radical-removal steps

$$RSH + H \rightarrow RS + H_2 \quad (30)$$

$$\rightarrow R + H_2S \quad (30a)$$

[RIESZ and BURR 1962], and

$$RSH + OH \rightarrow RS + H_2O \quad (31)$$

where reaction (29) occurs in competition with the conversion reaction (11) in acid solution. Deamination is not observed as an initial reaction in either evacuated or oxygenated solution.

The radiation chemistry of cystine in aqueous solution is also largely dominated by the sulfur moiety[1]. PURDIE [1967] has recently examined this system in detail and suggests that both e^-_{aq} and OH react with cleavage of the disulfide linkage

$$RSSR + e^-_{aq} \rightarrow RS^- + RS \quad (32)$$

$$RSSR + OH \rightarrow RSOH + RS. \quad (33)$$

However, some competition involving the α-carbon position appears to be involved,

[1] (ARMSTRONG and GRANT, 1963; BRDIČKA, SPURNÝ, and FOJTIK, 1963; FORBES and SAVIGE, 1962; GRANT, MASON, and LINK, 1961; MARKAKIS and TAPPEL, 1960; PURDIE, 1967).

since $G(NH_3) \sim 0.5$ for both evacuated and oxygenated solutions.

ARMSTRONG and GRANT [1963] find that deamination is the major chemical consequence of the radiolysis of cystine in dilute hydrochloric acid solution under which condition OH is converted to Cl via

$$OH + H^+ + Cl^- \rightarrow H_2O + Cl \qquad (34)$$

and of course e^-_{aq} is converted to H via reaction (11). With 4×10^{-3} M cystine in 0.02 M hydrochloric acid, the initial ammonia yield corresponds to $G(NH_3) \simeq 2.5$ for both evacuated and aerated solutions. We would suggest that in the evacuated case, both H and Cl attack preferentially through H abstraction at the α-carbon position and that these α-carbon radicals then disproportionate as described in eq. (8). In oxygenated solution, the H reaction is quenched whereas the α-carbon radicals formed by Cl attack are quantitatively removed via reaction (25) or (27).

Although methionine is a sulfur-containing amino acid, it does nevertheless yield ammonia (and a carbonyl) as major products on radiolysis in evacuated, $G(NH_3) = 2.0$, and in oxygenated solution, $G(NH_3) = 2.5$ [HOLIAN and GARRISON, 1967a]. Certainly, the reductive and oxidative reactions of e^-_{aq} and OH at the sulfur moiety are not negligible[1] but, the observed $G(NH_3)$ values suggest

[1] (KOPOLDOVA et al., 1958; OHARA, 1966; SHIMAZU, KUMTA, and TAPPEL, 1964).

that the α-carbon position is competing effectively as a locus of chemical change. Further work on the radiation chemistry of methionine appears warranted.

3. Chemical Criteria for Reductive Deamination

Although, as discussed in section 2.1, both the zwitterion and cation forms of the simpler α-amino acids undergo reductive deamination via reaction (5), there is still the interesting question as to whether or not a simple dipeptide such as glycylglycine undergoes an analogous reductive cleavage of the terminal N-C bond. In evaluating the experimental evidence it is convenient here first to compare the ammonia yields from glycine and from glycylglycine at pH 7 in oxygen-free solution under γ rays. We find [WILLIX and GARRISON, 1967] in fig. 7 that $G(NH_3)$ from both compounds increases abruptly with solute concentration and approaches limiting yields, under which conditions we may assume that all of the OH, H, e^-_{aq} formed in the radiation-induced reaction are quantitatively scavenged by the solute. The effects of added formate ion on these maximal ammonia yields from glycine and glycylglycine are shown in fig. 8. In both cases $G(NH_3)$ decreases with increasing formate concentrations and then levels off to a limiting value which remains essentially constant at the higher formate concentrations. With glycine, the ammonia yield levels off with increasing formate concentrations to give $G(NH_3) = 1.8$ as a measure of the reductive deamination reaction (5) in this system: the yield for conversion of e^-_{aq} to H through reaction (5b) corresponds to $G_{e^-_{aq}} - 1.8 = 0.7$, where $G_{e^-_{aq}} = 2.5$ represents the yield for production of e^-_{aq} by γ rays in the radiation-induced step 1. As noted in section 2.1, this production of H atoms with $G = 0.7$ contributes to the relatively high hydrogen yields observed in the γ radiolysis of neutral glycine. The limiting ammonia yield from glycylglycine in the presence of excess formate ion corresponds to $G(NH_3) = 2.5$ and we conclude in this case that the reaction of e^-_{aq} with the glycylglycine zwitterion via

reaction (5) is essentially quantitative. Conformation of the above formulation is found in the data of fig. 9, which shows the effect of a competing electron scavenger, chloracetate ion, on ammonia yields from oxygen-free 0.25 M glycylglycine. Chloracetate reacts rapidly ($k_{35} = 1.2 \times 10^9$ M^{-1} sec^{-1}) with the hydrated electron [ANBAR and HART, 1965], via

$$e^-_{aq} + RCl \rightarrow R + Cl^- . \qquad (35)$$

The ammonia yield drops rapidly with increasing chloracetate concentration and this decrease in $G(NH_3)$ is accompanied by a corresponding and stoichiometric increase in $G(Cl^-)$.

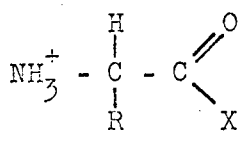
The formate technique has been used to measure the yield of reductive deamination in the γ radiolysis of a number of amino acids and amino acid derivatives in neutral oxygen-free solution. The results are summarized in table 3. We note first that of the compounds studied only those with an amino group at the carbon position α to a carboxyl, ester, or peptide linkage undergo reductive deamination as a major reaction. Certainly the implication here is that the unsaturated C=O group which is common to all of the above three types of linkage is somehow involved in the deamination reaction. This aspect of the subject is treated in a later section.

We note also from table 3 that it is with glycine and alanine that we observe the most pronounced decrease in $G(NH_3)$ on addition of the radical scavenger. As we have noted, the reactions of OH radicals with these simplest amino acids occurs almost exclusively at the α-carbon position to yield radicals of the type $NH_3^+\dot{C}R_2$. These α-carbon radicals have the property of disproportionating via reactions (7a, 8) to yield ammonia and carbonyl.

However, the more complex amino acids, valine for example, in table 3 offer competing loci for OH and H attack at the β, γ, etc. positions of the side chain. Radicals formed at these sites have the chemical properties of ordinary aliphatic radicals and undergo simple dimerization without the involvement of nitrogen chemistry. Hence, the quenching of these latter reactions by formate ion does not influence $G(NH_3)$.

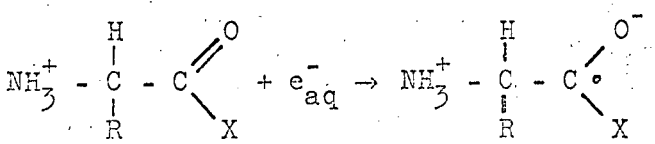
Similarly, the presence of high concentrations of formate ion has had a relatively small effect on $G(NH_3)$ from glycylglycine and glycine ethyl ester. In these cases, also, additional loci are available for OH attack and the evidence is that attack at the terminal carbon position to give the $NH_3^+\dot{C}R_2$-type radical is relatively unimportant. We have shown elsewhere (section 5) that peptides are susceptible to OH attack along the main chain to give radicals of the type $RCONH\dot{C}R_2$, which species undergo simple dimerization to yield the α-α'diamino acid derivatives. The present evidence is that reaction of OH with the simple peptide derivatives of glycine occurs preferentially at the C-H bond α to the peptide nitrogen.

We conclude that reductive deamination via reaction (5) is a general and characteristic reaction of compounds containing the grouping



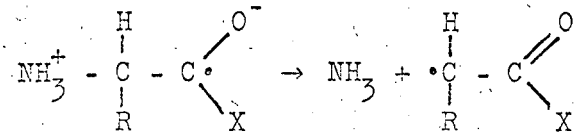
when X represents O, OH, NHR, etc. If there is more than one carbon unit between the amino and carbonyl groups reductive deamination does not occur. β-alanine and ε-aminocaproic acid are examples of amino compounds that do not

undergo reductive deamination. Now, RIESZ and MORRIS [1965] find that the simple aliphatic amine cations, methyl ammonium ion, for example, react with e^-_{aq} exclusively via the conversion reaction (5b) to yield H. Such reaction is analogous to the conversion of e^-_{aq} to H by NH_4^+ as observed by JORTNER et al. [1962], who also showed that the rates of conversion of e^-_{aq} to H by proton donors correlate to a first approximation with the pK values of the donor acids as implied by the Bronsted general acid catalysis law (the lower the pK the faster the reaction with e^-_{aq}). And, BRAAMS [1965,1966] has studied, by the method of pulse radiolysis, the rate of disappearance of e^-_{aq} in oxygen-free neutral solutions of a variety of simple amines, β-amino acids, α-amino acids, and peptides. He finds in all cases a reasonably good correlation between the dissociation constants of the protonated amino groups and their rate constants for reaction with e^-_{aq}. BRAAMS [1965] concludes without specifying the nature of the chemistry involved that the protonated amino group of all of these various classes of amino compounds represents the locus of the reaction with e^-_{aq}. It is likely that such is the case for those compounds that react with e^-_{aq} via the conversion reaction (5b) in accord with the earlier work of JORTNER et al. [1962]. It is not clear that the same correlation between pK and reaction rate in the reductive deamination reactions of the α-amino acids, and the ester and peptide derivatives, necessarily implies that e^-_{aq} is reacting at the locus of the amino group. In fact, the finding that an unsaturated double bond must be present α to the amino group for reductive deamination to occur, suggests the possibility that e^-_{aq} adds to the double bond [WEEKS, COLE, and GARRISON, 1965]



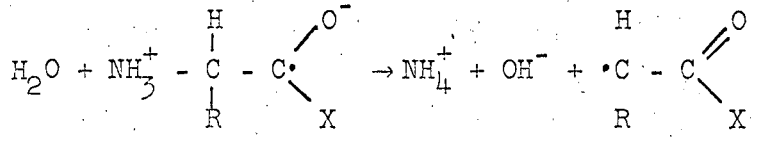
(36)

which is then followed by the dissociation



(37)

or the hydrolysis



(37a)

The recent finding [WILLIX and GARRISON, 1965b] that the glycine-Cu^{+2} chelate undergoes reductive cleavage of the N-C bond on reaction with e^-_{aq} as given by the over-all stoichiometry of eq. (16) is also consistent with the interpretation that e^-_{aq} reacts at the C=O bond of the ligand. The formulation (36,37) is in accord with the finding that the rate of reaction of e^-_{aq} with the zwitterion forms of the α-amino acids is quite low as compared to its rates of reaction with the cation, ester, and peptide forms (table 4), since the double bond character of the C=O group of the carboxylate ion is considerably less than that of the C=O group of the acid, ester, and peptide derivatives. While it is true that the rate of reaction of e^-_{aq} with isolated peptide linkage, N-ethylacetamide for example, is low, the presence of the NH_3^+ group in the α position induces a strong polarization in the C=O bond which would account for the enhanced reactivity of the α-amino acids towards e^-_{aq}. And, since this polarization is manifested in turn as an increase in the acid strength of the NH_3^+ group [GREENSTEIN and WINITZ, 1961], a correlation between the pK of the amine group and the rate of reaction of e^-_{aq} via reaction (5) would be expected.

The recent results of CLAY and KABI [1965] indicate that e^-_{aq} reacts with benzyldimethylamine cation and with benzyltrimethyl ammonium ion to yield dimethylamine and trimethylamine respectively. This suggests the reactive grouping in the general case corresponds to $R_2NH^+ - C(R_2) - C\begin{matrix}X\\Y\end{matrix}$.

4. Rates of Reductive Deamination

Rates of reaction of e^-_{aq} with the cation and zwitterion forms of the amino acids and derivatives that have been shown to undergo reductive deamination have been measured by the method of competition kinetics [WILLIX and GARRISON, 1967]. The data of table 4 are derived from an analysis of competitive kinetics involving the organo-nitrogen compound at a fixed concentration, 0.05 M,

$$e^-_{aq} + R_3N \rightarrow \text{product} \qquad (38)$$

and a second solute, chloracetic acid, in increasing concentration over the range 5×10^{-4} M to 5×10^{-2} M. The latter reacts with e^-_{aq} according to the stoichiometry [HAYON and ALLEN, 1961; HAYON and WEISS, 1958],

$$e^-_{aq} + RCl \rightarrow R + Cl^- \qquad (39)$$

to give chloride ion which is followed analytically.

All the compounds studied have pK values such that at pH 7 each solute exists almost exclusively as a single species, i.e., the zwitterion form of the α-amino acids, β-amino acids, and dipeptides, the cation form of the simple amines, and the anion forms of the acetylamino acids and the chloroacetic acid. For simplicity, we distinguish these proton-deficient species in terms of $\overline{R_3N}$ and $\overline{RCl}$ as defined by the equilibria

$$(\overline{R_3N})(H^+)/(R_3N) = K_{R_3N}$$

$$(\overline{RCl})(H^+)/(RCl) = K_{RCl}.$$

The reactions in neutral solution are written

$$\overline{R_3N} + e^-_{aq} \rightarrow \text{products}, \qquad (\overline{38})$$

$$\overline{RCl} + e^-_{aq} \rightarrow \overline{R} + Cl^-, \qquad (\overline{39})$$

to distinguish them from the corresponding reactions of the acid forms given by eqs. (38 and 39). For simple competition in these two solute systems at pH 7, we may derive the expression

$$\frac{1}{G(Cl^-)} = \frac{1}{G_{e^-_{aq}}} + \frac{1}{G_{e^-_{aq}}}\left(\frac{\bar{k}_{38}}{\bar{k}_{39}}\right)\left(\frac{\overline{R_3N}}{\overline{RCl}}\right) ,$$

where $G(Cl^-)$ represents the experimentally observed chloride yield, $(\overline{R_3N})$, and $(\overline{RCl})$ the concentration of the two solutes species in neutral solution, and $\bar{k}_{38}$ and $\bar{k}_{39}$ the respective velocity constants for reaction with e^-_{aq}. A plot of the reciprocal of the chloride yield as a function of $(\overline{R_3N})/(\overline{RCl})$ gives a straight line with slope $1/G_{e^-_{aq}}(\bar{k}_{38}/\bar{k}_{39})$, as shown by the typical data of fig. 10 [WILLIX and GARRISON, 1967].

The intercept value $1/G(Cl^-) = 0.36$ gives $G_{e^-_{aq}} \approx 2.8$, in reasonable agreement with published values. Taking $\bar{k}_{39} = 1.2 \times 10^9\ \underline{M}^{-1}\ sec^{-1}$ as determined by ANBAR and HART [1965] in pulse radiolysis studies, we obtain the values of $\bar{k}_{38}$ given in table 4.

A parallel series of experiments was run at pH 3 to obtain rate constants for reactions of e^-_{aq} with these organo-nitrogen compounds in the protonated form. Results are obtained in terms of the rate constant for reaction of e^-_{aq} with the undissociated chloroacetic acid molecule. Corrections must be included for removal of e^-_{aq} by the proton reaction of eq. (11),

$k_{11} = 2.2 \times 10^{10}\ \underline{M}^{-1}\ sec^{-1}$, and for the competition by $\overline{R_3N}$ and $\overline{RCl}$ at the concentrations determined by the equilibrium constants K_{R_3N} and K_{RCl}. It can be shown that the reciprocal yield relationship then takes the more complicated form

$$\frac{1}{G(Cl^-)} = \frac{1}{G_{e^-_{aq}}} + \frac{1}{G_{e^-_{aq}}}\left\{\frac{k_{38}(H^+) + \bar{k}_{38}K_{R_3N} + k_{11}(H^+)^2/(R_3N)}{k_{39}(H^+) + \bar{k}_{39}K_{RCl}}\right\}\left(\frac{R_3N}{RCl}\right).$$

Here again a plot of the reciprocal chloride ion yield versus $(\overline{R_3N})/(\overline{RCl})$ should give a straight line with intercept equal to $1/G_{e^-_{aq}}$, with the slopes given by

$$\frac{1}{G_{e^-_{aq}}}\left\{\frac{k_{38}(H^+) + \bar{k}_{38}K_{R_3N} + k_{11}(H^+)^2/(R_3N)}{k_{39}(H^+) + \bar{k}_{39}K_{RCl}}\right\}.$$

We may calculate the respective values of k_{38}, assuming $k_{39} = 6.6 \times 10^9\ \underline{M}^{-1}\ sec^{-1}$ as derived from the work of HAYON and ALLEN [1961]. Values of k_{38} so obtained are listed in table 4.

Some of the rate constants reported in table 4 have also been measured by pulse-radiolysis methods in which e^-_{aq} is followed spectrophotometrically [BRAAMS, 1965,1966; DAVIES, EBERT, and SWALLOW, 1965]. These values are included in parentheses under k_{38} in table 4. Agreement between the two methods is reasonably good. Rate constants for reaction of e^-_{aq} with the cation forms of the amino acids and simple peptides (k_{38}) are not available from pulse-radiolysis work. Presumably this is because of the very short lifetime of e^-_{aq} in aqueous solution at the low pH values needed to retain an appreciable fraction of the amino acid in the cation form. At pH 3, for example, assuming $k_{11} = 2 \times 10^{-10}\ \underline{M}^{-1}\ sec^{-1}$, the lifetime of e^-_{aq} corresponds to $1/(10^{-3})(2 \times 10^{10}) = 0.5 \times 10^{-7}$ sec.

5. Degradation of Substituted Amines

5.1. DEGRADATION REACTIONS

JAYSON, SCHOLES, and WEISS [1955] first showed that substituted aliphatic amines such as diethylamine are degraded to yield the aldehyde on radiolysis in diluted aqueous solution containing dissolved oxygen. At about the same time it was shown [JAYKO and GARRISON, 1956] that the primary amine is produced concomitantly with the aldehyde and, to account for these results, a simple reaction scheme was outlined involving the intermediate formation of a Schiff-base derivative

$$H_2O \rightarrow H + OH$$

$$H + O_2 \rightarrow HO_2$$

$$OH + RNHCH_2R \rightarrow RNH\dot{C}HR + H_2O \qquad (40)$$

$$O_2 + RNH\dot{C}HR \rightarrow RN{=}CHR + HO_2 \qquad (41)$$

$$H_2O + RN{=}CHR \rightarrow RNH_2 + RCHO, \qquad (42)$$

which gives the over-all stoichiometry

$$RNHCH_2R + O_2 + H_2O \rightarrow RNH_2 + RCHO + H_2O_2 . \qquad (43)$$

It was also proposed by JAYKO and GARRISON [1956] that peptides, as a particular class of secondary amines, would be expected to undergo analogous chemistry following OH attack at the C-H position α to the peptide nitrogen to give the over-all stoichiometry

$$RCONHCHR_2 + O_2 + H_2O \rightarrow RCONH_2 + R_2CO + H_2O_2 . \qquad (44)$$

And, by analogy, the oxidation of tertiary and quaternary nitrogen functions was represented in terms of

$$R_2N\text{-}CH_2R + O_2 + H_2O \rightarrow R_2NH + R_2CO + H_2O_2 \qquad (45)$$

$$R_3N^+CH_2R + O_2 + H_2O \rightarrow R_3N + R_2CO + H_2O_2 + H^+. \qquad (46)$$

While subsequent work[1] on the radiation chemistry of a wide variety of organonitrogen compounds has confirmed the essential correctness of these formulations regarding the lability of substituted amines in radiolysis, it is also clear that the intermediate chemistry of these processes is considerably more complex than was originally envisioned. In the following sections we consider the nature of some of these intermediate processes.

[1] (ATKINS, BENNETT-CORNIEA, and GARRISON, 1967; BENNETT-CORNIEA and GARRISON, 1959; GARRISON and WEEKS, 1962; GARRISON, JAYKO, and BENNETT-CORNIEA, 1962; HATANO, 1960; JAYKO and GARRISON, 1958; JAYKO, WEEKS, and GARRISON, 1958; LIEBSTER and KOPOLDOVA, 1966; NAKKEN and PIHL, 1966; SOKOL, BENNETT-CORNIEA and GARRISON, 1965; SOUTHERN and RHODES, 1965; WEEKS, KLAND-ENGLISH, and GARRISON, 1961).

5.2. N-ACETYL AMINO ACIDS

Ammonia is a relatively minor product in the radiolysis of the N-acetyl derivatives of the simpler α-amino acids, glycine and alanine, in oxygen-free solution. The major products are higher molecular weight compounds which, in the case of N-acetylglycine, have been shown to be predominantly the α-α N-acetyldiaminosuccinic acid derivative [WEEKS, KLAND-ENGLISH, and GARRISON, 1961; GARRISON and WEEKS, 1962]. The evidence is that with both N-acetylglycine and N-acetylalanine the attack of OH and H occurs predominantly at the α-carbon position.

$$RCONHCHR_2 + OH \rightarrow RCONH\dot{C}R_2 + H_2O \qquad (47)$$

$$RCONHCHR_2 + H \rightarrow RCONH\dot{C}R_2 + H_2 . \qquad (48)$$

The peptide radicals formed in reactions (47 and 48) then dimerize preferentially to give the α-α' diaminosuccinic acid derivative.

$$2\ RCONH\dot{C}R_2 \rightarrow \begin{matrix} RCONHCR_2 \\ | \\ RCONHCR_2 \end{matrix} . \qquad (49)$$

Disproportionation of these radicals to form the dehydropeptide

$$2\ RCONH\dot{C}R_2 \rightarrow RCONHCHR_2 + RCON{=}CR_2 \qquad (50)$$

would lead on subsequent mild hydrolysis to the formation of ammonia and keto acid

$$RCON{=}CR_2 + H_2O \rightarrow RCOOH + NH_3 + R_2CO . \qquad (51)$$

While mild hydrolysis of these irradiated systems does liberate small amounts of ammonia and keto acid, the chemical and physical evidence is that these products arise not from reaction (50), but rather from the reaction of the peptide radical $RCONH\dot{C}(R)$ with the small amounts of H_2O_2 formed in the radiation decomposition of water.

$$H_2O_2 + RCONH\dot{C}R_2 \rightarrow RCONHC(OH)R_2 + OH \quad (52)$$

$$RCONHC(OH)R_2 + H_2O \rightarrow RCOOH + NH_3 + R_2CO \quad (53)$$

to give $G(NH_3) \simeq G(R_2CO) \simeq G_{H_2O_2} = 0.8$. At pH 3, under which condition e^-_{aq} is converted essentially quantitatively to H via reaction ( ), the yield of diaminosuccinic acid from N-acetylglycine corresponds to G = 1.6. This value decreases with increasing pH in accord with the proposed scheme.

The effect of oxygen on these systems is to markedly increase the yield of "amide-like" ammonia to give $G(NH_3) \simeq 2.5 \simeq G_{OH}$. Under these conditions the peptide radicals formed by OH attack at the α-carbon in reaction (47) are scavenged by O_2

$$O_2 + RCONH\dot{C}(R_2') \rightarrow RCONHC(O_2)R_2. \quad (54)$$

Subsequent chemistry has been interpreted in terms of:

$$RCONHC(O_2)R_2 \rightarrow RCON{=}CR_2 + HO_2 \quad (55)$$

$$HO_2 + RCONHC(O_2)R_2 \rightarrow RCONHC(OOH)R_2 + O_2 \quad (56)$$

$$H_2O + RCONHC(OOH)R_2 \rightarrow RCONHC(OH)R_2 + H_2O_2 \quad (57)$$

where $RCON{=}CR_2$ represents the dehydropeptide and $RCONHC(OH)R_2$ the corresponding hydrate. Such compounds readily decompose on hydrolysis as described in reactions (51 and 53) above. The above reaction scheme for oxygenated solutions requires that the ammonia and carbonyl yields be in the relationship $G(NH_3) = G(R_2CO) = G_{OH}$ where the latter term represents the 100-eV yield for OH production in the radiation-induced step 1. We have measured ammonia and carbonyl yields in the γ ray induced oxidation of N-acetylglycine, glycine anhydride, N-acetylalanine, and alanine anhydride in oxygenated dilute solution and we find for each system, $G(NH_3) \simeq 2.5$. However, we also find that carbonyl production in these simple peptide systems is not in accord with the quantitative requirements of the proposed oxidation scheme; the initial carbonyl yields are uniformly low with $G(R_2CO) \leq 0.8$. There is then the question as to whether this apparent discrepancy arises from a) an incorrect formulation of the locus of initial OH attack or from b) unspecified complexities in the chemistry of removal of the peroxy radicals $RCONHC(O_2)R_2$.

To obtain specific information on this point, we have employed Fe(III) instead of O_2 as the scavenger of intermediate radicals formed in the radiolysis of N-acetylalanine and N-acetylglycine. Heavy metal ions such as Fe(III) and Cu(II) oxidize organic free radicals in aqueous solution by electron transfer and by ligand transfer[DE LAMARE, KOCHI and RUST,1963; BAXENDALE and SMITHIES, 1956]. Such reactions in the case of the peptide radical $RCONH\dot{C}R_2$ would correspond to:

$$RCONH\dot{C}R_2 + Fe(III) \rightarrow RCON{=}CR_2 + Fe(II) + H^+ \qquad (58)$$

$$RCONH\dot{C}R_2 + Fe(III) + H_2O \rightarrow RCONHC(OH)R_2 + Fe(II) + H^+ \qquad (59)$$

respectively. Note that the organic products of reactions (58 and 59) are identical with the postulated products of reactions (55 and 57).

Ammonia production (fig. 11) in 0.1 M acetylalanine increases abruptly from $G(NH_3) = 0.7$ to $G(NH_3) = 4.3$ with increasing concentrations of Fe(III) up to $\sim 10^{-3}$ M. The ammonia yield then falls gradually to a limiting value of $G(NH_3) = 3.3$ at the higher Fe(III) concentrations. We also find under these conditions that pyruvic acid and ammonia are formed in equal molar yields. Yields of glyoxylic acid and ammonia from acetylglycine also show this same quantitative relationship. Aldehyde yields from these systems are low, $G \simeq 0.1$.

At the higher Fe(III)/peptide ratios, the reducing species e^-_{aq} and H are preferentially scavenged by Fe(III) and the yield for peptide oxidation through OH attack is in accord with

$$-G(\text{peptide}) = G(NH_3) = G(RCOCOOH) \simeq 3.2 \simeq G_{OH} + G_{H_2O_2} .$$

Hydrogen peroxide formed in the radiation-induced step 1, reacts rapidly with Fe(II) to give an additional yield of OH radicals[1]

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH. \qquad (60)$$

The maximum in the yield curve shown in fig. 11 is attributed to the onset of the reaction

$$H + RCONHCHR_2 \rightarrow RCONH\dot{C}R_2 + H_2 \qquad (61)$$

[1] (See footnote 1, page 8).

in competition with

$$H + Fe(III) \rightarrow Fe(II) + H^+ \qquad (62)$$

at the lower Fe(III)/peptide ratios. The $RCONH\dot{C}R_2$ radicals from both reactions (47 and 61) are then available for oxidation by Fe(III). This effect is shown more clearly in fig. 12 which gives ammonia and pyruvic acid yields as a function of acetylalanine concentration over the range 10^{-3} M to 1.5 M, in the presence of 0.05-M Fe(III). The limiting value for peptide oxidation at the higher acetylalanine concentrations is given by

$$-G(\text{peptide}) = G(NH_3) = G(R_2CO) \simeq 4.0 \simeq G_{OH} + G_{H_2O_2} + G_H .$$

We conclude then that the reaction of OH (and H) radicals with these peptide derivatives of the simpler amino acids, glycine and alanine, occurs essentially quantitatively at the α position as formulated in reactions (47 and 61). The evidence also is that the oxidation of $RCONH\dot{C}R_2$ radicals by Fe(III) via reactions (58 and 59) is quantitative. In the case of acetylalanine such oxidation appears to occur almost exclusively through ligand transfer (reaction 59) since measurements of the optical absorption of the irradiated solutions (after removal of Fe(III)) reveal negligible absorption above 230 mμ when read differentially against unirradiated control solution. Absorption by control solutions containing authentic acetyldehydroalanine (ε_{240}=6050) show that G values of > 0.1 for reaction (58) would be detectable. To our knowledge the optical properties of acetyldehydroglycine have not been described.

The low carbonyl yields obtained when O_2 is used in place of Fe(III) as the radical scavenger we interpret then as evidence that other more complex

degradation reactions occur in parallel with the dehydrogenation and hydroxylation reactions (55 to 57). One possibility, of course, is

$$RCONHC(OOH)R_2 \rightarrow RCONHC(=O)-R + ROH. \qquad (57a)$$

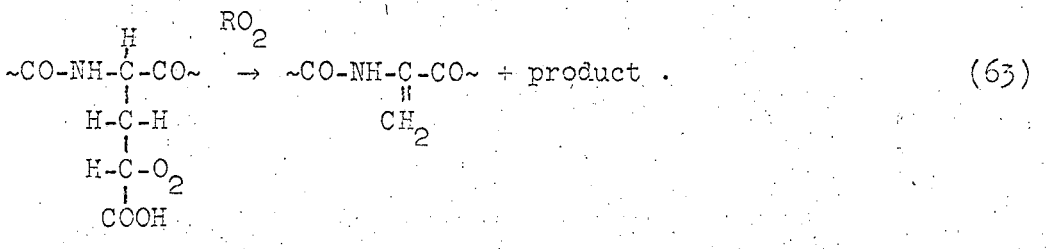
The specific nature of these various branching reactions is presently under study.

5.3. PEPTIDES AND POLYPEPTIDES

Gamma irradiation of poly-DL-alanine (MW 3000) in dilute (0.2%) aqueous solution (pH 7) saturated with oxygen results on mild hydrolysis in the formation of ammonia and carbonyl products with $G(NH_3) = 2.4$, $G(R_2CO) = 1.2$. The carbonyl is predominantly pyruvic acid plus a small amount of acetaldehyde. The relatively higher carbonyl yield from polyalanine as compared to acetylalanine suggests that the yield of the branching reaction as represented in eq. (57a) is somewhat lower if the carboxyl group α to the radical site is in the peptide form; we assumed that OH attack along the polypeptide chain is essentially random.

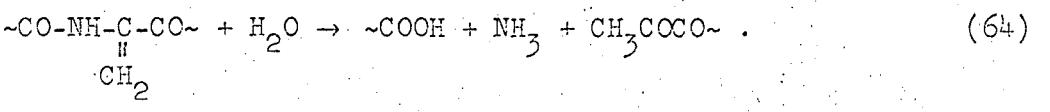
In early experiments [SOKOL, BENNETT-CORNIEA, and GARRISON, 1965] on the γ-ray induced oxidation of poly-α-L-glutamic acid in neutral oxygenated solution, we were surprised to find that the amide yield (again measured in terms of ammonia after hydrolysis) corresponds to $G(NH_3) \simeq 2.4$ which is essentially the same as that obtained with polyalanine. We had assumed that the C-H bonds of the glutamic acid residue would compete for OH radicals, (as observed in the case of the free α-amino acids, fig. 6). Analysis of the

carbonyl fraction revealed that, although α-keto glutaric acid is produced with $G \simeq 0.8$, this represents only a third or so of the total amide yield. The major organic product is pyruvic acid. After considering the possible chemical consequences of OH attack at each of the various C-H bonds of the glutamic acid residue, we proposed that pyruvic acid is produced through OH attack at the C-H bond α to the side-chain carboxyl group to give the γ-peroxy radical which degrades as



(63)

The acrylic acid residue is labile and on mild hydrolysis yields ammonia and pyruvic acid



(64)

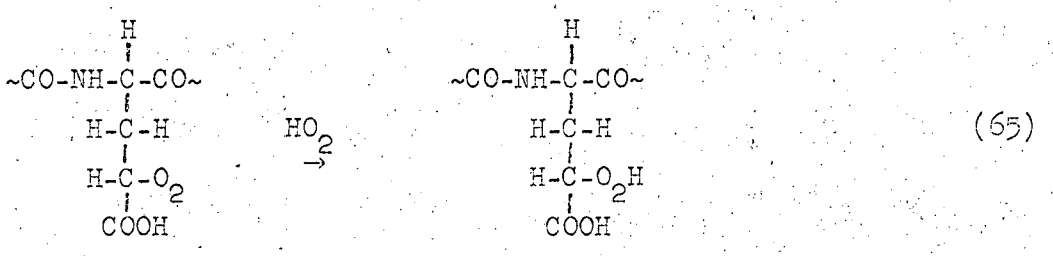
It would appear that this system represents a case in which radical attack at the γ position of the side chain leads to formation of a labile peptide linkage.

We also observed that the yields of amide ammonia and total carbonyl from polyglutamic acid exhibit a marked pH dependence as shown in fig. 13. The yield of ammonia and the combined yield of α-keto acids increase abruptly to their maximum values with increasing pH over the narrow range $pH \simeq 4.5$ to $pH \simeq 6$. That this effect is not a result of incomplete scavenging of OH

radicals at pH < 6 is shown by the fact that product yields at both pH 4 and 7 are independent of the polyglutamic acid concentration from 0.15 % down to at least 0.015 %. Nor does it appear that the sharp break in the pH-yield curves is directly related to changes in hydrogen-ion concentration or degree of ionization of side-chain carboxyl groups, per se. This is shown by results obtained with N-acetylglutamic-α methyl ester, a radiation-chemical model for the single-residue segment of the PGA chain; ammonia and carbonyl yields from 0.05-M solutions of this low molecular weight peptide derivative of glutamic acid are essentially independent of pH over the entire range, pH 3 to 8, with $G(NH_3) \simeq G(>C=O) \simeq 2$.

The data of fig. 13 show that G(pyruvic) increases sharply with $G(NH_3)$ with increasing pH from 4.5 to 6; whereas the yield of α-keto-glutaric, and hence the yields of reactions (55 to 57), are essentially pH independent. In interpreting this finding we should note first that a unique characteristic of the radiation chemistry of a macromolecular substance in aqueous solution is that each molecule undergoes reaction with a relatively large number of OH radicals even at the lowest practicable dosages. For example, with a 0.15-% solution of polyglutamic acid, a γ-ray dose of 3×10^{18} eV/gm produces but one OH per 100 glutamic acid residues but at the same time this corresponds to about 20 OH radicals per polyglutamic-acid molecule (140,000 MW). However, since polyglutamic acid above pH 6 has the random coil configuration, the various peroxy-radical sites are free to interact both intermolecularly and intramolecularly as shown in eq. (63), where RO_2 represents sites at both the α-carbon position and the γ-carbon position. Hence at pH > 6, we find no essential difference in the chemistry

of the macromolecule and the low molecular weight model. But, as the pH of the solution is decreased, polyglutamic acid undergoes the coil→helix transition over the pH range 6 to 4.5 [APPLEQUIST and BRESLOW, 1963], which as we have noted, is the significant pH range of fig. 13. With polyglutamic acid in the helix form, the RO_2 radicals are frozen in a fixed spatial arrangement and the relative importance of reaction (63) is decreased whereas the competing, terminating step involving HO_2

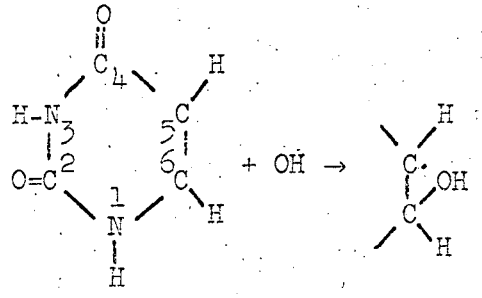


(65)

is uninhibited by the coil→helix transformation. The peroxide product of reaction (65) simply hydrolyzes to give the hydroxylated side chain. Hence, G(pyruvic acid) and $G(NH_3)$ decrease with decreasing pH as shown in fig. 13.

6. Reactions of Pyrimidine Bases

Studies of the radiation-induced oxidation of thymine, uracil, and cytosine in oxygenated solution have established that the 5,6 carbon-carbon double bond is an important locus of OH attack[1], e.g. for uracil,



(B) (ḂOH) (66)

In the presence of molecular oxygen, reaction (66) is followed by

$$\dot{B}OH + O_2 \rightarrow B(OH)\dot{O}_2$$

while e^-_{aq} is removed via (67)

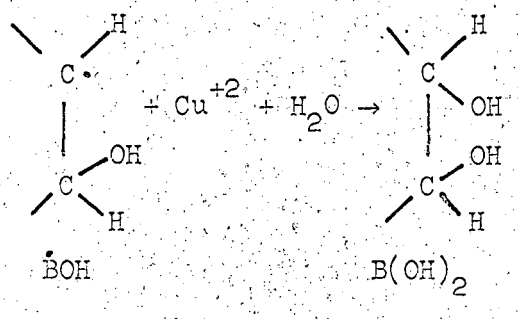
$$e^-_{aq} + O_2 \rightarrow O_2^-(HO_2) \,.$$

Subsequent interactions of the radicals $B(OH)\dot{O}_2$ and HO_2 lead to formation of a complexity of products which include: hydroxy hydroperoxides, glycols, and the barbituric acid derivatives. However, the combined yield of these oxidation products is considerably less than the yield for base destruction which for γ rays is approximated by $G(-B) = G_{OH} = 2.5$ [SCHOLES, 1963; WEISS, 1964].

Use of a transition metal ion such as Cu^{+2} or Fe^{+3} in the place of O_2 as the scavenger of intermediate radicals leads to a considerable simplification in the radiation chemistry of aqueous solutions of the pyrimidine bases

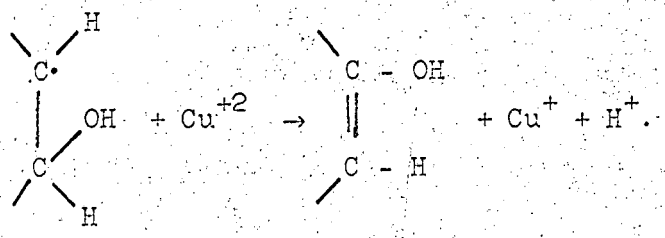
[1] (EKERT and MONIER, 1960; LATARJET, EKERT, and DEMERSEMAN, 1963; SCHOLES, WARD, and WEISS, 1960; SCHOLES and WEISS, 1960).

and provides direct chemical evidence for the yield of reaction (66) [HOLIAN and GARRISON, 1966]. The specific chemical effect of the metal ion involves the preferential oxidation of the hydroxy pyrimidyl radical, $\dot{B}OH$, formed through OH addition via reaction (66), i.e.



(68)

to give the corresponding glycol as the single major product of γ radiolysis with $G(B(OH)_2) \simeq G_{OH}$. Data for oxygen-free solutions of uracil and cytosine containing Cu^{+2} are given in table 5. Formation of the isobarbituric acid derivatives (5-hydroxy pyrimidines) with $G \sim 0.5$ in each case may be attributed to a parallel branching reaction



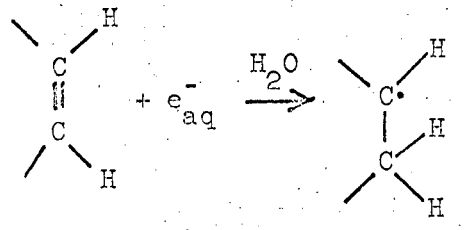
(69)

In any event, in the presence of Cu^{+2} the pyrimidine nucleus is quantitatively oxidized in accord with the stoichiometry $G(\text{glycol}) + G(\text{isobarbituric acid}) = G_{OH} + G_{H_2O_2}$, where the reaction: $Cu^{+1} + H_2O_2 \rightarrow Cu^{+2} + OH + OH^-$ provides the additional source of OH radicals[1].

[1] (See footnote 1, page 8).

The velocity constants for reaction of e^-_{aq} with Cu^{+2} and with the pyrimidine bases are such [GORDON et al., 1963; HART, THOMAS, and GORDON, 1964] that, for the low (base)/(Cu^{+2}) ratios of table 5, capture of e^-_{aq} is predominantly by $Cu^{+2} + e^-_{aq} \rightarrow Cu^+$. However, at the higher (base)/(Cu^{+2}) values e^-_{aq} is scavenged almost exclusively by the base: $B + e^-_{aq} \rightarrow \dot{B}H + OH^-$. Such reaction does not lead to net chemical change in the base, since reaction of the hydropyrimidyl radical $\dot{B}H$ with Cu^{+2} through $\dot{B}H + Cu^{+2} \rightarrow B + Cu^+ + H^+$ or through $\dot{B}H + Cu^{+2} + H_2O \rightarrow B(H_2O) + Cu^+ + H^+$ followed by $B(H_2O) \rightarrow B + H_2O$ leads simply to base regeneration.

In evacuated neutral solution the G value for base destruction is found to be about one third that observed in oxygenated solution. LATARJET, EKERT and DEMERSEMAN [1963] and EKERT [1962] report $G(-B) \sim 0.8$ and $G(-B) = 0.7$, respectively, for evacuated 10^{-3} M thymine solutions under γ rays; the data of PONNAMPERUMA, LEMMON, and CALVIN [1962] give $G(-B) = 0.9$ for aqueous cytosine under similar conditions. The velocity constants for reaction of OH and e^-_{aq} with the pyrimidine bases are such [SCHOLES et al., 1965] that both the oxidizing and reducing species are quantitatively scavenged by the base at the millimolar concentrations used in these studies. It would appear then that some type of reconstitution reaction is acting to reduce the G value for base destruction in these oxygen-free solutions. As we have noted, OH adds quantitatively to the 5,6 double to form the adduct $\dot{B}OH$. If the hydrated electron also adds to the labile 5,6 position, as



(70)

then the reconstitution reaction may be interpreted in terms of water regeneration[1]

$$\dot{B}H + BOH \rightarrow B + B(H_2O) \quad (71)$$

$$B(H_2O) \rightarrow B + H_2O. \quad (72)$$

Competing reactions would include[2]

$$2\dot{B}H \rightarrow B + BH_2 \quad (73)$$

$$\dot{B}H + H_2O_2 \rightarrow B + H_2O + OH \quad (74)$$

$$\dot{B}OH + H_2O_2 \rightarrow B(OH)_2 + OH \quad (75)$$

$$B(OH)_2 \rightarrow \dot{B}OH + H_2O \quad (76)$$

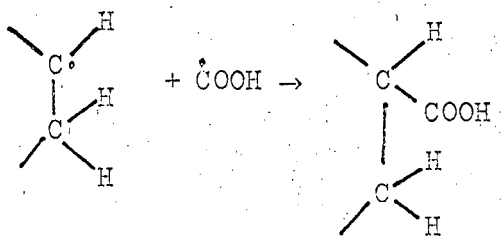
where the H_2O_2 shown in reactions (74 and 75) represents the molecular hydrogen peroxide yield of the radiation-induced step 1.

[1] It seems likely that the radiation-induced deamination of cytosine to give uracil in oxygen-free solution [PONNAMPERUMA, LEMMON, and CALVIN, 1962] occurs not through attack of e^-_{aq} or OH at the 4-amino position but rather through hydrolytic deamination of the hydrate intermediate.

[2] (EKERT, 1962; KAMAL and GARRISON, 1965; KHATTAK and GREEN, 1965, 1966a, 1966b; SCHOLES, 1963).

If the reconstitution reaction is indeed as indicated in eqs. (71-74), it follows that addition of a second organic solute, preferentially reactive towards OH via $RH + OH \rightarrow \dot{R} + H_2O$, would lead to the replacement of OH by R and to an enhancement in G(-B), since the possibility for self-protection through water elimination via reaction (72) would be excluded. The increase in G(-B) would correspond to an increase in the observed yield of products saturated at the 5,6 position. Cytosine was chosen for a study of this effect because the (dihydrocytosine)derivatives obtained on saturation of the 5,6 double bond hydrolyze readily to give the corresponding 5,6 dihydrouracil derivatives and ammonia, a product conveniently followed analytically. Sodium formate and ethanol were used as second solutes; each of these compounds is relatively inert towards e^-_{aq} and at the same time is extremely reactive towards OH via $HCO^- + OH \rightarrow \dot{C}O^- + H_2O$ and $CH_3CH_2OH + OH \rightarrow CH_3\dot{C}HOH + H_2O$. The effects of added formate and ethanol on ammonia yields in the γ radiolysis of oxygen-free 0.06 M solutions of cytosine at pH ~7 are shown in fig.14 [KAMAL and GARRISON, 1965]. We see that $G(NH_3)$ increases abruptly with increasing concentrations of either ethanol or sodium formate and reaches a limiting value of approximately $G(-B) = G(NH_3) = G_{OH} = 2.5$ at the higher scavenger concentrations.

Now, if the interpretation of this enhancement is correct, the hydrated electron e^-_{aq} is removed via reaction (70) and the OH radical is converted in the presence of formate to the $\dot{C}OOH$ radical, which in turn is removed via reaction (77)



(77)

$\dot{B}H$ B(HCOOH)

In accord with this formulation, the pyrimidine carboxylic adduct, B(HCOOH), is found to be produced with $G \simeq 2.5 \simeq G_{OH}$ [KAMAL and GARRISON, 1965]. More recently BROWN, CALVIN, and NEWMARK [1966] isolated the adduct $B(C_2H_5OH)$ formed in the γ radiolysis of dilute aqueous solutions of thymine plus ethanol.

The evidence is that with cytosine, uracil, and thymine, OH attack in acidic and neutral solution occurs exclusively by addition to the 5,6 double bond of the pyrimidine nucleus. In the case of thymine there is a change in the locus of attack as the pH is increased above pH ~9. MYERS et al. [1965] find that, as the pH is increased, the major site of chemical change in the radiolysis of air-saturated thymine solutions shifts from the 5,6 double bond to the 5-methyl group.

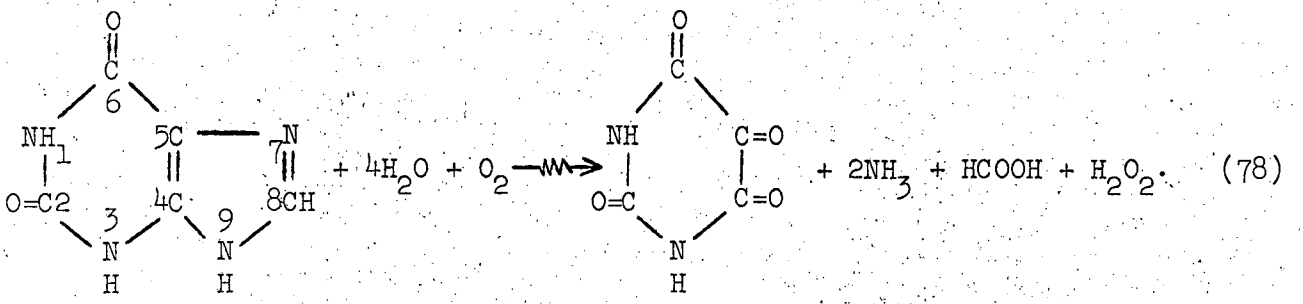
7. Reactions of Purine Bases

Because of the chemical complexity of the purines, our knowledge of their radiation chemistry both in evacuated and oxygenated solution has developed more slowly. Adenine has received the most attention and is reported by SCHOLES and WEISS [1952] to yield ammonia with $G \simeq 0.5$ on radiolysis with x rays in dilute solution under aerobic conditions. Miss CONLAY [1963] has isolated organic products from the same system after γ radiolysis and finds 8-hydroxyadenine and 4,5,6-triaminopyrimidine in yields corresponding to $G \leq 0.1$. The latter product is presumed to arise from the formamide pyrimidine reported by HEMS [1960] to be formed in low yield from the purine nucleus in oxygen-free solution. And PONNAMPERUMA et al. [1961] also find small amounts of hypoxanthine, $G \simeq 0.05$, produced in the γ radiolysis of adenine in oxygen-free solution. However, in view of the primary yields for water decomposition, $G_{OH} \sim G_{e^-_{aq}} = 2.5$, it is clear that no conclusions regarding the major loci of reaction of the purine nucleus can be made on the basis of the yields of these observed degradation products from adenine.

Now, let us assume that the OH radical adds to the carbon-carbon double bond of the purine nucleus and that the radical so formed is in turn scavenged by molecular oxygen. By analogy with the conventional chemistry of the purines [Howard, 1960] we might expect that such oxidation at the 4,5

HOWARD, G. A. 1960, in Chemistry of the Carbon Compounds vol. IV, chapt. XX, ed. by E. H. Rodd, Elsevier Publishing Co., Amsterdam.

position would produce labile species which in the case of xanthine, for example, would yield alloxan, ammonia and formic acid on mild hydrolysis

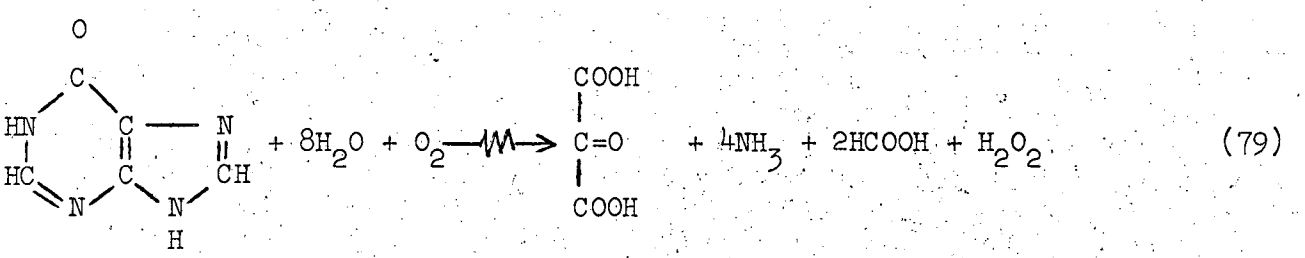


(78)

xanthine alloxan

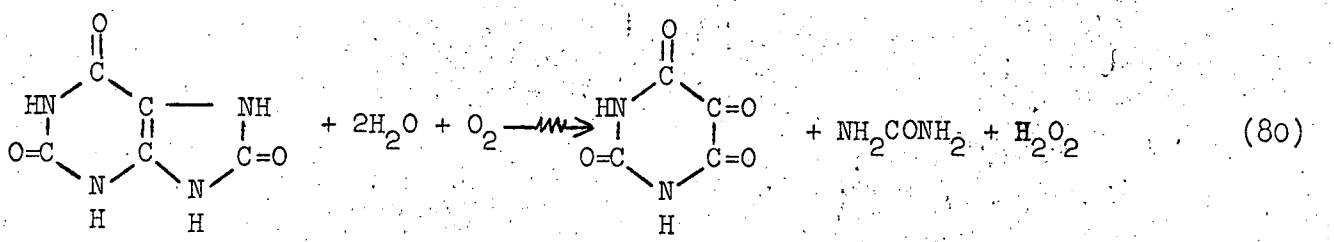
We find in fact for the γ-radiolysis of 2×10^{-3} M xanthine in oxygenated solution that $G(-B)\sim2.0$ and that on hydrolysis $G(NH_3)=2G(-B)=4.1$, $G(\text{alloxan})\sim2$ [HOLIAN and GARRISON 1967b, 1967c] as shown in table 6.

Degradation of hypoxanthine and of uric acid may be represented as follows



(79)

hypoxanthine mesoxalic acid



(80)

uric acid alloxan urea

Degradation yields in the γ-radiolysis of oxygen-saturated solutions of hypoxanthine and uric acid are included in table 6. It is seen in terms of the above hydrolysis steps that in each case there is a satisfactory agreement between $G(-B)$ and $G(NH_3)$ when the latter is measured after hydrolysis. The observed ammonia yield with uric acid, $G(NH_3)=0$, is quite consistent with the above formulation since urea released in reaction (80) is stable under the hydrolytic conditions employed in this study (24 hours in 2N NaOH at room temperature). The presence of urea in the irradiated uric acid solutions after hydrolysis has been substantiated by other chemical and enzymatic methods [HOLIAN and GARRISON, 1967c].

Apparently, OH addition to the carbon-carbon double bond of these purines via reaction akin to 66 (followed by steps 67) is essentially quantitative. Subsequent reactions of $B(OH)O_2$ and HO_2 lead to formation of the oxidation products. It is to be noted that the hydroxy hydroperoxide B(OH)OOH and indeed the hydroperoxide radical $B(OH)O_2$ could undergo various branching reaction with formation of a number of different degradation products. In point of fact such reaction may explain the observation that G(carbonyl) from hypoxanthine and uric acid is less than G(-B). However, the complexities of the rearrangement reactions in the conventional chemical oxidation of uric acid and other purines is well-known, and in view of this we consider the quantitative implications of the carbonyl yields with some reservation. As mentioned above, the main point here is: (a) that OH addition leads to oxygen substitution at both the 4 and 5 position with $G(-B)\simeq G_{OH}=2.5$, and (b) that the observed degradation may be formally represented in terms of a "glycol" mechanism.

The radiation-induced reactions of the amino purines, adenine and isoquanine, in oxygenated solution are somewhat more complicated. For example,

the observed values of G(-B) for these compounds are strongly dependent on the pH of the irradiated solution. In the case of adenine, G(-B)=2.2 at pH ~1, but this value gradually decreases to G(-B)=1.1 at pH 7. The reasons for this pH effect are not entirely clear neutral form of adenine reacts with the OH radical and that such reaction occurs in competition with OH addition to the unsaturated six-membered ring. However, at pH 1, OH addition to the ring appears to be essentially quantitative to give G(-B)=2.2, and, after mild hydrolysis, $G(NH_3)$=9.6; a small amount of urea is also present in the hydrolyzate, G(urea)$\sim$0.5. These results at pH 1 are consistent with the "glycol" mechanism, i.e., $G(NH_3)$ + 2G(urea)=10.1$\sim$5G(-B). However, only traces of the expected carbonyl products have been detected, G(mesoxalic acid)=0.2, and G(glyoxylic acid)=0.2. Oxalic acid has been tentatively identified as the major product of the oxidation. Apparently the 3-carbon element of the adenine glycol (or the hydroxy hydroperoxide precursor) undergoes more extensive degradation than that of simple hydrolysis.

8. Solid State Reactions of Amino Acids and Peptides

8.1. AMINO ACIDS

In the early studies of DALE, DAVIES, and GILBERT [1949], it was observed that the x radiolysis of solid glycine in vacuo produces ammonia in essentially the same yield as is obtained in the radiolysis of evacuated, neutral solutions of glycine at concentrations adequate to insure the quantitative scavenging of the oxidizing and reducing species derived from water. RAJEWESKY and DOSE [1957] identified ammonia and ketoacids as major products in the x radiolysis of glycine, alanine, and aspartic acid in the solid state. More recently, MESHITSUKA et al. [1964] reported the first detailed study of the reaction stoichiometries involved in the γ radiolysis of solid glycine in vacuo and find both acetic acid and glyoxylic acids as the major organic products formed concomitantly with ammonia. Their results are summarized in table 7. The reaction scheme proposed by MESHITSUKA et al. [1964] involves the homolytic step,

$$NH_3^+CH(R)COO^- \rightsquigarrow H^+ + \dot{N}H_2 + \dot{C}H(R)COO^- \qquad (81)$$

followed by the abstraction reactions

$$\dot{N}H_2 + NH_3^+CH(R)COO^- \rightarrow NH_3 + NH_3^+\dot{C}(R)COO^- \qquad (82)$$

$$\dot{C}H(R)COO^- + NH_3^+CH(R)COO^- \rightarrow CH_2(R)COO^- + NH_3^+\dot{C}(R)COO^-.$$

The latter provides a source of the stable, long-lived, α-carbon radicals observed

in the irradiated solid at room temperature by ESR methods[1]. The $NH_3^+\dot{C}(R)COO^-$ radicals are then removed via reactions (8) and (9) on dissolution of the solid in water.

$$2NH_3^+\dot{C}(R)COO^- \rightarrow NH_2^+=C(R)COO^- + NH_3^+CH(R)COO^- \qquad (8)$$

$$H_2O + NH_2^+=C(R)COO^- \rightarrow NH_4^+ + (R)COCOO^-. \qquad (9)$$

The role of reactions (8) and (9) in the radiolysis of the simpler α-amino acids in aqueous oxygen-free solution is described in section 2.1 [WEEKS and GARRISON, 1958]. The above scheme gives product stoichiometries in agreement with the data of table 7. However, it is unlikely that homolytic cleavage as formulated in reaction (81) can make a major contribution to the over-all chemistry since "cage-effects" in the solid phase favor the preferential recombination of such radical pairs. If cleavage of the N-C bond does occur as envisaged in reaction (81), it would seem necessary either that: (a) the deamination arises through a molecular rearrangement, or that (b) one of the indicated fragments is produced as a positively charged species.

There is evidence to suggest that a heterolytic process may indeed be involved in the radiation-induced cleavage of the N-C bond of the α-amino acids in the solid state. Now, the finding that the hydrated electron, e^-_{aq}.

[1] (BOX and FREUND, 1965; BOX, FREUND, and BUDZINSKI, 1966; COMBRISSON and UEBERSFELD, 1954; GHOSH and WHIFFEN, 1959; GORDY, ARD, and SHIELD, 1955; MESHITSUKA et al., 1964; MORTON, 1964; SINCLAIR and HANNA, 1967; UEBERSFELD and ERB, 1956; WEINER and KOSKI, 1963.

reacts with the simpler α-amino acids via reductive deamination (reaction (5)) prompted the suggestion [GARRISON, 1964] that reactions of the secondary electrons produced in the radiolysis of the solid amino acids may also lead to deamination—i.e., the electron of reaction (5) need not necessarily be "wet" for reductive cleavage to occur. If we represent the ionization act simply in terms of:

$$NH_3^+CH(R)COO^- \longrightarrow\!\!\!\!\!\!\sim\!\!\!\!\!\!> NH_3^+\dot{C}(R)COO^- + H^+ + e_s^- \quad , \qquad (83)$$

and accept the proposed reaction (5) as the fate of the secondary electron, e_s^-, then the abstraction reaction (7), which may be envisaged as occuring as readily in the solid as in solution, yields the observed fatty acid product. The α-carbon radicals formed in reactions (83) and (7) are then removed through the steps (8) and (9). This scheme, which is closely analogous to the mechanism proposed for the aqueous system[1] (section 2.1), gives

[1] The analogy between the proposed reaction schemes for the aqueous and solid systems appears even closer when one considers that reaction (83) is the stoichiometric equivalent of

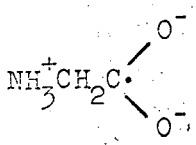
$$H_2O \longrightarrow\!\!\!\!\!\!\sim\!\!\!\!\!\!> H^+ + OH + e^-$$

$$OH + NH_3^+CH(R)COO^- \longrightarrow H_2O + NH_3^+\dot{C}(R)COO^- .$$

In fact, from the standpoint of over-all chemistry, the only difference between the proposed mechanisms for solution and solid is that in the solid case the conversion of e_s^- through the equivalent of reaction (5b) is negligible; $G(H_2) = 0.2$ from solid glycine (Cf. tables 1 and 7).

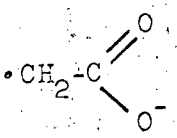
G(acetic acid) = G(glyoxylic acid); $G(NH_3)$ = G(acetic acid) + G(glyoxylic acid), and this is in essential agreement with the data of MESHITSUKA et al. [1964] as shown in table 7.

There are other more recent observations which are in support of the latter formulation. For example, we have noted (section 3) that, for reductive deamination by e^-_{aq}, to occur in aqueous solution, a carbonyl group (or other unsaturated linkage) must be at the α position. And the suggestion was made [WEEKS, COLE, and GARRISON, 1965] that: (a) the hydrated electron adds to the C=O linkage, and (b) subsequent rearrangement of the reduced intermediate via reaction (37 or 37a) yields ammonia and the corresponding fatty-acid radical. Now BOX, FREUND, and BUDZINSKI [1966] have studied the ESR spectrum of γ-irradiated solid glycine (single crystals) at 77°C and find that the observed radical corresponds to



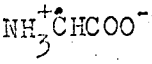
(I)

Furthermore, on warming to intermediate temperatures (~165°K) the spectrum changes to one which corresponds to the configuration



(II)

And, on further warming to room temperature the radical species II is transformed to III



(III)

(through the hydrogen-abstraction reaction (7)). BOX, FREUND, and BUDZINSKI [1966] conclude that (I) is converted to (II) through elimination of NH_3. Very similar results have been observed with α-aminoisobutyric acid [BOX and FREUND, 1966] and with alanine [SINCLAIR and HANNA, 1967]. One other piece of evidence that supports indirectly the above formulation for reductive deamination by e_s^- in the solid state may be derived from the fact that whereas $G(NH_3)$ from solid glycine and alanine approximates 5, the ammonia yield from solid β-alanine, $(NH_3^+CH_2CH_2COO^-)$, corresponds to the relatively low value of $G(NH_3) = 0.8$. As we have noted in section 3, β-alanine does not undergo reductive deamination in aqueous solution on reaction with e_{aq}^-. Values of $G(NH_3)$ obtained in the γ radiolysis of solid glycine, alanine, β alanine, serine, phenylalanine, and cystine (in vacuo) are summarized in table 8 [PETERSON and GARRISON, 1967]. The presence of the hydroxyl group at the β position in serine does not appear to affect appreciably the course of the deamination reactions. The $G(NH_3)$ values from phenylalanine and cystine are surprisingly high when one considers that the phenyl and sulfur moieties are generally assumed to dominate the chemistry of these amino acids.

The -SH linkage of cysteine does, however, appear to represent a major locus of chemical change in the γ radiolysis of the evacuated solid; $G(H_2) = 4.0$, $G(NH_3) = 1.8$, and $G(H_2S) = 0.65$; cystine appears as a major product [PETERSON and GARRISON, 1967]. If we accept the evidence that e_s^- in solid glycine and alanine escapes the parent ion and is subsequently captured at a distance via reaction (5), then we must conclude that a competing process for capture of e_s^- in cysteine leads to the formation of hydrogen as the major fragmentation product. As a tentative over-all mechanism

we proposed the ionization step,

$$HSCH_2CH(NH_3^+)COO^- \longrightarrow \dot{S}CH_2CH(NH_3^+)COO^- + H^+ + e_s^- , \qquad (84)$$

followed by removal of e_s^- predominantly through

$$e_s^- + HSCH_2CH(NH_3^+)COO^- \rightarrow \dot{S}CH_2CH(NH_3^+)COO^- + H_2 , \qquad (85)$$

where step 85 may involve the intermediate formation of H. Dimerization of $\dot{S}CH_2CH(NH_3^+)COO^-$ on dissolution of the irradiated solid yields cystine.

8.2. PEPTIDES

Although free ammonia is produced in high yield in the γ radiolysis of the solid α-amino acids (section 8.1), it is a relatively minor product in the γ radiolysis of the corresponding N-acetyl derivatives. We find, however, that a major chemical effect of the radiolysis of these simplest peptide derivatives is the formation of labile amide-like products which are readily degraded to yield ammonia on mild hydrolysis[1]. Initial G values for ammonia formation (total ammonia liberated on hydrolysis) in the γ radiolysis of a number of N-acetylamino acids in the evacuated polycrystalline state are summarized in table 8. [BENNETT-CORNIEA, and GARRISON, 1967; GARRISON et al., 1967]. The N-acetyl derivatives of all of the aliphatic amino acids studied, including N-acetylmethionine, undergo "deamidation" as a major radiation-induced reaction. Apparently, the aromatic ring of N-acetyl phenylalanine is effective in partially quenching the degradation.

[1] (JAYKO, BENNETT-CORNIEA, and GARRISON, 1965; GARRISON, 1966; GARRISON et al., 1967, GARRISON, JAYKO, and BENNETT-CORNIEA, 1964; GARRISON and WEEKS, 1962).

To obtain information on the mechanism of the radiation-induced degradation of the peptide chain, a detailed study has been made of concomitant products formed in the γ radiolysis of N-acetyl-DL-alanine. The data are given in table 9 [GARRISON et al., 1967].

The evidence is that the observed radiolytic degradation of acetyl-alanine cannot be represented simply in terms of the rearrangement

$$RCONHCH(CH_3)R \longrightsquigarrow RCONH_2 + \begin{matrix} H \\ | \\ C-R \\ \| \\ CH_2 \end{matrix} , \qquad (86)$$

since we find G(acrylic acid) < 0.1. The possibility that the N, O shift,

$$RCONHCH(CH_3)R \longrightsquigarrow R-\overset{\overset{NH}{\|}}{C}-O-\overset{\overset{H}{|}}{\underset{\underset{CH_3}{|}}{C}}-R , \qquad (87)$$

occurs in high yield and leads to ammonia formation through subsequent hydrolysis of the labile imino ester

$$2H_2O + R-\overset{\overset{NH}{\|}}{C}-O-\overset{\overset{H}{|}}{\underset{\underset{CH_3}{|}}{C}}-R \longrightsquigarrow RCOOH + NH_3 + CH_3CH(OH)R \qquad (88)$$

is negated by the fact that G(lactic acid) $\simeq$ 0.2. We conclude also that the formation of dehydropeptide via

$$RCONHCH(CH_3)R \longrightsquigarrow R-\overset{\overset{O}{\|}}{C}-N=\underset{\underset{CH_3}{|}}{C}-R + H_2(2H) \qquad (89)$$

does not represent the main source of the amide-like function since we find G(pyruvic acid) $\sim$ 0.4; the dehydropeptides are hydrolyzed quantitatively to yield carbonyl and ammonia

$$2H_2O + R\text{-}\overset{\overset{O}{\|}}{C}\text{-}N=\underset{\underset{CH_3}{|}}{C}\text{-}R \rightarrow RCOOH + NH_3 + CH_3COR \qquad (90)$$

under the conditions of hydrolysis employed here. Additional evidence that the dehydrogenation reaction (89) occurs in low yield is given by the gas-yield data which show $G(H_2) = 0.4$[1].

We do find that propionic acid is produced as a major product, G(propionic acid) $\sim$ 1.4. Although it is clear that main-chain scission occurs, we cannot, at the present time, distinguish between two possible reaction schemes both of which are consistent with the present observations. The first scheme involves a direct scission of the N-C bond via reaction of the type[2]

[1] Hydrogen yields from a variety of compounds containing the peptide bond are uniformly low. We have found initial $G(H_2)$ values of 0.45, 0.85, and 0.22 for polyalanine, nylon, and gelatin, respectively [JAYKO and GARRISON, 1966].

[2] Although this reaction sequence (reactions (91 to 93)) is formulated in terms of neutral free-radical species, we would presume, in view of the caging effects referred to in section 8.1, that the actual processes involve charged or "hot" intermediates. The detailed nature of the reaction intermediates can only be speculated upon at the present time.

$$RCONHCH(CH_3)R \longrightarrow\!\!\!\!\!\sim\!\!\!\!\!\rightarrow RCO\dot{N}H + \dot{C}H(CH_3)R \qquad (91)$$

followed by the abstraction reactions

$$RCO\dot{N}H + RCONHCH(CH_3)R \rightarrow RCONH_2 + RCONH\dot{C}(CH_3)R \qquad (92)$$

$$\dot{C}H(CH_3)R + RCONHCH(CH_3)R \rightarrow CH_2(CH_3)R + RCONH\dot{C}(CH_3)R \qquad (93)$$

to give amide, fatty acid, and the long-lived α-carbon radicals which have been observed in irradiated peptides at room temperatures through ESR spectroscopy [DREW and GORDY, 1963; FREUND and LILGA, 1961]. The alternative formulation involves the dissociation

$$RCONHCH(CH_3)R \longrightarrow\!\!\!\!\!\sim\!\!\!\!\!\rightarrow RCONH\dot{C}(CH_3)R + H\,, \qquad (94)$$

followed by

$$H + RCONHCH(CH_3)R \rightarrow RCONH_2 + \dot{C}H(CH_3)R\,, \qquad (95)$$

(or the equivalent heterolytic steps involving e^- and H^+), and by the abstraction reaction (93). The stoichiometry of reactions (94 and 95) followed by (93) is identical with that given by the reaction sequence (91, 92, and 93). On dissolution in water (oxygen-free), the α-carbon radicals, $RCONH\dot{C}(CH_3)R$, undergo simple dimerization to yield the α, α'-diaminosuccinic acid derivative [GARRISON and WEEKS, 1962].

We estimate from ESR measurements that the yield of the long-lived α-carbon radicals is roughly $G \sim 3$. This value and the propionic acid yield value of $G = 1.4$ are somewhat lower than would be predicted from the reaction sequence (91 to 93) on the basis of $G(\text{amide}) \sim 3.4$. The apparent

discrepancy may be understood if a fraction of the $\dot{C}H(CH_3)R$ radicals are removed through radical-radical interactions in the spur-regions of high radical concentration. That the "amide" type of radiolytic degradation of the peptide chain is not confined to the N-acylamino acid configuration is shown by the fact that the G values for ammonia and propionic acid from poly-DL-alanine are almost identical with those obtained with N-acetylalanine[1,2].

It has been suggested that the long-lived α-carbon radicals are produced in peptide radiolysis largely through side-chain cleavage [BRAAMS, 1966b;

[1] In the radiolysis of simple linear peptides such as glycylglycine (in the evacuated solid state) we find, as might be expected on the basis of the formulations of sections 8.1 and 8.2, that both deamination and deamidation are involved.

[2] Acetaldehyde which is produced in the γ-radiolysis of acetylanine with G=0.8 (table 10) is also produced in this same yield in the γ-radiolysis of polyalanine. This would suggest that these products of labile "amide" ammonia in the systems also involves the radiation-induced cleavage of C-C bonds with formation of products of the type $RCON=CH(CH_3)$ which would then hydrolyze via: $RCON=CH(CH_3) + 2H_2O \rightarrow RCOOH + NH_3 + CH_3CHO$. We find that the relative yields of the several classes of organic products represented in table 10 depend on the nature of the amino acid residues and on the over-all composition of the peptide.

RIESZ, WHITE, and KON, 1966], e.g., reaction (94) followed by

$$H + RCONHCH(CH_3)R \rightarrow RCONH\dot{C}(CH_3)R + H_2, \qquad (96)$$

where H, formed in reaction (94), may have excess kinetic energy. However, the low $G(H_2)$ values observed in the γ radiolysis of simple peptides, polypeptides, and protein [JAYKO and GARRISON, 1966] do not support the concept that reaction (96) represents a major source of α-carbon radicals.

HAYDEN, ROGERS, and FRIEDBERG [1966] have irradiated polyamino acids, fibrous, and globular proteins with γ rays in the evacuated solid state and find that polyglutamic acid, polylysine, and gelatin show lower intrinsic viscosities and lower number average molecular weight after radiolysis; G values for main-chain degradation of 1.8, 4.1, and 1.4, respectively, were calculated. Globular proteins such as creative kinase and ribonuclease, on the other hand, show relatively little change in molecular weight under identical conditions of radiolysis and dissolution. HAYDEN, ROGERS, and FRIEDBERG [1966] suggest that the secondary and tertiary structure of the globular protein favor fragment recombination. On the basis of the "amide" mechanism a main-chain break yields the amide and acyl functions at the locus of cleavage and the long-lived radicals[1] in close proximity. With the

[1] With the simpler polyamino acids, this radical corresponds to the α-carbon radical ~CONH-$\dot{C}$(R)~. With more complex polyamino acids and with protein, we do not preclude the possibility that the observed long-lived spin centers may be situated at side-chain loci.

irradiated globular protein, combination of these radicals on dissolution would be favored by the constraints imposed by the secondary and tertiary structure. With the polyamino acids and fibrous protein such constraints are minimal and the separation of radical sites on dissolution would be competitive with combination. We would predict on the basis of the "amide" mechanism that the globular proteins would show an increase in amide function even though there is no net gross fragmentation of the main chain.

Brief Summary

Oxidative deamination of amines and amino acids is induced by attack of OH radicals at the C-H linkage α to the amino group. The characteristic products are ammonia and a carbonyl.

Amino compounds containing the grouping $NH_3^+CH(R)COX$ where X represents O^-, OH, OR, NHR etc. undergo reductive deamination on reaction with e^-_{aq} to give the corresponding fatty acid derivative. If more than one carbon unit separates the amino and carbonyl groups, reductive deamination does not occur.

The chemistry of reductive deamination indicates that e^-_{aq} adds to the carbonyl double bond and that cleavage of the N-C linkage ensues on rearrangement of the reduced intermediate. Observed correlations between pK of the NH_3^+ group and the velocity constant for the e^-_{aq} reaction are in accord with this formulation.

Oxidative degradation of substituted amines including peptides is initiated by OH attack at the C-H linkage of the α-carbon atom.

The hydrated electron adds to the peptide bond but N-C cleavage does not ensue.

The reactions of OH and e^-_{aq} with the pyrimidine and purine bases occur almost exclusively at the carbon-carbon double bond.

The actions of ionizing radiations on solid glycine and alanine in the absence of oxygen indicates that the electron escapes the parent ion and is subsequently removed through addition to adjacent C=O groups. The reduced intermediate looses ammonia on rearrangement. There is a marked similarity in the radiation chemistry of the difunctional amino acids in

the solid state and in aqueous solution (oxygen-free). This analogy does not hold in the case of certain trifunctional amino acids, e.g., cysteine and cystine.

A major chemical effect of ionizing radiations on solid peptide derivatives of the aliphatic α-amino acids leads to the formation of amide and fatty acid through main-chain scission at the NH-CH(R) linkage. There is evidence that main-chain scission also occurs at the CHR-CO linkage.

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Table 1

Yields of major products in the γ radiolysis of oxygen-free solutions of glycine and alanine, 1 M pH 6.4 [MAXWELL, PETERSON and SHARPLESS, 1954; WEEKS, COLE and GARRISON, 1965]

product	yield, G	
	glycine	alanine
ammonia	4.3	4.3
keto acid	2.1	1.6
fatty acid	1.2	1.0
aldehyde	0.5	0.5
hydrogen	2.0	1.3

Table 2 Product Yields from 0.30 *M* Glycine 0.03-0.04 *M* Cu(II) Solutions [WILLIX and GARRISON, 1965b]

pH	$G(NH_3)$	$G(CHOCO_2H)$	$G(CH_2O)$	$G(CO_2)$	$G(H_2)$	$G(CH_3CO_2H)$	G(succinic acid)
3.0	2.2 ± 0.2	1.8	0.53	0.5		0.04	0.07
8.5	5.0 ± 0.2	1.9 ± 0.1	0.8 ± 0.1	3.7	0.45	0.12	0.24
8.4[a]	5.0 ± 0.2	2.2	0.54	3.1			

[a] Preformed 0.02 *M* bis(glycinato)copper(II).

Table 3

Effect of 0.8 M Sodium Formate on $G(NH_3)$ from Oxygen-Free Solutions of Amino Acids and Derivatives at pH 7 [WILLIX and GARRISON, 1967]

Compound[a]	$G(NH_3)$	
	Formate-free	1.0 M formate
alanine	4.3	2.5
glycine	4.0	1.8
glycine ethyl ester	2.7	2.6
bis-glycinato-CuII[b]	5.0	3.2
glycylglycine	3.0	2.5
valine[c]	1.8	0.8
ϵ-aminocaprioc acid	0.35	<0.35
β-alanine	0.75	0.45

[a] At the minimum concentration required to insure the quantitative scavenging of water decomposition-products (see Fig. 7).

[b] At pH 8.5.

[c] Measured at only one valine concentration, viz 0.25 M.

Table 4

Rate Constants for Reaction of e^-_{aq} with Amino Acids and Derivatives as Measured by Competition Kinetics[a] [WILLIX and GARRISON, 1965a,1967]

Compound	pH 6.7, $\bar{k}_{38}(M^{-1}\ sec^{-1})$	pH 3, $k_{38}(M^{-1}\ sec^{-1})$
glycine	1.4×10^7 (0.9×10^7)[b,c]	4.5×10^8
alanine	1.8×10^7 (0.6×10^7)[b,c]	8.0×10^8
bis-glycinato-CuII	3.5×10^8	---
glycine ethyl ester	1.0×10^9	---
diglycine	1.0×10^8 (2.5×10^8)[b]	8.9×10^8
triglycine	7.2×10^8 (9×10^8)[b]	3.0×10^9
N-ethylacetamide	1.7×10^7	1.5×10^7
N-acetyl alanine	1.1×10^7	1.2×10^8
ethyl amine		$\sim10^6$

[a] Based on $\bar{k}_{39} = 1.2 \times 10^9\ M^{-1}\ sec^{-1}$, $k_{39} = 6.6 \times 10^9\ M^{-1}\ sec^{-1}$.

[b] Pulse radiolysis [BRAAMS, 1965]

[c] Pulse radiolysis [DAVIES, EBERT, and SWALLOW, 1965]

Table 5

Product yields in the γ radiolysis of pyrimidine-Cu^{+2} solutions[HOLIAN and GARRISON, 1966]

	Base (mM)	Cu^{+2}(mM)	pH	G(glycol)	G(isobarbituric)	ΣG(Products)
uracil	30	2	5	2.3	0.50	2.8
	30	1	5	2.3	0.60	2.9
	30	0.5	5	2.3	~0.7	3.0
	10	10	3.5	2.4	0.45	2.85
cytosine	10	2	3.7	2.28	0.42	2.70
	20	1	3.1	2.25	0.45	2.70

Table 6

Product yields in the γ radiolysis of purine bases in neutral, oxygenated solution [HOLIAN and GARRISON, 1967c]

	G(-B)	$G(NH_3)$[a]	G(carbonyl)
Xanthine	2.0	4.1	2.0[b]
Hypoxanthine	2.4	8.5	1.2[c]
Uric acid	2.2	0	1.2[d]

[a] Treated with 2N NaOH in the cold for 24 hours.

[b] Alloxan.

[c] Mesoxalic acid plus glyoxylic acid.

[d] Alloxan.

Table 7

Product yields in the γ radiolysis of solid glycine (evacuated)[MESHITSUKA, et al., 1964]

product	yield (G)
Ammonia	4.8
Acetic acid	2.3
Glyoxylic acid	2.5
Hydrogen	0.2
Methyl amine	0.2
Carbon dioxide	0.2

Table 8

Ammonia yields in the γ radiolysis of solid amino acids (evacuated)[PETERSON and GARRISON, 1967]

compound	ammonia yield, G
glycine	5.2 (4.8)[a]
alanine	5.4
β-alanine[b]	0.8
serine	6.2
cystine	3.5
phenyl alanine	2.9
cysteine	1.8

[a] [MESHITSUKA, et al., 1964]

[b] β-aminopropionic acid

Table 9

Amide yields in the γ radiolysis of solid N-acetyl amino acids (evacuated) [GARRISON et al., 1967; BENNETT-CORNIEA and GARRISON, 1967]

N-acetyl derivative of:	yield of "amide" ammonia, [a] G
glycine	2.7
alanine	3.4
valine	3.0
leucine	2.7
methionine	2.3
phenyl alanine	0.8

[a] Determine as ammonia after treatment with 2N NaOH in the cold for 24 hours to affect the quantitative conversion of the amide.

Table 10

Product yields in the γ radiolysis of N-acetyl alanine (evacuated)[GARRISON, JAYKO, WEEKS, SOKOL, and BENNETT-CORNIEA, 1967]

product	yield, G
Ammonia	3.4[a]
Propionic acid	1.4
Pyruvic acid	0.4[a]
Acetaldehyde	0.8
Alanine	0.4
Lactic acid	0.2[a]
Acrylic acid	< 0.2
Hydrogen	0.4[b]

[a]After hydrolysis.

[b]After dissolution.

Fig. 1. Yields of ammonia (○), pyruvic acid (△) and acetaldehyde (▲) as a function of alanine concentration in oxygen-free solutions at pH 6.4 under γ radiolysis (Weeks, Cole, and Garrison, 1965).

Fig. 2. Ammonia yields from 1.0 M alanine (○) and 1.0 M glycine (●) as a function of sodium formate concentration in oxygen-free solution at pH 6.4 under γ radiolysis (Weeks, Cole, and Garrison, 1965).

Fig. 3. Product yields from 1.0 M alanine as a function of sodium formate concentration in oxygen-free solution of pH 6.4 under γ radiolysis. Ammonia (●), propionic acid (▲) and pyruvic acid (■) (Weeks, Cole, and Garrison, 1965).

Fig. 4. Effect of Cu(II) concentration on ammonia yields in the γ radiolysis of oxygen-free 0.3 M glycine solutions at pH 3.0 (●), pH 8.5 (■) (Willix and Garrison, 1965b).

Fig. 5. Effect of formate ion on ammonia yields in the γ radiolysis of 0.3 M glycine - 0.04 M Cu(II) solutions (oxygen-free) at pH 8.6 (●), pH 4.0 (†) (Willix and Garrison, 1965b).

Fig. 6. Ammonia yields in the γ radiolysis of a homologous series of α-amino acids in oxygenated solution, pH. Glycine (○), alanine (●), α-aminobutyric acid (◐), nor valine (◒), nor leucine (◓) (Holian and Garrison, 1967c).

Fig. 7. Effect of glycylglycine and glycine concentrations on ammonia yields from oxygen-free solutions at pH 6.5 under γ radiolysis (Willix and Garrison, 1967).

Fig. 8. Effect of formate concentration on ammonia yields in the γ radiolysis of 1.0 M glycine (○) and 0.20 M glycylglycine (●) in oxygen-free solution at pH 6.5 (Willix and Garrison, 1967).

Fig. 9. Effect of chloracetate concentration on ammonia (⊙) and chloride ion (▲) yields in the γ radiolysis of 0.20 M glycylglycine, oxygen-free, pH 6.5 (Willix and Garrison, 1967).

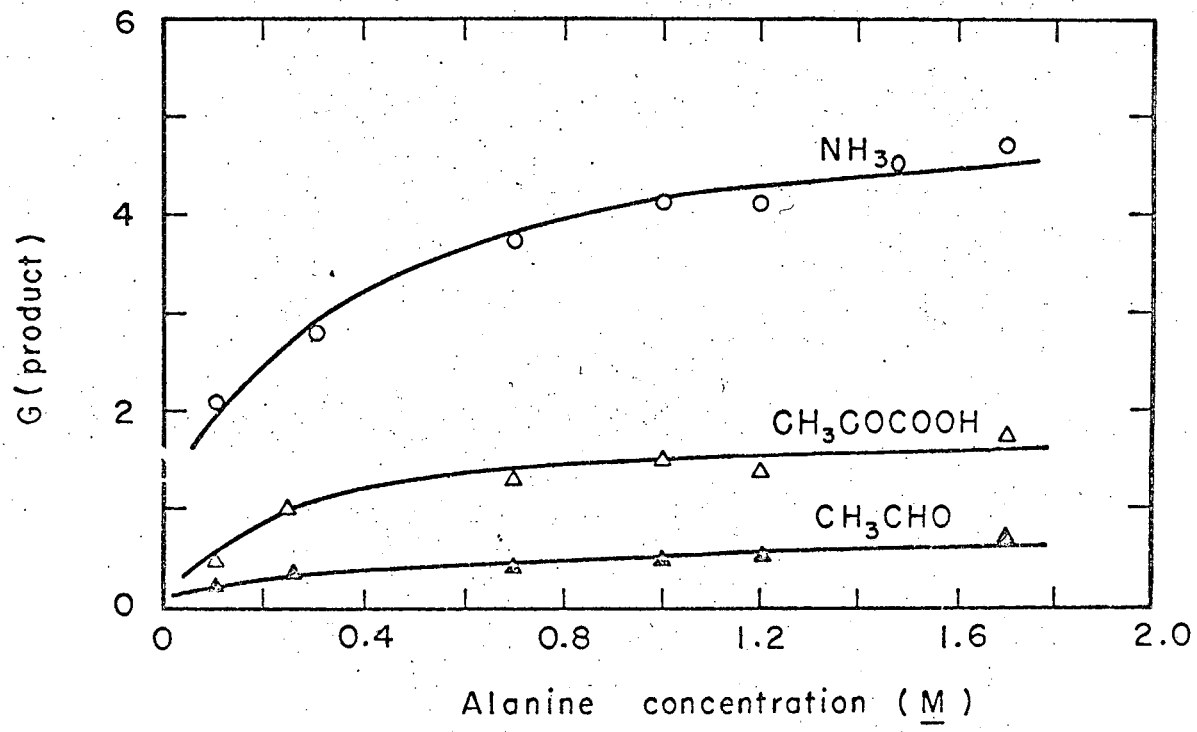
Fig. 10. Typical plots of the reciprocal chloride ion yields (γ radiolysis) as a function $(\overline{R_3N})/(\overline{RCl})$ or $(\overline{R_3N})/(\overline{RCl})$ for glycine zwitterion (△), glycine cation (▲), glycylglycine zwitterion (□), glycine ethyl ester (■), ethyl amine (⊙). (Willix and Garrison, 1967).

Fig. 11. Effect of Fe(III) concentration on the yields of ammonia (⊙) and pyruvic acid (▲) from 0.1 M acetylalanine and of ammonia (○) and glyoxylic acid (△) from 0.1 M acetylglycine (oxygen-free solution at pH 3 under γ rays)(Atkins, Bennett-Corniea and Garrison, 1967).

Fig. 12. Effect of acetylalanine concentration on yields of ammonia (⊙) and of pyruvic acid (▲) from solutions containing 0.05 M Fe(III). (Oxygen-free solutions at pH 3 under γ rays) (Atkins, Bennett-Corniea and Garrison, 1967).

Fig. 13. Effect of pH on the yield of ammonia (⊙), total α-keto acids (●) and α-ketoglutaric acid (○) in the γ radiolysis of oxygen-saturated solutions containing 0.15 percent poly-α-L-glutamic acid. (M.W. ~140,000) (Sokol, Bennett-Corniea and Garrison, 1965).

Fig. 14. Effect of a second-solute on ammonia yields in the γ radiolysis of 0.05 M cytosine, evacuated ph 7, formate (○), ethanol (⊙) (Kamal and Garrison, 1965).



MUB-5826

Fig. 1.

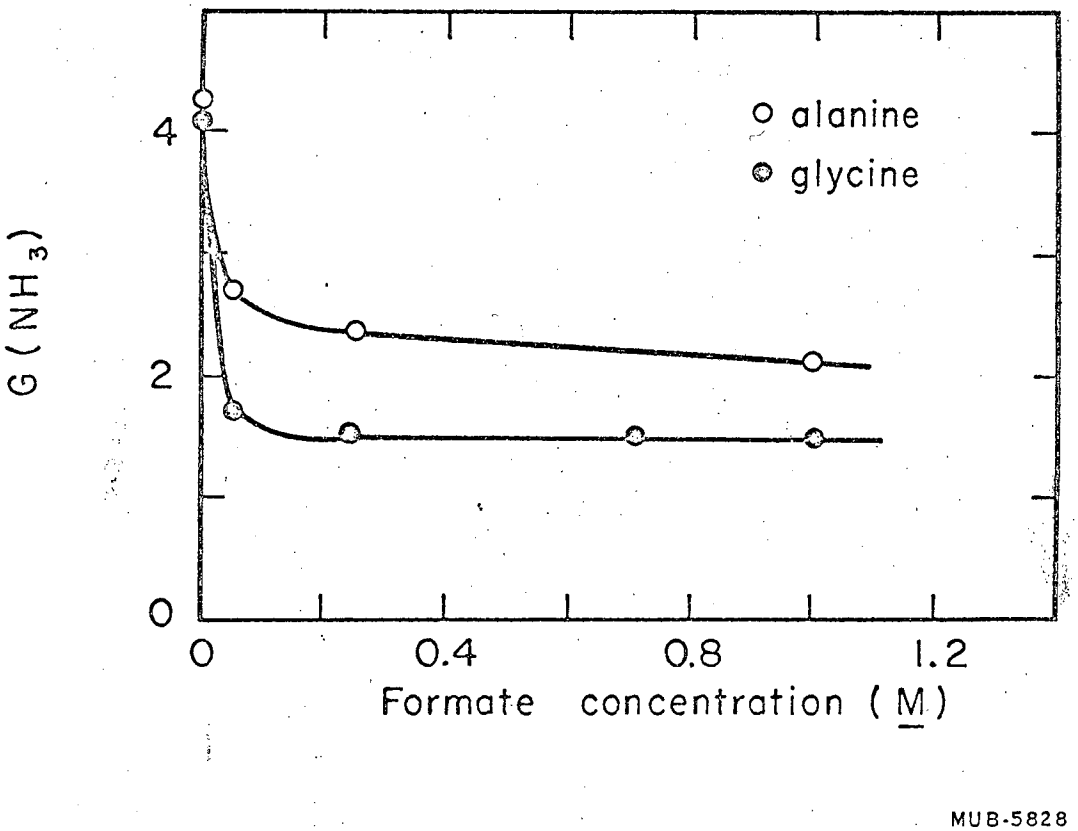

G (NH3)
4
2
0
alanine
glycine
0
0.4
0.8
1.2
Formate concentration (M)
MUB-5828

Fig. 2.

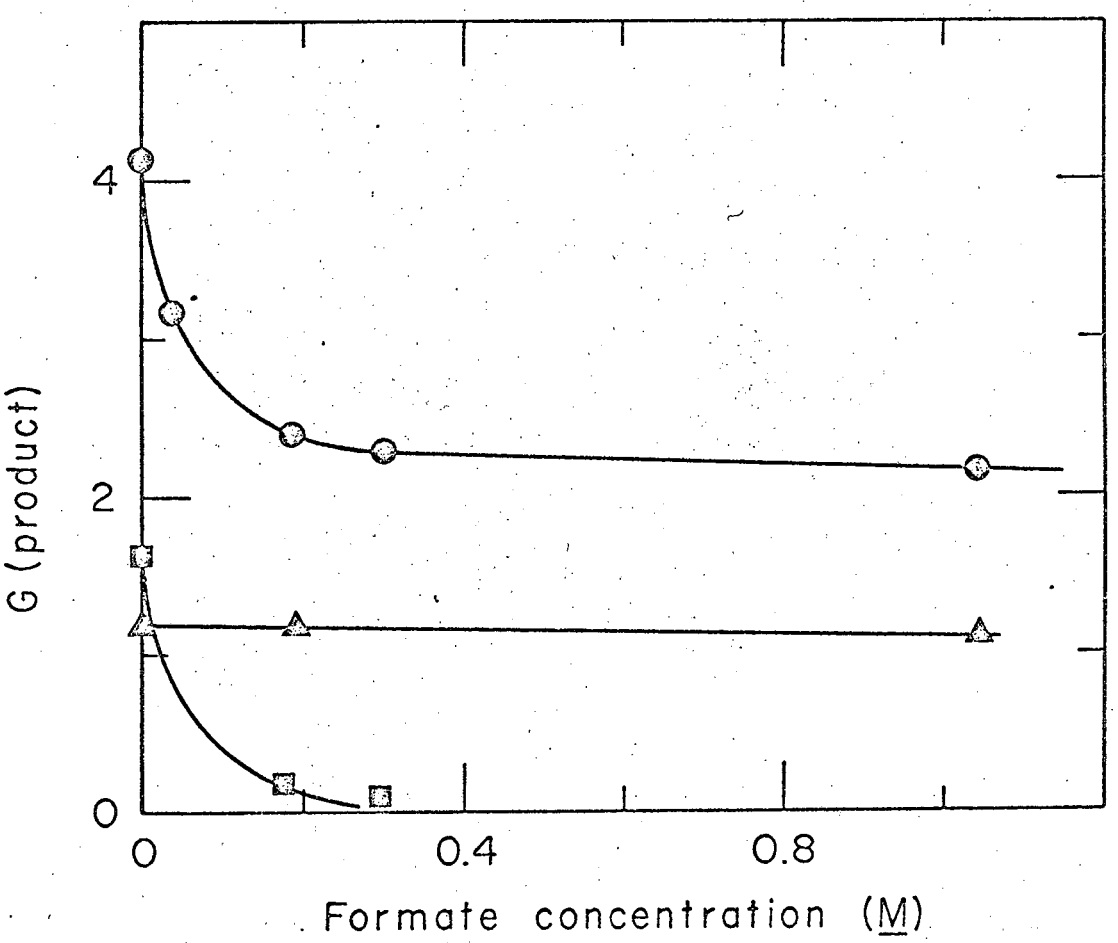


Fig. 3. XBL673-2201

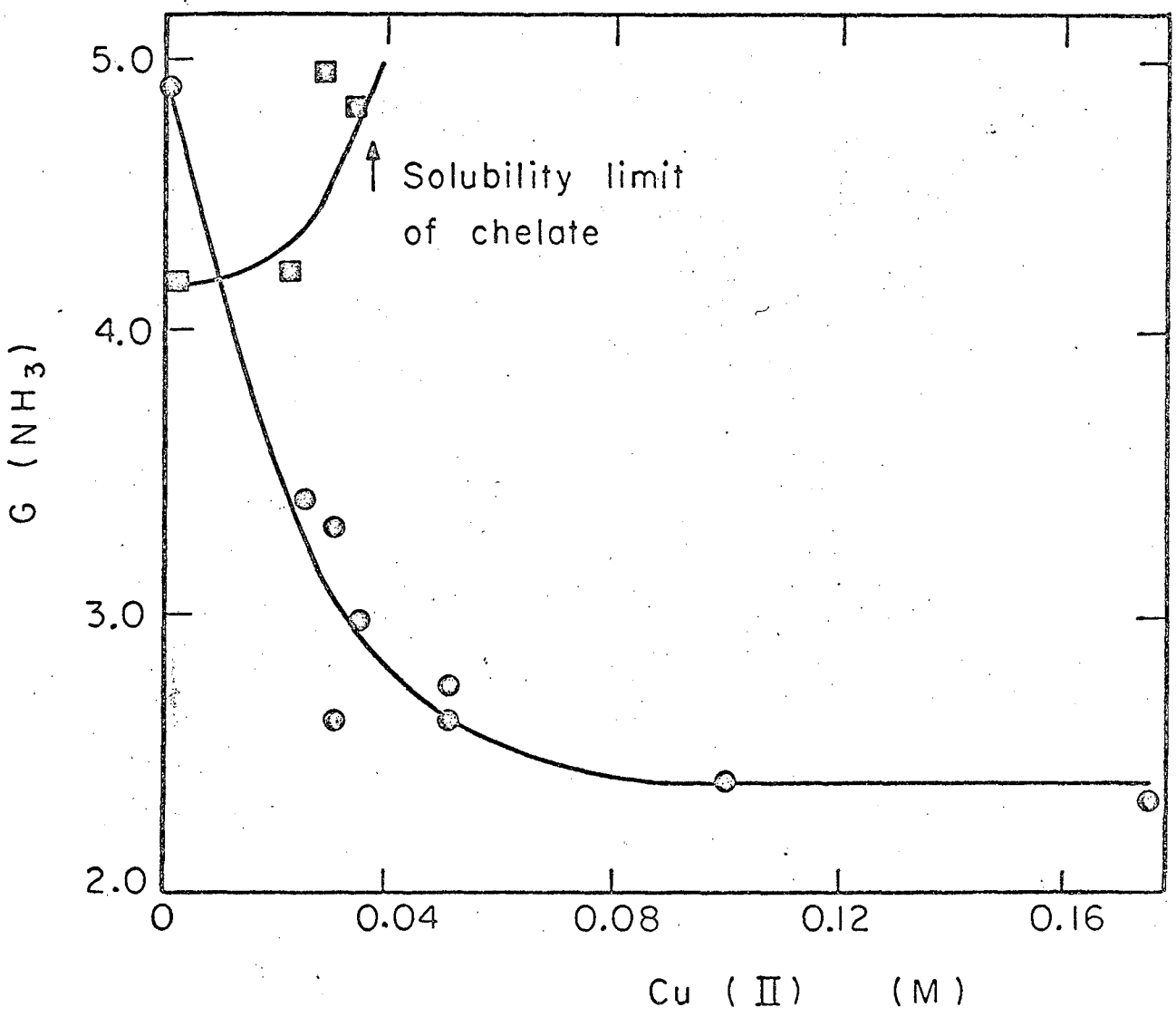


Fig. 4.

MUB-4309

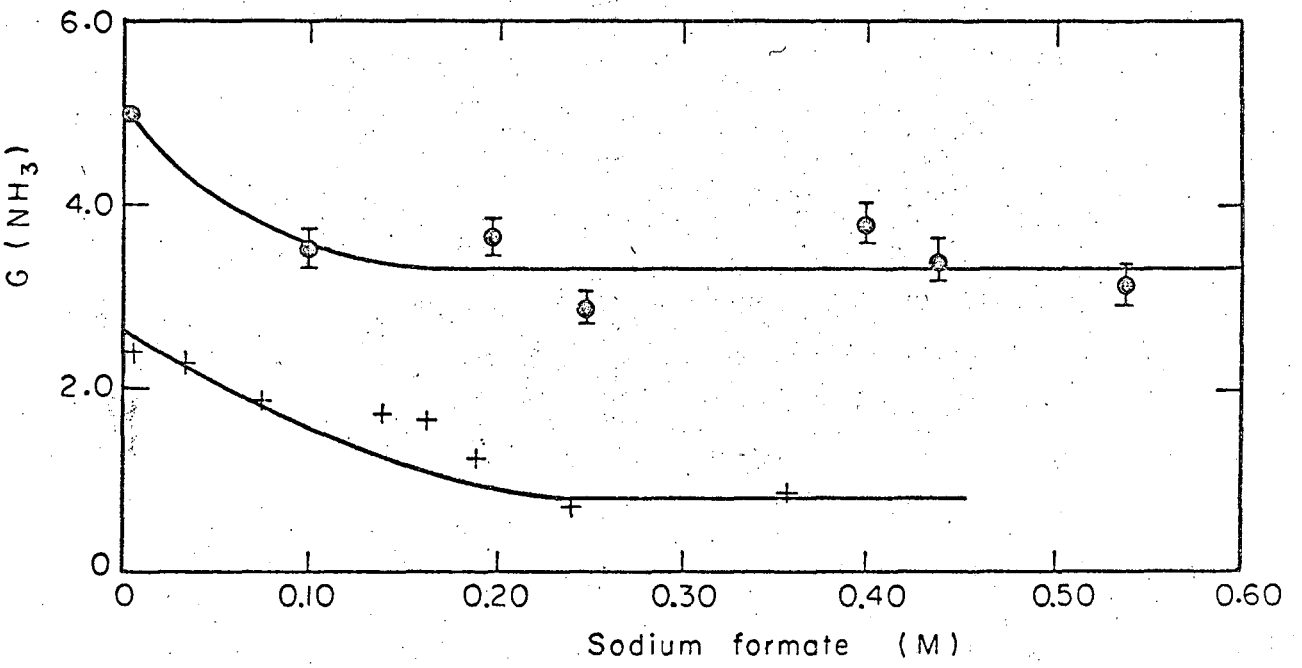


Fig. 5.

MUB-4308

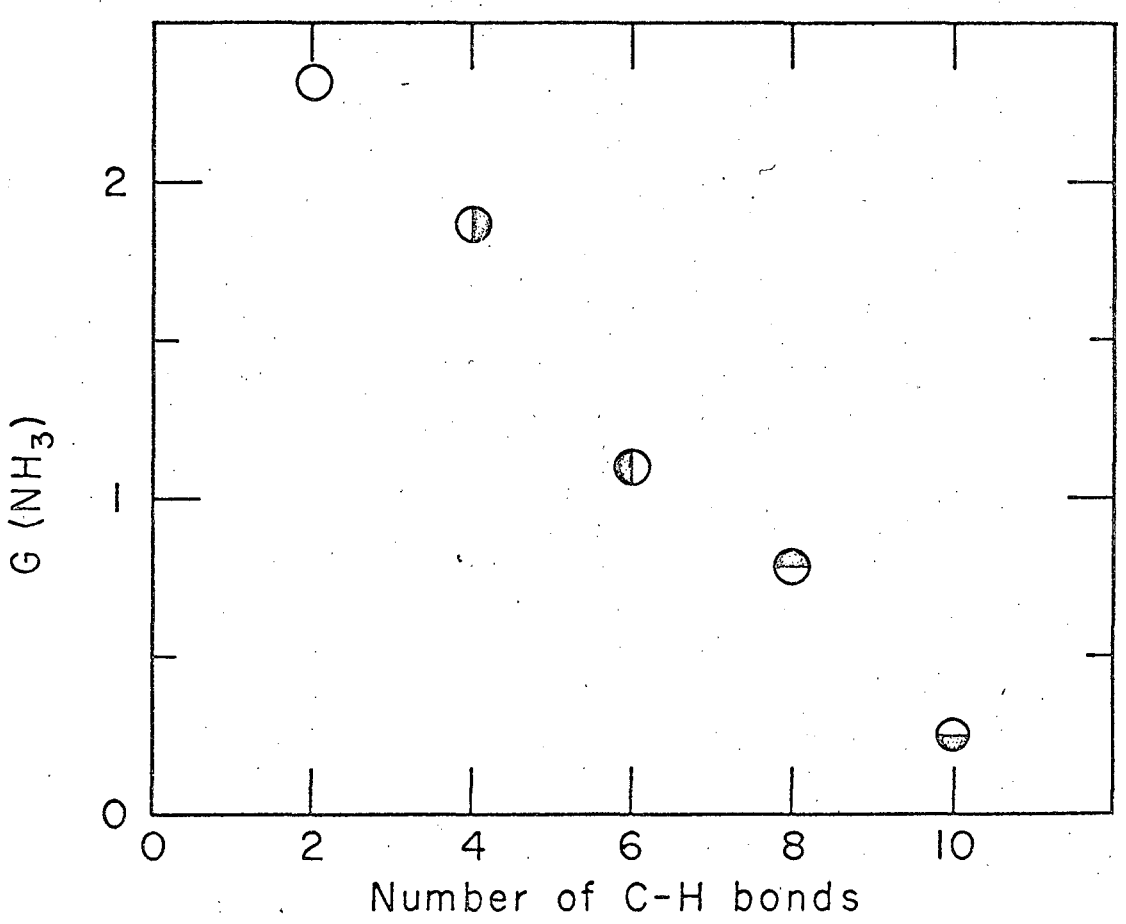


Fig. 6.

XBL673-2204

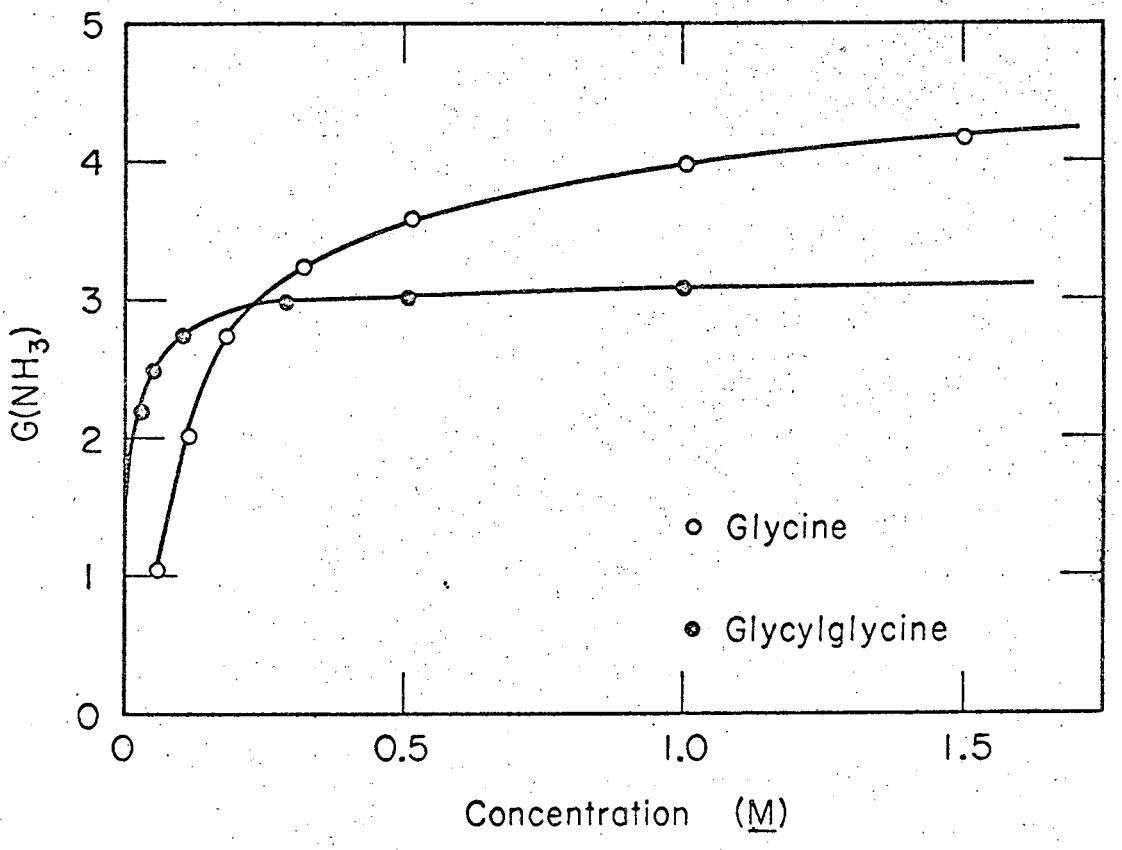


Fig. 7.

MUB-13910

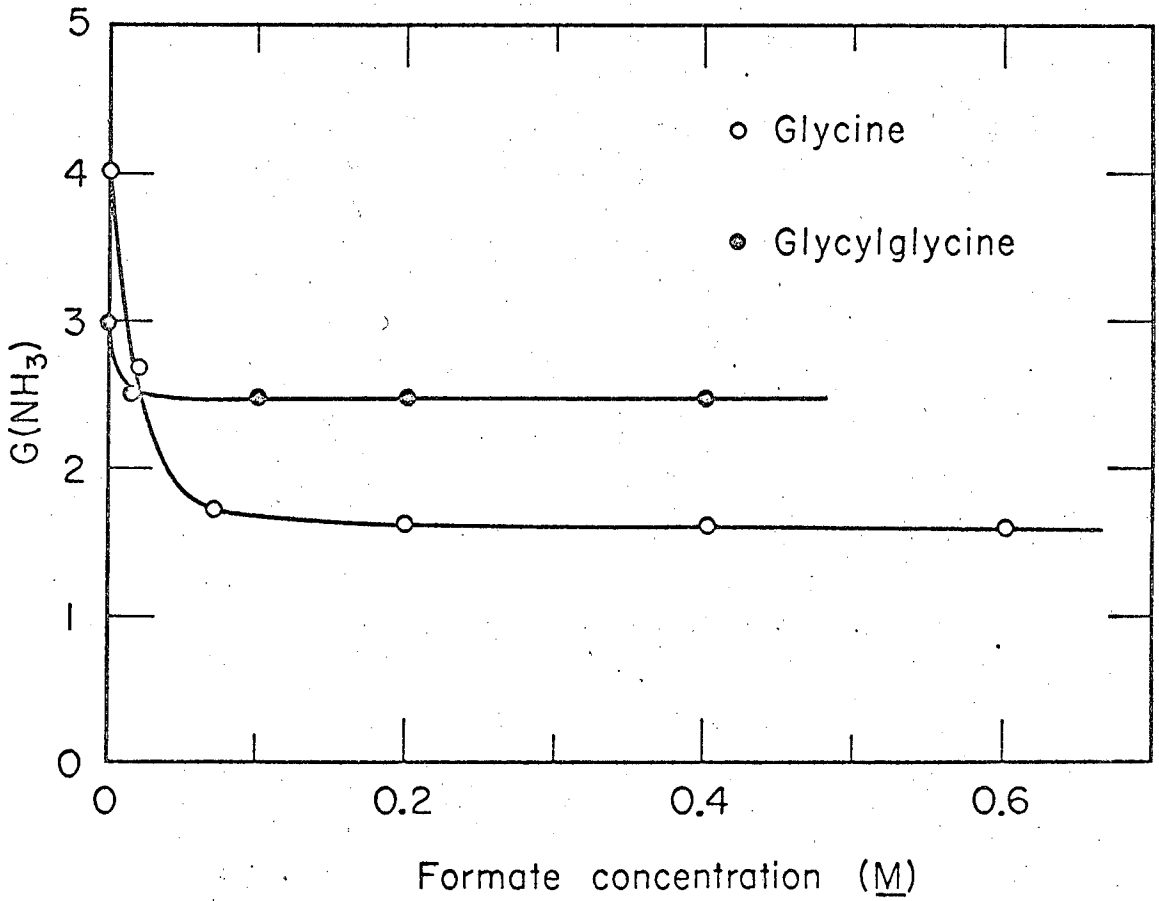


Fig. 8.

MUB-13911

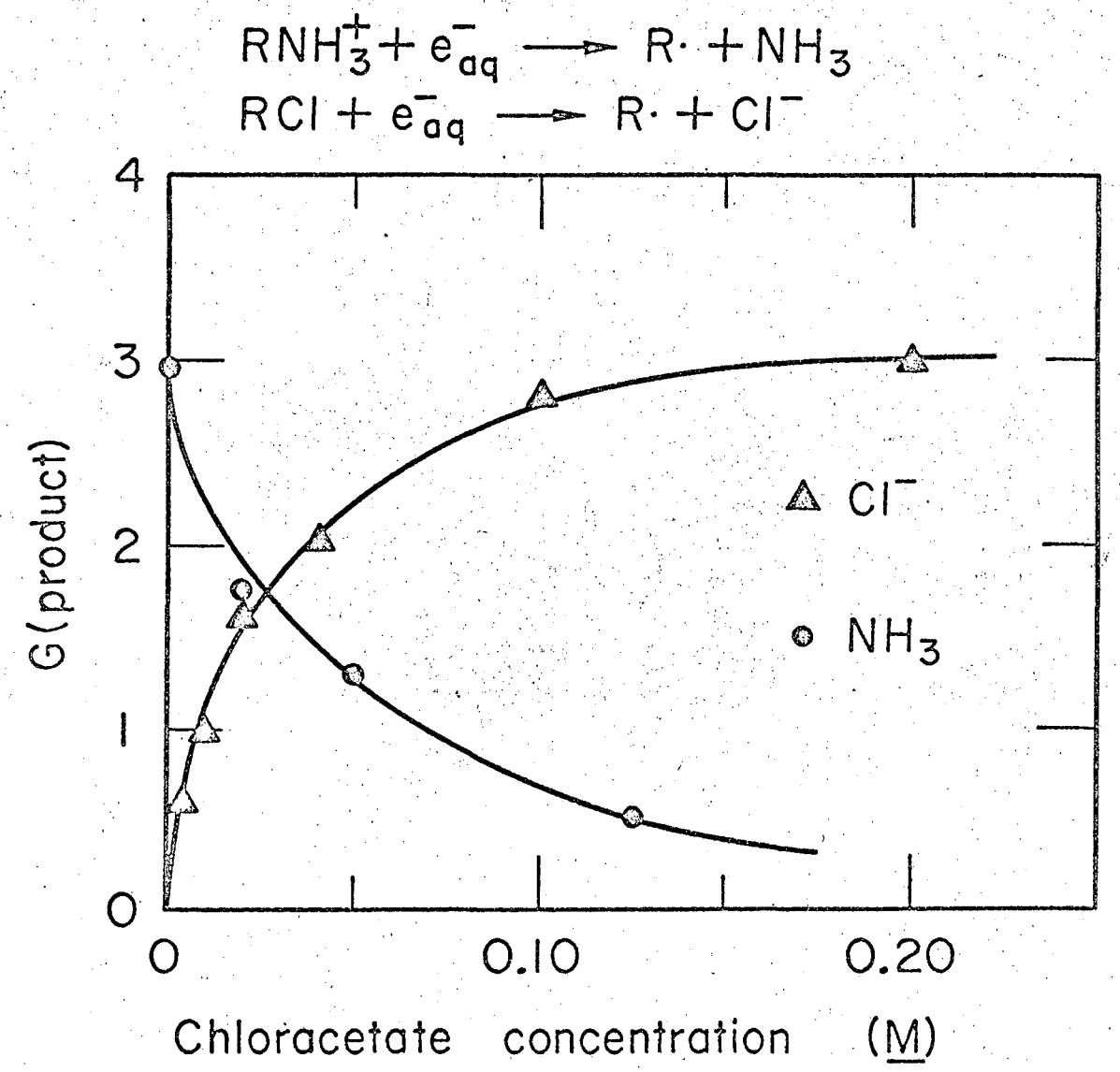


Fig. 9.

MUB-13912

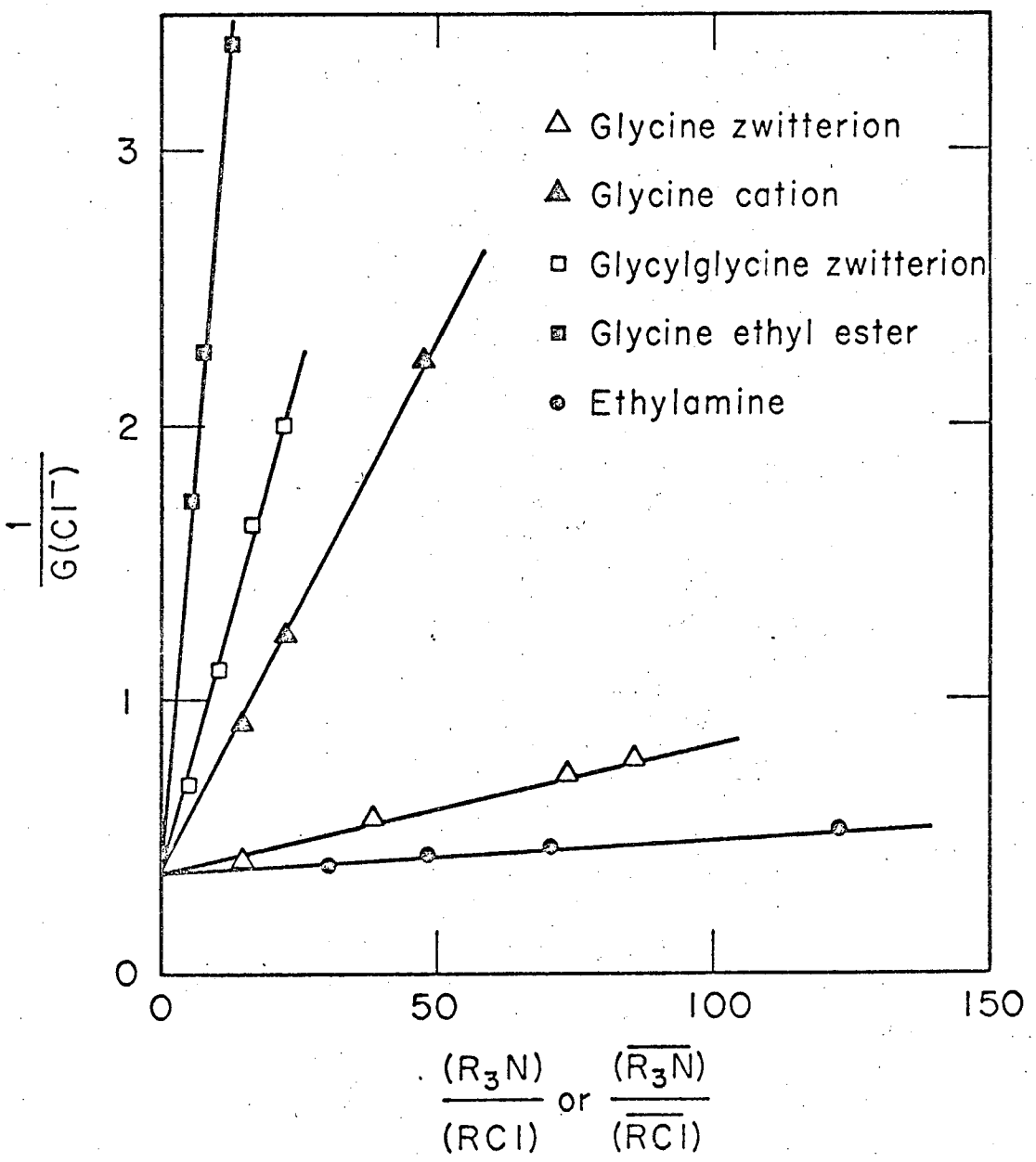

Glycine zwitterion
Glycine cation
Glycylglycine zwitterion
Glycine ethyl ester
Ethylamine
3
2
1
0
0
50
100
150
$\frac{1}{G(Cl^-)}$
$\frac{(R_3N)}{(RCl)}$ or $\frac{(\overline{R_3N})}{(\overline{RCl})}$
MUB-13909

Fig. 10.

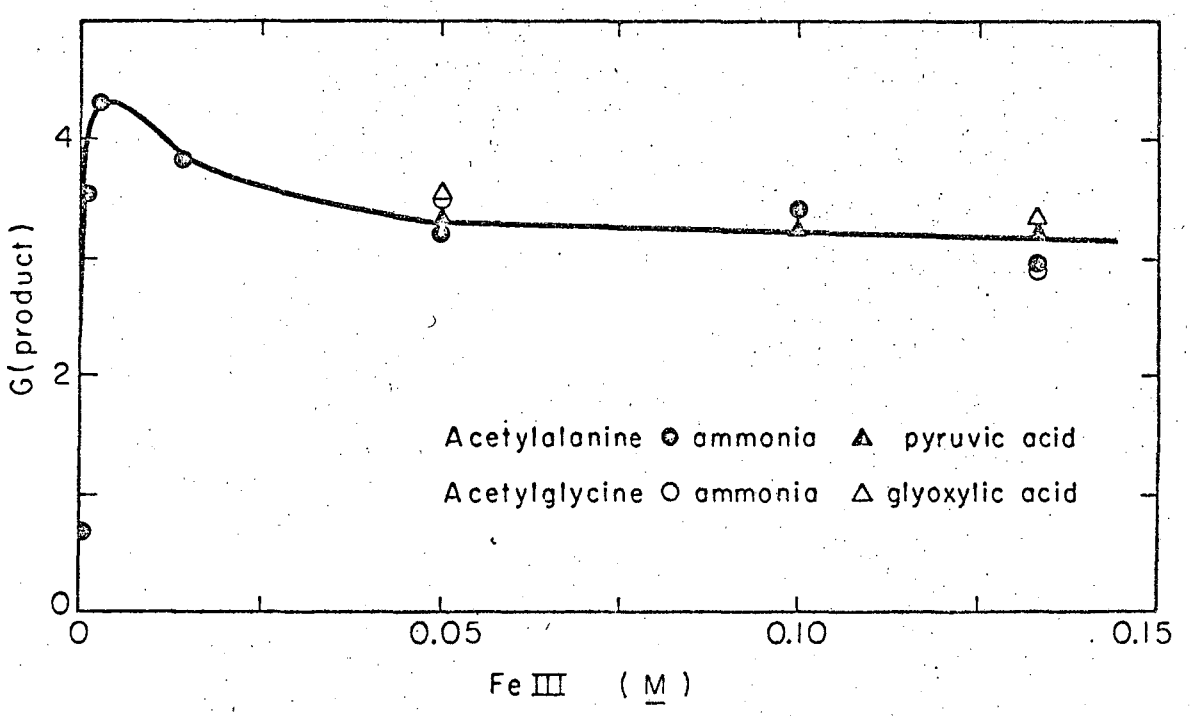

G(product)
4
2
0
0
0.05
0.10
0.15
Fe III (M)
Acetylalanine ⊙ ammonia ▲ pyruvic acid
Acetylglycine O ammonia △ glyoxylic acid
MUB-12106

Fig. 11.

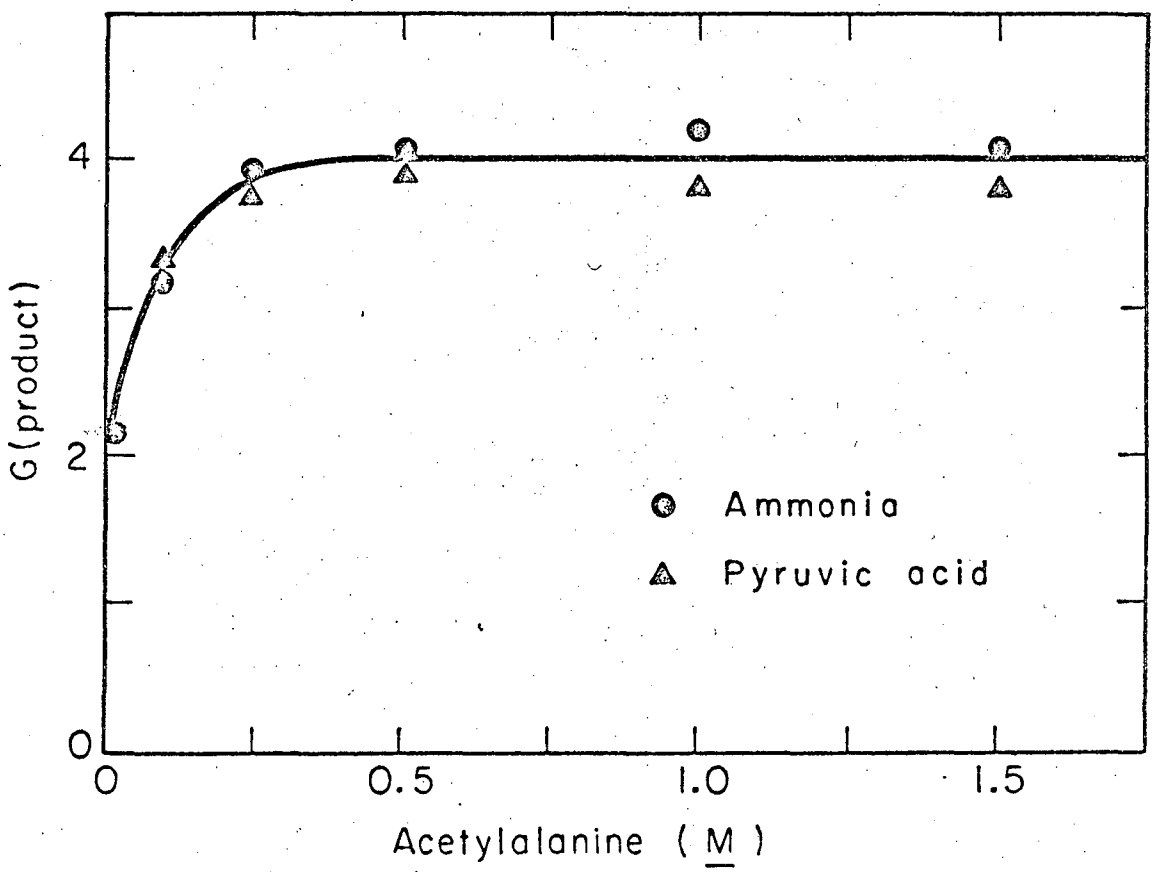


Fig. 12.

MUB-12107

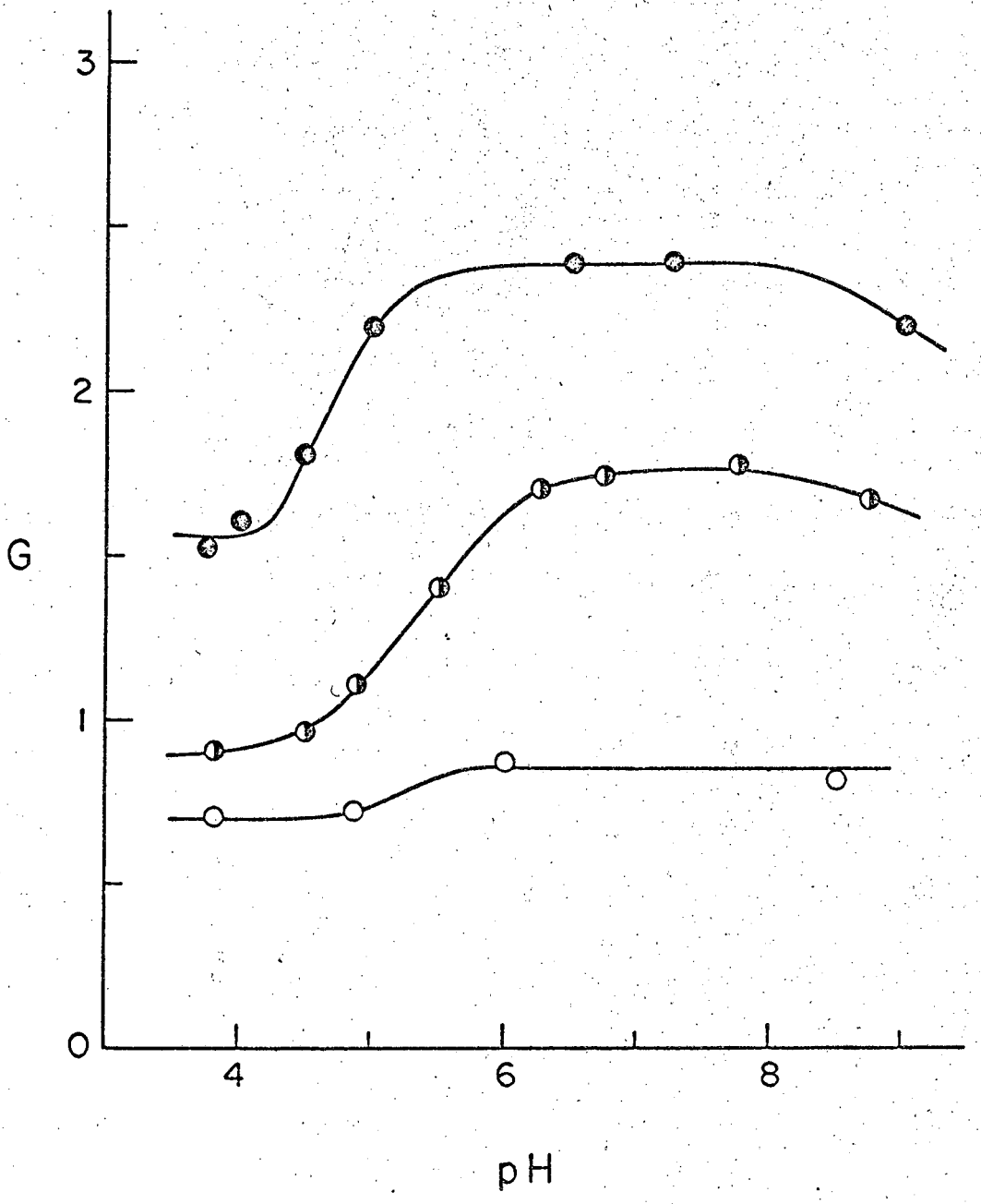


Fig. 13.

MUB-4299

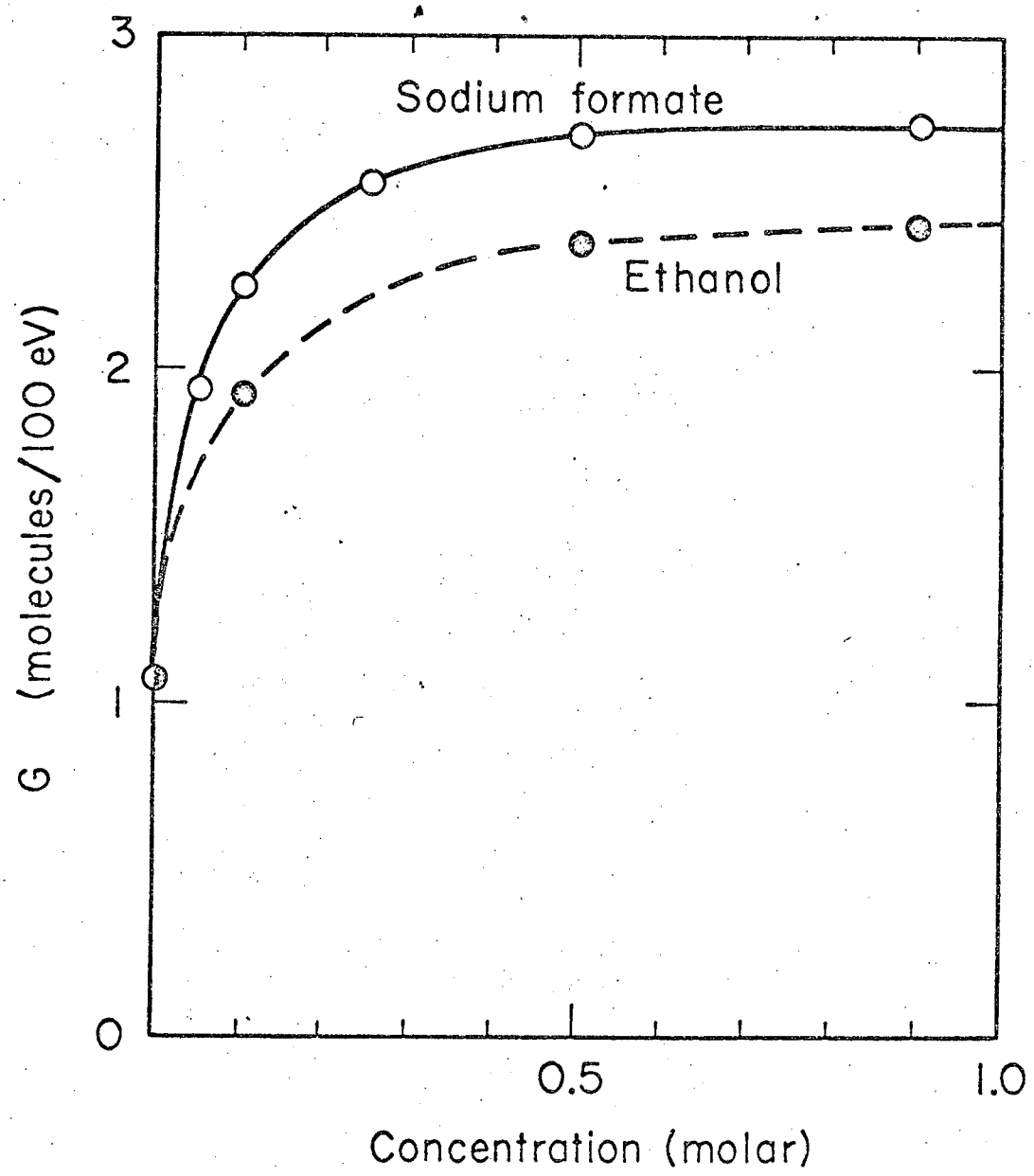


Fig. 14.

MUB-3540