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Radiolysis, Racemization, and the Origin of Optical Activity[1]

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An investigation has been undertaken to determine whether ionizing radiation might engender racemization (*radioracemization*) of optically active amino acids, along with their well-known radiolysis. We have exposed a number of solid and dissolved optically active amino acids to the ionizing radiation from a 3000-Ci ^{60}Co γ-ray source for periods of time which would engender substantial, but not total radiolysis. γ-Ray doses which caused 55–68% radiolysis of solid amino acids typically engendered 2–5% racemization. Aqueous solutions of the sodium salts of amino acids which underwent 53–66% radiolysis typically showed 5–11% racemization. The corresponding hydrochloride salts in aqueous solution, however, underwent little or no racemization. In aqueous solution both percentage degradation and percentage racemization were approximately proportional to γ-ray dosage within the range employed (1–36 $\times$ 10^6 rads). Mechanisms for the radioracemization of amino aicds in the solid state and as dissolved sodium salts are proposed, and the absence of racemization for dissolved hydrochloride salts is rationalized. Implications of these observations with regard to the origin of optical activity by the Vester–Ulbricht β-decay mechanism are discussed, as are their implications regarding the use of diagenetic racemization rates of ancient amino acid samples as criteria for geochronological and geothermometric calculations.

INTRODUCTION

The origin of optically active molecules in nature is a question which has puzzled and intrigued scientists for well over a century (*1*). Its importance is enhanced by its obvious relationship to the larger questions of the natural genesis of biopolymers of unique chirality and of the origin of life itself, and by the fact that in recent years optical activity has been suggested as perhaps the most valid criterion (the "Pasteur probe") for the recognition of life elsewhere in the universe (*2*). Since Pasteur's time a number of theories have been proposed to explain the origin of optical activity, some of which have been supported by experimental evidence (*3*). One of our recent interests in this problem has been to test experimentally the validity of a number of these proposed nonbiological hypotheses and thereby perhaps to develop model systems for the abiotic generation of chiral monomers and polymers from biologically realistic precursors. In this paper we shall review briefly earlier experiments by ourselves and others involving attempts to generate optical activity by means of ionizing β-radiation, and finally we shall describe our new observations of *radioracemization*, a phenomenon which may make

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questionable the *inevitable* efficacy of β-radiation or any other low-efficiency abiotic mechanism for the origin of molecular asymmetry in the biosphere.

In 1956 Lee and Yang (*4*) suggested that if the principle of parity were violated for the weak interactions involved in β-decay, then, as was soon thereafter verified experimentally, the β-particles from certain radioactive nuclides should be longitudinally polarized (*5*), and their subsequent γ-ray Bremsstrahlen photons should be circularly polarized (*6*, *7*). In 1959 Vester, Ulbricht, and co-workers (*8–10*) suggested a simple causal connection between this fundamental asymmetry of β-decay and the well-known chirality of biomolecules in nature, invoking the following mechanism: β-decay → longitudinally polarized electrons → circularly polarized photons → asymmetric photochemically induced reactions → optically active organic molecules.

Attempts to confirm the Vester–Ulbricht hypothesis experimentally (*8*, *10*) were unsuccessful until Garay (*11*) in 1968 reported that D-tyrosine in dilute alkaline solution containing 0.143 mCi of $^{90}SrCl_2$/ml underwent more extensive degradation than did L-tyrosine under similar conditions, after 18-month exposure to the ^{90}Sr radiation. Because of certain reservations about Garay's experiments, one of us (*12*, *13*) subsequently attempted to modify and extend them, irradiating a variety of racemic and optically active amino acids (both solid and dissolved) with the Bremsstrahlen from a 61.7-kCi ^{90}Sr–^{90}Y facility at Oak Ridge National Laboratory. After 1.34 years of irradition (4.1×10^8 rads) degradation of the samples had occurred to the extent of 13–49%, but no evidence whatsoever for optical activity was found on examining the undecomposed residues of the irradiated samples by means of both optical rotatory dispersion (250–630 nm) and analytical gas chromatography (gc) (*14*). It seemed likely (*12*) that these unexpected negative results might be due, among other reasons, to the fact that the majority of the ^{90}Sr–^{90}Y Bremsstrahlen intensity is found at the low energy end of the Bremsstrahlen spectrum (*15*) where the circular polarization of the photons is known to be low (*6*, *7*). We accordingly attempted to circumvent these difficulties by employing "artificial" longitudinally polarized, monoenergetic (120 keV) electrons produced in a specially designed linear accelerator (*16*, *17*). This system had the additional advantage over natural β-decay electrons in that either "natural" antiparallel-spin ("left-handed") electrons or "unnatural" parallel-spin ("right-handed") electrons could be employed at will, thus permitting the crucial test for the predicted reversal of any asymmetric effect by electrons of opposite chirality. In a number of replicate experiments irradiation with natural "left-handed" electrons preferentially destroyed D-leucine in a crystalline DL-leucine target, while, conversely, unnatural "right-handed" electrons preferentially destroyed the L-enantiomer (*16*, *17*). These experiments thus both established the validity of the asymmetric degradation effect by replication and confirmed it by demonstrating its reversal with electrons of mirror-image handedness. The magnitude of the enantiomeric excesses produced in these experiments was small but relatively reproducible, i.e., 0.6–1.4%. In connection with these experiments, Keszthelyi (*18*) and Walker (*19*) have since argued on energy grounds that such successful asymmetric degradations must be engendered by the impinging electrons and not by their Bremsstrahlen. More recently Darge and co-workers (*20*) have claimed an impressive 19% optical enrichment in the residue from the asymmetric radiolysis of DL-trypthophan, using the β-radiation from 0.63 mCi of [^{32}P]phosphate in frozen dilute aqueous solution. We are currently attempting to corroborate this remarkable observation.

Finally, we have recently examined (*21*) for gross degradation and possible asymmetric radiolysis a number of crystalline ^{14}C-labeled amino acids (both racemic and optically active) of high specific radioactivity which had been synthesized at the Lawrence Radiation Laboratory, University of California, in the 1950s (*22*). Despite self β-radiolysis as high as 67%, no asymmetric degradation was noted in any of the racemic samples, using gc criteria for the establishment of their enantiomeric compositions. Several of the optically active ^{14}C-labeled samples, however, gave gc analyses which suggested that radiation-induced racemization might have accompanied their overall radiolysis. Since the possibility of such *radioracemization* has not been considered in any of the above studies involving asymmetric effects engendered by β-radiation, and since such a phenomenon would clearly diminish the efficacy of the Vester–Ulbricht β-decay mechanism for the abiotic origin of molecular asymmetry, we have undertaken to investigate this phenomenon in greater detail.

EXPERIMENTAL

The samples irradiated consisted of 10 to 17 mg portions, either solid or dissolved, of the optically active amino acids (purest qualities available from Aldrich Chemical Co., Calbiochem, Mann Research Laboratory, Nutritional Biochemicals, or Sigma Chemical Co.) listed in the tables below. For hydrochloride salt irradiations the weighed amino acid was first dissolved in an equivalent amount of 0.10 *N* aqueous HCl solution, while for sodium salt irradiations each amino acid was dissolved in the equivalent volume of 0.10 *N* NaOH. The solid or dissolved samples were placed in 1 × 4-cm glass vials stoppered with Bakelite caps lined with Teflon gaskets, and the vials were installed in a 3000-Ci ^{60}Co γ-ray source at the Lawrence Berkeley Laboratory. This source was specially designed to permit delivery of high dose rates (ca. $4–10 \times 10^6$ rads/hr) to small samples, and irradiations were conducted for time periods (5–90 hr) sufficient to afford the total radiation dosages indicated in the following tables.

After irradiation the samples were quantitatively divided, solids by weighing and solutions by taking aliquots with a microsyringe, after which the latter aliquots were rotary-evaporated to dryness under vacuum. The quantitatively measured fraction of each irradiated amino acid (or salt) was then treated with a weighed quantity of the corresponding enantiomeric (or racemic) amino acids, so as to permit estimation of the percentage decomposition of each sample by the "enantiomeric marker" technique (*23*). The remaining portions of each solution sample were similarly vacuum-evaporated to dryness. All of the amino acid residues were thereupon converted to their *N*-trifluoroacetyl 2-propyl ester derivatives by treating first with refluxing HCl-saturated 2-propanol, stripping, and heating finally with trifluoroacetic anhydride (*14*). Each derivatized sample was then quantitatively analyzed gas-chromatographically for its enantiomeric composition, using 4–6 m × 0.05-mm stainless-steel capillary columns coated with the optically active phases, *N*-lauroyl- (*24*) or *N*-docosanoyl-L-valyl-t-butylamide (*25*). The columns were installed in a Hewlett–Packard 5700A Gas Chromatograph, and peak integration was performed with a Hewlett–Packard 3380A digital electronic integrator as previously described (*14*). Our first experiments involved irradiating crystalline D- and L-leucine with total doses of ca. 10^9 rads. This caused extensive racemization but essentially complete degradation, leading to poor reproducibility (Table 1). Subsequent doses were accordingly adjusted so as to give

TABLE 1

DECOMPOSITION AND RACEMIZATION OF CRYSTALLINE AMINO ACIDS ON γ-IRRADIATION

Amino acid	Radiation dose (rads × 10^{-8})	Decomposition (%)	Decomposition (G)[a]	Composition of residue (percentage of D)	Composition of residue (percentage of L)	Composition of residue ($\pm$SD)[b]	Racemization (%)
L-Alanine	8.1	38.6	5.2	1.9	98.1	0.1	3.8
D-2-Aminobutyric acid	8.1	55.8	6.5	99.2	0.8	0.1	1.6
L-Norvaline	8.1	66.1	6.7	1.6	98.4	0.1	3.2
L-Norleucine	8.1	63.1	5.7	1.3	98.7	0.1	2.6
D-Leucine	8.1	67.9	6.2	97.2	2.8	0.2	5.6
L-Leucine	8.1	68.0	6.2	2.5	97.5	0.1	5.0
D-Leucine	10.2	96.1	6.9	96.2	3.8	0.6	7.6
L-Leucine	10.2	93.2	6.8	6.8	93.2	0.2	13.6

[a] G = Molecules destroyed by 100 eV of absorbed radiation.

[b] Standard deviation from average of two or three gc analyses; same for Tables 2, 3, and 4.

TABLE 2

DECOMPOSITION AND RACEMIZATION OF AMINO ACID SODIUM SALTS (0.10 M AQUEOUS SOLUTION) ON γ-IRRADIATION

Amino acid	Radiation dose (rads × 10^{-8})	Decomposition (%)	Decomposition (G)	Composition of residue (percentage of D)	Composition of residue (percentage of L)	Composition of residue ($\pm$SD)	Racemization (%)
L-Alanine	1.7	65.8	3.8	5.8	94.2	0.0	11.6
D-2-Aminobutyric acid	1.8	58.0	3.1	96.0	4.0	0.1	8.0
D-2-Aminobutyric acid	1.8	59.4	3.2	95.8	4.2	0.0	8.4
L-Norvaline	1.7	59.6	3.4	4.2	95.8	0.1	8.4
L-Norleucine	1.7	52.8	3.0	5.3	94.7	0.0	10.6
L-Valine	1.7	55.3	3.2	5.5	94.5	0.2	11.0
L-Leucine	1.7	54.4	3.1	2.6	97.4	0.1	5.2
D-Leucine[a]	41.0	66.0	0.2	87.3	12.7	0.7	25.4
L-Leucine[a]	41.0	58.7	0.1	10.4	89.6	0.4	20.8

[a] Irradiated in 61.7-kCi ^{90}Sr–^{90}Y β-ray Bremsstrahlen source; 1.0 M Na salt solution containing 5% excess NaOH.

less gross degradation of the samples. Some of the experimental data and the results of these irradiations are summarized in Tables 1, 2, and 3.

In order to assess the effect of variation in radiation dosage on the extents of both gross degradation and racemization, a number of D- or L-leucine samples (15–17 mg) were dissolved as before in the equivalent volumes of 0.10 N HCl or 0.10 N NaOH, whereupon the solutions were irradiated in the above ^{60}Co γ-ray source for increasing time periods, so as to achieve the increasing radiation dosages listed in Table 4. A quantitative aliquot of each solution was removed and added to a weighed sample of L-

TABLE 3

DECOMPOSITION AND RACEMIZATION OF AMINO ACID HYDROCHLORIDE SALTS (0.10 *M* AQUEOUS SOLUTION) ON γ-IRRADIATION

Amino acid	Radiation dose (rads × 10^{-7})	Decomposition (%)	Decomposition (*G*)	Composition of residue (percentage of D)	Composition of residue (percentage of L)	Composition of residue (±SD)	Racemization (%)
L-Alanine	2.2	53.4	2.4	0.2	99.8	0.1	0.4
D-2-Aminobutyric acid	2.2	52.1	2.3	100.0	0.0	—	0.0
L-Norvaline	2.2	57.9	2.6	0.0	100.0	—	0.0
L-Norleucine	2.2	63.5	2.8	0.0	100.0	0.0	0.0
L-Valine	2.2	54.8	2.4	0.0	100.0	0.0	0.0
L-Leucine	2.2	55.3	2.4	0.0	100.0	0.0	0.0

TABLE 4

DECOMPOSITION AND RACEMIZATION OF LEUCINE SALTS (0.10 *M* AQUEOUS SOLUTION) AS A FUNCTION OF γ-RAY DOSAGE

Enantiomer	Salt	Radiation dose (rads × 10^{-6})	Decomposition (%)	Composition of residue (percentage of D)	Composition of residue (percentage of L)	Composition of residue (±SD)	Racemization (%)
D	Na	1.0	1.8	99,2	0.8	0.0	1.6
D	Na	3.0	9.4	99.2	0.8	0.1	1.6
D	Na	14.0	41.7	97.9	2.1	0.0	4.2
L	Na	17.3	54.4	2.6	97.4	0.1	5.2
D	Na	20.5	63.1	96.2	3.8	0.3	7.6
D	Na	27.0	73.6	95.4	4.6	0.1	9.2
D	HCl	1.0	3.3	99.8	0.2	0.0	0.4
D	HCl	3.0	7.6	99.7	0.3	0.0	0.6
D	HCl	14.0	32.5	99.2	0.8	0.1	1.6
D	HCl	22.0	55.3	0.0	100.0	0.3	0.0
L	HCl	22.1	57.6	—[a]	—	—	—
D	HCl	36.1	75.7	—[a]	—	—	—

[a] Gas-chromatographic traces obscured by peaks for degradation products.

(or D-) leucine as an "enantiomeric marker", and all solutions were evaporated to dryness and converted to their *N*-trifluoroacetyl 2-propyl ester derivatives as above. Gas-chromatographic analyses then gave the percentage degradation and racemization values summarized in Table 4.

Finally, two samples of aqueous sodium salt solutions of D- and L-leucine which had been irradiated for 1.34 years in the 61.7-kCi ^{90}Sr–^{90}Y β-ray Bremsstrahlen source at Oak Ridge National Laboratory (*12*) were examined in the same way for degradation and racemization. These data are included in Table 2.

The optically active amino acids in the above tables were examined gas

chromatographically as *N*-trifluoroacetyl 2-propyl ester derivatives prior to their irradiation, and each was found to be optically homogeneous (>99.98%). We have previously emphasized (*14, 23*) the advantages of the above gc technique over optical rotation or rotatory dispersion methods for determining the enantiomeric composition of undecomposed residues in the presence of other optically active or inactive decomposition products.

RESULTS

Tables 1 and 2 indicate that ^{60}Co γ-rays (and in Table 2 ^{90}Sr–^{90}Y Bremsstrahlen) cause not only the expected radiolysis of the amino acid samples, but also induce their significant racemization as well, both in the solid state and as their sodium salts in aqueous solution. Extensive radiolysis also occurs with amino acid hydrochloride salts in aqueous solution (Table 3), but interestingly, little or no concomitant racemization is observed. Expression of the radiolyses in Tables 1, 2, and 3 in terms of *G*-values (molecules destroyed per 100 eV of absorbed radiation) indicates no great differences in the stability of the amino acids studied toward γ-radiation. It is noteworthy (Table 2) that the sodium salt of leucine seems more susceptible to destruction by ^{60}Co γ-radiation than by ^{90}Sr–^{90}Y β-ray Bremsstrahlen, although a strict comparison is probably unwarranted because of the different solute concentrations involved. A rough indication of the overall reproducibility of the decomposition and racemization values in the tables is afforded by the duplicate data on the sodium salt of D-2-aminobutyric acid in Table 2. The samples employed in these two experiments were identical in size and were irradiated simultaneously.

Table 4 indicates that increasing radiation dosages cause increasing extents of radiolysis of leucine salts in aqueous solution, as well as increasing extents of racemization of the sodium salt. Again, the hydrochloride salt solutions suffered little or no racemization. A plot (Fig. 1) of the data in Table 4 shows that radiolysis is slightly faster for the sodium salt of leucine than for its hydrochloride and that for both salts the extent of radiolysis is approximately directly proportional to total dosage up to about 60% degradation. For a number of crystalline amino acids, Tolbert and co-workers (*26*)

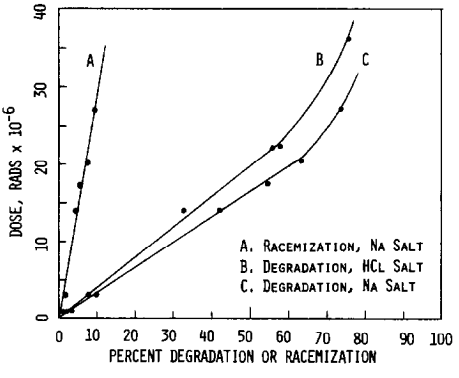


FIG. 1. Radiolysis and radioracemization of leucine salts (0.1 *M* aqueous) on γ-irradiation.

have found a similar initial linear relationship between radiolysis and dose, and we have likewise observed a linear decomposition–dose relationship for crystalline leucine during γ-ray radiolysis to the extent of 82% (*23*). Figure 1 also shows that an approximately linear relationship exists between the extent of racemization and dosage for the aqueous sodium salt of leucine within the dose range studied.

The 0.10 *N* leucine sodium salt solutions irradiated above had a pH of ca. 11.2, and the temperature within the ^{60}Co γ-ray source cavity was ca. 40°C. To satisfy ourselves that the degradations and racemizations observed above were in fact caused by the γ-radiation and not (in part) by simple thermal or pH effects, the following control experiment was performed. A similar 0.10 *N* aqueous solution of the sodium salt of L-leucine was placed in an oven at 40°C for a period (118 hr) longer than that involved in any of the above γ-irradiations, then was processed in the usual way. Customary gc analyses of the final *N*-TFA-2-propyl ester derivative indicated a complete lack of observable degradation or racemization within this time period in the absence of the ionizing radiation.

DISCUSSION

The racemization of optically active compounds by ionizing radiation (*radioracemization*) is a phenomenon which has been reported only sporadically in the past literature and has hitherto not been examined in detail. In 1952 Wright (*27*) observed that the optical activity of solid mandelic acid was reduced during its irradiation in pile, but it was not clear whether this was due to racemization or to simple radiolysis of the mandelic acid. In 1959 Feng and Tobey (*28*) investigated this question and found that mandelic acid in aqueous solution suffered both radiolysis and racemization on ^{60}Co γ-irradiation, results which were explained in terms of a conventional free-radical mechanism. Evans *et al.* (*29, 30*) have noted that partial racemization of a number of ^{3}H-labeled amino acids accompanied their self-β-radiolysis during storage of their aqueous solutions, but no conclusion was drawn as to whether the racemization was a purely chemical effect or was due totally or in part to the β-radiation. Bayly and Evans' earlier failure (*31*) to detect any racemization of optically active ^{14}C-labeled amino acids stored in aqueous solution suggested to them that the racemization of the ^{3}H-labeled samples must have been due to a chemical effect, "although a possible contribution from the β-radiation cannot be ruled out." More recently Ehrl (*32*) has studied the γ-irradiation of dilute aqueous solution of L-phenylalanine, L-tyrosine, and L-dopa and found a linear increase in "optical inactivation" with increasing dosage over the initial portions of the dose ranges studied. He concluded that the optical inactivation noted was due to "substrate inversion" rather than "decomposition of the asymmetry center" of the substrate or "optical inactivation of the irradiation products." Finally, the partial racemization of solid amino acids during their ^{3}H-labeling by the Wilzbach method (*33*) (exposing them to $^{3}H_2$ gas) has been reported by Parmentier (*34*). Garnett and co-workers (*35*) have since suggested that such racemization results from the radiolytic formation of an imine intermediate, RC(=NH)COOH, which subsequently adds $^{3}H_2$ to form the racemic saturated product, ^{3}H-labeled at its N- and α-C-atoms.

At the present time we have made no attempt to establish mechanisms whereby the observed radioracemizations of solid amino acids or their aqueous sodium salts might occur. The gc traces of the crude irradiated amino acids or their salts after derivitization as *N*-trifluoroacetyl 2-propyl esters showed several extraneous peaks along with the residual D- and L-amino acid enantiomers being quantitatively estimated (particularly in the cases of the sodium salts), but no effort has been made as yet to identify the degradation products responsible for these peaks. About all that can be done at present is to speculate as to how radioracemization might superimpose itself on the mechanisms which have heretofore been proposed for the radiolysis of amino acids in general, both as solids and in aqueous solution.

The radiolysis of crystalline amino acid has been discussed in detail by Garrison (*36–38*). He proposes that the initial ionization of the amino acid zwitterion, I, by the γ-photon produces heterolytically a proton, a secondary electron, e_s^-, and an α-radical, II

$$\underset{\text{I}}{H_3\overset{+}{N}CH(R)COO^-} \xrightarrow{\gamma\text{-photon}} \underset{\text{II}}{H_3\overset{+}{N}\dot{C}(R)COO^-} + H^+ + e_s^- \qquad [1]$$

$$e_s^- + \underset{\text{I}}{H_3\overset{+}{N}CH(R)COO^-} \longrightarrow NH_3 + \underset{\text{III}}{\dot{C}H(R)COO^-} \qquad [2]$$

$$\underset{\text{I}}{H_3\overset{+}{N}CH(R)COO^-} + \underset{\text{III}}{\dot{C}H(R)COO^-} \longrightarrow \underset{\text{II}}{H_3\overset{+}{N}\dot{C}(R)COO^-} + RCH_2COO^- \qquad [3]$$

$$2\,\underset{\text{II}}{H_3\overset{+}{N}\dot{C}(R)COO^-} \longrightarrow \underset{\text{IV}}{H_2\overset{+}{N}{=}C(R)COO^-} + \underset{\text{I}}{H_3\overset{+}{N}CH(R)COO^-} \qquad [4]$$

$$H_2\overset{+}{N}{=}C(R)COO^- + H_2O \longrightarrow NH_4^+ + RCOCOO^- \qquad [5]$$

(Eq. [1]). The electron then attacks and cleaves another amino acid molecule (Eq. [2]), giving ammonia and a deaminated α-radical, III. The latter in turn abstracts a proton from another amino acid zwitterion to regenerate the α-radical II and produce the observed carboxylate radiolysis product (Eq. [3]). The α-radical II produced in Eqs. [1] and [3] can further disproportionate as in Eq. [4], producing an α-imino acid zwitterion, IV, (stable in the crystal lattice) and regenerating the original amino acid zwitterion, I. On solution in water, the α-imino ion IV is hydrolyzed to produce an α-keto acid (Eq. [5]), one of the observed radiolysis products. To the extent that the disproportion reaction [4] is operative as part of the overall radiolysis scheme, the regenerated zwitterion I should be totally or substantially racemic. Similarly, if the α-radical II produced in Eqs. [1] or [3] were to abstract a hydrogen atom from any other source in the crystal lattice, one would again expect the regenerated starting material I to be substantially racemized. In fact, to the extent that reaction [1] occurs, its reversal by any mechanism should produce racemized starting material.

The principal effects of ionizing radiation on the simpler amino acids in aqueous (O_2-free) solution are, as in the solid state, reductive and oxidative deamination producing, respectively, fatty acids and α-keto acids as the major radiolysis products (*37, 38*). Such

processes are believed to be initiated by the hydrated electron, e^-_{aq}, and the HO· radical, which are produced upon radiolysis of the water solvent (*3*⁻, *38*) (Eq. [6]).

$$H_2O \xrightarrow{\sim\sim\sim} e^-_{aq} + HO\cdot + H^+ + H_2 + H_2O_2 \qquad [6]$$

Garrison argues (*37*, *38*) that the hydrated electron, e^-_{aq}, may attack the substrate I to initiate deamination and produce the α-radical III, just as does the secondary electron, e_s^-, in crystalline amino acids (Eq. [2]), whereupon the subsequent reaction [3] producing the fatty acid product may ensue. In addition, the HO· radical may abstract an α-H from the zwitterion I (Eq. [7]) producing the α-radical II, which may then react

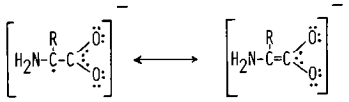
$$\underset{\text{I}}{HO\cdot + H_3\overset{+}{N}CH(R)COO^-} \longrightarrow \underset{\text{II}}{H_2O + H_3\overset{+}{N}\dot{C}(R)COO^-} \qquad [7]$$

as in Eq. [4] to yield ultimately the α-keto acid product. Clearly, the reversal of reaction (7) would provide an efficient mechanism for the racemization of zwitterion I in aqueous solution.

Our solution radiolyses were not conducted using examples of the zwitterion I as solutes, but rather their sodium salts (anion V, pH 11.2 for L-leucine) and their hydrochloride salts (cation VI, pH 1.8 for L-leucine). Tables 2 and 3 show that anions V

$$\underset{\text{V}}{H_2NCH(R)COO^-} \qquad \underset{\text{VI}}{H_3\overset{+}{N}CH(R)COOH}$$

suffered extensive radioracemization while cations VI racemized little if at all, even though each underwent roughly comparable radiolysis for roughly equivalent radiation doses. If radioracemization in aqueous medium can be achieved through formation of an α-radical by α-H abstraction (Eq. [7]), followed by reversal of the reaction, then the above results suggest either that radiolytically produced HO· radicals are not prevalent at low pH values or that the α-radical from V should be formed more readily (be more stable) than that from VI, and that the latter should form only with great difficulty. The former possibility can be eliminated, since Draganic and Draganic (*39*) have observed that there is no significant dependence of HO· yield on pH during the γ-radiolysis of water. Adams (*40*), on the other hand has pointed out that the rate constant for the reaction of and HO· radical with a given solute can be influenced by the effect of pH on the structure of the solute itself. Thus, aqueous formic acid (pH ~2) reacts much more slowly with HO· than does formate ion (pH ~6). A similar effect must clearly be responsible for the differing susceptibilities presently observed for ions V and VI toward α-H abstraction by the HO· radical. We suggest that the difference may be rationalized in terms of the enhanced stability of the highly symmetrical resonance hybrid VII of the α-radical anion obtained from V, a hybrid structure unobtainable for the α-radical cation from VI. Such an explanation may also apply to the observation in Fig. 1 that the



VII

sodium salt of leucine (V, R = isobutyl) undergoes gross radiolysis somewhat more rapidly than does the hydrochloride salt (VI, R = isobutyl). The fact that the extents of radiolysis of ions V and VI derived from the various amino acids in Tables 2 and 3 are nevertheless *roughly* comparable, however, suggests that the α-H attack by HO· radical (Eq. [7]) may be less important than the deamination attack by e^-_{aq} (cf. Eq. [2]) in initiating the gross radiolysis of leucine. A detailed mechanistic study involving careful product analysis might resolve these questions.

The racemization rates for natural amino acids under diagenetic environmental conditions are questions of considerable current interest to geochemists, paleontologists, and archeologists, since it has been widely assumed that the D/L ratios for residual amino acids isolated from paleontological or archeological specimens may provide a measure of the age of the specimen. If one assumes that all amino acids isolated from a prehistoric sample are of protein origin and were originally of the L-configuration and that the simple first-order kinetic equations applicable to racemization in solution are likewise applicable to the natural diagenetic racemization of these amino acids, it is possible, by establishing the D/L ratios for several amino acids from a sample of known age, to establish the specific rate constants for the diagenetic racemization of those amino acids. Assuming also a uniform temperature environment at the given geographical location within the geological epoch in question, one can then use the established rate constants to estimate the ages of other samples from the same area, again after simple determination of the D/L ratios for the same amino acids, isolated from the latter samples. In this manner measurements of such enantiomer ratios have been utilized during the past decade for the geochronological dating of ancient samples of geological sediments (*41*, *42*), shells (*43*), bones (*44*, *45*), teeth (*46*, *47*), and corals (*48*). Furthermore, having established the age of a prehistoric sample independently (e.g., by ^{14}C dating) and having made laboratory measurements of the temperature effects on the rates at which certain amino acids in modern bones racemize (or epimerize), Bada *et al.* (*49*, *50*) and others (*51*) have used the measurement of such enantiomer or epimer ratios to estimate the temperatures prevailing over past geological epochs, i.e., as paleotemperature indicators.

The validity of applying amino acid racemization criteria to geochronology and geothermometry has recently been critically questioned by Dungworth (*52*) and by Williams and Smith (*53*). The latter authors in particular point out the errors which can be introduced in age estimates by uncertainties in the average geological temperature of the samples, as well as by the state of the amino acids in the samples (free or bound as peptides). They also list (*53*) numerous environmental and other factors which may engender serious errors in the rate constants for diagenetic racemization, such things as the organism from which the amino acid samples originated (species effects), contamination of the samples by recent bacteria or by free amino acids introduced in ground waters, the undeterminable extents to which metal ions (particularly Cu^{2+} and Mg^{2+}) might have enhanced the racemization of the samples, and in particular, the racemizing influence of clay minerals. Kroepelin (*54*) has noted, for example, that L-leucine adsorbed on montmorillonite was catalytically racemized after heating in the dry state at 100°C for 100 hr (or 200°C for 6 hr), and one of us (*55*) has more recently observed that L-aspartic acid was racemized to the extent of 63% by mere heating to 90°C for 8 days with an aqueous suspension of kaolin.

To the above formidable list of pitfalls challenging the validity of amino acid enantiomer ratios as criteria for estimating geological ages and temperatures must now be added the possibility of radioracemization during the epoch in question. Clearly the proximity of radioactive minerals, either in an indigenous rock matrix or dissolved in underground waters, to the prehistoric amino acid or peptide samples during the geological period involved could induce indeterminate amounts of radioracemization in the samples in question, and thus suggest a spurious antiquity. The total radiation from radioactive material in the earths crust has been estimated (*56*) to be about 10^{20} cal/year, or about 0.5 rads/g of earth's crust/year. Thus the radiation doses employed in our racemization experiments above (ca. 10^8 rads) would be attainable within 200 million or so years of geological time. In addition, it seems possible that clay minerals (in conjunction with radioactive sources) might augment the effectiveness of radioracemization, as they apparently do for thermal racemization, thus shortening further the time required for the diagenetic racemization of amino acids. This possibility has not yet been investigated experimentally. Finally, from a cosmological viewpoint, the phenomenon of radioracemization may also render questionable any conclusion that a D=L composition for amino acids found in meteorites must inevitably indicate their abiotic origin.

In our original disclosures (*17, 57*) of some of the data in Tables 1–4, we pointed out that the phenomenon of radioracemization clearly jeopardizes the Vester–Ulbricht β-decay hypothesis as a viable mechanism for the abiotic origin of molecular asymmetry in nature. Dependening upon the relative rate of appearance of an enantiomeric excess in a racemate due to asymmetric radiolysis as compared to the rate of radioracemization of the excess enantiomer so produced, it is possible that in a particular case no net enantiomeric excess might acrue before gross radiolysis is complete. If we formulate the competing processes as in Eq. [8] and allow k_D to be the specific rate constant for the radiolysis of the D-enantiomer, k_L that for the L-enantiomer, and k and

$$\text{Product} \xleftarrow{k_D} \text{D} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{L} \xrightarrow{k_L} \text{Product} \qquad [8]$$

k' those for the radiation-induced inversion of the D and L enantiomers, respectively, then the rates of formation of the two enantiomers within the racemate are given by:

$$d[\text{D}]/dt = -k_D[\text{D}] - k[\text{D}] + k'[\text{L}]$$

$$d[\text{L}]/dt = -k_L[\text{L}] - k'[\text{L}] + k[\text{D}]$$

Now let us examine briefly the conditions under which these expressions will permit the formation of an excess of one enantiomer during radiolysis. If $k_D > k_L$ and (k_D and k_L) $\gg$ (k and k'), for example, then an excess of the L-enantiomer will result in the undecomposed residue before radiolysis is complete, whereas if $k_L > k_D$ the D-isomer will be left in excess. On the other hand if k_D and k_L are small compared to k and k', then the inversion processes will predominate. In the extreme, if k_D and $k_L = 0$ then $d[\text{D}]/dt = -d[\text{L}]/dt$, and no enantiomeric excess can be produced. Clearly the relative values of the several rate constants involved will determine if a significant excess of either enantiomer can be formed before gross radiolysis is complete. A similar simple

analysis indicates that comparable kinetic considerations, including competing radioracemization, apply to the β-radiation-induced asymmetric synthesis of optically active products from prochiral precursors (as opposed to asymmetric radiolysis) as a mechanism for the origin of optical activity via the Vester–Ulbricht mechanism. Finally, the phenomenon of radioracemization [as well as other types of diagenetic racemization, e.g., by the agency of clay minerals (*54*, *55*)] renders untenable the recent calculation of Keszthelyi (*58*) that the β-radiation-induced production of optically active molecules could necessarily survive racemizing influences during long geological epochs. Unfortunately, Keszthelyi's calculations are based only on the simple thermal racemization assumptions of Bada and Schroeder (*59*) and ignore the more probable and effective diagenetic racemization mechanisms involving specific ion or clay mineral catalysis and the effects of ionizing radiation.

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