by the oxygen when the methane does not contain interceptors such as propane or higher alkanes. As to the latter possibility, it may be interesting to point out that, according to Wolfgang and Pratt[47] and Wexler[48] the labeled ethyl ions give, upon neutralization, methyl and methylene radicals, according to the equation

$$C_2H_5^{*+} + e \longrightarrow CH_3^* + CH_2^* \qquad (10)$$

Both these labeled radicals could be scavenged by the oxygen present in our experiments.

It is not possible to establish the fate of the ethyl ions from our experiments. The latter indicate, however, that in presence of oxygen only a minor fraction of the ethyl ions form ethylene upon neutralization.

It is also interesting to note that the yield of CHT_3 is low in methane. This indicates that the hydride ion transfer

$$CT_3^+ + CH_4 \longrightarrow CHT_3 + CH_3^+ \qquad (11)$$

which has been found by von Koch[43] to occur in the mass spectrometer, is slow in comparison with reaction 2. This is not the case with other alkanes; in ethane, for instance, the reaction

$$C_2H_5^{*+} + C_2H_6 \longrightarrow C_2H_6^* + C_2H_5^+ \qquad (12)$$

has been shown to occur with high efficiency, owing to the lack of effective competitive processes.[32,49,50]

Conclusion

The chemical effects of the decay of a constituent atom of a tritiated methane molecule in systems at atmospheric pressure have been interpreted using the available mass spectrometric data concerning the decay-induced fragmentation of CH_3T, and taking into account the reaction of the resulting ions.

The model obtained in this way allows one to predict, with reasonable accuracy, the nature and the yields of the products formed by the decay of CT_4 in systems at 760 torr.

The results indicate that the fragmentation following the β decay of a tritium atom in a methane molecule is largely independent of the pressure and suggest that a technique based on the decay of multilabeled molecules is a valuable means to introduce into systems at high pressure, or even in the liquid state, a known amount of ions of defined nature, whose reactions can be followed through the radioactivity of the final products.

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(47) T. H. Pratt and R. Wolfgang, *J. Am. Chem. Soc.*, **83**, 10 (1961).

(48) S. Wexler, *ibid.*, **85**, 272 (1963).

(49) H. von Koch, *Arkiv Fysik*, **28**, 559 (1965).

(50) V. Aquilanti and G. G. Volpi, *J. Chem. Phys.*, in press.

Relative Reaction Rates of Hydrated Electrons with Krebs Cycle and Other Anions

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Abstract: Using the e_{aq}^-–chloroacetate reaction as a reference, competition kinetics have been employed to obtain relative reaction rates of e_{aq}^- with a number of organic anions including the di- and tricarboxylates of the Krebs citric acid cycle. The latter, arranged in the order of oxidation recognized as obtaining in mitochondria, show alternately high and low rates of reaction with e_{aq}^- over a major part and possibly the whole of the cycle, in agreement with observations in the gas phase by Lovelock. Relative rate measurements throughout the work are in general agreement with the available pulse-radiolysis data.

The hydrated electron e_{aq}^- is the simplest reducing agent. The reaction of a given compound with e_{aq}^- can unambiguously be considered as an ideal charge-transfer process whose rate is determined by the availability of a molecular orbital of sufficiently low energy to react with the hydrated electron in solution.[1] The basic chemical properties of a compound can therefore be examined by studying its reactivity with e_{aq}^-. Since hydrated electrons represent one of the chief links between the purely physical and the slower chemical processes involved in the absorption of the energy of ionizing radiation in living matter, it is also important to know both the rate at which they react with biochemicals and the nature of the products formed. Further, a knowledge of e_{aq}^- reactions may yield information which will help to explain certain biological processes in which free electrons are thought to play an important part (photosynthesis,[2,3] transfer of energy in mitochondria[4–6]).

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(1) M. Anbar and E. J. Hart, *J. Am. Chem. Soc.*, **86**, 5633 (1964).

(2) D. F. Bradley and M. Calvin, *Proc. U. S. Natl. Acad. Sci.*, **42**, 710 (1956).

(3) J. R. Platt, *Science*, **129**, 372 (1959).

(4) D. E. Green, *Discussions Faraday Soc.*, **27**, 206 (1959).

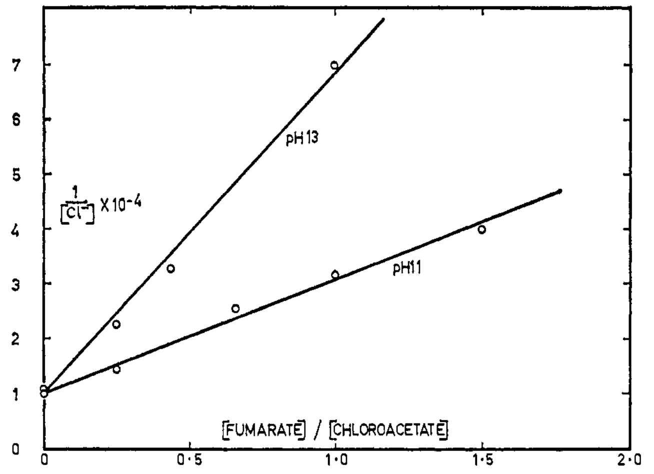

Figure 1. Chloride ion yields in 35-krad irradiation of deaerated 0.01 *M* fumarate–chloroacetate mixtures.

From gas phase measurements of the attachment of low-energy electrons to the volatile ethyl esters of some of the carboxylic acids, Lovelock[7] has suggested that an ordered series of reversible electron traps may operate round the Krebs cycle yielding a net electron transfer. Stockdale, Hurst, and Christophorou[8] have criticized the physical basis of these measurements on the ground that comparisons made between compounds may correspond to electron attachment at differing energies. However, Lovelock's results, showing a difference of the order of 10^5 in the "thermal electron absorption coefficient"[9] of alternate compounds in the cycle, continued to suggest that these acids and their close derivatives might fall into two clear classes on the basis of their rates of reaction with thermal electrons in the gas phase.

As part of a program concerned with the action of radiation on various systems, we have examined e_{aq}^- reaction rates with the Krebs cycle acids. For some of these, the absolute rates of reaction with hydrated electrons have been published.[10,11] It was necessary to find a method of determining the others as relative rate constants. Matthews and Sangster[12] have used competition kinetics to determine ·OH radical relative reaction rates. For electron rates, chloroacetic acid was used as the reference compound because in deoxygenated alkaline solution with methanol present as hydroxyl radical and hydrogen atom scavenger, the chloride produced appears to be specific for hydrated electron reaction.[13] Chloride can be readily estimated with a silver–silver chloride electrode. The method was checked by making measurements on compounds whose reaction rates had been determined by pulse radiolysis.

Experimental Section

Solutions and Irradiation. Triple-distilled water, reagent grade chemicals, and low-carbonate sodium hydroxide solution were used throughout. Chloroacetic, citric, succinic, benzoic, and salicylic acids were BDH AR grade; sodium pyruvate, sodium biphthalate, fumaric, malic, α-oxoglutaric, *p*-hydroxybenzoic acids, acrylamide, and *m*-nitrophenol were BDH laboratory reagent grade; *cis*-aconitic acid, trisodium DL-isocitrate, and oxalacetic acids were Fluka Puriss chemicals. Competitor concentration varied over the range 0–0.01 *M* for the less reactive and 0–0.001 *M* for the more reactive compounds, generally in mixtures with 0.001 *M* chloroacetate. For highly reactive compounds such as *m*-nitrophenol, the chloroacetate concentration was increased to 0.01 *M*. The pH was adjusted by adding enough sodium hydroxide to neutralize the acids present and the excess required to make 0.001 (pH 11) or 0.1 *M* (pH 13). Enough degassed methanol was added to make the solution 0.04 *M*. The Argonne degassing flask and syringe technique[10] was used for the preparation of solutions, and irradiations were carried out in the syringes. Dry cylinder nitrogen was used as the inert gas.

Irradiations were carried out at ambient temperature (18–25°), either in a cobalt-60 γ-ray source or in a pond containing spent reactor fuel elements. No differences were observed between irradiations carried out in the two facilities. In each case a dose of 35 krads was delivered at a dose rate of 3 krads/min and was measured by the Fricke ferrous sulfate dosimeter taking $G_{Fe^{3+}}$ as 15.5.

Chloride Estimation. Chloride was estimated after adding nitric acid to make the solution approximately 0.01 *N*. An electrode of silver wire was coated with silver chloride by making it the anode in a strong potassium chloride solution for a few minutes. The deposit was washed thoroughly in demineralized water and calibrated using chloride solutions of known strengths. The potential against a saturated potassium chloride–calomel–mercury electrode and concentrated potassium nitrate bridge was measured by a Radiometer pH meter Model 4. When the value for the blank was subtracted the points lay on a straight line, the slope, and voltage agreeing with theoretical and tabulated data. (The blank chloride concentration $\approx 10^{-5}$ *M* was rather higher than desirable. Distillation of the nitric acid over silver nitrate failed to improve this value.)

Results

It was found in preliminary experiments that $G_{Cl^-} = 2.9 \pm 0.2$ at both pH 11 and 13 and was independent of methanol concentration over the range 20–100 m*M*, of dose over the range 30–70 krads, and of chloroacetate concentration in the range 0.01–0.001 *M*.

In the presence of known hydrated electron scavengers the chloride yield decreased. The reciprocal of chloride concentration was plotted for each competitor against the ratio of the concentration of competitor to the concentration of chloroacetate. The ratio of the slope to the intercept on the $1/(Cl^-)$ axis gives the rate of reaction of the electron–competitor relative to that of electron–chloroacetate. Figure 1 shows typical results for fumarate as competitor at pH 11 and 13.

Relative rates obtained are listed in Table I. The carboxylates of the Krebs cycle, pyruvate to oxalacetate, are given in their accepted order of biological oxidation.[6] From repeated measurements on individual compounds, it is estimated that the figures listed are accurate to ±20%. For comparison, the values given in the literature for absolute reaction rates at pH 11 determined by pulse radiolysis have been divided by the only published absolute rate for the e_{aq}^-–chloroacetate reaction, 1.2×10^9 mole^{-1} sec^{-1} at pH 11, given by Anbar and Hart.[14] Absolute rates at pH 13 have been divided by

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(5) P. George and J. S. Griffith in "The Enzymes," P. D. Boyd, *et al.*, Ed., Academic Press Inc., New York, N. Y., 1959, p 397.

(6) A. L. Lehninger, "The Mitochondrion," W. A. Benjamin Inc., New York, N. Y., 1964, Chapters 3 and 6.

(7) J. E. Lovelock, *Nature*, **189**, 729 (1961).

(8) J. A. Stockdale, G. S. Hurst, and L. G. Christophorou, *ibid.*, **202**, 459 (1964); **203**, 1267 (1964).

(9) J. E. Lovelock, A. Zlatkis, and R. S. Becker, *ibid.*, **193**, 540 (1962).

(10) E. J. Hart, S. Gordon, and J. K. Thomas, *J. Phys. Chem.*, **68**, 1271 (1964).

(11) E. J. Hart, J. K. Thomas, and S. Gordon, *Radiation Res.*, *Suppl.*, **4**, 74 (1964).

(12) R. W. Matthews and D. F. Sangster, *J. Phys. Chem.*, **69**, 1938 (1965).

(13) E. Hayon and J. Weiss, *Proc. Intern. Conf. Peaceful Uses At. Energy, Geneva*, **29**, 80 (1958); E. Hayon and A. O. Allen, *J. Phys. Chem.*, **65**, 2181 (1961).

(14) M. Anbar and E. J. Hart, *ibid.*, **69**, 271 (1965).

Table I. Reaction Rates with e_{aq}^-

	Rel rates $k(e_{aq}^-$–competitor$)/k(e_{aq}^-$–chloroacetate$)$				Gas phase electron absorption[c] coefficient (for esters) divided by 570
	This work		Pulse radiolysis[a]		
Competitor	pH 11	pH 13	pH 11	pH 13	
Benzoate	2.2	2.6	2.6[b]		
Phthalate	1.4	1.3	1.0[f] (pH 6.8)	1.4[f]	
Cinnamate			5.7[f] (pH 7.2)	7.3[f] (pH 12.5)	
Trimesate			2.5[f] (pH 8.8)	3.2[f] (pH 12.4)	
p-Hydroxybenzoate	0.21		0.33[b]		
Salicylate	2.8		2.7[b]		
Acrylamide	0.19		15[d] (pH 7)		
n-Nitrophenol	14		21[b]		
Krebs cycle					
Pyruvate	2.2	4.5		5.1[d]	3.0
Citrate	0.06		0.01[e] (–)		
cis-Aconitate	0.18	0.36			
Isocitrate	0.02	0.02			
α-Oxoglutarate		6.1			
Succinate	0.02		0.01[e] (–)		2×10^{-5}
Fumarate	2.1	5.7		5.6[d]	2.6
Malate	0.05				2×10^{-5}
Oxalacetate	3.6	6.1			2.3

[a] Absolute reaction rates $k(e_{aq}^-$–competitor$)$ have been divided by 1.2×10^9 mole^{-1} sec^{-1} in the pH 11 column, but by 1.3×10^9 mole^{-1} sec^{-1} in the pH 13 column. [b] See ref 1. [c] See ref 7. [d] See ref 10. [e] See ref 11. [f] A. Szutka, J. K. Thomas, S. Gordon, and E. J. Hart, *J. Phys. Chem.*, **69**, 289 (1965).

the slightly higher figure of 1.3×10^9 mole^{-1} sec^{-1}. This figure was obtained by dividing the value 3.4×10^9 mole^{-1} sec^{-1} interpolated from published data[15] for the benzoate–electron absolute reaction rate by our experimental figure 2.6 for the relative rate. The relative values given by Lovelock for esters in the gas phase have been divided by 570 (chosen to normalize the figures of Lovelock to our relative rates at pH 11) for easy comparison and are given in the last column.

Discussion

Test of Method. From the published rate data,[14] it can be calculated that the other primary species, hydroxyl radicals and hydrogen atoms, react with 0.04 *M* methanol rather than with 0.001 *M* chloroacetate. This is confirmed by the chloride ion yields being independent of methanol concentration. Chloride ion production is thus specific for the chloroacetate–hydrated electron reaction, and the competitor studies give relative rates for hydrated electron reactions.

Table I shows that at pH 11 our relative rates for phthalate, benzoate, *p*-hydroxybenzoate, salicylate, and *m*-nitrophenol agree reasonably well with the estimated relative rates from pulse radiolysis, bearing in mind that the combined errors of the two experiments might account for differences of about 40%, or more in the case of relatively low reaction rates such as that for *p*-hydroxybenzoate.

Comparison between rates at pH 13 (pyruvate, fumarate, phthalate, and benzoate) also show reasonably good agreement. The relative rate we have obtained for acrylamide (0.19) is very much less than the pulse radiolysis estimate (14). This discrepancy is very likely due to polymerization of the acrylamide at the dose (35 krads) which we have used resulting in a decrease in monomer concentration and apparent reduction in rate. Armstrong, *et al.*,[16] have observed an analogous effect in H_2 yields from dilute deaerated acrylamide solutions irradiated with Co-60 γ rays at doses greater than 5 krads. Lower doses and much shorter irradiation times in pulse radiolysis experiments would preclude any polymerization effect.

Value of $G_{e_{aq}^-}$. All substances irradiated at both pH 11 and 13 produced straight-line graphs similar to Figure 1. Other measurements, which are not described in this paper, were made with various concentrations of the inert salts sodium sulfate and sodium perchlorate added to the sodium benzoate–chloroacetate–hydroxide–methanol deoxygenated aqueous system. The figure, $G_{Cl^-} = 2.9 \pm 0.2$, obtained by extrapolating these lines to zero competitor concentration, is the same as that resulting from irradiation of competitor-free deaerated 0.001 *M* chloroacetate aqueous solutions at pH 11 or pH 13 in the presence of 0.04 *M* methanol. These data imply that there is only a fairly small ($\approx 10\%$) increase in $G_{e_{aq}^-}$, if any, over this pH range, since under the conditions used it would be expected that $G_{Cl^-} = G_{e_{aq}^-}$. This constant G value is not in agreement with pulse radiolysis measurements of Dainton and coworkers[17] who report a 35% increase in $G \times \epsilon$ from pH 10 to pH 13 which corresponds to the increase in measured

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(15) M. Anbar and P. Neta, *Intern. J. Appl. Radiation Isotopes*, **16**, 227 (1965).

(16) D. Armstrong, E. Collinson, and F. S. Dainton, *Proc. Intern. Conf. Peaceful Uses At. Energy, Geneva*, **29**, 85 (1958).

(17) F. S. Dainton and W. S. Watt, *Proc. Roy. Soc.* (London), **A275**, 447 (1963); D. M. Brown, F. S. Dainton, J. P. Keene, and D. C. Walker, *Proc. Chem. Soc.*, 267 (1964); G. V. Burton and F. S. Dainton, *Proc. Roy. Soc.* (London), **A287**, 427 (1965).

reducing radical yield from 2.85 at pH 10.8 to 3.55 at pH 12.8 (ϵ being the extinction coefficient for e_{aq}^- absorption at 700 mμ). They ascribed this increase to the reaction

$$OH^- + H_2O^* \longrightarrow e_{aq}^- + \cdot OH + H_2O$$

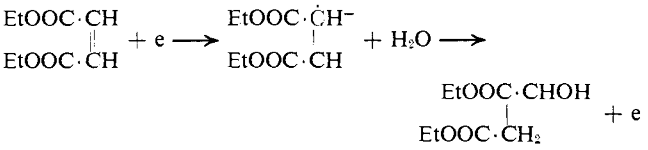
(* indicating an excited state or a radical pair H–OH). Cheek and Linnenboom,[18] however, in their studies on hypobromite found no increase.

According to the published rate constants,[15] one would expect most of the primary hydrogen atoms to react in solutions at pH 13 with hydroxyl ions giving more hydrated electrons than are found at pH 11. Jortner and Rabani[19] found such an increase when studying the reactions of hydrogen atoms generated in an electrodeless discharge and passed into alkaline chloroacetate, but no increase was observed in the present experiments.

Krebs Cycle Anions. Lovelock[7] has pointed out that alternate members of the Krebs cycle have one or other of the two electrophores –CO·CO– and –CO·CH::CH·CO–, and, in the gas phase, react readily with the electrons present under his experimental conditions. The remaining intermediates do not react readily. The figures for e_{aq}^- relative reaction rates listed in Table I show general agreement with this picture. *cis*-Aconitate shows an intermediate reactivity, although from its structure it would be expected to react readily with electrons. This anomaly is probably due to a polymerization effect similar to that mentioned in the case of acrylamide earlier in this paper. Fumarate, of similar structure to *cis*-aconitate but with a symmetrical double bond, apparently does not polymerize significantly under our conditions, since the relative rate obtained agrees with the estimated pulse-radiolysis figure. However, polymerization has been observed when the ester is irradiated in the liquid organic phase.[20]

Consideration of other compounds, such as acetone, styrene, and butadiene, shows that conjugated carboxyl groups and double bonds react readily with hydrated electrons, and for the aqueous phase it is not necessary to invoke the rather more complex electrophores. (However, they may be required in the explanation of measurements in the gas phase; acetone, for instance, though reacting readily with e_{aq}^- does not capture thermal or near-thermal electrons readily in the gas phase.[21]) The difference in reaction rate of the two classes, high and low reactivity, in the aqueous phase is approximately a factor of 100, that is, much less than the 10^5 reported in the gas phase measurements. However, the figures listed in Table I for some of the less reactive anions, notably isocitrate and succinate, may represent upper limits (owing to the possible presence of small amounts of contamination in the irradiated samples) rather than accurate reaction rates; nevertheless, it is unlikely that in aqueous solution the difference in reaction rate would be as great as 10^5.

The enzyme-catalyzed interconversions of the Krebs cycle acids are not understood in detail, though the over-all process is relatively well known. The conversion of citric acid to isocitric, for instance, may be a molecular rearrangement proceeding *via* a three-point attachment to the enzyme[22] and not involving *cis*-aconitate as a direct intermediate. Similarly, there has been some controversy over the simultaneous dehydrogenation and decarboxylation of isocitrate to α-ketoglutarate, the enzyme for the probable major pathway in mitochondria showing the capacity to decarboxylate or reduce added oxalosuccinate, although this possible intermediate has not been found.[6] The removal of *cis*-aconitate from Table I or the addition of oxalosuccinate would change the simple picture of alternates showing high reactivity with electrons; however, considerable regularity in this regard would still remain in the cycle ordering. (The rearrangement step citrate to isocitrate might be regarded as a single "unit" of low reactivity, and it should be noted that pyruvate is normally related to the cycle through external enzyme paths[6] and does not directly enter the cyclic order.) Hydrated electron reactions, perhaps involving subsequent rapid protonation (as in e_{aq}^- reactions with benzoate[23]), enzyme-catalyzed direct hydrogen atom transfer (as in reduction of the pyridino proteins of the respiratory chain[24]), enzyme-catalyzed molecular rearrangement, or combinations of these may be involved in the Krebs cycle. Beyond showing that the carboxylates fall into two classes on the basis of their reaction rates with e_{aq}^-, the results reported here may be of some interest in connection with radiation action, and the work will be extended by examining product yields in reactions of the carboxylic acids with e_{aq}^-. Of particular interest is Lovelock's recent observation[25] that thermal or near thermal electrons from a point discharge react with water-saturated diethyl fumarate in a liquid organic phase by a chain reaction, about 30 molecules being transformed to succinate, malate, and oxalacetate for every electron added to the liquid. He suggests the following mechanism.



$$\begin{array}{c}\text{EtOOC}\cdot\text{CH}\\ \| \\ \text{EtOOC}\cdot\text{CH}\end{array} + e \longrightarrow \begin{array}{c}\text{EtOOC}\cdot\dot{\text{C}}\text{H}^-\\ | \\ \text{EtOOC}\cdot\text{CH}\end{array} + H_2O \longrightarrow \begin{array}{c}\text{EtOOC}\cdot\text{CHOH}\\ | \\ \text{EtOOC}\cdot\text{CH}_2\end{array} + e$$

The diethyl fumarate–electron adduct undergoes hydration with the small amount of water present, and the product (diethyl malate) gives up the electron which is then available for further reaction, an electron chain reaction. Recently evidence has been published that electron transfer can occur in an organic phase from an adduct to another molecule with greater electron affinity.[26] If similar reactions had occurred in our dilute aqueous solutions, this would have been reflected in a decreased slope and a low apparent reaction rate.[27] The rate found is in good agreement with that found in pulse radiolysis and for similar compounds. This indicates that a chain reaction does not occur in aqueous solution under our experimental conditions.

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[18] C. H. Cheek and V. J. Linnenboom, *J. Phys. Chem.*, **67**, 1856 (1963).

[19] J. Jortner and J. Rabani, *ibid.*, **66**, 2078, 2081 (1962).

[20] J. Nosworthy, *Trans. Faraday Soc.*, **61**, 1138 (1965); A. Orszagh and H. Czarnadola, *Nukleonika*, **10**, 177 (1965).

[21] A. Zlatkis and J. E. Lovelock, *Clin. Chem. Suppl.*, [2] 11, 259 (1965); G. S. Hurst, J. A. Stockdale, and L. B. O'Kelly, unpublished data.

[22] D. D. Davies, J. Giovanelli, and T. Ap. Rees, "Plant Biochemistry," Blackwell, Oxford, 1964, Chapter 4.

[23] D. F. Sangster, *J. Phys. Chem.*, **70**, 1712 (1966).

[24] B. Vennesland, *Federation Proc.*, 17, 1150 (1958).

[25] J. E. Lovelock, *Nature*, **195**, 488 (1962).

[26] J. M. Fritsch, T. P. Layloff, and R. N. Adams, *J. Am. Chem. Soc.*, **87**, 1724 (1965).

[27] R. W. Matthews and D. F. Sangster, AAEC/TM282, 1965.