We gratefully acknowledge a research grant from the Department of Scientific and Industrial Research for the purchase of an infrared spectrophotometer. P. B. D. thanks the Medical Research Council for a research studentship.

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Photoregeneration of Faded Alkali Metal Solutions[1]

Sir:

Solutions of alkali metals in ammonia or amines decompose to form the corresponding amides. We have found that illumination of such faded solutions regenerates the metal or solvated electron components characteristic of the original metal–solvent combination. In addition to its general interest in connection with the fundamental properties of these systems, the regeneration reaction affords a convenient method for the preparation of metal solutions of controlled concentration, particularly in amine solvents.

In ethylamine solution, the absorption spectra of potassium or rubidium exhibit two bands near 650 and 850 mμ,[2,3] which we have previously shown to be due, respectively, to monomer and dimer species.[2] The 650-mμ band of potassium is associated with an e.s.r. spectrum having a characteristic hyperfine pattern.[2] Illumination of faded potassium or rubidium ethylamine solutions in the amide absorption region (~315 mμ) regenerates the original optical bands, as shown in Figure 1a,b. The e.s.r. patterns of the regenerated potassium solutions are likewise identical with the original spectra, and the fading rates are also similar. Repeating the cycle of fading and photorecovery gradually develops a strong absorption at 265 mμ, presumably due to some irreversible reaction associated with the process. This absorption does not correspond to *sym*-diethylhydrazine.

In ammonia, the photoregeneration of potassium solutions shows two phases, which are conveniently studied by flash technique, using apparatus previously described.[4] Flashing potassium amide solutions in ammonia, in the region 240–390 mμ, produces an infrared transient whose absorption spectrum (at least as far as 1000 mμ) closely resembles the solvated electron band of metal–ammonia systems.[5] Most of this transient disappears rapidly ($\tau \approx 40$ μsec.), but a residual absorption, having the same spectrum, persists for as long as a minute (Figure 1c).

Under the conditions of these experiments, the metal amides in ethylamine solution are probably highly ion-paired, and it appears that the relevant reactions are

$$(M^+)(Et\text{-}NH^-) \xrightarrow{h\nu} M + Et\text{-}NH\cdot$$

$$Et\text{-}NH\cdot + \text{solvent} \longrightarrow \text{products (265 m}\mu\text{)}$$

(1) This work was supported by a grant from the U. S. Atomic Energy Commission to Brandeis University (Grant No. AT(30-1)-2003).
(2) M. Ottolenghi, K. Bar-Eli, H. Linschitz, and T. Tuttle, *J. Chem. Phys.*, 40, 3729 (1964).
(3) R. R. Dewald and J. L. Dye, *J. Phys. Chem.*, **68**, 121 (1964).
(4) H. Linschitz, C. Steel, and J. A. Bell, *ibid.*, **66**, 2574 (1962).
(5) "Metal-Ammonia Solutions," G. Lepoutre and M. J. Sienko, Ed., Benjamin Publishing Co., New York, N. Y., 1963.

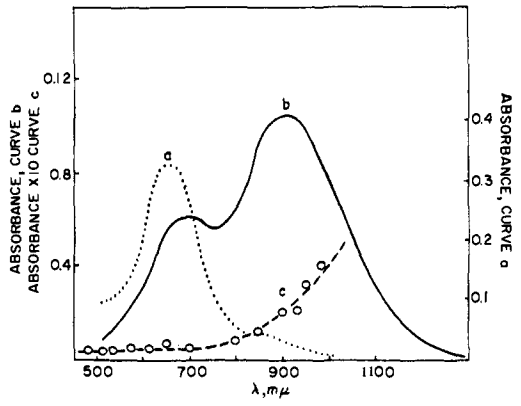

Figure 1. Photoregeneration of decomposed metal solutions (Hanovia Type HS (150 w.) quartz mercury arc, 10 cm. from sample): (a) absorption spectrum of decomposed potassium ethylamine solution (amide ~ 10^{-3} *M*), 5-min. irradiation, 1-cm. Pyrex cell, −78°; (b) decomposed rubidium ethylamine solution (amide ~ 10^{-3} *M*), 7-min. irradiation, 1-cm. quartz cell, room temperature; (c) flash transient spectrum, potassium amide in ammonia (~10^{-4} *M*), time after flash, 20 μsec., 5-cm. cell, −75°.

In ammonia, where ion-unpairing is more extensive,[6] it is possible that charge transfer occurs from excited amide ions directly to solvent to yield solvated electrons. This is evidently closely related to the formation of hydrated electrons by photoionization of negative ions in water.[7]

Quantitative aspects of this work will be presented shortly.

(6) W. H. Hawes, *J. Am. Chem. Soc.*, **55**, 4422 (1933).
(7) M. Matheson, W. A. Mulac, and J. Rabani, *J. Phys. Chem.*, **67**, 2613 (1963).

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The Catalytic Versatility of Carbonic Anhydrase from Erythrocytes. The Enzyme-Catalyzed Hydration of Acetaldehyde

Sir:

Although several workers have studied the catalytic effect of erythrocyte carbonic anhydrase (EC4.2.1.1) upon the reversible hydration of carbon dioxide,[1a-f] the accelerative effect of this enyzme in the hydration of other carbonyl systems does not appear to have been considered. *We have recently established that the catalytic effect of carbonic anhydrase is not limited to CO_2 hydration, but that the enzyme very powerfully and reversibly catalyzes the hydration of acetaldehyde and many other related carbonyl systems.* The hydration of acetaldehyde was studied in the ultraviolet by following the decrease in the carbonyl band at 278 mμ (ε 16.8). For the determination of the catalytic constant k_{enz} the initial concentration of acetaldehyde

(1) (a) F. J. W. Roughton and V. H. Booth, *Biochem. J.*, **40**, 319 (1946). (b) R. P. Davis, *J. Am. Chem. Soc.*, **80**, 5209 (1958); **81**, 5674 (1959). (c) H. De Voe and B. G. Kistiakowsky, *ibid.*, **83**, 274 (1961). (d) J. C. Kernohan, *Biochim. Biophys. Acta*, **81**, 346 (1964). In this paper imidazole buffers were employed but the rapid and reversible carbamate formation prior to hydration appears to have been ignored. (e) B. H. Gibbons, and J. T. Edsall, *J. Biol. Chem.*, **239**, 2539 (1964). (f) S. Lindskog and B. G. Malmstrom, *ibid.*, **237**, 1129 (1962).

Table I. Catalytic Constants $k_c = k_f + k_r$ (l. mole^{-1} min.$^{-1}$) for the Reversible Hydration of Acetaldehyde at 0.0° in H_2O and D_2O

Catalyst	$k_c^{H_2O}$	$k_c^{D_2O}$	$k_c^{H_2O}/k_c^{D_2O}$
Imidazole[a]	3.80	1.35	2.8[b]
Imidazolium ion	0.74		
Zn^{2+} in acetate buffer[c]	6.5 (pH 6.69)		
Zn^{2+} in imidazole buffer[d,e]	130 (pH 7.63)	88 (pD 8.16)	1.5[g]
Bovine carbonic anhydrase	16,000 (pH 5.64)	11,600 (pD 6.19)	1.4[g]
	25,000 (pH 6.09)	15,850 (pD 6.64)	1.6[g]
	38,000 (pH 6.50)	23,700 (pD 7.05)	1.6[g]
	41,000 (pH 6.59)	25,400 (pD 7.14)	1.6[g]
	62,400 (pH 7.15)		
	72,500 (pH 7.42)	50,500 (pD 7.97)	1.4[g]
	84,000 (pH 7.91)	55,000 (pD 8.46)	1.5[g]
Human carbonic anhydrase B[f]	11,300 (pH 7.15)		
Human carbonic anhydrase C[f]	45,200 (pH 7.15)		

[a] We were able to show that the rate of formation of the acetaldehyde–imidazole complex is appreciably faster than the hydration, so much so that the rates of hydration are determined after the initial equilibrium between free acetaldehyde and imidazole has been established: K = [A][Im]/[A·Im] = 7.6 mole l.$^{-1}$. [b] The relative rates of hydration in H_2O and D_2O for AcOH and AcO^- are 2.8 and 2.5, respectively (ref. 2b). [c] Probably refers to the catalytic activity of hydrated zinc ions. [d] We have determined that each Zn^{2+} binds four imidazoles; *cf.* also J. T. Edsall, G. Felsenfeld, D. W. S. Goodman, and F. R. N. Gurd, *J. Am. Chem. Soc.*, **76**, 3054 (1954). [e] We have shown that the catalytic constant of zinc ions dissolved in malonate buffers (pH 7.5) is also low. [f] We are indebted to Professor J. T. Edsall and Dr. J. Armstrong for supplying us with samples of human carbonic anhydrase B and C. Although the enzyme samples lost some of their original activity during transportation, as redetermined by a Wilbur and Anderson assay,[1e] we found the human enzymes to be highly effective catalysts for the hydration of acetaldehyde. [g] Reactions in H_2O *vs.* D_2O are compared in buffers with the same acid:base ratio.

throughout each series of runs was kept constant at about 0.0356 *M*. The hydration process is reversible, the fraction of hydration at 0.0° being approximately 0.70 in H_2O and 0.73 in D_2O. The reaction follows good pseudo-first-order kinetics, the rate coefficients of which refer to the sum of forward and reverse rate constants: $k_c = k_f + k_r$.

$$CH_3CHO + H_2O \rightleftharpoons CH_3CH(OH)_2;\ K_{H_2O} = [CH_3CH(OH)_2]/[CH_3CHO] = 2.3$$

$$CH_3CHO + D_2O \rightleftharpoons CH_3CH(OD_2);\ K_{D_2O} = [CH_3CH(OD)_2]/[CH_3CHO] = 2.7$$

The buffer for all experimental mixtures containing enzyme was 0.002 *M* in phosphate. The hydration of acetaldehyde is general-acid–general-base catalyzed,[2]

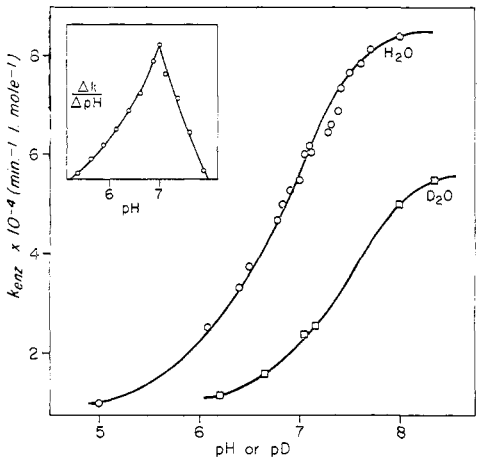

Figure 1. The bovine carbonic anhydrase catalyzed hydration of acetaldehyde in H_2O and D_2O.

and the various acids and bases present in phosphate buffer solutions catalyze the reaction independently.

$$k_{obsd} = k_{H_2O} + k_{OH^-}[OH^-] + k_{H_3O^+}[H_3O^+] + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] + k_{enz}[E]$$

Using catalytic amounts of bovine carbonic anhydrase several runs were made at constant pH, varying only the concentration of enzyme. A plot of k_{obsd} *vs.* enzyme concentration at a given pH gives a straight line[3] the slope of which we define as k_{enz}. With bovine carbonic anhydrase this procedure was followed at 20 different pH (and 6 different pD) values (Figure 1). The resulting pH–rate profile reveals a point of inflection at pH 7.0[4] strongly suggesting that histidine residues play an important role in the catalytic activity of the enzyme.

Since our pH- and pD–rate profiles suggest the catalytic importance of histidine residues in the enzyme, and earlier work[1f] indicates that the zinc ion associated with each molecule of carbonic anhydrase is an obligatory component for its catalytic activity, hydration experiments were carried out involving the use of imidazole, its conjugate acid, and zinc ions as catalysts. We have established that although catalysis by imidazole and its conjugate acid is moderate and that catalysis by zinc ions in acetate buffers (pH 6.69) and in malonate buffers (pH 7.5) is also mild, enhanced catalysis is observed when zinc ions are introduced into imidazole buffers (Table I). The similarity of D_2O kinetic solvent isotope effects ($k_c^{H_2O}/k_c^{D_2O}$) obtained for the enzymatically catalyzed hydration of acetaldehyde with that obtained for the zinc-imidazole complex is in accord with our suggestion that the active site of bovine carbonic anhydrase consists, at least in part, of a dynamic equilibrium between protein-bound zinc, one or more imidazole residues, and a water molecule, and also with our view that *hydrated* carbonic anhydrase acts both as a *general-acid–base and a nucleophilic catalyst.* This suggestion is in accord with the principle of microscopic reversibility with respect

(2) (a) R. P. Bell and B. de B. Darwent, *Trans. Faraday Soc.*, **46**, 34 (1950); R. P. Bell and J. C. Clunie, *ibid.*, **48**, 439 (1952); *Proc. Roy. Soc.* (London), **A212**, 33 (1952); (b) Y. Pocker, *Proc. Chem. Soc.*, 17, (1960).

(3) The reaction follows Michaelis–Menten kinetics and we purposely chose a concentration of substrate and a concentration range of enzyme such that k_{enz} is a linear function of [E].

(4) In addition to the major point of inflection (pH 7.0) a small perturbation is observed in the region pH 7.1–7.4.

to both enzyme-catalyzed hydration of CH_3CHO and dehydration of $CH_3CH(OH)_2$.

Acknowledgment. This investigation was supported by Public Health Service Research Grant GM 10181 from the National Institutes of Health.

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The Mechanism of Oxidative Decarboxylation with Lead(IV) Acetate

Sir:

Recently there has been increased discussion[1] concerning the mechanism of lead tetraacetate oxidation[2] of organic compounds, particularly oxygenated species. The conversion of Pb(IV) to Pb(II) has often been postulated to involve a direct 2-equiv. transformation with concomitant formation of carbonium ion or cationic intermediates. The case for the participation of free radicals has been made, although definitive studies are lacking.[3] We wish to present evidence that the *oxidative decarboxylation of carboxylic acids with lead(IV) acetate proceeds via a free radical chain process.*

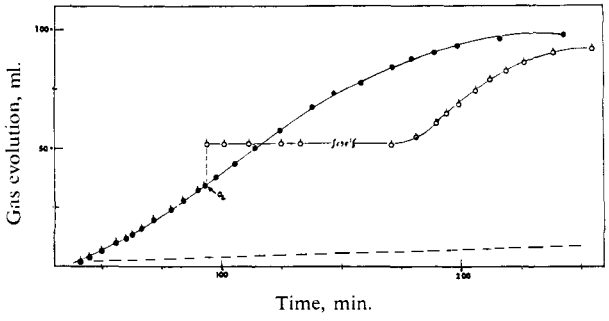

Figure 1. Decomposition of valeric acid with 0.2 *M* lead(IV) acetate in benzene at 81° (---------). Catalysis by pyridine (●). Inhibition by oxygen (♁).

The decarboxylation of aliphatic acids[4] is generally slow in refluxing benzene, but is catalyzed by pyridine.[5] Under both conditions, *n*-valeric acid is decomposed at 81° to butane, butene-1, carbon dioxide, *n*-butylbenzene, *sec*-butyl acetate, and valerate. The onset of reaction is characterized by an induction period which is drastically shortened by degassing. The rate of gas evolution in the presence of pyridine follows a typical sigmoid curve as shown in Figure 1. If oxygen is introduced at an intermediate point, the reaction stops abruptly and resumes spontaneously after an inhibition period (proportional to the oxygen present) or sooner, if oxygen is removed by flushing..

The decarboxylation also proceeds readily at 81° in the absence of pyridine if a catalytic amount of cupric salt is added and the oxygen scrupulously removed (Figure 2). The rapid decarboxylation under these conditions can be instantaneously interrupted with oxygen, and remains completely inhibited for prolonged (and indefinite) periods of time and not until the oxygen is removed, at which time it continues in a manner previous to its inhibition (Figure 2).

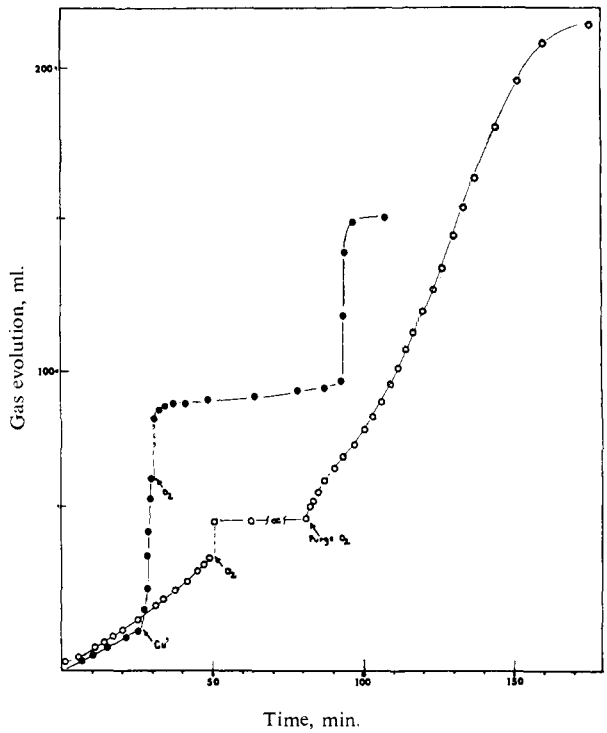

Figure 2. Decomposition of valeric acid with 0.2 *M* lead(IV) acetate catalyzed by copper salts. O, No pyridine, 0.045 *M* copper(II) acetate, inhibition by oxygen. ●, With pyridine, catalysis by 3.7×10^{-4} *M* copper(I) acetate, inhibited by oxygen.

The rate of decarboxylation of valeric acid by Pb(IV) in the presence of pyridine is additionally catalyzed in a remarkable manner by copper acetate as shown in Figure 2. However rapid this reaction is, it is strongly inhibited by oxygen (Figure 2), and remains quiescent until the added oxygen is consumed, and then resumes abruptly. The products of decomposition in the presence of copper salts are independent of the pyridine and the stoichiometry is given by eq. 1. No significant amounts of esters or butylbenzenes are formed under these conditions. A similar behavior is shown by iso-

$$CH_3CH_2CH_2CH_2CO_2H + Pb^{IV}(OAc)_4 \xrightarrow{CuX_n} CH_3CH_2CH{=}CH_2 + Pb^{II}(OAc)_2 + 2HOAc + CO_2 \quad (1)$$

valeric and 2-methylbutyric acids. The latter, being secondary, is less sensitive to oxygen effects and the length of inhibition (not retardation) is shorter. Moreover, the copper-catalyzed decarboxylation of the

(1) For leading recent references see: W. H. Starnes, Jr., *J. Am. Chem. Soc.*, **86**, 5603 (1964); S. Moon and J. M. Lodge, *J. Org. Chem.*, **29**, 3453 (1964); D. Hauser, K. Kalvoda, H. Heusler, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **47**, 1883, 1961 (1964).

(2) R. Criegee, *Angew. Chem.*, **70**, 173 (1958).

(3) Nuclear methylation and phenylation of aromatic compounds with lead(IV) acetate and benzoate have been noted (L. F. Fieser, *et al.*, *J. Am. Chem. Soc.*, **64**, 2043, 2052 (1942); D. H. Hey, *et al.*, *J. Chem. Soc.*, 2747 (1954); 3963 (1955); D. I. Davies, *ibid.*, 2351 (1963)]. However their significance with respect to oxidative decarboxylations in general[1,2,4] has not been delineated.

(4) W. A. Mosher and C. L. Kehr, *J. Am. Chem. Soc.*, **75**, 3172 (1953); D. Benson, L. Sutcliffe, and J. Walkley, *ibid.*, **81**, 4488 (1959); E. J. Corey and J. Casanova, Jr., *ibid.*, **85**, 165 (1963).

(5) C. A. Grob, M. Ohta, and A. Weiss, *Angew. Chem.*, 70, 343 (1958).