The effect of placing electronegative substituents in the aliphatic portion of alkyl azides appears quite reasonable. By simple inductive means, they would tend to lower the energy of the electrons on N_1, and thus increase the energies of both transitions. These are exactly the effects seen in the data of Tables II and III. According to this, the effect should fall off rapidly with the distance of separation of the electronegative group and N_1.

Acknowledgments.—Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. The authors thank Professor E. M. Kosower for the loan of some spectral equipment and for interesting and encouraging discussion.

Experimental

Solvents.—All solvents were of spectra-grade quality, or better. The 2,2,3,3-tetrafluoropropanol was received as gift from Dr. E. O. Langerak of the Organic Chemical Department, E. I. du Pont de Nemours and Company, and was purified according to the procedure of Kosower and Huang.[6b]

Measurements.—The low energy transitions of the alkyl azides were measured with a Cary recording spectrophotometer, Model 14, using matched 1.0-cm. cells. In general, the maxima were measured by running over the maximal absorption at the slowest speed at least three times and averaging the maxima thus obtained. The maxima could be duplicated to at least ±5 Å., and usually to ±3 Å. The extinction coefficients probably are accurate to within ±5%.

The high intensity maxima were measured in the same way, using a Cary Model 14 recording spectrophotometer and 0.01-cm. cells. The maxima could be duplicated to at least ±4 Å., and usually to ±2 Å.

A test of the obedience of the absorption maxima of *n*-butyl azide in isoöctane to Beer's law also was carried out. The results are shown in Table IV.

Materials.—*n*-**Butyl azide** was prepared by the method of Lieber, Chao and Rao[15]; b.p. 63–65° (200 mm.), $n^{20}D$ 1.4196 (lit.[15] b.p. 71° (225 mm.), $n^{20}D$ 1.4192).

Cyclohexyl azide was prepared as above; b.p. 66–68° (20 mm.), $n^{24}D$ 1.4681 (lit.[15] b.p. 72° (30 mm.), $n^{20}D$ 1.4693).

(15) E. Lieber, T. S. Chao and C. N. R. Rao, *J. Org. Chem.*, **22**, 238 1957).

TABLE IV

BEER'S LAW DATA FOR *n*-BUTYL AZIDE IN ISOÖCTANE

Concn., *M*	ϵ_{max}, 2161 Å.	ϵ_{max}, 2863 Å.
0.3150	468	..
.1575	465	..
.0788	474	24.7
.0394	[503]	24.7
.0197	466	24.5

t-**Butyl azide** also was prepared by the method of Lieber, *et al.*,[15] but could not be separated completely from contaminating *t*-butyl alcohol. The material finally used for spectral measurements had b.p. 70–74°, $n^{26}D$ 1.3955 (lit.[16] b.p. 68–71°, $n^{23}D$ 1.3865). The extinction coefficient of this material (for example in methanol at 2157 Å., ϵ_{max} was 258) indicated that it was at least 60% *t*-butyl azide. The small amount of *t*-butyl alcohol present would be unlikely to affect the position of the maximum greatly.

2-Chloroethyl azide was prepared by the method of Wiley and Moffat,[17] and had b.p. 39° (20 mm.), $n^{20}D$ 1.4660 (lit.[17] b.p. 45° (25 mm.), $n^{20}D$ 1.4658).

2-Hydroxyethyl azide was prepared by the method of Forster and Fierz,[18] and had b.p. 69–72° (20 mm.), $n^{25}D$ 1.4587 (lit.[19] b.p. 52–54 (5 mm.), $n^{25}D$ 1.4588).

Ethyl azidoacetate was obtained by Lieber's method[15] and had b.p. 72–73° (20 mm.), $n^{25}D$ 1.4358 (lit.[20] b.p. 74–75 (23 mm.), $n^{25}D$ 1.4341).

2-Acetoxyethyl azide was prepared by acetylating 2-hydroxyethyl azide with acetyl chloride in the presence of pyridine according to the method of Sarel and Newman.[21] It had b.p. 71–73° (20 mm.), $n^{25}D$ 1.4366.

Anal. Calcd. for $C_4H_7N_3O$: C, 37.21; H, 5.46; Found: C, 37.08; H, 5.62.

Hydrazoic acid in aqueous solution was obtained by acidifying with dilute hydrochloric acid a solution of sodium azide. Spectral measurements then were made directly on this solution. Solutions of hydrazoic acid in isoöctane and chloroform were obtained by shaking an aqueous solution of the acid with the appropriate solvent and drying the organic layer with sodium sulfate. Since the concentration of hydrazoic acid in these solvents was not known, no attempt was made to calculate the extinction coefficient in isoöctane or chloroform.

(16) D. H. R. Barton and L. R. Morgan, Jr., *J. Chem. Soc.*, 622 (1962).
(17) R. H. Wiley and J. Moffat, *J. Org. Chem.*, **22**, 995 (1957).
(18) M. O. Forster and H. E. Fierz, *J. Chem. Soc.*, **93**, 1865 (1908).
(19) H. O. Spauschus and J. M. Scott, *J. Am. Chem. Soc.*, **73**, 208 (1951).
(20) W. F. Huber, *ibid.*, **77**, 112 (1955).
(21) S. Sarel and M. S. Newman, *ibid.*, **78**, 5416 (1956).

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF ILLINOIS, CHICAGO 12, ILLINOIS, AND THE ARGONNE NATIONAL LABORATORY, ARGONNE, ILLINOIS]

Deuterium Isotope Effects in Transamination: L-Alanine and Pyridoxal[1,2]

BY M. I. BLAKE, F. P. SIEGEL, J. J. KATZ AND M. KILPATRICK

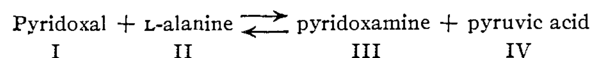
RECEIVED AUGUST 3, 1962

The reaction between pyridoxal and L-alanine at 100° depends on pH, the nature and concentration of added buffer constituents and metal ions. For the solvent H_2O, L-protio-alanine reacts with pyridoxal 2.4 times faster than the L-deuterio-alanine. For the solvent D_2O in similar buffer systems, L-protio-alanine reacts 2.9 times faster than L-deuterio-alanine.

In living systems a transfer of amino groups occurs between certain amino and keto acids. This important reversible process known as transamination has been reviewed recently by Snell,[3] who discusses the mechanism of the enzymatic and non-enzymatic reactions. Although a number of rate studies have been carried out, velocity constants have not been reported until recently for the non-enzymatic reactions.[4,5]

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.
(2) Presented at the 141st Natl. Meeting of the American Chemical Society, Washington, D. C., March, 1962.
(3) E. E. Snell and W. T. Jenkins, *J. Cellular Comp. Physiol.*, **54**, Supplement p. 161 (1959).
(4) B. E. C. Banks, A. A. Diamantis and C. A. Vernon, *J. Chem. Soc.*, 4235 (1961).
(5) G. M. Fleck, Dissertation, University of Wisconsin, 1961.

As L-deuterio-alanine was available from *Scenedesmus obliquus* grown in deuterium oxide,[6] it was of interest to design a set of relatively simple kinetic experiments to determine the kinetic isotope effect in the solvents H_2O and D_2O. The reaction chosen for study was



This reaction is not simple and both rapid and slow spectral changes occur on mixing the reactants. These changes are a function of temperature and pH and indicate a number of intermediates as well as species from the reactants. In addition, it is known that pyruvic acid hydrates and that pyridoxal may exist in solution

(6) M. I. Blake, H. L. Crespi, V. Mohan and J. J. Katz, *J. Pharm. Sci.*, **50**, 425 (1961).

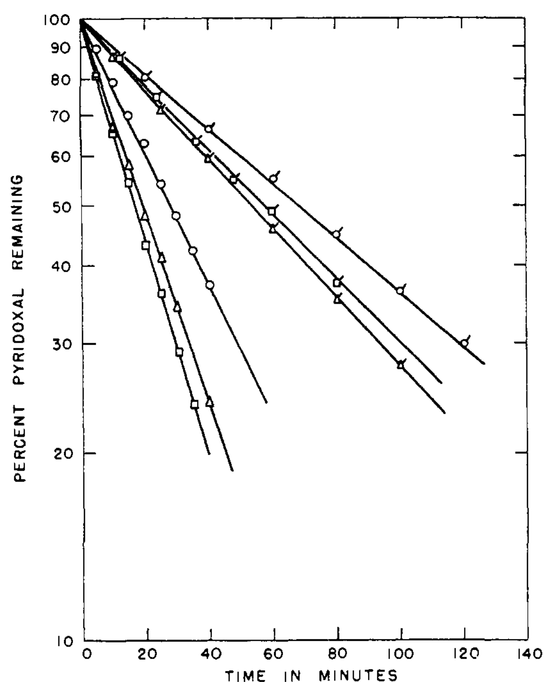


Fig. 1.—Test of equation 1, all solutions initially 0.010 M pyridoxal: O solvent H_2O, L-alanine 0.200 M; O- solvent H_2O, L-deuterio-alanine 0.200 M; Δ solvent D_2O, L-alanine 0.200 M; Δ solvent D_2O, L-deuterio-alanine 0.200 M; □ solvent H_2O, L-alanine 0.200 M, alum. 5 × 10^{-5} M; □- solvent H_2O, L-deuterio-alanine 0.200 M, alum. 5 × 10^{-5} M.

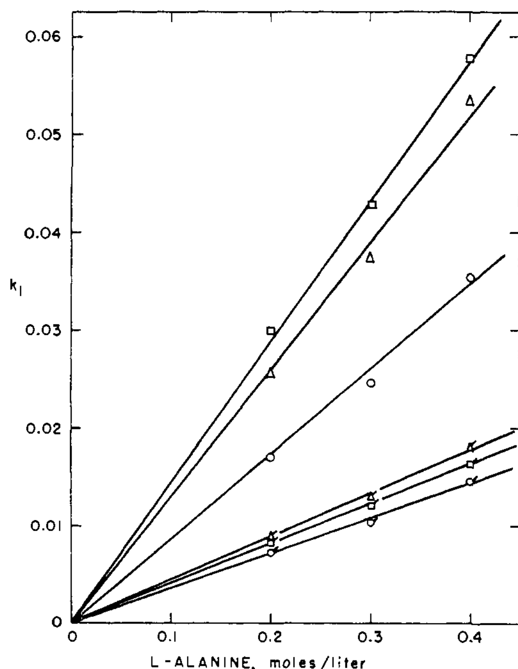


Fig. 2.—First order velocity constants *versus* alanine concentration: O solvent H_2O, L-alanine; O- solvent H_2O, L-deuterio-alanine; Δ solvent D_2O, L-alanine; Δ solvent D_2O, L-deuterio-alanine; □- solvent H_2O, L-alanine, alum. 5 × 10^{-5} M; □ solvent H_2O, L-deuterio-alanine, alum. 5 × 10^{-5} M.

in many forms including an internal hemiacetal.[7] Vernon and his co-workers, who studied carefully the kinetics of the reaction of pyridoxamine and pyruvic acid to pyridoxal and alanine, report a few experiments for the reverse reaction at 25° and pH 10. They find the initial reaction velocities for the production of pyridoxamine and pyruvate vary by a factor of two.[4] Fleck working at pH 8 and 25° and maintaining the ionic strength at 0.05 by means of sodium acetate failed

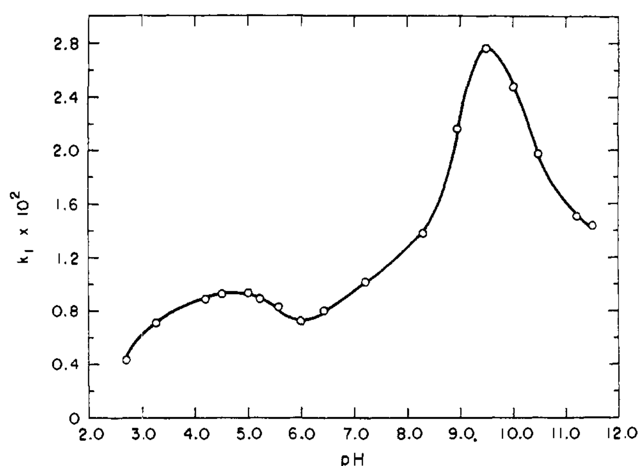


Fig. 3.—pH Profile, 0.01 M pyridoxal, 0.2 M alanine, T = 100°.

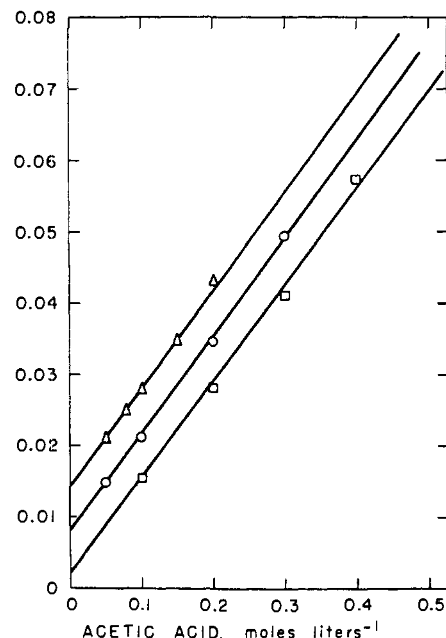


Fig. 4.—First order velocity constants *versus* acetic acid concentration: alanine 0.200 M; temp. 100°: Δ 1:2 HAc–Ac^- buffer, O 1:1 HAc–Ac^- buffer, □ 2:1 HAc–Ac^- buffer.

to find proportionality to the alanine concentrations and concluded further experiments are required.[5]

Our initial experiments were carried out at 100° with an excess of alanine in a 1:1 acetic acid–acetate buffer and the reaction was followed by determining the change of pyridoxal with time by an analytical method developed by Siegel and Blake.[8] As shown by typical results in Fig. 1, the reaction went practically to completion and the reaction was first order in pyridoxal

$$-\,d[I]/dt = k_1[I] \quad (1)$$

When k_1 is plotted against the alanine concentration (Fig. 2), proportionality indicates the reaction is also first order in alanine

$$-\,d[I]/dt = k_2[I][II] \quad (2)$$

The pH profile established in the absence of added buffer as given in Fig. 3 shows a broad maximum near pH 5 and a sharp peak at pH 9.5. Although equation 2 still applies, it is likely that the predominant species involved at pH 5 are not the same as those involved at pH 9.

To determine whether the concentration and nature of the buffer constituents have any effect on the velocity constants, three series of experiments were carried out

(7) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **77**, 2431 (1955).

(8) F. P. Siegel and M. I. Blake, *Anal. Chem.*, **34**, 397 (1962).

TABLE I

VELOCITY CONSTANTS FOR THE REACTION BETWEEN PYRIDOXAL AND ALANINE AT 100°

Initial concentrations: 0.01 M pyridoxal, 0.100 M acetate, 0.100 M acetic acid.

Initial concn. L-Alanine, mole/l.	k_1, min.$^{-1}$	k_2, l. mole^{-1} min.$^{-1}$	k_H/k_D
Solvent: Protium oxide			
H	0.0171	0.0856	
0.200			2.37
D	.00722	.0361	
H	.0247	.0823	
0.300			2.43
D	.0102	.0340	
H	.0353	.0883	
0.400			2.50
D	.0141	.0353	
No added buffer: Solvent, protium oxide			
H	0.00888	0.0444	
0.200 (pH = 4.15)			2.12
D	.00420	.0210	
H	.00866	.0433	
0.200 (pH = 4.85)			2.36
D	.00367	.01833	
Solvent: Protium oxide (5×10^{-5} M in alum.)			
H	0.0300	0.1500	
0.200			3.55
D	.00844	.0422	
H	.0430	.1433	
0.300			3.52
D	.0120	.0400	
H	.0577	.1443	
0.400			3.53
D	.0163	.0408	
Solvent: Deuterium oxide (99%)			
H	0.0255	0.1275	
0.200			2.93
D	.0868	.0434	
H	.0374	.1247	
0.300			2.92
D	.0128	.0426	
H	.0533	.1333	
0.400			2.96
D	.0180	.0450	

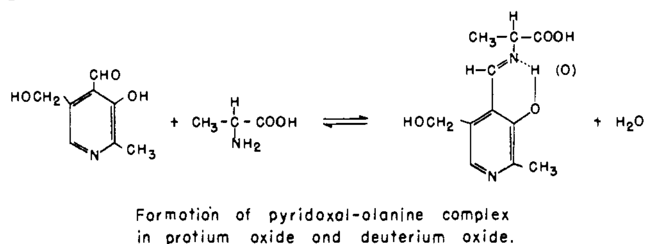
with 0.2 M L-alanine and 0.01 M pyridoxial n 1:2, 1:1 and 2:1 acetic acid buffers with an initial acetate concentration of 0.4 M. These buffers were diluted with sodium chloride solution to keep the ionic strength constant. Figure 4 shows the first order velocity constants are proportional to the acetic acid concentration with a catalytic constant $k_{HAc} = 0.65$. This plot would seem to indicate general acid catalysis, but the intercepts are in the wrong order for interpretation as hydrogen ion catalysis plus a water reaction and experiments in other buffer systems do not confirm simple general acid catalysis. The half time of the reaction at 100° for 0.2 M alanine and 0.01 M pyridoxal without added acetate buffer is 80 minutes and in the 1:1 acetic acid–acetate buffer at 0.4 M acetate is 12 minutes at 100°. The problem is complicated by the fact that the alanine present in excess is an acid with a first dissociation constant of 2×10^{-3} at 25° and is required at an initial concentration of not less than 0.2 M to maintain sufficient excess over the 0.01 M initial concentration of pyridoxal needed for the analytical method. Further detailed experiments will be required at lower substrate concentrations and at known hydrogen ion concentrations to clarify the considerable dependence of the rate of reactions on the nature and concentration of the added buffer.

Since the major interest of this paper is the isotope effect the experiments reported in Table I were carried out with constant initial concentrations of acetic acid and acetate. For comparison, a few experiments are reported without added buffer.

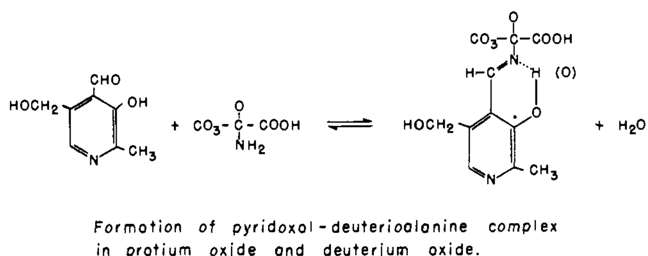
Discussion

From Table I it is evident that for the solvent H_2O L-protio-alanine reacts with pyridoxal 2.4 times faster than L-deuterio-alanine but 3.5 times faster when the solution contained aluminum ion. For the solvent D_2O the L-protio-alanine reacts 2.9 times faster than the L-deuterio-alanine.

In the absence of metal ions it is suggested that the intermediate is a hydrogen (or deuterium) bonded complex as shown

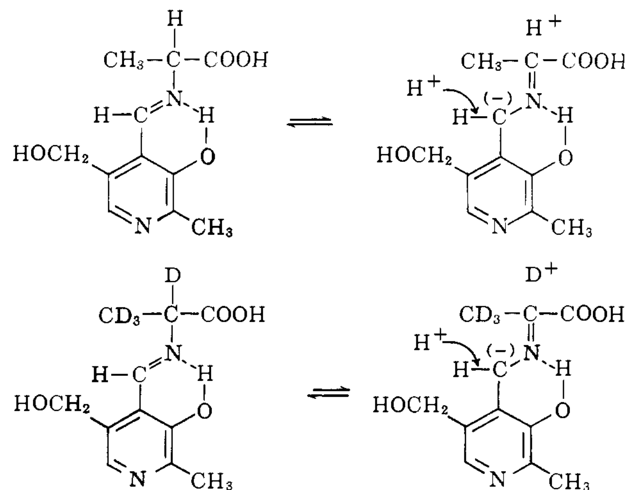


Formation of pyridoxal-alanine complex in protium oxide and deuterium oxide.



Formation of pyridoxal-deuterioalanine complex in protium oxide and deuterium oxide.

In the solvent D_2O the exchangeable hydrogens will be replaced by deuterium. The concentrations of the complex will vary with the solvent and will be different in the hydrogen bonded and deuterium bonded cases. The conjugated systems of double bonds extending from the α-carbon permits withdrawal of electrons and the weakening of the C–H or C–D bond at the α carbon of the amino acid



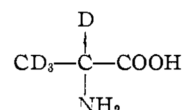
With the breaking of this bond, and loss[9] of the proton or deuteron to the solvent (or other base) a proton or deuteron is added at the formyl carbon. Subsequent hydrolysis yields pyridoxamine and pyruvic acid. When

(9) A. S. Konikova, M. G. Kritzmann and R. W. Teiss, *Biokhimiya*, **7**, 86 (1942).

the reaction involves a metal rather than hydrogen, the more stable chelate will further weaken the C–H (or C–D) bond of the α carbon of the amino acid by electron withdrawal and increase the rate of reaction, the effect being greater for the C–H than the C–D bond. This would qualitatively account for the greater ratio of k_H/k_D when a metal ion is present but the ratio could also be increased by an increase in concentration of the Schiff base.

Experimental

Isolation and Purification of L-Deuterio-alanine.—The isolation of L-deuterio-alanine from the algae *Scenedesmus obliquus* has been described elsewhere.[6] A modification for desalting the amino acid was carried out: The amino acid was dissolved in a small volume of one molar hydrochloric acid and passed through a 10-cm. column (0.9 cm. in diameter) of Dowex 50-X8 resin. The column was washed with water until the eluate was pH 7.0 and was chloride free. The column then was eluted with 15% aqueous ammonia and after the first drop of eluate was pH 8.0 a volume of 100 ml. was collected. The ammonium hydroxide removed the amino acid from the column without removing the sodium ions. The eluate was evaporated to dryness on a steam bath, the residue was decolorized with charcoal and recrystallized from ethanol. The identity of the deuterated amino acid was again established by chromatogramming 5 λ of a 1% solution, 5 λ of a 1% solution of ordinary amino acid, and a 5 λ aliquot of a mixed solution of both known and unknown compounds. Elemental analysis and optical rotation measurements indicated pure L-deuterio-alanine



Data obtained from a Spinco amino acid analysis indicated a purity better than 99%.

L-Alanine.—L-Alanine was obtained from the Nutritional Biochemical Corporation and the purity was checked by chromatographic analysis.

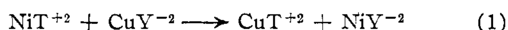
Pyridoxal.—Pyridoxal was obtained as the hydrochloride from the same source and its purity checked by analysis.[8]

Water.—Water was triply distilled and deionized.

D_2O.—D_2O of 99.6% concentration was distilled from a Barnstead conductivity still after digestion with hot alkaline permanganate. Other chemicals were analytical reagent grade. All chemicals were checked for metal content by spectrographic analysis.

Measurements of pH were made with a Beckman Model 76 expanded scale pH meter.

Kinetic Measurements.—Stock solutions were prepared for each run from weighed quantities of alanine and pyridoxal hydrochloride. This solution, with buffer added where required, was transferred to 5-ml. ampoules, sealed and placed in a boiling water bath at 100° at time zero. At suitable intervals ampoules were removed, plunged into ice water and the contents analyzed for pyridoxal at room temperature using a Coleman Junior spectrophotometer.[8] The stability of the pyridoxal at 100° was tested and found to be stable for periods of five hours over the pH range 3 to 9 in the presence and absence of added buffer.



[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY, LAFAYETTE, IND.]

Coördination Chain Reactions. Exchange between Ethylenediaminetetraacetatocuprate(II) and Triethylenetetraminenickel(II)

BY D. C. OLSON AND D. W. MARGERUM[1]

RECEIVED MAY 21, 1962

A chain reaction mechanism is proposed for the exchange of triethylenetetramine and ethylenediaminetetraacetate between their nickel(II) and copper(II) complexes. This exchange proceeds at a much faster rate than the aqueous dissociation rate of these complexes. The chain reaction is initiated by a trace of either of these multidentate ligands. In the chain-propagation steps one multidentate ligand displaces another from its metal complex and *vice versa*. Very sensitive control of the reaction rate is possible because the chain propagators are stable ligands whose concentration can be adjusted. Metal ions terminate the chain and the reaction can be used for trace metal determination.

Introduction

The kinetics of ligand (or metal) exchange between two multidentate ligand complexes is shown to proceed by a chain reaction mechanism. The exchange occurs between the complex ions triethylenetetraminenickel(II) and ethylenediaminetetraacetatocuprate(II), abbreviated NiT^{+2} and CuY^{-2}, respectively.

$$NiT^{+2} + CuY^{-2} \longrightarrow CuT^{+2} + NiY^{-2} \quad (1)$$

Triethylenetetramine (trien) occupies four sites in the metal coördination sphere[2,3] and its complexes with nickel(II) and copper(II) have stability constants with log K_1 values of 14.0 and 20.4, respectively.[4] Ethylenediaminetetraacetic acid (EDTA) occupies five coördination sites in the case of nickel[5] and its complexes with nickel(II) and copper(II) have stability constants with log K_1 values of 18.56 and 18.79, respectively.[6] These constants indicate that reaction 1 goes essentially to completion so that the reverse reaction can be neglected in the kinetic study.

The rate of dissociation of NiT^{+2} and its *p*H dependence has been studied by Clarke[7a] and Latterell.[7b] The rate of dissociation of CuY^{-2} can be calculated from its stability constant and its rate of formation.[8] The rate of reaction 1 is much faster than the rate of dissociation of either NiT^{+2} or CuY^{-2}, indicating that the exchange is not controlled by their dissociation rates.

The rate of attack of Ni^{+2} on CuY^{-2} and of Cu^{+2} on NiT^{+2} have been recently measured[7b,9] and are both too slow to contribute significantly to the reaction rate because of the low concentrations of the aquo metal ions in the reaction system. On the other hand, the rate of attack of EDTA on NiT^{+2} is known to be extremely rapid even at very low ligand concentration,[7a] and recent work in this Laboratory[10] has shown that in a similar manner the rate of trien attack on 1,2-diaminocyclohexanetetraacetocuprate(II) is much greater than the rate of dissociation of

(1) Correspondence to be addressed to this author.
(2) H. B. Jonassen and B. E. Douglas, *J. Am. Chem. Soc.*, **71**, 4094 (1949).
(3) H. B. Jonassen and A. W. Meibohm, *J. Phys. Colloid Chem.*, **55**, 726 (1951).
(4) G. Schwarzenbach, *Helv. Chim. Acta*, **33**, 974 (1950).
(5) G. S. Smith and J. L. Hoard, *J. Am. Chem. Soc.*, **81**, 556 (1959).
(6) G. Schwarzenbach, R. Gut and Z. Anderegg, *Helv. Chim. Acta*, **37**, 937 (1954).
(7) (a) J. F. G. Clarke, Ph.D. Thesis, Purdue University, 1960; (b) J. J. Latterell, M.S. Thesis, Purdue University, 1962.
(8) H. Ackermann and G. Schwarzenbach, *Helv. Chim. Acta*, **35**, 485 (1952).
(9) T. J. Bydalek and D. W. Margerum, *J. Am. Chem. Soc.*, **83**, 4326 (1961).
(10) D. W. Margerum and R. A. Libby, to be published.