The Zn appears to be evently distributed between the DNP and the surrounding cellular matter. However, considering that approximately 40% of the dry weight of the thymus cells consists of DNP, the results indicate that the surrounding cellular matter contains more than 3 times as much Fe as the DNP. Furthermore, the results show that essentially all of the Cu found in the thymus cells is concentrated in the DNP. Since all of the Cu found in the cells was recovered, apparently there was no significant loss during the extraction.

The values we report for Zn in DNP are consistent with those previously reported by Heath[13]. We did not detect Cr and Mn in calf thymus DNP as previously reported in DNA. The presence of these metals may be specific to the type of tissue.

These studies provide the basis for further investigation into the significance of the metals in DNP and DNA. Further studies are in progress which may reveal the function of transition metals in DNP and provide a better understanding of nucleic acid mechanisms.

Syntheses of amino acids from unsaturated aliphatic carboxylic acid by contact glow discharge electrolysis[1]

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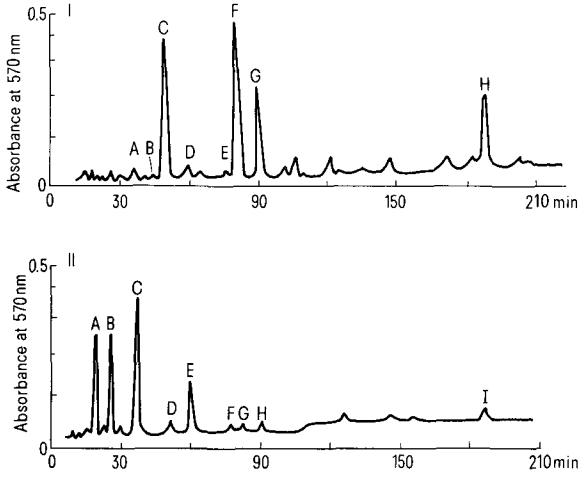
Summary. Hydroxy amino acids are synthesized from unsaturated aliphatic carboxylic acid using aqueous ammonia under conditions of contact glow discharge electrolysis.

Contact glow discharge electrolysis (CGDE) is a chemical change due to the glow discharge in a solution containing ions and an electrode in contact with the solution. Many reactions by CGDE have been studied mainly on inorganic compounds such as water, ammonia and metal ion in an aqueous solution[2,3]. Recently a few studies have been reported on the formation of various amino acids from aliphatic carboxylic acids[4,5] or aliphatic amines[5,6], using aqueous ammoniacal solutions or formic acid solutions, respectively. Urea, glycine and other amino acids were also formed in aqueous ammoniacal solutions by CGDE using a carbon rode as the anode[7].

In the present paper, the synthesis of hydroxy amino acid from unsaturated aliphatic carboxylic acid by CGDE is described. The CGDE was carried out in the reaction tube (a single tube equipped with platinum cathode and anode[5]) containing an ammoniacal solution (about 15 ml) of a substrate (0.005 moles) for 1 h under saturation of ammonia gas while stirring. The applied electric current was 50–60 mA at 400–600 V. The reaction temperature was kept at 10–15 °C by cooling the reaction tube in a methanol-dry ice bath. After the reaction was over, the solution was evaporated to almost dryness under reduced pressure and the residue was diluted appropriately for amino acid analysis (amino acid analyzer: Yanagimoto model LC-5S). The reaction mixture was also treated with 2,4-dinitrofluorobenzene, and the resulting dinitrophenyl (DNP)-amino acids were separated by celite column chromatography[8], followed by identification using a thin-layer chromatoplate. The major amino acid products were identified by comparing the Rf values with the authentic DNP-amino acids.

2 typical charts of the amino acid analyses of the reaction products are shown in the figure. The main amination products of acrylic acid are alanine (Ala, 2.6%) and β-Ala (1.8%). In addition to that, hydroxy amino acids, serine (Ser) and iso-Ser, are also formed in 3.1 and 2.1% yields, respectively. As the control experiments, aqueous ammoniacal solution of acrylic acid was kept at 10 °C for 1 h without CGDE. The amino acid formed in the reaction mixture was only β-Ala (0.2%). The main amino acids synthesized from maleic acid by CGDE are aspartic acid (4.4%), glutamic acid (2.2%), erythro-β-hydroxy aspartic acid (e-OH-Asp, 3.2%) and threo-OH-Asp (2.8%). The results of the amino acid formations from the unsaturated carboxylic acids by CGDE are summarized in the table (reactions No. 1–5), being compared with that of amino acid formation from the corresponding saturated aliphatic carboxlic acids (reactions No. 6–9). In the control experiments in reactions No. 2–5, no amino acids were



Aminations of acrylic acid and maleic acid by CGDE. I. Reaction of acrylic acid. *A* Aspartic acid (Asp); *B* threonine (Thr); *C* serine (Ser); *D* glutamic acid (Glu); *E* glycine (Gly); *F* alanine (Ala); *G* α-aminobutyric acid (α-ABA); *H* β-Ala. Other peaks are of unknown amino acids. Iso-Ser was analyzed by basic column. II. Reaction of maleic acid. *A* erythro-β-Hydroxyaspartic acid (e-OH-Asp); *B* threo-β-hydroxyaspartic acid (t-OH-Asp); *C* Asp; *D* Ser; *E* Glu; *F* Gly; *G* Ala; *H* α-ABA; *I* β-Ala. Other peaks are of unknown amino acids.

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2 A. Hickling and M. D. Ingram, J. Electroanalyt. Chem. *8*, 65 (1964).
3 A. Hickling, in: Modern Aspects of Electrochemistry, vol. 6, p. 329. Ed. J. O. Bockris and B. E. Conway. Plenum Press, New York 1971.
4 K. Harada and T. Iwasaki, Nature *250*, 426 (1974).
5 K. Harada, in: The Origin of Life and Evolutionary Biochemistry, p. 183. Ed. K. Dose, S. W. Fox, G. A. Deborin and T. E. Pavlovskaya. Plenum Press, New York 1974.
6 K. Harada and T. Iwasaki, Chem. Lett. *1975*, 185.
7 K. Harada and S. Suzuki, Nature *266*, 275 (1977).
8 A. Courts, Biochem. J. *58*, 70 (1954).

Formations of amino acids from unsaturated and saturated aliphatic carboxylic acids by CGDE

No.	Starting materials (0.005 moles)	Reaction products (yield percent) Asp	Thr	Ser	Glu	Gly	Ala	αABA	β-Ala	iso-Ser	e-OH-Asp	t-OH-Asp	e-OH-Glu*	t-OH-Glu*
1	CH_2=CHCOOH	+	+	3.1	+	0.1	2.6	0.5	1.8	2.1				
2	CH_3CH=CHCOOH	+	3.9	+	+	0.3	0.3	1.6	0.3	—				
3	HOOCCH=CHCOOH (cis)	4.4	—	0.1	2.2	0.2	0.1	0.1	0.2	—	3.2	2.8	—	—
4	HOOCCH=CHCOOH (trans)	4.5	—	0.1	2.2	0.2	0.1	0.1	0.2	—	3.3	3.0	—	—
5	HOOCCH$_2$CH=CHCOOH	—	0.2	—	1.3	0.1	+	—	+	—	—	—	0.9	0.8
6	CH_3CH_2COOH	0.1	—	0.1	—	0.1	1.2	+	0.9	+				
7	$CH_3CH_2CH_2$COOH	+	0.1	+	+	0.1	+	0.8	0.3					
8	HOOCCH$_2$CH$_2$COOH	1.6	—	+	0.2	+	+	—	0.2		+	+	—	—
9	HOOCCH$_2$CH$_2$CH$_2$COOH	+	—	—	1.8	0.1	+	—	—		—	—	+	+

Voltage 400–600 V; current 50–60 mA; time 1 h; temp. 10–15 °C. * e-OH- or t-OH-Glu: erythro- or threo-β-hydroxy glutamic acid.

identified. The CGDE of the unsaturated carboxylic acid gives more hydroxy amino acids than that of the saturated one. It could be considered that the hydroxy radicals generated from water attack the double bond of the unsaturated carboxylic acid. There is no difference in the species and the amounts of amino acids synthesized from maleic acid and fumaric acid by CGDE

The glow discharge electrolysis could be regarded as a simulation of lightning hitting the primitive sea which contains organic and inorganic compounds. Thus, the above findings suggest one possibility for the prebiotic synthesis of hydroxy amino acids from simple organic compounds under possible prebiotic conditions on the primordial earth.

Possible role of intestinal alkaline phosphatase activity in thiamine transport

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Summary. Thiamine deficiency caused a marked decrease of intestinal alkaline phosphatase (al-Pase) activity, but had no effect on the Ca^{++}-ATPase activity and Ca^{++}-absorption in rats. The al-Pase activity was significantly decreased 1 h after oral administration of ethanol at 0.5 and 2.5 g/kg. In contrast, Mg^{++}-, Ca^{++}- and $(Na^+ + K^+)$-ATPase activities did not change after the administration of ethanol. These findings show that the al-Pase activity, unlike the Ca^{++}-ATPase activity, is not related to Ca^{++}-absorption. A possible role of al-Pase activity in the active transport of thiamine in the intestine was discussed.

It is reported that intestinal Ca^{++}-binding protein[1,2], Ca^{++}-ATPase[3–5] and al-Pase[6–10] play functional roles in the transport of Ca^{++} in the intestine. A close relation between the Ca^{++}-ATPase and al-Pase activities under a variety of conditions suggested that activities of these enzymes might represent measure of the same enzyme, though a few reports[11,12] conflict with this idea.

Previously we showed that the al-Pase and thiamine diphosphatase (TDPase) activities of rat duodenum were markedly decreased by thiamine deficiency and suggested that intestinal TDPase activity was identical with the al-Pase activity[13]. These results led us to consider that thiamine deficiency possibly causes decreases of intestinal Ca^{++}-ATPase activity and Ca^{++}-absorption. The present study was carried out to clarify this point.

Finally, this paper reports the effect of ethanol, which is well-known to impair intestinal absorption of thiamine in human[14,15] and animals[16], on the activities of intestinal phosphatases to deduce a possible role of the al-Pase activity in the absorption of thiamine.

Materials and methods. Male Sprague-Dawley rats, weighing 80–100 g, were used throughout. Thiamine-deficient and pair-fed rats were prepared as described previously[17]. Rats were fasted overnight but allowed free

1 R. H. Wasserman and A. N. Taylor, Science *152*, 791 (1966).
2 S. Kowarski and D. Schachter, Am. J. Physiol. *229*, 1198 (1975).
3 D. L. Martin, M. J. Melancon, Jr, and H. F. Deluca, Biochem. Biophys. Res. Commun. *35*, 819 (1969).
4 M. J. Melancon, Jr, and H. F. Deluca, Biochemistry *9*, 1658 (1970).
5 S. Kowarski and D. Schachter, J. clin. Invest. *52*, 2765 (1973).
6 A. W. Norman, A. K. Mircheff, T. H. Adams and A. Spielvogel, Biochim. biophys. Acta *215*, 348 (1970).
7 M. R. Haussler, L. A. Nagode and H. Rasmussen, Nature *228*, 1199 (1970).
8 E. S. Holdsworth, J. Memb. Biol. *3*, 43 (1970).
9 E. L. Krawitt, Biochim. biophys. Acta *274*, 179 (1972).
10 R. G. G. Russell, A. Monod, J.-P. Bonjour and H. Fleisch, Nature New Biol. *240*, 126 (1972).
11 E. L. Krawitt, P. A. Stubbert and P. H. Ennes, Am. J. Physiol. *224*, 548 (1973).
12 F. A. De Wolf, Eur. J. Pharmac. *33*, 71 (1975).
13 H. Iwata, T. Matsuda and A. Baba, Experientia *32*, 1252 (1976).
14 P. A. Tomasulo, R. M. H. Kater and F. L. Iber, Am. J. clin. Nutr. *21*, 1340 (1968).
15 A. D. Thomson, H. Baker and C. M. Leeby, J. Lab. clin. Med. *76*, 34 (1970).
16 A. M. Hoyumpa, Jr, K. J. Breen, S. Schenker and F. A. Wilson, J. Lab. clin. Med. *86*, 803 (1975).
17 H. Iwata, S. Fujimoto, T. Nishikawa and K. Hano, Experientia *24*, 378 (1968).