Zinc is known to be a constituent of most foods and in small quantities is necessary for body nutrition (*3, 4*). Although small traces of zinc can occur in urine, the major portion of this element entering the body is excreted by way of the intestinal tract.

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Effect of Ethylenediamine Tetraacetic Acid on Adenosinetriphosphatase Activity

Milton Gross

Department of Biology, New York University, and *Department of Laboratories of the B. S. Pollak Hospital for Chest Diseases, Medical Center, Jersey City, New Jersey*

Calcium ion is considered indispensable for the enzymic function of myosin-adenosinetriphosphatase, the degree of activation depending on the concentration of adenosinetriphosphate (*1*). Reduction in the availability of calcium ion by citrate (*2*) or oxalate (*3*) results in decrease or complete inhibition of enzyme activity. Ethylenediamine tetraacetic acid (EDTA) is a noncolloidal, organic chelating agent that can deionize a system of its heavy metal and alkaline-earth ions through formation of stable unhydrolyzed complexes. Since EDTA has a strong affinity for calcium ions, the effect of this compound on calcium activated myosin-adenosinetriphosphatase of mouse heart homogenates was studied.

Adenosinetriphosphatase activity was estimated with the procedure of DuBois and Potter (*4*). The buffered substrate was prepared as follows: 305 mg adenosinetriphosphoric acid[1] was dissolved in 10 ml water; 50 ml 0.06 *M* Veronal buffer (pH 7.4) and 10 ml 0.06 *M* calcium chloride were added and the pH readjusted to 7.4 with *N* sodium hydroxide. The volume was made to 100 ml with distilled water and filtered. In the test, 1 ml buffered substrate was added to a 5-ml test tube containing 0.5 ml water or test solution. Five-tenths milliliter C-57 mouse heart homogenate was added and the tubes incubated for 15 min at 37.5°. The quantity of adenosinetriphosphatase that liberates 1 μg of phosphate from adenosinetriphosphoric acid in 15 min is taken as one D-P unit. In the table the activity is expressed as D-P units/milligram of fresh tissue.

A 0.04 *M* buffered stock solution of the disodium salt of ethylenediamine tetraacetic acid[2] was prepared in 0.03 *M* Veronal buffer (pH 7.4) and the pH readjusted with *N* sodium hydroxide. Lower concentrations of EDTA were obtained by diluting the stock solution with the appropriate amount of water and buffer. The solution of EDTA must be buffered to compensate for the hydrogen ions displaced by the combined calcium. In order to exclude pH variation as contributory to the effect on adenosinetriphosphatase, the pH of each complete incubation mixture was determined.

[1] Schwarz Laboratories.

[2] Obtained from Alrose Chemical Co., Providence 1, R. I., through the courtesy of H. M. Zussman.

TABLE 1

EFFECT OF ETHYLENEDIAMINE TETRAACETIC ACID ON ADENOSINETRIPHOSPHATASE ACTIVITY

Molarity EDTA in incubation mixture	Mouse No. 55			Mouse No. 73		
	pH of incubation mixture	Homogenate activity (D-P units)	Per cent original activity	pH of incubation mixture	Homogenate activity (D-P units)	Per cent original activity
0.010	7.1	2.08	8.1	7.2	4.08	13.3
0.008	7.1	4.56	17.1	7.2	5.74	18.5
0.006	7.0	5.68	21.2	7.2	8.14	26.2
0.004	7.2	9.92	37.2	7.2	9.32	30.0
0.002	7.2	41.84	156.0	7.2	50.12	161.2
0.001	7.4	34.96	130.0	7.3	41.12	132.3
0.0001	7.4	29.09	106.0	—	—	—
0.00001	7.4	26.89	100.0	—	—	—
0.0	7.4	26.88	100.0	7.4	31.02	100.0

One mole of EDTA can chelate an equivalent amount of calcium ion. Thus, since the concentration of calcium in the test mixture is 0.003 *M*, this concentration was expected to limit the activation of adenosinetriphosphatase. The results obtained are summarized in Table 1 and Fig. 1. Actually, when the level of EDTA is over 0.003 *M* the enzyme activity is diminished, and above 0.004 *M* this is linear. However, the stimulation observed between 0.001 and 0.003 *M* was not anticipated. With 0.002 *M* EDTA, the adenosinetriphosphatase was over 150% more active than in the original homogenate.

A series of tests was made with various concentrations of EDTA that had been combined with an equimolecular amount of calcium chloride, in Veronal and sufficient *N* sodium hydroxide, so that when added to the incubation mixture, the final pH was 7.3–7.4. The results are represented in Fig. 1, curve C. It is apparent that saturation of the chelation valences of EDTA before addition to the incubation mixture abolishes its effect on adenosinetriphosphatase activity. This indicates that the effects observed are due to the deionization capacity of EDTA and not to a direct toxic action of the compound on the enzyme itself.

The enzyme system utilized may contain toxic ionic species that do not permit maximum activity of adenosinetriphosphatase. EDTA, in concentrations

that are not high enough to limit the calcium activation, can enhance adenosinetriphosphatase activity by combining with these elements. Adenosinetriphosphatase is especially sensitive to minute traces of cupric ion, and it has already been suggested that the favorable effect of cyanide or glycine on the enzyme is due to removal of this ion (*1, 5*). Swanson (*6*) has reported similar experiences with magnesium activated pyrophosphatase and also considers the stimulation by EDTA in noninhibitory concentrations results from the protection of the enzyme from traces of heavy metals. Meyer and Rapport (*7*) found that 0.001 *M* EDTA was partially effective in counteracting the inhibition of hyaluronidase by 5×10^{-5} *M* ferric or cupric ion.

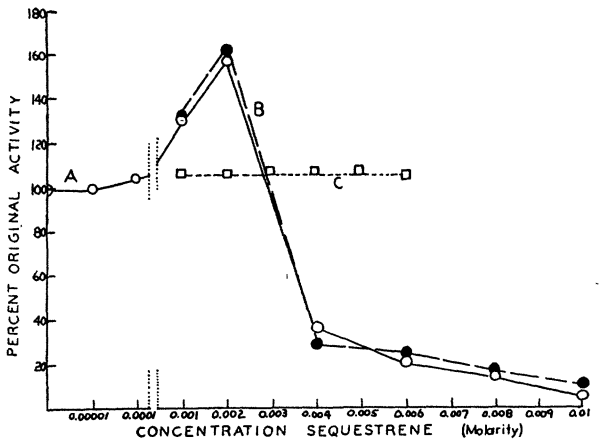

FIG. 1. Effect of ethylenediamine tetraacetic acid on adenosinetriphosphatase of mouse heart homogenate. Curve A, mouse 55; Curve B, mouse 73; Curve C, calcium ethylenediamine tetraacetate.

In any enzyme system affected by inorganic ions, chelation of these factors results in either elevation or diminution of the observed enzymic activity, depending on the relative availability of activating or inhibitory ions. It is conceivable that chelation is an important aspect in both the regulation of enzyme activity *in vivo,* and cellular ionic exchanges generally. Ethylenediamine tetraacetic acid and its effect on adenosinetriphosphatase may be taken as models for this concept. Reversible complex formation by naturally occurring chelating substances, such as keto-, hydroxy-, or amino acids, nucleic acids and proteins, could influence the activity of calcium activated adenosinetriphosphatase by controlling the level of ionic calcium.

Cellular ionic exchanges in general may involve preferential chelation. For example, since the preferential chelation of an ion from a mixture depends on the comparative stability of the ionic complex formed, calcium ion can be expected to displace magnesium from its EDTA complex whereas copper will displace the calcium (*8–10*). The instantaneous intracellular availability of a given free ion may be determined by the resultant equilibrium between competing ions, chelation, and other ion-binding reactions.

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Hydrolytic Enzymes in Hyaluronidase Preparations

Howard Chauncey, Fabian Lionetti, and Vincent Lisanti

Boston University School of Medicine* and *Tufts Medical and Dental School, Boston, Massachusetts

The increasing use of hyaluronidase preparations as adjuvants to local anesthetics, for hyperdermoclysis of medicants, resolution of injection hematomas, and for reduction of lymphedema and traumatic swellings, intensifies the necessity for a complete elaboration of the basic enzymic mechanisms involved. Reported differences in action, specificity, and end products produced by both testicular and bacterial preparations (*1–3*) have led us to suspect that hyaluronidase preparations available for use are actually enzyme mixtures capable of eliciting more physiological effects than the hydrolysis of the as yet incompletely characterized hyaluronic acid. The data enclosed, obtained by surveying commercial preparations for a spectrum of eight hydrolytic enzymes, give validity to this supposition.

Three commercial testicular, one commercial bacterial, and three laboratory streptococcal hyaluronidase preparations were assayed for acid and alkaline phosphatases, total esterases, pseudocholinesterases, lipases, β-D-galactosidases, β-glucuronidases, and sulfatases. The techniques were those of Seligman and co-workers (*4–8*), modified to substitute solutions of the test hyaluronidase preparation for the serum or tissue homogenate ordinarily used. Hyaluronidase assays were done viscosimetrically (*9, 10*). The principle of the method employed is the colorimetric determination of β-naphthol liberated from a variety of naphthyl esters serving as substrates for the enzymes tested, i.e., β-naphthyl acetate for esterases, β-carbonaphthoxycholiniodide for pseudocholinesterases. Table 1, therefore, is expressed as micrograms of β-naphthol liberated per milligram of hyaluronidase test preparation. Average tissue values from