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.

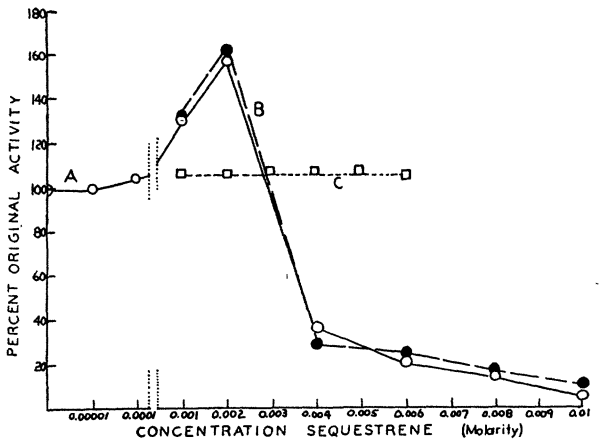

FIG. 1. Effect of ethylenediamine tetraacetic acid on adenosinetriphosphatase of mouse heart homogenate. Curve A, mouse 55; Curve B, mouse 73; Curve C, calcium ethylenediamine tetraacetate.

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

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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

TABLE 1

ENZYME CONTAMINANTS OF HYALURONIDASE PREPARATIONS

μg of β-Naphthol of Preparation/mg of Hyaluronidase Preparation

Enzyme	Incubation time hr	Testicular			Bacterial	Crude bacterial			Av comparative values in tissues*
		A	B	C	D	E	F	G	
Acid phosphatase	2	170	135	92.5	0.25	0.50	0.00	1.25	2.9
Alk. phosphatase	1	7.0	3.5	2.0	0.00	0.00	0.00	0.00	2.6
Total esterases	1	370	465	420	0.80	1.10	0.60	1.10	64
Pseudocholinesterase	1	3.75	5.15	1.90	0.35	0.75	0.75	0.65	41
Lipase	5	1.6	2.2	1.8	23.4	0.00	0.00	0.00	0.0
β-D-Galactosidase	2	12.0	47.0	5.0	0.00	0.00	0.00	0.00	2.7
β-Glucuronidase	4	4.2	0.0	0.4	0.00	0.00	0.00	0.00	—
Sulfatase	24	1.5	0.6	2.5	0.00	0.00	0.00	0.00	4.5/4 hr*
Hyaluronidase		13 V.R.U.	20 V.R.U.	12 V.R.U.	15 V.R.U.	4.8 V.R.U.	3.6 V.R.U.	3.2 V.R.U.	

A, B, C Commercial testicular hyaluronidase preparations.
D Commercial bacterial preparation.
E, F, G Laboratory preparations (streptococcal) beef brain-heart infusion broth (Difco) cultures, Seitz filtered, precipitated with $(NH_4)_2SO_4$, dialyzed, and lyophilized.

* Human serum values for all except β-D-galactosidase (rat liver and sulfatase [rat liver]) as μg β-naphthol liberated/mg of serum protein (based on 7.0 g %).

the literature (*4–8*) are given for the same test conditions.[1]

The data indicate the not surprising observation that testicular enzyme preparations contain appreciably more enzyme contaminants than the bacterial products. The testicular preparations were high in acid phosphatases, total esterases, and β-D-galactosidase, with measurable quantities of the others in most cases. The commercial bacterial hyaluronidase preparation was significantly high only in lipase activity. However, it is somewhat surprising that the relatively crude preparations of laboratory streptococcal material were comparatively free from these accompanying enzymes, whereas the magnitude of the contaminants in the testicular products was far greater than anticipated. For instance, permitting Seligman's assumption that the amounts of β-naphthol liberated are proportional to the times of incubation of reactants, it is seen that 30–50 times the titers of acid phosphatase of serum were found in comparable amounts of testicular enzyme protein tested. In these substances the alkaline phosphatases were of the same order of magnitude as the serum values and total esterases were 6 times as high. For β-D-galactosidase, in the absence of a serum value, an elevation of 2–15 times over that for liver tissue was found. Whereas bacteria are known which produce several of these enzymes, our procedure consisting of filtration, salt precipitation, dialysis, and lyophilization is ample to isolate a product relatively free of the hydrolytic enzymes tested. Accordingly, the variability in substrate specificity of both types of preparation may be attributable to such enzymes with the bacterial varieties being more specific. It is conceivable that the degradation of chondroitin sulfate produced by testicular hyaluronidase and not by bacterial hyaluronidase, as well as certain other differences, may be accountable by regarding the former as an unspecific mixture.

[1] The substrate for β-glucuronidase-8-aminobenzoylnaphthylglucuronide was supplied through the courtesy of Arnold M. Seligman, Beth Israel Hospital Surgical Research Department, Boston, Mass., method unpublished.

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The Influence of epi-F, a Stereoisomer of Compound F, on the Glycogenic Property of Compound F (17-Hydroxycorticosterone)

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All the known naturally occurring adrenal cortical steroids having a hydroxyl group on the eleven carbon are of the beta configuration. Two of these are corticosterone (Kendall's compound B) and 17-hydroxy-

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