Inhibition of Rigor Mortis by Ethylenediamine Tetraacetic Acid.* (31436)

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In summarizing the sequence of events in the development of rigor mortis, Bendall(1) pointed out that "contraction" during rigor is always weak and slow and extends over a period of hours. Locker(2) suggested that the mechanism of contraction with the onset of rigor mortis is in no way different from that of living fibers or from that induced in isolated myofibrils. In the molecular theory of muscle contraction(3), calcium released from the sarcoplasmic reticulum forms chelate links between actin and myosin, which results in the development of tension and shortening. Edman and Grieve(4) have given evidence favoring the hypothesis that the release of cellular calcium is an essential step in the initiation of contraction. Ethylenediamine tetraacetic acid (EDTA) forms a strong calcium chelate linkage in which the calcium becomes chemically unavailable to many reactions(5). Since Watanabe and Sleator(6) produced relaxation of contracted glycerol-treated fibers with EDTA, we were prompted to test the possibility that an intravenous ante-mortem injection of EDTA might inhibit the development of rigor mortis.

Methods. Eight rabbits weighing between 1.6 and 2.3 kg were randomly divided into 2 groups, one treated and one control with 4 animals in each. The treatment consisted of an intravenous injection of a lethal dose of the tetrasodium salt of EDTA in isotonic saline solution. The dosage consisted of 65 to 132 mg of EDTA per kg of body weight. The controls received an intravenous injection of 3 ml of an isotonic saline solution per kg body weight, so that both groups were injected with approximately the same volume of solution.

All rabbits were bled within 5 minutes of injection. Following exsanguination, the intact semitendinosus muscle was removed and shortening was recorded on a kymograph with an isotonic lever loaded with approximately 8 g/sq cm of muscle cross section. The muscle was held at 0°C over calcium-free Ringer's solution with nitrogen bubbling through it. The longissimus dorsi muscle from the right side was removed immediately after bleeding and frozen in liquid nitrogen. The longissimus dorsi muscle from the left side was removed after 20 hours at 0°C and frozen in liquid nitrogen. The frozen samples were then pulverized and stored at —30°C for subsequent pH and adenosine triphosphate (ATP) determinations.

ATP was determined by the bioluminescent enzymic method(7,8) using an Aminco-Bowman Spectrophotofluorometer. Duplicate pH measurements, using a 0.5 g sample homogenized for 0.5 minutes in 25 ml of 0.005 M sodium idoacetate, were made with a Corning pH meter Model 12.

Results and discussion. Fig. 1 shows that the intravenous injection of EDTA greatly inhibited shortening of the semitendinosus muscle. Any shortening occurring in the muscles of the treated rabbits was completed after 3 hours, whereas normal shortening con-

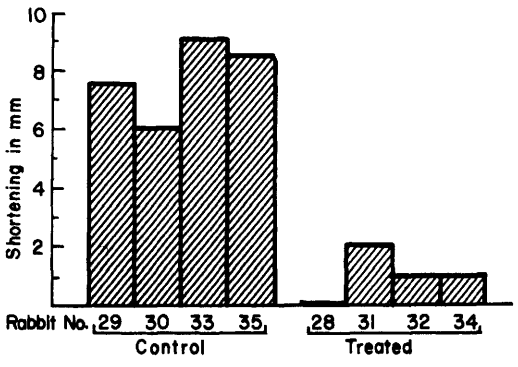

FIG. 1. Effect of EDTA on shortening of the semitendinosus muscle. The control consisted of an intravenous ante-mortem injection of isotonic saline. and the treatment consisted of an intravenous ante-mortem injection of EDTA in isotonic saline.

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tinued up to 7 hours for the controls. Observations show that 7 hours post-mortem the muscles from the control rabbits were rigid and inextensible (Table I). However, the muscles from the treated rabbits were elastic and extensible and could be easily stretched to about 40% of their equilibrium length and would still snap back to their original length on release. Results indicate that rigor mortis was inhibited in the semitendinosus muscle of the treated rabbit.

Davies(3) suggested that formation of the Ca-actomyosin complex develops tension, and if the sub-total of the forces developed at any one time by the ultramicro-contractions is sufficient to overcome the extended load and the resistance of the series of elastic elements, the actin filaments will be pulled along the myosin filaments into the A band and the muscle will undergo quantal contraction. The mechanism of action of chelating agents in an actomyosin system appeared to be the removal of a trace amount of calcium(9). Hasselbach(10) showed that the calcium content of the undissolved structural proteins from fresh muscle was diminished 50% by washing with EDTA, but that the magnesium content was not altered. In accord with these results, it appears that the EDTA removed enough free Ca++ to inhibit the shortening and to prevent the inextensibility that normally occurs within 7 hours post-mortem.

The data in Table II show that there is no appreciable difference in pH and ATP values of the longissimus dorsi muscle from the EDTA-treated rabbits and the control rabbits at either 0 hour or 20 hours. The values obtained in the present study are in agreement with results of other workers for rabbits(11,12).

The breakdown of ATP has been shown to be correlated with the onset of rigor in normal animals(13,14,11,15), but in this study the breakdown of ATP was not found to result in the development of shortening or inextensibility. Recently Lee(16) showed that a continuous supply of ATP is necessary for the relaxing factor to chelate calcium. Thus, the EDTA present in this experiment could bind free Ca++ on being released by the relaxing factor as the level of ATP declines. This work thus suggests that as the level of ATP declines after death, a certain level of free Ca++ is required for the development of rigor mortis.

TABLE I. Properties of Semitendinosus Muscle 7 Hours Post-Mortem.

Treatment	EDTA		Control	
Rabbit No.	32	34	33	35
Resting length (cm)	5.5	5.0	5.0	5.5
Stretched length (cm)	8.0	7.0	5.0	5.5
Condition of muscle	elastic	elastic	rigid	rigid

TABLE II. ATP and pH Values for the L. Dorsi Muscle.

		EDTA				
Rabbit No.		28	31	32	34	Avg
pH	0 hr	6.56	6.66	6.81	6.58	6.65
	20 "	5.67	5.66	6.12	5.48	5.73
ATP,	0 "	4.79	3.13	5.91	6.13	4.99
μM/g	20 "	.116	.169	.195	.170	.162
		Control				
Rabbit No.		29	30	33	35	Avg
pH	0 hr	6.68	6.69	6.56	6.71	6.66
	20 "	5.72	5.77	5.71	5.55	5.68
ATP,	0 "	4.26	4.27	5.20	6.99	5.18
μM/g	20 "	.220	.149	.219	.556	.286

Summary. A lethal intravenous injection of EDTA in rabbits inhibited the post-mortem shortening of the semitendinosus muscle and prevented the inextensibility that normally occurs during development of rigor mortis. It was also shown that no appreciable difference in pH and ATP values existed between the EDTA-treated rabbits and the control rabbits at either 1 hour or 20 hours. These results thus indicate that EDTA inhibited the onset of rigor mortis, but did not prevent the breakdown of ATP.

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Electrophoretic Heterogeneity of Leucocyte Alkaline Phosphatase in Normal Man and in Patients with Polycythemia Vera. (31437)

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Interest in the alkaline phosphatase activity of human polymorphonuclear leucocytes was stimulated by the now well-established finding that the activity of this enzyme falls to very low values in chronic myelogenous leukemia and rises to very high levels, well beyond the normal, in polycythemia vera(1). The speculation that the decrease in enzyme activity of the leukemic granulocyte is the result of partial deletion of the small acrocenter of chromosome 21, the Philadelphia chromosome, has been advanced(2,3). Evidence has also been presented(4,5) purporting to relate maturity and differentiation of the polymorphonuclear leucocyte with alkaline phosphatase activity. The potential for phosphatase synthesis is realized during differentiation of the granulocyte from its precursors, the myeloblast and myelocyte. Enzymes are now known to exist in multiple molecular forms and different isozyme patterns have been observed in different tissues (6). Heterogeneity of non-specific human serum alkaline phosphatase and of human placenta has been demonstrated(7). The purpose of the present investigation has been to describe the pattern of heterogeneity of normal human leucocyte alkaline phosphatase and to determine the influence of polycythemia rubra vera on this pattern.

Materials and methods. Five hundred ml of blood in ACD solution were obtained from 5 presumably normal donors immediately after removal in a commercial blood bank. The alkaline phosphatase assay of their leucocytes ranged between 29.6 and 83.0 mg phos/10^{10} polymorphonuclear leucocytes. In addition, 2 healthy, hematologically normal volunteers were phlebotomized of 500 ml of blood. The leucocyte alkaline phosphatase assay of these samples was 26 and 35 mg phos/10^{10} polymorphonuclear leucocytes. Similarly, 500 ml of blood were removed from 3 patients with well documented polycythemia rubra vera whose leucocyte alkaline phosphatase values were: 61, 192, and 465 mg phos/10^{10} polymorphonuclear leucocytes.

The leucocytes were isolated by means of differential sedimentation in 5% polyvinyl pyrrolidine and 2% bovine fibrinogen in normal saline(5). The leucocyte preparation was freed of most of the contaminating erythrocytes by exposure to hypotonic saline followed by restoration of tonicity with 3.5% saline (8). Six volumes of cold distilled water were added rapidly to 2 volumes of a saline suspension of the cells. After vigorous mixing for 30 seconds, tonicity was restored by rapid addition of 2 volumes of 3.5% saline. After one final washing with normal saline the leucocyte cell pack was resuspended in 1-2 volumes of water and stored at —20°C.

Upon thawing and homogenization of the cell pack in a tissue grinder, the enzyme was extracted by the method of Morton(9), as modified by Trubowitz *et al*(10). The extract