TABLE II. Inhibition of Peptic Digestion of Coagulated Egg White in Mett Tubes. Pepsin: 250 μg/ml in 0.056 N HCl. Incubation: 16-20 hr at 37°. d = length of egg white column digested.

		Polymer I			Polymer II			Polymer IV		
Inhibitor, μg/ml	None	500	250	125	500	250	125	500	250	125
d^2	2450	121	529	1764	169	1296	1681	121	1089	1521
	2500	121	676		1369	1369	2116	121	1156	1849
	1764	100	289	1190	812	812	1296	144	900	1521
	2304	121	504	1480						
% inhibition avg	0	95	81	32	95	49	24	94	53	27

might lead one to search for more powerful anti-pepsins, or anti-lysozymes in the hope of uncovering therapeutically more valuable compounds.

Summary. (1) Ability of 4 acidic polymers to inhibit lysozyme and/or pepsin has been measured. (2) Their anti-enzyme effect is compared to their action in the Shay rat.

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Force-Velocity Relationship of Tetanus Toxin Treated Skeletal Muscle.* (24600)

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The mechanical properties of muscles actively shortened by tetanus toxin injection vary from normal in a manner and to a degree not solely attributable to atrophy(1). These muscles exhibit increased tetanus-twitch ratio, shortened twitch relaxation time, as well as lower fusion frequency(2), and an augmented semi-dynamic stiffness, at short degrees of stretch, following bouts of tetanic stimulation(3). Such observations are not readily interpreted when considered as isolated facets of the mechanical behavior of such actively shortened muscle. To extend

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the interpretation of prior observations, an investigation of force-velocity relationship of these preparations was made. The theoretical and practical importance of this relationship has been stated by Wilkie(4). Energy released during shortening of muscle appears to govern the shape of the force-velocity curve. The constants a and b of Hill's(5) characteristic equation $(P + a)v = (P_o - P)b$ where a is the 'extra' heat/cm of shortening, and b is a constant with velocity dimensions can be derived equally well from studies of force and velocity, or of heat and length.

Methods. Thirteen male albino rats averaging 394 g in body weight (range 329-428) were used for the study. Active shortening of one gastrocnemius muscle of each animal was induced by unilateral injection of tetanus toxin into the popliteal space on that side. As a routine procedure, right and left muscles alternately were selected as experimental preparations. Maximum hyperextension of the limb without overt spread to the contralateral side was assured by careful adjustment of the toxin dosage. Gastrocnemii were exposed under chloralose anesthesia (15 mg/100 g body weight) 14 days after toxin injection(3). After measurement of the *in situ* resting length and severance of the insertion so as to include a portion of the os calcis, an 11 mm wound clip with attached nylon monofilament was fastened to the free tendinous end. The monofilament was connected to an isotonic myograph employing an electromechanical transducer(6). Muscle shortening produced a potential change which was registered on a Dumont 322A cathode ray oscilloscope as a trace that was preserved on 35 mm film. Maximum initial velocities were calculated from enlargements of these photographs. Stimulation of the preparation consisted of brief slightly supramaximal square wave pulses, frequency 105/sec., applied to the muscle through fine, sharply pointed silver wire electrodes. Four-minute intervals of rest were allowed between successive tetani. Subsequent to the testing procedures all muscles were carefully excised and their wet weights determined. Plots of $(P_o - P)/v$ against P, as described by Katz (7) were used to calculate the constants a and b of Hill's equation(5). The small sample t test was utilized in statistical analyses. Probabilities of 0.01 were accepted as evidence for a true difference in mean values.

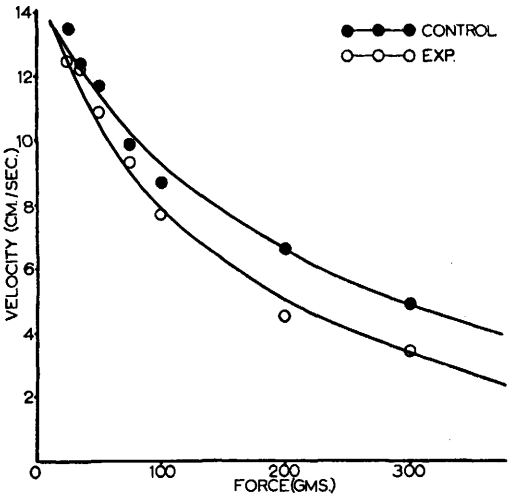

FIG. 1. Force-velocity curves of tetanus toxin shortened and contralateral control gastrocnemii. Circles are observed values; solid lines are theoretical curves.

Results. The solid lines in Fig. 1 represent the theoretical force-velocity curves calculated from Hill's characteristic equation, while the circles indicate actual observed values. Constants of the equation are presented in Table I.

In respect to maximum initial velocity, no statistically significant difference between toxin treated and control muscles can be demonstrated. Probabilities range from $P = .10$ to $P = .90$ for all observations. Although not within the accepted confidence limits, the probability ($<.02$) of a difference between velocity constants b suggests a trend toward slower contraction in the experimental muscle. On the other hand, there is a clear cut, significant difference between the 2 'extra' heats of shortening as evidenced by the constants a.

Discussion. The present evidence that an economy in energy expenditure for shortening is effected by toxin treated muscles, makes it

TABLE I. Means and Standard Errors of Constants in Hill's Equation Derived for Tetanus Toxin Shortened and Contralateral Control Gastrocnemii.

	Control	Exp.	P
a (g wt)	246.5 ±8.5	153.0 ±11.0	.001
b (cm/sec.)	2.94 ± .18	2.50 ± .19	.02
a/P_o	.21	.17	

appropriate to reconsider the several aspects of altered mechanical properties previously noted in these preparations. To the extent that all such observations can be reconciled, one with another, some speculations concerning the fundamental basis for the changes seems desirable and warranted if meaningful future inquiries are to be initiated.

Tension deficit, either twitch or tetanus, is greater than expectations based upon reported atrophy(1), in this case 14%. Because of the considerably shorter equilibrium length (87% of control) in the experimental muscles, an approximation of the cross sectional area from the value of volume/length suggests little change in this index. Certainly, the negligible increase in water content recorded at 14 days post-injection cannot adequately account for the tension deficit. More pertinent, of course, are the depletions of myosin and potassium with concomitant increase of sodium reported by Fischer and Skowlund(8).

A greater decrement in twitch tension than tetanus tension (twitch tension 51%, tetanus tension 73% of control) produces an increased tetanus-twitch ratio(2). What are the characteristics of a toxin treated muscle twitch and tetanus response that may account for the difference in ability to maintain tension development? In experimental muscles the absolute twitch relaxation time relative to the control muscles is shorter. This observation, however, can be misinterpreted unless the actual twitch tension attained is considered. The twitch relaxation time is 73% of control while the peak twitch tension subject to decay amounts to only 51% of control. Thus, in effect, the average unit tension decay is prolonged. This phenomenon is not irreconcilable with the reported increase in maximum proportional rate of relaxation ($-dP/dt/P_o$), since the element $-dP/dt$ by definition describes maximum decay rate and this rate is not well maintained throughout the relaxation period. Indeed, a marked slowing of relaxation rate was noted before complete tension dissipation(2). The observation and interpretation are in accord with the findings of Ranson(9). Moreover, such a delay is evident, as well, in the contraction phase of the twitch response. Contraction time amounts to 90% of control for a total tension, as previously noted, equal to only 51% of control.

The critical fusion frequency—lowest stimulation frequency yielding nearly maximal tension together with complete fusion of the myogram — has been used to calculate the duration of maintenance of the active state at maximum intensity(10). In toxin treated muscles, the active state intensity parameter probably is diminished, but the evidence for a lower fusion frequency(2) implies that the duration parameter is prolonged.

Additional suggestive evidence for prolongation of the active state derives from the observation of an augmented semi-dynamic stiffness in the experimental muscles following bouts of tetanic stimulation. This augmentation is more noteworthy because in the unstimulated condition the semi-dynamic stiffness of these muscles is less than in the contralateral control muscles, *i.e.*, they exhibit an increased compliance. Ritchie and Wilkie (11) showed that the active state in frog sartorius muscles was prolonged by previous stimulation and that prolongation as well as tension development were more marked when the muscle was allowed to contract in tetanus against an increased compliance. From this evidence, one may speculate that in toxin treated muscles the twitch tension is the maximum attainable for the available active state intensity, whereas, the tetanus tension is, in fact, the supplemented response of the tetanus-twitch ratio and is attributable to active state prolongation.

Normally there is little reason to expect a decrease in shortening heat concomitant with active state prolongation. Indeed, Hill's studies(12) on the effects of anions (Br, NO_3, I) on muscle suggest the opposite effect with the distinct difference, however, that in these cases there is an increase in intensity parameter of the active state. When the intensity parameter decreases, as it appears to do in tetanus toxin treated muscles, shortening heat might logically be expected to decline. Each of the above responses can be interpreted in terms of depletion of myosin linkages acting as centers for energy release.

Summary. Investigation of the force-velocity relationship of muscles actively short-

ened by tetanus toxin injection reveals that these preparations exhibit a significant decline in the *a* constant of Hill's characteristic equation. An attempt has been made to interpret the above change in terms of other reported mechanical and chemical changes.

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Study of Lymph Lipids Following Administration of Oleic-1-C^{14} Acid with or without Cholesterol.* (24601)

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It has long been known(1) and confirmed (2-6) that when fatty acids are ingested either as free acids or in various compounds, they appear in lymph principally as triglycerides, the remainder consisting of small amounts of phospholipids, sterol esters and free fatty acids. In these various fractions specific activity has been found to vary according to fatty acid administered, but has generally been less than half that of the fed acid. It thus appears probable that incoming lipids are diluted in lymph in varying degree by endogenous lipids. Since in almost all cases in which labeled free fatty acids have been administered, they were dissolved in a large proportion of vegetable oil, it is probable that this difference between fed labeled and unlabeled acids has had an influence on distribution of activity in lymph lipids. In our experiments, rats were fed oleic-1-C^{14} acid entirely as free fatty acid, either alone or mixed with cholesterol, to assess the differences in lymph lipids brought about by presence of the latter substance during absorption of fatty acids. Lymph lipids were separated into various fractions by chromatography on silicic acid and, where possible, were hydrolyzed to give free fatty acids, which were separated on the reversed phase column of Howard and Martin. From weights and activities of all fractions, it was possible to determine, at least qualitatively, the influence of cholesterol on fatty acid absorption and distribution in lymph.

Materials and methods. Adult male rats weighing about 250 g were fasted for 12 hours before operation. The thoracic duct cannulation procedure was a modification of that described by Bollman, Cain and Grindlay(7). After operation, rats were given 1 ml of either oleic-1-C^{14} acid‡ or a mixture of this acid with cholesterol (25 mol. %) by stomach tube.

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‡ Fed cleic acid consisted of a mixture of oleic-1-C^{14} acid(8) and containing 6.3 x 10^4 disintegrations/sec/mg diluted with inactive oleic acid to mixtures containing 9.0 and 37.6 disintegrations/sec/mg. Composition of the acid determined by reversed-phase chromatography was 90% oleic, 10% linoleic.