times during the first hour, and then at 3, 4, 5, 6, 24, and 48 hours after challenge.

In no case did recipients of normal serum or cells react to ovalbumin challenge. Mice receiving serum from sensitized donors developed edematous reactions of maximum mean diameter (10.6 mm) 3 hours after challenge, often accompanied by petechiae. These reactions lasted, somewhat diminished, through the sixth hour and had disappeared when the next (24-hour) reading was made. They never showed induration or necrosis. Hypersensitized tissue recipients, on the other hand, showed no reactions until the 6-hour reading, when induration, but no edema, was commencing. Induration continued to develop through the 48th hour when mean induration diameter was 1.6 mm and all mice showed central necrosis.

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23 March 1959

Paramyosin and Contraction of "Catch Muscles"

Abstract. The isotonic shortening of glycerol-extracted preparations of molluscan catch muscles is inhibited at *p*H's and ionic strengths at which extracted paramyosin crystallizes. The isometric tension development is hardly altered under the same conditions. Consequently, the "catch mechanism" is explained on the basis of the crystallization of paramyosin.

The unusual features of the mechanical responses and the metabolic activity of certain molluscan and annelid muscles led earlier investigators to postulate a "catch mechanism" (*1*) by means of which these muscles could remain contracted for long periods without a large expenditure of energy (*2*). These muscles can be brought into a condition which resembles a reversible rigor in isolated preparations also by the use of certain stimuli. Resistance to stretch is increased, and the muscle can sustain loads which exceed by several fold the maximal load which it can actively lift. The quick-release phenomenon is missing during this period (*3*). These muscles are also characterized by a periodicity revealed in small-angle x-ray diffraction (*4*) and electron optical studies (*5*). This unique fine structure has been attributed to the presence of paramyosin, a protein which, when extracted and viewed after drying and staining, has a great tendency to show periodic structures (*6*). Paramyosin has been obtained only from the so-called catch muscles, but its amino acid composition shows some similarities to that of proteins of the tropomyosin class (*7*) .

A connection between the presence of paramyosin and the "catch mechanism" is an intriguing possibility. In this report experiments are described which tend to support this view and which provide a model for the control of the elastic properties of "catch muscles" based on the behavior of extracted paramyosin.

For the preparation of paramyosin the adductor muscle of *Venus mercenaria* was blended for 1 minute in 0.1*M* KCl and washed three times in 20 volumes of 0.1*M* KCl. The residue was extracted in 3 volumes of 0.6*M* KCl, containing 0.04*M* *p*H 7.5 Tris buffer. To the extract 3 volumes of 95-percent ethanol was added. The precipitate was resuspended in 0.6*M* KCl containing 0.01*M* neutral phosphate buffer and dialyzed against 10 volumes of 0.6*M* KCl containing 0.01*M* neutral phosphate buffer. Paramyosin went into solution, while actomyosin, which is denatured by ethanol, remained insoluble, and was removed by centrifugation. Paramyosin was crystallized by dialyzing it against 6 volumes of 0.01*M* *p*H 6.0 phosphate buffer and redissolving in neutral 0.6*M* KCl. After recrystallization the protein was stored by lyophilizing it from 0.6*M* KCl solution.

For a second series of experiments, the anterior byssus retractor muscle of *Mytilus edulis* was prepared by removing the muscle, along with a piece of the shell, from the animal and stretching it slightly on a wooden frame. This unit was immersed in aerated sea water for 1 hour and then placed in a 50 percent (by volume) glycerol-water mixture at 0°C for 24 hours. These muscles were stored in 50 percent glycerol at −20°C for at least 2 weeks before use. Cell bundles 100 to 150 μ in diameter were stripped from the muscle for measurements of tension and shortening. Prior to these measurements, the bundles were

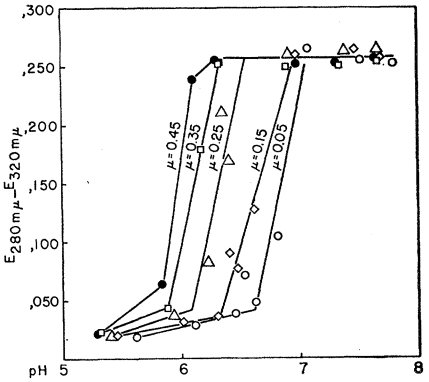

Fig. 1. Solubility of paramyosin. Paramyosin (0.9 mg/ml final concentration) was allowed to stand for 3 hours at room temperature. It was centrifuged, and the protein of the supernatant was measured by ΔO.D. 280 to 320.

equilibrated, first in 20 percent glycerol for ½ hour, and then for 15 minutes in an ATP-free solution of the desired ionic strength and *p*H. The fiber was finally transferred to the ATP-containing solution of identical *p*H and final ionic strength.

Crystallization is an outstanding property of paramyosin (*7, 8*). The protein forms needle shaped crystals—though with no faces and edges—in aqueous media which dissolve when the *p*H is changed by a relatively small amount. It was observed that a change of half a *p*H unit was sufficient to dissolve or reform the crystals (Fig. 1). The point of transition depended on the ionic strength, but stayed between *p*H 6 and 7.5 within the range studied (μ = 0.05 to 0.45), shifting toward the acid side with increasing ionic strength. Neither 0.01*M* $MgCl_2$ nor 0.01*M* $CaCl_2$, when added to the solution, had a measurable effect on the solubility of the paramyosin. Adenosine triphosphate (4m*M*) increased the solubility at lower ionic

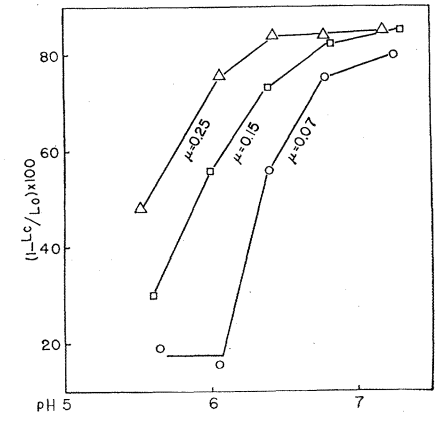

Fig. 2. Isotonic shortening of glycerinated preparations of anterior byssus retractor muscle of *Mytilus edulis*. Ordinate: *Lc,* contracted length; *Lo,* initial length.

strength ($\mu = 0.096$) and had no effect at the higher ones ($\mu = 0.15$ and 0.25) provided its contribution to the total ionic strength at various *p*H's is taken into account. The results were the same when histidine or acetate buffer was used in place of phosphate buffer. Crystallization of paramyosin thus appears to be most influenced by the *p*H of the solution.

The mechanical properties of "catch" muscles could be thus controlled by altering the *p*H of the medium. The control is simple and can be achieved under conditions which alter little the properties of actomyosin, since at low ionic strength the maximum tension and isotonic shortening of a glycerol-extracted psoas muscle fibril remains approximately constant between *p*H 6 and 7. If there is a connection between crystallization of paramyosin and the "catch" mechanism one would expect that the mechanical properties of glycerinated preparations of such muscles will show a *p*H dependence different from that of other muscles.

The isotonic shortening of glycerinated byssus fibers is greatly dependent on *p*H (Fig. 2). In solutions with a total ionic strength of 0.07, 0.15, 0.25, shortening is maximum above *p*H 6.7. Acidification causes a gradual inhibition. At *p*H 6.0 and ionic strength 0.07 the inhibition amounts to about 80 percent, while at higher ionic strengths inhibition occurs in more acid solutions. A comparison of the solubility curves of paramyosin and the data on isotonic shortening indicates that in conditions where paramyosin is crystallized shortening is inhibited. The correlation between cyrstallization and inhibition of shortening is reflected in the dependence on both *p*H and ionic strength. Fibers which failed to contract at low *p*H contracted readily when the *p*H of the solution was raised. Glycerol-extracted preparations of other catch systems, such as *Mytilus* adductor, *Venus* adductor—both tinted and white portions—*Ostrea* white adductor and *Pectin* white adductor, show essentially similar dependence of shortening on *p*H and ionic strength.

The inhibition of shortening was not caused by a reversible inactivation of the actomyosin system. The isometric tension did not show a considerable *p*H dependence and was nearly maximal under conditions where shortening was inhibited by about 80 percent. Thus one fiber developed 0.28 kg/cm² at *p*H 6.0 and another from the same bundle 0.32 kg/cm² at *p*H 7.2 at ionic strength of 0.07. Another fiber of the same bundle developed 0.42 kg/cm² tension at *p*H 6.0 and 0.25 ionic strength. There was, however, a difference in the rate of tension development at various *p*H's, the rate being greater at higher *p*H. Preliminary experiments on the *p*H-dependence of adenosine triphosphatase activity of breis prepared from glycerinated byssus muscles of *Mytilus* indicate that a peak occurs at *p*H 6.3 to 6.5 with little decline between *p*H 6.0 and *p*H 7.0. Although the optimal conditions for tension development in these preparations have not been worked out, the above results indicate little *p*H dependence of tension development, certainly not enough to produce up to 80 percent inhibition of shortening.

These experiments may be simply explained by assuming that actomyosin and paramyosin are at least functionally separated in catch muscles. The behavior of the actomyosin system does not differ much from the actomyosin system of other muscles. The different behavior of "catch muscles" could be explained by the presence of the paramyosin system, the crystallization of which "freezes" the muscle at any length or in any state, inhibiting shortening and increasing the resistance to stretch. It has been found recently that the 145-A periodicity, observed in electron micrographs, is predominantly associated with muscles in the catch state, suggesting that crystallization occurs in these muscles (*9*). In this way the tension developed by the actomyosin system may be preserved by the paramyosin system for an indefinite time without any further active process and without the need for a continuous expenditure of energy. In vivo, the two sytems may be activated independently and, even though it is not necessarily a *p*H variation which activates the catch system, it is of importance that essential features of the "catch mechanism" can be shown by glycerol-extracted preparations.

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Contamination of the Air by Radioactivity from the 1958 Nuclear Tests in the Pacific

Abstract. By the use of bomb-produced tungsten-185 tracer, debris from the 1958 nuclear tests (Hardtack) held at the U.S. Pacific Proving Ground have been identified as they appeared in the ground-level air along the 80th meridian. A large amount of radioactivity from these tests appeared in South America, particularly at the high-altitude collecting stations.

The Hardtack series of nuclear tests in the Eniwetok-Bikini area afforded a unique opportunity to identify radioactive debris in the atmosphere which were associated with a known series of tests, since a number of nuclear devices exploded during that series contained tungsten which became activated by the intense neutron flux. This radiotungsten activity, then, served as a specific radioactive tracer for debris from this one series of tests.

Ground-level air-filter samples from 18 collecting stations located principally along the 80th meridian from Coral Harbour, Northwest Territories, Canada, to Punta Arenas, Chile, were analyzed by radiochemical techniques for β-emitting W^{185} and for a number of high-yield fission products, among them Sr^{90}.

Figure 1 shows the concentration of W^{185} activity in disintegrations per minute per 100 cubic meters of air (corrected to sea-level pressure) for the period of May–July 1958. Background samples collected at all stations during April 1958 showed that at that time there was no background activity of W^{185} in the atmosphere. The rapid spread of this activity is particularly noteworthy. By the end of May it was detected at 10 stations along the 80th meridian from Columbia, South Carolina (34°N), to Antofagasta, Chile (23°S). By the end of June, it was detected from Moosonee, Ontario (51°N) to Punta Arenas (53°S). In July, it appeared at Coral Harbour, (64°N), our northernmost station. The highest W^{185} concentrations appeared initially at the high-altitude sites of Chacaltaya, Bolivia (5220 m), Huancayo, Peru (3353 m), Quito, Ecuador (2818 m), and Bogota,