Abwesenheit des Natriums dem Wert null zustrebt, scheint das extracelluläre Natrium für den Glycintransport essentiell zu sein. Diese Beobachtung erinnert an die Befunde anderer Autoren über die Natriumabhängigkeit der Zuckeraufnahme durch die Darmschleimhaut[6-11]. Inwieweit auch das intracelluläre Natrium für den Glycintransport von Bedeutung ist, lässt sich zur Zeit noch nicht übersehen.

Unsere Befunde bestätigen also nicht die oben Zitierte und von HEMPLING[12] schon kritisierte Hypothese über einen Austauschmechanismus für Kalium und Glycin. Sie sprechen dagegen für einen unmittelbaren Zusammenhang zwischen extracellulärem Natrium und Glycintransport. Über die Natur dieses Zusammenhangs lassen sich zur Zeit nur Vermutungen anstellen. Eine Komplexbildung zwischen Natrium und Aminosäuren ist unter den vorliegenden Bedingungen wenig wahrscheinlich. Dagegen könnte das Natrium eine für den Glycintransport entscheidende Reaktion aktivieren.

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Institut für vegetative Physiologie der Universität Frankfurt a. Main, Frankfurt a. Main (Deutschland)

H. KROMPHARDT
H. GROBECKER
K. RING
E. HEINZ

[1] E. HEINZ, *Biochemie des aktiven Transports*, Springerverlag, Heidelberg, 1961, S. 167.
[2] H. N. CHRISTENSEN, T. R. RIGGS, H. FISCHER UND J. M. PALATINE, *J. Biol. Chem.*, 198 (1952) 1.
[3] T. R. RIGGS, L. M. WALKER UND H. N. CHRISTENSEN, *J. Biol. Chem.*, 233 (1958) 1479.
[4] E. HEINZ, *J. Biol. Chem.*, 211 (1954) 781.
[5] E. HEINZ UND M. WALSH, *J. Biol. Chem.*, 233 (1957) 1488.
[6] E. RIKLIS UND J. H. QUASTEL, *Can. J. Biochem. Physiol.*, 36 (1958) 347.
[7] T. Z. CSÁKY UND M. THALE, *J. Physiol. London*, 151 (1960) 59.
[8] T. Z. CSÁKY UND L. ZOLLICOFFER, *Am. J. Physiol.*, 198 (1960) 1056.
[9] R. K. CRANE, D. MILLER UND J. BIHLER, *Symp. on Membrane Transport and Metabolism, Prag, 1960*, p. 286.
[10] J. BIHLER UND R. K. CRANE, *Biochim. Biophys. Acta*, 59 (1962) 78.
[11] J. BIHLER, K. A. HAWKINS UND R. K. CRANE, *Biochim. Biophys. Acta*, 59 (1962) 94.
[12] H. G. HEMPLING UND D. HARE, *J. Biol. Chem.*, 236 (1961) 2498.

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SC 2279

Effects of ultrasonics on the size and shape of L-myosin and the meromyosins

Recent advances in understanding the structure of L-myosin have resulted mainly from the discovery of meromyosins[1]. In addition to these enzymic subunits of L-myosin, much interest has been centered on its chemical subunits, for example, those obtained in guanidine hydrochloride[2]. This communication deals with the physical degradation of L-myosin and the meromyosins exposed to ultrasonic vibration.

L-Myosin, heavy meromyosin, and light meromyosin, 6–8 mg/ml, in 0.6 M KCl (pH 7.0) were vibrated at 20 kcycles at 2–10°. The effects of vibration on the myosins were followed in the Spinco analytical ultracentrifuge at 20°, and by viscometry.

The sedimentation coefficients (in Svedberg units) of L-myosin extrapolated to zero concentration were 6.4, 6.7, 7.1, 7.4, 7.7 S, those of heavy meromyosin 7.0, 6.6, 6.2, 5.7, 5.0 S, and those of light meromyosin 3.0, 3.0, 3.0, 2.9, 2.9 S for a vibration time of 0, 10, 20, 30, and 40 min, respectively. Fig. 1 shows the sedimentation coefficients of these proteins as a function of time of vibration at a single concentration, 4.8 mg/ml. Fig. 2 illustrates the ultracentrifugal patterns for 0 and 40 min of vibration time. Since the sedimentation of L-myosin increases, that of heavy meromyosin decreases, and that of light meromyosin remains nearly constant, one can conclude that the effects of ultrasonics on these proteins are entirely different.

These variations in the sedimentation were accompanied by a fall in the viscosity. The intrinsic viscosity values of L-myosin were 2.15, 1.44, 1.00, 0.66, 0.40, those of heavy meromyosin 0.42, 0.32, 0.27, 0.23, 0.20, and those light meromyosin 1.00, 0.90, 0.82, 0.75, 0.70 for a vibration time of 0, 10, 20, 30, and 40 min. In other words, ultrasonic treatment changes the viscosity of L-myosin extensively, but that of light meromyosin to a small extent.

The large decrease in viscosity of L-myosin, with the simultaneous increase in its sedimentation, indicates a change in the shape of the L-myosin molecule. Such

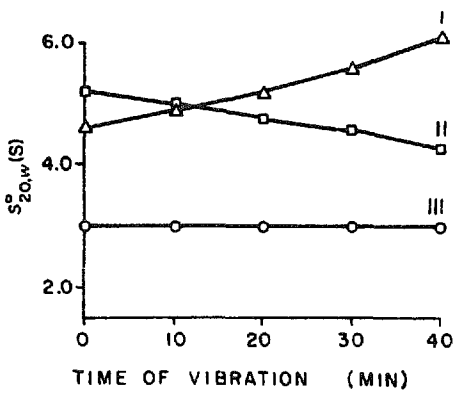

Fig. 1. The sedimentation coefficients as a function of time of vibration. i (△—△), L-myosin; ii (□—□), heavy meromyosin; iii (○—○), light meromyosin.

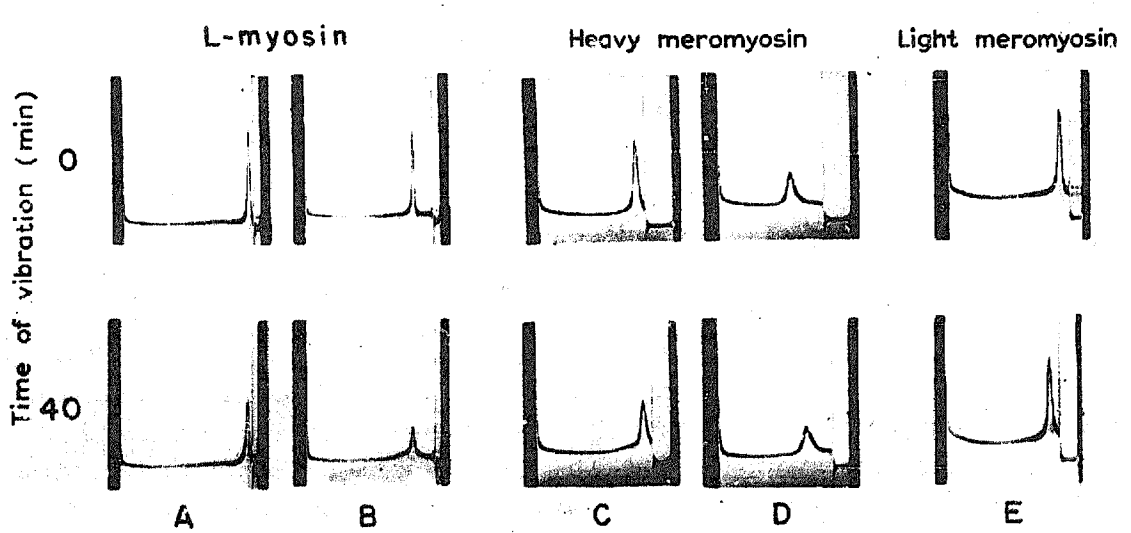

Fig. 2. Ultracentrifugal patterns of L-myosin (6.7 mg/ml), heavy meromyosin (6.3 mg/ml) and light meromyosin (6.5 mg/ml). Sedimentation proceeds to the left. Photographs were taken in rows A, B, C, D, E, after 10, 70, 20, 80 and 30 min, respectively.

a change can be caused by a coiling or by breaks of the L-myosin molecule and subsequent rearrangements of the fragments. To differentiate between these two possibilities, we treated the L-myosin sonicates with 5 M guanidine·HCl according to KIELLEY AND HARRINGTON[2]. Fig. 3 shows that the sedimentation coefficient of L-myosin and its sonicates in 5 M guanidine·HCl continuously decreases from 4.2 to 1.7 S during a vibration time of 0–40 min. This significant decrease of the sedimentation coefficient of sonicated L-myosin in guanidine·HCl, in contrast to its increase in the absence of guanidine·HCl (Fig. 3), excludes a mere coiling of the L-myosin molecule without cleavage of its backbone. On the other hand, it is well known that L-myosin has a pronounced tendency for side-to-side aggregation (*e.g.* see ref. 3). It appears, therefore, that the L-myosin molecules are split by sonic treatment and then some fragments combine with the parent molecule.

KIELLEY AND HARRINGTON[2] have concluded from their physicochemical studies that L-myosin does not have a uniform diameter. RICE[4] has shown by electron microscopy that the L-myosin molecule is rod-like with a wider globular head on one end. LOWEY AND COHEN[5] suggested that heavy meromyosin has a non-uniform mass distribution, whereas light meromyosin is uniform. The results of this study are in agreement with these data of the literature. The compact structure of light meromyosin is also reflected in its relative resistancy to ultrasonic treatment. The non-uniform heavy meromyosin, on the other hand, is readily fragmented by ultrasonics. It is reasonable to assume, therefore, that in an end-to-end arrangement of heavy meromyosin and light meromyosin parts in the L-myosin molecule, its heavy

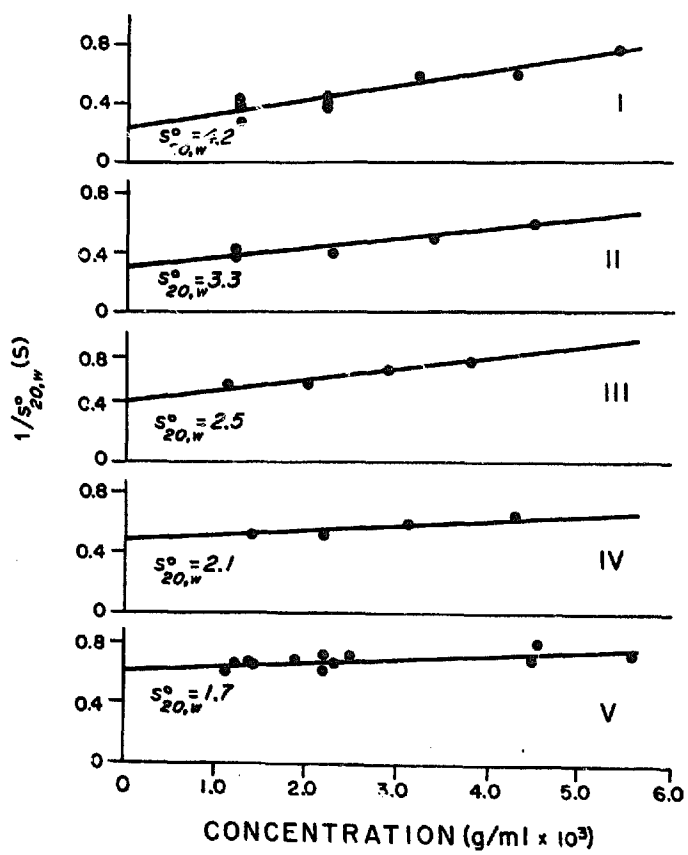

Fig. 3. Concentration dependence of the reciprocal of the sedimentation coefficients of L-myosin sonicates in 5 M guanidine·HCl. Time of vibration: zero for i, 10 min for ii, 20 min for iii, 30 min for iv, 40 min for v.

meromyosin-end is fragmented in the sonic field. These heavy meromyosin fragments will then be attached to the thinner portion of the L-myosin. In this way the molecule becomes less asymmetric, thus explaining the increase in its sedimentation and the simultaneous decrease in its viscosity.

It is interesting to compare mechanical and chemical degradations. SZENT-GYÖRGYI AND BORBIRO[6] treated the meromyosins with urea and found that light meromyosin depolymerized into small subunits, whereas heavy meromyosin did not undergo such a splitting. On the contrary, in case of ultrasonic treatment, the light meromyosin remained nearly unchanged compared with the pronounced breakdown of heavy meromyosin. These findings clearly indicate that the mechanism of the mechanical and chemical degradations are different.

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K. BÁRÁNY
M. BÁRÁNY
H. OPPENHEIMER
T. T. ANTONY

Institute for Muscle Disease, Inc.,
New York, N.Y. (U.S.A.)

[1] E. MIHÀLYI AND A. G. SZENT-GYÖRGYI, *J. Biol. Chem.*, 201 (1953) 189, 197, 211.
[2] W. KIELLEY AND W. F. HARRINGTON, *Biochim. Biophys. Acta*, 41 (1960) 401.
[3] S. LOWEY AND A. HOLTZER, *J. Am. Chem. Soc.*, 81 (1959) 1378.
[4] R. V. RICE, *Abstr. Biophys. Soc.*, 7th Annual Meeting, 1963, New York.
[5] S. LOWEY AND C. COHEN, *J. Mol. Biol.*, 4 (1962) 293.
[6] A. G. SZENT-GYÖRGYI AND M. BORBIRO, *Arch. Biochem. Biophys.*, 60 (1956) 180.

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Quantitative estimation of urokinase and bacterial plasminogen activators using a fibrin substrate formed from plasminogen-free fibrinogen

A quantitative fibrinolytic method for estimating bacterial activators of plasminogen (streptokinase and staphylokinase) has been described using as a source of fibrin commercial bovine fibrinogen[1]. Briefly, the method consists in clotting a mixture of the activator, bovine fibrinogen and human plasminogen with human thrombin. The clots are incubated at 37° for 1 h, activation of plasminogen to plasmin taking place simultaneously with fibrinolysis. Any undigested fibrin is dissolved in strong urea solution thus releasing from within the clot products of plasmin digestion. The fibrin is precipitated with trichloroacetic acid and acid-soluble products of fibrinolysis determined spectrophotometrically in the filtrate.