sulin target tissues. It should be emphasized, however, that although ILM exerts super-insulin effects on both insulin-sensitive and insulin-insensitive mammary cells, ILM exerts super-insulin effects only on insulin-refractory epididymal fat pad and diaphragm from C57Bl/6J ob^{++}/ob^{++} mice. With the corresponding tissues from lean littermates, the effect of ILM is no greater than that of insulin. It is evident that appropriate target tissue must be used in assessing superactivity of ILM. From these results it might be predicted that any palliative advantage of ILM would be to tissues that do not respond to insulin itself.

It has been shown recently (*5*) that ILM is an N^1,N^2-disubstituted guanidine in which bovine serum albumin and insulin are the substituents.

Superactive placental lactogen and prolactin have also been prepared from the corresponding Sepharose complexes (*6*). It may be possible to demonstrate superactive forms of other peptide hormones prepared in a similar way, particularly if they are tested on target cells that are deficient in their response to the native hormone.

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Adenosine Diphosphate Effect on Contractility of Human Muscle Actomyosin: Inhibition by Ethanol and Acetaldehyde

Abstract. *Magnesium adenosine triphosphate (Mg^{2+}-ATP) is known to produce dissociation of muscle actin and myosin in vitro, while its hydrolysis leads to reassociation. The interaction of purified actin and myosin from human muscle, in the presence of Mg^{2+}-ATP, was stimulated by minute amounts of adenosine diphosphate (ADP), a product of ATP hydrolysis. By contrast, the dissociation of the actomyosin complex was inhibited by ADP. These data suggest that ADP serves to modulate muscle contraction. Ethanol and its primary metabolite, acetaldehyde, inhibited these effects of ADP. The inhibition was reversible when the preparations were freed of these compounds. The effects of ethanol and acetaldehyde on the response of actomyosin to ADP may play a role in the pathogenesis of alcoholic myopathy and cardiomyopathy.*

The biochemical events of muscle contraction and relaxation involve association and dissociation of actin and myosin in the presence of Ca^{2+}, Mg^{2+}, and adenosine triphosphate (ATP) (*1*). The hydrolysis of high-energy ATP to adenosine diphosphate (ADP) by the adenosine triphosphatase activity of actomyosin provides the energy for contraction (*2*). Magnesium activates the hydrolysis of ATP, while calcium modulates this activity via the relaxing protein complex (*3*). Recently we postulated a modulatory function for ADP because of its ability to enhance the association of actin and myosin from human platelets, in the presence of Mg^{2+}-ATP, and to inhibit their dissociation (*4*). We therefore investigated the role of ADP in the association and dissociation of human muscle actomyosin in the presence of Mg^{2+}-ATP. In view of our previous demonstration that chronic ethanol consumption by human volunteers leads to alterations of skeletal muscle (*5*), we also studied the effects of ethanol and its primary metabolite, acetaldehyde, on this process.

Alcoholic myopathy and cardiomyopathy are well-defined syndromes, characterized principally by muscle weakness and occasionally by necrosis (*6*). In human volunteers, chronic ethanol ingestion, with an adequate diet, resulted in subclinical myopathy (*5*). Moreover, we have shown that, in vitro, ethanol inhibits Ca^{2+} transport into and out of the sarcoplasmic reticulum (*7*) and decreases the $(Na^+ + K^+)$-stimulated adenosine triphosphatase activity of muscle plasma membranes (*8*), functions intimately involved with muscle contraction and relaxation.

Fresh skeletal muscle (vastus medialis or vastus lateralis) was obtained from tissue incidentally removed from patients undergoing orthopedic procedures. The patients had no clinical or laboratory evidence of muscle disease or trauma to these muscles. The muscle appeared normal by light and electron miscroscopy. Actomyosin was prepared from the fresh muscle, as described previously (*9*). By sodium dodecyl sulfate disc electrophoresis, these preparations displayed four major bands, corresponding to myosin, actin, and components of the troponin-tropomyosin system (*10*). There was no myokinase activity or evidence of contamination by other constituents of the cell. Protein concentration was determined by the method of Lowry *et al.* (*11*). Superprecipitation, as an index of the association of actin and myosin (*12*), was measured by the change in absorbance at 620 nm in a Gilford recording spectrophotometer. In a final volume of 1 ml, before the addition of ATP, 0.5 mg of muscle protein was suspended in 0.15*M* KCl plus 1 μM Ca^{2+}, buffered with 15 to 25 m*M* tris(hydroxymethyl)aminomethane acetate (*p*H 6.8), to obtain a zero setting. Increasing concentrations of ADP were added to the protein suspension before the addition of Mg^{2+}-ATP. At zero time 1 m*M* ATP plus 2 m*M* Mg^{2+} were added to the suspension, and the reaction was allowed to proceed at 25°C. The effective Mg^{2+} and ATP concentrations varied from 0.5 to 3 m*M* and 0.5 to 5 m*M*, respectively. Clearing was observed as a decrease in absorbance (relaxation), whereas the succeeding superprecipitation caused increased absorbance (contraction) (*4*). When the reaction mixture contained added ADP, an increase in the ADP concentration (50, 75, and 100 μM) decreased both the duration and extent of the clearing phase (Fig. 1). The apparent Michaelis constant (K_m) for the action of ADP on clearing was 250 μM (Fig. 1). At the end of the clearing phase a rapid increase in turbidity indicated the beginning of superprecipitation. Increasing the concentration of ADP accelerated the onset of superprecipitation.

The inhibitory effects of both ethanol and acetaldehyde on superprecipitation were also evident in the presence of added ADP. Increasing amounts of ethanol or acetaldehyde (Fig. 1) proportionally inhibited both effects of ADP, namely the association and dissociation of actin and myosin. The inhibitory constants (K_i), half-maximal effects for both compounds determined from the double reciprocal plots, were 135 m*M* for ethanol and 170 μM for acetaldehyde. The effects of ethanol and acetaldehyde on muscle actomyosin were reversible; when they were removed from the protein by dialysis, the preparations were normally activated in the presence of ADP and were susceptible to inhibition by newly added ethanol or acetaldehyde. Methanol, propanol, and glycerine had no effect on either adenosine triphosphatase activity or superprecipitation at concentra-

Table 1. Effect of ethanol, acetaldehyde, and ADP on the adenosine triphosphatase activity of human muscle actomyosin. Determinations were carried out at 25°C for 15 minutes on 0.2 mg of human muscle actomyosin. The values obtained were from three different protein preparations, each assayed in duplicate. Ethanol (150 m*M*) and acetaldehyde (150 μM) were added 10 minutes before Mg^{2+}-ATP. When ethanol or acetaldehyde were added together with ADP, the concentrations were the same as when these compounds were added singly.

Preparation	Adenosine triphosphatase activity (nanomoles of P_i per milligram of protein per minute)					
	Mg^{2+}-ATP	Plus ethanol (150 m*M*)	Plus acetaldehyde (150 μM)	Plus ADP (100 μM)	Plus ethanol (150 m*M*) and ADP (100 μM)	Plus acetaldehyde (150 μM) and ADP (100 μM)
1	63.4	61.0	62.0	76.3	64.7	62.8
	61.7	59.0	60.0	77.4	66.7	63.9
2	57.0	55.0	54.0	67.0	57.4	55.1
	53.5	52.0	51.0	65.4	55.9	52.7
3	72.0	71.0	70.0	84.9	71.6	70.5
	76.3	73.0	74.0	85.8	74.2	73.1

tions comparable to those employed with ethanol.

The effect of ADP on the Mg^{2+}–adenosine triphosphatase activity of human muscle actomyosin was also determined. Adenosine triphosphatase activity was determined by the release of inorganic phosphorus (P_i) from ATP according to the method of Marsh (*13*), adapted to detect as little as 3 nmole of P_i (*14*). Each milliliter of final assay mixture contained 0.04*M* imidazole buffer (*p*H 6.8), 0.06*M* KCl, 0.5 m*M* ATP, 1 m*M* $MgCl_2$, and 0.1 m*M* ouabain. In some mixtures, ADP was added to the protein 5 minutes before addition of ATP. The amount of protein added to the final mixture was 0.2 mg. The reaction was stopped by adding 0.4 ml of 20 percent trichloroacetic acid (TCA) per milliliter of mixture. Blanks consisted of protein inactivated by TCA before the addition of ATP. Adenosine triphosphatase activity was measured by the difference between P_i at a particular time and P_i at zero time. The values obtained were comparable to those reported by others (*15*). Addition of 100 μM ADP produced a consistent increase in Mg^{2+}–adenosine triphosphatase activity (Table 1). Ethanol (*16*) or acetaldehyde did not alter the Mg^{2+}–adenosine triphosphatase activity of human muscle actomyosin (Table 1). Previous incubation of actomyosin with ethanol or acetaldehyde for 15 minutes also had no effect. Adenosine diphosphate (100 μM) increased Mg^{2+}–adenosine triphosphatase activity by about 20 percent (Table 1). Both ethanol and acetaldehyde blocked the stimulation of Mg^{2+}–adenosine triphosphatase activity by ADP.

To our knowledge, this is the first report in which ethanol and acetaldehyde have been found to influence the biological activity of structural proteins in muscle cells. The data suggest that both ethanol and acetaldehyde, in concentrations which may be found in the blood of human alcoholics, can alter the contractile activity of muscle actomyosin. These compounds apparently do not change the Mg^{2+}–adenosine triphosphatase activity of actomyosin or the response of this complex to ATP, but exert their inhibitory effects by blocking stimulation of contractile activity by ADP. The effects described apparently occur under relaxing conditions, since in the absence of clearing, minimal inhibition by these compounds is observed. This inhibition may reflect effects of ethanol and acetaldehyde on actin or myosin, or both. On the other hand, since the isolated actomyosin contains troponin and tropomyosin, ethanol and acetaldehyde may affect the regulatory function of this relaxing system.

These direct effects of ethanol and acetaldehyde may be superimposed on other chronic effects in the production of alcoholic myopathy and cardiomyopathy.

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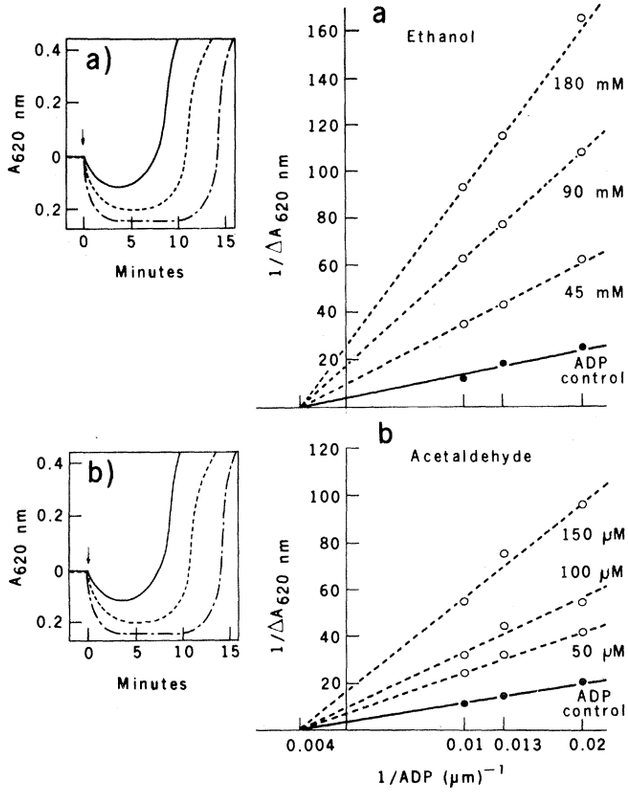

Fig. 1. Lineweaver-Burke plot of the effect of increasing concentration of (a) ethanol (45, 90, and 180 m*M*) and (b) acetaldehyde (50, 100, and 150 μM) on the clearing phase of actomyosin superprecipitation. The data are from the superprecipitation curves shown as insets on the left. The arrows indicate addition of Mg^{2+}-ATP. In the insets the solid line shows the effect of ADP; the dashed line, the effect of ADP plus ethanol or acetaldehyde; and the dotted-dashed line, the superprecipitation pattern of human actomyosin without ADP, ethanol, or acetaldehyde. Ethanol and acetaldehyde inhibited the effect of ADP noncompetitively. Abbreviation: *A*, absorbance.

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16. Barely detectable trace amounts of acetaldehyde were found by gas chromatography.
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