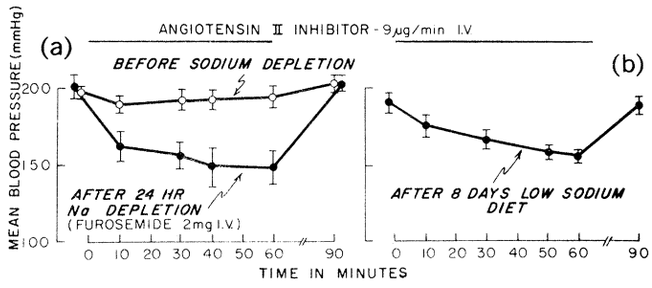

Fig. 2. (a) Blood pressure response to angiotensin II blockade 15 weeks after the clipping of one renal artery, before and after sudden sodium depletion. (b) Blood pressure response to angiotensin II blockade 15 weeks after the clipping of one renal artery and after the animals were deprived of dietary sodium for the 8 days preceding testing.

sure by 43.0 ± 7.6 mm-Hg ($P < .01$) after 10 minutes and a maximum fall of 55.0 ± 10.3 mm-Hg after 60 minutes, a similar response in pressure to that seen in group 1.

In the rats of group 3, 14 to 15 weeks after the animals were clipped and after they had been placed on a low sodium diet for 8 days prior to test, the angiotensin II inhibitor induced a significant fall in blood pressure of 28.1 ± 4.2 mm-Hg ($P < .01$) at 10 minutes and a maximum fall of 46.4 ± 6.4 at 60 minutes ($P < .01$) (Fig. 2b). Balance studies in five animals showed a mean sodium loss of 2.8 ± 0.3 meq after the final 8-day period. However, the body weight showed only a transient fall during the first 2 to 3 days, but then rose slightly toward the end of the 8-day period on a low sodium diet (428 ± 9 g as compared to the initial weight of 420 ± 10 g).

These experiments indicate that the two-kidney Goldblatt model of renovascular hypertension, often considered a typical model of renin dependent hypertension, is subject to a change in its mechanism, presumably resulting from changes in the sodium metabolism. Thus, infusion of the angiotensin blocker produced a lowering of blood pressure at 4 weeks, confirming that at this stage the hypertension is renin dependent (*2*). However, 10 weeks later the response to the blocker became negligible, indicating that angiotensin no longer appeared to play a role in the maintenance of the high blood pressure and that sodium retention with ensuing volume expansion was probably the main operating mechanism. Sodium depletion over a period of hours by the use of diuretics alone did not cause any fall in the blood pressure, probably because of stimulation of renin release, as suggested by the dramatic fall of blood pressure when infusion of the angiotensin blocker was repeated. More gradual sodium depletion of the same magnitude, achieved by dietary salt deprivation over a longer period, had the same effect of reinstituting the renin dependency of the hypertension.

This sequence of events in two-kidney Goldblatt hypertension thus resembles that already described in the one-kidney Goldblatt model (*6*). Both models exhibit a renin dependent early phase followed by a volume dependent phase later on, during which a latent role for renin can still be exposed by sodium deprivation. In the two-kidney model this transition occurs in several weeks and may be attributed to the presence of a normal contralateral kidney, which at first excretes sodium freely in reaction to the raised systemic blood pressure. However, the continuing exposure of this initially normal kidney to raised arterial pressure, perhaps combined with elevation in the circulating renin level, may produce secondary vascular damage in this kidney and lead to impaired sodium excretion, to volume expansion, and then to suppression of renin release from the clipped kidney (*10*); at this point removal of the clipped kidney no longer normalizes the blood pressure (*11*). In the one-kidney model a considerable loss of total renal function occurs immediately, resulting in a decrease of capacity for sodium excretion and thus earlier sodium retention (*4*).

A similar mechanism may be applicable in renal and even certain forms of essential hypertension in man. According to this hypothesis, patients exhibiting "normal" or even low renin levels might have actually had an elevation of renin levels earlier, which could have produced subtle kidney damage sufficient to sustain the hypertension with the subsequent lesser or latent participation of renin. In this regard, essential hypertensive patients with low renin levels have been found to be older than essential hypertensive patients with normal renin levels (*12*).

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13. Supported by PHS grant HL-14148.
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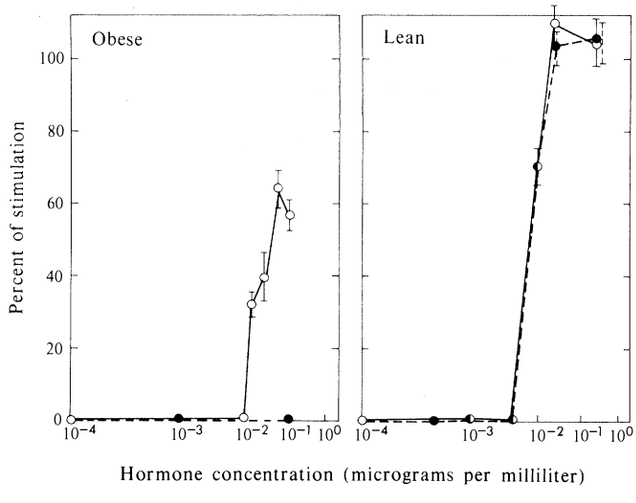
16 January 1975

Insulin-Unresponsive Tissues Respond To Superactive Insulin-Like Material

Abstract. *Insulin-like material prepared from insulin-Sepharose stimulates glucose oxidation by isolated diaphragm of C57Bl/6J ob/ob mice, but insulin does not. This material is much more effective than insulin on epididymal fat tissue from these mice. Insulin-like material and insulin are equipotent on the corresponding tissues from lean littermates.*

It has been reported (*1*) that soluble insulin-like material (ILM) can be produced by treating insulin-Sepharose with bovine serum albumin solution, and that the material solubilized in this manner elicits unusual insulin-like responses from mammary epithelial cells. The ILM exerts a greater stimulatory effect than insulin does on the accumulation of α-aminoisobutyric acid by mammary cells from pregnant



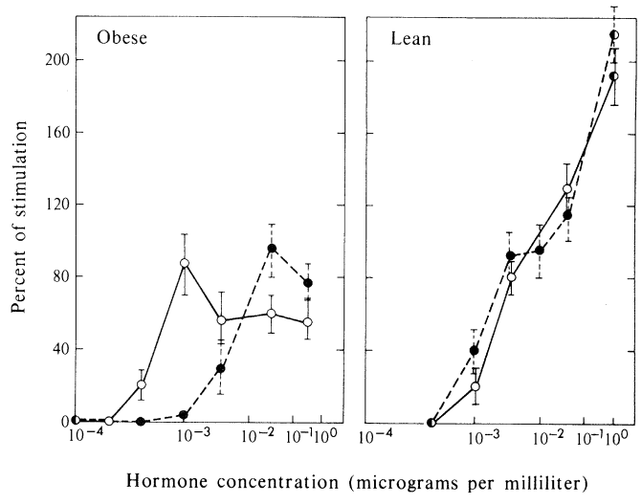


Fig. 1 (left). Effect of insulin and ILM on glucose oxidation in the diaphragm of C57Bl/6J mice. Obese and lean C57Bl/6J mice (2 to 4 months old) (Jackson Laboratory, Bar Harbor, Maine) were killed by cervical dislocation, and the diaphragms were removed, washed in medium 199 (Gibco), and cut into several pieces, each weighing 2.5 to 7 mg wet weight. The tissue was incubated in 25-ml Erlenmeyer flasks with 2 ml of medium 199 containing 2.5 percent crystalline bovine serum albumin, 0.040*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (*p*H 7.2), 0.1 to 0.2 percent glucose, and 0.5 μc of D-[1-^{14}C]glucose (New England Nuclear; specific activity, 7.99 mc/mmole), with the indicated concentration of ILM or insulin. The concentration of ILM, expressed as insulin-equivalent, was calculated from the protein content of, and the total radioactivity in, the [^{125}I]insulin-Sepharose, as described previously (*1*). The flasks were closed with a rubber cap from which a polyethylene well was suspended. The gas phase was air. The flasks were shaken in a water bath at 37°C for 60 minutes (for lean mice), and either 60 or 90 minutes (for obese mice), depending on which period corresponded to the greater effect of ILM. Incubations were terminated by injecting 0.2 ml of NCS (Nuclear-Chicago solubilizer; Amersham/Searle) into the center well and 0.5 ml of 5*N* H_2SO_4 into the incubation medium. Shaking of the vessels was continued for another 30 minutes, and then the NCS was quantitatively transferred to counting vials. Radioactivity was determined in a mixture of 2,5-diphenyloxazole, 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene, and toluene in a liquid scintillation spectrometer. The results are expressed as percent of stimulation of $^{14}CO_2$ production in the presence of varying concentrations of insulin or ILM, compared to controls without hormone. The controls varied from 150 to 650 count/min per 5 mg of tissue from obese mice and 200 to 650 count/min per 5 mg of tissue from lean mice. The results have been confirmed with hemidiaphragms, the usual system of choice. Each point represents the mean ± standard error of the mean (S.E.M.) of three to ten separate experiments. ○, With ILM; ●, with insulin. Fig. 2 (right). Effect of insulin and ILM on glucose oxidation in the epididymal fat pad of C57B1/6J mice. Obese and lean 2- to 4-month-old male littermates were used. The epididymal fat pads were cut into several pieces, each weighing 20 to 150 mg. Glucose oxidation was determined as described in the legend to Fig. 1. Incubation lasted 60 minutes. The values of controls without added hormone varied from 200 to 400 count/min per 10 mg of tissue in the case of lean mice and from 100 to 200 count/min per 10 mg of tissue in the case of obese mice. The results are expressed as percent of stimulation by insulin or ILM compared to the systems without added hormone. Each point represents the mean ± S.E.M. of three to six separate experiments. ○, With ILM; ●, with insulin.

mice, and it is also more efficacious than insulin as a stimulant of DNA synthesis and the combined activities of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and phosphogluconate dehydrogenase (E.C. 1.1.1.43). The specific biological activity of ILM on these cells is more than five times greater than that of insulin. Moreover, ILM stimulates the accumulation of α-aminoisobutyric acid by virgin mouse mammary cells—cells that are unresponsive to insulin itself. We have termed this material "super-insulin" (*1*).

It was of interest to inquire whether ILM could also evoke insulin-like responses from tissues of mutant strains of certain mice that are known to exhibit a more general insulin-resistance. To this end, tissues from C57Bl/6J $\frac{ob}{ob}$ mice have been used (*2*). It will be shown that ILM is more efficacious than insulin in these instances, too.

The effect of a wide range of concentrations of insulin and ILM on the oxidation of [1-^{14}C]glucose to $^{14}CO_2$ in the diaphragm of obese and lean mice is shown in Fig. 1. In agreement with earlier findings (*3, 4*), the tissue from obese mice was unresponsive to insulin at all the concentrations examined. In some instances production of $^{14}CO_2$ was even lower in the presence of insulin than in the absence of the hormone. The diaphragm from obese mice, however, did respond to ILM at a concentration as low as 2×10^{-2} μg/ml, and the maximum effect was produced at 5×10^{-2} μg/ml. In this respect, diaphragm from the obese C57Bl/6J mouse is almost as sensitive to ILM as the tissue from the lean mouse is to insulin (see below). In the diaphragm from lean mice insulin and ILM are equipotent. Both stimulated $^{14}CO_2$ production at a concentration of 10^{-2} μg/ml, and the stimulation was maximum at 2×10^{-2} μg/ml. It should be noted that the magnitude of response to ILM is greater in the tissue from lean mice than it is in tissue from obese ones.

Studies on the effect of insulin and ILM on glucose oxidation in the epididymal fat pad are shown in Fig. 2. Glucose oxidation in the tissue from obese mice was stimulated by insulin at a concentration as low as 5×10^{-3} μg/ml, and the maximum effect was produced at 5×10^{-2} μg/ml. These results are in contrast to those obtained with the diaphragm, which did not respond to insulin. Such a difference in tissue responsiveness has been reported previously (*4*).

The ILM also stimulated $^{14}CO_2$ production in the fat pad from obese mice, but did so at a much lower concentration than insulin. The minimum effective concentration was 5×10^{-4} μg/ml, about ten times lower than that for insulin. The maximum increase was obtained at a concentration of 10^{-3} μg of ILM per milliliter, which is about 50 times lower than the concentration of insulin required to produce the same effect. Thus ILM is much more potent than insulin in this tissue, too.

The fat pad from lean mice is very sensitive to both insulin and ILM. Both agents stimulated glucose oxidation equally over a large range of concentrations. The extent of response was again larger in tissue from lean mice than in that from obese animals.

The results of this and of an earlier report (*1*) indicate that the superactivity of ILM can be manifested on a variety of in-

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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12 November 1974; revised 15 January 1975

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-