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Some Characteristics of the Insulin Augmentation Phenomenon

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A serum protein fraction possessing the capacity to augment insulin action on adipose tissue *in vitro* has been isolated and studied. Factors responsible for augmentation have been found to be nondialyzable and susceptible to de-

struction by ethanol, alkali and heat. The augmentation phenomenon is probably dependent upon the presence of a protein moiety. (*Metabolism* 14: No. 9, September, 945-949, 1965)

FACTORS IN HUMAN SERUM capable of augmenting the action of crystalline insulin on the epididymal fat pad have been found to be most concentrated in the faster-moving gamma-globulin and beta-globulin proteins.¹ A pool of these globulins was isolated from the serum of normal subjects for the studies described in this report. The pooled globulin was treated by exposure to a wide pH range, to ethanol, and to heat; and the resultant effects of such pretreatment on the augmentation phenomenon then observed.

MATERIALS AND METHODS

Serum was obtained in the fasting state from 5 healthy, nonobese adults without family histories of diabetes and with normal carbohydrate tolerance. The serum was stored at -20 C. and within 3 weeks separated into albumin and globulin components by dialysis against ammonium sulfate. The globulin component was fractionated by elution from diethylaminoethyl (DEAE) cellulose as previously described.² Fraction no. 1 was discarded. Fractions nos. 2 and 3 were eluted, pooled, dialyzed against an infinite volume of distilled water, lyophilized and stored at -20 C. All studies were performed with the pooled material within 12 weeks of its isolation.

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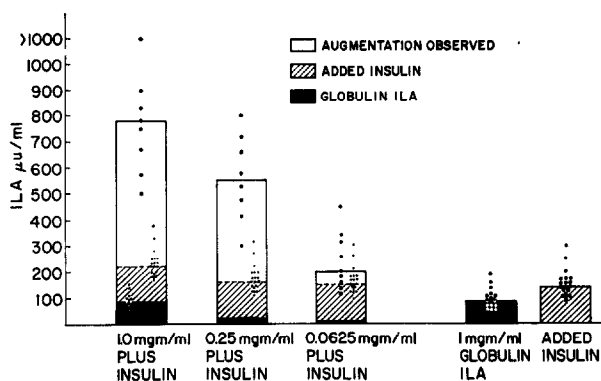


Fig. 1.—Dose response pattern of globulin augmentation of insulin activity. Three concentrations of globulin were used with a constant insulin concentration.

For each study, 3 independent variables were determined—the insulin-like activity (ILA) of the pooled protein fraction itself, the insulin activity of the added insulin in each study, and the insulin-like activity of a solution containing both pooled protein fraction and crystalline insulin. Augmentation is identified in the illustrations by the open area at the top of each column. It represents the difference between the sum of the individual activities (determined separately) and the ILA actually obtained when they were combined in the same solution. The method of assay has been previously reported.³

RESULTS

Dose Response Pattern of Augmentation

Three concentrations of the pooled protein fraction—1.0 mg./ml.; 0.25 mg./ml.; and 0.0625 mg./ml.—were assayed in Krebs bicarbonate buffer containing a standard amount of crystalline insulin and a dose response effect on the augmentation phenomenon was demonstrated (fig. 1).

Effect of Heat Pretreatment

Pooled protein fraction was dissolved in 0.05 M phosphate buffer pH 7.4. An aliquot was held at room temperature as a control. Other aliquots were placed in a water bath at 75 C. for 1 minute, 2 minutes and 5 minutes. All aliquots were then dialyzed against an infinite volume of distilled water at 2 C. for 24 hours, lyophilized and assayed for both ILA and their capacity to augment. Heating to 75 C. reduced both the insulin-like activity and augmentation capacity of the protein fraction (fig. 2).

Effect of pH Pretreatment

Aliquots of the pooled protein fraction were dissolved in 0.05 M phosphate buffer pH 7.4, then dialyzed at 2 C. for 24 hours with agitation against an infinite volume of 0.05 M phosphate buffer at pH 1.0, 4.0, 7.4, 9.0 and 11.0. Extremes of pH were achieved by addition of HCl or NaOH to the buffer bath. All aliquots were then dialyzed for 24 hours more at 2 C. against an infinite volume of distilled water, lyophilized and assayed for ILA and augmentation

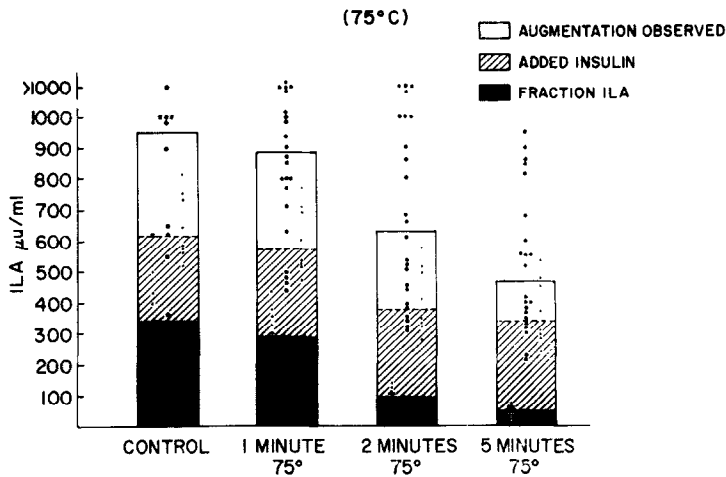


Fig. 2.—Effect of heat pretreatment of globulin on its capacity to augment insulin activity. Globulin aliquots in solution were exposed to 75 C for 3 periods of time.

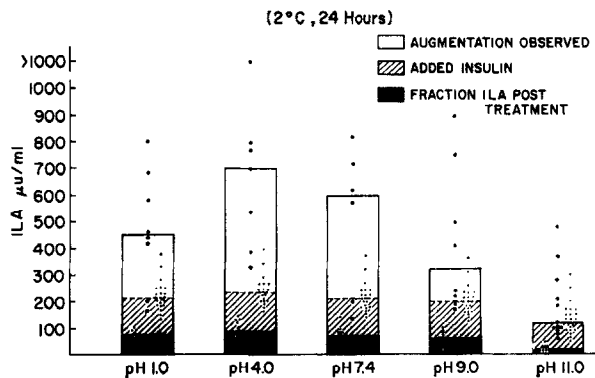


Fig. 3.—Effect of pH pretreatment of globulin on its capacity to augment insulin activity. Globulin aliquots in solution were dialyzed against 5 pH concentrations at 2 C for 24 hours.

(fig. 3). The acid pH was without significant effect on ILA or augmentation but at pH 11.0 both were destroyed.

Effect of Ethanol Pretreatment

Aliquots of the pooled protein fraction were dissolved in normal saline, and two concentrations of ethanol (40 and 70 per cent) in normal saline. Exposure took place at 25 C. for 1 hour, after which the fractions were dialyzed 24 hours at 2 C. against an infinite volume of distilled water, lyophilized and assayed for ILA and augmentation (fig. 4). This treatment led to a considerable reduction in augmentation and less reduction in pooled globulin ILA. This is the first indication of a differential susceptibility of the two properties, augmentation appearing to be more susceptible to ethanol denaturation than ILA itself.

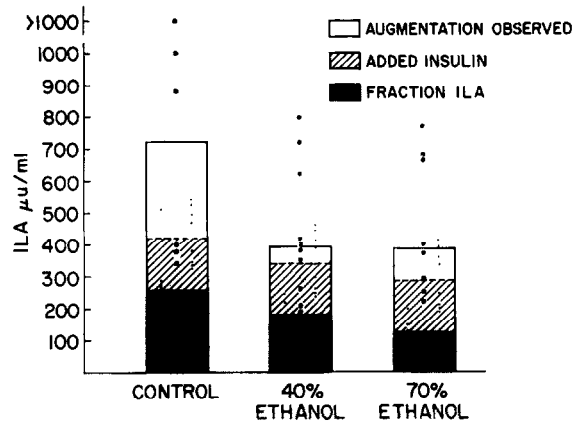


Fig. 4.—Effect of ethanol pretreatment of globulin on its capacity to augment insulin activity. Globulin was exposed to 2 concentrations of ethanol at 25 C for 1 hour.

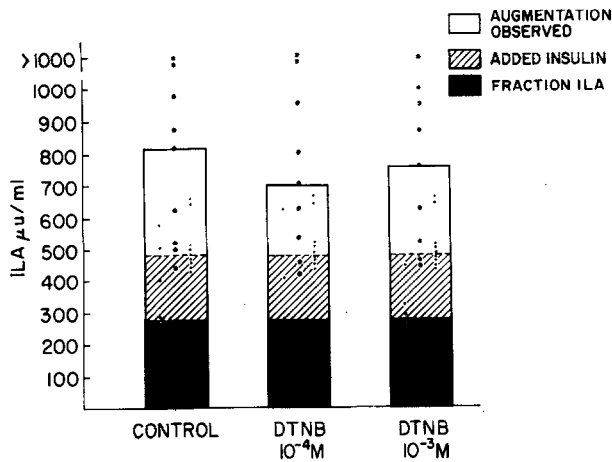


Fig. 5.—Effect of exposure of globulin to the sulfhydryl blocking agent dithionitrobenzoate on its capacity to augment insulin activity. Two concentrations of DTNB were used at pH 8.0 and 25 C for 30 minutes.

Effect of Pretreatment with a Sulfhydryl Blocking Agent

Aliquots of the pooled protein fraction were dissolved in 0.05 M phosphate buffer pH 8.0 and added to a solution of dithionitrobenzoate (DTNB) to final concentrations of 10^{-4} Molar DTNB and 10^{-3} Molar DTNB. After 30 minutes exposure at room temperature, all aliquots were dialyzed against an infinite volume of distilled water at 2 C. for 24 hours with agitation, lyophilized, and assayed for ILA and augmentation (fig. 5). Under these experimental conditions, neither ILA nor augmentation was affected by DTNB.

DISCUSSION

A reasonable dose response relationship has been demonstrated between the augmentation factor(s) and insulin concentration in the present studies.

Insulin-like activity of the pooled protein fraction and augmentation activity of the pooled protein fraction were comparably resistant to acid pH and comparably susceptible to heat and to alkaline pH. The differential behavior of the two properties on exposure to 40 per cent ethanol is of interest, and could provide a basis for distinction in future studies.

The augmentation capacity behaves like a phenomenon involving a protein moiety. It is not dialyzable and is somewhat acid resistant. It is susceptible to denaturation by alkali, ethanol, and heat. The attempt to interfere with augmentation using a sulfhydryl blocking agent DTNB was motivated by previous speculation that sulfhydryl-disulfide associations might be operative in the augmentation effect.⁴ The present failure to demonstrate interference does not eliminate this possibility, since alternative methods were not explored and optimum conditions for its demonstration may not have been provided.

REFERENCES

1. Power, L., Lucas, C., and Conn, J. W.: Further studies on insulin augmentation capacities of various serum proteins. *Metabolism* 14:845, 1965.
2. —, Reyes-Leal, B., and Conn, J. W.: A comparison of the insulin-like activity contained in the serum proteins of normal subjects and diabetic patients. *Diabetes* 14:71, 1965.
3. —, —, and —: Serum insulin-like activity in genetic and experimental diabetes mellitus. *Metabolism* 13:1297, 1964.
4. —, Lucas, C., and Conn, J. W.: A globulin system from human serum that augments the activity of crystalline insulin on the epididymal fat pad: deficiency in diabetes mellitus. *Metabolism* 14:104, 1965.