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Further Studies on Insulin Augmentation Capacities of Various Serum Proteins

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The ability of human serum protein to augment the action of crystalline insulin on the epididymal fat pad has been further evaluated. Serum proteins were fractionated by elution from DEAE cellulose and the capacity of individual fractions to augment insulin action determined. The property was found to be present in all fractions, but concentrated to a slightly greater extent in the faster-moving gamma globulins and beta globulins. The augmentation effect has again been found to be deficient in diabetes mellitus.

WHEN CRYSTALLINE INSULIN is added to undiluted human serum, its activity is completely recovered in the epididymal fat insulin assay system. When insulin is added to diluted human serum in the same system, however, its activity has been found to be augmented.[1] This augmentation effect has further been found to be glucose-responsive, to be present in the serum globulins, and to be deficient in the serum of patients with diabetes mellitus.[1,2] The present study was undertaken to make a more definitive separation of the serum protein fractions than had been made previously,[2] in an effort to determine which fraction contains the greatest quantity of augmentation activity.

MATERIALS AND METHODS

Normal Subjects and Diabetic Patients

Six healthy nonobese young adults, from 21 to 28 years of age, without family histories of diabetes and with normal carbohydrate tolerance, served as control subjects. Six maturity-onset type diabetic patients from 23 to 45 years of age, with family histories of diabetes and with fasting blood sugars of 110 mg. per cent or less, comprised the experimental group. Two exceeded ideal weight by 10 per cent and one by 15 per cent. The remaining 3 were of normal weight. None had been treated. Blood was drawn in the fasting state and the serum stored at −20 C.

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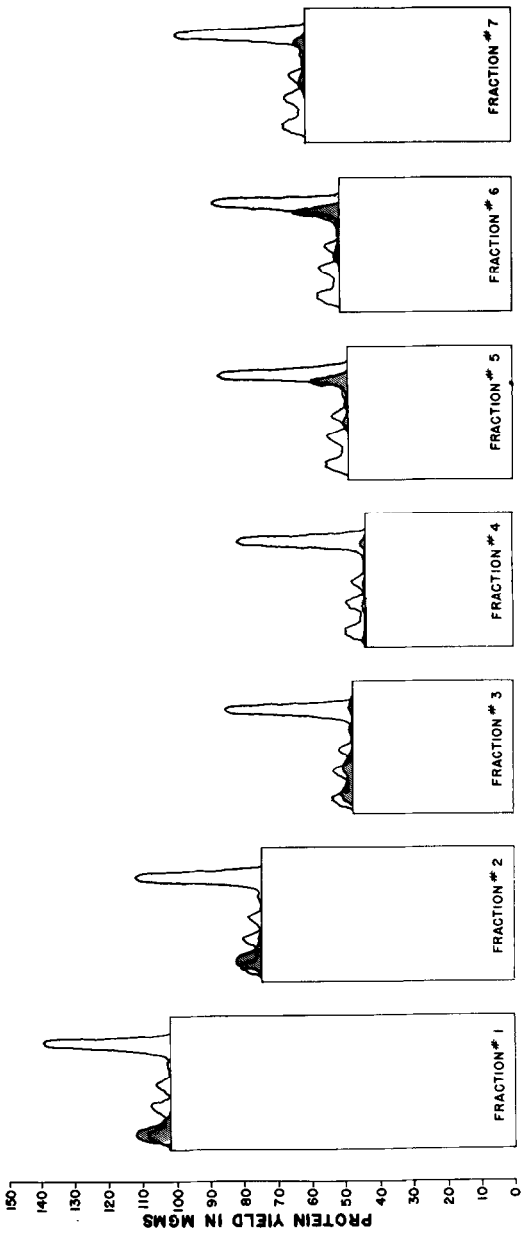

Fig. 1.—Electrophoretic patterns of serum protein fractions eluted from DEAE cellulose.

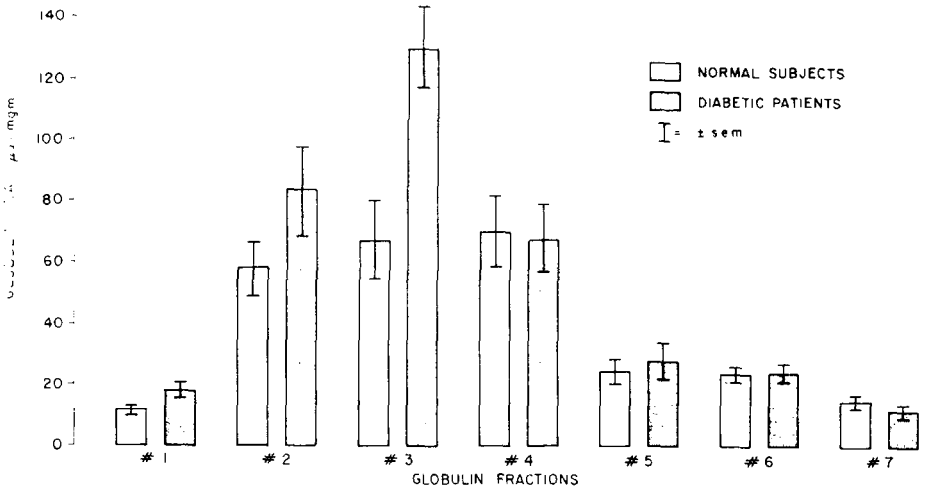

Fig. 2.—Distribution of ILA in serum protein fractions of normal and diabetic subjects in the fasting state.

Serum Fractionation

Within 3 weeks of collection, serum was separated into albumin and globulin components by dialysis against ammonium sulfate, and the globulin component further separated by elution from diethylaminoethyl (DEAE) cellulose. Seven fractions were derived from each globulin component.[3] Each was dialyzed against an infinite volume of distilled water, and lyophilized. For assay, each fraction was dissolved in Krebs bicarbonate buffer at a concentration of 1 mg./ml. Crystalline insulin on a gelatin carrier was added to each as well as to buffer alone for simultaneous assay of its activity.[1]

Results

The electrophoretic composition and insulin-like activity (ILA) of the separated globulin fractions are shown in figures 1 and 2. These results have been previously reported.[3] The protein composition of each fraction was determined by paper electrophoresis. Their ILA was measured using a modification of the epididymal fat pad method.[4] Separation of serum into albumin and globulin by dialysis against ammonium sulfate was incomplete and heavy albumin concentrations appeared in the later cellulose fractions, as can be seen in figure 1.

The effect of adding approximately 200 μU of crystalline insulin to each mg. of protein from the individual fractions is shown in figures 3 and 4. The solid-colored base of each column represents the ILA of a mg. of protein from that fraction and the hatched areas represent the separately-measured activity of the added insulin. The open areas above represent the extent of augmentation as determined by measurement of the ILA of a solution of both the particular fraction and the added insulin. The augmentation effect is seen in all fractions from the normal subjects as well as in those of the diabetic patients. A slight peak of activity is apparent in that fraction containing the faster-moving gamma globulins, and perhaps some beta globu-

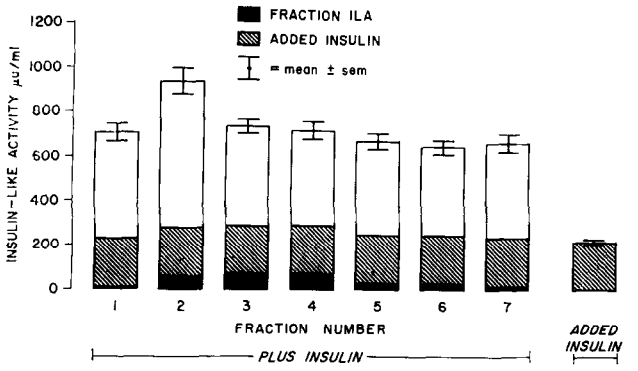

Fig. 3.—Augmentation of insulin action by serum protein fractions of fasting normal subjects.

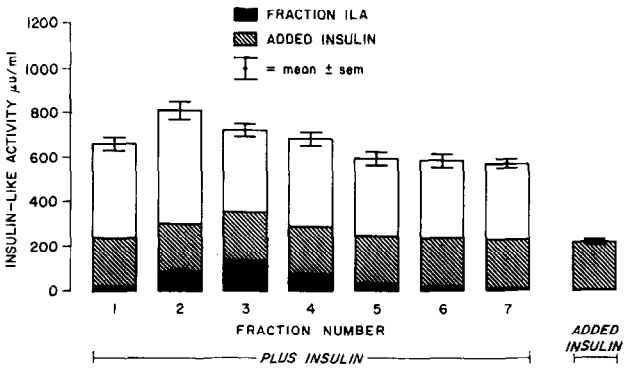

Fig. 4.—Augmentation of insulin action by serum protein fractions of fasting untreated diabetic patients.

lins as well. Diabetic patients showed a consistently impaired augmentation capacity compared to normal subjects ($p < .01$).

Discussion

These studies have extended previous observations on the behavior of a factor in human serum capable of augmenting the action of insulin on adipose tissue and muscle in vitro. Augmentation activity has been found in all the serum protein fractions with a slightly increased concentration in the gamma-beta globulins. Because previous studies had indicated this property to be glucose-responsive and to be deficient in patients with diabetes mellitus, it was suggested that it might be related to a difference in the circulating insulin moiety.[2] If such were the case, and if ILA were a single moiety, augmentation would then be expected to parallel the concentration of ILA in any given protein fraction. As these studies show, however, augmentation is highest in fraction no. 2, while ILA is highest in fractions nos. 3 and 4. Thus in these experiments, ILA and augmentation capacity seem unrelated. Three major

possibilities exist: (1) that there are several entities in serum protein with insulin-like activity but different augmentation capacities; (2) that there is only one entity in serum protein with ILA and augmentation capacity but separatory technics have partly denatured it and led to the different distribution of ILA and augmentation; and (3) that augmentation is not a property of the circulating insulin moiety, but a previously unrecognized factor or factors.

A more serious objection to the hypothesis that augmentation is a property of circulating endogenous insulin is the diffuseness of its distribution in the serum proteins. A discrete entity generally associates itself with one of the serum proteins to a greater degree than that observed in the present study. This consideration leads to the possibility that the serum proteins have been altered by the manipulations and that augmentation results from an unrecognized artifact. Such an artifact, however, would have to be induced by manipulations of serum as gentle as dilution; diabetic serum would have to be less susceptible to the induction of artifact than normal; and normal serum would have to be more susceptible after glucose loading. These considerations argue against this possibility.

Were the insulin moiety a small protein molecule, it could exhibit the wide distribution among serum proteins that has been found. Under such circumstances, its slight affinity for the gamma-beta globulins might reflect a capacity of the latter to bind nonspecifically, rather than a capacity of the small moiety to be specifically bound.

A final consideration arising from the present study, derives from our demonstration of the augmentation phenomenon in fractions composed predominantly of albumin, an observation in line with the findings of Alp et al.[3] and in contrast to earlier findings of our own.[2] The easiest explanation for the discrepancy is a difference in methods, the "albumin" of the different studies being isolated by different technics and containing different proteins. An alternative explanation can be developed from a property peculiar to the augmentation effect in whole serum. Intact serum does not augment the activity of added insulin, yet it contains this capacity. In a similar way, augmentation may be masked in some of the more heterogeneous serum protein fractions. Separation of serum globulins in the present work has revealed a greater total capacity to augment than was suspected from previous studies of unseparated globulin.[2] That these findings reflect the existence of a blocking property eventually eliminated by technical manipulations, remains a possibility.

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