Insulin-like Activity of Serum Protein Fractions

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There have been recent studies indicating that specific insulin-like activity is associated with discrete serum protein fractions.[1-3] Insulin-like activity in these studies was determined by a bio-assay technic utilizing blood glucose changes in hypophysectomized-alloxanized mice and rats. Serum protein fractions were obtained by Cohn Methods six, nine, and twelve. The present communication is concerned with further investigations of this subject employing different methods of bio-assay and serum protein fractionation. A new method of assay for insulin-like substances has been devised relating blood glucose decrement of intact 20 to 30 gm. mice to insulin dosage. Serum protein fractions were obtained by preparative electrophoresis performed with a Continuous Flow Electrophoresis Cell.

METHODS

The present method of insulin bio-assay utilized blood glucose decrement of intact 20 to 30 gm. mice as its parameter. Anesthesia was performed by intraperitoneal injection of pentobarbital sodium (Nembutal) in a dosage of 60 to 70 mg. per kg. The initial blood specimen was obtained from the mouse tail and intraperitoneal injection of the test substance was performed simultaneously. The second tail blood specimen was secured one hour later. Blood glucose levels were determined by the Somogyi-Nelson method.[4] Insulin dilutions* were carried out in 5 per cent albumin. Blood glucose values were deleted if the initial blood glucose level was not in the 50 to 150 mg. per cent range.

Serum protein fractionation was performed with a Spinco CP Continuous Flow Electrophoresis Cell. This apparatus operated by permitting a vertical constant regulated flow of buffer and serum downward over a hanging sheet of filter paper which had a horizontal electrical field. The serum fractions, diluted in buffer, flowed from serrated filter paper drip points at the lower border of the hanging filter paper sheet into test tubes. Barbital buffer, *p*H of 8.6 and 0.02 M, was employed. Electrical power of 30 to 60 ma. was supplied by Heathkit, Duostat, or Constat units. The total duration of runs varied from 16 to 36 hr.

Material obtained by this method of preparative electrophoresis was concentrated by dialysis against 20 per cent polyvinylpyrrolidone (PVP) for 2 to 4 hr. One-milliliter volumes of concentrated fractions were assayed in the intact mice. Improved separation of the various serum protein fractions was obtained by previous dialysis of the whole serum against PVP. The greater current of 50 to 60 ma. obtained with the Constat (Spinco) also seemed to favor more satisfactory serum protein electrophoretic separation.

RESULTS

The method of assay employed in this study appears to be sensitive to a minimum of one milli-unit of insulin (figure 1). There is a definite log-dose relationship in

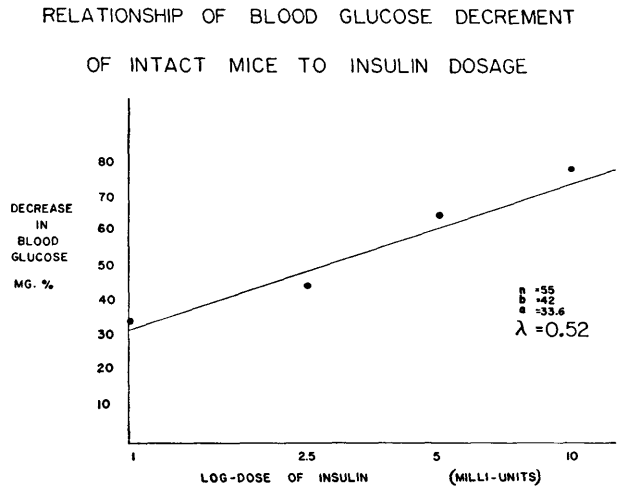

FIG. 1. Relationship between dose of crystalline insulin in 5 per cent albumin and blood glucose decrement (ordinate), calculated one hour following intraperitoneal injection of insulin. Insulin dosage of 1-10 mu. is plotted on log $_{10}$ scale (abscissa). Lambda (index of precision) = s/b, where s is the standard deviation about the regression line and b is the slope of the log-dose line.

*Recrystallized insulin was contributed by the U.S. Pharmacopoeia through the courtesy of Dr. Lloyd Miller.

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the one to ten milli-unit range, but, because of the marked standard deviation about the curve, the index of precision, lambda (λ)* is 0.52.

Discrete serum protein fractions were obtainable by this method of preparative electrophoresis (figure 2). The barbiturate buffer employed for the electrophoretic separation was selected as the logical control. Beta globulin possessed predominant insulin-like activity (figure 3, table 1). The mean glucose decrease associated with beta globulin was 32 mg. per cent as compared with 20 mg. per cent for the barbiturate buffer controls, 20 mg. per cent for a component having a mobility intermediate between that of beta and gamma globulins, 19 mg. per cent for alpha globulin, 12 mg. per cent for gamma globulin, and 7 mg. per cent for albumin. The glucose decrement in response to beta globulin was significantly greater than that associated with the barbitu-

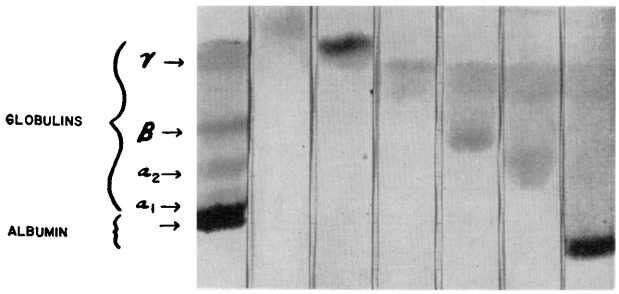

FIG. 2. Delineation by analytic electrophoresis (routine Durrum procedure) of serum protein components of various fractions obtained by preparative electrophoresis (Spinco Continuous Flow Electrophoresis Cell). Strip on left represents analytic electrophoretic pattern of pooled whole serum preceding preparative electrophoresis. Remaining paper strips denote analytic electrophoretic patterns of discrete pools of serum fractions obtained by preparative electrophoresis of the same whole serum. Gamma globulin, a component migrating between gamma and beta globulin, beta globulin, alpha globulin, and albumin each appears to be sole component of successive serum fraction pools (pools composed of contents of one or more of the thirty-two test tubes receiving the fractionated serum).

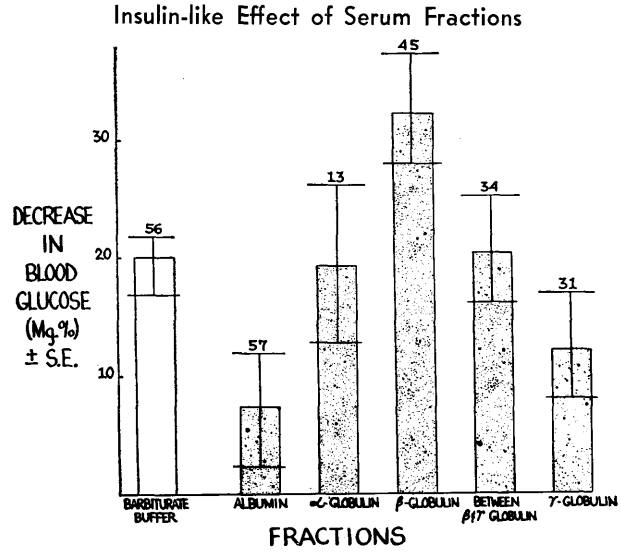

FIG. 3. Effect of discrete serum protein fractions on blood glucose decrement of 20 to 30 gm. intact mice. Mean blood glucose decrement ± standard error of mean (ordinate) calculated as mg. per cent decrease in blood glucose one hour following intraperitoneal injection of 1 ml. of concentrated serum protein fraction or barbiturate control. Numbers above bars represent number of determinations.

* λ = s/b. s is the standard deviation about the regression line; b is the slope of the log-dose line.

TABLE 1

Blood glucose decrement of intact mice in response to serum protein fractions*

Fraction	Number of Determinations	Blood Glucose Decrement (Mg.%) Mean ± S.E.†	Significance of Difference Between Means: P Value Calculated by "T" Test Beta Globulin t	P	Barbiturate Buffer t	P
Beta globulin	45	32 ± 4.6			2.08	>.02,<.05
Alpha globulin	13	19 ± 6.2	1.68	>.05,<.1	0.14	>.8
Gamma globulin	31	12 ± 4.4	3.14	<.01	1.39	>.1,<.2
Between beta and gamma globulin	34	20 ± 4.8	1.81	>.05,<.1	0	>.9
Albumin	57	7 ± 4.4	3.84	<.001	2.20	>.02,<.05
Barbiturate buffer control	56	20 ± 3.7	2.08	>.02,<.05		

*Initial blood glucose level 50 to 150 mg. per cent.
†Standard Error of Mean.

rate buffer control ($P > .02, < .05$), and an even more decisive effect was evident as beta globulin was compared to gamma globulin ($P < .01$) and to albumin ($P < .001$). However, neither alpha globulin nor the component with mobility intermediate between beta and gamma globulins, neither having any appreciable effect on blood glucose decrement as compared with the barbiturate controls, demonstrated statistically significant differences with beta globulin ($P > .05, < 0.1$).

DISCUSSION

The method of insulin assay described above does not possess sufficient sensitivity or reproducibility to constitute an effective insulin assay. However, it is a relatively simple technic and serves as an adequate screening procedure.

Serum protein fractions can be effectively separated in quantity with the Continuous Flow Electrophoresis Cell. Dialysis of the serum against PVP before electrophoresis and employment of a constant, powerful current in the 50 to 60 ma. range appeared to enhance electrophoretic separation.

This study demonstrates that beta globulin has predominant specific insulin-like activity as compared with albumin and gamma globulin. The somewhat increased effect on blood glucose of alpha globulin and of the component migrating between beta and gamma globulins, as compared with gamma globulin and albumin, may be attributable to slight "contamination" by the adjacent beta globulin.

The results of the present investigation support earlier reports that serum fractions containing principally alpha and beta globulins possess insulin-like activity.[1-3] Increased significance may be attached to both these past observations and the present report since quite different methods of serum protein fractionation have been utilized, viz., Cohn Methods six, nine, and twelve in the earlier studies, and electrophoretic separation in the present investigation. Also, the bio-assay procedures differed greatly. The previous technic related diminution of blood glucose increment in hypophysectomized-alloxanized rats and mice, following gavage with dextrin, to insulin dosage. The present method utilized blood glucose decrement of intact 20 to 30 gm. mice as the parameter.

The greater mean glucose decrement associated with the barbiturate buffer controls as compared with albumin and gamma globulin may suggest a nonspecific "insulin-like" effect of the barbiturate. However, the alternative possibility should be considered: that the barbiturate buffer substances may actually constitute the most accurate controls. The diminished insulin-like effect of albumin and gamma globulin as compared with barbiturate buffers may reflect anti-insulin activity of these serum proteins.

It is important to realize that total biological effects are being demonstrated in studies such as these. A number of discrete insulin-like and anti-insulin substances may be involved. Conceivably, some factors totally unrelated to insulin may be operative.

SUMMARY

Serum protein fractions obtained by preparative continuous flow paper electrophoresis were tested by a simplified insulin assay for insulin-like effect utilizing intact mice. The predominant insulin-like effect was associated with beta globulin.

SUMMARIO IN INTERLINGUA

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ACKNOWLEDGMENT

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