Insulin-like Effects of Serum Albumin and Globulin Fractions on Glucose Uptake by Rat Epididymal Adipose Tissue

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A method of bio-assay for insulin-like material utilizing glucose uptake by rat epididymal adipose tissue demonstrates sensitivity to as little as 10 micro-units of insulin per milliliter.[1] In this type of bio-assay, "nonspecific" insulin-like factors must be considered. This paper presents investigations of such possible activity associated with protein. Specificity of insulin-like activity is further elucidated in this paper by studies with serum protein fractions.

METHODS

The method of insulin assay, utilizing glucose uptake by rat adipose epididymal tissue, has been described in detail.[1] Concentrations of commercial human serum albumin,* varying from 0.2 per cent to 5 per cent concentration, have been tested in this system to determine the effect of protein on glucose uptake by adipose tissue. Adipose tissue segments weighing 40 to 100 mg., from rats in the 100 to 200 gm. weight range, were incubated in Krebs-bicarbonate buffer with an initial glucose content of 2.0 to 2.6 mg./beaker. Incubation proceeded for two to four hours in a Dubnoff metabolic shaker under 95 per cent O_2-5 per cent CO_2, at a temperature of 36.5°.

Human serum protein fractions were tested under the same conditions, except that heavier animals weighing 180 to 300 gm. were employed. Generally, lower glucose uptake determinations were obtained with adipose tissue from these heavier animals. The serum protein fractions were obtained from pooled normal sera by preparative electrophoresis, as described previously.[2] A continuous flow electrophoresis apparatus† was utilized and the fractions analyzed by the Durrum analytic electrophoresis method.

Adequate controls, containing no insulin and insulin in 10 to 1,000 micro-unit/ml. concentrations, were employed in each series of determinations. Only satisfactory assays have been analyzed in this study. These have been selected arbitrarily on the basis of a minimum increment of 0.100 (log 10) by the mean of insulin over the noninsulin controls for each animal. Series of determinations obtained from adipose tissue of animals not meeting this requirement were deleted.

RESULTS

No nonspecific "insulin-like" effect of commercial human serum albumin was evident. The highest mean glucose uptake, 4.7 mg. glucose per gram adipose tissue, was obtained with no protein in the incubation medium (table 1, figure 1). The higher protein concentrations tested, 1 per cent, 2 per cent, and 5 per cent, demonstrated glucose uptakes which were, possibly, diminished to a significant extent. The mean glucose uptake values were 3.3, 3.6, and 3.1 respectively (table 1). Mean glucose uptakes of the smaller protein concentrations, 0.2 per cent and 0.5 per cent, were 4.3 and 3.9 respectively. These values did not differ significantly from the nonprotein control.

Two-tenths per cent egg albumin demonstrated approximately the same mean glucose uptake, 4.3 mg. glucose/gram adipose tissue, as 0.2 per cent commercial serum albumin.

As indicated previously, adipose tissue from heavier animals was utilized in the study of human serum fractions, with lower glucose uptakes as a consequence.[1] The only significant insulin-like effect was associated with beta globulin and adjacent fractions (table 2, figure 2). The mean glucose uptake associated with beta globulin was 4.7 mg. glucose/gram adipose tissue; 4.2 mg. was the mean glucose uptake for the fraction having a mobility between beta and gamma globulin, and 3.8 mg. was the uptake for the fraction with a mobility midway between alpha-$_2$ and beta globulin. None of the remaining fractions demonstrated significant insulin-like activity as compared with the nonprotein control. This control was composed principally of Krebs-bicarbonate buffer containing a small amount

* Normal serum albumin (human), salt poor, USP, Hyland Laboratories, Los Angeles.

† Spinco C-P Continuous Flow Electrophoresis Apparatus.

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TABLE 1

Effect of protein concentration on glucose uptake*

Protein concentration (Per cent)†	Number of determinations	Glucose uptake (mg. glucose/gm. adipose tissue) Mean‡	90 per cent confidence limits	Significance of protein mean glucose uptake compared with non-protein control mean uptake t	P
None	21	4.7	4.0-5.5		
0.2	12	4.3	3.5-5.4	.5	>.5
0.5	18	3.9	3.4-4.4	1.6	>.1,<.2
1.0	17	3.3	2.7-4.2	2.2	>.02,<.05
2.0	15	3.6	3.0-4.3	2.0	>.05,<.1
5.0	6	3.1	1.8-5.3	2.1	>.02,<.05

*Rat weight 100-240 gm., adipose tissue weight 40-100 mg., 2.0-2.6 mg. glucose/beaker.
†Human serum albumin (commercial).
‡Antilog of geometric mean.

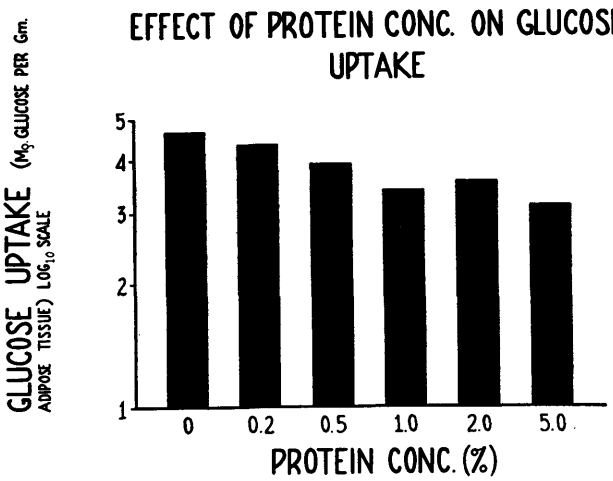

FIG. 1. Effect of varying concentrations of protein (0-5 per cent) on glucose uptake by rat adipose tissue (mg. glucose/gm. adipose tissue—log 10 graph).

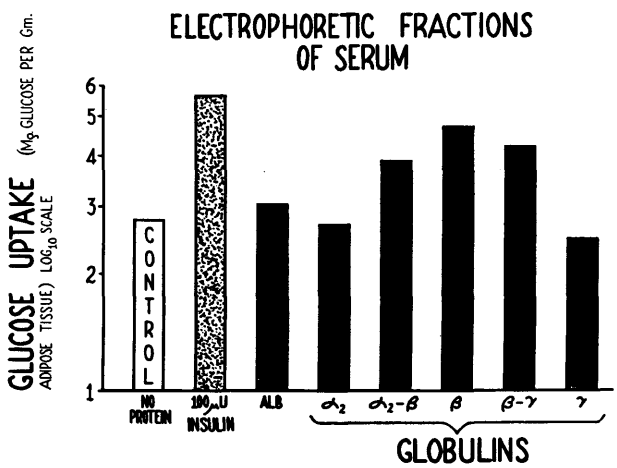

FIG. 2. Effect of serum protein fractions obtained by preparative continuous flow electrophoresis on glucose uptake by rat adipose tissue (mg. glucose/gm. adipose tissue—log 10 graph).

of the barbiturate buffer employed in the preparative electrophoretic serum protein fractionation. The mean glucose uptake of this control material was 2.8 mg./gram adipose tissue. The albumin fraction obtained concurrently by this technic of preparative electrophoresis was associated with a mean glucose uptake value of 3.0. Alpha-$_2$-globulin and gamma-globulin fractions demonstrated mean glucose uptakes of 2.7 and 2.4 respectively. Commercial human serum albumin (0.2 per cent) tested in this series of experiments had a mean glucose uptake value of 2.9. There was a significant increment of mean glucose uptake by insulin concentrations of 100 micro-units/milliliter and greater. The mean uptake for the 100 micro-unit per milliliter concentration of insulin was 5.6 mg. of glucose/gram adipose tissue.

DISCUSSION

There has been some evidence that a nonspecific stimulation of glucose uptake by rat diaphragm may be associated with plasma protein fractions.[3,4] Such a nonspecific protein stimulating effect has not been demonstrated with the adipose tissue insulin bio-assay method. There is even some statistical evidence suggesting a possible diminution of glucose uptake related to concentrations of protein greater than 0.5 per cent.

The present study of human serum fractions completely confirms the previous investigations from this laboratory utilizing the same technic of serum protein fractionation and a method of insulin bio-assay relating insulin concentration to blood glucose decrement of intact mice.[2] Both studies indicate that beta globulin and

TABLE 2

Effect of serum protein fractions on glucose uptake*

Fraction	Number of determinations	Glucose uptake (mg. glucose/gm. adipose tissue) Mean‡	90 per cent confidence limits	Significance of protein fraction mean glucose uptake compared with control mean uptake* t	P
Nonprotein control	21	2.8	2.4-3.2		
Alpha$_2$ globulin	4	2.7	2.1-3.5	0.1	>.9
Between alpha$_2$ and beta globulin	19	3.8	3.1-4.8	2.2	>.02,<.05
Beta globulin	33	4.7	4.2-5.3	4.8	<.001
Between beta and gamma globulin	31	4.2	3.6-5.0	3.0	<.01
Gamma globulin	11	2.4	1.6-3.6	0.9	>.3
0.2 per cent commercial serum albumin	25	2.9	2.5-3.3	0.5	>.6
100 μU/ml. insulin	6	5.6	4.4-7.1	3.8	<.001

*Rat weight 180-300 gm., adipose tissue weight 40-100 mg., total glucose 2.0-2.6 mg./beaker.
†Antilog of geometric mean.

adjacent serum protein fractions possess endogenous serum insulin-like activity. A recent report from the Netherlands of Bolinger and others[5] also stated that beta globulin contains the predominant endogenous serum insulin-like activity. These workers employed glucose uptake by the rat diaphragm as the insulin bio-assay method and fractionated serum proteins by starch-column electrophoresis. They also indicated that radioactive labeled insulin I^{131} was recovered in the beta-globulin fractions. Randle and Taylor[6,7] utilizing similar technics noted "bound" insulin to be associated with serum protein migrating in the beta-gamma globulin zone. They also observed that "free" insulin appeared to migrate with the slow-moving segment of albumin or with alpha$_1$-globulin. These results are consistent with the findings of Antoniades[8] and of Berson and Yalow.[9]

The difference in electrophoretic mobilities between "free" and "bound" insulin must be interpreted with caution. The "bound" insulin supposedly represents endogenous insulin. However, methods of insulin bio-assay are not specific for insulin, and substances of biological importance other than insulin may possess "insulin-like" activity, as has been previously emphasized.[2] It has been shown that an endogenous plasma insulin-like factor, unlike crystalline insulin, can be adsorbed by a cationic exchange resin from which it may be subsequently eluted.[10,11] More recently, Walaas and others[12] have described a dialyzable serum insulin-like factor of small molecular weight that is probably not insulin. Furthermore, they have noted "insulin-like" activity of certain individual amino acids at low concentrations. Leonards,[13] employing the rat epididymal fat pad for measurement of insulin-like activity, has been unable to extract the serum factor responsible for this activity with acid-alcohol or to block this activity with specific insulin anti-sera.

SUMMARY

Various concentrations of commercial human serum albumin demonstrate no nonspecific insulin-like activity as tested by a method of insulin bio-assay utilizing glucose uptake by rat epididymal adipose tissue. Serum protein fractions obtained by preparative continuous flow electrophoresis have been assayed in this system. Endogenous serum insulin-like activity appears to be associated with beta globulin and adjacent fractions containing beta globulin.

SUMMARIO IN INTERLINGUA

Effectos Insulinoide Exercite per Albumina Seral e per Fractiones de Globulina Super le Acceptation de Glucosa per Histos Adipose Epididymal del Ratto

Varie concentrationes de human albumina seral de provenientia commercial demonstrava nulle nonspecific activitate insulinoide in tests effectuate per medio de un methodo de bio-essayage de insulina basate super le studio del acceptation de glucosa per histos adipose epididymal de rattos. Fractiones de proteina seral, ob-

tenite per electrophorese a fluxo continue esseva essayate in iste systema. Il pare que le endogene activitate insulinoide in le sero es associate con globulina beta e fractiones adjacente que contine globulina beta.

ACKNOWLEDGMENT

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REFERENCES

[1] Beigelman, P. M.: Insulin-like activity of normal and diabetic human serum. Diabetes *8*:29-34, 1959.

[2] Beigelman, P. M.: Insulin-like activity of serum protein fractions. Diabetes 7:365-67, 1958.

[3] Park, C. R., and Bornstein, J.: Inhibition of glucose uptake by the serum of diabetic rats. J. Biol. Chem. *205*:503, 1953.

[4] Randle, P. J.: Insulin in blood in Hormones in Blood, Boston, Little, Brown, and Co., 1957, pp. 129-30.

[5] Bolinger, R. E., Van Der Geld, H., and Willebrands, A. F.: Electrophoretic separation of insulin activity of normal plasma and plasma with added insulin-I^{131}. Metabolism *8*:39, 1959.

[6] Randle, P. J., and Taylor, K. W.: Insulin in protein fractions of serum from healthy people and from insulin-treated diabetics. Lancet *275*:996-97, 1958.

[7] Randle, P. J., and Taylor, K. W.: The insulin activity of protein fractions of normal human serum. J. Endocrin. *17*: 387, 1958.

[8] Antoniades, H. N.: Preparation of human plasma protein concentrate with insulin activity. Science *127*:593, 1958.

[9] Berson, S. A., and Yalow, R. S.: Ethanol fractionation of plasma and electrophoretic identification of insulin-binding antibody. J. Clin. Invest. *36*:642, 1957.

[10] Beigelman, P. M., Antoniades, H. N., Goetz, F. C., Renold, A. E., Oncley, J. L., and Thorn, G. W.: Insulin-like activity of human plasma constituents. II. Biologic assay of human plasma fractions for insulin-like activity. Metabolism *5*:44, 1956.

[11] Antoniades, H. N., Beigelman, P. M., Pennell, R. B., Thorn, G. W., and Oncley, J. L.: Insulin-like activity of human plasma constituents. III. Elution of insulin-like activity from cationic exchange resins. Metabolism 7:266, 1958.

[12] Walaas, O., Haavaldsen, R., and Barrebak, B.: Characterization of the insulin-like activity of muscle by dialysate from blood plasma. Fourth International Congress of Biochemistry (Abstracts), pp. 103, 1958.

[13] Leonards, J. R.: Insulin-like activity of blood. What is it? Fed. Proc. *18*:272, 1959.

One of the cornerstones of contemporary biochemical thinking is the concept of the individual enzyme as the unit of enzymatic action. According to this view, every biochemical reaction is catalyzed by a specific enzyme; and all multistep processes can be accounted for by the collaboration of a group of discrete, specific enzymes. Furthermore, the characteristics of any enzyme-catalyzed sequence can be predicted on the basis of a detailed knowledge of the properties of the individual enzymes which are concerned in this process. Any number of examples appear to vindicate fully these working assumptions. The successful reconstruction of highly complex processes, such as glycolysis, fatty acid oxidation, purine synthesis, and the pentose and urea cycles, with combinations of isolated enzymes can be evaluated as impressive support for the thesis that certain multi-enzyme processes are merely the sum of the individual enzymatic reaction.

By contrast, this approach has utterly failed when applied to enzymatic processes which are intimately bound up with subcellular structure. Processes such as electron transport, oxidative phosphorylation, and photosynthesis have been found to be wedded to mitochondrial or chloroplast structure, and it has not been possible to deal with these processes by classical methods.

From an article by David E. Green and Johan Jarnefelt in *Perspectives in Biology and Medicine,* Vol. 2, page 163, 1959.

Biologists realize more keenly now than they did not more than a decade ago the crucial part played by phosphorus in the processes of life. Practically every form of energy exchanged inside the living cell is now recognized to involve the making and breaking of what are called "high energy bonds" that link oxides of phosphorus to carbon compounds or carbon nitrogen compounds. Every biological or physiological event involves gain or loss of energy, and accordingly we are entitled to infer that these "energy" rich phosphorus bonds have always played a crucial role, even from the very start of the most primitive manifestation of life.

From "Phosphorus as a Factor in the Origin of Life," by Addison Gulick, in *The American Scientist,* volume 43, page 479.