Studies on the Mechanism of Insulin Antagonism by Albumin in Rat Diaphragm

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SUMMARY

Utilizing a modification of the rat diaphragm technic, studies of the mechanism of action of the albumin-associated antagonist were carried out using human fraction V. It was demonstrated that the antagonist could not be washed off of the diaphragm, that prior exposure of the diaphragm to insulin did not fully protect it from subsequent antagonism of the insulin effect and that, although antagonism was proportional to albumin concentration, large excesses of insulin could not completely overcome small concentrations of the antagonist. The intracellular accumulation of AIB was inhibited by the antagonistic albumin but not by a nonantagonistic one (bovine fraction V). Both albumins depressed radioactive glycine incorporation into muscle protein. The antagonist was shown to inhibit glucose uptake much more than glycogen synthesis.

These data indicate that the albumin-associated antagonist primarily blocks insulin effects on transport by either an extremely strong binding to the muscle cell or an irreversible alteration of the diaphragm's response to insulin without binding. DIABETES *15*:373-79, June, 1966.

Factors extraneous to the pancreas were first implicated in the pathogenesis of diabetes mellitus by the classical experiments of Houssay and Biasotti[1] who were able to produce an amelioration of the diabetic state by hypophysectomy in dogs. The demonstration that adrenalectomy also alleviates the severe diabetes caused by total pancreatectomy soon followed.[2,3] When purified growth hormone became available, diabetes mellitus could be produced in normal cats[4] and dogs[5] by administration of this hormone. More recent evidence has been the demonstration of increased levels of circulating insulin in untreated adult-onset and early cases of juvenile diabetes by immunoassay,[6] rat diaphragm[7] and adipose tissue[8] assays. All of this suggests that insulin antagonism may play a fundamental role in diabetes mellitus.

Although many insulin antagonists have been demonstrated,[9] the one associated with albumin[10] has several characteristics which indicate its possible importance in the pathogenesis of human heredofamilial diabetes. It is present in higher concentrations in untreated diabetics than normal controls,[11] dependent on an intact pituitary gland,[12] and is active against insulin effects on muscle but not adipose tissue.[13] This latter property is especially interesting since any hypothesis concerning the etiology of diabetes must account for the obesity and lack of ketosis in the vast majority of adult-onset diabetics. Consequently, this antagonist was chosen for investigation of its mechanism of action against insulin. The purpose of this paper is to present the results of these studies which demonstrate that the albumin-associated antagonist primarily blocks insulin effects on transport. This blockade is proportional to albumin concentration but cannot be overcome by large excesses of insulin suggesting a noncompetitive type of inhibition.

MATERIALS AND METHODS

Assay of insulin antagonism. The method used for most of these studies was detailed in a previous publication.[14]

Albumins. Human fraction V (Pentex) was used in a 5 per cent concentration throughout these experiments as the antagonist unless stated otherwise. Bovine fraction V (Pentex) in the same concentration was used as a nonantagonistic albumin. Their concentrations were determined by the biuret protein method.

Alpha-aminoisobutyric acid (AIB) transport. One to two μc of C-14-labeled AIB* were added to the second-hour flasks. At the end of the incubation period, the hemidiaphragms were washed in cold KRB, blotted, weighed, homogenized in 3.0 ml. of cold distilled water and centrifuged. One-hundred lambda aliquots in duplicate of the supernatant were dried on filter paper

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*Purchased from New England Nuclear Corporation.

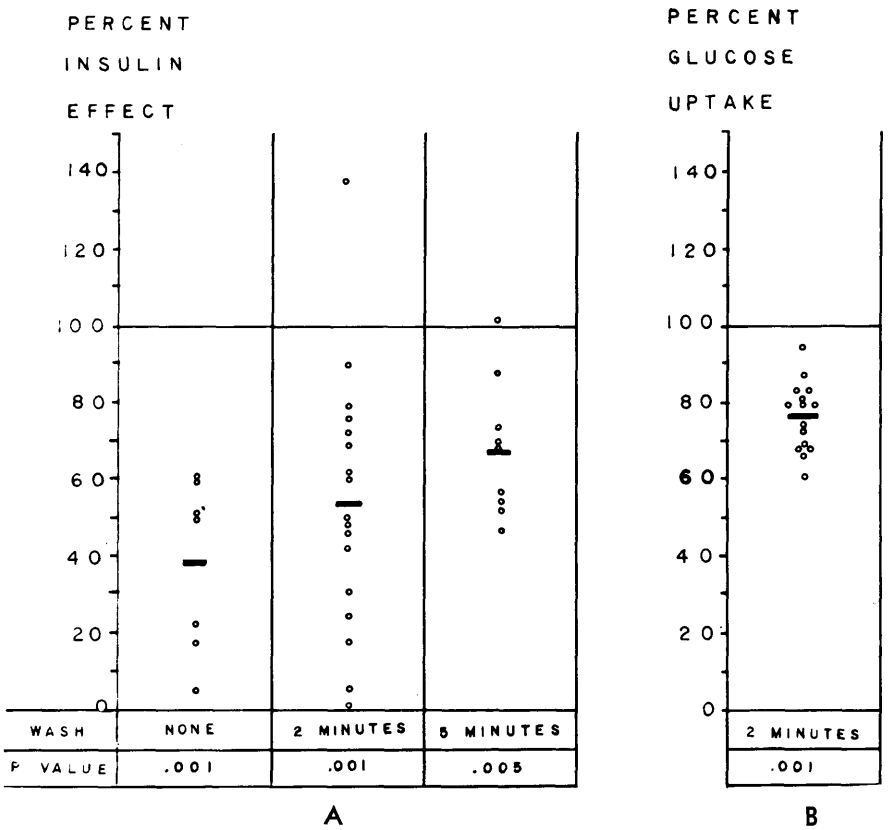

FIG. 1A. The effect of washing on hemidiaphragms after exposing to albumin before incubating in a glucose-insulin-containing media (500 μU./ml.). Insulin effect is the difference in glucose uptake between the second- and first-hour flasks. One hundred per cent is the insulin effect on the control hemidiaphragm in any one rat. Each experimental point represents the percentage of this effect attained by the paired hemidiaphragm exposed to albumin. These results show that after pre-exposure to human fraction V, the hemidiaphragms were not able to respond fully to insulin, and this diminished response could not be restored with up to five minutes of washing.

FIG. 1B. Effects of incubating hemidiaphragms in an albumin-glucose-containing media after exposure to insulin. One hundred per cent is the total glucose uptake of an insulin-exposed control hemidiaphragm during the subsequent hour of incubation. Each experimental point represents the per cent of this uptake attained by the paired hemidiaphragm treated similarly except that albumin was present during the final hour of incubation. The results show that prior exposure to insulin does not protect the response of the hemidiaphragm when subsequently incubated with human fraction V.

and counted in a liquid scintillation detector. Twenty lambda aliquots of the media were similarly counted. The slice medium (S/M) ratio of AIB radioactivity was then calculated assuming a water content of the diaphragm of 75 per cent[15] and an extracellular space of 20 per cent[16,17] of the initial tissue weight (S/M = CPM per μl intracellular water/CPM per μl incubation medium).

Glycogen synthesis. One to two μc of uniformly labeled C-14 glucose* were added to the second-hour flasks. At the end of the incubation period, the hemidiaphragms were weighed in the usual fashion and glycogen-C-14 was determined by methods previously published[18] except that carrier glycogen was not added.

Protein synthesis. One to two μc of glycine-2-C-14* were added to the second-hour flasks. After the period of incubation, the hemidiaphragms were washed in cold KRB, blotted, weighed and homogenized in 3.0 ml. of cold 10 per cent trichloroacetic acid (TCA). The radioactive protein was determined as published previously.[18]

The *t* test for differences in paired observations was used on the data in the washing, initial exposure to insulin, and stoichiometric relationship experiments. The *t* test for differences between means was used in the AIB, glycogen and protein experiments.

*Purchased from New England Nuclear Corporation.

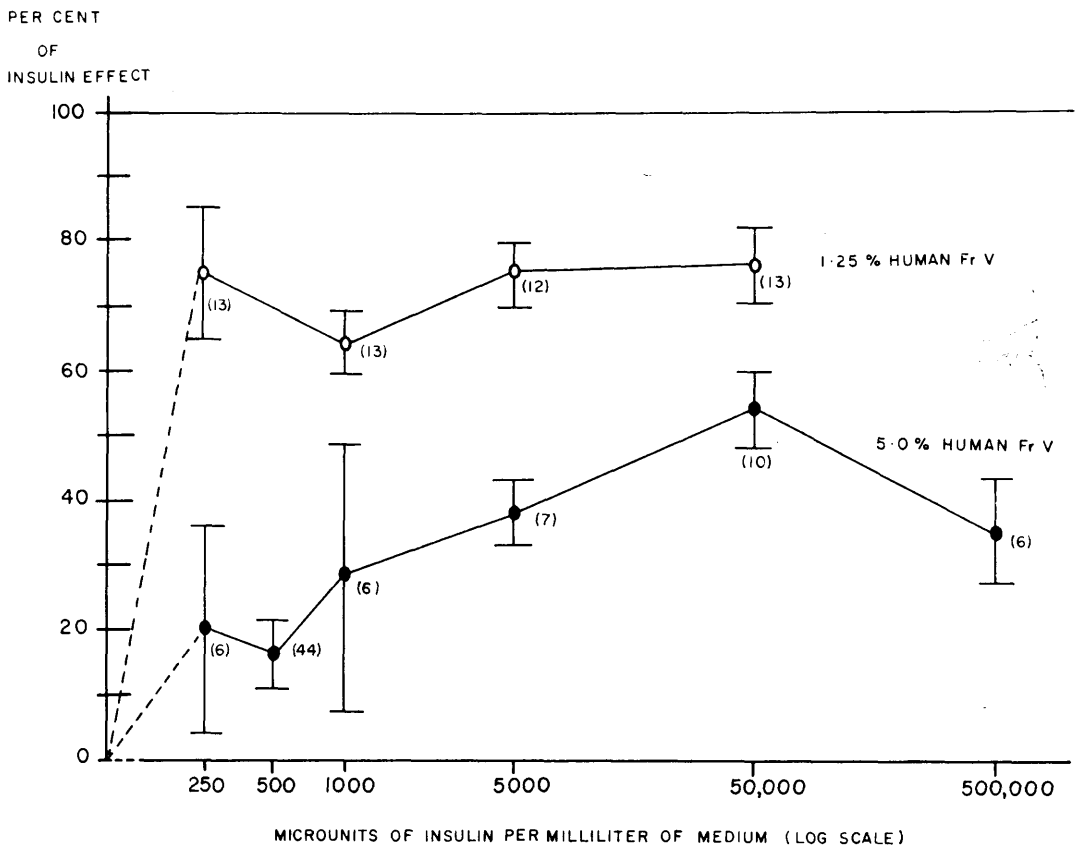

FIG. 2. The stoichiometric relationship between insulin and two concentrations of the antagonist. The per cent of insulin effect attained by a hemidiaphragm exposed to albumin, compared to its paired control, is plotted against the log of the insulin concentration. Each point on the graph is highly significant, the largest p value being 0.025. The results show that large excesses of insulin will not overcome the antagonist even when the albumin is diluted to 1.25 gm. per cent.

RESULTS

Washing. The design for these experiments was:

B	I
A	I

Diaphragms were washed between the first and second hour of incubation for either two or five minutes in approximately 2 ml. of gassed KRBG in a Dubnoff shaker at 37.5° C. at 100 cycles per minute. The results are shown in figure 1A and indicate that after initial exposure to human fraction V up to five minutes of washing will not restore full insulin sensitivity to the diaphragm.*

Initial exposure to insulin. After the initial hour of preincubation, the hemidiaphragms were incubated under the usual conditions in 0.5 U./ml. of insulin for fifteen minutes. They were then washed for two minutes as in the previous washing experiments and finally incubated in KRBG alone or with albumin for an hour. (This higher concentration of insulin was chosen because it afforded maximal glucose uptake during the subsequent hour whereas preincubation with 500 μU./ml. did not. Stadie et al.[20] have reported similar findings.) The results are shown in figure 1B and indicate that prior exposure of the diaphragm to insulin does not fully protect it from subsequent antagonism of the insulin effect.

Stoichiometric relationship. Human fraction V at 1.25 gm. per cent and 5 gm. per cent was tested against varying concentrations of insulin in the standard way:

B	I
A	A + I

The results are shown in figure 2 and indicate that the antagonist cannot be overcome by excessive concentrations of insulin. Even a 1.25 gm. per cent solution

*Using a similar system, Stadie et al.[19] were the first to show insulin antagonism by albumin. They demonstrated that preincubation of the diaphragm in a 2.5 per cent Cohn fraction V solution for five minutes before exposure to insulin for one minute resulted in diminished glycogen synthesis during the subsequent period of incubation. However, no measurements of glucose uptake were made.

TABLE 1

Effect of albumin on AIB transport

			Human Fx 5		Bovine Fx 5	
	B	I	A	A+I	A	A+I
S/M* ±S.E.	3.7±0.2	5.2±0.3	1.4±0.1	1.6±0.1	4.0±0.5	5.7±0.7
n	16	20	4	10	4	10
	B vs. I		B vs. A	I vs. A + I	B vs. A	I vs. A + I
	$p<.001$		$p<.001$	$p<.001$	n.s.	n.s.

*S/M = slice/media ratio.

TABLE 2

Effects of albumin on glycine incorporation into protein

			Human Fx 5		Bovine Fx 5	
	B	I	A	A+I	A	A+I
CPM/mg./ 10^5 cts. std.	94.4	103.2	31.2	35.0	12.9	10.3
±S.E.	±7.2	±6.5	±1.4	±3.2	±2.0	±2.1
n	9	11	4	6	5	5

shows significant antagonism against 50,000 μU./ml. of insulin.

The remainder of these studies are concerned with the measurement of various radioactive substances. This was done under basal conditions using the experimental design:

B	B*
A	A*

and in the presence of insulin:

B	I*
A	A + I*

Although comparisons of the insulin effect on glucose uptake could be made in the usual way from the same rat, the insulin effect on these various radioactive parameters had to be calculated using basal values from one rat and insulin-stimulated values from another. In order to minimize the day-to-day variation, the experiment on any one day included determinations under both basal conditions and in the presence of insulin.

AIB transport. The effects of human fraction V on insulin-stimulated AIB transport are shown in table 1. Not only is this effect of insulin on amino-acid transport antagonized, but the transport of AIB under basal conditions is also depressed. A nonantagonistic albumin had no significant effect on either basal or insulin-stimulated transport.

*Radioactive material present in second-hour flask only.

TABLE 3

Effect of human fraction V on glycogen synthesis under basal conditions

	Glucose uptake* (μg./mg./hr.±S.E.)	Equiv. μg. to glycogen±S.E.†	*n*
B	2.0±0.13	0.11±0.001	14
A	1.6±0.21	0.24±0.024	14

*Glucose uptake is expressed as μg. glucose per mg. wet weight of diaphragm per hour incubation and represents total uptake in the second-hour flask.

†Glycogen synthesis is expressed as equivalent μg. of glucose going into glycogen and is calculated using total chemical glucose and specific activity of radioactive glucose.

TABLE 4

Effect of human fraction V on glycogen synthesis in the presence of insulin

	Insulin effect* μg./mg./hr.±S.E.	Equiv. μg. to glycogen±S.E.†	*n*
I	2.42±0.24	0.63±0.06	15
A+I	0.36±0.13	0.33±0.03	15

*Insulin effect is the difference in glucose uptake under basal conditions and in the presence of insulin.

†The expression of glycogen synthesis is the same as in table 2.

Glycine incorporation into protein. Table 2 shows that glycine-2-C-14 incorporation into protein was markedly depressed by both human fraction V and bovine fraction V under both basal conditions and in the presence of insulin ($p < .001$ in all instances).

Glycogen synthesis. Table 3 contains the results of glucose-C-14 incorporation into glycogen under basal conditions. Although glucose uptake is slightly decreased, glycogen synthesis is more than doubled when the diaphragm is incubated with albumin alone ($p < .001$) suggesting in itself that glycogen synthesis is not blocked by albumin. Table 4 shows the results when insulin is added to the media. Although the insulin effect on glucose uptake is markedly impaired by albumin, most (92 per cent) of the additional uptake over basal is channeled into glycogen synthesis. A comparison of glycogen synthesis in the presence of albumin in tables 3 and 4 reveals that insulin is still able to stimulate glycogenesis over the increase by albumin alone ($p < .025$). All of these data suggest that the albumin-associated antagonist exerts no specific action against the intracellular glycogen pathway.

DISCUSSION

The planning and interpretation of experiments concerning insulin antagonists will obviously be enhanced by a detailed knowledge of the mechanism of insulin

action. Although this hormone was first isolated in 1921, no clear and unchallenged view of the way in which its many effects are mediated has emerged. It is known that insulin will accelerate the transport of glucose and certain other monosaccharides from the extracellular water into the cell interior[21] and it had been assumed for many years that insulin effects on fat and protein metabolism were secondary to this phenomenon. However, insulin in vitro, in the absence of glucose, has recently been shown to stimulate 1. the transport of certain amino acids into muscle cells,[16,22,23] 2. the incorporation of amino acids into protein,[24,25] 3. the synthesis of RNA,[26,27] and 4. to decrease the lipolysis of adipose tissue.[28] The increase in protein synthesis is independent of the transport of amino acids as well as glucose.[29,30] Finally, there is evidence that insulin affects glycogen synthesis over and beyond its effect on glucose transport by activating glycogen synthetase.[31,32]

Thus, for the purposes of this discussion, the effects of insulin stimulation may be grouped into two general categories: 1. the transport of certain monosaccharides and amino acids, and 2. the intracellular synthesis of fats, protein, glycogen and nucleoprotein, all of which are independent of transport. (Studies with puromycin and actinomycin D have demonstrated that insulin-stimulated protein, fatty acid, and RNA synthesis is independent of insulin-stimulated glucose, galactose, AIB and xylose transport.[33-37]) Our results clearly indicate that a primary effect of the albumin-associated antagonist is to block insulin-mediated transport since glucose uptake and intracellular accumulation of AIB, a nonutilizable amino acid, were markedly depressed by human fraction V.

However, the interpretation of insulin effects on intracellular pathways is difficult when the transport of substrate for the reactions under consideration are insulin-dependent. Similarly, demonstration of insulin antagonism specifically on these intracellular pathways is hampered when substrate delivery is depressed secondary to the compromised transport step as shown above. Since insulin-stimulated protein synthesis can be dissociated from the transport of amino acids,[29,30] it was hoped that studying the effect of albumin on glycine-2-C-14 incorporation into protein would give definitive evidence concerning the site of action of this insulin antagonist. Unfortunately, both nonantagonistic as well as antagonistic albumin inhibited this incorporation, probably through nonspecific amino acid competition. Therefore, another approach had to be taken to determine if intracellular pathways were specifically affected by the albumin-associated antagonist. The experiments on glycogen synthesis suggest that this pathway is relatively unaffected by the antagonist. Although human fraction V depressed basal glucose uptake slightly, it increased glucose-C-14 incorporation into glycogen by 118 per cent. Secondly, in the face of a marked reduction of the insulin effect by albumin, 92 per cent of the additional glucose uptake over basal is channelled into glycogen. And finally, although glycogen synthesis is stimulated by albumin alone, insulin was still able to increase it another 46 per cent in the presence of the antagonist.* In evaluating these data, it is important to realize that, in the absence of insulin, increasing the glucose concentration in the media will result in greater glucose uptake but no increase in glycogen synthesis by the diaphragm.[38,39] Studies on RNA biosynthesis are currently being carried out to determine if other insulin-stimulated intracellular pathways are unaffected by the albumin-associated antagonist.

Recently, two papers have appeared in abstract form which deal with the mechanism of action of albumin antagonism against insulin. Buse and Buse[40] studied glucose uptake, lactic acid production, glycogen synthesis and D-xylose transport in the cut rat diaphragm preparation. Insulin-stimulated glucose uptake, glycogen synthesis, and D-xylose transport were partially inhibited whereas the insulin effect on lactic acid production was completely abolished by a 5 per cent solution of human serum albumin. They concluded that both membrane transport and glycolysis were affected and further postulated that free fatty acids (0.75 to 0.90 μEq./50 mg. albumin) may partly explain this antagonism. Our findings definitely support their conclusion that membrane transport is affected by the albumin antagonist although evidence has been presented that free fatty acids do not play a role here.[14] The inhibition of glycolysis is difficult to interpret because, in contrast to glycogen synthesis, lactic acid and CO_2 production from glucose are increased when glucose transport is enhanced by non-insulin means (raising either the concentration of glucose[39,41] or the total osmolality of the medium[39]). Therefore, antagonism of glucose transport alone might be expected to result in a parallel reduction of glycolysis.

Jervel,[42] using 5 per cent albumin solutions pre-

*This dissociation of the antagonist's effect on insulin-stimulated glucose uptake and glycogen synthesis is another link in the recent chain of evidence for intracellular sites of insulin action independent of transport.

pared from human plasmas by different technics, studied glucose uptake and glycogen synthesis in cut rat diaphragm and D-xylose transport in the intact preparation. His results differed from both ours and those of the above investigators. He found inhibition of glycogen formation "to the same degree" as glucose uptake and was unable to show any reduction by albumin of the insulin-stimulated increase in D-xylose transport. His conclusion that an intracellular aspect of glucose metabolism is affected rather than membrane transport seems to be based solely on the lack of antagonism of D-xylose penetration, a finding that could not be confirmed by Buse and Buse.[41]

The experiments discussed up to this point were designed to find out *where* the antagonist acts; the following studies were done to determine *how* it acts. Stadie et al.[20] has shown that initial exposure of the diaphragm to insulin followed by washing will still result in an insulin effect when the diaphragm is subsequently incubated in a glucose-containing media. From these results it was postulated that a chemical combination or binding of insulin to the muscle cell occurred, that this binding was not easily reversible, and that this combined insulin exerted its customary metabolic effects during the subsequent incubation period. Similarly, exposing the diaphragm to albumin followed by washing before incubation in a glucose-insulin media results in a moderate reduction of the insulin effect. This would indicate that either the antagonist is so strongly bound to the muscle cell that insulin is unable to completely displace it within the following hour or that the initial contact with the antagonist irreversibly alters the diaphragm's response to insulin. On the other hand, prior incubation of the diaphragm with insulin does not fully protect it from a subsequent diminution of the insulin effect by albumin. This suggests that either the antagonist is able to displace insulin from the binding sites or that the response of the diaphragm can be altered even after insulin is bound. An investigation of the stoichiometric relationship between the albumin-associated antagonist and insulin revealed that reducing the albumin concentration 75 per cent did not abolish antagonism against an insulin concentration 1,000 times that sufficient for a maximal response of the diaphragm. All of these data are certainly inconsistent with competitive inhibition and suggest either an extremely strong binding of the antagonist to the muscle cell or an irreversible alteration of the diaphragm's response to insulin even after it has been primed with this hormone.

ADDENDUM

After this manuscript was submitted for publication, Alp and Recant[43] published observations on the insulin-inhibitory effect of human albumin which are consistent with our findings. These investigators found that in every comparison there was greater antagonism of insulin-stimulated glucose uptake (mean of 64 per cent inhibition) as compared to glycogen synthesis (mean of 41 per cent inhibition). They interpreted these data as evidence for "no obvious impairment of phosphorylation of glucose taken up by the tissue." These findings are also consistent with our contention that further steps in the glycogen synthetic pathway are relatively unaffected by albumin antagonism. Alp and Recant also demonstrated more antagonism against the same insulin concentration with increasing amounts of albumin and suggest that there "appears to be some type of competitive inhibition." Our data also show increased antagonism against the same amount of insulin at two different albumin concentrations but further studies using excessive amounts of insulin and the washing experiments indicate that albumin-associated antagonism does not act through competitive inhibition as usually defined. And finally, the amount of radioactive insulin bound to the diaphragm in buffer was not significantly different than when the tissue was incubated with either antagonistic or nonantagonistic albumin. Taken along with our data, this would imply that the albumin-associated antagonist acts by an irreversible alteration of the diaphragm's response rather than a displacement of bound insulin.

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REFERENCES

[1] Houssay, B. A., and Biasotti, A.: The hypophysis, carbohydrate metabolism and diabetes. Endocrinology *15*:511, 1931.

[2] Hartmann, F. A., and Brownell, K. A.: Relation of adrenals to diabetes. Proc. Soc. Exp. Biol. *31*:834, 1934.

[3] Long, C. N. H., Lukens, F. D. W., and Dohan, F. C.: Adrenalectomized-depancreatectomized dogs. Proc. Soc. Exp. Biol. *36*:553, 1937.

[4] Cotes, P. M., Reid, E., and Young, F. G.: Diabetogenic action of pure anterior pituitary growth hormone. Nature *164*:209, 1949.

[5] Campbell, J., Davidson, I. W. F., and Lei, H. P.: Production of permanent diabetes by highly purified growth hormone. Endocrinology *46*:588, 1950.

[6] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157, 1960.

[7] Vallance-Owen, J., Hurlock, B., and Pease, N. W.: Plasma-insulin activity in diabetes mellitus measured by the rat diaphragm technic. Lancet *2*:583, 1955.

[8] Steinke, J., Soeldner, J. S., Camerini-Davalos, R. A., and Renold, A. E.: Studies on serum insulin-like activity (ILA) in prediabetes and early overt diabetes. Diabetes *12*:502, 1963.

[9] Segal, B. M.: Insulin inhibition and antagonism. Metabolism *13*:753, 1964.

[10] Vallance-Owen, J., Dennes, E., and Campbell, P. N.: Insulin antagonism in plasma of diabetic patients and normal subjects. Lancet *2*:336, 1958.

[11] Vallance-Owen, J., and Lilley, M. D.: Insulin antagonism in the plasma of obese diabetics and prediabetics. Lancet *1*: 806, 1961.

[12] Vallance-Owen, J., Dennes, E., and Campbell, P. N.: The nature of the insulin-antagonist associated with plasma-albumin. Lancet *2*:696, 1958.

[13] Lowy, C., Blanshard, G., and Phear, D.: Antagonism of insulin by albumin. Lancet *1*:802, 1961.

[14] Davidson, M. B., and Goodner, C. J.: Assay of insulin antagonism by serial incubation of paired rat hemidiaphragms. Diabetes *15*:380-87, June 1966.

[15] Vallance-Owen, J., and Wright, P. H.: Assay of insulin in blood. Phys. Rev. *40*:219, 1960.

[16] Akedo, H., and Christenson, H.: Nature of insulin action on amino-acid uptake by the isolated diaphragm. J. Biol. Chem. *237*:118, 1962.

[17] Kostyo, J. L., Hotchkiss, J., and Knobil, E.: Stimulation of amino-acid transport in isolated diaphragm by growth hormone added in vitro. Science *130*:1653, 1959.

[18] Goodner, C. J.: Studies of anterior pituitary tissue in vitro: comparison of carbohydrate metabolism in glands from male and female rats. Endocrinology *75*:846, 1964.

[19] Stadie, W. C., Haugaard, N., Hills, A. G., and Marsh, J. B.: Hormonal influences on the chemical combination of insulin with rat muscle (diaphragm). Amer. J. Med. Sci. *218*:275, 1949.

[20] Stadie, W. C., Haugaard, N., Marsh, J. B., and Hills, A. G.: The chemical combination of insulin with muscle (diaphragm) of normal rat. Amer. J. Med. Sci. *218*:265, 1949.

[21] Levine, R., and Goldstein, M. S.: On the mechanism of action of insulin. Recent Progr. Hormone Res. *11*:343, 1955.

[22] Kipnis, D. M., and Noall, M. W.: Stimulation of amino acid transport by insulin in the isolated rat diaphragm. Biochim. Biophys. Acta *28*:226, 1958.

[23] Manchester, K. L., and Young, F. G.: The effect of insulin in vitro on the accumulation of amino acids by isolated rat diaphragm. Biochem. J. *75*:487, 1960.

[24] Wool, I. G., and Krahl, M. E.: Incorporation of C-14-amino acids into protein of isolated diaphragm: an effect of insulin independent of glucose entry. Amer. J. Physiol. *196*: 961, 1959.

[25] Manchester, K. L., and Young, F. G.: The effect of insulin on incorporation of amino acids into protein of normal rat diaphragm in vitro. Biochem. J. *70*:353, 1958.

[26] Wool, I. G.: Insulin and incorporation of radioactivity into nucleic acid fraction of isolated diaphragm. Amer. J. Physiol. *199*:719, 1960.

[27] Wool, I. G.: Effect of insulin on nucleic acid synthesis in isolated rat diaphragm. Biochim. Biophys. Acta *68*:28, 1963.

[28] Jungas, R. L., and Ball, E. G.: Studies on the metabolism of adipose tissue. XII. The effects of insulin and epinephrine on free fatty acids and glycerol production in the presence and absence of glucose. Biochem. J. *2*:383, 1963.

[29] Manchester, K. L., and Krahl, M. E.: Effect of insulin on the incorporation of C-14 from C-14-labeled carboxylic acids and bicarbonate into the protein of isolated rat diaphragm. J. Biol. Chem. *234*:2938, 1959.

[30] Wool, I. G., and Krahl, M. E.: An effect of insulin on peptide synthesis independent of glucose or amino acid transport. Nature *183*:1399, 1959.

[31] Villar-Palasi, C., and Larner, J.: Insulin-mediated effect on the activity of UDPG-glycogen transglucosylase of muscle. Biochim. Biophys. Acta *39*:170, 1960.

[32] Villar-Palasi, C., and Larner, J.: Insulin treatment and increased UDPG-glycogen transglucosylase activity in muscle. Arch. Biochem. *94*:436, 1961.

[33] Lukens, F. D. W.: Insulin and protein metabolism. Diabetes *13*:451, 1964.

[34] Carlin, H., and Hechter, O.: Insulin action and protein synthesis in diaphragm muscle. Proc. Soc. Exp. Biol. *115*:127, 1964.

[35] Eboué-Bonis, D., Chambaut, A. M., Volfin, P., and Clauser, H.: Action of insulin on the isolated rat diaphragm in the presence of actinomycin D and puromycin. Nature *199*: 1183, 1963.

[36] Gellhorn, A., and Benjamin, W.: Insulin action in alloxan diabetes modified by actinomycin D. Science *146*:1166, 1964.

[37] Fritz, G. R., and Knobil, E.: In vitro stimulation by insulin of alpha-amino-isobutyric acid transport in the absence of protein synthesis. Nature *200*:682, 1963.

[38] Larner, J., Villar-Palasi, C., and Richman, D. J.: Insulin-stimulated glycogen formation in rat diaphragm. Ann. N. Y. Acad. Sci. *82*:345, 1959.

[39] Kuzuya, T., Samols, E., and Williams, R. H.: Stimulation by hyperosmolarity of glucose metabolism in rat adipose tissue and diaphragm in vitro. J. Biol. Chem. *240*:2277, 1965.

[40] Buse, M. G., and Buse, J.: Insulin antagonism associated with albumin. Proceedings of the Fifth Congress of the International Diabetes Federation. 1964, p. 169.

[41] Chain, E. B.: Recent studies on carbohydrate metabolism. Brit. Med. J. *2*:5154, 1959.

[42] Jervell, J.: The effect of human plasma albumin on the insulin stimulation of glucose uptake, glycogen formation, and membrane transport in the isolated rat diaphragm. Biochem. J. *89*:31, 1963.

[43] Alp, H., and Recant, L.: Studies of the insulin-inhibitory effect of human albumin fractions. J. Clin. Invest. *44*:870, 1965.