

# Insulin Antagonists

JOHN A. OWEN, JR.

*Department of Internal Medicine, University of Virginia  
School of Medicine, Charlottesville*

Ten years ago, in 1955 the "Metabolic Establishment" first began to hear what might be called the "Great Diabetes Paradox:" impaired glucose tolerance can occur side by side with normal concentrations of blood insulin or insulin-like activity. This sounded like endocrinologic heresy but it has been confirmed by almost every worker in the field, using different assay methods. Since the paradox is true, it is provocative, for it suggests that an important truth may be hidden beyond it.

One line of approach to this problem has been to look for insulin antagonists which might prevent normal amounts of insulin from accomplishing their physiological tasks. Much work has been done in this area, some of it controversial, all of it exciting. It is not easy to prepare a critical evaluation without beginning to seem to be against progress. But true progress requires that controversy be resolved.

## INSULIN ANTAGONISTS

In 1954, John Vallance-Owen and Barbara Hurlock described a method for the assay of insulin-like activity (ILA) in plasma, using as an index of response the amount of glucose disappearing from the medium in which was incubated a rat hemi-diaphragm. These workers found a low level of ILA in fasting normals, with a brisk rise after glucose ingestion. Crystalline beef insulin added to plasma was recovered quantitatively.

The next year Vallance-Owen and co-workers (1955) reported

similar values in obese diabetics requiring no drug treatment, but found no detectable ILA in the plasma of uncontrolled, insulin-requiring diabetics; crystalline insulin added in vitro had little or no effect. Later these authors showed that if plasma from such patients were diluted 1:4, ILA could be again demonstrated and added insulin recovered quantitatively. They concluded that such plasma contained ILA all along, but that some other plasma component antagonized the action of both the ILA and added crystalline insulin (Vallance-Owen et al., 1958a). It has never been clear why the antagonism disappears when both ILA and the antagonist are diluted to the same degree.

The next step was to isolate and identify the antagonist (Vallance-Owen et al., 1958b). Fractionation of serum proteins showed the antagonist to be associated with albumin and because it could be eluted off the albumin on column chromatography, it was called "synalbumin antagonist." Serum albumin from both normal and diabetic patients antagonized 1,000  $\mu$ u per ml of beef insulin when added in a 3.5 to 5.5% concentration to the medium containing rat hemi-diaphragm. When the same albumin fractions were added in only 1.25% concentration, there was no inhibition with normal albumin but persisting, almost complete, inhibition with diabetic albumin. A fair appraisal would be that everyone has synalbumin antagonist, but that insulin-requiring diabetics have more of it than others.

Other disease entities were soon admitted to the not-so-exclusive ranks of synalbumin excess. In 1961, obese maturity-onset diabetics and pre-diabetics with normal glucose tolerance were shown to have antagonist at the 1.25% concentration in blood (Vallance-Owen and Lilley). In 1963, synalbumin antagonist was found in 19 of 28 patients with fresh myocardial infarction and no family history of diabetes. This finding persisted after recovery from infarction, and contrasted with an incidence in only 6 of 28 control patients without vascular disease or positive family history (Vallance-Owen and Ashton). Then the antagonist was found to be present in 6 of 10 grossly obese women without diabetic "chemistries" or family history (Vallance-Owen, 1965). Further, Alp and Recant (1965) have demonstrated the presence of synalbumin antagonist (at levels between 1.25 and 3.50%) in ten pregnant women in the third trimester, again without family history of diabetes.

#### EFFECT ON METABOLISM IN ADIPOSE TISSUE

It was soon learned that synalbumin antagonist did not antagonize the effect of insulin on adipose tissue (Vallance-Owen and Lilley, 1961; Lowy, Blanshard, and Phear, 1961). Vallance-Owen has emphasized this (1964a, 1964b), and has speculated that obesity may be a manifestation of diabetes rather than the other way around. Investigation of this phenomenon by Alp and Recant (1964) revealed that the synalbumin antagonist, like insulin, actually stimulated the oxidation of glucose by adipose tissue. The similarity was particularly close in that anti-insulin antibody could abolish this effect, a finding considered by many to be proof of identity with insulin. Furthermore, the effect of crystalline insulin was enhanced by the presence of synal-

bumin. Fourteen diabetic albumin samples in 1.25% concentration produce on the average 86% inhibition of the effect of 1000  $\mu$ u per ml of insulin on rat diaphragm. The same albumin contributed on the average 41% of the stimulation of glucose uptake by adipose tissue which was ascribed to insulin-like activity. It is perfectly clear that such a metabolic picket line could divert a great deal of blood glucose away from muscle and into fat.

These divergent actions have not been investigated by other workers, who have concentrated their efforts on the study of the diaphragm effect. Sherman (1965) has recently reported confirmation of the findings of synalbumin antagonist in normal and diabetic plasma in the same concentration as originally noted. Using the Stadie technique, Davidson and Goodner (1965) found that after diaphragm was dipped in solution containing synalbumin antagonist, no amount of washing would enable it to respond properly to insulin. Conversely, prior dipping in an insulin solution partially but not completely prevented subsequent action of the antagonist. They also found that a huge excess of insulin did not wholly overcome the effects of a small amount of synalbumin, and inferred that the inhibition is not competitive, contrary to the report of Alp and Recant (1965) that it was partially competitive. Of special interest was the finding that synalbumin also antagonized the insulin effect of intracellular accumulation of aminoisobutyric acid, and to a lesser degree of glycogen synthesis, which actually tended to increase proportionally as overall glucose utilization declined. Both antagonistic human serum albumin and non-antagonistic beef serum albumin depressed the incorporation of glycine-2-C<sup>14</sup> into muscle protein. Davidson and Goodner concluded that the synalbumin antagonist either binds strongly to the cell membrane, precluding contact with insulin, or irreversibly alters

the membrane's biochemical response to insulin.

#### CHEMICAL IDENTITY OF ANTAGONIST

During the last three years, Vallance-Owen and his colleagues have been attempting to identify synalbumin. Preliminary studies suggested that it was probably a polypeptide. In 1963, Ensinnck et al. found that when insulin is cleaved enzymatically *in vitro*, the 30-amino acid B chain appears to associate with albumin. Ensinnck and Vallance-Owen (1963) reported at the same time that purified B chain readily unites with non-antagonistic albumin *in vitro*, and thereby restores the usual antagonism to 1000  $\mu$ u per ml. They have demonstrated 10 biochemical similarities between isolated synalbumin antagonist and B chain, with no reported dissimilarities (Vallance-Owen, 1964b). In 1964, Ensinnck et al. incubated I<sup>131</sup>-labelled insulin with the hepatic enzyme that cleaves insulin, glutathione-insulin transhydrogenase. They dialyzed the material and found that most of the radioactivity remained attached to the albumin on electrophoresis. In 1965, Ensinnck, Mahler, and Vallance-Owen repeated this study with non-labelled insulin in order to test the effects of various alkylating agents. Reduced or sulfated B chain was antagonistic as usual, but if the molecule were oxidized, or its thiol groups alkylated with iodacetamide or N-ethylmaleimide, the antagonism disappeared. It is generally accepted that the insulin molecule attaches to a receptor site on the cell membrane by forming disulfide bonds. Reduced B chain, with its own "dangling" disulfide bonds, could conceivably attach to the cell membrane and effectively compete with insulin for attachment sites by the mechanism of steric hindrance. Ensinnck et al. (1965) have found that albumin-B chain complex incubated with diaphragm or cell-free muscle extracts

become non-antagonistic, so apparently B chain has a greater affinity for cell membrane than for the albumin molecule.

Vallance-Owen (1946b) has presented the idea that hepatic glutathione-insulin transhydrogenase cleaves insulin to a greater or lesser extent corresponding to the activity of the pituitary-adrenal axis, but concedes that there is no direct support for this theory. As indirect support he cited earlier work on cats; ordinarily cats do not have the synalbumin antagonist (Vallance-Owen and Lukens, 1957), but, it can be demonstrated after pancreatectomy, although not after combined pancreatectomy and hypophysectomy.

#### ROLE IN PATHOGENESIS OF DIABETES

The presumed role of synalbumin antagonist in the pathogenesis of diabetes mellitus can now be traced as follows: Due to a genetic predisposition, there is excessive hepatic glutathione-insulin transhydrogenase activity (perhaps due to decrease in inhibitor), which is exaggerated by pituitary-adrenal hyperactivity. Insulin secreted by the pancreatic B cells reaches the liver via the portal vein and much of it there is cleaved to the A and B chains. The B chain binds to albumin, circulates in the blood, and detaches from albumin at the cellular level in order to attach to membrane binding sites. In adipose tissue the B chain actively supports the uptake of glucose; in muscle it merely inhibits the attachment of the intact insulin molecule. Therefore, glucose tolerance diminishes, blood sugar rises, the cells become initially hypersecretory and eventually exhausted, and permanent pancreatic diabetes ensues (Vallance-Owen, 1964a and b).

Vallance-Owen has been quite interested in using the presence of synalbumin antagonist as a biochemical marker for the genetic diabetic predisposition (1964a).

Most recently (1965), he studied 94 people in 9 families. Of these 38 had normal amounts of antagonist, and 56 had an increased amount equivalent to that found in diabetes; 18 of these had frank diabetes, and 3 more had spontaneous hypoglycemia. He feels that the diabetic predisposition is inherited as a Mendelian dominant, that perhaps 25% of the world population is constituted as diabetic, but that probably only 10% of this segment will develop frank diabetes during life.

#### CRITICISMS OF ROLE OF ANTAGONIST

This review summarized a body of evidence assembled over a twelve-year period. On the other side of the argument, there is the following evidence:

1) Berson and Yalow (1965) have challenged the experiment using  $^{131}\text{I}$ -labelled insulin and the hepatic enzyme (Ensink et al., 1964), on the grounds that the radioactivity bound to albumin can occur without enzymatic intervention and probably represents damaged insulin as a result of irradiation.

2) Some workers have found that albumin is not antagonistic to glucose uptake by muscle (Cameron, Keen, and Menzinger, 1964) or, if it is, only to an extent that could be explained by the association of free fatty acids (Buse and Buse, 1964).

3) A more troublesome question is to explain the presence of synalbumin antagonist in pancreatectomized animals who do not possess the antagonist in the intact state? Vallance-Owen (1964b) has explained this by saying that previously secreted insulin still circulates and contributes B chain to form the antagonist. He argues that hypophysectomy prevents this by suppressing the activity of the hepatic enzyme that cleaves insulin, and that administration of steroids reverses this suppression. It is difficult to understand how a metabolite

of insulin could appear only after insulin production has been extinguished.

4) One feels uneasy about the basic premise of this concept of diabetes; can we reasonably base our entire theory on the presence of a factor which can only be demonstrated as something in *human* plasma which antagonizes the effects of *beef* insulin on *rat* diaphragm? This is several steps removed from the clinical situation, and is subject to criticism since beef albumin does not antagonize beef insulin under these conditions (Davidson and Goodner, 1965).

5) The same objection might be made to a specific pathogenetic role of an antagonist which occurs in such a large segment of the population. If everyone possesses synalbumin antagonist, as demonstrated on rat diaphragm, and 25% possess an excessive amount, why should only 2.5% ever develop even chemical diabetes? Explaining this selectivity is really not much easier than explaining the incidence of clinical diabetes without ever invoking synalbumin at all. A factor which is invoked to explain not only diabetes, but also myocardial infarction, obesity, and the third trimester of pregnancy does not really explain anything.

6) Even if the synalbumin antagonist were of metabolic importance to man, how would its excess lead to diabetes? According to Alp and Recant (1964, 1965), the increased glucose uptake by adipose tissue ought to help counteract the decreased uptake by muscle, especially as the bulk of adiposity increases. If so, should not progressive obesity lead to progressive improvement in glucose tolerance? And in that case, why should cell secretion become exhausted? On this point the theory does not agree with clinical facts.

7) Berson (1965) has recently quoted Mirsky as showing that continuous intravenous infusion of reduced B chain in dogs had no effect on glucose tolerance.

CONCLUSION

In conclusion, the synalbumin antagonist exists, and may be important in the pathogenesis of human diabetes, but its role has not been proved. The proof will require elucidation of the problem of species specificity, and ability to measure accurately the levels of synalbumin antagonist.

REFERENCES

- ALP, H., AND L. RECANT. Effect of the insulin-inhibitory albumin fraction from normal and diabetic subjects on adipose tissue. *Metabolism* 13: 609-619, 1964.
- ALP, H., AND L. RECANT. Studies of the insulin-inhibitory effect of human albumin fractions. *J. Clin. Invest.* 44: 870-882, 1965.
- BERSON, S. A., AND R. S. YALOW. Some current controversies in diabetes research. *Diabetes* 14: 549-572, 1965.
- BUSE, M. G., AND J. BUSE. Insulin antagonism associated with albumin. Program of the Fifth Congress, International Diabetes Federation, Toronto, 1964, p. 169 (abstr 336).
- CAMERON, J. S., H. KEEN, AND G. MENZINGER. Insulin activity of normal plasma and plasma acid-ethanol extracts. *Lancet* 1: 74-76, 1964.
- DAVIDSON, M. B., AND C. J. GOODNER. Studies on the mechanism of insulin antagonism by albumin in rat diaphragm. R. H. Williams Laboratory, King County Hospital and University of Washington, Seattle (Defense Documentation Center, Bull. AD618508, 1965).
- ENSINCK, J., G. COOMBS, M. NYDICK, AND R. H. WILLIAMS. The transport of "A" (glycyl) and "B" (phenyl alanyl) of insulin in human serum. *Diabetes* 12: 353, 1963 (abstr).
- ENSINCK, J., AND J. VALLANCE-OWEN. Antagonism of insulin by the albumin-bound "B" chain of insulin. *Diabetes* 12: 353-354, 1963 (abstr).
- ENSINCK, J. W., G. J. COOMBS, R. H. WILLIAMS, AND J. VALLANCE-OWEN. Studies in vitro of the transport of the A and B chains of insulin in serum. *J. Biol. Chem.* 239: 3377-3384, 1964.
- ENSINCK, J., R. J. MAHLER, AND J. VALLANCE-OWEN. Antagonism of insulin action on muscle by the albumin-bound B chain of insulin. *Biochem. J.* 94: 150-159, 1965.
- LOWY, C., G. BLANSHARD, AND D. PHEAR. Antagonism of insulin by albumin. *Lancet* 1: 802-804, 1961.
- RECANT, L., AND H. ALP. Insulin inhibitors and adipose tissue metabolism. *Ann. N. Y. Acad. Sci.* 131: 334-343, 1965.
- SHERMAN, L. Reproducibility and reliability of results obtained with Vallance-Owen ("synalbumin") insulin antagonist. *Proc. Can. Soc. Clin. Res.* 38: 72-73, 1965.
- VALLANCE-OWEN, J. Insulin antagonists and inhibitors. *Advan. Metabol. Dis.* 1: 191-215, 1964a.
- VALLANCE-OWEN, J. Synalbumin antagonism in obesity and maturity onset diabetes mellitus. *Ann. N. Y. Acad. Sci.* 131: 315-323, 1965.
- VALLANCE-OWEN, J. Synalbumin insulin antagonism and diabetes. *Ciba Found. Colloq. Endocrinol.* 15: 217-239, 1964b.
- VALLANCE-OWEN, J., AND W. L. ASHTON. Cardiac infarction and insulin antagonism. *Lancet* 1: 1226-1228, 1963.
- VALLANCE-OWEN, J., E. DENNES, AND P. N. CAMPBELL. Insulin antagonism in plasma of diabetic patients and normal subjects. *Lancet* 2: 336-338, 1958a.
- VALLANCE-OWEN, J., E. DENNES, AND P. N. CAMPBELL. The nature of the insulin-antagonist associated with plasma-albumin. *Lancet* 2: 696, 1958b.
- VALLANCE-OWEN, J., AND B. HURLOCK. Estimation of plasma-insulin by the rat diaphragm method. *Lancet* 1: 68-70, 1954.
- VALLANCE-OWEN, J., B. HURLOCK, AND N. W. PLEASE. Plasma-insulin activity in diabetes mellitus measured by the rat diaphragm technique. *Lancet* 2: 583-587, 1955.
- VALLANCE-OWEN, J., AND M. D. LILLEY. Insulin antagonism in the plasma of obese diabetics and prediabetics. *Lancet* 1: 806-807, 1961.
- VALLANCE-OWEN, J., AND F. D. W. LUKENS. Studies on insulin antagonism in plasma. *Endocrinology* 60: 625-633, 1957.