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# Diabetes Mellitus and synalbumin insulin antagonism : an etiological concept

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# DIABETES MELLITUS

# AND

# SYNALBUMIN INSULIN ANTAGONISM: AN ETIOLOGICAL CONCEPT

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Historically diabetes has been recognized as a medical problem for more than 2,000 years. Polyuria was recorded in the Ebers papyrus from ancient Egypt even before Celsus (30 B.C. to 50 A.D.) described it unmistakably as a disease of polyuria without pain but with emaciation. (1) Aretaeus in the first century gave diabetes its name and outlined its principal presenting symptoms, progressive nature and fatal outcome.

In the second century A.D., Claudius Galenus introduced the concept that diabetes was caused by kidney weakness and that drink was excreted unchanged. This error persisted and thwarted the understanding of diabetes for 1,500 years. In the Far East, the great Chinese physician, Tchang Tchong-king, described diabetes in the year 200 A.D. as the disease of thirst.

The hereditary character of diabetes was noted as early as the sixth century. The Arab physician, Avicenna (980 to 1037 A.D.) first described diabetic gangrene and some translations credit him also with the first hypothesis of a nervous origin of diabetes and the first theory of the role of the liver.

During the eighteenth century, John Rollo theorized that diabetes was a primary and peculiar affection of the stomach, best treated by a nauseating diet of old meats, animal fat and milk, to which lime water, cathartics and opium were added. The great physiologist, Claude Bernard (1813 to 1878) postulated that the

elevated blood sugar concentration in diabetes resulted from overproduction of sugar by the liver.

The Italian, Arnoldo Cantani (1837 to 1893), discovered on histological examination of necropsy material that atrophy and fatty change were more frequently found in the pancreases of diabetic patients than in those of normal patients. In 1867, Paul Langerhans described the islet formations of the pancreas although it was not until 1893 that the French histologist, Gustave E. Laguesse, gave them significance by suggesting their endocrine function and calling them 'islands of Langerhans'. In 1889,Oscar Minkowski and Joseph Von Mering reported that pancreatectomy produced diabetes, that the pancreas was an organ of internal secretion and was the organ involved in diabetes.

Shortly after the turn of the century, Opie and Sobolev advanced independently the theory that diabetes resulted from pathological changes in the islet tissue and that the islets were needed for control of carbohydrate metabolism. In 1907, M. L. Lane described the distinguishing featues of two types of islet cells, Alpha and Beta.

With the epoch-making discovery of insulin by Banting and Best in 1921, a most important era in the concept of diabetes began. Since insulin was a hormone, the possible relationship of other hormones to diabetes was a natural part of the research studies which were stimulated. Preeminent among these was the observation

of B. A. Houssay that removal of the pituitary gland ameliorated diabetes and reduced the insulin needs in the depancreatized dog.

## CHANGING CONCEPTS

The continuous goal of researchers and clinicians regarding diabetes mellitus clearly has been the adequate diagnosis and treatment of the aberrant carbohydrate metabolism so characteristic of the disease. Earlier the relationship of proper diet to normal blood and urine sugar, and, more recently, the role of insulin administration in the control of these same parameters were appreciated and valued as indispensible factors in the proper treatment of overt diabetes. Indeed, the carbohydrate metabolic abnormality of diabetes was considered to be the sole basic feature of the disease, and all clinical and research activities were directed toward this monolithic theory. However, although entirely cognizant of the dramatic changes wrought by the introduction of insulin, it became increasingly apparent that many unresolved problems still remain. For example, insulin is neither a cure nor a preventative of the disease. Significantly, many of the complications historically associated with diabetes are still far too prevalent and continue to plague the diabetic patient. (2)

More recently oral hypoglycemic agents have come into use and have simplified life for those fortunate few whose disease is controllable by oral medication. These drugs, however, have

inherent, stringent limitations and deficiencies. They are relatively therapeutically impotent in acute stressful situations such as trauma, surgery and severe infections, and, like insulin, they neither cure nor prevent diabetes.

Hence, despite the many basic advances in diabetes, the treatment remains largely symptomatic control; the ability to cure, prevent or forestall complications of the disease is still entirely lacking.

In light of clinical observations, it is necessary to reassess the significance of diabetes. With the ability to control the hyperglycemia and glycosuria and the specific complications resulting from their lack of control, other aspects of diabetes come into sharper focus and, with this, the awareness that they may not necessarily be dependent entirely on carbohydrate abnormality. A notable example was the observation that normal mothers, destined to become overtly diabetic sometime in the future, would manifest the identical complications of diabetic pregnancy, not only in the same degree of severity but in the same degree of frequency. (3) These complications included stillbirths, gigantism, neonatal fatalities and hypertrophy of the Islets of Langerhans. It must be stressed that these complications could occur independent of or prior to the onset of overt carbohydrate metabolic abnormality. (4) Soon all other complications of diabetes were noted antecedent to overt disease including retinopathy, nephropathy, neuropathy and

vascular abnormalities. These observations indicate that diabetes is, in fact, a complex, multi-faceted disease process which is more than a simple insulin deficiency.

## PREDIABETES DEFINED

It is generally accepted that diabetes mellitus is diagnosed on the basis of the first carefully performed glucose tolerance test, the results of which are abnormal. The recognition of a disease process preceding the discovery of the first abnormal glucose tolerance curve requires the formulation of a new concept, that of prediabetes.

> "Prediabetes connotes the state of a person during the period before he or she becomes plainly and clinically diabetic, in which, however, there is a latent abnormality which may show itself under certain specific conditions". (5)

Prediabetes is not a dormant period but rather it is an active dynamic phase when many metabolic and structural changes are taking place, providing the nidus for microangiopathy and neuropathy, and becoming visible only after the passage of a varying period of time. (6) The prediabetic patient is capable of maintaining a normal carbohydrate status for many years. Because of this factor, early recognition of the prediabetic patient is most difficult and must be based on a high index of suspicion on the part of the clinician. Since diabetes is hereditary, and therefore familial, an investigation of members of families of known

diabetic persons would result in the detection of many diabetic relatives. Because of the increased frequency of stillbirths and very large babies in mothers predestined to develop diabetes, investigation of such mothers would bring to light still more. Further, the presence of obesity in both of these categories would yield a higher percentage of diagnoses. The increased sensitivity of the cortisone-augmented glucose tolerance test has been an additional aid in uncovering latent and potential cases, although its reliability in people over the age of forty-five years has been seriously questioned. (7) Once again, operating on a high degree of suspicion, the clinical diagnosis of diabetes still rests on evidence of abnormalities in carbohydrate metabolism and its secondary effects. Until recently no characteristic deviation from normal metabolism was known in the prediabetic state.

## METABOLIC CHANGES

Research and investigation is revealing certain consistent alterations in metabolism among prediabetics, juvenile diabetics and adult-onset diabetics. For example, serum insulin-like activity is found to be increased in these three groups of diabetics as compared to normal individuals. Measurements of fasting serum insulin-like activity by Steinke et. al. (8) have demonstrated a definite and considerable increase in the juvenile prediabetic patient. They also found that previously unknown, untreated,

severely ketotic, juvenile diabetic persons demonstrated elevation of serum insulin-like activity. (9) Comparable findings in adult diabetic patients have been supplied by Beigelman and Tranquada (10) who demonstrated normal or elevated fasting levels of serum insulin-like activity in nontreated, nonketotic adult-onset diabetics. Patients whose diabetes begins in maturity have higher levels of serum insulin one to two hours after oral glucose loading than the normal subject as measured by Yalow and Berson.(11)

## INSULIN ANTAGONISM

The presence of increased insulin activity in the serum of prediabetic or overtly diabetic patients is a paradox which is at least partially resolved by the discovery of serum insulin antagonists. Of particular interest is that insulin antagonist demonstrated by John Vallance-Owen, M.A., M.D., Department of Medicine, University of Newcastle-upon-Tyne, England.

In 1957, Vallance-Owen (a Rockefeller Travelling Fellow at the George S. Cox Medical Research Institute, University of Pennsylvania in Philadelphia) and Lukens (12) reported their investigation of apparent insulin antagonism in man. They noticed that one group of diabetic patients, besides having hyperglycemia and glycosuria, usually develop ketosis and rapidly lose weight unless given insulin treatment. In a study of plasma insulin activity in these patients, it was shown that if the blood sugar

was elevated at the time of the test, no plasma insulin activity was found. Moreover, when insulin was added to the plasma of these patients in vitro, its activity was apparently inhibited. (13)

Earlier, normal human subjects were demonstrated to have a low level of plasma insulin activity in the fasting state which rose significantly following a meal. When insulin was added in vitro to the plasma from normal subjects, there was no measurable insulin antagonism as proved by complete recovery of the activity of the added insulin. (14) Moreover, the plasma from obese diabetic patients, who show no tendency to develop ketosis unless their diabetes is complicated by infection and who ordinarily do not require insulin therapy, shows insulin activity without antagonism. (15)

When ketotic, diabetic patients were controlled, however, plasma insulin activity was found essentially in the normal range and the activity of added insulin was not diminished. Because these diabetics were receiving up to but usually less than 100 units of insulin daily, no insulin resistance was indicated.

> "Insulin resistance may be arbitrarily defined as the inability to obtain satisfactory regulation of diabetes with 200 units or more of insulin per day". (16)

Insulin sensitivity, or inhibition by antibodies, was ruled out because no allergic manifestations were noted. Also, several severely diabetic persons had such plasma insulin antagonism before they had ever had any insulin treatment.

The studies in the insulin-requiring diabetics suggested that these patients required insulin to overcome some antagonist circulating in their plasma. Then to achieve control, sufficient insulin must be injected to counteract this antagonism, yet leaving sufficient active insulin to carry out its normal metabolic functions.

In order to investigate the nature of this inhibition, normal, depancreatized, depancreatized-hypophysectomized (Houssay) and depancreatized-adrenalectomized (Long-Lukens) cats were studied, with and without various hormonal replacements. Estimation of plasma insulin activity and of insulin antagonism is based on an assay method developed and described by Vallance-Owen and Hurlock in 1954, (17) and which is discussed in the following pages. This is a modification of a similar test originally developed by Gemmill. (18) This method depends on the fact that small amounts of insulin increase the uptake of glucose by the isolated rat diaphragm and that a quantitative relationship exists between the concentration of insulin in the incubation medium and its effects on the glucose metabolism of the diaphragm. By rigorous control of the experimental conditions this method can be used for the estimation of plasma insulin activity, or the effective insulin concentration of plasma-insulin, i.e. the sum of the biological activity of insulin and its synergists on the one hand and its

antagonists on the other; it can therefore also be used to estimate antagonism in plasma or protein fractions.

Briefly, the assay method is as follows: From carefully selected rats, the thin anterior hemidiaphragms are obtained under sterile surgical conditions and placed in ice-cold balanced salt solution. To another balanced salt solution (B.S.S.) is added enough glucose to bring the final concentration to 300 mgm. per 100 ml.

Insulin is dissolved in distilled water previously adjusted to pH 2.8, making a final concentration of 50 units insulin per ml. This solution is diluted with glucose B.S.S. to an insulin concentration of one unit per ml. and serial dilutions are made with glucose B.S.S. down to $10^{-5}$ units per ml. These constitute the standard insulin solutions.

One hemidiaphragm is added to two ml. of standard insulin solution in a Warburg cup. The cups are exposed to a controlled oxygen-carbon dioxide atmosphere, then incubated at constant temperature and oscillated at a constant rate. At the end of this time the residual glucose is estimated in each cup by Harding's modification of the Schaffer-Hartman method with duplicates for each cup.

The hemidiaphragms are removed, rinsed in water, dried in an oven at 105° C. for two hours and then weighed. The final

glucose uptake over the incubation period is calculated in mgm. per 100 ml. per 10 mgm. dry weight of diaphragm.

Venous blood is drawn from the animals in a heparinized syringe, the blood sugar estimated, and the plasma separated and cooled. Plasma is added to a previously weighed quantity of glucose to make a final concentration of 300 mgm. per 100 ml. Hereafter the plasma is handled in the same way as the standard insulin solutions.

Results of basal glucose uptake of each hemidiaphragm are fairly constant for any one experimental day, provided the rats and the hemidiaphragms obtained from them are reasonably comparable in weight. The maximum difference between replicate estimations did not exceed 3.5%

The normal, depancreatized, Long-Lukens and Houssay cats, with and without hormonal replacement, were studied. The mean plasma-insulin activity in normal fasting cats was 114 micro-units per ml. and there was no inhibition of insulin added in vitro. In the plasma of depancreatized cats there was no recoverable insulin activity. The inhibition of added insulin was indicated by the fact that 1,000 micro-units per ml. added, only 15% was recovered. This inhibition within plasma of depancreatized cats was removed either by hypophysectomy or adrenalectomy. In either Long-Lukens or Houssay cats, there was again no plasma

insulin activity, but now the activity of insulin added with plasma was fully recovered. Treatment with cortisone or hydrocortisone for four days restored the inhibiting properties of the plasma of Long-Lukens cats. The same treatment for two days did not restore this inhibition. Treatment with hydrocortisone for four to nine days did not restore the inhibition to the plasma of the Houssay animals. Growth hormone injected for four days into either Houssay or Long-Lukens animals also failed to restore the inhibiting properties to the plasma.

It was therefore concluded that the insulin antagonism found in the plasma of depancreatized cats was the result of the combined activity of the pituitary and adrenal cortical steroids. Preliminary observations of electrophoretic properties indicated that the antagonist resided within the globulin fractions of the plasma protein but that it was not a lipo-protein.

## SYNALBUMIN

In an attempt to determine the chemical nature of the insulin antagonist and to elucidate its mode of action, Vallance-Owen, Dennes and Campbell (19) carried on further experiments using venous blood from uncontrolled insulin-requiring diabetics and non-fasting normal subjects. Plasma proteins were obtained from the whole plasma by salt fractionation using sodium sulfate at various concentrations ranging from 15% to 22%. The albumin

fraction was obtained by using a modification of the trichloroacetic acid-ethanol method of Debro et. al. The resulting precipitate was centrifuged and the extract was dialysed and freeze-dried. The fractions were subjected to paper electrophoresis and insulin antagonism was determined in the freeze-dried fractions by the rat diaphragm assay procedure.

Fractionation by saturation with 18% sodium sulfate solution produced a precipitate which was free of antagonistic activity whereas the supernatant had such activity. Paper electrophoresis demonstrated complete separation of the gamma-globulin and albumin fractions, i.e. all the gamma-globulin was in the precipitate and all the albumin in the supernatant. The other globulin components were present in both fractions. It was concluded that the antagonistic activity was not associated with the gamma-globulin fraction.

Attempts to fractionate the antagonistic activity of the supernatant from 18% sodium sulfate by higher concentrations of sodium sulfate were not successful. These results were consistent with the idea that the activity was associated with the albumin in the diabetic plasma. Albumin was, therefore, prepared from diabetic plasma by the trichloroacetic acid-ethanol method and this albumin antagonised the effect of added insulin. The inhibition of the added insulin was complete when the concentration of albumin in the buffer was between 3.5% and 5.5% which includes

the physiologic range. When the concentration of albumin was reduced to 1.25% there was still distinct antagonism of the added insulin. Paper electrophoresis of these albumin fractions failed to reveal any other plasma-protein.

Fractionation of the plasma-protein of normal subjects was accomplished by identical methods and insulin antagonism was determined, once again by the rat diaphragm method. The normal albumin in physiologic concentrations, i.e. 3.5% to 5.5% also had antagonistic activity. However, unlike albumin from diabetic plasma, a concentration of 1.25% failed to show any such antagonism. The possibility that this activity of normal plasma-albumin is due to a change in the albumin associated with the trichloroacetic-ethanol method of preparation was tested by examining the activity of two samples of ether-fractionated albumin and one sample of ethanol-fractionated plasma albumin (Cohn fraction V). These three samples were found to have similar activity. See Tables I and II attached.

TABLE I. - INSULIN ANTAGONISM OF FRACTIONS FROM DIABETIC PLASMA*

| Fraction | Mean glucose uptake above basal level ± standard error of the mean (mgm. per 100 ml. per 10 mgm. diaphragm) | |
|---|---|---|
| | Buffer + 1,000 micro-units per ml. insulin. | Fraction in buffer + 1,000 micro-units per ml. insulin |
| Ppt. from 18% $Na_2SO_4$ | 12.70±0.91 (4) | 13.17±1.54 (4) |
| Supernatant from 18% $Na_2SO_4$: | | |
| Ppt. from 22% $Na_2SO_4$ | 12.68±0.64 (6) | 3.02±1.57 (6) |
| Supernatant from 22% $Na_2SO_4$ | 11.43±1.02 (9) | 2.30±1.14 (9) |
| Albumin: | | |
| 3.5% - 5.5% | 12.22±1.08 (4) | -0.37±0.23 (4) |
| 1.25% | 12.32±1.05 (4) | 3.55±0.67 (4) |

Number of observations in parenthesis.

*Vallance-Owen, J., Denness, E., and Campbell, P. N., Insulin antagonism in plasma of diabetic patients and normal subjects. Lancet, 2:336-37, 1958.

TABLE II. - INSULIN ANTAGONISM OF FRACTIONS FROM NORMAL PLASMA*

| Fraction | Mean glucose uptake above basal level ± standard error of the mean (mgm. per 100 ml. per 10 mgm. diaphragm) | |
|---|---|---|
| | Buffer + 1,000 micro-units per ml. insulin. | Fraction in buffer + 1,000 micro-units per ml. insulin |
| Albumin (T.C.A.-ethanol) | 11.43±0.62 (6) | 0.50±0.48 (6) |
| 3.5% - 5.5% | | |
| 2.5% | 12.63±1.04 (3) | 6.60±1.01 (3) |
| 1.25% | 11.32±0.74 (4) | 11.17±0.89 (4) |
| Albumin (Ether frac.) | | |
| 5% | 12.50±1.40 (2) | 2.75±0.45 (2) |
| Albumin (Cohn Frac. V) | | |
| 3.5% | 12.40 (1) | 2.40 (1) |

Number of observations in parenthesis.

*Vallance-Owen, J., Denness, E., and Campbell, P.N., Insulin antagonism in plasma of diabetic patients and normal subjects. Lancet, 2:336-37, 1958.

More recently, Alp and Recant have confirmed the presence of a potent insulin antagonist associated with the albumin fraction of plasma. (20) A considerable spread of activity through the normal and diabetic groups was observed although a separation could be made between the two groups in that albumin from normal subjects was not inhibitory at concentrations of 2% or less. This concentration is in the same order of magnitude as the differentiating concentration of 1.25% reported by Vallance-Owen.

These observations suggest that the previously described plasma insulin antagonism resides in the albumin fraction. There is apparently similar antagonism in the plasma-albumin from normal subjects, although it is less active. Previously Bornstein and Park (21) and Hendley et. al. (22) found antagonistic activity in the lipoprotein fraction of plasma from alloxan-diabetic rats. Owing to the method of preparation of the albumin used in this experiment, it seems most unlikely that the antagonistic activity is associated with a lipoprotein. Moreover, the electrophoretic homogenicity of the trichloroacetic-ethanol albumin preparations excludes the possibility that the activity is due to the $alpha_1$-globulin plasma protein fraction which was described by Field et. al. (23) in the plasma of severely ketotic diabetic patients. The distribution of insulin itself in fractions of human serum protein has been determined by zone electrophoresis which demon-

strates maximal activity in the region of the alpha$_1$-globulin and also some insulin-like activity associated with the beta and gamma-globulins.

Question concerning the actual residence of the antagonistic activity, i.e. whether in the albumin itself or in an associated substance, was answered by study of a series of normal, hypophysectomized and diabetic human subjects. (24) Three hypophysectomized patients were studied. Two of the subjects who had carcinomatosis had their pituitaries ablated by trans-nasal implants of yttrium-90 into the sella turcica. These patients were cortisone dependent. The third patient was diabetic and the pituitary was removed by surgery. Albumin was prepared from the plasma of these patients and tested by the methods previously described. The plasma-albumin fractions from all these patients had no demonstrable antagonistic activity. In each case, the albumin fraction was tested at least twice.

Moreover, the albumin from normal and diabetic patients was prepared, dissolved in borate-phosphate buffer and passed through a column of cellulose. The protein was eluted with buffer, the eluent extensively dialysed and the protein freeze-dried. When the activity of this protein, which was electrophoretically identical with albumin, was tested, the original activity against insulin was found to have been removed by passage through the column.

Together, both these lines of evidence indicate that the activity of the albumin fraction from normal subjects and diabetic patients against insulin is not due to albumin per se. In view of the increased antagonistic activity of the diabetic plasma protein, this substance is probably present in larger amounts on diabetic albumin than on normal albumin. The presence of this substance with the albumin fraction gives rise to the name "Synalbumin" insulin antagonism. The results of hypophysectomy indicate that the antagonistic activity is related possibly indirectly to the pituitary gland.

The role of the adrenocortical steroids was clarified when the study of albumin from the plasma of two patients, on whom bilateral adrenalectomy had been performed and in whom cortisone therapy was discontinued for at least fifty hours, demonstrated no insulin antagonism. (25) In one of these patients, subsequently stabilized with cortisone, insulin antagonism was found on follow-up examination of the plasma-albumin fraction. These findings suggest a dependency of active insulin antagonism in humans on a competent pituitary-adrenal axis, and tend to confirm the data previously obtained using depancreatized cats.

A most significant discovery concerning insulin antagonism in prediabetics was reported by Vallance-Owen and Lilley in 1961. (26) Five obese diabetic patients were studied, all having

severe glycosuria but no ketonuria, and either fasting blood sugar levels above 150 mgm% or typical diabetic glucose tolerance tests. In addition, six prediabetic females, ranging in age from six to forty-one years, were examined. All had a family history of diabetes mellitus, normal glucose tolerance or only marginal impairment, but who had had typical diabetic curves during pregnancy, infection or after cortisone or prednisolone orally. One patient had had two stillbirths; one needed insulin during the latter part of pregnancy, which was complicated by toxemia necessitating a caesarean section. Blood was drawn in the non-fasting state, plasma albumin prepared and assayed by the rat diaphragm method in the usual manner. The results appear in Table III.

At a concentration of 1.25% both the prediabetic and obese diabetic albumins were antagonistic to insulin, to the same extent as albumin prepared from the plasma of insulin-requiring diabetics and to a considerably greater extent than that of normal albumin, which is inactive at this concentration. At this point no increased antagonism has been found with plasma-albumin from obese, but otherwise normal, persons. Moreover, patients with the diabetic syndrome, i.e. patients suffering from definite pancreatic disease such as hemochromatosis or acute pancreatitis, or who have undergone total pancreatectomy, have no increased antagonism to insulin associated with their plasma albumin. (27)

TABLE III. - INSULIN ANTAGONISM OF PLASMA-ALBUMIN FROM OBESE DIABETICS AND PREDIABETICS**

| | Mean glucose uptake above basal level* ± S. E. M. (mgm. glucose per 100 ml. per 10 mgm. rat diaphragm) | |
|---|---|---|
| | Buffer + 1,000 micro-units per ml. insulin | Fraction in buffer + 1,000 micro-units per ml. insulin. |
| Obese diabetic<br>Albumin 1.25% | 13.44±0.62<br>(5) | 4.43±1.34<br>(5) |
| Prediabetic<br>Albumin 1.25% | 15.20±0.77<br>(6) | 6.05±0.99<br>(6) |
| Normal<br>Albumin 1.25% | 11.32±0.74<br>(4) | 11.17±0.89<br>(4) |
| Insulin-requiring<br>Diabetic Albumin 1.25% | 12.32±1.05<br>(4) | 3.55±0.67<br>(4) |

Number of observations in parenthesis.

*Amount of glucose taken up by diaphragm when no insulin is added to medium.

**Vallance-Owen, J. and Lilley, M.D., Insulin antagonism in the plasma of obese diabetics and prediabetics. Lancet, 1:807, 1961.

The biochemical nature of the plasma-albumin insulin antagonist was given preliminary study by Vallance-Owen and Lilley. (28) The insulin antagonist can be separated from normal human albumin and rendered dialysable by heat coagulation of an albumin solution. There appears to be some inactivation of the antagonist by heat.

Removal of lipid material from albumin by refluxing with ethanol results in complete loss of antagonism with neither the extract nor the residual protein containing any activity.

Extraction of long-chain fatty acids with a mixture of iso-octane and acetic acid does not lead to concentration of the antagonist in this fatty acid fraction.

In addition, chloroform fails to extract the antagonistic substance from an albumin solution. Studies with Sephadex G-25 suggest a molecular weight of less than 4,000.

These observations suggest that the antagonist is of low molecular weight, and is unlikely to be a free lipid, fatty acid or steroid-type compound. It may be a polypeptide or a simple carbohydrate but lability under certain conditions supports a polypeptide structure.

Evidence is mounting that the "B" chain of insulin is transported in serum as an albumin-bound protein. (29) Recently Ensinck et. al. (30) established that after incubation of $^{131}I$-insulin in serum with preparations of purified glutathione-insulin

transhydrogenase, two radioactive compounds result. These have characteristics similar to the "A" and "B" chains of insulin and the radiolabeled component corresponding to the "B" chain is combined with serum albumin whereas the compound corresponding to the "A" chain is unassociated with any serum macromolecule. It is postulated that the two chains are transported in mammalian extracellular fluids and that serum albumin may act as a carrier protein for the "B" chain. It is theoretically possible that a part of the insulin molecule might compete with the whole molecule in metabolic processes.

Ensinck and Vallance-Owen have shown that the "B" chain of insulin, when complexed with albumin, previously rendered non-antagonistic by passage through a partially acetylated column, is capable of inhibiting the effect of insulin as determined by the rat diaphragm assay method. (31) Several additional physico-chemical characteristics are common to the synalbumin antagonist and to the radio-labeled "B" chain of insulin. Notable among these are:

(a) nondissociation from albumin by N/6 acetic acid.

(b) elution from Sephadex G-25 as molecular weight compounds below 4,000

(c) elution from cation exchange resin (Dowex 50 x 2) by 0.2M formate buffer pH 3.1.

(d) elution from anion exchange resin (DEAE cellulose) by 0.2M ammonium bicarbonate pH 7.7.

Such a high degree of similarity strongly suggests that the "B" chain of insulin and the synalbumin antagonist are synonymous.

## ESSENTIAL DIABETES

Consideration of all these observations and lines of investigation strengthens the concept that a fundamental abnormality in essential diabetes is increased synalbumin antagonism to insulin...an exaggeration of the normal. This allows the formulation of a basic etiological concept of essential diabetes and more clearly defines the position of prediabetes. The time of discovery of the first abnormal glucose tolerance test, currently the yard-stick of diagnosis of overt diabetes mellitus, will depend on the degree of antagonism and on the resilience or reserve capabilities of the beta cells of the pancreas. On this basis, abnormal glucose tolerance may, indeed, never occur, provided the beta cells are functioning to the degree that insulin production always out-strips insulin antagonism. If and when impaired glucose tolerance occurs it must be considered a late sign of a condition present and progressing since birth. The synalbumin antagonism is apparently profoundly affected by the pituitary-adrenal system and changes in physical health or environment involving this system will alter the degree of antagonism.

Therefore, systemic insults or stresses caused by pregnancy, infection, anxiety, growth spurt and menopause, or the administration of exogenous adrenocortical steroids, will precipitate the diabetic state of carbohydrate intolerance in susceptible individuals or aggravate the pre-existing condition.

This theory contradicts any idea that failure of the pancreas is primarily involved in diabetes mellitus. In fact, the mean size of islets in juvenile diabetics dying of the acute disease is greater than that of controls, (32) although both the mean size and weight of islets are decreased in proportion to the degree and duration of overt adult disease. Moreover, juvenile and obese diabetics have been shown to have high concentrations of insulin, and prediabetics have more circulating insulin than normal.

Regarding the action of insulin on various tissues when antagonized by synalbumin, Lowy et. al. (33) have demonstrated a differential effect on muscle and adipose tissue. Synalbumin inhibits the insulin response of rat diaphragm but not the response of rat epididymal fat. The differing effects may result from differences of glucose metabolism in fat and muscle. In muscle, a larger proportion of the glucose is converted to glycogen and breakdown is almost entirely by the glycolytic pathway. In fat, approximately equal amounts of glucose are metabolized by the glycolytic and pentose-phosphate pathways, and only a small proportion is converted to glycogen. Because the conversion

of carbohydrate to fat appears unimpeded by synalbumin, while protein metabolism is compromised, it is quite possible that obese diabetics are obese because they are diabetic and not vice versa. Newly diagnosed diabetics, over the age of forty years, are appreciably over-weight...the mean excess being almost 12% in each sex. (34) Abbas and Tovey (35) have demonstrated preliminary evidence that albumin can traverse placental tissue from the maternal to the fetal circulation, providing a route of transmission of synalbumin and possibly explaining the characteristic hypertrophy of the islets and the gigantism of the stillborn fetus of prediabetic mothers.

Diabetes mellitus has long been considered to be an hereditary disorder, transmitted by a single recessive gene according to Pincus and White. (36) Using the demonstration of excessive synalbumin antagonism as a biochemical marker, the relatives of diabetic patients have been studied. Plasma insulin antagonism rather than abnormal glucose tolerance was employed as the diagnostic criterion for essential diabetes. Of the sixty-one members of six families studied, twenty-five were synalbumin negative and thirty-six were synalbumin positive, i.e. had plasma insulin antagonism at concentration of 1.25%. Of the latter, only eleven had overt carbohydrate intolerance while two others had recurrent, spontaneous episodes of hypoglycemia.

> "The excessive synalbumin antagonism was found in familial concentrations which strongly suggested a 'dominant' mode of inheritance". (37)

The concept of genetic dominance is questioned by Alp and Recant (38) who observed two diabetics who showed little insulin inhibitory activity and who had diabetic siblings, one of whom was an identical twin who showed considerable inhibitory activity. Also, two of four prediabetics noted were not significantly inhibitory at 2% concentration of albumin. In addition, family history of diabetes did not seem to play a role in those pregnant women who showed greater than normal inhibitory activity.

The incidence of cardiovascular complications usually associated with long-standing diabetes and/or old age was studied among patients with acute cardiac infarctions who had no known disease or family history of diabetes mellitus. (39) Demonstrable antagonism in the acute series and results of subsequent tests are listed in tables IV, V and VI. Follow-up was considered essential to rule out the possibility of acute antagonism on the basis of shock associated with infarction. When twenty-eight unselected patients with cardiac infarction were compared with twenty-eight controls, nineteen of the former group were synalbumin positive as opposed to six of the latter. The groups were matched for age and sex. From the data, it is suggested that many patients with cardiac infarction are constituted as essential diabetics, only a small proportion ever demonstrating carbohydrate intolerance.

TABLE IV. - ANTAGONISTIC AND NONANTAGONISTIC PATIENTS WITH CARDIAC INFARCTIONS - ACUTE SERIES*

| | Acute | | After Recovery | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| Antagonistic | 9 | 4 | 9 | 4 |
| Nonantagonistic | 6 | 1 | 5** | 1 |
| Total | 15 | 5 | 14** | 5 |

**One patient died before re-examination was due.

TABLE V. - SYNALBUMIN ANTAGONISM TO INSULIN IN PATIENTS WITH CARDIAC INFARCTION AND THEIR CONTROLS, MATCHED FOR AGE AND SEX.*

| STATUS | INFARCTS | CONTROLS |
|---|---|---|
| Antagonistic | 19 | 6 |
| Nonantagonistic | 9 | 22 |
| Total | 28 | 28 |

$x^2 = 12.526$; $p = 0.001$

*Vallance-Owen, J., Synalbumin insulin antagonism. Diabetes, 13:243-4, (May-June) 1964.

TABLE VI. - ALBUMIN ASSAYS OF DIFFERENT GROUPS*

| Origin and status of albumin tested at 1.25 per cent. | Mean glucose uptake above basal level** standard error of mean (mgm. glucose per 100 ml. per 10 mgm. rat diaphragm. | |
|---|---|---|
| | Buffer + 1,000 micro-units per ml. insulin | Albumin in buffer + 1,000 micro-units per ml. insulin |
| Acute infarction: | | |
| Antagonistic | 13.23±0.45 (13) | 4.49±0.63 (13) |
| Nonantagonistic | 14.40±1.02 (7) | 13.68±1.07 (7) |
| Three months or more after recovery: | | |
| Antagonistic | 14.62±0.62 (13) | 3.08±0.56 (13) |
| Nonantagonistic | 11.67±1.08 (6) | 11.45±1.27 (6) |
| "Chronic" cases 6 mos.-4 yrs. after infarction: | | |
| Antagonistic | 12.14±0.97 (5) | 4.23±0.89 (6) |
| Nonantagonistic | 15.40±0.25 (2) | 15.30±0.70 (2) |
| Controls | | |
| Antagonistic | 11.65±0.56 (5) | 4.87±0.84 (6) |
| Nonantagonistic | 11.39±0.55 (12) | 11.84±0.44 (22) |

Number of observations in parenthesis.

*Vallance-Owen, J., Synalbumin insulin antagonism. Diabetes, 13:245, (May-June) 1964.

**Amount of glucose taken up by diaphragm when no insulin is present in incubation medium.

Alp and Recant (40) discovered highly significant levels of inhibitory activity in nephrotic urine albumin. This may be important in the understanding of the alterations in carbohydrate metabolism. Urine obtained from two diabetic nephrotics showed insulin inhibitory activity at 1.25%. Serum albumin from one nephrotic patient showed 56% inhibition; her urine albumin showed a 45% inhibition when tested at the same concentration. The loss of a potent insulin antagonist might well play a role in the decreased insulin requirements of these patients.

## SUMMARY

A brief history of the progress in knowledge and understanding of diabetes mellitus was provided by way of introduction. A review of the changing concepts of diabetes was presented with emphasis on the definition and significance of prediabetes. Certain aberrations of insulin and carbohydrate metabolism were sited which led to the better understanding of the relationship between the pituitary-adrenal system and glucose tolerance. The method of bioassay of insulin activity using the rat diaphragm procedure was described.

The isolation and identification of an insulin antagonist residing in the albumin fraction of human plasma was discussed. A quantitative difference in the ability of this substance to inhibit insulin was noted among normal subjects, prediabetics,

juvenile diabetics and adult-onset diabetics. A few of the biochemical properties of the synalbumin insulin antagonist were listed and the similarity between the synalbumin antagonist and the "B" chain of insulin was noted.

A basic etiological concept of diabetes mellitus was stated, stressing that pancreatic failure was not the prime causation. The differential effect of synalbumin antagonism on muscle and adipose tissue was mentioned with thoughts concerning its role in obesity associated with diabetes. The familial predisposition and heredity of diabetes was discussed with a suggestion of a "dominant" mode of inheritance, using synalbumin as a biochemical marker. The correlation of positive synalbumin antagonism to acute cardiac infarction was noted. Preliminary observations regarding synalbumin insulin antagonism in nephrosis were mentioned.

## CONCLUSIONS

The presence of a substance in human plasma, antagonistic to insulin, is beyond question. This insulin antagonism has been demonstrated by many observers. Its precise residence among the plasma proteins has been much debated. However, recent studies by Ensinck, et. al. confirm the earlier findings by Vallance-Owen, et. al. that the substance is consistently isolated in the albumin fraction of human plasma. Bioassay of albumin fractions from the

plasma of normal subjects, prediabetics, juvenile diabetics and adult-onset diabetics demonstrates consistent and quantitative differences in insulin antagonistic activity using solutions of standardized concentrations. The performance of insulin antagonism depends at least in part on the integrity of the pituitary-adrenal system as evidenced by the lack of insulin antagonism in hypophysectomized and adrenalectomized human patients when hormonal replacement therapy was withheld.

Although the biochemical properties of the synalbumin antagonist and the "B" chain of insulin show many similarities, they cannot be equated at the present time.

The discovery of synalbumin insulin antagonism allows the formulation of a new etiological concept of diabetes mellitus. When synalbumin insulin antagonism progresses beyond the normal limits, an overload is placed upon the pancreas. The seriousness of the disease will then depend upon the degree of antagonism and the resilience of the beta cells of the pancreas. If antagonism exceeds insulin production, carbohydrate intolerance and clinical diabetes occurs. However, overt intolerance need never occur but rather the prediabetic can live for many years in an exaggerated state of normal, i.e. greater than normal insulin production in response to greater than normal insulin antagonism. Impaired glucose metabolism may therefore be considered a late sign of a condition present since birth. Acute stresses such as infection,

pregnancy, anxiety, growth spurt and surgery may precipitate overt disease in the borderline patient.

Obesity in diabetes is probably the effect of synalbumin insulin antagonism rather than the cause of acquired carbohydrate intolerance. Synalbumin impedes the metabolism of glucose by muscle but not by adipose tissue.

Vallance-Owen suggests a "dominant" mode of inheritance of diabetes and therefore of positive antagonism. Certain exceptions to such "dominance" have been noted, e.g. inconsistencies in insulin antagonistic activity, family histories and bioassay system. It follows that clarification of the variety of non-genetic factors that may influence this inhibitory activity should first be made. Moreover, it is difficult, on a theoretical basis, to assign an all or none characteristic to a material which is present to varying degrees in most persons. Thus one must conclude that at this time it is premature to characterize synalbumin as a genetic marker.

There is a high correlation between synalbumin activity and cardiac infarction, suggesting a predisposition to this disease determined by the degree of atherosclerosis commonly associated with diabetes.

The mechanism of insulin inhibition appears to be competitive and is not quantitatively related to the free fatty acid content

of albumin fractions. The suggested equation of synalbumin and the "B" chain of insulin, and the subsequent possibility of a part of the insulin molecule competing with the whole molecule remains in doubt.

The role of albumin as a carrier protein for the "B" chain is still in question.

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REFERENCES


1. A monograph on diabetes. The Upjohn Co., 1961, p. 94-97.

2. Ellenberg, Max, Diabetic complications without manifest diabetes. J.A.M.A., 183:926, (Mar. 16) 1963.

3. Miller, H. C., The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant. J. Ped., 29:455, 1946.

4. Ellenberg, op. cit. number 2.

5. Jackson, W. P. U., Prediabetes - a synthesis. Post. Grad. Med. J., 35:287-96, 1959.

6. Ellenberg, Max, Clinical concept of prediabetes. New York State Med. J., 64:2885-90, (Dec.1) 1964.

7. Jackson, W. P. U., The cortisone glucose tolerance test with special reference to the prediction of diabetes. Diabetes, 10:33, 1961.

8. Steinke, J. and others, Studies on serum insulin-like activity (ILA) in prediabetes and early overt diabetes. Diabetes, 12:502, (Nov.-Dec.) 1963.

9. _____, Insulin and insulin antagonists in the serum of untreated juvenile diabetics. Lancet, 1:30, 1961.

10. Beigleman, P.M. and Tranquada, R. E., Serum insulin-like activity in untreated diabetics. Diabetes, 11:179, (May-June) 1962.

11. Yalow, R. S. and Berson, S. A., Plasma insulin concentrations in non-diabetic and early diabetic subjects. Diabetes, 9:4, 1960.

12. Vallance-Owen, J. and Lukens, F. D. W., Studies on insulin antagonism in plasma. Endocrinology, 60:625-33, 1957.

13. _____, Plasma-insulin activity in diabetes mellitus, measured by the rat diaphragm technic. Lancet, 2:583-87, 1955.

14. _____, Estimation of plasma insulin by the rat diaphragm method. Lancet, 1:68-70, 1957.

15. Vallance-Owen, J. and Lukens, F. D. W., op. cit. number 13.

16. Harrison, T. R. and others, Principles of Internal Medicine. New York, McGraw-Hill, 1958, p. 615.

17. Vallance-Owen, J. and Lukens, F. D. W., op. cit. number 14.

18. Gemmill, C. L., The effect of glucose and of insulin on the metabolism of the isolated diaphragm of the rat. Bull. Johns Hopkins Hospital, 68:329-36, 1941.

19. Vallance-Owen, J. and others, Insulin antagonism in plasma of diabetic patients and normal subjects. Lancet, 2:336-38, 1958.

20. Alp, H. and Recant, L., Studies of the insulin-inhibitory effect of human albumin fractions. J. Clin. Invest., 5:870-881, 1965.

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

22. Hendley, E. D. and others, Inhibition of muscle glucose uptake by diabetic rat sera of varying glucose and lipoprotein content. J. Biol. Chem., 226:459, 1957.

23. Field, J. B. and others, Further characterization of an insulin antagonist in the serum of patients in diabetic acidosis. J. Clin. Invest., 36:1588, 1957.

24. Vallance-Owen, J. and others, The nature of the insulin-antagonist associated with plasma albumin. Lancet, 2:696-97, 1958.

25. Vallance-Owen, J. and Lilley, M. D., An insulin antagonist associated with plasma-albumin. Lancet, 1:804-06, 1961.

26. Vallance-Owen, J., Insulin antagonism in the plasma of obese diabetics and prediabetics. Lancet, 1:806-7, 1961.

27. _____, Diabetes mellitus - causation. Proc. Roy. Soc. Med., 55:207-10, 1962.

28. Vallance-Owen, J. and Lilley, M. D., op. cit. number 25.

29. Ensinck, J. and others, The transport of "A" (glycyl) and "B" (phenylalanyl) chains of insulin in human serum. Diabetes, 12:353, 1964.

30. _____, Studies in vitro of the transport of the A and B chains of insulin in serum. J. Biol. Chem., 10:3377-83, 1964.

31. _____, Antagonism of insulin by the albumin-bound "B" chain of insulin. Diabetes, 12:353, 1964.

32. MacLean, N. and Ogilvie, P. F., Observations on the pancreatic islet tissue of young diabetic subjects. Diabetes, 8:83-91, 1959.

33. Lowy, C. and others, Antagonism of insulin by albumin. Lancet, 1:802-4, 1961.

34. Pyke, D. A., Aetiological factors in diabetes. Post. Grad. Med. J., 35:261-65, 1959.

35. Abbas, T. M. and Tovey, J. E., Proteins of the liquor amnii. Brit. Med. J., 1:476-78, 1960.

36. Pincus, G. and White, P., On the inheritance of diabetes mellitus. I. An analysis of 675 family histories. Amer. J. Med. Sci., 186:1-14, 1933.

37. Vallance-Owen, J. and Ashton, W. L., Cardiac infarction and insulin antagonism. Lancet, 1:1226-28, 1963.

38. Alp, H. and Recant, L., op. cit. number 20.

39. Vallance-Owen, J. and Ashton, W. L., op. cit. number 37.

40. Alp. H. and Recant, L., op. cit. number 20.