ANOMALY OF AN INSULIN-LIKE SUBSTANCE IN JUVENILE DIABETICS AND THEIR RELATIVES

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The common cause of diabetes mellitus - a disease entity adequately described by Aretacus in the first century A.D., remains obscure to this day. The classical observations of Von Mering and Minkowski (1), and Banting and Best (2), established that the pancreas (in particular the islet tissues of Langerhans) was essential for sugar homeostasis, and that the disease itself appeared correctable by the injection of 'insulin'derived by simple chemical means from this organ.

This initial momentous discovery was hailed as the first specific treatment of a disease in man. As a corollary, the idea that diabetes might be due to lack of insulin arose. This view was slowly modified as the progress of other components of the syndrome of diabetes, such as vascular disease, was found to be not necessarily dependent on the degree of control of hyperglycemia with insulin, or the amount of insulin required for this control (3). Diabetes mellitus as a part of other disease was increasingly recognized. Hyperfunction of the cortex and medulla of the adrenal (or similar neural tissues), the thyroid gland and the anterior pituitary, has been associated with a mellituria similar to that found in diabetes. Processes destroying the pancreas such as pancreatitis, hemochromatosis, or surgical removal of the organ, may produce a disturbance of carbohydrate metabolism similar to diabetes mellitus.

Duncan et al (4), and Becker and Miller (5), in reviewing case reports of diabetes resulting from such pancreatic causes, showed that some of these individuals may develop diabetic vascular lesions. However, another report (6) stressed the absence of vascular changes.

The hyperglycemic responses which the adrenals may evoke (7-10), were discovered, as were the similar actions of the pituitary (11, 13, 14) and thyroid (12), giving rise to the concept that these endocrine glands modify the action of insulin (15). The role of the pituitary gland in the causation of diabetes generally remains unsolved (16-21), and there is little evidence that there is increased adrenal corticoid production (22) in the disease overall.

Lack of insulin as a cause of the disease was unproven until methods of measuring the extremely small amounts of insulin in blood became available. Such a method was the response of adrenalectomized, hypophysectomized alloxan-diabetic rats (23) to the insulin-like effect of human serum. In a group of obese adult mild diabetics, this activity of serum appeared slightly lower than normal, but in the diabetic in severe ketotis, plasma insulin activity was absent. Indeed, animals receiving such sera were resistant to the hypoglycemic effect of subsequently administered insulin (24).

The ability of isolated organs such as rat hemidiaphragm (25), rat epididymal fat pad (26), and frog sartorius muscle (27), to respond to small amounts of insulin in vitro, made the measurement of insulin-like activity of serum a practical procedure. However, the concentrations of insulin found in normal human sera differ according to the organs used, suggesting that different substances were being measured (28).

Using these in vitro bioassay techniques, it was generally shown that non-ketotic or mild, untreated, diabetics had normal or even supranormal concentrations of insulin-like substance in their plasma, whereas uncontrolled severe and ketotic diabetics showed the low concentrations (29-37). Vallence-Owen (29) recorded little insulin activity in juvenile onset diabetics' sera using the rat diaphragm assay. Steinke et al, however, in addition to confirming this finding, showed when using rat epididymal fat, the opposite results were obtained (38).

MacLean and Ogilvie (39) showed that islet tissue in the pancreases of diabetic subjects early in their disease may be normal or even increased in amount, and Wrenshall et al (40) showed slightly reduced insulin in pancreatic tissue from early or adult onset diabetics. These latter workers showed little or no insulin in pancreases from juvenile onset diabetics who had had their disease for a long time.

In certain situations, it appeared that diabetes was not a disease directly related to lack of insulin. To accommodate these findings, a search arose for plasma substances which would inhibit the action of insulin.

From the above observations it appeared that a substance present in the serum of severe uncontrolled ketotic diabetics, but absent in the serum of controlled or non-ketotic diabetics, might be found.

Bornstein and Park (41) found that glucose uptake was about 25% lower in diaphragms incubated in sera from alloxan-diabetic rats than in those incubated in normal sera. These differences were not attributed to the presence of insulin in the normal sera since adrenalectomy or hypophysectomy of the diabetic rat serum-donors restored glucose uptake to normal. It was concluded that the serum of diabetic animals contained an inhibitor of glucose uptake which was subsequently thought to be a peptide fraction of growth hormone (42). Measuring the increase in glycogen deposition in the rat diaphragm (43), Field et al (44-47), have studied a serum insulin antagonist that appears during the stage of acute diabetic acidosis and disappears within a few hours following insulin therapy. This antagonist is a non-lipoprotein component of the α_1 globulins (45-47), resistant to the action of trypsin, but destroyed by chymotrypsin (46), and by heating at 100°C (45).

Using an ethanol fractionation of normal human serum (48), Vargas et al found that α_2 and βglobulins from fraction IV and VI inhibited glucose uptake of the rat diaphragm, and diminished the effect of added insulin, and could thus be regarded as insulin antagonists (49).

Vallence-Owen and co-workers, using the insulin induced glucose uptake of isolated rat diaphragms as an assay system, described a property of diabetic serum which antagonised the action of insulin in vitro (29). His earlier work was questioned as it became known that antibody production to insulin was an inevitable result of heterologous insulin treatment of diabetics (50, 51). These antibodies are capable of binding insulin and thus neutralizing its biological effect (29). The antagonist, however, was found not in the globulin fraction of serum (52), but in the albumin fraction (52-55). The antagonism could also be found to a lesser extent in normal serum, even after pancreatectomy (56), but could be abolished by adrenalectomy and hypophysectomy (56). Lowy et al (58) could not confirm this latter finding. Ensinck et al (59) suggested that the B chain of insulin was the substance whose effects Vallence-Owen had described, which does not accord with the antagonist being present in the serum of pancreatectomized cats (56). Keen (57) was unable to demonstrate antagonism to the action of insulin on diaphragm by 'synalbumin' extracts similar to Vallence-Owen's, and indeed found the contrary.

Pav et al (60) described a complement consuming antibody in most diabetics who had not received insulin. These findings have been questioned (28, 72).

'Insulinase', a soluble enzyme described by Mirsky (61, 62) and subsequently defined as an insulin-glutathione-transhydrogenase (63-67) is present in many tissues (68-70), and is an obvious

physiological antagonist of insulin. Excessive destruction of endogenous insulin in diabetics remains unproven (71), though the greater effect of an insulinase inhibitor (indole-acetic acid) in lowering blood glucose in the diabetic compared with normal, is an argument in favour of this possibility (I24). The disappearance rate of insulin injected intravenously into diabetic individuals is not however, accelerated (83).

Radioimmunoassay of insulin became available in 1959 (73), and was subsequently modified to make a simple assay of insulin antigen in serum (74). The assumption inherent in this technique is that only substances reacting with anti-insulin antibody will be detected, and that all forms of circulating insulin will react with the antibody in a manner similar to the purified insulin standards used. Despite these unverified assumptions, and the interference which complement in test plasma may cause (75), estimates of daily secretion in man using this method correspond well with the insulin requirements of humans recently pancreatectomized. Biological activity of insulin in vivo correlates better with immunoassay than bioassay on isolated tissues in vitro (76).

Radioimmunoassay soon revealed that not all diabetics lacked insulin antigen in their sera. Juveniles with ketosis invariably had low concentrations (77, 78) and responses of these concentrations, to glucose (79, 80), or tolbutamide (81, 82), became lessened, with increasing duration of the disease. Maturity onset diabetics, particularly in

the early phases of the disease, may have normal (87,88) or elevated (83-86) insulin concentration responses to glucose. Individuals with diabetes resulting from pancreatitis showed low concentrations of insulin in their sera (77). It appeared that diabetes in all cases is not based on insulin lack.

To accommodate some of these findings, experiments conducted by Samaan and associates, and Antoniades and associates, claimed to demonstrate serum binding of insulin, reducing the biolocical potency of the total insulin in serum. The former workers showed that 'typical' insulin was neutralized by antisera, whereas 'atypical' insulin was a form of insulin bonded to another substance in serum which rendered it nonimmunoreactive, but which had biological activity on the epididymal fat of the rat (89,92). This bound form of insulin was said to be present in excess in untreated obese adult diabetics (90), and could account for apparent lack of insulin-like effects in tissues other than fat in the diabetic. These views have been criticised (91), as serum substances of non-pancreatic origin may have an insulin-like effect on the rat epididymal fat (93-96). Antoniades and associates found that a fraction of serum with different ionic characteristics to insulin had insulin-like activity on rat epididymal fat, but not rat diaphragm, unless extracts of epididymal fat were added (97-101). This fraction was called 'bound' insulin, and failure of conversion of 'bound' to 'free' insulin was proposed as a cause of diabetes (102). There is evidence that treatment of diabetic serum with similar fat pad extracts does not release immunologically active insulin (76), and the same

criticism may be levelled at Samaan's work.

All of these insulin 'antagonists' have not been either found, or looked for, in relatives of diabetics, with the exception of Vallence-Owen's 'synalbumin' antagonist. The propensity to produce this substance is said to be inherited as an autosomal dominant (103).

INHERITANCE OF DIABETES IN HUMANS

The disease is known to have a strong familial incidence, but the mode of inheritance remains obscure.

Steinberg (104) has suggested that diabetes results from the homozygous inheritance of a recessive gene of variable expression. Whilst this hypothesis is most in accord with family studies, the extreme clinical difference between the usual juvenile onset diabetic, and the usual maturity onset diabetic, is difficult to reconcile with this hypothesis. In a group of juvenile diabetics and their parents (105), Simpson suggested that early onset diabetes may not be a result of gene disorder at the same locus as late onset diabetes. This author extended this work to a study of first degree relatives of diabetics, and the offspring of parents who were both diabetic, and concluded that the hereditary basis for diabetes is multifactorial (107).

Even the definition of diabetes has been questioned as an entity. Crombie (106) has suggested that the disease entity diabetes is a "semantic myth, or at best an arbitrary definition - ..lying.. anywhere

between the minimum requirement of 'diabetic' glucose tolerance and the maximal of a syndrome in which all the possible components have been in evidence at some time". Large scale testing of glucose tolerance in relatives of 'diabetics' has revealed a higher incidence of elevated blood glucose response than in the general population. Pincus and White in 1934 (108), studied 103 offspring and siblings of known adult diabetics by routine blood sugar examinations and oral glucose tolerance tests. A group of 125 subjects without a family history of diabetes mellitus was used as a control group. Their findings indicated that if both parents were diabetic, 25% of their children had hyperglycemia; if one parent was diabetic, 17.9% had an abnormal test, and if neither of the parents was known to have diabetes, only 6.8% had hyperglycemia.

In a carefully controlled study of the oral glucose tolerance in siblings of children with diabetes, Burkeholder et al (109) showed an 18% incidence of 'abnormal' glucose tolerance tests. In families in which both parents had 'diabetic' glucose tolerance tests, 56% of their offspring had abnormal tests.

Fajans and Conn (110) found abnormal tolerance tests in 19% of first degree relatives of diabetics. This percentage rose to 25% when cortisone was exhibited to the group, compared with suitable normal controls (111). However, the latter author stressed that "an abnormal test is not a sine qua non for the eventual development of overt diabetes, and a normal test does not exempt the child from the development of diabetes in the future."

Indeed, an increasing incidence of sustained hyperglycemic response to oral loading with glucose occurs with ageing (112), with an incidence of 25% over the age of seventy. This suggests that if glucose 'intolerance' is a marker of a genetic predisposition to 'diabetes', the gene frequency is high, with a low order of expression as the clinical disease (approximately 1% of a large population) (113).

Conn (114), Williams (115), and Schwartz and Hechter (116), suggested that diabetes might, as a theoretical possibility, arise from a disorder in the synthesis of insulin, resulting in a hormone with low biological activity. Such an abnormality, if genetically transmitted, would provide evidence of the mode of transmission of the disease, independent of tests of carbohydrate tolerance.

This thesis presents original experimental evidence that an abnormality of an insulin-like substance exists in juvenile diabetics, and in their relatives.