Some studies in the metabolic consequences of diabetes and the management of chronic pain.

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Some Studies in the Metabolic Consequences of Diabetes and in the Management of Chronic Pain

by

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Abstract

This submission details work published between 1979 and 1989 into some of the metabolic consequences of diabetes and the management of chronic pain. The results of these studies are to be found in the 26 published research papers appended.

1. **The metabolic consequences of diabetes:** studies in this area are various but fall into several distinct sections, a) the use of cortisol creatinine ratio as a marker for nocturnal hypoglycaemic episodes, these studies looked at the possibility that the stress response to hypoglycaemia could be used to identify episodes of nocturnal hypoglycaemia in insulin treated diabetics. b) investigations of the phenomenon of chlorpropamide alcohol flushing. These studies were designed to investigate the possibility that the tendency of some non-insulin dependent diabetic patients to flush when taking alcohol concurrently with chlorpropamide was important as a marker of their susceptibility to the long term complications of the disease. c) other studies involving the metabolic aspects of diabetic complications. The role of magnesium in glucose disposal; urinary albumin as a marker of renal disease; haemoglobin glycosylation and retinopathy; hormones in pregnancy.

2. **The management of chronic pain:** this work arose as a natural continuation of the work with cortisol as a marker for hypoglycaemic stress and looked at the stress response to other clinical interventions such as surgery and in subjects who experienced chronic pain and sought to discover how this might affect recovery from surgery and general patient well being. As a consequence of these studies a more general assessment was made of pituitary function before and after alcohol destruction of the pituitary as a treatment for the relief of chronic pain.

Much of the work required the development of new analytical rests for various analytes e.g. chlorpropamide, free insulin, insulin antibodies, plasma acetate and urinary albumin.

1.0 **Summary**

The publications contained within this portfolio represent original work undertaken between 1979-1989 and in collaboration with clinical and scientist colleagues in the fields of diabetes and pain. The main theme is that of analysis of diabetes but with minor excursions into the area of the metabolic and endocrine responses to pain. During these collaborations my role was largely that of analytical scientist; devising original techniques and experimental designs, as well as evaluating and performing the analytical and experimental procedures necessary to support the research hypotheses, but also contributing to the evaluation and interpretation of results and writing of material for publication. The results of these studies contributed to the development of our understanding of some areas of both diabetes and pain, to the improvement and development of analytical techniques for their study, and to the management of patient care.

2.0 Unrecognised Nocturnal Hypoglycaemia.

Before the discovery of insulin by Banting and Best in 1921 diabetes mellitus was invariably fatal, sufferers rarely surviving beyond early adulthood. Once universal insulin treatment had removed the immediate spectre of mortality it became apparent that insulin treatment alone was not a cure for the disease but the intermittent nature of insulin delivery would itself lead to unique metabolic consequences resulting in an increased mortality and morbidity associated with diabetes. In particular, insulin-dependent diabetics would often suffer from nephropathy, neuropathy, and a range of micro- and macro-vascular complications. It has now become an accepted aim of insulin treatment should be to establish 'good glycaemic control' and that control of the blood glucose to near normal levels should reduce the incidence of diabetic complications. Long term studies have subsequently confirmed the veracity of this belief. An unfortunate consequence of the maintenance of good glycaemic control is that, in the absence of normal feedback control, the insulin dose required to reduce the glucose to normal may under some circumstances be in excess thus causing an increase in hypoglycaemic episodes[1]. Indeed,

hypoglycaemia induced by insulin treatment may be the most frequently observed metabolic emergency[2].

In 1979, before blood glucose measurement at home was possible, it was usual to determine insulin dosage via a combination of patient records of the qualitative assessment of morning urine glucose and random blood glucose measurements taken in a diabetic clinic. When the diabetes seemed uncontrollable patients were hospitalised so that monitoring of the blood glucose during the day could be carried out. Clearly undertreatment with insulin will result in a raised blood glucose level especially during the overnight period when individuals are sleeping. As early as 1959, however, Somogyi [3] had advanced the theory that insulin overtreatment, causing unrecognised nocturnal hypoglycaemia, could result in the secretion of counter-regulatory hormones, such as adrenaline and cortisol, whose effects would oppose the actions of insulin and mobilise glucose stores such that the fasting glucose level in a patient upon waking would be raised. Clearly this posed a serious clinical dilemma since if this theory was correct both undertreatment and overtreatment with insulin could result in a raised fasting blood glucose making decisions regarding treatment changes most difficult.

This was the stimulus for a group of us to begin to consider ways in which it might be possible to determine if a raised fasting blood glucose could be easily attributed to either over or undertreatment with insulin. Insulin lowers blood glucose by inhibition of glycogenolysis and by stimulating the uptake of glucose by the peripheral tissues. As the blood glucose falls other glucose regulating hormones, notably glucagon and adrenaline, are released to 'balance' the effect of insulin and restore the blood glucose, these effects being so efficient as to maintain the fasting blood glucose within narrow limits. Both glucagon and adrenaline have an acute effect on the maintenance of the blood glucose. Where hypoglycaemia is prolonged both cortisol and growth hormone are also secreted and contribute to the insulin resistance which is seen after hypoglycaemic episodes [4].

Thus the hypothesis was proposed that in response to the hypoglycaemia caused by insulin overtreatment, counter-regulatory hormones to be released, and thus their measurement on waking may be an indication of the mode of the nocturnal hypoglycaemia. In order to be clinically useful the determination of insulin overtreatment would have to be sufficiently rapid

to allow for immediate changes in treatment and, for patient acceptance, should also be minimally invasive. At the time we began to consider this problem the measurement of hormones by immunoassay was still very much in its infancy and the measurement of glucagon and adrenaline was impractical whilst growth hormone assays required overnight incubations and therefore would not give the required information in adequate time. This left cortisol as a hormone for consideration. Cortisol is usually present in blood associated with the transport protein, cortisol binding globulin (CBG). At that time the measurement of plasma cortisol still required extraction from binding proteins before complex fluorimetric analysis thus making the assays lengthy and invasive if blood samples were to be used. It was, therefore, decided to try to use the measurement of cortisol in a randomly sampled morning urine, collection of which would be minimally invasive, and where measurements would be unaffected by protein binding. The technique chosen initially was the competitive protein binding method devised by Beardwell and Burke[5] utilising pregnancy serum as a source of binding protein and tritium-labelled cortisol as the tracer. The method was improved by altering the bound/free separation step and omitting the extraction of urines with dichloromethane. The assay was validated against the existing fluorimetric method with extraction. As a result of these ideas three studies were performed and subsequently published.

1. A preliminary study (#3) was performed to establish whether excretion of cortisol overnight could be used to demonstrate a nocturnal hypoglycaemic event. Because the samples were random urines it was decided to express the results as a ratio with the urinary creatinine in order to account for varying diuresis. The cortisol to creatinine ratio in an early morning urine sample was compared in four groups of patients; healthy subjects, non-diabetic hospital patients, diabetics without presumptive hypoglycaemia, and diabetics in whom hypoglycaemia was strongly suspected. In this study there was a clear distinction between the healthy subjects, the hospitalised non-diabetics, the symptom-free diabetics, and those with frank hypoglycaemia, allowing the establishment of a unitless ratio with a cut off level of $>55 \times 10^{-6}$ as indicative of a hypoglycaemic event.

2. The second study (#4) reported the use of the cortisol/creatinine ratio in the outpatient clinic. The results supported the argument regarding the prevalence of nocturnal hypoglycaemia

in the diabetic population. During the study of 62 patients attending the diabetic clinic and encompassing 200 early morning urines, 26 had cortisol/creatinine ratio in excess of 55×10^{-6} giving a prevalence of 26% for nocturnal hypoglycaemia, a figure which was similar to others reported at that time.

3. The third paper (#5) considered the use of the cortisol/creatinine ratio to aid in the detection of unrecognised nocturnal hypoglycaemia and in the optimisation of insulin dose in a group of diabetic in patients. The ratio was used to optimise the insulin dose such that if the fasting glucose was above 7 mmol l^{-1} the long acting insulin was increased, unless the cortisol/creatinine ratio was above 55×10^{-6} in which case it was reduced. Of the 43 patients studied 9 were stabilised by insulin reduction of insulin dose whilst 34 were stabilised by increasing the insulin dose.

It is reasonable to suggest that the use of cortisol/creatinine ratio to determine nocturnal hypoglycaemia represented an original contribution to the debate concerning nocturnal hypoglycaemia and the Somogyi effect. Other workers reported variable experiences with the test, some found it useful [6,7,8], others not [9,10]. Since this time, however, considerable sophisticated work has been performed such that the scientific concepts underlying the Somogyi effect have been called into question. Whilst there is undoubtedly a rise in glucose between 5 am and 8 am this is caused by the normal diurnal changes in growth hormone secretion which occur irrespective of nocturnal hypoglycaemia. When nocturnal hypoglycaemia does occur, although there is a counter-regulatory response to hypoglycaemia in diabetics, it is insufficient to cause a rebound elevation of glucose in the presence of insulin. Further, it has also been shown that the counterregulatory response to hypoglycaemia is impaired in patients with diabetes[11]. This has been shown mainly to influence glucagon and adrenalin with disagreement as to the effect of circulating insulin level on the cortisol response to hypoglycaemia. However, a decreased response of cortisol to CRF at elevated levels of insulin has been shown for healthy subjects[12]. Any effect of increased insulin level is likely to be further exacerbated by the presence of circulating insulin antibodies which act as a resevoir for insulin.

Nonetheless attempts to maintain normoglycaemia mean that the diagnosis of nocturnal hypoglycaemia still represents a serious clinical problem. The existence or otherwise of the Somogyi effect is a subject around which there is still considerable debate [13].

3.0 The endocrine response to pain.

As a result of the above studies a strong collaboration was established with members of the Oxford Regional Pain Relief Unit who were interested in the possible endocrine and metabolic responses to surgery and to pain. This collaboration resulted in four publications (#6,#8,#9,#13) between 1981 and 1983. In each of these studies my contribution involved the design and planning of experiments, liason with the clinical team, collection of samples, storage and handling of samples and data, the conduct of the laboratory investigations, analysis of data and the production and presentation of the finished work.

It had been recognised that surgical stress resulted in some well defined metabolic and hormonal responses which were characterised by altered carbohydrate metabolism, net protein loss and lipolysis. It had been suggested that improved post-surgical morbidity could be achieved by reducing the stress response to the surgery and strategies to control postoperative pain were designed with this end in view. These studies, however, had been mainly concentrated on gynaecological procedures and had neglected to consider the possibility of a difference related to sex. The first study (#6) aimed to consider the endocrine and metabolic response to anaesthesia and surgery in subjects undergoing total hip replacement. This study looked at the metabolic and endocrine response (as determined by the plasma glucose, cortisol and prolactin), during and following surgery in 12 men and 8 women treated with the synthetic opiate, buprenorphine, immediately before and 3 hours post operation.

A stress response to the operation was clearly evident with all three measured substances showing a post surgical elevation. What was interesting was the finding that there were significant differences in the response for men compared with those in women. Men showed a fall in cortisol after the second dose of buprenorphine while women had a greater rise in prolactin than the men. Attempts to produce stress free surgery still continue today. The current debate mainly concerns the possible benefits of epidural analgesia[14,15] and patient controlled analgesia[16] in reducing the stress response to surgery.

We continued this line of enquiry, albeit some years later, by examining whether the cortisol/creatinine ratio technique we had devised earlier could be used to observe a stress response induced by pain alone. (#13). A total of 67 patients attending the Pain Relief Unit were studied compared with a control group comprising 33 subjects who were either laboratory staff or convalescent in-patients at the Radcliffe Infirmary, Oxford. The subjects had pain from a variety of causes and were receiving a number of therapies. Of these 31% showed a raised cortisol /creatinine ratio. None of these could be related to pain score, cause of pain or drug therapy. Given the relatively small numbers and many variables this is perhaps not so surprising. What is important, however, is that this study contributed to the debate that pain itself could be considered a stress and that the treatment of pain should also be aimed at reducing the hormonal component of the stress response. The debate as to how best to modify the stress response to pain continues today.[17]

This interest in the endocrine responses to stimuli led to my development of a number of immunoassays for pituitary hormones, especially growth hormone, lutenising hormone, follicle stimulating hormone and thyroid stimulating hormone. A collaboration then evolved between myself and a group of surgeons who were performing hypophysectomy for the relief of terminal cancer pain. It was known that alcohol ablation of the pituitary could relieve cancer pain in about 70% of patients[18] but the mechanism was unknown[19]. The degree of pituitary functional loss, and its effects, were also unknown at this time. Thus, it was decided to study the effect of hypophysectomy on pituitary function. Pituitary function was tested before; and 6 and 12 weeks after, pituitary ablation, by measuring the response at 30 minute intervals for 2 hours to releasing hormone stimulation and hypoglycaemic stress. The results showed again that pain relief was achieved, but that destruction of the pituitary was variable. Pain relief was not related to loss of any particular hormone or to the degree of pituitary destruction. The mechanism for this effect remains controversial[20].

4.0 Chlorpropamide Alcohol Flushing

Non-insulin dependent diabetic patients have been prescribed oral hypoglycaemic agents for over 30 years and chlorpropamide has always been one of the most popular of these agents[21]. Chlorpropamide treatment has always been associated with certain side effects; particularly

hyponatraemia and hypoglycaemia, especially in the elderly[22], hypertension,[23] and an unusual reaction which is characterised by an embarrassing facial flushing after alcohol ingestion (Chlorpropamide Alcohol Flushing, CPAF) not unlike that seen with disulfiram. This flushing phenomenon was studied extensively during the 1980's as possibly genetically derived and thus a potential marker for subsequent diabetic complications. It is no longer considered to be of any pathophysiological significance but nonetheless engendered considerable debate at the time. Dr Rowan Hillson was particularly interested in this phenomenon and I was approached with a view to setting up a team to study the mechanism underlying the facial flushing. This collaboration was to yield three publications over the next few years (#11, #12, #24). Dr Hillson had developed a technique for measuring cheek temperature rise in response to a measured dose of alcohol. Although we were able to relate this to the dose of the drug we particularly wanted to try to relate the cheek temperature rise to the circulating level of the drug. My first assay for chlorpropamide involved solvent extraction from serum followed by methylation using diazomethane and gas-liquid chromatography (GLC) with flame ionisation detection. This was quite successful and the first studies were performed using this assay. It was , however, quite time consuming and the methylation step meant that we often had poor recoveries. The introduction into the laboratory of a high performance liquid chromatograph allowed the development of a new assay which permitted direct measurement of chlorpropamide on this instrument using UV absorption. This eliminated the requirement for derivatisation in the GLC method, and at the same time enabled the assay of the drug to be determined with greater speed. Subsequent interest in the possible role of acetaldehyde and aldehyde dehydrogenase in this phenomenon led to my development of assays for erythrocyte chlorpropamide and for aldehyde dehydrogenase within the red cell. As previously my role in this group extended also to planning (and often to the performance) of experiments, collection and storage of samples, collation of data and the writing of publications.

The first publication (#11) reported the findings from a cross over study involving six subjects given either chlorpropamide or placebo. Red cell aldehyde dehydrogenase activity failed to correlate with plasma chlorpropamide or with the flushing phenomenon. This finding was confirmed during a more extensive study (#24) involving 21 subjects on long term chlorpropamide therapy. The attempt to link the flushing phenomenon to the activity of

aldehyde dehydrogenase was logical since it was known that levels of acetaldehyde were raised in those who exhibited flushing. In retrospect, however, these findings are can now be understood since this phenomenon is mediated via inhibition of hepatic aldehyde dehydrogenase by an as yet unknown metabolite of chlorpropamide[24]. The other publication (#12) reported the observations in two groups; one which had noted flushing and one which had not. The extent of the facial flush as measured by cheek temperature rise, was correlated to the plasma chlorpropamide level (irrespective of group) and to the daily dose of the drug. Thus the hypothesis that CPAF was a marker for diabetic complications would seem to have been something of a confounder with all subjects, even non-diabetics[25], exhibiting the phenomenon to some degree.

5.0 The Oxford Prospective Study.

During the time at the Sheikh Rashid Diabetes Unit there was an ongoing prospective study of non-insulin dependent diabetes (NIDDM) for which I became responsible for the day to day organisation of samples and assays. This study aimed to look prospectively at patients diagnosed with NIDDM between 1973 and 1976. The diagnostic criteria for entry to the trial were glucose disposal rate constant (K_G) < 1.2 during a 20 g/m^2 body surface intravenous glucose tolerance test, or a preceding blood glucose > 8.5 mmol l^{-1}. Many clinical and biochemical parameters (e.g. K_G, glucose, Body Mass Index, blood pressure, glycerol, lactate, insulin, ECG and vibratory sensory threshold) were measured at diagnosis and 3 and 5 years post hoc in order to assess their possible contributions to the morbidity associated with the disease. Interim studies and reports were published from these studies using this valuable database.

5.1 Magnesium and diabetes.

During the early 1980's there was increasing interest in the role of certain metal ions in the development of diabetes and its complications. In particular, magnesium had been shown to be associated with impared glucose disposition, retinopathy, platelet dysfunction, and hypertension. Thus, Dr Nick Ward at the Imperial College Reactor Centre was invited to collaborate in a study of the role of metal ions in diabetes using the existing sample database.. The attraction of this collaboration was that Dr Ward used the technique of neutron activation analysis, a method capable of the determination of a large number of ions from the same sample.

From the analysis the results could be fed into the database and associations sought, using multiple linear regression analysis, between the various clinical and biochemical parameters. My role in this study involved being responsible for the sample collections, integrity of samples, collation of data and various biochemical analyses. I was also required to validate the elemental analysis technique. Because Neutron Activation Analysis was a specialised technique which had not been used in this area previously it was necessary to check the associations found using more traditional techniques. Thus, I set up atomic absorption spectrophotometric techniques for several of the atoms of interest, especially magnesium, calcium, vanadium, aluminium and zinc, using liquid aspiration and graphite furnace techniques. This was necessary in order to validate the results found by neutron activation analysis. A strong correlation was established between magnesium and glucose disposal which became the basis for publication #14.

In this study 32 insulin treated and 55 non-insulin treated as well as 30 non diabetic subjects were investigated. No differences were found in plasma magnesium between diabetic and non-diabetic subjects taken together. However, when analysed according to those taking insulin and those who were not, it was found that those who received insulin had lower magnesium, while those who did not, had higher magnesium than the controls. Plasma magnesium was found to be inversely related to both glucose and haemoglobin A_{1C} (an integrated index of long term glucose control) and directly related to the rate constant, K_G, even allowing for the previous relationship to glucose. These findings have recently been confirmed[26].

Serum contains only 0.3% of the total body magnesium. Various methods have been used to assess magnesium status; erythrocyte magnesium, leucocyte magnesium, 24 hour urinary magnesium excretion as well as, more recently, methods based on ion-selective electrode assessment of ionised magnesium and methods based on nuclear magnetic resonance[27]. A recent review[28] confirms these findings and notes that hyperglycaemia appears to induce hypomagnesaemia via osmotic diuresis. The study raised the question of the possible benefits of magnesium supplementation, but the advantages of this are still unresolved[29,30]

5.2 Mortal factors in NIDDM

The second publication to arise from the Oxford Prospective Study (#17) related to factors which predicted death five years from diagnosis. It reflects a substantial volume of data

collected over many years with significant contributions from several individuals including myself. The paper discusses the outcomes of 214 patients at five years post diagnosis of NIDDM and established, by a stepwise logistic regression proceedure, those factors which predispose to death and an overall prediction factor which may be used to target those most at risk. Five factors were determined; long duration of symptoms and a) increased glucose intolerance b) higher systolic blood pressure, c) less obesity, d) higher blood glycerol; all judged at one year after diagnosis. Using the predictive mortality index gave a sensitivity of 90% and a specificity of 83%. These findings were confirmed recently at ten year review [31,32] and extended in that the two major associations (glucose tolerance and blood pressure) appeared to form two independent interrelated groups: a metabolic group and one associated with 'degenerative conditions'. The search to identify risk factors and assess their contributions to disease continues today.[33,34,35]

5.3 Retinopathy and glycosylated haemoglobin (HbA)

A major complication of diabetes is the development of retinopathy that manifests as tiny microvascular aneurysms on the retina of the eye which can give rise to blindness. Part of the Oxford Prospective Study involved a collaboration with members of the Oxford Eye Hospital to monitor retinopathy in study subjects and to identify any predisposing factors. It had been recognised for some time that there was a relationship between the prevalence of retinopathy and glycaemic status as judged by fasting blood glucose measurements. The use of glycosylated haemoglobin measurements as a long term index of glycaemic control afforded the potential for a more useful index. This enabled the comparison of prevalence and progression of retinopathy to be related to glycosylated haemoglobin within the study. For this reason we set about developing a method for the routine assay of HbA Several methods had been described for this measurement; ion-exchange chromatography, affinity chromatography on boronic acid gels, iso-electric focussing and agarose gel electrophoresis utilising the electroendosmotic flow principle. Each method was developed and validated within our laboratory. I found that the agarose gel technique was offered the best analytical and clinical performance. At this stage we prepared our own gel plates and buffer systems and despite the introduction of commercial systems for this analysis we continued to use our own method for the duration of this study.

The paper from this work (#23) showed that, whilst the prevalence of retinopathy may be related to glycaemic control, once disease is established, progression of the disease is then unrelated to glycaemic control. This was very much in accord with the observation that scrupulous glycaemic control did not improve established retinopathy. A small but significant relationship was observed between the progression of retinopathy and the level of non-esterified fatty acids.

Recent studies continue to show glycaemic control as central to the development of retinopathy in both IDDM and NIDDM[36], although some studies show that progression is also related to the level of hyperglycaemia[37]. It is possible that the variable progression to retinopathy seen in individuals has a genetic component with the finding of a relationship to the immunoglobulin Gm allotype. [38]

6.0 The measurement of Free Insulin

Insulin treatment has been the obligatory treatment for juvenile onset diabetes since its first introduction in 1921. Until the introduction of recombinant human insulin, almost all of the insulin used has been obtained from either porcine or bovine pancreas. In 1944 Lowell [39] reported the presence of antibody -like insulin neutralising factors in serum. That these factors were indeed antibodies was confirmed by Yalow and Berson and co-workers[40]. Such antibodies are to be found in the serum of almost all insulin treated diabetics within a few weeks of the commencement of insulin treatment. Theoretically insulin antibodies could give rise to several deleterious effects *in-vivo*; among them insulin resistance, hypoglycaemia due the inappropriate release of insulin and vascular complications due to circulating immune complexes. In practice, however, their presence seems to have few discernible clinical effects. Plasma insulin levels in diabetic patients previously treated with insulin cannot be measured by conventional immunoassay methods because of the presence of the human anti-insulin antibodies in the plasma interfere with the antibodies in the assay. These endogenous antibodies can combine with the added labelled insulin and interfere between the binding of labelled and unlabelled ligand. At the Sheikh Rashid Unit we were anxious to develop methods suitable for the measurement of the free biologically active insulin and I was fortunate to be allowed to spend some time at the Novo Institute in Copenhagen learning the polyethylene glycol (PEG)

precipitation technique for assay of free insulin. This technique involved the precipitation of anti-insulin antibody with attendant insulin thus leaving the free unbound insulin in the supernatant fluid following centrifugation. Once the method was introduced into our laboratory I became intrigued why the precipitated insulin antibody should continue to bind insulin. I reasoned that the PEG precipitation procedure should destroy the tertiary structure of the insulin antibody and thus the binding site for insulin. I decided to apply the method of Steady State Gel Filtration (SSGF) to this problem. SSGF had been developed by Burke [41] for the measurement of free steroid hormones and seemed equally applicable to this problem.[42] The results of this study were submitted as part contribution to the degree of MSc [43]. Two other publications arose as a result of my work in this area (#1, #2), the first formed a chapter in "Methods in Diabetes Research" where the current techniques for free insulin measurement were described and the second was an invited review article on the effects of insulin antibodies and the various methods for the measurement of free insulin.

7.0 Analytical Publications

This section outlines four different publications which may be described as being purely analytical topics. In each case my role was related to assay and experimental design, data interpretation and to the writing of the final publication.

7.1 Thyroxine

This paper #10, describes a simple method developed at our laboratory at the Radcliffe Infirmary for the analysis of thyroxine by radioimmunoassay. At this time (1982) commercial assay kits were becoming freely available and whilst previously immunoassay had been the province of larger institutions with our expertise the advent of commercial reagents offered the opportunity for smaller laboratories to perform what were hitherto rather specialist tests. There was considerable debate as to the merits of commercial reagents especially in terms of cost. This paper aimed to demonstrate that analytically sound, cost effective assays could be created simply using commercially available individual components rather than purchasing the reagents in expensive kit form.

7.2 Urinary albumin (#16)

Around 1982 Viberti [44] reported that the finding of low concentrations of albumin (microalbuminuria) in the urine of diabetic subjects could act as a marker of subsequent renal complications. In order to investigate this observation for our own patients I designed a radioimmunoassay for albumin in urine which we used for various subsequent studies and then routinely in the management of patients. We collaborated with a group from the Institute of Urology in London who wanted to set up an assay for microalbumin but who did not have access to radioimmunoassay equipment. We decided to develop an electroimmunoassay in order to retain the benefits imparted by the antibody technology without the problems associated with radioimmunoassay. This novel approach had two main drawbacks; electroimmunoassay was traditionally slow, insensitive and is less suited to large sample numbers. A more useful assay was, however, designed, which compared favourably with our existing in house assay and which offered a new approach for laboratories with a small diabetic population who wished to offer this measurement.

7.3 Plasma Acetate

It was common practice at the Sheikh Rashid Diabetes Unit to measure various intermediary metabolites during studies on diabetic subjects, in particular the free fatty acids and ketone bodies. We were interested in the measurement of acetate for several reasons. Whether the high turnover of fatty acids found in diabetic subjects was likely to cause an increase in acetate. Because of the relationship of acetate to alcohol metabolism, and thus CPAF. And what effect the higher circulating levels of glucose found in diabetic subjects would have on the level of circulating acetate. There were at that time no simple methods available for the measurement of acetate in plasma. The extant methods were either complex enzymatic methods or gas liquid chromatography. The recent commercial availability of the enzyme acetate kinase allowed us to modify the method of Trivin [45] to a relatively simple coupled assay system and to adapt the method for analysis by centrifugal analyser in order to facilitate a high throughput of samples. We realised early on that the commercial preparations of acetate kinase could be contaminated by hexokinase and would need to be checked before use. My studies showed that indeed, acetate levels were higher in diabetic subjects compared to non-diabetics. Furthermore acetate levels

were correlated to glucose, lactate and acetoacetate levels. Other than observations in rats, [46] this was probably the first report of increased plasma acetate in diabetic subjects. This method was subsequently the mainstay of several studies which increased our knowledge regarding the metabolism of acetate in diabetes. [47-52]

7.4 Insulin and haemolysis

It had been reported that haemolysis affected insulin assay when charcoal was used as the mechanism for the separation of bound from free label. A group of us involved in measuring insulin were interested whether this was an observation unique to the separation method or one that was general to measurement of insulin. The paper (#22) reports the results of a study in which samples split and then deliberately haemolysed were assayed by either using charcoal separation or separation using a double antibody technique. C-peptide was also measured. The study clearly showed that a reduction in immunoreactive insulin occurred independently of the assay method, and that C-peptide was not so affected. These findings were of importance to anyone involved in the analysis of plasma insulin.

8.0 Miscellaneous.

This section comprises three publications which represent collaborations with other research groups.

8.1 Albumin Creatinine Ratio.

This publication (#18) was a collaboration between myself and members of the John Radcliffe Hospital Department of Paediatrics and Northamption General Hospital. My contribution was to provide the laboratory analyses, particulary the urinary albumin, to be responsible for the samples and liason during the exercise testing. I was involved in the data collection and contributed to its interpretation and to the final construction of the paper. Since it was known that whilst morphological changes could be observed in the kidneys of virtually all diabetics, but that only about 35% would subsequently progress to clinical nephropathy, the measurement of 'microalbuminuria' was seen as a possible marker for this subgroup. Furthermore it had been suggested that strict metabolic control could delay progression to nephropathy in this group. Thus the aims of this experiement were to see if exercise could unmask latent glomerular damage in children before overt microalbuminuria appeared and thus define the at risk subgroup at the

earliest possible time post diagnosis. Members of the University Department of Paediatrics performed a standardised exercise test on 40 children with IDDM and 21 non-diabetic similar children. Samples were collected pre and post exercise. The data was expressed either as Albumin Excretion Rate (AER) or as albumin/creatinine ratio. There was no difference between the diabetic and control groups before exercise but a quarter of the diabetic subjects showed an increased albumin excretion post exercise when compared to the non-diabetics. None of the 25% of diabetic subjects would have been characterised as abnormal by previously established criteria. Longer term studies[53] have confirmed these immediate post exercise findings but failed to find an increase in prediction of future renal damage.

8.2 Water Balence in the Elderly (#21).

Reduced renal excretion of water in the elderly leading to oedema and hyponatraemia represents a serious clinical problem. Dr Martin Crowe was particularly interested in this problem and assembled a group to perform a study of the problem. The aim of the study was to investigate the effects of age on thirst, vasopressin and renal responses to an oral water load and to determine whether the ability to excrete excess water is impaired in the elderly. My involvement in this study extended from planning and study design, the measurement of osmolar, creatinine and free water clearances, and writing of the published article. Six elderly water replete men were compared to two control groups of 6 water replete and six water deprived young men. The study clearly demonstrated a defective renal water excretion and thirst in elderly men compared to young men. This study extended the work of Lindeman[54] who examined the effects of age on free water clearance during maximal water diuresis but who was unable to measure plasma sodium, osmolality or vasopressin. These were important findings which increased our knowledge of water balence in the elderly and had significant outcomes in terms of patient care.

8.3 Hormonal changes in Diabetic Pregnancy (#20)

Pregnancy poses a considerable diabetogenic stress. Since many of the metabolic changes in pregnancy could be reproduced by administration of growth hormone (GH) it was decided to study the relationship between GH and Human Placental Lactogen (HPL) and the

development of diabetic complications during pregnancy. 3 groups of subjects were studied during 2nd and 3rd trimester until post-natel examination. 14 were non-diabetic, 15 insulin-dependent and 8 gestational diabetic. I was involved in all aspects of this study, particularly the evaluation of the HPL assay and the checking of antibody specificities between the closely related GH and HPL. I was responsible for the performance of the other assays (Urinary albumin, glycosylated Hb etc.) for the maintenance of the samples and for the collection of data. The study found no increase in tissue damage amonst the subjects studied. The urinary albumin rose in the immediate post-natel period in the IDDM group. Systolic blood pressure also rose in the 3rd trimester and pre-delivery in both the diabetic groups. This was the first study to show that diabetic subjects had higher GH levels than controls during pregnancy. Much work has continued in this area. It is now recognised that the placenta also secretes GH[55] and that this may regulate the secretion of maternal IgF-1[56] during pregnancy. It has further been suggested that the diabetogenic stress associated by GH may in fact be due to a 17-kilodalton fragment (hGH)-(44-191) rather than intact GH[57].

9.0 Learned Societies

During the period covered in this submission I was an active member of both the Medical Research Society and the British Diabetic Association (I remain a member of the MRS). During this time I attended a number of National and International Scientific Conferences to present papers and posters. Publications #7,15, 25, 26 and others (see Appendix 1) are submitted as evidence of an active contribution to the scientific community.

10.0 Appendix 1

1. Intravenous glucose tolerance and the accompanying insulin response corellate with plasma concentrations of group II elements in non-insulin treated diabetic patients
   Hockaday TDR, **Smith R.F,** Ward N, Dhar H, Yajnik CS
   Diabetologia (1983) 25: (2) 165

2. Does the Beckman glucose analyser overestimate glucose
   Smith R.F and Humphries S
   Diabetic Medicine (1985) 2: 514

3. Leukocyte Sodium Fluxes in normal plasma and tissue culture fluid
   Ng LL, **R.F Smith** and Hockaday TDR
   Clinical Science (1985) 69: S12 83

insulin gene.

R.F. Smith, Wainscoat J.S, Ng LL, and Hockaday TDR
Clinical Science (1986) 71: S15 62

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