THE ABC OF THE DIABETIC HAND – ADVANCED GLYCOSYLATION END PRODUCTS, BROWNING AND COLLAGEN

As long ago as 1912, Maillard foresaw the importance of his observation of the reaction of reducing sugars with amino acids to produce brown discolouration and CO_2 [1]. Although the food and brewing industries took particular interest in this reaction and were able to apply it to their advantage, nearly 60 years were to pass before its relevance to medicine was realized through work on haemoglobin and collagen. When products of the Maillard reaction were found in aging and diabetic tissue, Maillard's supposition was confirmed [2].

Now known more commonly as 'browning' or non-enzymatic glycosylation, the Maillard reaction is, in fact, a series of reactions. The initial step of non-enzymatic condensation of a reducing sugar with an amino acid forms a Schiff base or early glycosylation product. The rate of this step is dependent on the concentration and type of sugar. It is rapidly reversible if sugar concentration falls below a critical level, otherwise the Schiff bases re-arrange themselves to become more stable compounds, known as Amadori products (glycosylated haemoglobin is an example). The level of these reflect average glycaemia over the previous 5 to 6 weeks as well as the rate of protein degradation. The Amadori product may then be oxidized to form an irreversible lysine residue or form reactive intermediates which propagate the Maillard reaction and lead to its termination by the formation of heterocyclic protein adducts with intra- and inter-molecular cross-links. These products are known as advanced glycosylation end products (AGEs). As well as having brown discolouration some of these products are fluorescent which can be quantified and thus provide a measure of the extent of browning.

Whereas the browning of long lived proteins such as collagen in diabetics appears to be an acceleration of the normal aging process, partial starvation may retard some of these processes [2]. The fluorescence of the pentosidine cross-link increases exponentially with age [3] but the fluorescence of diabetic collagen compares with that of non-diabetics twice their age [4]. Glycation rate of collagen can be reduced after 4 months of intensive insulin therapy but once browning has occurred the changes are probably irreversible [5]. In contrast, collagen browning and cross-linking can be increased in experimental hyperglycaemia [6] and type IV collagen production can be increased by a factor of 2 to 3 in cultured glomerular epithelial, endothelial and mesangial cells when exposed to high concentrations of glucose [7].

Collagen-linked fluorescence from skin biopsy specimens was found by Monnier and colleagues to correlate with the severity of retinopathy, arterial wall stiffness and hand joint stiffness [8]. There was a positive trend between the degree of fluorescence and severity of nephropathy but this did not reach significance. The age-adjusted fluorescence was twice as high in the diabetics and there was an interactive relationship between retinopathy, arterial wall stiffness, systolic blood pressure and age-adjusted fluorescence. More recently specific AGE-receptors on macrophages have been identified from which a radioreceptor assay for AGE-modified proteins has been developed [9] which promises to be a more sensitive and specific method to quantify AGEs. These authors measured AGE content in arterial wall collagen and serum AGEs in both a low molecular weight peptide fraction and a high molecular weight protein fraction. Tissue levels were four times higher in diabetics, particularly those with end-stage renal disease who had levels twice as high as those diabetics without renal disease. Moreover, serum levels of both fractions were elevated in diabetics and the peptide fraction appeared to parallel the severity of renal impairment. Haemodialysis was able to reduce the peptide fraction by 24% (compared to a creatinine reduction of 75%) but renal transplantation in two patients resulted in normalization of AGE peptide serum levels within days. This suggests that the normal kidney is able to clear the circulating low molecular weight peptide fraction and therefore limit their tissue accumulation. However, the lack of a consistent independent association between limited joint mobility and early nephropathy, in contradistinction to retinopathy [10,11], might suggest that high molecular weight AGEs are accumulating before renal damage is overt, but once renal damage occurs this process accelerates and involves the low molecular weight fraction as well.

AGEs are able to trap proteins including low density lipoprotein [12], and the accumulation of these not only impedes degradation but they may also induce a cascade of events including transendothelial monocyte chemotaxis, secretion of platelet-derived growth factor [13] and monokines such as interleukin-1 and tumour necrosis factor [12].

In a histological study of joint capsular tissue from a group of type 1 diabetics with frozen shoulder, proliferation of fibroblasts and myofibroblasts identical to those seen in Dupuytren's disease and vascular changes suggestive of diabetic microangiopathy were found and the authors postulated that release of platelet-derived growth factor from abnormal vessels might be responsible [14].

The oxidation reactions between reducing sugars and proteins may in themselves induce an escalating cycle of damage to both proteins and lipids with increasing production of free radicals and saturation of normal scavenger systems [15]. The finding of increased concentration of substrates able to produce superoxide-free radicals in idiopathic Dupuytren's disease and the benefit of allupurinol [16] is of particular interest.

The inter-relationship between these processes is likely to be complex but the result of these alterations to collagen in the diabetic hand is to produce a stiff hand. A number of terms are used including cheiro-

arthropathy, diabetic contractures or limited joint mobility. 'The syndrome of limited joint mobility' seems to be the favoured term by our North American colleagues and simply 'limited joint mobility' here. The clinical features are of flexion contractures affecting the interphalangeal joints, particularly the proximal, and the metacarpophalangeal joints, along with thickening and tightening of the skin. These changes may not be confined to the hand but the hand appears to be the initial target. Palmar changes including fascial thickening, induration, nodules and tendon contractures (i.e Dupuytren's disease) should be absent. In practice I believe this distinction is difficult and possibly even unnecessary given the known association with diabetes and that the underlying aetiopathogenesis may be similar.

The clinical assessment of flexion contractures is based on 'the prayer sign' and 'the table top' tests—the subject attempts to either oppose the palmar surfaces of the hands or place the hand flat on a table top, in both cases with the fingers fanned. Severity is then graded as mild, moderate or severe depending on the number of joints involved in the hand and whether larger joints are involved. The most frequently quoted methods of assessment are based on either Rosenbloom *et al.* [17] or Grgic *et al.* [18]. Although both methods emanate from the same centre the former is based on the prayer sign and the latter on the table top test. Rosenbloom includes passive extension and states that the proximal interphalangeal joint should extend to at least 180° and the metacarpophalangeal joint to 60°. Both Rosenbloom and Grgic have based their work on children with diabetes and using their assessment in adults may well be invalid. In fact, these tests are not infrequently abnormal in 'normals' and frequently abnormal in patients who have even mild degrees of hand OA (A. H. Isdale, unpublished). Other methods of assessment include using a photocopier or painting the palmar surface of the hand and applying it to paper. One of the problems with all these methods is the force the subject uses in their attempt to flatten the hand and although a fixed weight can be applied to the back of the hand in the techniques other than the prayer manoeuvre they lack sensitivity. In our own unit we have developed a portable fixed torque goniometer and we have been able to demonstrate significantly reduced range of motion at the metacarpophalangeal joint in diabetics (A. H. Isdale *et al.*, unpublished). Whilst this gives an objective measure of joint stiffness, our attempts to develop a similar instrument to measure skin stiffness non-invasively has proved rather less sensitive. Although we have developed an instrument that exerts a torque on the skin and we were able to demonstrate a trend towards increasing skin stiffness with duration of diabetes this did not reach significance and was confounded by the effects of age (A. H. Isdale *et al.*, unpublished). Commercial 'extensometers' are available but have not been formally assessed in the diabetic hand; our results were not reproducible and the apparatus was cumbersome to use.

There are a number of difficulties in measuring the stiffness of skin. Skin is anisotropic due to the orientation of collagen fibres (as demonstrated by Langer's lines) and stiffness will vary depending on the direction of the strain that is applied. Skin is also a viscoelastic material and the strain rate of the measuring device will influence the result. Another important factor is prestress which will vary depending on factors such as joint position and skin turgor.

An assessment of collagen changes by skin biopsy in conjunction with a skin extensometer would allow a more valid appraisal of the instrumentation and hopefully provide us with an easily applied simple objective clinical tool. We would then be able to equate 'the ABC' with the clinical entity of the diabetic hand.

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ANNOUNCEMENTS AND CALENDAR FOR 1993

October	14–15	Core Course. LIVERPOOL (Dr R. N. Thompson)
October/ November	31–5	SR Travelling Fellowship. LEEDS (Prof. V. Wright).

Further information about these events from Ms. Anne Mansfield, British Society for Rheumatology, 3 St Andrew's Place, Regent's Park, London NW1 4LE.