N’-(carboxymethyl)lysine, N’-(carboxymethyl)hydroxylysine, and the fluorescent cross-link pentosidines are formed by sequential glycation and oxidation reactions between reducing sugars and proteins. These compounds, termed glycoxidation products, accumulate in tissue collagen with age and at an accelerated rate in diabetes. Although glycoxidation products are present in only trace concentrations, even in diabetic collagen, studies on glycation and oxidation of model proteins in vitro suggest that these products are biomarkers of more extensive underlying glycative and oxidative damage to the protein. Possible sources of oxidative stress and damage to proteins in diabetes include free radicals generated by autoxidation reactions of sugars and sugar adducts to protein and by autoxidation of unsaturated lipids in plasma and membrane proteins. The oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage, and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress. Structural characterization of the cross-links and other products accumulating in collagen in diabetes is needed to gain a better understanding of the relationship between oxidative stress and the development of complications in diabetes. Such studies may lead to therapeutic approaches for limiting the damage from glycation and oxidation reactions and for complementing existing therapy for treatment of the complications of diabetes. *Diabetes* 40:405–12, 1991

Oxidative stress may be defined as a measure of the steady-state level of reactive oxygen or oxygen radicals in a biological system. A hypothetical sequence of events by which oxidative stress may be linked to tissue damage and the development of pathophysiology is outlined in Fig. 1. According to this scheme, increased oxidative stress may result from overproduction of precursors to reactive oxygen radicals and/or decreased efficiency of inhibitory and scavenger systems. The stress then may be amplified and propagated by an autocatalytic cycle of metabolic stress, tissue damage, and cell death, leading to a simultaneous increase in free radical production and compromised inhibitory and scavenger mechanisms, which further exacerbate the oxidative stress.

For practical reasons, neither the rate of oxidant production nor the steady-state levels of reactive oxygen species are easily measured in biological systems. Thus, oxidative stress must be inferred from measurements of oxidative damage as estimated from the kinetics of formation, the steady-state levels, or the extent of accumulation of oxidation products in tissues, plasma, or urine. However, the detection of increased levels of oxidation products in tissues is not, per se, sufficient to implicate oxidative stress in the pathology unless the damage can be logically and quantitatively related to the development of pathology and until it can be shown that inhibition of oxidative damage prevents or retards the disease process.

The concept I develop in this article is that oxidative stress may be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes. Mechanisms that contribute to increased oxidative stress in diabetes may include not only increased nonenzymatic glycosylation (glycation) and antioxidative glycosylation but also metabolic stress resulting from changes in energy metabolism, alterations in sorbitol pathway activity, changes in the level of inflammatory mediators and the status of antioxidant defense systems, and localized tissue damage resulting from hypoxia and ischemic reperfusion injury. The goal of this article is to focus more on the common pathway, the role of oxidative stress and damage in the development of complications, rather than on the array of contributory mechanisms. For

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more background on the relevance of oxidation in biological systems, see ref. 1. There are also excellent reviews on the role of free radicals in the etiology of diabetes (2), on the possible role of altered antioxidant defenses in the development of complications (2–4), and on the role of oxidation of plasma lipids and lipoproteins in the development of atherosclerosis in diabetes (5). For further development of my viewpoints on oxidative stress in diabetes, see ref. 6.

APPROACHING THE QUESTION

If we accept that the complications of diabetes are in some way an indirect manifestation of metabolic stresses resulting from altered insulin homeostasis and energy metabolism, then the critical questions from the viewpoint of this perspective are: 1) Do these metabolic stresses lead to increased oxidative stress in diabetes? 2) If so, is the resulting structural and/or functional damage sufficient to induce the development of complications? I approach these questions in a reverse order by asking first, What is the nature of the tissue changes and damage associated with the development of complications in diabetes? and then, Is oxidative stress a likely source of damage? Because of the limited information on oxidative damage to nucleic acids and glycoconjugates (glycolipids, glycoproteins, glycosaminoglycans) in diabetes, the discussion will focus on oxidative modifications of proteins and lipids, with emphasis on the role of modifications of collagen in the development of vascular and basement membrane pathology in diabetes.

NATURE OF COLLAGEN MODIFICATIONS IN DIABETES

There is no evidence that once oxidative damage occurs it may be reversed, for example, by chemical or enzymatic reduction of the oxidized species back to the native form. In the case of DNA, repair enzymes act by excision and replacement of the modified base or nucleotide. For proteins, lipids, and RNA, the kinetics of turnover of the molecule appears to be the critical factor limiting the accumulation of oxygen radical damage. However, for long-lived unrepairable protein molecules such as the collagens, products of oxygen radical reactions may accumulate with time, and through alterations in protein structure and function, these oxidation products may contribute to the development of pathology. Long-lived proteins therefore constitute a unique sensor for exposure to oxidative stress and provide a convenient source for identification of products formed during oxidative modifications of proteins.

Modifications of long-lived extracellular proteins (e.g., crystallins, collagens, elastins, laminin, myelin sheath proteins) and structural changes in tissues rich in these proteins (lens, vascular wall, basement membranes) are associated with the development of complications in diabetes such as cataracts, microangiopathy, atherosclerosis, and nephropathy. The chemical and physical changes characteristic of collagen in diabetes are summarized in Table 1. The physical changes in collagen are directly related to the underlying chemical modifications of the protein. Similar changes, both chemical and physical, develop gradually during the normal aging of collagen, but the process appears to be accelerated.

### TABLE 1

<table>
<thead>
<tr>
<th>Chemical Changes in Collagen in Diabetes</th>
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<tbody>
<tr>
<td><strong>Chemical</strong></td>
</tr>
<tr>
<td>Increased glycation</td>
</tr>
<tr>
<td>Increased pentosidine</td>
</tr>
<tr>
<td>Increased carboxymethylation</td>
</tr>
<tr>
<td>Increased cross-linking</td>
</tr>
<tr>
<td>Maturation of reducible cross-links</td>
</tr>
<tr>
<td>Resistance to enzymatic digestion</td>
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in diabetes, depending on the severity and duration of disease. Alterations in collagen synthesis and turnover also occur, and the structural changes are accompanied by morphological and functional alterations in collagen-rich tissues in diabetes, such as the thickening of basement membranes, altered vascular permeability, decreased joint mobility, and impaired wound healing.

LACK OF DIRECT EVIDENCE FOR INCREASED OXIDATIVE MODIFICATION OF COLLAGEN IN DIABETES

For two reasons, it is not possible to make a firm statement about the significance of oxidation in the chemical modification of collagen in diabetes: first, there is limited information on the nature of the chemical changes that occur in proteins exposed to oxidative stress; second, there is even less information on the nature of the chemical changes that occur in diabetic collagen. Carbonyl compounds are known to be formed from amino acids during metal-catalyzed oxidation of proteins in vitro and in vivo (7). However, carbonyl compounds are unstable in biological systems and do not accumulate; they may react with amines or be further oxidized to carboxylic acids. Some stable oxidation products, such as aspartate, produced on oxidation of histidine, are indistinguishable from the natural amino acids in protein, so that evidence of oxidative damage is not readily detected. Other products, particularly those derived from tryptophan, may be destroyed during hydrolytic work-up of the protein. In those cases where unique and stable oxidation products are formed, e.g., α-tyrosine or m-tyrosine by hydroxylation of phenylalanine or dityrosine by oxidative dimerization of tyrosine, there is no information on their rate of accumulation or concentration in diabetic compared with control collagen. In summary, there are few clearly defined stable chemical markers of oxidative damage to proteins, and those that have been characterized have not been measured or shown to increase in collagen in diabetes. This problem is not unique to diabetes because little is known about the oxidation of proteins, even in pathologies in which oxidative stress is considered to have a more definitive role, such as atherosclerosis and rheumatoid arthritis.

INDIRECT EVIDENCE FOR INCREASED OXIDATIVE DAMAGE TO COLLAGEN IN DIABETES

Despite the lack of information on amino acid oxidation products, studies on glycation of proteins and Maillard reactions of glycated protein have yielded indirect evidence for increased oxidative modification of collagen in diabetes. Thus, in addition to the Amadori adducts, fructoselysine (FL) and fructoselysyllysine, formed on glycation of lysine and hydroxyllysine residues in collagen, there are three carbohydrate-derived oxidation products that are increased in diabetic compared with age-matched nondiabetic collagen: N^α-(carboxymethyl)lysine (CML), N^ε-(carboxymethyl)-hydroxylysine (CMHL), and pentosidine (Fig. 2). CML and CMHL are formed by oxidative cleavage of Amadori adducts (8,9), whereas pentosidine is a fluorescent (excitation/emission 328/378 nm) cross-link formed between lysine and arginine residues in protein (10,11). All three of these compounds are autooxidation products, i.e., formed in reactions in which the oxidant is a reactive form of oxygen. Thus, the formation of CML and CMHL from Amadori compounds is inhibited under anaerobic conditions and by metal ion chelators and oxygen radical scavengers in aerobic systems (8,12). The formation of pentosidine during browning of proteins by sugars or during synthesis from carbohydrate and amino acid precursors is also inhibited under anaerobic conditions. Although the terms Maillard and browning reaction are often used synonymously to describe events occurring after the glycation reaction, CML and CMHL are colorless and have been described as products of nonbrowning pathways of the Maillard reaction (12). In contrast, pentosidine is a true browning product, with maximum absorbance at 328 nm, and its concentration correlates strongly with total fluorescence in collagen, measured at either 328/378 or 370/440 nm. The presence of these oxidation products in glycated collagen is not surprising, because it has been known for decades that the Maillard reaction in vitro is stimulated by oxygen and catalysts of oxidation reactions such as phosphate and traces of transition metal ions and inhibited by reducing agents such as ascorbate, bisulfite, and thiol compounds (13).

The increased concentrations of CML, CMHL, and pentosidine in diabetic collagen provide indirect evidence for a diabetes-related increase in oxidative damage to the protein (11). The evidence is indirect because it is not the amino acids in protein that have become oxidized but rather the attached carbohydrate. However, the assumption that the increase in carbohydrate oxidation products signifies an increase in underlying oxidative damage to the protein is reasonable because glycation of proteins in vitro may be accompanied by oxidative fragmentation of the protein and peroxidation of associated lipids (4,14,15). Amadori adducts are also a ready source of superoxide, e.g., in the fructosamine assay (16,17), providing experimental support for the argument that glycation of protein enhances its potential exposure to oxidative damage. Glycation also enhances the development of fluorescence during oxidation of proteins (18), and the wavelength maxima of fluorescence generated during browning of proteins in vitro are similar if not indistinguishable from that found in oxidized proteins (19,20). Thus, it is possible that much of the increase in collagen-linked fluorescence observed in diabetes is the result of a glycation-dependent enhancement of autooxidative reactions. This argument is supported graphically by the three-dimensional (3-D) fluorescence spectra shown in Fig. 3. The

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similarities in the 3-D spectra of natural skin collagen and browned RNase support the involvement of Maillard reactions in the browning of protein in vivo (21; Fig. 3, A and B). The similarities between these spectra and those of RNase oxidized by exposure to oxygen radicals generated either by ionizing radiation (Fig. 3C) or metal-catalyzed oxidation (Fig. 3D) suggest that much of the fluorescence in browned proteins may be formed by secondary oxidation reactions. Admittedly, the spectra are somewhat featureless, and the structures of the fluorescent products are largely unknown, but those products that are known to accumulate in collagen in diabetes (CML, CMH, and pentosidine) are, in fact, products of oxidation reactions, suggesting that other oxidation products, both fluorescent and nonfluorescent, may also be formed.

**AUTOXIDATIVE GLYCOSYLA TION AND GLYCOXIDATION**

Wolff (4) introduced the term autoxidative glycosylation to describe the proposed role of reducing sugars as catalysts of the oxidative chemical modification and cross-linking of proteins (14). Autoxidative glycosylation is initiated by the oxidation of an aldose or ketose to a more reactive dicarbonyl sugar (glucosone), which would then react with protein to form a ketoamine adduct. This adduct is related to but more reactive than the ketoamine adduct formed by the Amadori rearrangement and would also initiate further Maillard or browning reactions. The reduced oxygen products formed in the autoxidation reaction include superoxide and hydrogen peroxide (16,17,22), which, in the presence of metal ions, would cause oxidative damage to neighboring molecules. Therefore, autoxidative glycosylation is a reasonable mechanism for the production of free radicals, leading to fragmentation of proteins (4,14) and oxidation of associated lipids (14) during glycation reactions.

Thus far, specific carbohydrate-derived products of autoxidative glycosylation, such as the ketoamine adduct to protein, have not been identified in proteins either in vitro or in vivo, so that the significance of this pathway remains controversial (23,24). On the other hand, regardless of their origins or mechanism of formation, by autoxidative glycosylation or otherwise, CML, CMH, and pentosidine are sugar-derived autoxidation products, which have been identified in tissue proteins. They are formed by free radical oxidation reactions and may also participate in the initiation and propagation of damaging free radical reactions. These three compounds and total fluorescence increase coordinately in collagen with age and at an accelerated rate in diabetes (9–11,13), and thus they provide evidence of increased oxidative damage to collagen in diabetes. Because of the interplay between glycation and oxidation in their formation, we have termed these compounds glycoxidation products (21). Because they are not formed by reactions of proteins with malondialdehyde or peroxided lipids, glycoxidation products may be considered biomarkers of carbohydrate-dependent damage to protein and indicators of the extent of underlying chemical modification, oxidation, and cross-linking of tissue protein caused by reducing sugars. Furthermore, because these products accumulate in collagen normally as a function of age and at an accelerated rate in diabetes, diabetes may be legitimately described, at the chemical level, as a disease characterized by accelerated aging of collagen by both glycative and oxidative mechanisms. Individual differences in the accumulation of glycoxidation products in collagen (2- to 3-fold ranges at ages 60–80 yr in both diabetic and nondiabetic populations) suggest a wide variation in individual susceptibility to damage, an observation that might yield insight into the basis for individual differences in susceptibility to development of complications.
IS GLYCOXIDATIVE DAMAGE SUFFICIENT TO CAUSE OBSERVED STRUCTURAL CHANGES IN COLLAGEN?

The Amadori adduct FL is the major Maillard reaction product identified in collagen but accounts for, at most, 2–3% of the lysine residues in the protein or ~1 mol FL/mol triple-stranded collagen, even in poorly controlled diabetes. There is no evidence that this extent of modification is harmful, and correlations between the level of glycation of collagen and the presence of complications are weak (25,26), indicating that glycation alone is not sufficient to cause complications. In contrast, although glycoxidation products accumulate gradually and irreversibly in collagen, consistent with a possible role in the development of complications, they are found in only trace concentrations in the protein. For example, CML is present at <10% of the level of FL, even in patients with long duration of diabetes. This level of CML would have minimal effects on the charge properties of collagen, and because CML is not involved in cross-linking, it is not likely to affect the protein’s physical properties or resistance to proteases (Fig. 1). Although the pentosidine content and fluorescence of collagen are correlated with the presence of complications (11,27), there are even lower levels of pentosidine in collagen, ~0.01 mol pentosidine/mol triple-stranded collagen in the skin of elderly diabetic patients. This is probably <1% of the level of natural enzymatic cross-links in skin collagen, and although up to 10-fold higher levels of pentosidine are found in collagens in other tissues (10), it is unlikely that pentosidine cross-links are sufficient to cause significant changes in the properties of the protein.

Although pentosidine is present in only trace amounts in collagen, it represents 25–40% of the fluorescence at 328/378 nm in human skin collagen. Pentosidine also accounts for ~80% of the fluorescence in RNase or lysozyme browned by incubation with glucose in vitro (250 mM glucose for 1 mo at 37°C in 0.2 M phosphate buffer), thus it is a major fluorophore in both natural and artificially browned proteins. Despite its prominence, however, the pentosidine content of protein dimers isolated from glycation reaction mixtures accounts for only a small fraction of the total intermolecular cross-links in the dimerized protein (21), i.e., <0.01 mol pentosidine/mol lysozyme dimer, whereas there must be at least 1 mol intermolecular cross-link/mol protein dimer. By an aggressive extrapolation from experiments with model proteins in vitro, we might conclude that pentosidine also accounts for <1% of the carbohydrate-dependent nonenzymatic cross-links present in collagen. Multiplying the pentosidine content of collagen by 100 would yield a level of nonenzymatic cross-links in collagen approaching that of the enzymatic cross-links present in the protein. Thus, the trace concentration of pentosidine in collagen suggests an underlying level of nonenzymatic cross-linking and perhaps other chemical modifications, clearly sufficient to affect the physical and chemical properties of the protein. Although their structures are unknown, most of the nonenzymatic cross-links must be relatively nonfluorescent, perhaps colorless compounds; otherwise, they would be more apparent in the absorbance and fluorescence spectrum of the protein. Furthermore, if they are formed by oxygen radical reactions initiated by reducing sugars or glucose adducts to protein, it is possible that the carbon skeleton of glucose may not be directly involved in their structure; i.e., the cross-links may be glucose-dependent but not glucose-derived structures. These are not trivial considerations, because efforts aimed at characterization of fluorescent products in browned proteins may miss the major products involved in cross-linking of the protein, and similarly, efforts to label the cross-links with radioactive glucose may yield only traces of radioactive products in highly cross-linked proteins. In summary, although glycoxidation products (Fig. 2) and fluorescence (Fig. 3) provide insight into the relative level of glycative and oxidative damage to protein, those products characterized thus far are not present at concentrations high enough to have a significant impact on the physical or chemical properties of collagen (Fig. 1). However, the presence and accumulation of these compounds in collagen suggest the presence of a larger fraction of unidentified cross-links and suggest a catalytic and structural role for glucose in the oxidation and cross-linking of collagen in diabetes. Continued analysis of glycated proteins and collagen will be necessary to characterize the quantitatively more significant cross-links, to determine their origin and concentration in proteins, and then to assess their significance in diabetes.

LIPID PEROXIDATION IN DIABETES

Significant changes in lipid metabolism and structure also occur in diabetes, particularly in patients with vascular complications (28). In these cases, the structural changes are clearly oxidative in nature, and oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the development of vascular disease in diabetes (recently reviewed by Lyons [5]). However, epidemiological studies suggest that the level of lipid peroxides in human plasma is more associated with hypertriglyceridemia and vascular disease itself rather than directly with diabetes (29). In diabetic rats, increased lipid peroxidation was also associated with hypertriglyceridemia, but the oxidation and resultant toxicity of the oxidized lipoproteins were inhibited by administration of the lipophilic antioxidant probucol without an effect on the hyperlipidemia (30). Probucol also inhibited the development of atherosclerosis in nondiabetic hyperlipidemic rabbits (31), but its effectiveness in the treatment of diabetic vascular disease has not been tested. These experiments suggest that diabetes and hyperlipidemia are not alone sufficient to induce vascular disease and argue that oxidative stress may be an independent risk factor in the development of vascular disease.

Research on the role of lipid peroxidation in diabetic complications is hampered by the complexity of products formed (32) and limitations in the various assays for measuring the status and products of lipid peroxidation (33). Furthermore, although there are numerous reports of increased peroxidation of lipids in plasma lipoproteins, in erythrocyte membrane proteins, and in various tissues in diabetes, the relative importance of enzymatic versus nonenzymatic sources of the lipid peroxidation in diabetes is unknown (3,5). Increased lipid peroxides in plasma might result from enzymatic processes activated by generalized vascular inflammation, leading to increased levels of prostaglandins and lipoxygenase products. Alternatively, lipid peroxides might be formed by nonenzymatic reactions of unsaturated lipids with superoxide radical, hydrogen peroxide, and adventitious metal ions in the circulation or extravascular space or at the
surface of endothelial and phagocytic cells. The distinction between enzymatic and nonenzymatic (autoxidative) oxidation of lipids in vivo is not absolute. Thus, the enzymatic synthesis of prostaglandins may be stimulated by lipid peroxides derived from nonenzymatic pathways, and enzymatically generated lipid peroxides may also react with metal ions to initiate autoxidation reactions. Hydrogen peroxide and superoxide, intermediates in the autoxidative pathway, are also produced by both enzymatic and nonenzymatic pathways. Some lipid peroxidation products may be formed by both pathways, and degradation products, measured by the thiobarbituric acid assay, may also be derived from products of either pathway. Hayaishi and Shimizu (34) showed that a significant decrease in total lipid peroxides in rabbit plasma occurred within a few hours after aspirin administration. Similar experiments have not been conducted in humans, but the observation illustrates the probable involvement of both enzymatic and nonenzymatic pathways of lipid peroxidation in diabetes and the sensitivity of lipid peroxidation to anti-inflammatory agents. In general, studies on lipid peroxidation are consistent with studies on glycoxidation of proteins in diabetes; i.e., increased oxidation of both lipids and proteins is associated with the development of complications. However, comparative studies on oxidation of lipids and proteins in diabetes have not been reported.

It is difficult to conclude whether increased lipid peroxidation is a cause or effect of complications in diabetes (5), and it is probably more appropriate to consider lipid peroxidation as part of a continuous cycle of oxidative stress and damage. Lipid peroxidative damage may not be limited to the lipid compartment because lipid peroxides may cause browning and cross-linking of collagen (35) and contribute to the development of fluorescence in plasma proteins (and possibly collagen) in diabetes (20,36). This crossover between the oxidative chemistry of lipids and proteins is reminiscent of experiments discussed earlier in which glycation of proteins causes oxidation of associated lipids (14,15) and enhances the generation of fluorescence during oxidation of proteins (18). Thus, increased glycation of collagen and plasma proteins in diabetes may stimulate the oxidation of lipids, which may in turn stimulate autoxidative reactions of sugars, enhancing damage to both lipids and proteins in the circulation and the vascular wall, continuing and reinforcing the cycle of oxidative stress and damage. In this case, it is less important to fix the blame and more important to focus on the development of various possible therapeutic approaches for intervening in the cyclic process.

OTHER EVIDENCE FOR ROLE OF OXIDATIVE STRESS IN CROSS-LINKING OF COLLAGEN

There are indications in the literature that suggest that antioxidant or anti-inflammatory therapy may limit damage to proteins by glycation reactions. Thus, aspirin and salicylate inhibited the increase in tail collagen cross-linking in diabetic rats, as measured by effects on thermal rupture time (37). This effect was observed without an effect on glycation, suggesting that the drugs might be acting as inhibitors of oxidation and oxidative cross-linking reactions through their inhibition of cyclooxygenase activity. The drug dosages used in these experiments were probably too high for therapeutic use in humans, but similar effects were observed with the lipoxygenase inhibitors indomethacin and naproxen at doses within the therapeutic range (38), again without an effect on glycation. The action of these drugs could result either from inhibition of enzymatic pathways of lipid peroxidation or by their action as oxygen radical scavengers. The impressive therapeutic effect of sorbinil, an aldose reductase inhibitor, on collagen-linked fluorescence (39) and vascular permeability (40) in experimental animals could also be interpreted as the result of their antioxidant activity, either directly by their action as oxygen radical scavengers or indirectly by their effects on cellular redox potentials and NADPH and glutathione concentrations (4,40). Rutin, an aldose reductase inhibitor and, based on its structure, probably a transition metal ion chelator and radical scavenger, also inhibited the development of collagen-linked fluorescence in diabetic rats (41). Other effects of nonsteroidal anti-inflammatory and antioxidant agents may be more general, such as inhibition of neutrophil activation by inflammatory stimuli (42), which would limit the systemic production of free radicals and initiation or propagation of oxidative damage by both carbohydrate-dependent and lipid-dependent mechanisms.

Studies on the mechanism of action of aminoguanidine (AG) also suggest that autooxidation reactions are involved in the cross-linking of collagen by glucose. AG is the one agent specifically designed to inhibit the browning and cross-linking of protein by glucose during advanced stages of the Maillard reaction (43). It works well in vitro and in vivo, inhibiting the development of fluorescence, the formation of pentosidine, and the cross-linking of collagen (43) and model proteins (21) by glucose but is without an effect on glycation of the proteins (41,43,44). AG is not an antioxidant, based on the fact that it does not inhibit the formation of superoxide from Amadori compounds or the oxidation of F to CML in vitro. However, we have observed that oxygen accelerates the cross-linking of collagen by glucose in vitro, indicating that autooxidation reactions may be important in the formation of fluorescent and cross-linking products in collagen in vivo. During its inhibition of cross-linking, AG forms characteristic carbohydrate adducts in solution, and these compounds appear to be derived from its reaction with products of oxidation of glucose. These products are formed at similar rates in the presence or absence of collagen, suggesting that AG is trapping dicarbonyl intermediates formed by autooxidation of glucose. Further studies on the mechanism of action of AG should clarify the role of autooxidation reactions in the cross-linking of proteins by glucose in vitro and also suggest methods for evaluating the role of sugar autooxidation in vivo.

CONCLUSIONS

To the landscape artist, "perspective" deals with the convergence of lines to portray relationships between objects. This article has dealt with convergence and relationships, presenting the hypothesis that oxidative stress may be a common pathway relating diverse seemingly distinct mechanisms proposed for the pathogenesis of complications in diabetes. I have tried to argue not that oxidative stress is increased in diabetes, which then leads to the development...
of complications, but primarily that diabetes with complications is associated with increased chemical modification of proteins and lipids and that this "damage" appears to be largely oxidative in origin and is sufficient to explain the altered function of proteins in the extracellular matrix. There are many possible causes of increased oxidative stress in diabetes, but the source of the oxidative stress, if there is one primary source, may be extremely difficult to determine, especially if cyclic, autocalyotic, and reinforcing processes are involved. At this stage, fundamental information is needed on the nature of products formed during oxidation of proteins and of products accumulating in collagen in diabetes, so that the relationship between oxidative stress and the development of complications in diabetes can be addressed more directly. It would also be worthwhile to identify discrete products whose level in blood proteins could be used as short-term or medium-term integrators of oxidative stress in the manner in which glycation of plasma proteins and hemoglobin is used as an index of glycemic stress. These products, distinct from long-term integrators such as glycoxidation products in collagen, would provide an indication of the current status of oxidative stress rather than cumulative oxidative damage. They may also be useful for identifying patients at risk or with incipient disease and for assessing responses to antioxidant therapy. Eventually, these studies may lead to the development of effective strategies for limiting the damage from glycation and oxidation of proteins or for complementing other therapeutic approaches to the treatment of complications in diabetes.

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