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The Role of Aidose Reductase in Diabetic Retinopathy: Prevention and Intervention Studies

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CHAPTER 10

The Role of Aldose Reductase in Diabetic Retinopathy: Prevention and Intervention Studies

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1. INTRODUCTION

Diabetic retinopathy is the major ocular complication of diabetes, both in terms of incidence and irreversible visual impairment. In spite of modern procedures for strict blood glucose control (DCCT, 1993), laser treatment (ETDRS, 1991f; Aiello, 1994), vitrectomy (Gardner and Blankenship, 1994; Glaser, 1994b), and classical pituitary ablation (Kohner et al., 1976; Speakman et al., 1966; Poulsen, 1953), it is a serious threat to normal vision. It is mainly a vascular disorder, primarily involving microvessels (Garner, 1970). Retinal capillaries undergo multiple, extremely complex structural alterations in response to the unavoidable hyperglycemia of long-term diabetes. Because many of the angiopathies occur on microscopic vessels before retinal changes can be detected clinically, differentiating primary from secondary events in the etiology of diabetic retinopathy has been difficult. Histopathological studies are critical for determining the earliest microscopic changes that may cause the subsequent, clinically discernable, constellation of lesions comprising this disorder. Identification of the primary, triggering factors and the development of an appropriate animal model are necessary for the design and evaluation of therapies to prevent the many debilitating effects of this diabetic complication.

A summary of the salient clinical and histopathological features of diabetic retinopathy is presented first (Sections 2–5) to provide a basis for subsequent discussions of experimental models (Sections 6 and 7), currently available therapeutics (Sections 8–10), possible underlying mechanisms (Sections 11 and 12) and the significance of the galactose-fed rat model (Section 13). There has been a deliberate attempt to keep Sections 2–5 free of any reference to animal data so that the true status of current information on the human condition can be evaluated as accurately and independently as possible before assessing the relevancy of animal models. Emphasis throughout will be placed on the initial pathologies and their possible prevention or delay.

There is evidence that the primary triggering event of diabetic retinopathy is the increase in tissue aldose reductase activity, which results from elevated circulating levels of glucose and other hexoses. The
Aldose Reductase and Diabetic Retinopathy

reduction of excess glucose results in marked intracellular accumulation of sorbitol and unleashes a cascade of biochemical and structural anomalies. Clear causative relationships have been demonstrated between increased aldose reductase activity and the earliest structural changes of diabetic retinopathy: capillary basement membrane thickening and intramural pericyte loss. These, in turn, are probably causally linked to various subsequent retinal microangiopathies such as endothelial cell proliferation, microaneurysms, dilated channels and overt neovascularization.

2. DIABETIC RETINOPATHY: INCIDENCE AND CLINICAL CHARACTERISTICS

Diabetic retinopathy is the main cause of blindness by a single systemic disease in Great Britain (Garner, 1970) and the leading cause of blindness in the United States in 20–74 yr-old individuals (Klein et al., 1984a,b; Klein and Klein, 1985). The majority of individuals who have had diabetes mellitus for ≥ 15 yr have developed some clinically visible signs of diabetic retinopathy: approximately 98% if they became diabetic before reaching 30 yr of age (Klein et al., 1984a) and 78% if they became diabetic after reaching 30 yr of age (Klein et al., 1984b). After 15 yr of Type I diabetes, 50% of the subjects have proliferative retinopathy (Klein et al., 1984a). Blindness is 25–29 times more common in diabetic than in non-diabetic individuals (Fong and Rand, 1994; Klein and Klein, 1985). It was estimated almost two decades ago (National Advisory Eye Council, 1977) that 300,000 people in the United States were at risk of blindness from diabetic retinopathy. Because the prevalence of diabetes continues to increase (Warram et al., 1994), the number of people at risk is also increasing, perhaps now approaching 500,000. Since insulin was introduced as a therapeutic agent for systemic diabetes mellitus (Banting and Best, 1922), many lives have been saved, but the incidence of diabetic complications has risen, especially for complications that usually take several years to develop, as does diabetic retinopathy (Klein and Klein, 1985; Krolewski et al.,

Fig. 1. Etiology of diabetic retinopathy. Diagram modified from Garner (1993) to depict primary roles for capillary basement membrane thickening and pericyte degeneration, and to show the probable sequential interrelationships among the main clinical and histopathological lesions of diabetic retinopathy. Bm, basement membrane; IRMA, intraretinal microvascular abnormalities (Section 5). Modified, by permission and courtesy of Dr Alec Garner and The Royal College of Ophthalmologists.
1987; White, 1960). Whereas diabetes accounted for only 1.0% of new blindness in the United States in 1930, it was responsible for 15% by 1960 (Winter, 1960).

Although some neurological changes occur (Bresnick et al., 1984), vascular pathology, primarily involving the retinal microvessels (capillaries) constitutes the main component of diabetic retinopathy (Cogan and Kuwabara, 1967a,b; Deruaz, 1969; de Venecia et al., 1976; Garner, 1970). Blindness (defined as a best-corrected vision of 20/200 or worse) results from one of the following, all of which are indicative of compromised vessels: (1) macular edema — when the increased permeability of damaged intraretinal vessels contributes to severe thickening at the center of the macula; (2) non-resolving vitreous hemorrhage — when new, pre-retinal and intravitreal vessels rupture; (3) traction retinal detachment — when the fibrovascular proliferations and associated vitreous elements undergo sufficient contraction and/or displacement to detach the retina (Aiello, 1994; Davis, 1988; ETDRS, 1991d; Patz and Fine, 1977).

The spectrum of retinal angiopathies that precedes these final conditions is very broad (Fig. 1; Sections 5 and 11), involving not only a constellation of capillary lesions, which are probably primary, but changes in arterioles, venules and various rheological parameters of the retinal circulation (Ashton, 1983; Cogan et al., 1961; Cunha-Vaz, 1991; ETDRS, 1991a,d,f; Garner, 1970, 1993).

Most of the clinical signs and potential diagnostic characteristics of diabetic retinopathy were described in the 19th century, though not fully linked to diabetes (James, 1980). Ophthalmoscopically, no particular sequence of changes occurs consistently. Nevertheless, usually the first clinical manifestation of diabetic retinopathy is the appearance of one or more small, often clustered, microaneurysms usually limited to the posterior pole. The posterior pole is defined as the central retina surrounding the optic disc and including, roughly, the area enclosed by the superior and inferior temporal vessels (Cogan, 1974). Moderate dilations of veins may accompany or precede microaneurysm detection (L'Esperance, 1989). Frequently, small 'dot and blot' hemorrhages are intercalated between the microaneurysms and can be indistinguishable from them. Another relatively early change observed by central fundus examination is the appearance of white to yellowish spots with rather distinct borders in the deep retinal layers. These so-called hard exudates consist of concentrated derivatives of plasma that escaped from the compromised vessels (Garner, 1994). They are found most frequently in association with macular edema, microaneurysms and other microangiopathies. A more advanced condition is marked by the formation of various intraretinal microvascular abnormalities, or IRMA (Section 5.6), which may be difficult to distinguish from intravitreal neovascularization. They represent irregular focal dilations and varicosities of capillaries, which have become large enough to be resolved ophthalmoscopically. IRMA are most clearly visible when they occur in the middle of the capillary plexus, where all other vessels (capillaries) are too small to be detected by fundus exam. Here they appear as isolated islands of enlarged, tortuous vascular segments ranging in size from barely visible to 30 μm or more in diameter. Cotton-wool spots (sometimes called soft exudates) occur only in the superficial layer of the retina. They are indicative of nerve damage resulting from ischemic conditions (Section 5.7). While their presence suggests advancing diabetic angiopathy, their severity is a relatively weak predictor of retinopathy progression (ETDRS, 1991d,f). They can occur at any time, even with relatively mild retinopathy (Brown et al., 1985; Kohner et al., 1969). Venous beading and macular edema are almost always associated with the severe non-proliferative stage. Although perivenous sheathing, perivenous exudates and arteriovenous nicking are minor lesions with respect to the frequency of their occurrence, they are included in the grading systems used in clinical trials (DRS, 1981b).

The proliferative stage of diabetic retinopathy is the most threatening with respect to the potential for causing severe loss of vision (ETDRS, 1991d,f). The new vessels usually arise on the surface of the optic disc or from veins near arteriovenous crossings (Michaelson, 1948; Taylor and Dobree, 1970). The areas of proliferation are often associated with regions of ischemia (Garner, 1994). The new vessels may grow directly into the vitreous or remain at least temporarily between the inner limiting membrane and the retina. Extraretinal neovascularization is usually followed soon by pre-retinal hemorrhages and fibrous proliferations, which form pre-retinal
membranes. Repeated hemorrhaging, retinal edema and/or retinal detachment may lead to irreversible visual loss.

3. CLASSIFICATION AND GRADING OF MAIN FUNDUS CHANGES

Classification systems of the clinically discernable retinal lesions are similar in complexity to the lesions themselves. Attempts to assign angiopathies to stages or use quantitative grading systems to assess the severity of diabetic retinopathy have undergone continual updating. Often, two broad categories have been utilized — background and proliferative diabetic retinopathy (Garner, 1994; L'Esperance, 1989). Occasionally a pre-proliferative division has been added between the two (Benson et al., 1988; DRS, 1978; Sahel et al., 1994). However, many refinements have been developed as more precise information has become available and as modifications have been needed to meet the requirements of particular investigations (DRS, 1976, 1978, 1981a,b; ETDRS, 1991c,d,f; Goldberg and Fine, 1969; L'Esperance, 1989). Now, essentially all grading systems utilize a set of standard fundus photographs to make precise and reproducible assessments (DRS, 1981a,b; ETDRS, 1991d,f). The lesions have been subdivided and grouped based on how predictive they are of the proliferative stage and ultimate blindness (Aiello, 1994; ETDRS, 1991a,d,f). As a result, each category contains eyes with similar prognoses, and there is an orderly progression of risk with increasing category (ETDRS, 1991f).

The findings that have emerged from the grading efforts tend to confirm early notions that the different retinal microangiopathies form a continuum: one stage ordinarily leading to another, after having arisen from a previous stage (L'Esperance, 1989). This assumption is generally accepted with the caveat that some patients may never develop more than non-proliferative retinopathy. The current systems (Aiello, 1994; ETDRS, 1991a,d,f; L'Esperance, 1989) classify the lesions into mild, moderate, severe and very severe stages of non-proliferative diabetic retinopathy and mild, moderate and high-risk stages of proliferative diabetic retinopathy. Modified classification systems have been developed to utilize information gained from retinal fluorescein angiography (ETDRS, 1991c,g). Although not utilized for diagnosis, a pre-retinopathy stage was proposed to accommodate evidence of some early retinal lesions, including vessel leakage, which are observed by vitreous fluorophotometry to occur before angiopathies are detectable by fundus photography or fluorescein angiography (Cunha-Vaz et al., 1975).

There are marked changes in blood components and flow characteristics in diabetes (Feke et al., 1994; Frank, 1994; Hatchell and Sinclair, 1994; Little, 1976), but none has yet been utilized in standardized classification systems (Aiello et al., 1985; ETDRS, 1991b).

4. ROLE OF TECHNOLOGICAL DEVELOPMENTS IN CORRELATING HISTOPATHOLOGIES

Advancements in technological procedures have had a major impact on all aspects of investigation on the nature of diabetes-related retinal microangiopathies. Microaneurysms were first described in 1877 by Nettleship in making a pathological report on a case for MacKenzie and were definitively associated with diabetes soon after (James, 1980). However, their rediscovery as an important entity in pathological material was delayed until the 1940s. By then, techniques of fundus photography (Bedell, 1939; James, 1980) and specialized procedures for vessel staining and injection with contrast media followed by examination of flat retinal whole mounts permitted histopathological correlations. These demonstrated that microaneurysms and minute, perfectly round hemorrhages are among the earliest changes of diabetic retinopathy (Ballantyne, 1945; Ballantyne and Loewenstein, 1944; Friedenwald, 1949, 1950; Ashton, 1949, 1950).

The important role of pericytes in early microangiopathy (Kuwabara and Cogan, 1963; Cogan and Kuwabara, 1967a) was not recognized until improved histopathological procedures (Kuwabara and Cogan, 1960) permitted enzyme digestion and removal of essentially all non-vascular tissues, leaving relatively intact vascular beds, which could be mounted and stained. Although various enzyme combinations were used including pepsin plus trypsin...
### Table 1. Spectrum of Retinal Microangiopathies in Diabetic Retinopathy Compared to Other Disorders

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<th>Acquired Immunodeficiency Syndrome</th>
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<th>Arterial Occlusion</th>
<th>Coat's Disease</th>
<th>Collagen and Cardiac Vascular Disease</th>
<th>Diabetic Retinopathy</th>
<th>Eales Disease/IV Drug User Embolism/Vasculitis</th>
<th>Hypertension</th>
<th>Juxtafoveal Retinal Telangiectasia</th>
<th>Leukemia</th>
<th>Macroglobulinemia</th>
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**Footnotes:**

- ▲ = The 2 earliest histopathologic lesions of diabetic retinopathy (Ashton, 1974; 1983; Cogan and Kuwabara, 1967; Speiser et al., 1968)
- ▼ = The 3 basic histopathological changes which correlate with clinical findings of diabetic retinopathy (Ashton, 1983)
- ● = The 5 basic clinical pathologic processes of diabetic retinopathy (Aiello et al., 1989; Davis, 1988)

**Symbols:**

- = absent or rare
- □ = present but not similar to diabetic retinopathy
- ● = characteristic of diabetic retinopathy

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References:

See Sections 6 and 11 and these references for details and additional examples.
and collagenase plus trypsin (Addison et al., 1970; Ashton, 1963; Ashton and Tripathi, 1975), use of the crude pancreatic trypsin extract originally reported (Kuwabara and Cogan, 1960) became standard in most ocular pathology laboratories for the next 30 yr. Recently, Laver et al. (1993) demonstrated that the most important component of the crude trypsin extract is elastase and that purified elastase alone in appropriate buffers provides cleaner preparations and more consistent results. The elastase digestion procedure permits the isolation of intact vascular beds from retinas previously resistant to processing owing to extraordinary vessel fragility.

5. UNIQUENESS OF LESIONS TO DIABETIC RETINOPATHY

Two histopathological lesions are essentially unique to diabetic retinopathy, and several others occur as a characteristic group of lesions, which itself forms a unique spectrum (Aiello, 1994; Benson et al., 1988; Cogan and Kuwabara, 1967b; Garner, 1993). The two unique lesions — (1) a diffuse thickening of capillary basement membranes superimposed on age-related thickening; and (2) a selective loss of intramural pericytes — are not only unique in their occurrence, but they are the two earliest lesions to occur in diabetic retinopathy (Addison et al., 1970; Ashton, 1974; Bloodworth, 1967; Cogan and Kuwabara, 1967a; Kuwabara and Cogan, 1963; Sahel et al., 1994; Speiser et al., 1968; Yanoff, 1966). All subsequent lesions may occur in various other retinopathies, but even so, they usually are not expressed in the same way and are not accompanied by many of the other lesions of the typical ‘diabetic retinopathy spectrum’ (Table 1).

Subsequent to capillary basement membrane thickening and selective pericyte loss, the most important histopathological lesions have been identified as the following: (1) endothelial proliferation; (2) capillary microaneurysms; and (3) capillary closure (Ashton, 1963). These correlate well with the five basic clinical pathological processes: (1) capillary occlusion; (2) permeability; (3) microaneurysms; (4) vessel-glial proliferation; and (5) glial-vitreal contraction (Aiello et al., 1985; Davis, 1988). Table 1 summarizes the salient histopathological and clinical lesions that form the spectrum of retinal microangiopathies characteristic of diabetic retinopathy and indicates their occurrence and relative similarities in other retinal vasculopathies. It is clear that no other ocular or systemic disorder exhibits the same spectrum of retinal lesions in the same way as does diabetic retinopathy. No other disorder shows such a close association in the posterior pole among retinal areas of pericyte loss, non-perfusion, punctate microaneurysms, ‘dot and blot’ hemorrhages, hard exudates, cotton-wool spots and IRMA (Cogan and Kuwabara, 1967b; Benson et al., 1988). Figure 1 shows the probable sequences and inter-relationships

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**Table 1 (continued)**

of microangiopathies in the etiology of diabetic retinopathy.

5.1. Capillary Basement Membrane Thickening and Selective Pericyte Loss

A marked, diffuse thickening of the basement membranes which ensheathe capillaries is a hallmark of diabetic microangiopathy (Ashton, 1974, 1983; Williamson and Kilo, 1977). The basement membranes form a thin covering around the endothelial tube and completely envelop the intramural pericytes in capillaries of essentially all body tissues. A progressive, diffuse thickening also occurs with the normal aging process (Table 1), but it does not develop as rapidly or to the same extent as in diabetes. Whereas the basement membranes of retinal capillaries are normally approximately 80–120 nm in thickness, they may become 3–5 times thicker in diabetic compared to non-diabetic subjects of comparable ages (Ashton, 1974, 1983; Bloodworth, 1962, 1963, 1967; Toussaint and Dustin, 1963). The basement membrane thickening reported in other disorders is either focal rather than diffuse or only involves larger than capillary size vessels. Qualitative changes also occur in basement membrane components, but few analytical studies are available (Carlson, 1989; Carlson and Bjork, 1990; Toussaint and Dustin, 1963).

There is no firm evidence for a selective loss of capillary pericytes in any condition other than diabetic retinopathy (Table 1). Normal aging may involve a selective loss of capillary endothelial cells (Kuwabara et al., 1961) or loss of both pericytes and endothelial cells, equally, but not a loss of pericytes alone. Sugi (1966) using data obtained from 170 autopsy eyes of non-diabetic individuals with various other diseases or advanced age alone demonstrated significant loss of capillary cells, but no preferential loss of pericytes. Speiser et al. (1968) analyzed 209 autopsy eyes: 46 of which were from diabetic persons, 126 from patients with various chronic disorders and 37 from individuals dying of non-disease-related causes. A statistically significant difference ($p = 0.0001$) was found in mean ratios of endothelial cells to pericytes in non-diabetic persons free of other diseases (1.1, range 0.52–1.56) compared to diabetic patients (5.2, range 0.62–17.54), indicating a selective loss of pericytes. The ratios for persons with other diseases ranged from 1.3 to 1.8. These investigators also provided evidence that there was no increase in the number of endothelial cells per capillary length, thus demonstrating that the change in ratio did indeed result from a selective loss of pericytes.

In spite of a few reports to the contrary (de Oliveira, 1966), early supernormal thickening of capillary basement membrane and selective loss of intramural pericytes are considered hallmarks of diabetic microangiopathy and unique to diabetic retinopathy (Ashton, 1983; Bloodworth, 1967; Cogan and Kuwabara, 1967a; Deruaz, 1969; Speiser et al., 1968; Sugi, 1966; Toussaint and Dustin, 1963; Williamson and Kilo, 1977; Yanoff, 1966). Even so, identification of the earliest time when these lesions occur or which of the two lesions occurs first in human diabetic retinopathy has never been determined unequivocally. Both pericyte loss and capillary basement membrane thickening are microscopic changes that develop during the clinically silent phase. They do not match the criteria for the pre-retinopathy stage, which has been utilized by Cunha-Vaz (1991) for functional lesions. Whether they should be assigned to a new, 'microscopic stage' remains to be determined.
5.2. Dilations and Endothelial Cell Hypertrophy

A moderate, uniform dilation of all the capillaries of the plexus occurs during the early stages of diabetic retinopathy (Cogan, 1974). Later, many varicosities and extraordinarily large dilations develop in localized regions, accompanied by endothelial cell hypertrophy. Many of the larger dilations form hypercellular channels that traverse large acellular areas of the plexus. Others develop as 'islands' isolated in the middle of capillary beds where they must depend on much smaller capillaries for perfusion (Kohner and Henkind, 1970; Section 5.6). The cell proliferation involved has been considered to be an expression of intraretinal neovascularization involved in microaneurysm and IRMA formation (Kohner and Henkind, 1970; Wise, 1957). Commonly in diabetes, but only rarely in other diseases (Table 1), capillaries that have undergone endothelial cell hypertrophy, capillary dilation and varicose transformations are found side-by-side with non-perfused capillaries.

5.3. Permeability and Hard Exudates

The hard exudates, observed ophthalmoscopically as white to yellowish spots, indicate plasma leakage from compromised vessels. These exudates are aggregations of lipoprotein, cholesterol crystals and foamy histiocytes (Garner, 1994). Their topographical association with microaneurysms, capillary dilations and acellular capillaries in the posterior pole is characteristic of diabetic retinopathy (Table 1). Although the exudates of radiation retinopathy are very similar, they differ in that they are associated with disc edema.

5.4. Capillary Non-perfusion, Occlusion and Acellularity

Capillary non-perfusion resulting from occlusion and associated with acellularity is a prominent feature and is also considered a hallmark of diabetic retinopathy (Ashton, 1963; Benson et al., 1988; Bresnick, 1994; Bresnick et al., 1976; Garner, 1994). Retinal areas of capillary occlusion were first demonstrated by India ink injections of autopsy specimens (Ashton, 1950) and were later associated topographically with the areas of acellularity observed histopathologically (Cogan and Kuwabara, 1967b; Kuwabara and Cogan, 1965) and with the areas of non-perfusion observed clinically (de Venecia et al., 1976; Kohner and Henkind, 1970; Bresnick et al., 1976). In diabetes, unlike most other disorders (Table 1), capillary non-perfusion is characteristically confined to the posterior pole and closely associated topographically with microaneurysms, dilated hypercellular capillaries, IRMA, hard exudates and cotton-wool spots (Ashton, 1959; Cogan and Kuwabara, 1967b; Cogan et al., 1961; Kohner and Henkind, 1970; Toussaint, 1968).

5.5. Microaneurysms and Intraretinal Hemorrhages

Small, 'dot and blot' intraretinal hemorrhages and microaneurysms are usually the first funduscopic signs of diabetic retinopathy (Ballantyne and Loewenstein, 1944; Davis, 1988; Michaelson, 1980; ETDRS, 1991d,f). Although they are barely distinguishable clinically, they are very distinct in structure when examined in histological sections. Many of the entities originally interpreted clinically to be small, well-defined hemorrhages ('dot and blot' type) were revealed to be microaneurysms by histopathological exam (Ashton, 1949). Clinically, microaneurysms are the main hallmark of diabetic retinopathy (Table 1), although they do occur in other diseases and tissues (Garner, 1994). Examination of retinal digest preparations permits identification of occluded as well as patent microaneurysms. Summarizing their numerous histopathological studies of diabetic retinopathy Cogan and Kuwabara (1967b, p. 61) stated that "...microaneurysms are much more numerous than would have been suspected clinically."

5.6. IRMA, Shunts and Dilated Capillary Meshworks

Intraretinal microvascular abnormalities (IRMA) originated as a clinical term although the
histopathological correlates had already been documented using various names for the individual components (Bloodworth, 1967; Cogan and Kuwabara, 1963; Cogan et al., 1961; Kohner and Henkind, 1970; Toussaint, 1968). IRMA were defined as: "...the abnormal vascular structures within the retina variously interpreted as 'shunt' vessels, dilated pre-existing capillaries, fusiform aneurysms, or intraretinal neovascularization" (Davis et al., 1969, p. 13). Transections of IRMA demonstrate a wide variety of changes that capillaries are capable of undergoing. The extent of capillary dilation, shape change and wall abnormality is truly extraordinary. The dilations can be greater than 10-fold and involve several capillary branches. The dilations can give rise to the formation of minute or large fusiform bulges, sausage-shaped varicosities, loops, complex coils, channels and networks, by various distortions of capillary walls (Cogan et al., 1961; de Venecia et al., 1976; Wise, 1957). IRMA are not limited to diabetic retinopathy, but tend to be most prominent in this disorder as it develops to the severe non-proliferative stage (Table 1).

5.7. Cotton-Wool Spots and Ischemia

The cotton-wool spot (sometimes called soft exudate) is located in the nerve fiber cell layer. It is not an exudate at all: histologically, the cotton-wool spot is composed of an aggregate of several spherical to oblong bodies called cytoid bodies due to their resemblance to cells (Ashton and Harry, 1963; Garner, 1994; Wolter, 1959). By electron microscopy, each cytoid body was shown to contain accumulations of remnant debris from degenerated axoplasmic organelles including mitochondria, endoplasmic reticulum and neurofilaments (Ashton, 1970). The cytoid body probably results from obstruction of orthograde or retrograde axoplasmic transport (McLeod et al., 1977) and swelling with accumulation of cell organelles in a disrupted ganglion cell axon. Clinically, the cotton-wool spot is indicative of regions of non-perfusion and ischemia, often associated with arteriole occlusion (Destro and Gragoudas, 1994; Garner, 1994; Kohner and Dollery, 1969). Its development may be preceded by arteriolar occlusion, which may (Kohner and Dollery, 1969; Kohner et al., 1969) or may not (Michaelson, 1980) be preceded by capillary dilations and occlusions. Though not permanent, cotton-wool spots persist for relatively long periods in diabetes, having half-lives of 8.1–17.2 months (Kohner et al., 1969), compared to an average disappearance time of 6.9 weeks in the acquired immunodeficiency syndrome (Mansour et al., 1990a). Their expressions in other disorders are noted in Table 1.

5.8. Extraretinal Vessel-Glial Proliferation

The neovascularization on the surface of the retina and/or optic disc is a hallmark of proliferative diabetic retinopathy (Davis, 1988). The new vessels arise from the venous circulation (Norton and Gutman, 1967) and are accompanied by development of fibrotic tissue, which becomes permanently connected to the strands of vitreal collagen and plays an important role in subsequent events. There is nothing completely specific about the process, but in other disorders it is not accompanied by the other lesions typical of diabetes and it is less likely to be limited to the posterior pole (Table 1).

5.9. Extraretinal Hemorrhages, Glial-Vitreal Contraction and Macular Edema

These three processes, any of which is capable of precipitating irreversible blindness in diabetic patients (Benson et al., 1988; Patz and Fine, 1977), differ little in their expression in other severe retinal vascular disorders. They are probably a general expression of a highly damaged and traumatized retina.

6. EXPERIMENTAL MODELS OF DIABETIC RETINOPATHY

The importance of animal models for studies of diabetic retinopathy cannot be overstated. Unless adequate amounts of tissue with detailed records are available, the correct timing and inter-relationships of etiological events as well as the causative factors
cannot be determined. Retinal biopsies from diabetic patients are not feasible. Presently, donor eyes from diabetic patients have become more available, but their usefulness is limited. The clinical histories are often incomplete and the variables introduced by postmortem autolytic processes are hard to minimize. Only appropriate animal models permit the collection and proper preservation of adequate amounts of tissue under experimentally-controlled conditions.

Until recently, experimental studies on the basic mechanisms underlying the complete spectrum of angiopathies of diabetic retinopathy, and the testing of potential therapeutic agents have been severely hampered by the lack of reliable and convenient animal models (Engerman et al., 1982; Robison and Laver, 1993). Many animal models are available for diabetes as a systemic disease (Karasik and Hattori, 1994; Shafrir, 1993), but the same cannot be said for diabetic retinopathy, which is not the disease itself, but one of its many complications. In fact, investigators who have evaluated many animal models of diabetes as a systemic disease have drawn attention to the striking lack of adequate models of diabetic retinopathy (Engerman et al., 1982; Patz and Maumenee, 1962). Some promising models are now emerging. These will be discussed here, whereas human disorders and animal systems that provide insight into certain elements of diabetic retinal angiopathies will be covered in Section 11.

6.1. Diabetic Rodents

Theoretically, strains of laboratory mice and rats should provide ideal sources for models of both systemic diabetes and its tissue complications, whether studying hereditary or induced diabetes. The genetic homogeneity, economy and handling conveniences are very favorable. There are, in fact, numerous mouse and rat models of systemic diabetes mellitus, and other rodent models are continually being defined (Cohen and Rosenmann, 1990; Doi et al., 1990; Karasik and Hattori, 1994; Michaelis et al., 1988; Mori et al., 1991; Shafrir, 1993), including the recently developed transgenic mouse models for studies of tissue-specific aspects of diabetes (Erickson et al., 1990; Lee and Sarvetnick, 1993; Lutty et al., 1994; Stewart et al., 1993).

However, the same is not true for diabetic complications. Neither the mouse nor the rat, whether its diabetes is hereditary or induced, has provided a completely satisfactory model for the entire set of diabetes-related retinal vascular complications of long-term hyperglycemia in man (Engerman et al., 1982; Robison et al., 1991b). Several types of diabetic rats are good models for diabetic cataracts and diabetic keratopathy (Kinoshita and Nishimura, 1988; Kinoshita et al., 1990; Robison and Laver, 1993), but they usually only manifest some of the early lesions of diabetic retinopathy, such as capillary basement membrane thickening, intramural pericyte degeneration, endothelial cell proliferation and acellularity (Kern and Engerman, 1994; Papachristodoulou and Heath, 1977; Robison et al., 1991b; Sima et al., 1983, 1985). They rarely exhibit microaneurysms (Agrawal et al., 1966; Boot-Handford and Heath, 1980; Cohen et al., 1972; Cohen and Rosenmann, 1990; Engerman et al., 1982; Papachristodoulou et al., 1976; Robison et al., 1991b; Rosenmann et al., 1975; Yanko et al., 1971, 1975). Except for rare reports such as that by Toussaint (1968), they have not been demonstrated to develop IRMA or proliferative retinopathy (Engerman et al., 1982; Kern and Engerman, 1994; Robison et al., 1991b; Cohen and Rosenmann, 1990).

Significant thickening of retinal capillary basement membrane has been reported in streptozotocin-induced diabetic rats by several investigators: Fischer and Gärtnert (1983) after 12 months of hyperglycemia; Tilton et al. (1986) after 6–9 months; McCaleb et al. (1991) after 6–8 months; and Kojima et al. (1985) by as early as 3 months. Pericyte ghosts have been documented in micrographs by several investigators in both retinal digests and electron micrographs (Chakrabarti et al., 1987; Robison et al., 1991b). Pericyte ghosts, endothelial cell proliferation, capillary acellularity and varicose loops were documented in three different rat models with 6–8-month durations of diabetes (Robison et al., 1991b). Two were models for Type I diabetes: one by inheritance (BB Wistar diabetic); the other by streptozotocin injection (45 mg/kg). The third was a model for Type II diabetes (SHR/N-cp diabetic rat). No microaneurysms, IRMA or more advanced lesions were found. Probably the relatively short life-spans of diabetic rats and/or their spontaneous reversion to euglycemia...
(Kern and Engerman, 1994) preclude the development of lesions of the severe non-proliferative or proliferative stages of diabetic retinopathy. Such lesions are slow to develop, taking a minimum of 4 yr and often taking more than 10 yr in humans (Krolewski et al., 1987). This would explain why only the early lesions, such as capillary basement membrane thickening and pericyte degeneration, occur consistently in diabetic rats.

6.2. Diabetic Cats

The domestic cat has a retinal vascular pattern very similar to humans, but only a few reports of diabetic retinopathy in cats have appeared (Gepts and Toussaint, 1967; Toussaint, 1968; Mansour et al., 1990b). Gepts and Toussaint (1967) studied five spontaneously diabetic cats, some of which had documented durations as long as 7 months. They found that some had pericyte ghosts, focal acellularity, and/or varicose capillaries. Although many attempts to induce diabetes in cats have failed, diabetes can be achieved by procedures involving partial pancreatectomy alone or in combination with local injections of alloxan into the artery supplying the remaining pancreas (Mansour et al., 1990b; Reiser et al., 1987). In cats rendered diabetic by such methods, Mansour et al. (1990b) documented a significant increase in retinal capillary basement membrane thickness following 3–10 months of diabetes. Hatchell et al. (1994a) reported hemorrhages, microaneurysms, non-perfusion and IRMA as early as 7.5 yr in diabetic cats followed for 9 yr. Funduscopic examination was facilitated since cats do not develop diabetic cataracts.

6.3. Diabetic Non-human Primates

Non-human primates should be good models for diabetic retinopathy because their retinas have a fovea and a pattern of vascularization and capillary structure almost indistinguishable from that of human retinas. However, developing diabetic retinal microangiopathies in monkeys on a consistent basis has been difficult. In spite of the many approaches which have been used to induce diabetes, retinal lesions have been observed only rarely, even after 10 yr of diabetes, and the results are inconsistent (Frank, 1994). There is no clear documentation of pericyte loss or basement membrane thickening. Jonasson et al. (1985) found only the mild non-proliferative stage of diabetic retinopathy in a large series of monkeys with up to 12 yr of streptozotocin-induced diabetes. Microaneurysms have been reported occasionally, but seldom with supporting micrographs (Bresnick et al., 1976; Danis and Wallow, 1986; Gibbs et al., 1969). Several investigators have expressed their frustrations with the difficulty of inducing diabetic retinopathy in non-human primates. Nevertheless, recent preliminary results (Laver et al., 1994) suggest that a spontaneously diabetic monkey colony described and maintained by Hansen et al. (1991, 1994) may provide a source of meaningful models for several diabetic retinal microangiopathies characteristic of the non-proliferative stages. A classical Type II non-insulin dependent diabetes mellitus develops in middle-aged rhesus monkeys of this colony. After diabetes durations of 1–8 yr, these monkeys had varied amounts of pericyte degeneration, statistically significant thickening of retinal capillary basement membranes, capillary dilation, acellularity, occlusion, endothelial cell proliferation and a few microaneurysms (Laver et al., 1994). Because of the potential importance of a reliable non-human primate model for diabetic retinopathy, such leads merit further investigation.

6.4. Diabetic Dogs

A spontaneously diabetic dog was the first experimental model of a near complete spectrum of angiopathies characteristic of diabetic retinopathy (Patz and Maumenee, 1962). Its discovery dispelled the concept which had been developing for some time that diabetic retinopathy might be a diabetic vascular complication unique to human diabetes. A dog that had spontaneous diabetes for approximately 3 yr demonstrated pericyte degeneration with ghost formation, microaneurysms, acellularity, endothelial cell proliferation and putative exudates, along with cataracts and kidney lesions. Apparently, some microaneurysms exhibited loss of pericytes and proliferation of endothelial cells, while others demonstrated hyalinization (Patz and Maumenee, 1962). No IRMA or proliferative retinopathy
Aldose Reductase and Diabetic Retinopathy

6.5. Galactose-fed Dogs and Rats

A more complete diabetic-like spectrum of retinal microangiopathies can be induced in non-diabetic dogs and rats fed a galactose diet than has been reported in any other animal model (Engerman and Kern, 1984; Frank et al., 1983; Kador, 1990; Kador et al., 1988, 1990, 1994; Robison et al., 1983, 1986, 1988, 1989a, 1990c, 1991a; Robison and Laver, 1993; Takahashi et al., 1992). This discovery has not only provided a great asset to research on diabetic retinopathy, but it offers insight into the underlying mechanism of diabetic retinopathy. Galactose-fed animals have normal plasma glucose levels, but they share an important abnormality with diabetic humans. They accumulate high tissue levels of polyol (Sections 12.4 and 13.2), indicative of increased aldose reductase activity, which has been implicated in many diabetic complications (Dvornik, 1987; Frank, 1994; Fukushi et al., 1980; González et al., 1984; Kinoshita and Nishimura, 1988; Nishimura et al., 1988a). So, while galactose-fed animals would not be expected to manifest the full complement of physiological changes characteristic of diabetes, they would be expected to develop aldose reductase-related tissue complications. In fact, the complications should generally occur sooner and/or be more severe since aldose reductase has a higher affinity for galactose than for glucose (Hayman and Kinoshita, 1965), resulting in more polyol synthesis under hypergalactosemic than hyperglycemic conditions. Also, unlike sorbitol, galactitol is not metabolized by the cell, so polyol accumulates more rapidly (Fig. 2) and reaches much higher levels in tissues of galactose-fed animals than diabetic animals (Dvornik, 1987; Dvornik et al., 1988, 1994; Stewart et al., 1968; Section 12.4). As expected, polyol-related tissue complications such as cataracts and keratopathy do, indeed, occur sooner in galactose-fed animals (Dvornik, 1987; Kinoshita and Nishimura, 1988; Robison et al., 1990a; Robison and Laver, 1993). Sugar cataracts develop in galactose-fed rats in 2–3 weeks compared to 2–3 months in diabetic rats (Fukushi et al., 1980; Hu et al., 1983). The rate of cataract development is directly related to the percent of dietary galactose, severity of galactosemia and lens polyol accumulation in galactose-fed rats (Dvornik, 1987; Simard-Duquesne and Dvornik, 1973; Simard-Duquesne et al., 1985).

Based on its potential for accelerating the development of aldose reductase-related diabetic complications, galactose was fed to animals with the hope that diabetes-related retinal microangiopathies developed. Later, Patz et al. (1965) reported several early lesions of non-proliferative retinopathy in seven out of 12 spontaneously diabetic dogs. Engerman and Bloodworth (1965) reported non-proliferative retinopathy in two out of five dogs with metasomatotropin-induced diabetes and in one out of five with alloxan-induced diabetes. They added intraretinal hemorrhage to the list of the lesions of non-proliferative retinopathy which can be produced in diabetic dogs. Although not identified as such by them, their so-called 'oversize hypercellular capillary' could be classified as IRMA because of its size and large, irregular branches. Although retinas were examined histologically after 9, 15, 34, 37, 41, 43, 53, 67 and 69 months of diabetes, only those from the three dogs with durations of more than 43 months developed the retinal microangiopathies. Gepts and Toussaint (1967) reported typical non-proliferative diabetic retinal microangiopathies in 22 out of 30 diabetic dogs. The microangiopathies included pericyte ghosts, focal loss of endothelial cells as well as pericytes, generalized acellularity of capillaries, irregularly-dilated (varicose) capillaries, and typical microaneurysms. Sibay and Hausler (1967) documented saccular microaneurysms and hypercellular dilated capillaries, which could be classified as IRMA, in a dog that was diabetic for 5 yr, while only dilated capillaries were observed in a dog that was diabetic for 2 yr. Several additional studies (Bloodworth, 1967; Bloodworth and Molitor, 1965; Engerman et al., 1977; Toussaint, 1968) have confirmed the validity of the diabetic dog as a model of diabetic cataracts and essentially all the non-proliferative stages of diabetic retinopathy. However, in all these studies, microangiopathies required 48–60 months to develop, and proliferative stages have never been reported. Identification of the earliest retinal lesions in diabetic dogs has not been determined. Although some evidence suggests capillary basement membrane thickening and pericyte loss occur early (Stitt et al., 1994), just when they happen and which takes place first is not established.
would also occur sooner. The positive results obtained suggested that diabetic retinopathy, like cataracts, is also a tissue complication related to aldose reductase activity. Not only dogs fed 30% galactose (Engerman and Kern, 1984; Kador, 1990; Kador et al., 1988, 1990, 1994), but also rats fed either 30 or 50% galactose (Frank et al., 1983; Kern and Engerman, 1995; Robison et al., 1983, 1986, 1989a, 1990c, 1991a; Robison and Laver, 1993; Laver and Robison, 1993) responded to elevated tissue polyol levels by developing diabetic-like retinal lesions (Section 7).

7. SIMILARITIES BETWEEN GALACTOSE MODELS AND HUMAN DIABETIC RETINOPATHY

Although the retinas of dogs and rats have no fovea and their major vessels form unique arrays, their capillaries are similar in diameter, meshwork patterns and ultrastructure to human retinal capillaries (Figs 3–5). As in human diabetes, the capillaries of the posterior, central retina were the first structures to show signs of retinopathy in galactose-fed animals (Kador et al., 1990; Robison et al., 1991a; Robison and Laver, 1993; Takahashi et al., 1992). The types of microangiopathies and the sequence patterns, which developed through the high-risk proliferative stage in the dog and the mild proliferative stage in the rat, were extraordinarily similar to those of diabetic retinopathy in humans.

As in diabetic patients, the earliest histopathological retinal lesions (Figs 4 and 5) are a diffuse thickening of capillary basement membranes and degeneration of the intramural capillary pericytes of the central retina (Robison et al., 1983, 1986, 1988, 1989a, 1990c; Robison and Nagata, 1988; Robison and Laver, 1993; Kador, 1990; Kador et al., 1988, 1990; Takahashi et al., 1992). The earliest significant increase in retinal capillary basement membrane thickness has not been clearly documented in humans or these animal models. Previously, it was reported to occur as early as 7 months in rats fed 50% galactose (Robison et al., 1983, 1986), but is now known to take place as early as 6 months (Fig. 5; Robison et al., 1993). As in humans (Carlson, 1989; Carlson and Bjork, 1990; Toussaint and Dustin, 1963), there are qualitative as well as quantitative changes in capillary basement membranes in galactose-fed animals. The thickened basement membranes have a greater tendency to become multilaminar and vacuolated as well as acquire various electron-opaque inclusions such as fibrous collagen and degenerated pericyte remnants (Frank et al., 1983; Robison et al., 1983, 1986, 1988).

When the pericytes degenerate, they leave empty-appearing pockets in the ensheathing basement membranes, often called pericyte 'ghosts' (Figs 4 and 5). Pericyte ghosts first appear in galactose-fed
Fig. 3. Similarity of microvessels in human and rat retinas. Retinal vessels from a rat (A) fed 50% galactose for 6 months beginning at weaning; and from an 86-yr-old diabetic person (B). All arteries (A), veins (V) and capillaries (C) appeared normal at this magnification. Note the typical avascular zone of the macular (M) region in the human. PAS/hematoxylin stain. Both calibration bars represent 500 μm. Reproduced from Robison and Laver (1993) by permission and courtesy of Smith-Gordon and Co. Ltd.
Fig. 4. Intramural pericyte loss in human retinal capillaries. A, Light micrograph of capillaries from the perimacular region of Fig. 3B, showing intramural pericyte ghosts (pg) and nuclei of normal endothelial cells (e) and pericytes (p). B, Electron micrograph of a capillary from a similar region of the companion eye, showing a normal red blood cell (rbc), lumen (L) and endothelial cell lining (e), but abnormally thickened basement membrane (bm) and degenerated cytoplasmic remnants in all the intramural pericyte compartments (pg). The calibration bars represent 10 and 1.0 μm, respectively.
dogs after 19 months (Kador et al., 1988) and in galactose-fed rats after 6 months or less (Fig. 5). In humans, the first appearance of pericyte ghosts has not been determined with certainty (Section 5.1), but it occurs before clinical signs of retinopathy (Figs 3B and 4). The possible mechanisms of pericyte loss and its relation to subsequent clinical lesions will be discussed in Section 12.4.

In galactose-fed animal models, as in humans, there are two different ways that capillaries respond

Fig. 5. Intramural pericyte loss in rat retinal capillaries. Retinal capillaries from rats fed 50% galactose for 6 months beginning at weaning showing, by light microscopy with PAS/hematoxylin stain, A, an intramural pericyte ghost (pg), without a nucleus, as well as endothelial cells (e) and pericytes (p) with normal nuclei; and, by electron microscopy, B, a capillary with a normal lumen (L) and endothelial cell lining (e), but abnormally thickened basement membrane (bm) and degenerated cytoplasmic remnants representing pericyte ghost (pg) cytoplasm in all the intramural pericyte compartments. The calibration bars represent 10 and 1.0 μm, respectively.
Fig. 6. Diabetes-related microangiopathies in rats and humans. Retinal vessels from a rat fed 50% galactose for 24 months beginning at weaning, A, and from a 62-yr-old diabetic person who had utilized insulin for 15 yr, B. In both cases, many microaneurysms (ma) and acellular capillaries (ac) are present and some of the smaller arteries (A) and veins (V) have become tortuous. However, the most striking changes, when compared to the normal appearing structures observed in Fig. 3, are capillaries that have become irregularly dilated to extraordinary diameters to form tortuous, hypercellular channels and dilated meshworks (dm and arrowheads). PAS/hematoxylin stain. Both calibration bars represent 500 μm.
Fig. 7. Diabetes-related microangiopathies in rats and humans. Retinal vessels from a rat fed 50% galactose for 24 months beginning at weaning, A, and from a 58-yr-old diabetic individual who had utilized insulin for more than 13 yr, B. In both cases, intramural pericyte ghosts (pg), lightly-stained acellular capillaries (ac) and darkly-stained, apparently occluded capillaries (oc) are present. However, the most striking changes, when compared to the normal appearing structures seen in Fig. 3, are capillaries which have become irregularly dilated (dc) to extraordinary diameters to form cylindrical microaneurysms (cm), tortuous, hypercellular channels and dilated meshworks (dm) in association with both arteries (A) and veins (V) and accompanied by perivascular glial proliferations. PAS/hematoxylin stain. Both calibration bars represent 50 μm.
Fig. 8. Types of microaneurysms in rat retinas. Retinal vessels from rats fed 50% galactose for 24 months, showing several types of microaneurysms (ma), including saccular, A, fusiform with hyalinization, B, cylindrical, C, and irregular, D. The microaneurysms are accompanied by dilated capillaries (dc), capillaries with pericyte ghosts (pg) and acellular capillaries (ac), many of which appear to be occluded (oc), as judged by their degree of PAS staining. The microaneurysms can occur near the middle of the capillary plexus or in close association with arteries or veins (V). PAS/hematoxylin stain. The calibration bar represents 30 μm for all micrographs.
to pericyte loss. Some capillaries, in which pericytes have degenerated, exhibit a proliferation of endothelial cells and dilate to several times their original diameters, thus approaching the diameters of the major retinal vessels (Kador et al., 1990; Robison et al., 1989a, 1991a; Robison and Laver, 1993). Focal dilations result in microaneurysms, whereas more extensive dilations create hypercellular channels, shunts and other IRMA, some of which form dilated meshwork patterns (Cogan and Kuwabara, 1963; Cogan et al., 1961). By contrast, other capillaries, which are often adjacent, respond in an essentially opposite way. Instead of dilating, these capillaries maintain a normal diameter and lose their endothelial cells as well as their pericytes. As in humans (Ashton, 1983; Kuwabara and Cogan, 1965), such capillaries become acellular, non-functional and eventually are filled with glial cell cytoplasm (Robison and Laver, 1993; Robison et al., 1990c).

The areas of acellularity occur mainly in the central retina and are often closely associated with hypercellular channels, microaneurysms and IRMA (Figs 6 and 7) — a pattern very characteristic of human diabetic retinopathy.

Microaneurysm formation in the polyol-induced retinopathy of dogs and rats appears to be very similar to that in human diabetic retinopathy. Microaneurysms form by 27 months in galactose-fed dogs and as early as 16 months in galactose-fed rats. The types of microaneurysms include saccular, fusiform, cylindrical (tubular) and irregularly-shaped as well as many aggregations of the same (Fig. 8). Some are hyalinized, but most are patent. These exhibit the characteristic microaneurysm structure in transection, having an endothelial cell lining devoid of pericytes as in humans (Yanoff, 1969), and a lumen filled with erythrocytes (Fig. 9). Saccular microaneurysms are often observed in galactose-fed

![Fig. 9. Patent microaneurysm in a rat retina. Retinal micrograph from a rat fed 50% galactose for 28 months showing a transected microaneurysm (ma) in the inner nuclear layer (INL) with red blood cells (arrows) in the lumen and an endothelial cell lining (en) devoid of pericytes. The microaneurysm has a diameter far exceeding the normal diameter (arrowhead) of vessels in the inner nuclear layer, and is accompanied by a partially disrupted outer nuclear layer (ONL) with many surviving rod (m) and cone (cn) nuclei. The calibration bar represents 25 μm. Reproduced from Kinoshita et al. (1990).](image-url)
rats (Fig. 8A), but occur less frequently in rats than in dogs or humans (Robison et al., 1989a; Robison and Laver, 1993). The cylindrical microneurysms are often associated with perivascular glial proliferation (Fig. 7).

Capillary dilations that involve longer segments have been variously called tubular microaneurysms, hypercellular channels (Kohner and Henkind, 1970) or capillary ensheathing (Cogan et al., 1961) in descriptions of human diabetic retinopathy. Similar structures occur in both galactose-fed dogs and rats (Kador et al., 1990; Robison et al., 1989a, 1991a; Robison and Laver, 1993). When the hypercellular channels involve relatively extensive capillary segments and include several branches and anastomoses in the plexus network (Figs 6 and 7), they have been called dilated meshworks (Robison et al., 1989a). All these anomalies are the histological correlates of the clinically visible group of lesions called IRMA, which probably involve intraretinal neovascularization (de Venecia et al., 1976) and may act as shunts (preferential channels). In humans, they are characteristic of the severe non-proliferative stage of diabetic retinopathy and are highly predictive of proliferative retinopathy (Davis, 1991). In galactose-fed rats, IRMA are more prominent than microaneurysms in that they occur more frequently and involve more of the total capillary length.

'Dot and blot' hemorrhages occur by 33 months in galactose-fed dogs and can be observed clinically. In galactose-fed rats, hemorrhages occur by 24 months, but histological sections are required for their demonstration because the galactose-induced lens opacity is greater in rats. Likewise, hard exudates and cotton-wool spots occur in galactose-fed rats as well as dogs, but in rats they can be observed only in sectioned retinas. As in humans, veins become dilated and tortuous in galactose-fed rats (Fig. 6; Robison et al., 1989a).

Recently, extraretinal neovascularizations were documented in galactose-fed dogs after 72 months (Kador et al., 1994; Takahashi et al., 1992) and galactose-fed rats after 24–28 months (Laver and Robison, 1993), suggesting even greater similarity with humans and usefulness of these models of diabetic retinopathy. As in humans, the new vessels tend to develop first on or surrounding the optic disc. It is noteworthy that the regular occurrence of diabetic-like retinal microangiopathies through the severe non-proliferative stage in galactose-fed rats has been confirmed by a completely independent laboratory (Section 13.5).

As described (Sections 5 and 11), a specific spectrum of lesions is considered to be unique to human diabetic retinopathy. Although any one of the typical microangiopathies or some combination may occur in certain other retinal dysfunctions (Table 1) and some experimental models (Section 6), until now, the complete combination and pattern of lesions had not been demonstrated in any animal model. Presently, the entire spectrum of human-like diabetic retinal angiopathies can be produced on a regular basis in both galactose-fed dogs and rats, two very distinct animal models.

Besides diabetic retinopathy, the retinopathy that most closely simulates the spectrum of lesions found in the galactose-fed dog and galactose-fed rat models is radiation retinopathy (Table 1; Sections 5 and 11). Radiation retinopathy occurs when patients receive a total dose of 6000 rads or more (Archer et al., 1991; Chaudhuri et al., 1981; Hayreh, 1970; Maguire and Schachat, 1994). Like diabetic retinopathy, radiation retinopathy has a silent phase between the initial insult and the clinical expression of retinal microangiopathies, but this is significantly shorter. The latent period usually extends from 18 months to 3 yr following radiation treatment. Like diabetic retinopathy, radiation retinopathy is mainly a vascular disease involving microaneurysms, intraretinal hemorrhages (mainly superficial), telangiectases, hard exudates, cotton-wool spots, macular edema, capillary non-perfusion, neovascularization, vitreal hemorrhages and retinal detachment. However, radiation retinopathy differs from diabetic retinopathy in that it has fewer microaneurysms, and there is a selective loss of capillary endothelial cells rather than pericytes. Although basement membrane thickening occurs, it only affects vessels larger than capillaries (Chaudhuri et al., 1981). The galactose-fed animals experienced no radiation, and their early selective loss of pericytes, capillary basement membrane thickening, and frequent microaneurysm formation, best match the microangiopathies observed in human diabetic retinopathy.

Both the galactose-fed dog and the galactose-fed rat have distinct advantages over genetic or chemically-induced models of human diabetic retinopathy. The changes induced by galactose...
feeding are more advanced and more like human diabetic lesions than those which develop in long-term diabetic animals. Not only do galactose-fed animals demonstrate lesions earlier, but they are otherwise more healthy and have normal life-spans. Proliferative retinopathy has not been reported in diabetic dogs or rats. Diabetic rats usually do not live long enough to demonstrate more than capillary basement membrane thickening and pericyte loss (Robison et al., 1991b). By contrast, galactose-fed animals can develop retinopathy through the proliferative stages (Kador et al., 1994; Laver and Robison, 1993; Robison and Laver, 1993). Galactose-fed animals can be taken off the diet and returned to a normal physiological state within a few days. Thus, the effectiveness of intervening with therapeutic agents can be compared with complete removal of the physiological insult. This approach is not completely matched in any other animal model.

The dog and rat models provide unique contributions for particular studies. The galactose-fed dog permits clinical monitoring of the progression of diabetic retinopathy by ophthalmoscopic examination whereas the rat does not. Although cataracts are induced by galactose feeding in both models, some visualization of the fundus is still possible in the dog model since incomplete lens opacity and lens resorption are common in the dog, but not in the rat. Alternatively, the rat develops retinopathy sooner, and is more practical and economical to maintain as well as handle. The fact that clinical evaluation is unfeasible in the rat is partially compensated by the potential for greater availability of necropsy material, owing to the larger numbers of rats, which can be utilized within given limits of space and funds.

Galactose-fed animal models and diabetic humans have two important features in common: (1) their abnormal accumulations of tissue polyol and (2) their extraordinarily similar patterns of retinal microangiopathies. This commonality plus the majority of other evidences suggest that the reduction of excess intracellular hexoses to their corresponding polyols by aldose reductase is the triggering event of diabetic retinopathy.

8. POTENTIAL THERAPEUTICS: PREVENTION STUDIES

Evidence that aldose reductase is, indeed, involved in the retinopathy of these newly available animal models of diabetes and that drug therapy is feasible emerged from studies involving treatment of galactose-fed animals with aldose reductase inhibitors (Kador, 1990; Kador et al., 1988, 1990; Robison et al., 1989a,b, 1990c; Robison and Laver, 1993; Takahashi et al., 1992).

<table>
<thead>
<tr>
<th>Table 2. Effect of an Aldose Reductase Inhibitor (M79175) on Incidence* of Dog Retinal Angiopathies, Cataracts and HGB A1C Levels</th>
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<tr>
<td>Lesion</td>
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<td>Pericyte ghosts</td>
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<td>Microaneurysms</td>
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<td>Mean number per eye</td>
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<tr>
<td>Cataract severity†</td>
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<td>(6-36 month means)</td>
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<tr>
<td>(5-38 month means)</td>
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<tr>
<td>Hemorrhage</td>
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<td>HGB A1C levels‡</td>
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* Incidence expressed as the proportion of dogs showing the lesion.
† Cataract severity classified by slit-lamp exam on a scale of 0–4, with 4 being the most advanced cataract.
‡ Summary of HGB A1C levels determined by HPLC at 3-month intervals for the 4 dogs utilized in each group expressed as means ± standard deviations. Modified from Table 2, Kador et al. (1994) with permission from the authors and the publisher.
A set of positive studies on dogs utilized a homogeneous population of 9-month-old normal males from an inbred laboratory strain of beagles, which were age-matched. Eyes were enucleated at different times, ranging from 19 to 38 months, permitting observation of diabetic-like retinal microangiopathies from the earliest signs through the formation of microaneurysms in untreated galactose-fed dogs (Kador, 1990; Kador et al., 1988, 1990, 1994; Takahashi et al., 1992). The treated dogs were given different doses and combinations of the aldose reductase inhibitors sorbinil (Pfizer, Groton, Ct.) and M79175 (Eisai, Tokyo, Japan) administered by oral tablets both before and after a single daily 30% galactose meal. The sorbinil doses ranged from 59 to 105 mg/kg/day, while the doses of M79175 ranged from 0.5 to 16 mg/kg/day. The body weights remained equivalent in treated and untreated groups throughout the experiment. The levels of galactosemia in all groups of galactose-fed dogs was also the same throughout, as determined by measurements of blood galactose and glycated hemoglobin levels at 3–4 month intervals (Table 2).

Treatment with the higher doses of aldose reductase inhibitors (M79175 at 5–16 mg/kg/day) completely prevented ‘dot and blot’ hemorrhages through 38 months and significantly delayed all other diabetic-like retinal microangiopathies (Table 2). Pericyte ghosts normally occurred as early as 19 months and microaneurysms as early as 27 months following commencement of galactose feeding in untreated dogs. Their appearance was delayed for several months in treated dogs, the degree of delay being directly correlated with drug dose and potency.

A clear ameliorative effect of inhibitor treatment was demonstrated by detailed analyses of several parameters (Takahashi et al., 1993). Although the lower doses delayed rather than prevented the retinal microangiopathies, the dose-related ameliorative responses suggest that complete prevention rather than simply a delay of ocular complications in galactose-fed dogs is possible if enough aldose reductase inhibitor is maintained at the cellular level. The similar glycated hemoglobin (HGB A1C) levels in untreated and inhibitor-treated dogs (Table 2) demonstrates that aldose reductase activity, and not glycation (Section 12.3), caused the diabetic-like retinopathy. The prevention of ‘dot and blot’ hemorrhages and dose-dependent delays of other retinal microangiopathies were directly correlated with amelioration of sugar cataracts in these same dogs (Kador, 1990; Kador et al., 1994; Sato et al., 1991; Takahashi et al., 1992).

Cataracts were completely prevented in one-third of the dogs treated with doses of 10–16 mg/kg/day (M79175), and their progression was significantly delayed by lower doses, in a dose-related manner. The complete prevention of cataracts in some of the inhibitor-treated dogs was important because the lens provides a ‘built-in control’ in diabetic and galactose-fed animal models: the maintenance of a clear lens gives the assurance that enough aldose reductase inhibitor reaches the lens on a regular basis to block polyol formation (Section 13.5). In the galactose-fed dogs, there was a direct correlation between the severity of cataracts and the degree of retinal damage. Those dogs which had clear lenses due to aldose reductase inhibitor treatment had minimal retinal microvascular change. Unless the conditions are met, in which inhibitor treatment is capable of ameliorating or completely preventing cataracts, there is no reason to expect complete prevention of other ocular complications (Robison and Laver, 1993, 1994).

Negative findings with respect to the efficacy of aldose reductase inhibitor treatment of retinal damage in galactose-fed dogs have also been reported (Engerman and Kern, 1989, 1991, 1993; Kern, 1991; Kern and Engerman, 1991). These studies differed significantly in experimental design and interpretations from those used by Kador’s group, as indicated in a recent evaluation (Robison, 1994). Instead of using aldose reductase inhibitors in such a way as to provide the highest levels of inhibition, only one, relatively low dose of aldose reductase inhibitor was employed. Instead of adjusting the inhibitor administration for ideal pharmacokinetics, the feeding of galactose was ad libitum and tablets with aldose reductase inhibitor were administered once in the morning and once in the evening. By contrast, in Kador’s studies, galactose ingestion was limited to a single feeding and aldose reductase inhibitors were administered in three equal doses: 1 hr before feeding, 1 hr after feeding and 8 hr after feeding. Probably sufficient levels of the aldose reductase inhibitor were not attained in the target tissues in the Engerman and Kern studies.

Some of the problems, which make a meaningful
Fig. 10. Prevention of diabetes-related retinal microangiopathies. Whole mounts of retinal vessels from rats fed a diet with 50% galactose for 24 months and treated, A, or untreated, B, with an aldose reductase inhibitor, sorbinil (65 mg/kg/day) for the duration of the experiment. Note that the normal structure of the capillary meshwork (m) was maintained along with a normal (ca. 1:1.6) ratio of pericyte (p) to endothelial (e) cell nuclei in the sorbinil-treated retina, whereas various microangiopathies including dilated capillaries (dc), seemingly occluded capillaries (oc), microaneurysms (ma), dilated meshworks (dm) and various complex microvascular abnormalities, which would be recorded clinically as intraretinal microvascular abnormalities (IRMA) developed in the untreated galactose-fed rat. ×300. The calibration bars represent 50 μm. Reproduced from Robison and Laver (1993) by permission and courtesy of Smith-Gordon and Co. Ltd.
interpretation of the negative studies difficult (Robison, 1994), can be summarized as follows: (1) utilization of a single inhibitor dose; (2) lack of histological information on retinas until 42 and 60 months following disease onset, when it is too late to see the delays in microangiopathies documented to occur between 20 and 38 months (Akagi and Kador, 1990; Kador, 1990; Kador et al., 1988, 1990, 1994); (3) failure to prevent retinal capillary basement thickening (Engerman and Kern, 1991), which is a well-established effect of adequate doses of aldose reductase inhibitors (Frank, 1994); (4) interpretation of polyol measurements on the whole retina given that the most important concentrations probably occur in the capillary pericytes, which would be grossly under-represented in whole retinas; (5) determination of retinal polyol levels only at termination of the experiment, when the fewest pericytes would be present; (6) lack of clear documentation of retinal microangiopathies by either micrographs or by quantitative morphometric analyses; and (7) failure to completely prevent cataracts in either the diabetic or galactose-fed dogs by the drug dose and/or administration used. Probably, the experimental design precluded finding a positive effect of inhibiting aldose reductase activity.

Findings from inhibitor-treated, galactose-fed rats were very conclusive. Inhibitor treatment not only prevented cataracts, but also prevented capillary basement membrane thickening and the entire spectrum of diabetic-like microangiopathies through the mild proliferative stage (Fig. 10). In three studies totalling 115 rats (23 of which were galactosemic for > 24 months), galactose-fed rats had high blood levels of galactose (ca. 200 mg/dl) and polyol (ca. 35 mg/dl) at 6, 18 and 24 months, but were relatively healthy and had normal life-spans. The retinas of untreated rats which were galactosemic for 24 months or more exhibited the full range of diabetic-like retinopathies through the mild proliferative stage (Section 7). All the lesions were essentially prevented with AL-3152, sorbinil, or tolrestat, three structurally distinct aldose reductase inhibitors. The inhibitors were evenly mixed with fresh diet at 0.1, 0.05 and 0.05% w/w, respectively (Laver and Robison, 1993; Robison and Laver, 1993; Robison et al., 1983, 1986, 1988, 1989a,b, 1990c, 1991a). Aldose reductase inhibitor-treated galactosemic rats had lowered blood polyol levels (< 3.0 mg/dl), but did not differ significantly from untreated galactosemic rats in blood glucose, galactose, or glycohemoglobin levels (Section 13.2). The treated rats also remained free of cataracts throughout a period of 28 months, indicating that the continual oral administration of an aldose reductase inhibitor was sufficient to prevent tissue damage (Section 13.5).

These clear findings on the efficacy of aldose reductase inhibitors in preventing retinopathy in galactose-fed rats provide strong support for the positive effects of aldose reductase inhibitors in galactose-fed dogs and diabetic rats (Kojima et al., 1985). Together, these independent studies on the rat and dog models suggest that diabetic retinopathy in humans may be ameliorated by treatment with a sufficiently potent aldose reductase inhibitor, if treatment begins at the time of disease onset.

9. POTENTIAL THERAPEUTICS: INTERVENTION STUDIES

Since few clinical trials can be purely prevention studies, interventions in animal models are of inestimable value for determining how late therapy can be implemented and still have an effect. Now that diabetic-like retinopathy can be induced within a relatively short time in the new, galactose-fed animal models, intervention studies that were unfeasible previously can be undertaken.

Thus, a combined prevention and intervention study was performed using a relatively new aldose reductase inhibitor (AL-3152) on galactose-fed rats (Robison et al., 1993) to test the possibilities of: (1) preventing diabetic-like retinopathy by constant inhibitor treatment from initiation of 50%-galactose feeding, and (2) delaying or halting diabetic-like retinopathy in spite of postponing intervention by addition of inhibitor or removal of galactose until 6 months after galactosemia was induced. From rats killed at 6, 18 and 24 months, whole mounts of the retinal vasculature and transected whole retinas were used for morphometric analyses with a computerized image analysis system designed for 1024 × 1024 × 8 bit resolution. The amount of capillary basement membrane thickening, pericyte loss, capillary dilation, microaneurysms, acellularity and total length of capillary were quantitated for each rat. Then the rats were scored from 0 to 10 based on the numbers
of lesions in all categories, with the Grade 0 indicating no retinopathy and Grade 10 the maximum observed.

At 6 months, when intervention was begun, the untreated galactose-fed rats exhibited a 30%, statistically significant \((p < 0.01)\) increase in capillary basement membrane thickness, pericyte degeneration and Grade 1 retinopathy overall. By 18 months, the same group had Grade 7 retinopathy whereas the rats receiving intervention with either AL-3152-enriched or galactose-free diet exhibited only Grade 2 retinopathy, and the rats fed control diet or galactose plus AL-3152 throughout the 18 months demonstrated no retinopathy. At 24 months, the untreated galactose-fed rats exhibited Grade 10 retinopathy, while those receiving intervention by galactose removal had a Grade 8.5, and those receiving intervention with AL-3152 had a Grade 9. At 6, 18, and 24 months, all the rats that received galactose for 6 months or more had cataracts, except those that were treated from the beginning with AL-3152. All the galactose-fed rats had high levels of glycated (galactosylated) hemoglobin, whereas only the untreated galactose-fed rats had high plasma galactitol levels and developed diabetic-like retinopathy. In rats receiving intervention by removal of galactose, the retinopathy had progressed even though glycated hemoglobin and plasma polyol levels had been normalized. Because only rats which had high polyol levels for at least 6 months also exhibited retinopathy, it would appear that this diabetic-like complication is probably initiated by polyol accumulation and not by excessive tissue glycation, although increased glycation occurs with galactose feeding. The levels of glycohemoglobin did not correlate with either cataracts or retinopathy. In conclusion, cataracts and diabetic-like retinopathy are both prevented in galactose-fed rats if treatment with AL-3152 is started simultaneously with the galactose insult. But, intervention after 6 months by removal of galactose or addition of AL-3152 provides only a delay albeit a significant one.

The findings in galactose-fed rats are consistent with intervention studies in diabetic animals (Engerman and Kern, 1987; McCaleb et al., 1991), which also indicate that reversal does not occur and halting of damage is limited. The effect of intervention therapy was studied in diabetic dogs receiving poor glycemic control for 2.5 yr followed by good glycemic control for another 2.5 yr (Engerman and Kern, 1987). No microaneurysms developed in the 2.5 yr of poor glycemic control. However, in spite of intervention with good glycemic control for the next 2.5 yr, many microaneurysms formed and the overall vessel damage was greater than that found in diabetic dogs receiving good glycemic control for 5 yr. Intervention in humans by laser photocoagulation (Aiello, 1994; ETDRS, 1991d,f) or pituitary ablation (Speakman et al., 1966) decreases hemorrhages and proliferative changes, but does not reverse all the lesions of diabetic retinopathy. Pancreas transplantation in humans must be relatively early in order to demonstrate a clear effect (Petersen et al., 1990).

The cumulative evidence indicates that probably only a delay or halting of ongoing degenerations is all that can be effected once diabetes-related retinal vessel damage has progressed significantly. Perhaps there is a damage threshold beyond which reversal is impossible, as in sugar cataracts (Hu et al., 1983; Simard-Duquesne and Dvornik, 1973). Once an intramural pericyte has degenerated, it would be replaced very slowly if at all (Engerman et al., 1967). So, while prevention therapy could completely prevent loss of pericytes, intervention therapy would only be expected to prevent any further loss of pericytes. Assuming that pericyte loss initiates subsequent vessel lesions (Section 12.4), halting of pericyte loss by intervention would not halt subsequent lesions in those capillaries that had already lost several pericytes. The disease progression would be expected to continue until the effects of lost pericytes were exhausted. Evidence of beneficial effects of intervention therapy may be masked for a long time, until such existing tissue damage completely reveals its consequences.

### 10. CLINICAL TRIALS

Insofar as plasma glucose can be maintained at normal levels, the complications of diabetes should be preventable. Insulin is a miracle drug for an increasing number of people whose lives depend on artificially maintaining blood glucose levels in a near normal range. Insulin treatment, when designed to provide the tightest possible blood glucose control by multiple injections or continuous subcutaneous insulin infusion pumps (DCCT, 1993; Reichard et
al., 1993; Santiago, 1992), has significantly delayed the progression of diabetic complications, including diabetic retinopathy. This is what would be expected if hyperglycemia and/or the metabolic products of glucose are the initiating factors. However, tight glucose control methods are not able to bring the blood glucose below supernormal levels nor completely normalize glycosylated hemoglobin levels in all patients (DCCT, 1993; Reichard et al., 1993; Santiago, 1992; Zinman, 1989). Tight control brings problems of its own, such as higher risks of increased body weight, hypoglycemic shock and ketoacidosis (DCCT, 1993; Reichard et al., 1993; Santiago, 1992).

The methods used to obtain tight glucose control would be difficult to implement in the general population of diabetic patients without significant risks. A new approach to preventing diabetic complications is needed.

Aldose reductase inhibitors used in addition to conventional insulin treatment have the potential of blocking the toxic effects of the supernormal levels of glucose, which the best insulin therapy is unable to avert. A 3-yr, multi-center clinical trial to test the aldose reductase inhibitor sorbinil as a complementary treatment of diabetic retinopathy demonstrated no clear beneficial effect, although it did indicate "...a slightly slower progression rate in the microaneurysm count among patients assigned to take sorbinil..." (Sorbinil Retinopathy Trial Research Group, 1990, p. 1234). Why were negative results found in the clinic when the animal data (Sections 8 and 9) predicts potential beneficial effects? There are at least three possible explanations: (1) less than adequate drug dosing; (2) intervention too late with respect to disease progression; and/or (3) study too short to distinguish between the full expression of existing damage and beneficial effects of therapy.

Drug levels probably played a very important role, as suggested by animal studies, which demonstrated a direct correlation between drug dose and the ameliorative effects of aldose reductase inhibitors (Section 8). Intervention time and length of the study were undoubtedly very important also. Prevention studies can be designed to be straightforward in animals (Section 8). However, most clinical trials are intervention studies by nature and, as such, are plagued with many unknown variables related to the degree of tissue damage present at the time of intervention (Section 9). Intervention with good glycemic control in diabetic dogs led to the conclusion that "...retinopathy may be preventable but tends to resist arrest even in its incipient stages, before more than the first few aneurysms have appeared..." (Engerman and Kern, 1987, p. 808). The acceptance criteria for the sorbinil trial included patients with as many as five clinically-visible (patent) microaneurysms and an unknown number of occluded microaneurysms and microscopic lesions such as pericyte loss, capillary basement membrane thickening and endothelial cell proliferation. Concerns regarding the amount of such existing damage at the time of first clinical detection of diabetic retinopathy were expressed years ago, when retinal flat mounts first permitted histopathological correlations: "...it has not previously been realized how surprisingly numerous micro-aneurysms are and the picture is a depressing one for one wonders how it can ever be possible to reverse such a gross and widespread process by the administration of drugs or the control of diet. At best we can only hope to prevent the development of such lesions or, once the condition is established, to attempt to control the hemorrhages..." (Ashton, 1950, p. 41). The sorbinil study was relatively short compared to the time required for diabetic retinopathy to develop. It is possible that a beneficial effect of sorbinil would have been distinguishable from the progression of existing damage had the study lasted longer than 3 or 4 yr. This appears to be the most plausible explanation why relatively short-term studies on the efficacy of tight blood glucose control (Kroc Collaborative Study Group, 1984) failed to demonstrate an ameliorating effect on diabetic retinopathy. Recent long-term trials (DCCT, 1993; Reichard et al., 1993; Santiago, 1992) demonstrated a clear benefit, which would have been missed if the studies had been terminated earlier.

Findings from recently completed clinical trials and studies on animal models of diabetic retinopathy suggest that future clinical trials would benefit by including some of the following: (1) earliest possible intervention; (2) more potent and/or higher doses of inhibitors; (3) inhibitors with better pharmacokinetics; (4) longer trials; and (5) means for earlier diagnosis. A current multicenter trial to test the efficacy of tolrestat (Macleod et al., 1992) has incorporated many of these features.

Tolrestat has pharmacokinetics (Dvornik et al.,
1988, 1994) consistent with a once-a-day dosing in humans (plasma half-life of 12 hr), has been demonstrated to be efficacious in preventing diabetic-like retinopathy in rats (Robison et al., 1989a, 1990c), is well-tolerated in humans (Sima et al., 1993; Santiago et al., 1993), and has been demonstrated to interrupt the diabetes-induced progressive loss of nerve function in a double-blind, placebo-controlled clinical trial involving 372 patients with a long history of diabetic neuropathy (Santiago et al., 1993). This effect on nerve function was associated with reduction in structural signs of axonal degeneration, decreased incidence of myelin abnormalities and increased evidence of fiber regeneration. Currently tolrestat is being evaluated in long-term clinical studies in patients with diabetic nephropathy and retinopathy.

11. SUBORDINATE HYPOTHESES: COMPONENTS OF THE PATHOPHYSIOLOGICAL SPECTRUM

Although there is a spectrum of retinal microangiopathies unique to diabetic retinopathy (Section 5), various facets of the spectrum are exhibited in other disorders and may develop from secondary factors held in common. A single hypothesis probably defines the initial lesion (Section 12.4), but several subordinate hypotheses may be needed to explain the individual secondary changes in the triggered chain of events leading to the final complexity of vessel damage in diabetic retinopathy. Probably, the more advanced the lesion, the greater the possibility of it having a multi-factorial origin. As expressed by Bloodworth (1964, p. 81), "Diabetic retinopathy is a combination of degenerative, proliferative, and exudative lesions of vascular and parenchymal tissues." Indeed, many hypotheses have been proposed and variously modified over the years to explain the component retinal angiopathies individually or as groups. It has been proposed that microaneurysm formation may involve more than one mechanism acting in concert, with pericyte dysfunction being the primary aberration (Fryczkowski et al., 1991). Biochemical studies on the mechanisms of diabetic retinopathy have been classified into two categories (Frank, 1994) as follows: (1) those determining the earliest lesions (triggering events) and (2) those that might contribute to retinal-vitreal neovascularization. A discussion of all the hypotheses proposed to date is beyond the scope of this review, so only a select number will be covered with emphasis on the ones that might explain some of the major component angiopathies (Sections 11.1–11.5) or define the primary event (Section 12). Neovascularization will not be discussed since it is a late event and is the subject of many recent reviews (Bobik and Campbell, 1993; Casey et al., 1994; Davis, 1994; D'Amore, 1994; Forrester et al., 1993; Glaser, 1994a; Grant et al., 1993; Klagsbrun and D'Amore, 1991; Sunderkötter et al., 1994). The sum of the evidence in this section suggests that, while various vessel occlusive factors and perfusion pressure contribute to the spectrum of lesions characteristic of diabetic retinopathy, they probably do not represent the initiating event or provide an explanation for all the changes that develop. Capillary lesions are involved in the greatest number of angiopathies, suggesting that they are closer to a primary event than arteriolar and venular aberrations (Fig. 1).

11.1. Rheological Anomalies Related to Occlusions

Many characteristics of retinal blood flow become altered in diabetes and have been considered to be candidates for underlying factors in the development of diabetic retinopathy (Feke et al., 1994; Frank, 1994). Hatchell and Sinclair (1994) proposed that abnormal leukocyte rheology could be a prime cause of diabetic retinal microangiopathies. They suggested that leukocyte activation alone could initiate enough endothelial cell damage to induce capillary occlusion, whereas platelet aggregation, increased whole blood viscosity or decreased erythrocyte deformability would not produce sufficient change. They hypothesized that leukocyte activation may gel the cytoplasm and result in decreased cell deformability and increased leukocyte adherence to endothelial cells.

Naturally occurring human disorders provide insight regarding certain diabetes-related angiopathies. One example, which relates to a potential role of increased blood viscosity and flow resistance in angiopathies, is the sickling cell
condition. This disease involves an inherited change of erythrocyte shape and flow characteristics (Cohen and van Houten, 1994; Goldberg, 1976; Lutty et al., 1994; Stefansson et al., 1983). The sickle cell retinopathy that develops (Garner, 1994) includes hemorrhages, vessel tortuosity, arteriolar occlusions, arteriolar-venular anastomoses, areas of non-perfusion, cotton-wool spots and proliferative retinopathy. Compared to diabetic retinopathy, the hemorrhages tend to be much larger, the arteriolar occlusions are mainly equatorial and peripheral, the cotton-wool spots are extremely rare, and the neovascularization is mainly in the peripheral retina. Notably absent are microaneurysms and hard exudates.

11.2. Capillary Occlusion and Ischemia

Capillary occlusion has been suspected as a primary cause of diabetic retinal lesions. Radiation retinopathy induced in monkeys (Irvine and Wood, 1987) could be considered a test for the potential role of capillary occlusion and ischemia. As in diabetic retinopathy, the first changes occur in the small capillaries of the deep plexus. As in human radiation retinopathy (Archer et al., 1991; Chaudhuri et al., 1981; Hayreh, 1970; Maguire and Schachat, 1994) and diabetic retinopathy, there is a delay ('silent' period) before any effects of the insult can be detected. After approximately 12 months, there is a focal loss of endothelial cells and then pericytes from the capillary walls. These are followed by capillary occlusions and subsequent occlusions of larger vessels with accompanying non-perfusion and cotton-wool spots. Finally, microaneurysms, increased permeability, intraretinal neovascularization and rubecosis iridis develop. The lesions are similar to those of diabetic retinopathy except that there are fewer microaneurysms, no intravitreal neovascularization, and the first lesion is the selective loss of endothelial cells rather than pericytes.

Retinal ischemia was induced experimentally in cats by raising the intra-ocular pressure, by photocoagulation of individual arteries or by embolization of capillaries with sterile fat emulsions (Reinecke et al., 1962). The retinal capillaries responded similarly to all three methods. Ischemia lasting less than 90 min resulted in endothelial cell proliferation in the capillaries, while ischemia for longer periods caused an initial increase in endothelial cell number, then a loss of endothelial cells, and finally pericyte degeneration, leaving acellular capillaries. Laser-induced occlusion of retinal arterioles in rats resulted in increased numbers of neutrophils in retinal capillaries (Hatchell et al., 1994b). This suggests involvement of neutrophils in capillary plugging and a possible contribution to the non-perfusion observed following acute retinal ischemia.

11.3. Arterial and Arteriolar Occlusions

Michaelson (1980) suggested that arteriosclerosis is a prominent feature of diabetic retinopathy. Ashton (1953, 1963) presented histological evidence for narrowing of arterioles in eyes with diabetic retinopathy and suggested that ischemia is the basic problem of diabetic retinopathy. Focal occlusions of small arterioles are common in diabetic retinopathy and occur mainly in the posterior pole where other diabetic angiopathies develop. The microinfarctions result in the clinical appearance of cotton-wool spots (Destro and Gragoudas, 1994) which may occur very early in diabetic retinopathy (Kohner et al., 1969).

The retinal effects of arterial occlusion are demonstrated by naturally occurring disorders such as ocular ischemic syndrome and retinal obstructive disease. The characteristics of these disorders are dilated veins, hemorrhages (often in macula), microaneurysms in the midperiphery, cotton-wool spots, vessel leakage, and neovascularization on the disc and elsewhere (Brown, 1994a,b). Notably absent are numerous microaneurysms in the posterior pole, tortuous vessels and IRMA, when compared to diabetic retinopathy. Cogan (1974) reported that a diabetic patient developed typical non-proliferative retinopathy in one eye, but had no funduscopically visible diabetic microangiopathies in the contralateral eye, which previously had an occlusion of the central retinal artery.

Experimental occlusion of retinal arteries in cats induced the formation of many arterio-arteriolar and veno-venous shunts as well as capillary acellularity (Henkind, 1966). However, no arterio-venous shunts or microaneurysms developed, both of which are very characteristic of diabetic retinopathy.
11.4. Venous and Venular Occlusions

Many, but not all, aspects of diabetic retinopathy are mimicked by venous occlusion. Experimental venous occlusion was produced in a non-diabetic monkey eye by a laser burn accurately applied to avoid retinal artery damage (Hamilton et al., 1974). The resulting closure of the retinal vein induced essentially all the lesions that occur in human retinal venous occlusion (Cogan, 1974; Weinberg and Seddon, 1994), but did not exhibit all the angiopathies of diabetic retinopathy. There were intraretinal hemorrhages (but mainly small and flame-shaped), capillary dilations, preferential (enlarged and tortuous) channels within the capillary bed, tortuous venules, areas of capillary non-perfusion, secondary arteriolar and venule occlusions and eventual revascularizations (Hamilton et al., 1974). Notably missing were microaneurysms, pre-retinal neovascularization and cotton-wool spots. Other experimental models of venous occlusion (Wallow et al., 1991) have developed capillary and venous collaterals within the retina, similar to those characteristic of diabetes. However, no microaneurysms developed and the intramural pericytes remained unchanged or increased in their coverage of the capillary surface (Wallow et al., 1991).

11.5. Vessel Dilation and Vascular Perfusion Pressure

It has been proposed that retinal vessel dilation and wall stretching caused by increased retinal blood flow, increased perfusion pressure and/or vessel occlusion could cause many diabetic retinal microangiopathies involving endothelial cell proliferation and neovascularization (Stefansson et al., 1983). It was suggested that added luminal pressure could cause the endothelial cells of venules and capillaries to stretch, leak at junctions, and proliferate, producing microaneurysms, capillary dilation, and neovascularization. Hypertension retinopathy can serve as a test of hypotheses involving increased tension (Anand and Tasman, 1994; Brown and Benson, 1984; Garner, 1994). It results in retinal hemorrhages, cotton-wool spots, marked papilledema, hard exudates and capillary non-perfusion. It differs from diabetic retinopathy in having many exudates not confined to the posterior pole, having fewer microaneurysms mainly associated with the arterial side of the retinal circulation, having mainly flame-shaped rather than punctate hemorrhages, and having basement membrane thickening of arterioles, but not capillaries. In addition, there is no selective loss of pericytes. Collateral channels induced by experimental occlusion of retinal arteries developed no microaneurysms in spite of carrying blood under pressure probably in excess of that normally present in the capillary plexus (Henkind, 1966).

12. MAJOR HYPOTHESES: UNDERLYING MECHANISMS OF DIABETIC RETINOPATHY

The major hypotheses proposed to explain the underlying mechanism and possible triggering lesion of diabetic retinopathy are the following, in approximate order of greatest supporting evidence: (1) increased aldose reductase activity (Frank, 1991a, 1994; King and Banskota, 1994); (2) non-enzymatic glycation (Frank, 1991a, 1994; King and Banskota, 1994) (3) altered redox potential (King and Banskota, 1994) (4) the diacylglycerol–protein kinase C pathway (King and Banskota, 1994).

12.1. Key Involvement of Hyperglycemia

Certainly hyperglycemia (or excess in any blood hexose) must play the principal role in the development of diabetic retinopathy and must be central to any hypothesis proposed. The importance of hyperglycemia has been recognized by several investigators (Ashton, 1959; Davis, 1988; Frank, 1994; Garner, 1994). Strong supporting evidence includes the following: (1) relation of glucose control to retinopathy in diabetic dogs (Engerman and Kern, 1987; Engerman et al., 1977); (2) a similar relationship in epidemiological studies (Klein et al., 1988); (3) demonstration that diabetic retinopathy is directly related to blood glucose levels (DCCT, 1993); (4) the fact that Type I and Type II diabetes are very different in many ways, including origin, development, carbohydrate metabolism, fat metabolism and insulin levels (Fong and Rand, 1994),
yet they share similar retinopathies and chronic hyperglycemia (Cogan, 1974); and (5) the fact that diabetic-like retinopathy can be induced in normal animals by a galactose diet (Sections 6.5 and 7). The galactose-fed animal permits isolation of the effects of high blood hexose from all other complicating factors of the systemic disease. This permits a clear distinction between systemic diabetes and the complication (diabetic retinopathy). In galactose-fed animals, the plasma levels of insulin and glucose remain normal, yet diabetic-like retinopathy develops (Sections 6.5 and 7). The retinopathy must result solely from high hexose (galactose) or the products of its metabolism.

12.2. Altered Redox Potential and Diacylglycerol–Protein Kinase C Pathway

Both of these hypotheses are secondary to the increased activity of aldose reductase that is induced by hyperglycemia. The additional flux through the polyol pathway that occurs in diabetes increases the intracellular ratio of NADH/NAD (King and Banskota, 1994; Williamson et al., 1993), creating a condition called pseudohypoxia (Williamson et al., 1993) because the NADH/NAD ratio is also altered when oxygen deficits prevent oxidation of NADH to NAD⁺. Not only would the redox potential be increased, but there could be an increased flux through the diacylglycerol–protein kinase C pathway, explaining the increased protein kinase C activity observed in diabetic tissues (King and Banskota, 1994). However, consideration of these hypotheses as primary would fail to explain evidence from galactose-fed animals. The proposed alteration of the redox potential requires not only increased reduction of excess hexose to polyol by aldose reductase, but also the oxidation of the polyol (sorbitol to fructose in diabetes) by sorbitol dehydrogenase. Since, unlike sorbitol, galactitol is not metabolized by the cell, no NADH is produced by the polyol pathway in galactose-fed animals, yet they develop a retinopathy indistinguishable from diabetic retinopathy (Section 7). Glycolysis, another potential source of an increased ratio of NADH/NAD, has not been demonstrated to occur in galactose-fed animal models, although preliminary data has been mentioned (King and Banskota, 1994). Recent evidence (Geisen et al., 1994; Lee et al., 1995) indicates that inhibition of sorbitol dehydrogenase in diabetic animals accelerates cataract formation, a finding expected with polyol involvement, but not expected if altered NADH/NAD ratio were the primary event. In order for altered redox potential or increased flux through the diacylglycerol–protein kinase C pathway to be considered primary causative factors, independent of aldose reductase activity, the source of increased NADH/NAD ratio would have to be demonstrated to occur prior to aldose reductase action and the glucose-induced increase in redox potential should not be prevented by aldose reductase inhibitors.

12.3. Glycation (Non-enzymatic Glycosylation)

Glycation is an important direct cumulative effect of hyperglycemia. It involves the non-enzymatic covalent bonding between free aldehyde groups on any of several sugars and amino groups of proteins, usually lysines (glucose — glycosylation; galactose — galactosylation; fructose — fructosylation; and so on). The initial products of glycation are unstable Schiff bases that become stabilized by undergoing a reduction. Then they develop susceptibility to oxidation, undergoing repeated glycation-oxidation cycles and becoming highly crosslinked in irreversible reactions involving other compounds (Amadori rearrangements). Both intermediate and advanced end-products of glycosylation (AGE), such as ketoamides (early products of the Maillard, or browning reaction) resist turnover and accumulate in multiply-crosslinked moieties. The accumulation of glycoxidation products precedes continually, as a facet of the normal aging process, and is significantly accelerated in diabetic subjects (Baynes, 1991; Bunn et al., 1978; Sternberg et al., 1985). In non-diabetic individuals, the glycation products accumulate mainly in extracellular matrix components such as tissue collagens. However, under diabetic conditions, significant accumulations also occur intracellularly, in cells that do not require insulin for glucose uptake (Chiu et al., 1980; Nagaraj and Monnier, 1992; Nagaraj et al., 1991). Since glycation is a chronic, non-enzymatic process directly related to glucose levels, which causes marked accumulations of irreversible reaction products (advanced glycation end-products), it has been attractive to propose its involvement in diabetic complications (Brownlee,
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1990; Brownlee et al., 1988; Perry et al., 1987). However, hard evidence for a cause–effect relationship between glycated end-products and diabetic retinopathy is not yet available.

The main report which claims to have demonstrated prevention of retinal microangiopathies by inhibiting the formation of advanced glycation end-products (Hammes et al., 1991) is not accompanied by adequate supporting data. Although 37.5% (three out of eight) of the untreated diabetic rats and none of the aminoguanidine-treated rats were reported to have microaneurysms, the micrograph presented (their Fig. 2c) as raw data does not demonstrate a microaneurysm, but a twisted capillary, or perhaps a capillary loop (Garner, 1970) combined with an adjacent twist. Therefore, the numerical data presented are of questionable value and do not provide the support needed to identify glycation as an initiating cause of diabetic retinopathy. The increase in glycation end-products reported in the capillary basement membranes of diabetic rats is expected based on human studies (Sternberg et al., 1985), but there is no unequivocal evidence that the increased glycation contributes to the thickening of basement membranes in humans or animals. A thickened basement membrane would be expected to become more heavily glycated solely on the basis of the additional material available for glycation. The only agents known to unequivocally prevent diabetes-related basement membrane thickening are inhibitors of aldose reductase (Das et al., 1990; Frank et al., 1983; Robison and Nagata, 1988; Robison et al., 1983, 1986, 1988), which have no significant effects on plasma glucose levels in diabetic rats (Kemper and Dvornik, 1986; Yue et al., 1984) or on blood hexose and glycohemoglobin levels in galactosemic rats (Robison et al., 1989a, 1993; Yue et al., 1984; Section 13.2).

Aspirin has been reported to inhibit glycation and delay cataract formation in streptozotocin diabetic rats (Cherian and Abraham, 1993; Swamy and Abraham, 1989), but it is not efficacious in preventing diabetic cataracts or delaying the progression of retinopathy in diabetic patients, according to recent clinical trials (Seddon et al., 1991; ETDRS, 1991b). Perhaps glycation should be considered to be a possible factor in rendering some of the diabetes-induced alterations in vascular structure irreversible. However, long-term studies on animal models of diabetes are needed to determine the efficacy of various guanidines, including aminoguanidine, and/or other agents that inhibit formation of glycoxidation products (Fu et al., 1994) or block the recently proposed link with nitric oxide formation (Tilton et al., 1993).

Meanwhile, the bulk of the evidence is consistent with a primary role for aldose reductase activity in ocular complications of diabetes (Section 12.4), but would not be expected if glycation were the initiating event: (1) although blood glucose levels can be very high (ca. 500 mg/dl) in the diabetic rat, the blood galactose levels remain relatively low (ca. 200 mg/dl) in the galactose-fed rat (Robison et al., 1989a), yet sugar-induced cataracts and retinopathy take much longer to develop in the diabetic than in the galactose-fed rat (Datiles et al., 1982; Robison et al., 1989a, 1990c, 1991b); (2) congenitally hyperglycemic mice with high glycation levels do not develop cataracts, even when fed galactose, apparently because their lenses exhibit only one-tenth of the aldose reductase activity found in rat lenses (Varma and Kinoshita, 1974); (3) the degu rodent, which is essentially normo-glycemic (150 mg/dl blood glucose) but has high lens aldose reductase activity, develops a cataract after ingesting normal laboratory rat chow and develops a cataract much sooner than any other rodent (within 10–12 days) if made diabetic (465 mg/dl blood glucose) by streptozotocin injection (Varma et al., 1977); (4) glycated hemoglobin levels remain unchanged in galactose-fed dogs and rats treated with aldose reductase inhibitors (Kador, 1990; Kador et al., 1990, 1994; Engerman and Kern, 1991; Kern and Engerman, 1991, 1995; Robison and Laver, 1993; Robison et al., 1993; Section 13.2), yet cataract development and retinal vascular abnormalities are ameliorated (Kador et al., 1994; Robison and Laver, 1993, 1994); (5) inhibition of aldose reductase prevents the galactose-induced cataracts in rats without decreasing the elevated glycosylation levels of the lens proteins (Chiou et al., 1980); (6) inhibition of aldose reductase prevents hexose-induced retinal capillary basement thickening (Frank et al., 1983; Robison et al., 1983, 1986) and advanced retinal microangiopathies in rats without altering the blood insulin, glucose, galactose (Robison et al., 1989a, 1990c; Robison and Laver, 1993) or glycohemoglobin levels (Robison et al., 1993).
Although pentosidine starts accumulating soon after galactose feeding (Nagaraj et al., 1994), it remains to be demonstrated that advanced glycation end-products could accumulate fast enough to cause the rapid development of some complications of hyperglycemia such as the sugar cataracts (Section 12.4), which correlate closely with the incidence of diabetic retinopathy (Sections 8 and 13.5); and (8) transgenic mice that express aldose reductase activity in the lens develop cataracts at rates directly correlated with the amounts of polyol that accumulate in diabetes and galactosemia (Lee et al., 1995), leaving little doubt that increased aldose reductase activity causes sugar cataracts.

### 12.4. Aldose Reductase Activity

Rapid accumulation of intracellular polyol, resulting from increased aldose reductase activity, is one of the first and most dramatic tissue responses to hyperglycemia in cells that do not require insulin for glucose uptake (Dvornik, 1987; Dvornik et al., 1988, 1994; Kinoshita and Nishimura, 1988; Simán et al., 1993). Polyol accumulation is accompanied by tissue damage. In sugar cataracts, the lens epithelium, which is the cell layer with the highest concentration of aldose reductase in the lens, incurs the earliest ultrastructural damage, correlated directly with the rapid accumulation of polyol (Robison et al., 1990a,b).

In a series of experiments (Robison et al., 1990a,b), Sprague-Dawley rats were made diabetic by streptozotocin injection (50 mg/kg body weight in tail vein) or galactosemic by feeding a 30 or 50% galactose diet and were treated with one of two aldose reductase inhibitors (AL-1576: 4.0 mg/kg/day or sorbinil: 65 mg/kg/day). Plasma sugar levels were monitored and rats were killed at several intervals during the first 6-120 hr after injection or diet administration, and at a few intervals up to 3 months later. Polyol levels were measured in epithelium dissected free of contaminating fibers. Three to four individual epithelial layers were pooled from different rats for each of the three to six samples analyzed in each group. The intracellular levels of sorbitol and galactitol were made using a modified liquid chromatography procedure, which permits detection in the picomole range (Dickerson and Lou, 1990). Whereas mature sugar cataracts took from 3 weeks to 3 months to develop in 50% galactosemic and diabetic rats, respectively, the epithelial cells demonstrated rapid changes in both polyol levels and ultrastructure. The lens epithelial cells of streptozotocin-induced diabetic rats exhibited a 24-fold increase in polyol (sorbitol) accumulation within 48 hr after streptozotocin injection compared to controls (Fig. 2A). The lens epithelium of rats fed 30% galactose after a 24-hr fast exhibited a 17-fold increase in polyol (galactitol) accumulation within only 36 hr of commencing the diet (Fig. 2B). Analysis by light and electron microscopy revealed that the first detectable histopathologies of the lens (as early as 36 hr in the 50% galactose-fed rats) occurred in the anterior, central lens epithelial cells. The salient lesions were cell edema, apparent dilution of cytoplasm, rounding of nuclei, aberrant intracellular vacuoles and loss of normal tortuosity of cell boundaries. No changes were detectable in the controls or in any of the rats treated with an aldose reductase inhibitor. The findings indicate a major role for aldose reductase in hyperglycemic damage to the lens epithelium. This layer is the principal site of the important regulator mechanisms that are lost early in sugar cataractogenesis.

In retinal capillaries, the highest level of aldose reductase activity is in the intramural pericyte. Aldose reductase, its mRNA and its product (polyol) have been localized in pericytes, where one of the first histopathological lesions of diabetic retinopathy occurs. Immunohistochemical staining of aldose reductase in pericytes was demonstrated in trypsin-digested capillary whole mounts of dogs (Akagi et al., 1986) and humans (Akagi et al., 1983). Messenger RNA encoding for aldose reductase was identified in cultured human retinal pericytes by Northern blot hybridization (Nishimura et al., 1988b). Aldose reductase activity was demonstrated in isolated dog retinal capillaries (Kern and Engerman, 1985), cultured monkey (Buzney et al., 1977) and human (Hohman et al., 1989) retinal pericytes. The pericytes demonstrated rapid accumulation of polyols, vacuole formation and decreased proliferation when incubated in relatively high concentrations (≥ 30 mM) of hexose sugars, but demonstrated control levels of polyols and no damage when an aldose reductase inhibitor was included. The recent in vitro demonstration of a 5.5-fold greater glucose transport activity in pericytes...
compared to endothelial cells and a selective down-regulation of glucose transport activity in pericytes (Mandarino et al., 1994) suggests a possible relation to the increased aldose reductase activity in pericytes and their selective loss in diabetes. Since aldose reductase is concentrated in a cell that is so strategically located in the capillary and is the first to degenerate, it is highly likely that polyol formation by aldose reductase activity is the primary triggering lesion of diabetic retinopathy.

The mechanistic link between the toxic effects of aldose reductase activity in the pericyte and the histopathologies that develop subsequently is not completely understood. However, current evidence reveals close relationships between aldose reductase, early thickening of capillary basement membranes, early dysfunction and/or loss of intramural pericytes, endothelial cell proliferation and the formation of many proliferation-related vessel lesions. Basement membrane thickening is directly related to the polyol pathway and not to glycation. A diabetic-like basement membrane thickening was demonstrated in retinal capillaries of rats fed 50% galactose for 7 months (Robison et al., 1983, 1988), 11 months (Robison et al., 1983), 22 months (Robison et al., 1986) or 24 months (Robison et al., 1993) and in rats fed 30% galactose for 21 months (Frank et al., 1983). The thickening was prevented with three structurally-distinct aldose reductase inhibitors in two independent laboratories. Although data on glycated hemoglobin was not included in those studies, it has been demonstrated subsequently (Robison et al., 1993; Section 13.2) that glycation levels are not altered by treatment of galactose-fed rats with aldose reductase inhibitors, consistent with earlier findings on lens proteins (Chiou et al., 1980). Not only retinal capillary basement membrane thickening, but loss of pericyte–endothelial cell contacts, and the proliferation of retinal capillary endothelial cells were prevented with aldose reductase inhibitors in galactose-fed rats (Robison and Laver, 1993; Robison et al., 1989b).

There is an intriguing relationship between capillary basement membrane thickening and pericyte–endothelial cell interaction. A 2.4-fold increase in retinal capillary basement membrane thickening is accompanied by a 70% decrease in the number of cell-to-cell contact regions between pericytes and endothelial cells in rats fed 50% galactose for 28 months (Robison et al., 1989b). Since each of the contact regions contains several different types of cell membrane junctional complexes, the loss of a cell contact fenestra could mean loss of various functions. Upon finding that pericyte degeneration, the primary lesion of human diabetic retinopathy, was "...accompanied by proliferation of vessels within the retina and into the vitreous...", Kuwabara and Cogan (1963, p. 498) proposed that pericytes normally control endothelial cell proliferation and thus prevent neovascularization. Crocker et al. (1970) observed that the endothelial cell proliferation, which results in new capillary growth during normal wound healing, ceased as the new vessels became ensheathed by pericytes and as cell contacts were formed. They hypothesized that a form of contact inhibition like that reported in cell culture might explain the decrease in cell proliferation with increasing numbers of cell contacts. Bovine retinal pericytes co-cultured with endothelial cells (Orlidge and D'Amore, 1987) inhibited their proliferation through the action of transforming growth factor β (TGF-β). The loss of pericyte–endothelial cell contacts plus dysfunction of such a growth-factor-mediated mechanism may be involved in the endothelial cell proliferation that is characteristic of diabetic retinopathy (Cogan et al., 1961; Kohner and Henkind, 1970). It has been suggested that pericytes and their products might be involved in normal blood flow, capillary permeability and phagocytosis (Miller and Sims, 1986; Sims, 1991; Sims et al., 1990), all of which would be lost with pericyte degeneration. Consideration of all the evidence suggests there is a link between pericyte aldose reductase activity and endothelial cell proliferation, a major contributor to subsequent intraretinal anomalies such as microaneurysms and IRMA.

13. SIGNIFICANCE OF THE GALACTOSE-FED RAT

The galactose-fed rat provides the best evidence to date that aldose reductase activity is firmly linked to all the retinal lesions of diabetic retinopathy. The findings indicate that the reduction of excess hexose by aldose reductase is not only the triggering event,
but that it causes a cascade of cellular and tissue responses that ultimately result in all the microangiopathies of diabetic retinopathy through the mild proliferative stage. This model permits the dissociation of the effects of polyol accumulation from those of elevated blood glucose, elevated glycation levels, decreased insulin production and/or recognition, as well as other metabolic and genetic factors which might be involved in the development of diabetic complications. Only polyol-related complications and associated aspects should be manifest in a galactose-fed rat. Because of its greater accumulation of polyol, the galactose-fed rat model also permits accelerated development of polyol-related diabetic complications, a real advantage in studies of complications that take a long time to develop i.e. retinopathy.

The fact that the galactose-fed rat closely mimics human diabetic retinopathy through the mild proliferative stage (Robison et al., 1983, 1986, 1989a, 1990c; Robison and Laver, 1993; Laver and Robison, 1993) strongly suggests that the activity of aldose reductase is the primary causative factor. However, galactose feeding not only increases tissue polyol accumulation, but also glycation levels. Prevention of the retinopathy with aldose reductase inhibitors eliminates the possibility that glycation could be a major factor, since the inhibitors are specific and do not alter the plasma or tissue levels of hexose or glycation in the galactose-fed rat (Chiou et al., 1980; Kern and Engerman, 1995; Robison et al., 1989a, 1993). A complete prevention of cataracts and essential prevention of diabetic-like retinopathy has been documented in rats fed a 50% galactose diet plus one of three different aldose reductase inhibitors (AL-3152, sorbinil or tolrestat) for 24-28 months (Robison et al., 1989a, 1990c, 1993; Robison and Laver, 1993). Kern and Engerman (1995) demonstrated pericyte ghosts within 15 months in retinal capillaries of rats fed either 30 or 50% galactose. Figure 5 demonstrates that pericyte ghosts not only occur in galactose-fed rats, but can be formed as early as 6 months after administration of a 50% galactose diet.

13.1. Retinal Pericyte Ghosts

The early occurrence of pericyte ghosts in any rat model of diabetes has been questioned (Frank, 1994; Frank and Das, 1992; Frank et al., 1983; Tilton et al., 1986). Nevertheless, abundant evidence supporting pericyte loss in rats has been presented by several independent laboratories. Pericyte ghosts, many of which have been documented by published high resolution light and electron micrographs, have been reported in retinal capillaries of various types of diabetic rats (Cohen and Rosenmann, 1990; Kern and Engerman, 1994; Kojima et al., 1975; Robison et al., 1991b; Sima et al., 1985) and in the galactose-fed rat model (Robison et al., 1989a, 1990c; Robison and Laver, 1993). Kern and Engerman (1995) demonstrated pericyte ghosts within 15 months in retinal capillaries of rats fed either 30 or 50% galactose. Figure 5 demonstrates that pericyte ghosts not only occur in galactose-fed rats, but can be formed as early as 6 months after administration of a 50% galactose diet.

13.2. Galactitol and/or Glycation Levels

The limited measuring of polyol levels in galactose-fed rats has raised concerns (Engerman and Kern, 1993). Blood was the only tissue measured in the original report of diabetic-like retinopathy through the severe non-proliferative stage in a galactose-fed rat. The average non-fasting blood polyol was 36 mg/dl in the untreated rats, 2.9 mg/dl in the treated and less than 1.0 mg/dl in the controls (Robison et al., 1989a, p. 2290). Although total polyol rather than galactitol alone was reported, it is well established that essentially all the blood polyol is galactitol in galactose-fed rats (Dvornik, 1987). The remainder is sorbitol at a concentration of < 1.0 mg/dl. Measurements in tissues other than blood have been reported subsequently. The very rapid galactitol accumulation, which occurs in lens epithelium (Fig. 2B) is discussed in Section 12.4.

A major concern in the galactose-fed rat is that the same levels of galactosemia be maintained in both untreated and treated groups of animals throughout the experiment. In the original experiments on the galactose-fed rat model (Robison et al., 1989a, 1990c), the blood galactose levels were the same in both untreated and treated rats (216 and
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235 mg/dl), and were undetectable in controls at the termination of the experiment (Robison et al., 1989a). However, a much better assessment of galactosemia is accomplished by measuring the glycohemoglobin levels during the experiment. In a more recent experiment (Robison et al., 1993), glycohemoglobin levels measured at 6, 18 and 24 months demonstrated no statistically significant differences between galactose-fed rats whether untreated or treated with the aldose reductase inhibitor AL-3152. A more rigorous monitoring of glycohemoglobin levels was performed by Kern and Engerman (1995) in their galactose-fed rats. Two important findings were demonstrated: (1) that an aldose reductase inhibitor (sorbinil) had no effect on glycohemoglobin levels, and (2) that there was no need to modify the feeding regimes (ad libitum in both) to maintain an equivalent level of glycohemoglobin in untreated and treated galactose-fed rats. These findings suggest that all studies, which assure ad libitum feeding of a diet with a constant per cent of galactose will maintain comparable levels of glycohemoglobin regardless of whether they receive an aldose reductase inhibitor or not. However, the repeated monitoring of glycohemoglobin levels serves as a double check on feeding behavior, when performed.

13.3. Microaneurysms in Rat Retinas

Concerns were raised by investigators who have been unable to demonstrate microaneurysms in rats fed a 30% galactose diet for 21 months (Frank, 1991b, 1994; Frank and Das, 1992). Nevertheless, the majority of evidence indicates that microaneurysms do develop (Section 7), along with the other lesions characteristic of diabetic retinopathy through the mild proliferative stage (Figs 6–9). Confirmation that pericyte ghosts, acellular capillaries, hypercellular vessels (IRMA) and some microaneurysms in retinas of galactose-fed rats do, in fact, develop was recently reported by an independent laboratory. Using either a 50% or a 30% galactose diet (Kern and Engerman, 1995), multiple diabetic-like capillary lesions were demonstrated after 15–26 months. Only the definition and interpretation of microaneurysms differed slightly. A narrow criterion for what was accepted as a true microaneurysm was employed: the capillary aberration had to be saccular to be considered a microaneurysm by Kern and Engerman (1995). The fact that other types of microaneurysms occur in humans, perhaps even more commonly than the saccular type, was not taken into account. Recently, the different types of microaneurysms in human diabetics were quantified from retinal digest preparations (Ghose et al., 1994). It was determined that 17% of the microaneurysms were saccular, 24% were irregularly shaped and 59% were spherical.

Although more representative data are needed, it appears that the saccular type of microaneurysm is not necessarily the predominant type in humans or rats. The distinction between microaneurysms and IRMA is somewhat ambiguous in rats, in which no standard definitions have yet been formulated. Therefore, the findings of Kern and Engerman (1995) are basically consistent with our earlier report (Robison et al., 1989a) that most of the microaneurysms of galactose-fed rats are fusiform, cylindrical (tubular) or irregular in shape rather than saccular. Even if many of the irregular microaneurysms were classified as IRMA, this would not invalidate the galactose-fed rat as a model of human diabetic retinopathy because IRMA are among the most predictive lesions of progression to proliferative retinopathy (Davis, 1991; ETDRS, 1991).

Many of the microaneurysms in rats have more capillary connections than the two which are thought to be characteristic, representing the afferent and efferent portions of a single capillary. However, humans with diabetic retinopathy also commonly exhibit microaneurysms with multiple capillary connections in digest preparations. They have been clearly documented by some investigators (Fryczkowski and Sato, 1986; Fryczkowski et al., 1991) and appear to be present in the micrographs published by several others. Bloodworth (1962, Fig. 21; Bloodworth, 1964, plate X) demonstrated a human microaneurysm with at least three clear connections and a possible forth. Wolter (1961, Figs 12 and 13) also demonstrated microaneurysms with three and four connecting capillaries, in addition to several fibrous strands. The excellent scanning electron micrographs presented by Fryczkowski et al. (1991) leave no ambiguity. They demonstrate human diabetic retinal microaneurysms with three and four capillary connections. Therefore, contrary
to previous reports (Frank and Das, 1992), it appears that rats fed either a 30 or a 50% galactose diet are reliable models for the complete spectrum of diabetic retinopathies.

13.4. Galactosemia in Humans

Essentially all patients with congenital galactosemia have cataracts, as would be predicted from studies of galactose-fed rats. However, the clinical manifestation of retinopathy in these patients is uncommon, probably because galactosemia is strictly controlled and/or the patients usually die before significant retinal changes would be expected.

13.5. Can the Prevention Findings be Repeated?

Kern and Engerman (1995) confirmed that galactose feeding induces increased tissue polyol, and a concomitant diabetic-like retinopathy through the severe non-proliferative stage, but they were unable to demonstrate a prevention with the aldose reductase inhibitor sorbinil. Nevertheless, several studies have demonstrated an essential prevention of all the galactose-induced retinal microangiopathies using any one of three different aldose reductase inhibitors (Robison and Laver, 1993, 1994). It is important to note that the rat studies, which have demonstrated a positive effect of aldose reductase inhibitors in the retina (Sections 8 and 9), have routinely found complete prevention of cataracts (no sign of opacity by slit lamp, gross exam, or histology) for at least 24 months in all galactose-fed rats treated with most of the aldose reductase inhibitors tested, including sorbinil. The only exception has been statil, which delays rather than prevents cataracts (Robison et al., 1992). By contrast, Kern and Engerman (1995) were unable to completely prevent cataracts.

The failure to prevent cataracts suggests the key to interpreting the negative results in the retina. The lens, which retains all its cells throughout life, provides an accurate history of any past irreversible cellular insult, and thus serves as a good built-in control against experimental errors (Section 8). Sorbinil at the dose claimed is known to prevent cataracts for up to 24 months in rats fed 50% galactose (Dvornik, 1987; Robison and Laver, 1993, 1994). Cataract formation in the sorbinil-treated rats (Kern and Engerman, 1995) indicates that the expected target tissue levels of inhibitor were not obtained, probably due to a problem in sorbinil administration. Possibly uneven mixing or inadequate freshness of the sorbinil diet were factors. In any case, the inadequate dosing must have continued for at least 6 contiguous days during the experiment, since cataracts are reversible within 5 days by sorbinil treatment (Hu et al., 1983) or removal of the galactose diet (Simard-Duquesne and Dvornik, 1973).

Regardless of how the aldose reductase inhibitor studies might be interpreted, it is clear that the galactose-fed rat as well as the galactose-fed dog, is a useful model of diabetic retinopathy.

14. SUMMARY AND CONCLUSIONS

Diabetic retinopathy is the major cause of blindness in adults (20–74 yr-old) in the industrialized countries. Whereas systemic diabetes mellitus results from lowered availability and/or cellular recognition of insulin, the complications of diabetes such as diabetic retinopathy are caused by the chronic hyperglycemia itself. Although conventional insulin therapy lowers blood glucose levels enough to preserve life, it does not permit complete euglycemia nor prevent the long-term complications of chronic supernormal levels of blood glucose. Recent extensive clinical trials demonstrate that even intensive insulin treatment only delays diabetic complications, and it causes a 2−3-fold increase in severe hypoglycemia. This report presents experimental animal evidence for the efficacy of a novel approach to preventing diabetic complications, which would be used in addition to conventional insulin therapy, not to control blood glucose, but instead, to decrease the toxic effects of hyperglycemia on cells.

The studies were made possible by the creation of a reliable and convenient animal model for diabetic retinopathy — the galactose-fed rat. Probably the earliest toxic effect of elevated glucose levels in diabetic patients is increased aldose reductase activity, which leads to an intracellular accumulation of sorbitol, the polyol of glucose. By taking advantage of the fact that aldose reductase has a higher affinity for galactose than for glucose, much higher levels of polyol (galactitol) were attained in the cells of normal rats fed a 50% galactose diet than occurs in diabetic rats or humans (Fig. 2). The
extraordinarily high polyol level accelerated sugar cataract formation and permitted the development of all stages of diabetic retinopathy through the mild proliferative stage within 24 months in galactose-fed rats (Figs 6 and 7) in contrast to diabetic rats, which never show more than the early stages. The galactose-fed rat model permits the dissociation of the effects of polyol accumulation from those of elevated blood glucose, insulin, and glycosylation levels, as well as other metabolic or genetic factors that might influence the development of diabetic complications. Only polyol-related or glycation-related complications should be manifest in untreated galactose-fed rats and only glycation-related complications should develop in galactose-fed rats treated with an aldose reductase inhibitor. Since three inhibitors of aldose reductase with very different structures (Alcon 3152, sorbinil and tolrestat) prevented diabetic-like retinopathy (Fig. 10) without changing glycohemoglobin levels (Robison et al., 1993), the activity of aldose reductase is probably the initiating event of diabetic retinopathy. Whether or not glycation could be involved as a secondary factor remains to be established.

Substantial evidence indicates that abnormal accumulation of sorbitol resulting from activation of the enzyme aldose reductase of the polyol pathway is not only the best candidate for the initiating event of diabetic retinopathy, but also of many other ocular complications of diabetes. Prevention of sorbitol accumulation by oral administration of aldose reductase inhibitors has prevented sugar cataract formation, corneal edema, clouding and healing defects in both diabetic and galactose-fed rat models of diabetes. These findings identify a treatment that should be beneficial in humans, if used to complement conventional insulin therapy. Aldose reductase inhibitors would protect against the toxic effects of the relatively low, yet supernormal levels of glucose that cannot be eliminated with insulin therapy. However, treatment should begin early, long before clinical signs of the complications; drug pharmacokinetics should be considered thoroughly; and treatment should be continued on a long-term basis in order to prevent complications which take a long time to develop.

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