THE NATURAL HISTORY OF THE FIRST CLINICALLY VISIBLE FEATURES OF DIABETIC RETINOPATHY*

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INTRODUCTION

DIABETES MELLITUS IS A LEADING CAUSE OF VISUAL LOSS IN ADULTS IN America today.[1,2] Although many aspects of the visual system can be affected by diabetes mellitus, the major contributor to visual impairment is retinopathy.[3,4] In some situations, the more advanced stages of diabetic retinopathy can be influenced by medical and/or surgical techniques.[5,6]

There have been numerous attempts at intervention early in this disorder to prevent the development of these more advanced, and visually threatening, stages. One example of this approach is the Diabetes Control and Complications Trial (DCCT), a large multiple medical center collaborative study. It was an attempt to use rigorous metabolic control to try to influence retinopathy progression.[7] The DCCT data are being analyzed at this time and should be published in the near future. Another recent study involved a pharmacologic agent that was thought to inhibit diabetic retinal vascular changes.[8] So far, however, none of these techniques have influenced the development of such problems in most patients with diabetes.

The following is an effort to increase our knowledge about the earliest clinically visible aspects of diabetic retinopathy. Previous studies have not been directed toward an examination of diabetic retinopathy at this preliminary phase of its development. This may have been because the amounts of pathology in each eye were too small to permit the more common procedures available for the evaluation of diabetic retinopathy. However, in the recent past, a quantitative and reproducible methodology to measure the lesions in such eyes has been developed and was used in this study.

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A renewed interest in the biochemical pathways that may participate in the initiation of diabetic retinopathy resulted in this special attention to the earliest clinically measurable features. Many investigators believe that the development of the first clinically detectable diabetic changes should be able to be influenced by various therapeutic regimens. To assist their research efforts, independent fundus photo reading techniques that were unbiased, and could be masked to ensure the security of several coexisting studies, were needed. This resulted in the evolution of the diabetic retinopathy photographic grading procedures used in this report. The greatest amounts of work of this variety, at this time, involve studies of the aldose reductase inhibitors. One needs an understanding of the presumed role of aldose reductase inhibitors in the origin of diabetic retinal vascular disease to be fully aware of the features that were evaluated.

The biochemical studies of aldose reductase in ocular tissues progressed from an analysis of lens metabolism. In 1959, it was found that diabetic rats developed high concentrations of sugar alcohols in their lenses.[9] Soon afterward, it was demonstrated that this was caused by the activity of the enzyme aldose reductase in the lens epithelium.[10] In those experimental animals, the lens epithelial cells do not contain a mechanism to remove excess intracellular glucose; the intracellular glucose levels rise to approach extracellular levels by diffusion. At these high levels of glucose concentration, aldose reductase acts to convert glucose to sugar alcohols, such as sorbitol. Since those sugar alcohols cannot diffuse out of the cells, as sugar itself can, their osmotic effects lead to cell death and cataract formation.

Other cells in the eye, however, do not develop high enough sugar alcohol concentrations to produce cell death; in that situation, alternative pathways and problems are found. One of the diabetic retinopathic events that predates clinically visible changes in humans is a thickening of retinal capillary basement membranes.[11,12] Similar retinal capillary basement membrane thickening was found in experimental animals fed diets high in galactose.[13,14] This capillary basement membrane thickening was able to be prevented by the addition of an aldose reductase inhibitor to the experimental animal's diet.[15]

Another diabetic retinopathic event that occurs before the onset of clinically visible changes in humans is the loss of pericytes from the retinal microcirculation.[16] The identification of aldose reductase in these human cells implied that biochemical events that result in sugar alcohols could occur.[17] In the experimental animal model of diabetes, similar changes in retinal capillary pericytes were found; however, these were preventable with the use of aldose reductase inhibitors.[18]

A number of other corresponding research findings resulted in questions about the role of aldose reductase inhibitors in the prevention of the earliest clinically measurable diabetic retinopathic changes in humans. Since these agents appear to benefit animal models of diabetes, several drug manufacturers initiated preliminary experiments in human volunteers. By establishing a cooperative venture with various pharmaceutical investigators, experimental techniques were designed so that the natural history cohort populations could demonstrate these earliest stages of diabetic retinopathy and their rates of change. The report that follows is compiled, in part, from data collected in this manner. Since the work of these other investigators and the study reported here were able to overlap without compromising their interests, they were willing to cooperate in this activity. Those parts of these studies, however, that are within their proprietary interests will not be included in this report. The subjects of this report, therefore, are those members of the preliminary untreated control populations that remained as members of the natural history cohort populations.

HISTORIC REVIEW

One attempt to measure some of the early stages of diabetic retinopathy was described at the Airlie House Symposium on the treatment of diabetic retinopathy in 1968.[19] The discussions at that symposium indicated that those investigators were aware of the lack of sensitivity in their methods. Nevertheless, since they wanted to include all aspects of diabetic retinopathy in one grand scheme, this was not considered a major problem. However, at the same time, some investigators were aware that one could relate visual prognosis to the earliest stages of what was called background diabetic retinopathy.[20] During the years that followed the Airlie House Symposium, the techniques and tools that evolved from that meeting became the standards for measuring diabetic retinopathy and its progression.[21]

One large-scale test of the evolving methodology for the measurement of diabetic retinopathy was the Diabetic Retinopathy Study (DRS).[22] The DRS was a multiple medical center collaborative treatment trial initiated by the National Institutes of Health. It involved 1,758 patients, and they were followed with serial examinations and color stereo photographs at frequent intervals between 1972 and 1979.[22] Those investigators compared their clinical photos to a series of preexisting photos to determine if the retinal pathology was less than, equal to, or greater than, that seen in those standardized photos.[23-27] The large volume of data accumulated in the DRS required a modification of the original Airlie House Classification System for ease in data management.[21]

The next major evolutionary change of this methodology occurred when the National Institutes of Health initiated a treatment trial for diabetic retinopathy at an earlier stage of its development. This project, the Early Treatment Diabetic Retinopathy Study (ETDRS), was another multiple medical center collaborative study. To handle the new data that was expected from the ETDRS, and relate it to the data already accumulated from the DRS, further modification of the Airlie House Classification system was needed.[28] However, before the ETDRS could be completed, several additional treatment trials were able to evaluate these developing techniques.[29-34] The ETDRS and these other studies used classification schemes that expanded the range of measurable diabetic lesions. Some attempted to subdivide the early diabetic retinal lesions identified in patient photos.[29,30] This resulted in new diabetic retinopathy categories, such as "no classifiable retinopathy" (NCR); this term was used to categorize an eye that had some background retinopathy but not enough to be within one of the other defined groups. Specifically, NCR was described as "the presence of microaneurysms or red dots <125 μm in diameter without macular edema or hard exudates equal to or greater than a standard photo."[29] The next category was P_0, which was "at least one microaneurysm or red dot <125 μm in diameter, plus macular edema represented by either retinal thickening or hard exudates greater than, or equal to, a standard photo."[29] The remaining categories included more microaneurysms and hemorrhages, along with an increased frequency of soft exudates, venous beading, intraretinal microvascular abnormalities, and neovascularization.

Although these studies were able to widen the range of their grading systems, it was not until the ETDRS methodology came into common usage that a greater understanding of the early diabetic lesions evolved.[35,36] One of the features that made the ETDRS supersede other retinopathy measurement schemes was its strategy to differentiate microaneurysms from small retinal hemorrhages.[28] In the ETDRS, a microaneurysm was described as a red spot in the retina that was <125 μm in its longest dimension and had sharp margins. Any red spot that was ≥125 μm in its longest dimension was to be called a hemorrhage, unless it had sharply defined, round, smooth margins and a central light reflex, in which case it was a large microaneurysm. All other red spots that looked like microaneurysms, but did not fulfill these definition requirements, were called hemorrhages.

As an extra refinement, the ETDRS included a system for counting these lesions in each photographic field.[28] The photographic field was given a grade of 0 if there was no evidence of such lesions, a grade of 1 if there was a question in the mind of the photo reader that such a lesion was present, and higher grades if such lesions were visible. However, that scheme stopped

counting numbers of lesions when more than two lesions were present in the same photographic field.[28] In other words, if there were more than two microaneurysms, and/or other lesions, they were considered to be too numerous to count.

The next steps in the evolution of grading schemes for early diabetic retinopathy were built upon this ETDRS methodology.[37,38] However, the studies that used these procedures had visual acuity and overall retinopathy as end points and therefore did not emphasize the early measurements. In one study,[37] retinopathy levels were defined for each eye as follows: level 1, no retinopathy; level 1.5, retinal hemorrhages only and no microaneurysms; level 2, microaneurysms only; and higher levels related to the presence of other lesions, such as exudates. In another study,[38] the data from the two eyes were combined to achieve an overall patient retinopathy level. Nevertheless, the early diabetic retinopathic data available from these studies was limited because of the wealth of other information of interest to those investigators. Similar problems were found in the further analyses of the Kroc Collaborative Study data.[39] Even with these issues influencing data analyses, these studies emphasized the fact that the earliest clinically observable diabetic retinopathic changes seemed to be hemorrhages, microaneurysms, and exudates. In addition, these reports illustrated the presumption that some kinds of lesions appear before others, as if in a temporal cascade.

As this increased understanding developed, it was implied that microaneurysms could be one of the earliest diabetic retinopathic lesions detectable by clinical examination. However, there was concern that features associated with microaneurysms were being misinterpreted, because these lesions could be quite small and difficult to see against the red coloration of the retina itself. For this reason, some investigators chose to study microaneurysms with fluorescein angiography. That technique permitted a measurement of microaneurysms as small as 20 mμ in diameter.[40] Although that revealed additional information about microaneurysms, it also demonstrated that microaneurysms tend to become fewer in number, and/or disappear, over time.[40] It was thought that this reduction in microaneurysms may be due to capillary closure and/or blood shunting around the area containing the microaneurysm. How this related to the initiation of diabetic retinopathy, and the sequence of coexisting events, remained uncertain.

On a more practical basis, however, these findings resulted in some confusion among clinicians and research investigators. To verify data accumulated in scientific studies, some investigators believed that it was necessary to evaluate microaneurysms with two techniques: counting their numbers in color photographs and counting their numbers in fluorescein angiograms of select fields.[31-34,37,39-41] As determined by the bias of the investi-

gator, this would result in either a paradox or a dilemma. The more accurately one measured microaneurysms (ie, with fluorescein angiography), the fewer microaneurysms one could measure (owing to field size limitations). For most investigators and clinicians, this problem was resolved by an early publication of the DCCT.[42] That report described a comparison of data from both techniques and resulted in the following understanding: "Angiography allows a modest increase in sensitivity to the earliest signs of retinopathy, a gain potentially useful in some research applications, although not of demonstrated value in patient management."[42]

This, then, brings us to the work reported here. As mentioned in the introduction, this study is devoted to clinically visible changes in humans. It is restricted to those items that can be detected by any ophthalmologist with commonly available tools. Features that require specialized techniques, such as angiography, are omitted. For this reason, it may appear that the reported results are associated with a reduced sensitivity. However, since the same sensitivity level is present in all phases of this study, this potential error should be reduced to an inconsequential amount. Some recent work appears to confirm that presumption,[43] and certain features that support this concept will be described in greater detail in this paper.

METHOD

The study population consisted of individuals that were known to have insulin-dependent diabetes mellitus.[44] They were recruited originally into a series of studies by their regular medical doctors, since their primary physicians were regional investigators in various drug trials. Because many medical centers were involved in these pharmacologic studies, the clinical investigators at each site had to obtain the approval of each local Institutional Review Board (IRB) before enrolling patients. At enrollment, each participant was required to sign a local IRB consent form that allowed the accumulated clinical data to be used in the manner reported here, providing that individual identifiers were removed.

The patients who volunteered to enter these studies could be female without childbearing potential or male. This restriction was required by the pharmaceutical coinvestigators because of the uncertain teratogenic influence of the agents they planned to study. In female patients, this lack of childbearing potential had to be demonstrated by a natural, or surgically induced, postmenopausal status or by the presence of a surgically induced sterility, confirmed by the clinical investigator.

At the time of their original enrollments, these patients had to be between 18 and 54 years of age as of their most recent birthdays. Their insulin-

dependent diabetes mellitus had to have been diagnosed before they were 40 years of age, and the known duration of their insulin-dependent diabetes had to be more than 2 years and less than 15 years. At entrance into the studies, each patient had to weigh within –20% and +20% of the "normal" values for height and age as established from current actuarial tables. The diabetes control had to be with conventional injections of insulin (ie, with two, or fewer, injections of insulin each day); "rigid control" regimens, continuous subcutaneous infusions, and oral hypoglycemic agents were excluded.

At the time of entrance into these studies, each patient had to have evidence of clinically stable diabetes mellitus for at least 3 months before initial enrollment. This had to be shown by (1) the absence of ketoacidosis and symptoms attributable to markedly uncontrolled diabetes mellitus, (2) a history of an absence of significant hypoglycemic symptoms, (3) an overall impression to the primary physician of stability as ascertained by the total clinical presentation, and (4) chemical stability for 3 months, or more, before enrollment as judged by the clinical investigator at the site of enrollment.

In addition, patients were excluded from this study if they had any of the following medical problems: evidence of hepatic disease, cerebrovascular disease, decompensated or unstable cardiovascular disease, uncontrolled systemic hypertension, history or presence of a malignancy (excluding successfully resected basal cell skin cancer), mental illness, alcohol or drug abuse, history of pituitary surgery or pituitary irradiation, or a requirement for chronic antiplatelet drug or anticoagulant therapy. Patients were excluded if there was evidence of renal disease as manifested by a serum creatinine level of 2 mg/dl or greater.

Ocular inclusion criteria required the presence of two eyes and the visual acuity to be correctable to 20/30, or better, in each eye. Color stereo photographs of each eye had to be of a quality that would permit the accurate and reproducible grading of diabetic lesions. The original goal was to approach the criteria for the ETDRS.[28] The photos had to document that there was diabetic retinopathy in at least one eye of each patient, and if there was any diabetic retinopathy present, it was manifested by the presence of a total of 5 or fewer microaneurysms, or small round intraretinal hemorrhages, in the most involved eye.

Patients were excluded if there were any ocular problems that prevented good-quality color stereo photographs. In addition, patients were excluded from this study if any of the following were present: evidence of previous photocoagulation of the retina; iris neovascularization as defined by new vessels in the anterior chamber angle, or on the iris surface, or at the

pupillary margin; aphakia; a history of any previous ocular surgery; glaucoma requiring medication; an intraocular pressure of more than 25 mm Hg; or any requirement for chronic ocular medication.

After the clinical investigators at the study sites verified that the patients fulfilled the criteria for these studies, an agreement was reached with each patient regarding participation in the research activity, and the local IRB requirements were satisfied. Then, the patients had color stereo photographs taken of each eye. To achieve uniformity of in photographic quality, the following techniques were used:

EQUIPMENT

A modified fundus camera was used to perform stereo photography. Although many cameras could perform this activity, it was necessary to have a 30-degree photographic field to maintain uniform quality in this study. The most commonly used cameras sometimes needed to be modified in a minor way to take satisfactory photographs.[45] The stereo photographic techniques described by Allen[46] were recommended. The use of a mechanical stereo separator was optional, provided that it was documented that stereo photographs of a satisfactory quality could be produced in a consistent manner.

SEVEN STANDARD FIELDS OF THE FUNDUS

The seven standard stereo photographic fields of the fundus for both the right and left eyes were defined specifically for these studies. Other studies have used different definitions of standard photographic fields, and these were not interchangeable with this study. To verify the accuracy of the accumulated data, the seven standard fields had to be reproduced precisely by photographic technique. The following description of our seven standard photographic fields was based on the assumption that there were two cross-hairs in the camera ocular (one vertical and the other horizontal). Cameras that did not have cross-hairs in the oculars could be used if the photographic fields corresponded with that described in the following paragraphs.

Field 1—Disc. The center of the optic disc was at the intersection of the cross-hairs of the ocular.

Field 2—Macula. The center of the macula was immediately adjacent and contiguous to the intersection of the cross- hairs of the ocular. (Note: If the cross hairs were immediately in the center of the macula, the foveal light reflex could obstruct some areas of visualization.)

Field 3—Temporal to the Macula. The nasal end of the horizontal cross-hair corresponded with the center of the macula.

Field 4—Superior Temporal. The lower edge of this field was tangent to a horizontal line passing through the upper edge of the optic disc; the nasal edge of this field was tangent to a vertical line passing through the center of the optic disc.

Field 5—Inferior Temporal. The upper edge of this field was tangent to a horizontal line passing through the lower edge of the optic disc; the nasal edge of this field was tangent to a vertical line passing through the center of the disc.

Field 6—Superior Nasal. The lower edge of this field was tangent to a horizontal line passing through the upper edge of the optic disc; the temporal edge of this field was tangent to a vertical line passing through the center of the disc.

Field 7—Inferior Nasal. The upper edge of this field was tangent to a horizontal line passing through the lower edge of the optic disc; the temporal edge of this field was tangent to a vertical line passing through the center of the disc.

METHODS OF PROCESSING, MOUNTING, AND LABELING PHOTOGRAPHS

In this study, Kodachrome #25 Daylight Film was used and processed at the Kodak laboratories that were convenient to the local clinical sites. Other varieties of film, and other processing laboratories, may have been satisfactory. However, preliminary tests with other brands of film and processing techniques revealed potential problems in color perception by the photo readers if differing sources were used. The choice of one particular product and laboratory guaranteed a standard of reliability and reproducibility.

The photographic transparencies were mounted in standard cardboard 2×2 ready mounts. These transparencies were identified by a coded label to attempt to prevent bias by the photograph graders. The photographic transparencies were stored in clear plastic sheets containing 20 pockets per sheet. One sheet of 20 pockets was used for each eye. The transparencies were placed in the pockets so that the openings were toward the person mounting the slides. The transparencies were oriented in the plastic sheet for ease of stereo viewing with field 1 in the upper left, field 2 immediately below that, field 3 below that, field 4 below that, field 5 in the bottom left, field 6 in the upper right corner, and field 7 immediately beneath that.

PHOTOGRAPHIC QUALITY AND PATIENT ELIGIBILITY

The photographs were used to determine whether or not the patients satisfied eligibility requirements. The inclusion of findings sufficient to

satisfy eligibility requirements and the absence of exclusionary lesions had to be evident within the photographs. The photographs were graded for the presence of clinically relevant findings, the lack of exclusion criteria, and photographic quality. The results of these evaluations were sent to each investigational site. If patient eligibility could not be determined because of poor photographic quality, the investigational sites had to supply new photographs that were satisfactory or the patient was removed from the studies.

Since the results of these studies were to be determined by photographic documentation of retinal lesions, all photographs were graded for quality on the basis of the following features: how closely the photo corresponded with the definition of the photographic field; clarity and focus (how well the reader could identify retinal structures); stereoscopy (were the stereo pairs satisfactory to identify the depth of a lesion within the retina). Poor quality was defined as photographic fields that were more than one disc diameter away from definition, clarity too poor to permit identification of retinal structures, and/or no stereoscopic effect. A requirement to repeat the photos, or to remove the patient from the study, would develop if two or more stereo photo pairs (out of a total of seven pairs per eye) were of poor quality.

OPHTHALMIC PHOTOGRAPHERS

The photographs of the retina were the most important items needed for documenting these ocular features. Without quality photography these studies could not be performed. For this reason, each investigational site was encouraged to use the same photographer for all patients in these studies. If the original site photographer could not be available for all patients, patients were required to return for separate visits for photographs when that photographer was available.

FUNDUS PHOTOGRAPHY READING LABORATORY

This is a research laboratory designed as a core facility for several ongoing retinal studies. The functions of this laboratory in these diabetic studies were as follows: to determine patient eligibility, to monitor photographs for quality control, and to grade the photographic data.

The eligibility of patients for inclusion within these studies was determined at two points. The first was by the clinical investigators and the second was at the Fundus Photography Reading Laboratory. If there was disagreement between these two evaluations, telephone communication was initiated. However, unless photographic documentation could demonstrate eligibility for these studies, patients could not be enrolled. Therefore, although patients may have appeared to satisfy the requirements of these

studies at the original clinical sites, unless there was satisfactory photographic documentation to verify the eligibility, the patients could not be entered into these studies.

The photographs were graded by the readers at the Fundus Photography Reading Laboratory. These readers were masked as to patients and clinics in the making of these assessments. If there was a discrepancy between the investigational sites and the Fundus Photography Reading Laboratory in the eligibility or rating of a photographic series, the senior investigator adjudicated this difference of opinion.

There is an ongoing program to continually monitor and improve quality within the Fundus Photography Reading Laboratory. Periodically, duplicate masked photographs are sent to the same photographic reader to determine if the interpretation is being performed in the same manner.[47] This information compares the photographic reader's original interpretation with the second interpretation. If a discrepancy is determined, retraining of the photographic reader is initiated. In addition, there is a masked internal test performed in a periodic manner to determine if all photographic readers are interpreting data in a similar manner. To further ensure quality work by the Fundus Photography Reading Laboratory, periodic visits by outside consultants are made. These individuals use sets of classified photographs from outside these studies for an evaluation of the readers. Additional training programs are established if warranted by the evaluation results. In the specific series of photos reported here, there was some concern about a "temporal drift" in photographic interpretation. The photos used for this study were taken over an interval of 4 years. Some of the internal tests implied that there could be a small change of interpretation associated with the increased experience and knowledge developed by the photo readers during this interval; the photo readers may have become better at their jobs when they had 4 years' additional experience. Therefore, all of the photos in this report were re-graded within a 6-month period at the end of this study to avoid this interpretation error.

PHOTOGRAPHIC FEATURES OF GRADED LESIONS

Originally, these studies were designed to measure the features examined in the ETDRS.[28] The ETDRS Manual of Operations, chapter 18 (in the edition revised in March 1981), was used as a guide during the development of this work. However, instead of comparisons to standardized photos, the work reported here used descriptions of the most relevant anatomic and pathologic aspects of each suspect lesion. In this manner, while the ETDRS compared a lesion in a study eye to a similar lesion in a standard photo, the photo reading system used in this report described lesions in more anatomic

terms (eg, it used narrative descriptions of the lesions, counted the numbers of lesions, measured their sizes).

Some items graded in the ETDRS needed to be omitted or modified because the ETDRS was restricted to a different sample patient population. The data reported here, as compared with the ETDRS, were from patients with much less severe diabetic retinopathy. Some lesions, which were graded in detail in the ETDRS, were not expected to be seen. At the same time, different kinds of information needed to be gathered to make gradations at these very mild levels. Therefore, certain modifications were necessary to include these features and to gather data not included in the ETDRS. The following is a description of the techniques used in data collection for this report; it also refers to the corresponding sections of the ETDRS Manual of Operations,[28] when such a correlation exists.

Count of Microaneurysms in the Area of the Seven Standard Fields

This was an attempt to get an overview of the number of intraretinal red spots that appeared to be microaneurysms in the area covered by our seven standard fields. These red spots had to have round and sharp margins and had to be 125 μm or smaller in diameter. The total could not be calculated by merely adding the numbers of microaneurysms found in each field, because there was an overlap of the individual photographic fields. The error rate in this technique becomes a concern, since these fields overlap one another. For that reason, the photo reader used this method for initial screening only. The statistical data were analyzed from the "per field" information that is described next.

Count of Microaneurysms Per Field

There is some evidence that a patient with many microaneurysms in the area of our seven fields will have other diabetic lesions. Therefore, in this section the exact number in each photographic field was reported when it was less than, or equal to, 20, but was considered "too numerous to count in that photographic field" if greater than that. This corresponded with part of the description in the ETDRS Manual of Operations, chapter 18.4.1.

Retinal Hemorrhages and/or Large Microaneurysms in the Area of the Seven Standard Fields

This was a means of identifying and counting all red spots not captured above. All features previously recorded were excluded from this category. In this section, one counted all round red lesions that were greater than 125 μm, along with any of the red spots that were less than 125 μm that were not thought to be microaneurysms. In a manner similar to that already described, the photo reader used this method for initial screening only. For statistical purposes, the "per field" data were used.

Count of Retinal Hemorrhages and/or Large Microaneurysms Per Field

This was the same procedure as already described in regard to microaneurysms, but was restricted to hemorrhages and large microaneurysms. All red spots that were not captured earlier were included in this section. There is some evidence that a patient with many retinal hemorrhages in the area of the seven fields will have other diabetic lesions. Therefore, in this section the exact number was reported in each field when it was less than, or equal to 20, but was considered "too numerous to count in that photographic field" when there were more.

Total Hemorrhages and Microaneurysms (H/Ma)

In this item, the total number of H/Mas in each field was counted. This served as an internal confirmatory step. The features in this category approximate those of ETDRS Manual of Operations, chapter 18.4.2. when there are many lesions; when there are fewer lesions, this resulted in finer gradations than in the ETDRS.

Soft Exudates

These were described for these studies as superficial white, or pale yellow to white, areas with ill-defined "feathery" margins. In these studies, these lesions were measured in each photographic field. The cross-sectional diameter of a single hypothetical lesion that combined all of these exudates present in that one photographic field was calculated and used to represent the findings in that field. This part of these studies corresponded with the ETDRS Manual of Operations, chapter 18.4.5.

Preretinal Hemorrhages

This was defined as a red lesion on the surface of the retina that appeared (by stereo-optical techniques) to be in the preretinal, or subhyaloid, space and did not invade the overlying vitreous. Since one could measure the area of hemorrhage, this feature could be established with some accuracy. These lesions, if present, would be similar to those described in ETDRS Manual of Operations, chapter 18.4.17.

Vitreous Hemorrhage

This item was measured with the same techniques as those previously described. However, in this situation, vitreous invasion had to be demonstrated. This abnormality had to be within the vitreous as demonstrated by color stereo photographs. However, if present, these lesions were to be measured in cross-sectional diameter only. This is similar to the ETDRS Manual of Operations, chapter 18.4.18.

Intraretinal Microvascular Abnormalities

These were to be items that looked like early neovascular changes but were within the body of the retina when stereo-optical techniques were used. These represent intraretinal microvascular loops of varying widths. This is the same as the features described in the ETDRS Manual of Operations, chapter 18.4.8.

Hard Exudates

Hard exudates were defined as small white-to-yellow deposits with sharp margins. If present, they would be shining or glistening. One would expect these to appear to be within the body of the retina and to be deeper within the retina than the soft exudates. The numbers of these lesions were counted as in the ETDRS Manual of Operations, chapter 18.4.4.

Venous Beading

This would represent localized areas of symmetric increases of venous caliber where the swelling extends evenly on all sides of the involved vessels. The total number of venous beads seen in photographic fields 3 through 7 was to be counted. This corresponds with the ETDRS Manual of Operations, chapter 18.4.6-A.

Neovascularization Elsewhere

This was described as any new blood vessel on the surface of the retina, or into the vitreous, except for those that were within one disc diameter (1,500 mμ) of the edge of the optic nerve. This grading was based on the total area of retina covered by the new vessels in that photographic field. This corresponds with ETDRS Manual of Operations, chapter 18.4.11.

Fibrous Proliferation

This was to represent "fibrous" tissue that was made of multiple fine lines that were opaque and on the surface of the retina or extending into the vitreous cavity. This was to be graded in terms of the area involved. This corresponds with the ETDRS Manual of Operations, chapter 18.4.14.

New Vessels on the Disc

"On the disc" was interpreted in this system, and in the ETDRS, as meaning "on the disc or within one disc diameter (1,500 mμ) of the disc." This lesion was to be graded in field 1 only. An involved area with a diameter equal to one-third disc diameter was assumed to represent an area with a cross section diameter of 500 μm. This is described in the ETDRS Manual of Operations, chapter 18.4.10.

Clinically Significant Macular Edema

In light of the results from the ETDRS, this finding was reported, if present, in each eye. This item corresponded to any one of the three criteria for "clinically significant macular edema" established by the ETDRS.[48] These criteria are as follows: (1) the finding of an area of thickening of the retina either at, or within, 500 mμ, of the center of the macula, (2) the identification of hard exudates either at, or within, 500 mμ of the macular center when associated with retinal thickening, (3) the presence of one or more zones of retinal thickening, either one disc diameter in size or larger, when some part of the area of thickening is within 1,500 mμ of the macular center.

RESULTS

This study measured the changes in diabetic retinopathic features found in photographs of the eyes of 82 patients, 63 men and 19 women. Patient ages ranged from 18 to 54 years at the beginning of this study, and each patient was followed for 4 years. The initial patient data are listed in Table I.

Patients were excluded from this study if either eye was found to have more than five microaneurysms at the start. Table II lists the mean number of microaneurysms (and standard errors) in each photographic field of each eye at the start and at the end of 4 years. Table III describes the changes in microaneurysm counts for each photographic field found at the end of the study.

In every instance, the mean number of retinal microaneurysms in each patient was greater at the end of 4 years than at the start. This progressive increase of microaneurysm counts was not uniform. When this study began, some patients had microaneurysms in one eye only; however, by the end of the study, these patients had microaneurysms in both eyes. At the same time, a few of the patients that had bilateral involvement when this study began ended the study with a reduced number of microaneurysms in one eye and an increased number in the fellow eye.

Those patients that had microaneurysms in one eye and no abnormalities in the fellow eye at the start of the study had changes that were different from those in patients who began with bilateral microaneurysms. At the beginning, there were 11 patients with unilateral microaneurysms in the right eye (mean microaneurysm count for eyes with microaneurysms, 1.27; SD, 0.47; range, 1,2); and 19 with unilateral microaneurysms in the left eye (mean, 1.53; SD, 0.60; range, 1,3). When this study began, the eyes with microaneurysms from patients with unilateral involvement appeared to be the same as the eyes from patients who had visible pathology in both eyes.

TABLE I: INITIAL PATIENT DATA

PATIENT NO.	AGE*	SEX	DID	GH
1	18	Male	3.0	8.8
2	18	Male	3.5	10.6
3	20	Male	6.0	7.4
4	22	Male	4.16	9.2
5	23	Male	2.5	11.7
6	23	Male	3.0	9.2
7	23	Male	11.16	6.9
8	24	Male	2.0	5.6
9	24	Male	4.0	7.9
10	25	Male	13.0	8.1
11	26	Male	14.0	5.9
12	26	Male	4.0	8.2
13	26	Male	4.33	8.0
14	26	Male	9.0	8.0
15	27	Male	3.0	8.9
16	27	Male	2.08	5.6
17	27	Female	9.08	9.2
18	27	Female	14.0	9.5
19	27	Female	7.5	7.2
20	28	Male	3.0	12.4
21	29	Male	5.0	7.9
22	29	Male	2.25	9.6
23	29	Male	9.0	6.1
24	30	Male	2.0	5.4
25	30	Female	13.0	7.6
26	31	Female	6.0	8.3
27	31	Male	5.25	8.0
28	31	Male	5.0	9.6
29	31	Male	2.0	7.3
30	31	Male	8.0	8.7
31	32	Male	6.0	8.4
32	33	Male	11.42	7.5
33	33	Male	6.0	8.5
34	33	Male	4.0	10.8
35	33	Female	3.0	8.5
36	34	Female	2.0	4.8
37	34	Male	3.25	8.7
38	34	Male	3.0	10.5
39	34	Male	2.25	6.2
40	35	Male	3.75	5.6
41	35	Female	8.0	8.6
42	35	Male	2.25	10.6
43	35	Female	2.42	8.1
44	36	Male	5.5	6.3
45	36	Male	11.92	7.8
46	36	Male	3.0	8.1
47	36	Male	3.0	8.4
48	36	Male	6.0	6.7
49	36	Female	9.0	9.6
50	37	Male	2.25	7.5

TABLE I: INITIAL PATIENT DATA (CONT'D)

PATIENT NO.	AGE*	SEX	DID	GH
51	37	Male	5.0	7.3
52	38	Male	8.0	8.8
53	38	Male	2.75	8.1
54	38	Male	9.0	7.0
55	38	Male	6.0	7.8
56	38	Male	4.0	9.5
57	39	Male	7.0	9.2
58	39	Male	2.0	8.0
59	39	Female	4.0	10.4
60	39	Male	6.0	8.7
61	39	Female	2.33	6.4
62	40	Male	14.92	7.3
63	40	Male	2.5	6.4
64	41	Female	6.0	9.6
65	42	Male	7.75	9.4
66	42	Female	3.0	7.5
67	42	Male	4.0	8.7
68	43	Male	15.0	9.4
69	43	Male	4.0	8.9
70	44	Male	4.5	10.7
71	45	Male	10.0	10.4
72	45	Male	8.25	10.2
73	45	Male	9.0	8.8
74	45	Female	6.5	9.7
75	46	Female	10.0	6.2
76	46	Male	3.0	9.0
77	46	Male	7.0	6.8
78	47	Male	12.0	5.4
79	47	Female	10.58	10.3
80	47	Female	14.0	6.1
81	48	Female	12.25	9.0
82	54	Male	12.0	7.7

DID, duration of insulin-dependent diabetes mellitus at entrance into this study; GH, glycosylated hemoglobin level at entrance to study.
*Age of patient at most recent birthday before entering study.

At the end of 4 years, however, those that started with unilateral right eye involvement developed a right eye mean of 2.18 (SD, 0.87; range, 1,4), and those that started with unilateral left eye involvement were found to have a left eye mean of 1.83 (SD, 0.83; range, 1,4). During this same interval, those patients that started with bilateral microaneurysms ended the study with a right eye mean of 5.16 (SD, 4.75; range, 1,22), and a left eye mean of 5.91 (SD 5.71, range 1,22). By *t*-test analysis, these populations were different; those eyes that belonged to patients with bilateral microaneurysms at the start developed more new microaneurysms than those that began with uni-

TABLE II: MEAN MICROANEURYSM COUNTS

	MEAN COUNT AT START (SE)	MEAN COUNT AT 4 YR (SE)
Right eye		
Field 1	0.15 (0.04)	0.96 (0.29)
Field 2	0.22 (0.05)	1.45 (0.31)
Field 3	0.12 (0.04)	1.29 (0.31)
Field 4	0.16 (0.04)	1.29 (0.32)
Field 5	0.04 (0.02)	0.95 (0.26)
Field 6	0.02 (0.02)	0.77 (0.23)
Field 7	0.09 (0.03)	1.18 (0.32)
Left eye		
Field 1	0.12 (0.05)	1.26 (0.37)
Field 3	0.24 (0.06)	1.63 (0.34)
Field 2	0.20 (0.05)	1.01 (0.26)
Field 4	0.12 (0.04)	0.98 (0.30)
Field 5	0.12 (0.04)	1.20 (0.29)
Field 6	0.12 (0.04)	0.83 (0.19)
Field 7	0.12 (0.04)	1.56 (0.42)

TABLE III: INCREASE IN MICROANEURYSM COUNTS AT 4 YEARS FOR ALL EYES

	MEAN COUNT INCREASE (SE)
Right eye	
Field 1	0.82 (0.28)
Field 2	1.23 (0.31)
Field 3	1.17 (0.30)
Field 4	1.13 (0.32)
Field 5	0.92 (0.26)
Field 6	0.74 (0.23)
Field 7	1.10 (0.31)
Left eye	
Field 1	1.13 (0.37)
Field 2	1.39 (0.34)
Field 3	0.82 (0.26)
Field 4	0.85 (0.31)
Field 5	1.07 (0.29)
Field 6	0.70 (0.19)
Field 7	1.44 (0.41)

lateral involvement (P=.0003). At the beginning, for those with bilateral involvement, there was a correlation between right and left eyes for microaneurysms in photographic field 2 (Spearman rank correlation, r=.71, P = .0002); at the end of 4 years, a correlation remained (Spearman rank correlation, r=.61, P=.0001).

Some eyes seemed to have little change in overall counts at the end of this 4-year interval; however, when the serial photographic fields were examined in detail, it was found that there were times when some counts per field increased, while others were unchanged and others decreased. Table IV shows the percentage of photographic fields of each eye that were found to have increased numbers of microaneurysms at 4 years when compared with the start of the study. When those photographic fields that demonstrated increases in microaneurysm counts were examined alone, a much greater change in mean microaneurysm counts was seen; these findings are listed as Table V.

Most of the eyes that had photographic evidence of diabetic retinopathy had that feature characterized by microaneurysms in photographic field 2. Changes in the total numbers of microaneurysms in each eye, the numbers of microaneurysms in the other photographic fields, as well as all other aspects of diabetic retinopathy measured, appeared to follow those changes seen in photographic field 2. In those few eyes (four right, six left) that appeared, at the end of 4 years, to have had an improvement (ie, a reduction in microaneurysm counts) in field 2, most of the other fields had no change,

TABLE IV: PERCENTAGE OF PHOTOGRAPHIC FIELDS WITH INCREASED COUNTS AT END OF 4 YEARS

PHOTOGRAPHIC FIELD	% WITH INCREASE RIGHT EYE	% WITH INCREASE LEFT EYE
Field 1	31	34
Field 2	38	44
Field 3	35	23
Field 4	31	28
Field 5	32	32
Field 6	32	29
Field 7	35	38

TABLE V: CHANGE IN MICROANEURYSM COUNTS PER PHOTOGRAPHIC FIELD IN THOSE WITH INCREASES AT 4 YEARS

PHOTOGRAPHIC FIELD	RIGHT EYE MEAN (SE)	LEFT EYE MEAN (SE)
1	2.84 (0.78)	3.57 (0.94)
2	3.38 (0.66)	3.38 (0.62)
3	3.48 (0.66)	4.22 (0.74)
4	3.88 (0.83)	3.26 (0.92)
5	2.96 (0.68)	3.38 (0.69)
6	2.38 (0.63)	2.73 (0.44)
7	3.24 (0.73)	3.87 (0.95)

but some became better and a few became worse (Table VI). When there was no change (ie, there were the same numbers of microaneurysms at the end of 4 years as at the beginning) in field 2, there was no change in about 84% of the other fields, while an additional 13% became worse (Table VII). However, when photographic field 2 became worse, (ie, had more microaneurysms), most of the other fields became worse (Table VIII).

TABLE VI: FIELD 2: CHANGE FOR THE BETTER

	RIGHT EYE % CHANGES IN OTHER FIELDS			LEFT EYE % CHANGES IN OTHER FIELDS		
	BETTER	NO CHANGE	WORSE	BETTER	NO CHANGE	WORSE
Field 1	50	50	0	33.3	66.7	0
Field 3	0	75	25	50	50	0
Field 4	25	75	0	0	0	0
Field 5	25	75	0	0	83.3	16.7
Field 6	0	100	0	0	100	0
Field 7	0	100	0	0	100	0

TABLE VII: FIELD 2: NO CHANGES

	RIGHT EYE % CHANGES IN OTHER FIELDS			LEFT EYE % CHANGES IN OTHER FIELDS		
	BETTER	NO CHANGE	WORSE	BETTER	NO CHANGE	WORSE
Field 1	0	95.7	4.3	0	87.5	12.5
Field 3	4.3	85.1	10.6	5	95.0	0
Field 4	4.3	80.8	14.9	5	85	10
Field 5	2.2	87.2	10.6	2.5	90	7.5
Field 6	2.2	78.7	19.1	7.5	82.5	10
Field 7	4.3	76.6	19.1	2.5	80	27.5

TABLE VIII: FIELD 2: CHANGE FOR WORSE

	RIGHT EYE % CHANGES IN OTHER FIELDS			LEFT EYE % CHANGES IN OTHER FIELDS		
	BETTER	NO CHANGE	WORSE	BETTER	NO CHANGE	WORSE
Field 1	6.4	19.4	74.2	8.3	27.8	63.9
Field 3	9.7	16.1	74.2	8.3	41.7	50
Field 4	3.2	38.7	58.1	5.6	41.7	52.7
Field 5	0	32.3	67.7	8.3	30.6	61.1
Field 6	3.2	41.9	54.9	5.6	41.7	52.7
Field 7	6.5	29	64.5	5.6	38.9	55.5

Retinal hemorrhages were less common than microaneurysms in this study. At the start, it was rare to find any hemorrhages; and those that were present appeared as isolated "flame-shaped" lesions with "feathery margins." In the original photos, retinal hemorrhages were found in five right eyes and nine left eyes. One left eye had two hemorrhages in photographic field 2, while all of the others were seen as single spots in individual eyes. At the end of 4 years, retinal hemorrhages were present in 20 right eyes and 35 left eyes. At the conclusion of the study, every patient had at least one eye that contained retinal microaneurysms, but only 43% of patients had retinal hemorrhages.

At the end of 4 years, there were soft exudates in both eyes of three patients, and one additional patient had soft exudates in the right eye only. All of these soft exudates were near the center of the photographic field 2 of the involved eyes and occupied areas of less than 500 mμ in diameter. None of these involved eyes had microaneurysms in field 2 of the entrance photos, but they had between 4 and 16 microaneurysms in the field 2 photos at the end of 4 years.

Hard exudates were present in two left eyes only, and in no right eyes, at the end of 4 years. One eye had a ring of six hard exudates in the superotemporal portion of photographic field 2, and the other eye had a ring of ten hard exudates in the inferotemporal portion of photographic field 2. In addition, one of these individuals had bilateral soft exudates as described in the previous paragraph.

At the time of the 4-year photographs, one patient had intraretinal microvascular abnormalities (IRMA) in both eyes, while two other patients had IRMA in the left eye alone. All of the areas of IRMA occupied regions that were less than 500 mμ in diameter. The one right eye had this lesion in photo field 3, and one left eye had this lesion in photo field 3; the others were located in photo field 2, temporal to the fovea, and immediately central to the inferior temporal vascular arcades. These three involved individuals were the same ones identified earlier with soft exudates in both eyes.

Venous beading was found in both eyes of one individual at the 4-year examination. These lesions were seen in photo field 3 only, and the patient had all of the previously described lesions in his eyes.

During the 4 years of this study, there was no evidence, in any eye, of preretinal hemorrhages, vitreous hemorrhages, neovascularization, fibrous proliferation, or clinically significant macular edema. There was, however, a clustering of microaneurysms, exudates, IRMA, and venous beading in a small group of patients; their data are presented in tabular form as Table IX.

Patients were excluded from entrance into this study if either eye was found to have more than five microaneurysms. For this reason, it was

TABLE IX: PATIENTS WITH EXUDATES AND OTHER LESIONS

AT ENTRANCE				
CASE NO	SEX	AGE (YR)	DIABETES DURATION (YR)	GLYCOSYLATED HEMOGLOBIN
6	Male	23	3	9.2
51	Male	37	5	7.3
71	Male	45	10	10.4
82	Male	54	12	7.7

AT 4 YEARS								
	RIGHT EYE				LEFT EYE			
CASE NO.	SOFT EXUDATES	HARD EXUDATES	IRMA	VENOUS BEADING	SOFT EXUDATES	HARD EXUDATES	IRMA	VENOUS BEADING
6	+	0	0	0	+	0	+	0
51	+	0	0	0	0	+	0	0
71	+	0	+	+	+	+	+	+
82	+	0	0	0	+	0	+	0

+, Present; 0, absent; IRMA, intraretinal microvascular abnormalities.

expected that the amounts of initial pathology would be too small to relate to features seen in later photographs. This was examined with ANOVA techniques. When restricted to photographic field 2, there was no relationship identified (P=.77). However, when later photographs of an eye were compared with the photographic field of that eye with the most microaneurysms at the start, it appeared that a slight relationship may exist (P=.026).

Demographic features were examined to determine if they were related to changes in microaneurysm counts. Because of the gender bias in recruitment, more than 75% of the patients in this study were male; nevertheless, Fisher's exact test techniques indicated that there were no differences determined that were related to the sex of the patients (P=.9). The age of the patients at entrance into the study, a type of continuous variable, did not appear to be related to any increase in the number of aneurysms at the end of the study (ANOVA, P=.39).

The length of time that each patient had diabetes before entrance into this study appeared to be related to the changes in microaneurysm counts. Because of the small numbers and amounts of changes seen in other photographic fields, this finding achieved significance for photographic field 2 alone. As indicated earlier, patients could enter this study if there was a total of five, or fewer, microaneurysms in either eye at the start. At the beginning of the study, the numbers of microaneurysms in field 2 had no relationship to the duration of diabetes before the start (Student-Newman-Keuls [SNK] Test for Variable, P=.23). However, at the end of the study,

those eyes that had no apparent change in microaneurysm counts in photographic field 2 had a mean duration of diabetes of 8.87 years, while those with increased counts had a mean duration of diabetes of 11.99 years (SNK Test for Variable, $P=2\times10^{-4}$).

Glycosylated hemoglobin levels were used to examine these findings in more detail. At the start of this study, the glycosylated hemoglobin level of each patient was measured. The patients could then be divided into four groups of nearly equal sizes; those with levels of less than 7.3, 7.3 to 8.4, 8.5 to 9.3, and greater than 9.3. At the start, there was no relationship between glycosylated hemoglobin levels and microaneurysm counts on a "per eye" basis, or on a "per photographic field" basis. Multiple combinations were examined and positive findings were detected at the end of 4 years only. The relationship of eye findings and glycosylated hemoglobin levels that achieved significant statistical value was detected only when that group of patients with the lowest glycosylated hemoglobin levels at the start were compared with the highest group. With the use of the Fisher's exact test, the microaneurysm counts in field 2 at the end of the study were related to the initial glycosylated hemoglobin levels (lowest group was different from highest at $P=.0326$). When the same test was used to examine the highest and lowest groups with a comparison of the photographic fields that had the most microaneurysms at the end of the study (that is, not restricted to field 2), a more powerful relationship was found ($P=.015$).

During the time of this study, the internal quality control analyses of the Fundus Photography Reading Laboratory was designed to parallel the ETDRS. The ETDRS used "pathology levels" and measured agreements for that feature for all sets of photographs. At the time that the photos from this study were included with all other photos at the Fundus Photo Reading Laboratory, there was a perfect agreement for "pathology levels" in 96.8% of eyes, and an agreement within one level in 99.5% of eyes. This resulted in a kappa statistic of 0.958 and a weighted kappa (Kw) of 0.981. However, the counting of microaneurysms represents a more exacting activity. For this reason, the photographic interpretations used in this study were reassessed for that feature alone. In that situation, the kappa statistic was 0.654, and the Kw was 0.807. This interobserver agreement for the counting of microaneurysms is not as good as that found for "pathology levels." However, with the use of the standard benchmarks for interobserver agreement, this is an "almost perfect" strength of agreement,[47] and represents an exact agreement in more than 75% of the photographic fields evaluated in this study.

DISCUSSION

Although there are many aspects of this report that may warrant discussion, this will be limited to those features that appear to have the greatest clinical relevance. In addition, since some diabetic retinopathic features, such as venous abnormalities, vitreous hemorrhage, fibrous proliferation, and "clinically significant macular edema," are well described elsewhere,[5,6,35,48] and were rarely seen in this study, these features will not be discussed here.

Microaneurysms are the earliest clinically visible features of diabetic retinopathy. Although they had been well described in the English literature,[49] it was the work of Friedenwald[50] and Cogan and associates[51,52] that made most American investigators aware of their significance. A popular current theory about the origin of microaneurysms implies that they develop after the loss of retinal capillary pericytes.[51-53] This pericyte loss is not a clinically visible feature.[54] However, this cell loss results in a weakened capillary wall,[55] and that is thought to result in the development of the saccular bulges that are detectable by clinical examination.[54] The relationship between numbers of microaneurysms and diabetic retinopathy severity has been a puzzle; this was further confounded by the discovery that many diabetic microaneurysms become clinically invisible as they age.[54] However, the results of the Kroc Collaborative Study[40] and a Wisconsin population-based study[56] appeared to demonstrate the correlation of greater numbers of microaneurysms with more severe diabetic retinopathy.

Retinal hemorrhages can develop, in theory, from any blood vessel within the retina or from tissues adjacent to the retina. However, it is most likely that the hemorrhages seen in these studies originated from the degenerated capillaries that had lost their mural pericytes and/or the rupture of distended microaneurysms. Those that are rounded, or "blot-shaped," are often deep within the retina, while those that have "feathery-margins," or "flame-shaped," appear to be more superficial, as has been described in the earlier literature.[5,19,28,35,49,50,57] However, since these hemorrhages represent extravascular erythrocytes, they tend to disappear over time, as do the microaneurysms previously described.[54]

Soft exudates represent localized infarctions of the nerve fiber layer of the retina. This results in a coagulative necrosis of these isolated retinal foci.[58-60] However, as with retinal microaneurysms and hemorrhages, the natural regenerative activity of the retina results in their gradual reabsorption; such exudates are transient and disappear over time. The duration (time from onset to complete resolution) of soft exudates in young diabetic retinopathy patients varies between 2 and 18 months; however, 50% disappear in 8 months.[61] Nevertheless, when identified in the eye, they may be a hallmark of the start of a progressive change in diabetic retinopathy; since

soft exudates represent one manifestation of microinfarction, wide areas of subclinical retinal microvascular disease must predate their onset.[36,58-61]

IRMA were defined in this study as intraretinal microvascular loops of varying widths. They could have the clinical appearance of early neovascular growth within the retina. In the ophthalmic literature, the description of "IRMA" avoids an exact anatomic definition.[19] In the past, a popular theory implied that these were dilated and distended preexisting capillaries that were acting as vascular shunts.[62] More recent studies, however, appear to confirm a coexisting, but less popular, theory[57]; they imply that some IRMA may represent the earliest stages of neovascular growth.[63]

In this study, the most common lesions identified were microaneurysms in photographic field 2; this was both at the beginning of the study and at the end of 4 years (Table II). When both eyes were involved, there was a strong correlation regarding the numbers of microaneurysms in photographic field 2 of each eye. During the 4 years of the study, the changes in microaneurysm counts in photographic field 2 appeared to be the guide to all other changes in all of the other photographic fields (Tables VI, VII, and VIII). This corresponds quite well with the work of Klein and associates,[56] which implied that most microaneurysms will be in field 2 when there are just a few in an eye and there will be a tendency to distribute microaneurysms outside of field 2 when the number increases. The numbers of other diabetic retinopathic lesions (that is, other than microaneurysms) that were measured in this study were too few to permit statistical analysis; however, the Kroc Collaborative Study[40] revealed that such lesions, when present, correspond with increased numbers of microaneurysms. For these reasons, the numbers of microaneurysms in field 2 appear to be a good guide to the presence, and severity, of the earliest stages of diabetic retinopathy in an eye. In large population studies, such as epidemiologic and public health surveys, it would seem that stereocolor photographs of field 2 would be sufficient to obtain satisfactory data.

The earliest clinically detectable evidence of diabetic retinopathy was found in those patients who had microaneurysms in only one eye. In all of these patients, microaneurysms in field 2 were the first features to develop in their fellow eyes. This group of patients appeared to be behind the others in the rate of development of all aspects of their retinopathy. However, before the end of 4 years, all of these patients had microaneurysms in both eyes and were no longer a unique subpopulation.

Other studies have indicated that there may be a relationship between initial glycosylated hemoglobin levels and the rate of progression of diabetic retinopathy.[64,65] This was confirmed in this study but was noted at the extreme comparison of lowest to highest glycosylated hemoglobin levels.

There may be several possible reasons for this finding. The most obvious appears to be the relatively good diabetes control required of all patients entering this study. The other reports[64,65] described mean glycosylated hemoglobin levels well beyond the highest levels seen in this study population. One must assume that the lower glycosylated hemoglobin levels found at entrance may have been associated with relatively good diabetes control for the duration of the study.

The duration of diabetes was found to have the strongest correlation with the amount of diabetic retinopathy that was measured at the end of 4 years ($P=2\times10^{-4}$). This appeared to be independent of all other features, such as age, sex, amount of retinopathy at entrance into the study, and glycosylated hemoglobin levels. Although this study was limited to insulin-dependent diabetics, similar findings have been reported by others for all diabetics.[66-68]

SUMMARY AND CONCLUSIONS

Microaneurysms are the first features of human diabetic retinopathy that can be detected with common clinical techniques. These are found, most often, in photographic field 2 (that is, an area occupying 30 degrees of the ocular fundus centered on the middle of the macula). After the first microaneurysms develop, there will be a tendency for more to appear; however, over time many of the original microaneurysms will become no longer visible with clinical techniques, while other, newer, microaneurysms mature.

After the onset of microaneurysms, several years may pass before any other diabetic retinopathic lesions develop. Lesions other than microaneurysms were uncommon in this study; the following is a list in decreasing frequency: retinal hemorrhages, soft exudates, IRMA, hard exudates, and venous beading. During the 4 years of this study, there were no other diabetic retinopathic lesions detected.

The duration of insulin-dependent diabetes mellitus was related to the rate of change in microaneurysm counts. The age and sex of patients did not affect this rate of change. The accuracy of metabolic control, as determined by glycosylated hemoglobin levels, may influence this rate of change; however, this was detected only at the extremes of measurement in this study.

The equipment available to most ophthalmologists can detect the earliest clinical aspects of diabetic retinopathy. These features can be quantified in a reproducible manner with standardized photographic techniques to permit satisfactory data analysis.

REFERENCES

1. Kahn HA, Moorhead HB: *Statistics on Blindness in the Model Reporting Area, 1969-1970.* Washington, DC: US Dept of Health, Education, and Welfare; 1973. Public Health Service publication NIH 73-247.
2. Operational Research Department of the National Society to Prevent Bliness: *Vision Problems in the US: A Statistical Analysis.* New York, National Society to Prevent Blindness, 1980, pp 1-46.
3. Klein R, Klein BE: Vision disorders in diabetes, in R Hammon, MW Harris (eds): *Diabetes in America.* Bethesda, MD, National Institutes of Arthritis, Diabetes, Digetive and Kidney Diseases; 1985. US Dept of Health and Human Services publication NIH 85-1468.
4. Feman SS (ed): *Ocular Problems in Diabetes Mellitus.* Boston, Blackwell Scientific, 1992.
5. Diabetic Retinopathy Study Research Group: Diabetic retinopathy study design, methods, and baseline results. *Invest Ophthalmol Vis Sci* 1981;21:149-209.
6. ———: Early vitrectomy for severe vitreous hemorrhage in diabetic retinopathy: Four year results of a randomized trial. *Arch Ophthalmol* 1990;108:958-964.
7. Diabetes Control and Complications Trial Research Group: Results of feasibility study. *Diabetes Care* 1987;10:1-19.
8. Sorbinil Retinopathy Trial Research Group: A randomized trial of Sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. *Arch Ophthalmol* 1990;108:1234-1244.
9. Van Heynigen R: Formation of polyols by the lens of the rat with "sugar" cataract. *Nature* 1959;184:194-195.
10. Kinoshita JH: Cataracts in galactosemia. *Invest Ophthalmol Vis Sci* 1965;4:786-799.
11. Yamashita T, Becker B: Basement membrane in human diabetic eyes. *Diabetes* 1961; 10:167-174.
12. Ashton N: Vascular basement membrane changes in diabetic retinopathy. *Br J Ophthalmol* 1974; 58:344-349.
13. Engerman RL, Kern TS: Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 1984;33:97-100.
14. Takahashi Y, Wyman M, Ferris F, et al: Diabetes-like preproliferative retinal changes in galactose fed dogs. *Arch Ophthalmol* 1992;110:1295-1302.
15. Robison WG, Kador PF, Kinoshita JH: Retinal capillaries: Basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. *Science* 1983;221:1177-1179.
16. Cogan DG, Toussaint D, Kuwabara T: Retinal vascular patterns: IV. Diabetic retinopathy. *Arch Ophthalmol* 1961;66:366-378.
17. Akagi Y, Kador PF, Kuwabara T, et al: Aldose reductase localization in human retinal mural cells. *Invest Ophthalmol Vis Sci* 1983;24:1516-1519.
18. Robison WG, Nagata M, Laver N: Diabetic-like retinopathy in rats prevented with aldose reductase inhibitor. *Invest Ophthalmol Vis Sci* 1989;30:2285-2292.
19. Davis MD, Norton EW, Myers FL: The Airlie classification of diabetic retinopathy, in MF Golderg, SL Fine (eds): *Symposium on the Treatment of Diabetic Retinopathy.* Washington, DC: US Dept of Health, Education, and Welfare; 1968. US Public Health Service publication 1890:7-22.
20. Caird FI, Burditt AF, Draper GJ: Diabetic retinopathy: A further study of prognosis for vision. *Diabetes* 1968;17:121-123.
21. Diabetic Retinopathy Study Research Group: Report Number 7. A modification of the Airlie House classification of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 1981;21:210-226.
22. Diabetic Retinopathy Study: Report Number 6. Design methods and baseline results. *Invest Ophthalmol Vis Sci* 1981;21:1-200.
23. Diabetic Retinopathy Study Research Group: Preliminary report on effects of photocoagulation therapy. *Am J Ophthalmol* 1976;81:383-396.
24. ———: Photocoagulation treatment of proliferative diabetic retinopathy. *Ophthalmology* 1978;85:82-106.

25. ———: Four risk factors for severa visual loss in diabetic retinopathy. *Arch Ophthalmol* 1979;97:654-655.
26. ———: Photocogulation treatment of proliferative diabetic retinopathy: Clinical applications of Diabetic Retinopathy Study findings. *Ophthalmology* 1981;88:583-600.
27. ———: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. *Dev Ophthalmol* 1981;2:248-261.
28. Early Treatment Diabetic Retinopathy Study Group (ETDRS): Manual of Operations. Baltimore: ETDRS Coordinating Center, University of Maryland. Available from National Technical Information Service, 5285 Port Royal Rd, Springfield, VA 22161; Accession No. PB85-223006/AS.
29. Puklin JE, Tamborlane WV, Felig P: Influence of long-term insulin infusion pump treatment of Type I diabetes on diabetic retinopathy. *Ophthalmology* 1982;89:735-747.
30. Kroc Collaborative Study Group: Blood glucose control and the evolution of diabetic retinopathy and albuminuria. *N Engl J Med* 1984;311:365-372.
31. Sharma NK, Archer DB, Hadden DR: Morbidity and mortality in patients with diabetic retinopathy. *Trans Ophthalmol Soc UK* 1980;100:83-89.
32. Palmberg P, Smith M, Waltman S, et al: The natural history of retinopathy in insulin-dependent juvenile-onset diabetes. *Ophthalmology* 1981;88:613-618.
33. Frank RN, Hoffman WH, Podgor MG, et al: Retinopathy in juvenile-onset Type I diabetes of short duration. *Diabetes* 1982;31:874-882.
34. Doft BH, Kingsley LA, Orchard TJ, et al: The association between long-term diabetic control and early retinopathy. *Ophthalmology* 1984;91:763-769.
35. Early Treatment Diabetic Retinopathy Study Group: Grading diabetic retinopathy from stereoscopic fundus photographs: An extension of the modified Airlie House classification (ETDRS-Report No. 10). *Ophthalmology* 1991;98:786-806.
36. ———: Fundus photographic risk factors for progression of diabetic retinopathy. *Ophthalmology* 1991;98:823-833.
37. Klein R, Klein BE, Moss SE: Visual impairment in diabetes. *Ophthalmology* 1984;91:1-9.
38. Klein BE, Davis MD, Segal P, et al: Diabetic retinopathy: Assessment of severity and progression. *Ophthalmology* 1984; 91:10-17.
39. Kroc Collaborative Study Group: Diabetic retinopathy after two years of intensified insulin treatment: Follow-up of the Kroc Collaborative Study. *JAMA* 1988;260:37-41.
40. Kohner EM, Sleightholm M, Kroc Collaborative Study Group: Does microaneurysm count reflect severity of early diabetic retinopathy? *Ophthalmology* 1986;93:586-589.
41. Diabetes Control and Complications Trial Research Group: The Diabetes Control and Complications Trial: Design and methologic consideration for the feasibility phase. *Diabetes* 1986;35:530-545.
42. ———: Color photography vs. fluorescein angiography in the detection of diabetic retinopathy in the Diabetes Control and Complications Trial. *Arch Ophthalmol* 1987;105:1344-1351.
43. Leonard-Martin TC, Feman SS: A quantitative variation of the modified Airlie House classification of diabetic retinopathy. *Invest Ophthalmol Vis Sci* (suppl) 1993;34:1181.
44. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *J Am Diabetes Assoc* 1979;28:1039-1057.
45. Stenstrom WJ: A modification of the Zeiss fundus camera. *Arch Ophthalmol* 1960;64:935-938.
46. Allen L: Ocular fundus photographs. *Am J Ophthalmol* 1964;57:13-28.
47. Landis JR, Koch GG: The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159-174.
48. Early Treatment Diabetic Retinopathy Study Group: Photocoagulation for diabetic macular edema: Early treatment (ETDRS Report No. 1). *Arch Ophthalmol* 1985;103:1796-1806.
49. Ballantyne AJ, Loewenstein A: Diseases of the retina: I. The pathology of diabetic retinopathy. *Trans Ophthalmol Soc UK* 1943;63:95-115.
50. Friedenwald JS: Diabetic retinopathy. *Am J Ophthalmol* 1950;33:1187-1199.

51. Cogan DG, Toussaint D, Kuwabara T: Retinal vascular patterns: IV. Diabetic retinopathy. *Arch Ophthalmol* 1961;66:366-378.
52. Cogan DG, Kuwabara T: Capillary shunts in the pathogenesis of diabetic retinopathy. *Diabetes* 1963;12:293-300.
53. Speiser P, Gittelsohn AM, Patz A: Studies on diabetic retinopathy: III. Influence of diabetes on intra-mural pericytes. *Arch Ophthalmol* 1968;80:332-337.
54. deVenecia G, Davis M, Engerman R: Clinicopathologic correlations in diabetic retinopathy: I. Histology and fluorescein angiography of microaneurysms. *Arch Ophthalmol* 1976; 94:1766-1773.
55. Herman IM, D'Amore PA: Microvascular pericytes contain muscle and nonmuscle actins. *J Cell Biol* 1985;101:43-52.
56. Klein R, Meuer SM, Moss SE, et al: The relationship of retinal microaneurysm counts to the 4-year progression of diabetic retinopathy. *Arch Ophthalmol* 1989;107:1780-1785.
57. Yanoff M: Ocular pathology of diabetic retinopathy. *Am J Ophthalmol* 1969;67:21-38.
58. Wolter JR: Pathology of a cotton-wool spot. *Am J Ophthalmol* 1959;48:473-485.
59. Madson EC, Chester EM: The pathogenesis of the cotton-wool spot in diabetic retinopathy. *Doc Ophthalmol* 1974;37:217-224.
60. Ashton N: Pathophysiology of retinal cotton-wool spots. *Br Med Bull* 1970;26:143-150.
61. Kohner EM, Dollery CT, Bulpitt CJ: Cotton-wool spots in diabetic retinopathy. *Diabetes* 1969;18:691-704.
62. Cogan DG, Kuwabara T: Capillary shunts in the pathogenesis of diabetic retinopathy. *Diabetes* 1963;12:293-300.
63. Muraoka K, Shimizu K: Intra-retinal neovascularization in diabetic retinopathy. *Ophthalmology* 1984;91:1440-1446.
64. Klein R, Klein BE, Moss SE, et al: Glycosylated hemoglobin in a population-based study of diabetes. *Am J Epidemiol* 1987;126:415-428.
65. ———: Glycosylated hemoglobin predicts the incidence and progression of diabetic retinopathy. *JAMA* 1988;260:2864-2871.
66. Chen MS, Kao CS, Chang CJ, et al: Prevalence and risk factors of diabetic retinopathy among non-insulin dependent diabetic subjects. *Am J Ophthalmol* 1992;114:723-730.
67. Constable IJ, Knuiman MW, Welborn TA, et al: Assessing the risk of diabetic retinopathy. *Am J Ophthalmol* 1984;97:53-61.
68. Jerneld B, Algvere P: Relationship of duration and onset of diabetes to prevalence of diabetic retinopathy. *Am J Ophthalmol* 1986;102:431-437.