Angioscopic Changes in the Smaller Blood Vessels in Diabetes Mellitus and their Relationship to Aging

By Jørn Ditzel, M.D.

Observations with the stereoscopic dissecting microscope of the bulbar conjunctiva disclose vascular changes before pathologic changes are seen with the ophthalmoscope. In diabetic subjects lesions concomitant with aging are accelerated and, in addition, specific abnormalities characteristic of diabetes are observed.

UNTIL recently the vascular lesions in diabetes mellitus have been considered "arteriosclerotic" in origin. It was said that the high incidence of malignant vascular disease was due to an accelerating effect of diabetes upon the development of arteriosclerosis and normal aging.1, 2

Lately, there has been a change in opinion on this point and more investigators now regard the diabetic vascular syndrome as a specific entity characteristic of the disease.3, 4 This belief is mostly based on the fact that diabetic retinopathy and intercapillary glomerulosclerosis, both involving small blood vessels, show some characteristic morphologic features easily distinguishable from those of arteriosclerosis.5, 6 Evidence at present suggests that the diabetic lesions in the retina and the kidney are various manifestations of one generalized, slowly developing disease in the smaller blood vessels (diabetic microangiopathy). The increased fragility of the small blood vessels in the subcutaneous tissue of long-term diabetic subjects indicates the extensiveness of this microangiopathy.7, 8

Further information as to the nature of these changes can be obtained by studying other vascular beds of diabetic subjects. The bulbar conjunctiva offers unique possibilities for direct biomicroscopic observations of the smaller blood vessels and the circulating blood.9 These vessels can reasonably be considered representative of those of the subcutaneous tissue, since the 2 areas are closely related embryologically and anatomically.

The purpose of the present investigation was to evaluate the morphologic and hemodynamic changes occurring in the conjunctival vascular bed of diabetic and "healthy" subjects of various ages in order to determine whether changes in the diabetics differ from those occurring with normal aging.

METHODS AND MATERIAL

The material was obtained from observations of 220 diabetic and 175 "healthy" subjects, chosen with the object of providing an approximately even sex distribution in 4 convenient age groups: group 1, children, ages 4 to 15; group 2, young adults, ages 16 to 35; group 3, middle age, ages 36 to 55; and group 4, old age, ages 56 to 75.

The selected diabetic and control subjects formed comparable age and sex groups. The data on the number, sex, age, duration of diabetes, incidence of retinopathy, and insulin dose are given in table 1. The observer was unaware of the diagnosis and of the medical history of the subjects, since the selection of both diabetic and nondiabetic individuals was made by a cooperating investigator. The single exception was in the case of the children in whom the presence or absence of diabetes was known, but no other details of the medical history or chronologic age, etc., were revealed to the observer. Excluded from the study were all individuals who suffered from conditions that might cause interfering changes in the vascular bed: local infection or irritation, allergies, congestive heart failure, or infections. Furthermore, all diabetic subjects had normal blood pressure, defined in the present work as a systolic pressure below 150 and a diastolic below 100. They all were receiving insulin daily and all were in a state of fairly good control at the time.
Table 1.—Pertinent Data on All Individuals Investigated

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Number</th>
<th>Age Range (years)</th>
<th>Average Age (years) mean ± s.d.</th>
<th>Duration of Diabetes (years) mean ± s.d.</th>
<th>Incidence of Retinopathy</th>
<th>Insulin Dose (internat. units) mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>M</td>
<td>35</td>
<td>4-15</td>
<td>11.3 ± 3.3</td>
<td>5.6 ± 3.4</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>35</td>
<td></td>
<td>10.7 ± 2.6</td>
<td>4.3 ± 2.7</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>Young adults</td>
<td>M</td>
<td>25</td>
<td>16-35</td>
<td>26.3 ± 6.3</td>
<td>11.4 ± 7.8</td>
<td>7</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>25</td>
<td></td>
<td>26.4 ± 6.1</td>
<td>10.2 ± 8.5</td>
<td>8</td>
<td>46</td>
</tr>
<tr>
<td>Middle age</td>
<td>M</td>
<td>25</td>
<td>36-55</td>
<td>48.0 ± 4.9</td>
<td>11.1 ± 8.6</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>25</td>
<td></td>
<td>46.4 ± 6.3</td>
<td>10.5 ± 8.9</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>Old age</td>
<td>M</td>
<td>25</td>
<td>56-75</td>
<td>61.4 ± 4.2</td>
<td>6.8 ± 4.2</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>25</td>
<td></td>
<td>63.5 ± 5.7</td>
<td>11.2 ± 8.0</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Control Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>M</td>
<td>56</td>
<td>4-15</td>
<td>10.6 ± 3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>29</td>
<td></td>
<td>9.7 ± 3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young adults</td>
<td>M</td>
<td>15</td>
<td>16-35</td>
<td>27.8 ± 3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>15</td>
<td></td>
<td>24.3 ± 5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle age</td>
<td>M</td>
<td>15</td>
<td>36-55</td>
<td>47.0 ± 6.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>15</td>
<td></td>
<td>44.3 ± 4.5</td>
<td></td>
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<td></td>
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<tr>
<td>Old age</td>
<td>M</td>
<td>15</td>
<td>56-75</td>
<td>63.6 ± 5.1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>15</td>
<td></td>
<td>61.8 ± 6.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of the examination, i.e., none had acidosis. The groups are approximately comparable as to duration of diabetes and insulin dose except for the children, who obviously had to have a lesser duration of the disease. Only 41 diabetics showed any retinopathy and these individuals were distributed rather generally throughout the groups.

The subjects were placed in the supine position while the observations were being made. The stereobinocular microscope of the Grenough type (Leitz) was used to examine the lateral part of the bulbar conjunctiva of each eye. Normally the observations were made with a magnification of 48 × or 64 ×. The use of the Shahan ophthalmic lamp provided maximal illumination with negligible production of heat. A further detailed description of this method is given by Bloch. *

Immediately after each observation, information concerning vascular, perivascular, and intravascular changes was recorded. Dynamic alterations in the tone of the minute vessels were evaluated from the ratio between the diameter of the arterioles and the accompanying larger venules at various sites. Morphologic vascular changes were assessed from configuration of the arterioles, the capillaries, and the venules. These changes included irregularities, sacculations, and tortuosities. Perivascular changes were indicated by the presence of edema, localized or diffuse “hyaline” infiltration, and hemorrhages or pigment from old hemorrhages in the conjunctival tissue. Intravascular changes were based on a description of the hemodynamics in different parts of the vascular compartment. This included grading of the degree of aggregation of the red blood cells.

Examples of the observed changes were photographed by a specially developed technic.10

RESULTS

Table 2 illustrates the results of the study in the diabetic as compared to the control subjects according to vascular and perivascular changes observed in the conjunctival vascular bed.* Separate analysis of the findings has been made to evaluate the influence of age upon the vascular bed in the “healthy” and in the diabetic subjects. A comparison was also made between the significant† changes in the diabetic

* No distinction has been made for sex in the figures quoted, since no significant differences were found. The small figures in the lower right-hand corner in table 2 indicate the incidence of the changes in the control subjects.

† Unless otherwise indicated, whenever the terms “significant” or “statistically significant” are used throughout the paper, the significance has been calculated from the standard error of the difference, which equals

\[
\frac{p_1 - p_2}{\sqrt{\frac{s_1}{n_1} + \frac{s_2}{n_2}}}
\]

If the difference between the 2 observed percentages is 3 or more times the standard error of the difference, the 2 groups have been considered significantly different (p < 0.01).
and in the control subjects in order to determine the effect of diabetes alone upon the vascular bed exclusive of the aging process.

**Vascular Changes**

*Arterioles.* Jagged irregularities in the configuration of the terminal arterioles were occasionally observed in both diabetic and nondiabetic subjects. The number of nondiabetic cases that showed this lesion was small, and the incidence was only significantly correlated to age when the individuals in the 2 younger groups were compared with the 2 older groups. As may also be seen from table 2, the incidence of this lesion in the diabetic groups increased significantly with increasing age ($x^2 = 25.01$, $p < 0.01$, $n = 3$). When compared with their controls, the diabetic subjects showed a higher incidence of arteriolar lesions, which was, however, significant only in the old subjects (group 4). It therefore may be concluded that the lesion in the terminal arterioles is an aging change, and diabetes may possibly accelerate it.

*Capillaries.* The capillaries in the bulbar conjunctiva of the “healthy” subjects for the most part followed a smooth course (fig. 1). However, capillary elongation, appearing in the venous part as mixed angularities and tortuosities, was often encountered among the young diabetic subjects (group 1, 76 per cent; group 2, 72 per cent) but significantly less frequently in the older age groups (groups 3 and 4, 30 per cent) (fig. 2). When compared to the incidence in the control subjects, approximately 10 per cent, this capillary anomaly was found to be highly characteristic ($p < 0.01$) of the young diabetic subjects, while the difference in incidence in the older diabetic persons just reached the limit of significance. Consequently, this capillary change is not due to aging, but is a characteristic feature of diabetes, particularly in those individuals with onset of the disease early in life.

*Venules.* The venular changes (fig. 3) were categorized in 3 ways: venular irregularities, venular sacculations, and arteriolar-venular ratio.

Wavy and jagged venular irregularites occurred in the smaller venules (12-60 microns) of both nondiabetic and diabetic subjects. The incidence of this irregularity increased significantly with age in both the nondiabetic ($x^2 = 55.56$, $p < 0.01$, $n = 3$) and in the diabetic subjects ($x^2 = 26.00$, $p < 0.01$, $n = 3$). The incidence of venular irregularities was significantly higher in the diabetic than in the nondiabetic subjects in the 3 younger age groups. Thus the per cent of this change appearing in the conjunctiva of the diabetic children (group 1) was approximately equal to the non-
The conjunctival vascular bed of a "healthy" adult (52 years) shows an irregular network and numerous venular irregularities (v). 48X.

Table 2. The Degree of Vascular and Perivascular Changes in the Bulbar Conjunctiva Correlated to Age in Diabetic Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>10-15 Years</th>
<th>16-30 Years</th>
<th>31-50 Years</th>
<th>51-70 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar irregularities</td>
<td>5%</td>
<td>8%</td>
<td>18%</td>
<td>34%</td>
</tr>
<tr>
<td>Venular irregularities</td>
<td>76%</td>
<td>72%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Arteriolar sacculations</td>
<td>50%</td>
<td>68%</td>
<td>86%</td>
<td>94%</td>
</tr>
<tr>
<td>Venular sacculations</td>
<td>8%</td>
<td>16%</td>
<td>46%</td>
<td>36%</td>
</tr>
<tr>
<td>Arteriole venule A/V ratio</td>
<td>47%</td>
<td>48%</td>
<td>28%</td>
<td>6%</td>
</tr>
<tr>
<td>Edema</td>
<td>57%</td>
<td>40%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>&quot;Venous&quot; involvement</td>
<td>16%</td>
<td>62%</td>
<td>60%</td>
<td>74%</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>2%</td>
<td>24%</td>
<td>34%</td>
<td>29%</td>
</tr>
</tbody>
</table>

The small figures in the lower right-hand corner indicate the incidence of the changes in the control subjects.

diabetic members of group 3. Venular irregularities seem, therefore, to be part of the normal aging process and to be accelerated by diabetes.

Venular sacculations were observed in the larger venules measuring 60 microns or more in diameter. The data given in table 2 show that this lesion occurred less frequently than the smaller irregularities. However, a significant increase in incidence occurred commensurate with age in both nondiabetic (χ² = 19.71, p < 0.01, n = 1) and in diabetic individuals (χ² = 26.30, p < 0.01, n = 3). There was a significantly higher incidence of venular sacculation among the diabetic groups than in the nondiabetic groups and thus this venular change seems also to be accelerated by diabetes.

In order to evaluate dynamic changes occurring in the tone of the arterioles and venules the average A/V ratio was estimated. This ratio was determined by measurements of the diameter of the arterioles and accompanying larger venules. It was found that this ratio was 1:3 to 1:2 in all but 1 of the "healthy" subjects. In many diabetic subjects the larger venules showed marked distention, elongation, and congestion, and this change was often accompanied by a slight constriction of the accompanying arteriole. The A/V ratio in diabetic persons was found to reach values as low as 1:10. The change in the A/V ratio was particularly notable in the 2 younger groups (group 1, 47 per cent; group 2, 48 per cent) while less so in the older age groups (group 3, 28 per cent; group 4, 6 per cent). A χ² test indicated that this change, like capillary elonga-
tion, was inversely correlated with age ($\chi^2 = 28.21, p < 0.01, n = 3$). The venular distention and arteriolar constriction are highly characteristic of diabetes.

**Perivascular Changes**

The perivascular changes appear to be related to leakage of plasma and red blood cells through the walls of the venous part of the capillaries and the smaller venules.

*Edema.* Edema was encountered in only 4 cases among the "healthy" subjects and can be regarded as being inconsistent with good health and a normal functioning vascular bed. Microscopic and occasionally macroscopic edema of the conjunctival tissue was, however, often found in the younger diabetic subjects (group 1, 57 per cent; group 2, 40 per cent) while the occurrence of this change in the diabetic persons above the age of 35 was significantly less frequent (group 3, 10 per cent; group 4, 10 per cent; $\chi^2 = 44.03, p < 0.01, n = 3$). The edema appeared to be closely related to the presence of venular distention. Both these changes were present in many young diabetic persons without any evidence of retinopathy and nephropathy.

"Hyaline" infiltration. "Hyaline" infiltration is a common and easily observed senile change in the bulbar conjunctiva. In the nondiabetic individuals it appeared as a grayish or slightly yellowish spot in the perivascular tissue and was situated some distance from the limbus. This change was not found among the children and in only 2 cases (7 per cent) among the young adults, while its occurrence in the subjects above the age of 35 was significantly higher (group 3, 33 per cent; group 4, 37 per cent). In the young diabetic patients the "hyaline" infiltration appeared microscopically somewhat different from that of the nondiabetic subjects, but it appeared to be similar in the older diabetic and nondiabetic individuals. The "hyaline" infiltration in the young diabetic patients was extensive, cotton-wool-like, and diffuse (fig. 4). The incidence of "hyaline" infiltration was high in all 3 diabetic groups above the age of 15 (group 2, 62 per cent; group 3, 60 per cent; group 4, 74 per cent), while only 16 per cent of the children showed this tissue change. There appeared to be an abrupt rise in incidence at the age of approximately 25. When the incidence of the change was compared in the diabetic and the nondiabetic subjects, there was a markedly significant difference in all age groups. It therefore appears that even though the "hyaline" infiltration of the conjunctival tissue may be related to aging, the presence of diabetes has a markedly accelerating effect upon its formation.

*Hemorrhages.* Small blotchy hemorrhages or pigment of old hemorrhages was seen in approximately the same number of cases among the 3 older groups of diabetic subjects (group 2, 24 per cent; group 3, 34 per cent; and group 4, 29 per cent), while it was only observed in 1 case among the diabetic children. There was a significantly higher incidence of small hemorrhages among the diabetic than the nondiabetic subjects. It appears therefore that diabetes has an accelerating effect on the formation of hemorrhages in the conjunctiva.

*Over-all Vascular and Perivascular Changes*

In order to give an over-all picture of the relationship between age and the degree of vascular and perivascular damage in the nondiabetic individuals, the findings were graded

![Fig. 4. The conjunctival vascular bed of a young diabetic subject (23 years) shows extensive "hyaline" infiltration (h) surrounded by edema (e) and a distended venule (v). 64X.](http://circ.ahajournals.org/)
according to an arbitrary scale that has been used before (fig. 5). With an increase in years, there appear progressively more changes in the vascular bed. A $\chi^2$ test demonstrated that this relationship was statistically significant ($\chi^2 = 63.98, p < 0.01, n = 9$). None of the subjects fall into grade 3 because this combination of changes has not been found in healthy nondiabetic individuals.

No single grading system of the over-all vascular and perivascular changes can be employed in discussing the vascular pathology in diabetic individuals of various ages because different lesions appear in the smaller blood vessels, depending upon the age at onset of diabetes. Although a grading system cannot be used, all the single features such as arteriolar and venular irregularities characteristic of normal aging were found significantly more frequently in the diabetic than in the control subjects, as was shown above. Each group of diabetic subjects also showed, besides normal aging changes, alterations more characteristic of the diabetes itself, and these latter alterations are most obvious in the "juvenile" diabetic individuals with onset prior to ages 20 to 25.

Since the anomalies such as capillary elongation characteristic of diabetes are most obvious in the younger age groups, an evaluation of the effect of the duration of diabetes upon the vascular bed is also inaccurate if diabetic persons of various ages (4 to 75 years) are grouped together. An analysis made of the data from the children only (group 1) established that the degree of vascular change was positively correlated to the duration of the disease.

**Intravascular Changes**

The most easily recognizable intravascular change is the aggregation of red blood cells associated with slowing of blood flow. In order to take into consideration the size, placement, and physical properties of these aggregates, a grading system was used:

- $0 = $ Completely unaggregated blood stream.
- $1 = $ Small aggregates in the smaller venules.
- $2 = $ Consistent aggregation in the smaller venules, with occasional aggregation in the larger ones and in the capillaries.
- $3 = $ Aggregation in the arterioles, with or without intermittent plugging of the tip of the arterioles and capillaries. (Consistent heterogeneous aggregation appeared in the capillaries and venules.)
Table 3.—The Relationship between the Degree of Intravascular Erythrocyte Aggregation (E.A.) and Age in Diabetic and “Healthy” Subjects

<table>
<thead>
<tr>
<th>Grading of E.A.</th>
<th>Group 1 Children 4-15 years</th>
<th>Group 2 Young Adults 16-33 years</th>
<th>Group 3 Middle Age 36-55 years</th>
<th>Group 4 Old Age 56-75 years</th>
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</thead>
<tbody>
<tr>
<td>Diabetic subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 %</td>
<td>13</td>
<td>4</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>1 %</td>
<td>40</td>
<td>10</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>2 %</td>
<td>27</td>
<td>46</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>3 %</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>“Healthy” subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 %</td>
<td>86</td>
<td>73</td>
<td>60</td>
<td>37</td>
</tr>
<tr>
<td>1 %</td>
<td>10</td>
<td>23</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>2 %</td>
<td>4</td>
<td>3</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>3 %</td>
<td>0</td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3 illustrates the result of the grading of the intravascular erythrocyte aggregation in the nondiabetic and in the diabetic subjects. In the vast majority of “healthy” children and “healthy” young adults, there was no aggregation present in any vascular segment. On the other hand, various degrees of aggregation in the venules and capillaries were seen in 40 per cent of the middle age group and 63 per cent of the old age group. There was also a tendency for the “healthy” elderly subjects to show more severe degrees of aggregation. A $\chi^2$ test showed that the aggregation phenomenon was significantly increased in the 2 older age groups as compared to the 2 younger ($\chi^2 = 26.85$, $p < 0.01$, $n = 2$).

The finding that red cell aggregates were not observed in the arterioles of “healthy” subjects is of particular significance. Only 2 cases, both in the older age groups, were inconsistent with this finding. These may have had a disease process not revealed by the preliminary interview.

However, intravascular aggregation of the red cells with a marked decrease in the rate of blood flow was observed in a very high percentage in all the diabetic groups (87, 96, 92, and 98 per cent from the young to older groups, respectively). In all the diabetic groups except for the children, the severity of the aggregation was of the same degree. In the children (group 1) the aggregation phenomenon was significantly less severe than in the other age groups (groups 2, 3, and 4) ($\chi^2 = 26.46$, $p < 0.01$, $n = 2$). Thus, the diabetic subjects showed little evidence of change due to aging, while the nondiabetic subjects showed a significant relationship of the degree of aggregation to age. A striking difference was present between the incidence and degree of erythrocyte aggregation in the diabetic and in the nondiabetic subjects ($\chi^2 = 185.71$, $p < 0.01$, $n = 2$). This difference is particularly brought about by the presence in the diabetic patients of aggregates that could be seen to plug intermittently the tips of the terminal arterioles (grade 3). This aggregation was associated with particularly marked slowing of the rate of blood flow in the capillaries and venules.

**Discussion**

The low range of magnification provided by the ophthalmoscope makes impossible observations of the capillaries and the small branches of arterioles and venules in the retina. Thus, the initial pathologic changes in the development of diabetic retinopathy cannot be observed. By using the stereoscopic dissecting microscope on the easily accessible bulbar conjunctiva, this disadvantage can be overcome. The changes in the conjunctival vessels may be qualitatively or quantitatively different from those lesions found in the retina and in the kidney glomeruli. However, it is possible that such differences in vascular pathology may be explained by local factors such as the specific morphology of the retinal and kidney vascular beds and the high metabolic rate of both these tissues.

The description of the alterations in the conjunctival vascular bed has been based only on clearly definable and recognizable changes. Therefore the data present an incomplete microscopic picture. Despite this fact, it is felt that classification and analysis give an approximate outline of the changes that occur in this tissue in response to age and to diabetes.

By the study of “healthy” children in whom the aging changes have not influenced and distorted the vascular components, it was possible to outline the characteristics of the normal,
healthy, functioning vascular bed. The smaller blood vessels showed a regular configuration in which the ratio between the diameter of the arterioles and the accompanying larger venules was 1:3 to 1:2. The blood flow was optimal without red cell aggregation, and there was no edema present in the perivascular tissue (fig. 6).

As indicated by the study of "healthy" older nondiabetic subjects, the degenerative changes in the peripheral vascular bed apparently caused by normal aging were widespread and involved alterations in the vessel walls, in the perivascular tissue, and in the characteristics of the blood flow in the capillaries and smaller venules. All these changes became progressively severe with increasing age. However, there appeared to be an abrupt acceleration of the aging changes between the ages of 40 and 50. This fact was exemplified in the study by the significant increases in venular irregularities, sacculations, perivascular "hyaline" infiltration, and aggregation of the circulating blood in the subjects above the age of 35.

The irregularities in the configuration of the terminal arterioles were probably evidence of arteriosclerosis because this change was persistent and was related to aging. The only definable capillary change was a characteristic angular tortuosity of the venous part. This change was not related to advancing years. However, it should not be concluded that a general distortion in the capillary network is not a part of senescence. It was apparent that the capillary network in children and young adults was orderly and regular, while in the older patients the network became progressively more irregular. Functionally, this implies that in older individuals the capillaries are not evenly distributed and hence may not provide the tissues with normal supplies of oxygen and metabolites. The frequent and marked changes in the configuration of the venules of older subjects may influence the rate of blood flow, thus causing a situation that is favorable to the settling of the red cell aggregates and may lead to formation of microthromboses.

"Hyaline"-like deposits in the conjunctival tissue are a common macroscopic and microscopic sign of senescence and occur most often in the exposed triangular part of the conjunctiva at some distance from the limbus. Fuchs\textsuperscript{13} paid considerable attention to this senile change and found that patho-anatomically it consisted of an accumulation of amorphous "hyaline" material in the connective tissue ground substance associated with some hyaline degeneration of the collagen fibers. The "hyaline" material showed staining characteristics similar to fibrin. This change appears to be produced by 2 interrelated factors; senile alterations in the tissue and prolonged exposure to external and internal stimuli that produce vasomotor reactions.

In recent years attention has been drawn to the intravascular erythrocyte aggregation. The importance of this phenomenon in pathologic processes has been alternately supported, questioned, and rejected by various investigators.\textsuperscript{17} Some of the disagreement seems to have been based on the inadequate grading of the intravascular clumping and on the confinement of control observations to subjects of advanced age. In this study a grading system was used, the validity of which was supported by the finding of a significant relationship between the in vitro aggregation as measured by the sedimentation rate and the degree of in vivo aggregation in the same subjects.\textsuperscript{18} The progression with advancing age of erythrocyte aggregation in the capillaries and venules associated with slowing blood flow may explain the discrepancy in various opinions. Recently, Gillens and Morgan\textsuperscript{19} have shown that sedimentation rate increases with age. The aggregation phenomenon appears to be related to increases in relative or absolute concentrations of the globulin and fibrinogen fractions in the blood.\textsuperscript{19, 21, 22} The normal protein pattern becomes progressively altered with aging, and possibly it is the cause of the intravascular erythrocyte aggregation in older subjects.\textsuperscript{23} Of particular importance is the present finding that intravascular erythrocyte aggregation was not observed in the arterioles of healthy subjects. This supports the original statement by Bloch\textsuperscript{24} following his observations on more than 3000 healthy and diseased individuals: "To date no person has been seen in whom there is a significant and continuous retardation of blood
flow by erythrocyte aggregates through the arterioles who is not ill.”

Diabetes appears to affect the small blood vessels in 2 different ways: (1) by producing lesions that are indistinguishable from those of normal aging and (2) by producing capillary and venular changes that are characteristic of diabetes and unrelated to the aging process. Both kinds of alterations are present in diabetic subjects of all ages, but their incidence is influenced strongly by the age of onset of diabetes and the chronologic age of the subject. Thus, the vascular lesions occurring in the older patients consist predominantly of the changes indistinguishable from those of aging, while in the younger patients the lesions consist mostly of the characteristic diabetic capillary and venular alterations.

The aging changes in the diabetic subjects are the same as those in the “healthy” subjects, i.e., arteriolar and venular irregularities, “hyaline” infiltration of the perivascular tissue, and intravascular aggregation of red cells with slowing of blood flow. However, all these changes appear earlier in the diabetic than in the nondiabetic subjects, and in all diabetic groups the incidence is significantly increased. Thus, the aging process appears to be accelerated. This is particularly evident in the comparison of young diabetic subjects with their controls and becomes less striking in the older age groups.

The characteristic changes of diabetes are observed particularly in the young individuals. The vascular alterations consisted of tortuous elongations of the venous part of the capillaries and of the venules, as well as changes in the arteriolar-venular ratio associated with the presence of edema in the perivascular tissue (fig. 6). In order to elucidate further the nature of these characteristic changes, serial observations were made in a large number of young diabetic subjects for a period of 10 months. In this study it was demonstrated that the changes in the caliber of the arterioles and venules (A/V ratio) associated with exudation through the venular walls were a reversible pathologic vasomotor response. The elongation of the venous part of the capillaries and venules was apparently an irreversible change and most likely caused by a proliferation of the endothelial cells associated with degeneration. It thus appears that proliferation and pathologic vasomotor responses that particularly involve the venules take place to a greater extent in the young diabetic than in the older diabetic subjects. It should be noted that the retinal pathology appears to parallel this finding. During recent years it has become evident that the retinopathy of young long-term diabetic subjects differs somewhat from that of middle-aged and elderly diabetic subjects. While the retinopathy in the elderly diabetic patients tends more to show exudative benign changes, the retinopathy in the young patients is characterized by marked degenerative changes in the veins, formation of capillary microaneurysms and proliferation of vessels. Lundbaek also demonstrated that the venous lesions were statistically related to the young long-term diabetic subjects as compared to the older long-term diabetic ones.

Capillary microaneurysms of the type present in the retina of diabetics were not observed in the bulbar conjunctiva. According to Ashton such lesions are apparently not present in tissues other than the retina and possibly the glomeruli of the kidneys. This suggests that the formation of microaneurysms is associated with local factors in the retinal tissue such as special capillary morphology,

**Fig. 6.** The vascular bed of a young diabetic subject as compared to a normal vascular bed.
arrangement, functional need, or high pressure. The elongation of the venous part of the capillaries, which was found to be an early change in the conjunctiva, has likewise been demonstrated as an early finding in the retina, and studies suggest that the proliferation of new vessels start from such elongated and looped capillaries. 27 Thus, some of the capillary changes in the conjunctiva appear similar to those of the retina. The correlation between the degree of conjunctival changes in the diabetic children and the duration of the disease, 13 as is also true for diabetic retinopathy, supports the concept that the changes in the smaller blood vessels of the conjunctiva and of the retina are related. The pathologic vasomotor responses in the smaller conjunctival vessels are present very early in the diabetic condition, many years before retinopathy can be observed with an ophthalmoscope. 28 Observations suggest that the reversible responses are fundamental to the mechanism leading to the formation of the diabetic microangiopathy. 29

The most obvious difference between the diabetic and the "healthy" subjects was the rate of blood flow through the capillaries and the venules. In the diabetic subjects discontinuous and slow blood flow was brought about by the venular distention and a severe degree of intravascular erythrocyte aggregation, plugging the tips of the arterioles. The intravascular erythrocyte aggregation seems to be related to changes in proteins in the plasma of diabetic patients. There is evidence 18, 19, 22, 23 to indicate that certain globulins, when in excess in the plasma, form a surface film on intravascular phase boundaries by which aggregates of erythrocytes are formed. Changes in the physical characteristics (viscosity, tensile strength) of this surface film may affect the sizes of the erythrocyte aggregates and furthermore interfere with the passage of oxygen and metabolites through the capillary and venular walls. Changes in the serum proteins and their conjugates have been found not only in diabetic subjects with well-established vascular disease but also in diabetic subjects without clinical evidence of "complications." These changes are characterized by an increase in α2-globulin and in β-lipoprotein and a decrease in albumin. 22-34

The stasis associated with distention of the venules leads to intermittent seepage of plasma through the venular walls out into the perivascular tissue. Depending upon the degree and duration of exudation and the rate of resorption, proteins may be deposited. This may explain, in part, the early and extensive appearance of "hyaline" infiltration in the conjunctival tissue of young diabetic subjects.

This study strongly supports the supposition that the vascular lesions in the retina and glomeruli of the kidney are manifestations of one slowly developing, generalized disease in the smaller blood vessels. Even though the normal age changes appear to be accelerated in diabetes, the characteristic capillary and venular alterations in the vascular bed of the diabetic subjects emphasize that the vascular changes occurring in this disease are different from those of normal aging and "arteriosclerosis."

**Summary**

A comparative biomicroscopic study has been made of the bulbar conjunctival vascular bed of 220 diabetic and 175 "healthy" subjects of various ages. Vascular, perivascular, and intravascular alterations taking place in diabetic and nondiabetic subjects along with aging have been classified and analyzed.

The study of healthy children, in whom aging changes have not influenced and distorted vascular components, helped define the characteristics of a normal, healthy, functioning vascular bed. The smaller blood vessels showed a regular configuration with a ratio between the arterioles and the accompanying larger venules of 1:3 to 1:2. The blood flow was optimal without red cell aggregation and there was no edema in the perivascular tissue. The significant vascular changes along with aging occurred as irregularities in the terminal arterioles and in the larger and smaller venules. "Hyaline" infiltration in the perivascular tissue was correlated to age as was intravascular erythrocyte aggregation associated with slowing of blood flow in the capillaries and venules. Thus, all the degenerative changes in the peripheral vascular bed became progressively more severe with increasing age.

The significant vascular changes in the dia-
betic subjects consisted of (1) lesions that were indistinguishable from those of normal aging and (2) capillary and venular alterations, exudation, and arteriolar plugging of erythrocyte aggregates that were characteristic of diabetes and were unrelated to the normal aging process.

Both kinds of changes were present in diabetic subjects of all ages, but their distribution was influenced strongly by the age at onset of diabetes. Thus the vascular lesions occurring in the older patients consisted predominantly of changes indistinguishable from those of aging, while in the young patients the lesions consisted mostly of the characteristic diabetic alterations.

The diabetic capillary venular characteristics were of 2 types, i.e., reversible and irreversible changes. The reversible changes were seen as various degrees of venular distention and arteriolar narrowing and formed a set of pathologic vasomotor responses. The irreversible lesions consisted of elongations of the venous part of the capillaries and venules accompanied by evidence of degeneration.

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Summary in Interlingua

Un comparative studio biomicroscopico esseva execute in le vasculatura del conjunctiva bulbar de 220 diabeticos e 175 subjectos normal de varie etates. Le alterationes vascular, peri-vascular, e intravascular que occurre in diabeticos e non-diabeticos in le curso del progresso de etate esseva classificate e analysate.

Le studio de juveniles normal, in qui alterationes debite al etate ha non ancora influente e distorquite le componentes vascular, esseva de adjuta in defin le caracteristicas de un normal vasculatura. Le minor vasos sanguinee monstrava un configuration regular con un proportion de 1 a 3 o 1 a 2 inter arteriolas e le plus grande venulas accompaniante. Le fluxo de sanguine esseva optimal sin aggregations erythrocytic, e il habeva nulle edema in le histos perivascular. Le significative alterationes vascular que occurreva in le curso del progresso de etate se manifestava como irregularitates in le arteriolas terminal e le venulas major e minor. Infiltrationes hyalin in le histos perivascular esseva correlationate con le etate. Le mesmo vale pro le aggregation erythrocytic intra vascular associate con relentamento del fluxo sanguine in capillares e venulas. Assi omne le alterationes degenerative in le vasculatura peripheric deveniva progressivemente pejor in le curso del progresso de etate.

Le alterationes vascular que esseva significative in le individuos diabetic consisteva de (1) leesiones non distinguibile ab leesiones occurrente in individuos normal in le curso del progresso de lor etate e (2) alterationes capillar e venular, exudation, e obstruction arteriolar per aggregatos erythrocytic que esseva caracteristic de diabeticos e que non esseva connectite con le normal processo del avantiamento del etate.

Ambe typos de alterationes esseva presente in diabeticos de omne etates, sed le distribution de iste alterationes esseva fortemente influentiata per le etate del patiente al tempore del declaration de su morbo. Assi le leisiones vascular in patientes de etate plus avantiata consisteva predominantemente de alterationes que non esseva distinguibile ab illos debite al progresso de etate, durante que le leisiones in le patientes plus juvane consisteva predominantemente de alterationes caracteristicamente diabetic.

Le caracteristicas capillar e venular in diabeticos esseva de 2 typos, i.e. illos esseva (1) alterationes reversibile o (2) alterationes irreversibile. Le alterationes reversibile se manifestava como varie grados de distension venular e de restriction arteriolar e formava un serie de pathologic responsas vasomotor. Le alterationes irreversibile consisteva de elongationes del parte venose del capillares e del venulas es esseva accompaniante per signos evidente de degeneration.

References


Angioscopic Changes in the Smaller Blood Vessels in Diabetes Mellitus and their Relationship to Aging

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