Etiologic Considerations in Peripheral Vascular Diseases of the Lower Extremity with Special Reference to Diabetes Mellitus

By Herman T. Blumenthal, Ph.D., M.D., Aline W. Berns, A.B., Sidney Goldenberg, M.D., and Paul W. Lowenstein, M.D.

During the past several years the existence of thromboangiitis obliterans as a distinctive disease entity has been seriously questioned. This evidently began with the suggestion by Fisher1 that the peripheral manifestations of so-called Buerger's disease might actually represent atherosclerosis of the large arterial trunks with distal thrombosis. A more comprehensive study by Wessler et al.2 appears to support Fisher's suggestion, although such a view has been contested by others.8-7

This controversy represents only one facet of a larger, and perhaps more important, problem. In recent years there has developed an ever increasing tendency to consider almost all occlusive disease of the major arterial trunks as atherosclerosis attributable to faulty lipid or lipoprotein metabolism. An alternate concept8 holds that sclerotic disease (including lipid deposition) of the large arterial trunks may represent the common end-stage of pathologic changes produced by a variety of etiologic factors. This common end-stage is considered to be due to limitations in the response capabilities of the various tissue components that constitute the structure of the artery wall. Thus the pathologic characteristics of the end-stage lesion may provide no clue as to etiology.

Probably a better indicator of etiology is the changes in small peripheral and intraparenchymal vessels on the basis that changes in these small vessels reflect etiologic factors operative also in adjacent trunk arteries. In these small vessels, as shown in previous studies,9-18 it is possible to separate lesions on the basis of certain pathologic characteristics. Moreover, lesions of small vessels may be responsible for clinical manifestations of disease often attributed to occlusion of a major arterial trunk. An example of such a situation is seen in diabetes in which small patchy areas of gangrene of the lower extremity evidently occur more often than large demarcating areas14 and appear to be due to disease of small peripheral vessels, since the large trunk arteries often do not exhibit concomitant advanced occlusive disease.

In diabetes it has been found that microangiopathic changes occur which appear to represent an immune response to an altered insulin.15 It has also been suggested that such lesions may create a peripheral resistance leading to an intensification of arteriosclerosis of the arterial trunks. A similar relationship may exist in respect to thromboangiitis obliterans and perhaps other instances of peripheral vascular disease of the lower extremities.

Because of such considerations we have carried out a study of the pathologic changes in small vessels of 186 amputated lower extremities. These include a tabulation of various histopathologic lesions and a study of the reactions of certain of these lesions to immunofluorescent reagents.

Methods

The 186 amputation specimens were obtained...
from 162 patients. Pathologic examination included not only a study of the large cognate system arteries, but also the accompanying veins as well as samples of muscle and skin. These materials were fixed in 10 per cent formalin, embedded in paraffin and sectioned at 6 \( \mu \). Sections were stained for conventional light microscopic examination with hematoxylin-eosin, the Verhoeff-van Gieson stain, and by the PAS and colloidal-iron technics as in previous studies.\(^9-13\) Additional sections from selected paraffin blocks were stained with fluorescein-conjugated insulin and with fluorescein-conjugated rabbit antihuman globulin (RAHG) for examination by fluorescence microscopy. When positive insulin binding was obtained a control inhibition preparation was examined. This consisted in first immersing the slide in a normal insulin solution to saturate the insulin-binding sites followed by staining with fluorescent insulin. Evidence for specific inhibition consisted in complete or marked quenching of the fluorescent reaction. An additional control for nonspecific protein binding consisted in staining sections with fluorescein-conjugated snake-venom protein. The details of the technics used in these fluorescent studies have been described previously.\(^16\)

Cases were grouped according to previous clinical and pathologic diagnosis, and lesions of small vessels were tabulated as shown in table 1. The criteria used for the pathologic classification shown in table 1 have also been previously described.\(^9-13\) In the immunofluorescence studies a somewhat different clinical grouping was used as shown in table 2; the cases selected for such studies were based on findings with conventional light microscopy.

### Results

#### Histopathology of Small Vessels

The data in table 1 show no significant difference in the frequency of inflammatory lesions of small arteries or veins in the various clinical categories, with the exception of osteomyelitis. The over-all frequency of inflammatory lesions was about 37 per cent. Similarly, there is no significant difference in the frequency of thrombotic lesions with the exception of osteomyelitis; thrombotic lesions occurred with an over-all frequency of 15 per cent. Atheromatous lesions of small arteries were encountered only rarely, the over-all frequency being only 6 per cent. Of the 11 cases with atheromatous lesions, five were instances of atheromatous embolism as described by others\(^17-18\) in amputated lower ex-

<table>
<thead>
<tr>
<th>Clinical disease</th>
<th>Inflammatory</th>
<th>Thrombotic</th>
<th>Atheromatous*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics with arteriosclerosis</td>
<td>25/71 (35%)</td>
<td>4/9 (44%)</td>
<td>3/8 (38%)</td>
</tr>
<tr>
<td>Non-diabetics with arteriosclerosis</td>
<td>13/88 (15%)</td>
<td>6/71 (9%)</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Thromboangiitis obliterans</td>
<td>8/1/8 (13%)</td>
<td>0/4 (0%)</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>Osseous sarcoid of lower extremity</td>
<td>1/6 (17%)</td>
<td>0/6 (0%)</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>Soft tissue of lower extremity</td>
<td>1/6 (17%)</td>
<td>0/6 (0%)</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>Total</td>
<td>29/186 (15%)</td>
<td>11/186 (6%)</td>
<td>145/180 (78%)</td>
</tr>
</tbody>
</table>

*There were five cases of atheromatous embolism, three in the diabetic group and two among nondiabetic subjects.
tremities. Hemodynamic lesions were encountered most often (78 per cent). They were most common in the nondiabetic group with arteriosclerosis and were next most frequent in the diabetic group with arteriosclerosis, and in the group with a previous diagnosis of thromboangiitis obliterans. They were least frequent in the other three categories. Proliferative endothelial lesions were found in slightly over one half of the cases; they were most frequent in the diabetic group (80 per cent) and in the group with osteogenic sarcoma (88 per cent), and only slightly less frequent in cases with a prior diagnosis of Buerger's disease, osteomyelitis, or frostbite. Proliferative lesions of small arteries and veins were found least often in nondiabetic subjects with arteriosclerosis.

**Immunopathology of Small Vessels**

Binding of fluorescein-conjugated insulin was found in 70 per cent of cases with a proliferative angiopathy and arteriosclerosis which were tested; the same reaction was noted in 30 per cent of 10 diabetic subjects with arteriosclerosis but without proliferative lesions of small vessels. Thus insulin binding of small vessels was found in about 54 per cent of 26 diabetic subjects. The same reaction was observed in only about 15 per cent of nondiabetic subjects with a variety of other diseases involving the vascular system. In all of the cases showing fluorescent insulin binding, this reaction could be inhibited by prior immersion of sections in nonfluorescent insulin.

Fluorescent insulin binding was thus noted in a few otherwise normal vessels, in some tabulated as hyaline (hemodynamic) lesions, and most often in vessels with endothelial proliferation. The corresponding vessels as seen by conventional light and fluorescence microscopy are shown in figures 1 to 6.

In 56 per cent of diabetic subjects small vessels bound fluorescent RAHG. This same reaction was found in 22 per cent of nondiabetic subjects with a variety of other diseases involving the vascular system. In none of the

<table>
<thead>
<tr>
<th>Clinical disease</th>
<th>FL conjug. insulin</th>
<th>FL conjug. RAHG*</th>
<th>FL conjug. snake venom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes with arteriosclerosis and prolif. microangiopathy</td>
<td>11/16 (70%)</td>
<td>10/16 (63%)</td>
<td>0/11</td>
</tr>
<tr>
<td>Diabetes with arteriosclerosis and no prolif. microangiopathy</td>
<td>3/10 (30%)</td>
<td>12/23 (52%)</td>
<td>0/5</td>
</tr>
<tr>
<td>Nondiabetic with arteriosclerosis and prolif. microangiopathy</td>
<td>2/14 (14%)</td>
<td>3/10 (30%)</td>
<td>0/5</td>
</tr>
<tr>
<td>Nondiabetic with arteriosclerosis and no prolif. microangiopathy</td>
<td>5/30 (17%)</td>
<td>3/9 (33%)</td>
<td>0/6</td>
</tr>
<tr>
<td>Other diseases†</td>
<td>2/15 (13%)</td>
<td>3/12 (25%)</td>
<td>0/14</td>
</tr>
<tr>
<td>Total</td>
<td>23/85 (27%)</td>
<td>31/70 (44%)</td>
<td>0/41</td>
</tr>
</tbody>
</table>

*RAHG = Rabbit antihuman globulin.
†These include thromboangiitis obliterans, osteogenic sarcoma, osteomyelitis, and frostbite as listed in Table 1.

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**Table 2**

*Immunopathologic Analysis of Microangiopathies of the Lower Extremity*

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![Figure 1](http://circ.ahajournals.org/)

_Figure 1_

*Normal small artery in subcutaneous adipose tissue of a patient with arteriosclerosis and diabetes. Hematoxylin-eosin stain; magnification approximately 500 X. Note flat endothelial cells lining the internal surface of the wall, and sparsity of nuclei throughout the media and adventitia.*

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cases tested was there positive binding of fluorescein-conjugated snake-venom protein.

Discussion

As in previous similar studies in other organs,9–13 the present data show no significant differences between various disease categories in respect to inflammatory, thrombotic, or atheromatous lesions of small arteries and veins of the dermis, subcutaneous fat, muscle, or vasa vasorum. The frequency of hemodynamic lesions, as might be expected, is high in extremities with advanced sclero-

Figure 2
Normal small artery from same patient as in preceding figure. Section was stained with fluorescein-conjugated insulin and photographed with ultraviolet light; approximately 500×. Note fluorescence of cytoplasm and cell membrane of two endothelial cells; dark centers in these cells represent nuclei. There are two concentric circles of less intense fluorescence, the inner probably representing basement membrane of endothelium and the outer a basement-membrane layer separating media from adventitia. Outer intensely fluorescent irregular masses are artifacts.

Figure 3
Proliferative vascular lesion of subcutaneous tissue of a patient with arteriosclerosis and diabetes. Hematoxylin-eosin stain; approximately 500×. Note enlargement of endothelial cells and nuclei lining the lumen as compared with figure 1. There is also an increase in size and number of nuclei and cells in the remainder of the wall.

Figure 4
Proliferative vascular lesion from same patient as in figure 3. Section stained with fluorescein-conjugated insulin and photographed with ultraviolet light; approximately 250×. Note network of fluorescent material throughout the wall.

Figure 5
Proliferative and hyalinizing lesions of subcutaneous adipose tissue of a patient with arteriosclerosis and diabetes. Hematoxylin-eosin stain; approximately 500×. The upper vessel shows an enlargement of endothelial lining cells similar to that in figure 3. There are, however, fewer medial cells, and some degree of hyalinization has taken place. The lower vessel shows more marked hyalinization of the wall, and the endothelial nuclei are considerably reduced in size.
sis of cognate system arteries with accompanying gangrene, and in thromboangiitis obliterans. Hemodynamic lesions of small vessels are those in which the most prominent changes consist of thickening of the intima by fibrocollagenous tissue with some associated elastosis and hyalinization of the media; lipid deposition is not a prominent feature in these small vessels although they probably contain some lipid and lipoprotein. Also as in previous studies diabetic subjects with arteriosclerosis show a frequency of endothelial proliferative lesions of about 80 per cent as compared with about 20 per cent in nondiabetic arteriosclerotic subjects. On the other hand, similar proliferative lesions were found with an almost equally high frequency as in diabetics, in cases of Buerger’s disease, osteogenic sarcoma, osteomyelitis, and frostbite.

The binding of fluorescein-conjugated insulin was observed in slightly over half of the diabetic subjects, and with a frequency of about 70 per cent in diabetic subjects with proliferative angiopathy. This is somewhat lower than that found in the kidney and pancreas, in which similar immunofluorescent studies have been carried out. On the other hand, such insulin binding was also found in about 15 per cent of nondiabetic subjects. Of the nine cases in the latter group, seven were patients with advanced arteriosclerosis and gangrene and two had been diagnosed as Buerger’s disease.

These results can be grouped into several categories. In the first, there are diabetic subjects in relatively small percentage who show neither proliferative vascular lesions nor fluorescent insulin or RAHG-binding reactions. Presumably these individuals have developed arteriosclerotic gangrene similar to that present in a majority of nondiabetic subjects. A second group, consisting of nondiabetic individuals with various nonarteriosclerotic forms of peripheral vascular disease as well as with arteriosclerosis, show insulin binding in small percentage and RAHG binding in higher percentage; some of these show a proliferative angiopathy, but others do not. Those that show both insulin and RAHG binding are discussed below. Those that show only RAHG binding might have developed peripheral vascular disease on some immunologic basis other than insulin sensitivity (see below). It is also possible that this may represent an autoimmune response to necrosis of vascular wall analogous to the autoimmunity to myocardial muscle protein following myocardial infarction. In this regard it is noteworthy that Pokorny and Jezkova have found antibodies against antigens from normal and arteriosclerotic arteries in patients with peripheral obliterating vascular disease. Until the specific antigens that may be responsible for the globulin-binding reaction can be detected, it will not be possible to resolve this problem. Yet it may hold the key which may resolve the controversy regarding thromboangiitis obliterans.

In the case of peripheral vascular disease most often associated with diabetes, there is some indication as to the antigen that may be involved. Recently, we have reviewed evidence showing that there is a progressive deterioration in glucose tolerance with age, and have presented a concept of maturity onset diabetes as due to an age-related autoimmune reaction to an altered insulin; the reactions described here in small vessels are one
component. If this concept is a valid one, then it is to be expected that the frequency of insulin binding in tissues should increase with age even in some individuals who do not manifest the biochemical abnormalities of diabetes. Moreover, if such binding reactions are related to an autoimmune state, it should be possible to show a progressive increase with age in the frequency of insulin antibodies.

It has been reported that retinal vascular aneurysms, neuropathy of the type seen in diabetes, and nodular glomerulosclerosis believed to be characteristic of diabetes all may antedate the onset of hyperglycemia and glycosuria. This may also be the case in respect to hyalinization of the islets of Langerhans found in so-called nondiabetic subjects. In regard to peripheral gangrene, Lukens has noted that arteriosclerosis of the arteries of the feet may also appear before glycosuria, and is most common at an age when diabetes is most likely to occur. Moreover, Bartels and Rullo have studied a group of 100 patients with evidence of peripheral vascular disease of the lower extremities in whom previous diabetes mellitus had not been suspected; 20 per cent of these patients had definite evidence of diabetes mellitus, and "only 23 per cent of the entire group showed normal results on all aspects of the glucose tolerance test." The latter observations suggest that, in any large group of so-called nondiabetic individuals with arteriosclerotic gangrene, there may in fact be a considerable proportion of frank or borderline diabetic subjects.

Two studies utilizing a complement-consumption procedure for insulin antibody show a considerable percentage of positive tests in nondiabetic and in diabetic subjects who have never received exogenous insulin, thus eliminating the possibility of an induced insulin immunity. Despite such results in their own studies, Chetty and Watson believe that positive tests in these groups may represent insulin binding by nonimmune globulins. Such an interpretation appears highly unlikely in view of the fact that this procedure measures the fixation of complement.

The significance of vascular lesions characterized by proliferation of endothelial lining cells is also noteworthy. The occurrence of this lesion in association with diabetes has been confirmed by some investigators, whereas some reports have noted a failure to find such lesions. In some reports lesions are illustrated that we would consider as manifesting endothelial proliferation although the authors make no mention of this; one of these is in association with amyloid in a pancreas containing an islet-cell adenoma. Several investigators have particularly stressed basement-membrane thickening of small vessels and one report notes enlargement of pericytes. Although all these lesions may be associated with peripheral vascular disease in diabetes, it has not been established that any are "specific" in the sense that they occur only in this disease. This is also true of the endothelial proliferative lesion, which occurs in about 20 per cent of nondiabetic subjects. The latter lesion is considered by us to be associated with immune reactions of many types, and it is possible that some endothelial cells retain the capability of their more undifferentiated antecedents to synthesize antibody. The binding of insulin and antihuman globulin by some endothelial cells of otherwise normal vessels (fig. 2) may represent the earliest stage of the proliferative lesion; this reaction is similar to that noted in plasma cells in other immune states. Although not conclusive, this observation is compatible with antibody synthesis by some vascular endothelial cells. An alternate possibility is that these cells take up antibody from the circulating blood. The binding of fluorescent insulin and RAHG by some hyalinized vessels (fig. 6) is similar to that observed in pancreas and kidney. Initially there is an increase in PAS-positive basement-membrane material, and much of the media may later show this same histochemical reaction; at the latter stage fluorescent insulin binding of the vessel wall is most intense. Later collagenization of the lesion occurs and both the PAS and insulin-binding reactions fade out. A similar sequence of events has been noted in the hyaline nodules of the glomerulus.
The reported enlargement of pericytes in the peripheral vessels of diabetic subjects also bears on this problem. No data are given as to the occurrence of similar lesions in other forms of peripheral vascular disease. The enlarged pericyte, as with other vascular lesions in diabetes, is associated with an increased deposition of basement-membrane material. While pericytic enlargement and endothelial proliferation may both occur in diabetes, it would appear most likely that the increased synthesis of basement-membrane material would be linked with an increased activity of endothelial cells. Moreover, Cogan et al. have noted that in the vessels of the retina in diabetic subjects at the stage when endothelial proliferation of vessels is most marked and retinal vascular aneurysms are developing, the pericytes or mural cells are disappearing. While it is possible that retinal and peripheral vessels differ in regard to these reactions, this would appear unlikely.

Particular emphasis has been placed here on the nonarteriosclerotic component of peripheral vascular disease in diabetes because this appears to have been studied most intensively. Nevertheless, certain of these findings may apply, in principle, to other forms of peripheral vascular disease. It is not the aim of this report to present evidence supporting or negating the validity of thromboangiitis obliterans as a disease entity; no doubt some cases with such a diagnosis have been in error. Our primary objective, rather, has been to present evidence which supports the occurrence of immune reactions in some types of peripheral vascular disease, and to point out that such immune reactions may have etiologic or pathogenetic import. From a statistical standpoint, it would appear likely that the peripheral vascular manifestations associated with diabetes may be the most common form of such disease. It is also possible that some cases diagnosed as Buerger's disease fall into this category, although two cases in the present series with such a clinical diagnosis showed both insulin and antihuman globulin binding, and these may have been cases of pre- or potential diabetes.

On the other hand, it would also not appear valid to conclude that because large cognate system arteries contain atheromatous deposits, such lesions are always the result of faulty lipid or lipoprotein metabolism. Lesions of immune origin of the types discussed here may cause an increased peripheral resistance and intensified arteriosclerosis of trunk arteries. When such lesions occur in the vasa vasorum they may cause impairment of local nutrition of the wall of such arteries leading to the formation of atheromatous plaques. Both of these effects can contribute to sclerotic disease of cognate system arteries even in the absence of demonstrable abnormalities in lipid metabolism.

Summary

A histopathologic and immunopathologic study of changes in small vessels has been carried out on 168 amputated lower extremities. No difference was found in respect to the frequency of thrombotic or atheromatous lesions among the various clinical disease categories necessitating amputation. An increased frequency of inflammatory lesions of small vessels was found only in cases of osteomyelitis. The frequency of hemodynamic lesions was highest in nondiabetic subjects with arteriosclerosis, and second highest in diabetic subjects with arteriosclerosis, followed by thromboangiitis obliterans. Proliferative endothelial lesions were encountered most frequently in diabetes with arteriosclerosis, in osteogenic sarcoma, and in osteomyelitis. Such proliferative lesions were encountered in 80 per cent of diabetic subjects and in 20 per cent of nondiabetic individuals.

Fluorescent insulin binding by small vessels was found in 70 per cent of diabetic subjects with proliferative vascular lesions and in only 30 per cent of diabetic individuals without this proliferative microangiopathy. Such insulin binding was observed in 54 per cent of diabetic subjects and in only about 15 per cent of nondiabetic patients. The binding of fluorescent rabbit antihuman globulin was observed in 56 per cent of diabetic subjects and in 22 per cent of nondiabetic patients.
These findings are discussed in relation to the thesis that there may be diseases of immune origin primarily involving small peripheral vessels. The peripheral vascular disease of diabetes is particularly stressed here because it is probably the most common form of such disease of immune origin. Such pathologic processes in small vessels may secondarily involve large cognate system arteries, either by creating an increased peripheral resistance to the flow of blood or when lesions involve the vasa vasorum, there may be impairment of local nutrition. Either or both of these phenomena could intensify the development of arteriosclerosis of large trunk arteries.

Although no attempt has been made here to justify or negate the existence of thromboangiitis obliterans as a disease entity, it has been pointed out that at least some cases with this diagnosis may have a similar immune origin.

References

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