Basement Membrane Changes in Myocardial and Skeletal Muscle Capillaries in Myxedema

By P. M. McFadden, B.S., and G. S. Berenson, M.D.

SUMMARY

Nineteen dogs were given $^{131}$I and later administered appropriate amounts of methimazole or propylthiouracil daily. Over a period of 4–7 years several developed severe clinical features of myxedema. The myocardium and skeletal muscle of these severely myxedematous dogs displayed an array of morphologic changes on an ultrastructural level. Particularly interesting was the two- to threefold increase in the thickness of the myxedematous capillary basement membranes over those of normals. Increased tortuosity, lamination, and perivascular fibrosis were also observed as part of the myxedematous changes. These studies also demonstrated severe microcellular modifications, such as mitochondrial disruption, loss of cristae, lipid inclusions, and the presence of myelin figures, all a part of the diffuse systemic cardiovascular connective tissue pathology in myxedema.

Additional Indexing Words:
Hypothyroidism Cardiovascular connective tissue Electron microscopy

ALTHOUGH CLINICAL experience and pathologic observations indicate that severe cardiovascular changes develop in hypothyroidism, the precise nature of the cardiovascular lesions has yet to be clearly defined. 1,2 Diffuse alterations, such as interstitial edema, mucoid infiltration, and patchy fibrosis, are the most commonly reported findings in the myocardium and skeletal muscle of myxedematous patients. 3–5 These changes, however, are nonspecific for myxedema, and much controversy still exists over what histopathologic findings are consistently associated with this disease. Because of the success of replacement therapy and the difficulty of obtaining cardiac and skeletal muscle tissue from patients dying with uncomplicated myxedema, an experimental model of myxedema in dogs was chosen for study. An objective of the study was to determine what specific morphologic changes occur at an ultrastructural level. Rather prominent were the findings noted in the basement membranes of myocardial and skeletal muscle capillaries.

Materials and Methods

Nineteen mongrel male dogs were prepared according to the method described by Lippincott, Lewallen, and Shellabarger. 6 The dogs were starved for 1 week and administered 5 mc of $^{131}$I. Later, they were placed on a daily diet containing 100 mg of powdered methimazole (Tapazole). Methimazole was discontinued after 2 years and administration of propylthiouracil at 100 mg/kg of body weight was initiated.

Although most of the dogs developed metabolic evidence of hypothyroidism with an increase of serum cholesterol, over a period of 4–7 years only a few developed severe myxedema. Tissues for study were obtained at necropsy from the ventricular myocardium and pectoralis muscles of three of the myxedematous dogs and, similarly, tissues were also obtained from four normal dogs as controls. Tissues serving as controls were obtained from normal adult, male animals being

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maintained in a similar manner except for shorter periods and without administration of drugs. The tissue samples were placed immediately in 6% glutaraldehyde, washed with Millonig's buffer, and fixed in a 50:50 mixture of 2% OsO₄ and double-strength Millonig's buffer. The fixed tissues were dehydrated in graded alcohols and propylene oxide, embedded in Araldite resin, and stored 5 days in a 60°C oven.

Thin sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome, mounted on uncoated copper grids, and stained with uranyl acetate followed by lead citrate. Sections were studied with a Picker AE EM 6B electron microscope at 60 kv.

Photographs were made of the first 10 capillaries encountered that could be included in the frame at a single tap setting and magnification of 7,500. A transparent plastic grid consisting of 20 equidistant radiating lines, similar to that proposed by Siperstein, Unger, and Madison, was employed to obtain average widths of capillary basement membranes. The basement membrane was defined as consisting of both the outer lamina densa and the inner lamina lucida when observed under the electron microscope. Individual measurements were made with a 7x Bausch and Lomb hand objective. The pictures were discarded when less than 15 measurements were obtained per capillary. This procedure provided between 181 and 197 measurements on each tissue studied.

Results

Myocardial Capillaries

Under the electron microscope, normal myocardial capillary basement membranes

Figure 1

Electron micrograph of a normal dog myocardial capillary. The capillary lumen (L) is surrounded by a single endothelial cell (E). A thin, uniform, nontortuous basement membrane (BM), with a well-defined outer lamina densa and inner lamina lucida, surrounds the capillary. The pericapillary space (S), bounded by myocardium (M), contains sparse amounts of collagen and extracellular debris. Original magnification, X 7,500.
(fig. 1) appeared as relatively thin, nontortuous, filamentous structures of rather constant widths. The outer lamina densa and inner lamina lucida of the basement membranes were well defined. The pericapillary space was relatively "clean" and contained only sparse amounts of collagen and other extracellular debris.

In contrast to the normal findings, the myocardial capillary basement membranes of myxedematous dogs (fig. 2) were thickened, often tortuous, and quite variable in width. The inner zona lucida was often absent with only a thick, dense, filamentous zone comprising the membrane. Occasionally the membranes appeared laminated, similar to those commonly observed in skin capillaries and in diabetes mellitus. The pericapillary spaces were not as clear as those of controls and contained large amounts of collagen and other extracellular material. A schematic representation of the observations made on myocardial capillary basement membranes is shown in figure 3, and a scattergram of the actual

![Figure 2](attachment:image.png)

**Figure 2**

Electron micrograph of a myocardial capillary from a myxedematous dog. A red blood corpuscle (R) lies in the capillary lumen, surrounded by an indistinct endothelium (E). A thickened, tortuous, laminated basement membrane (BM) surrounds the capillary. There is no evidence of an inner lamina lucida in the membrane. A muscle cell (M) appears adjacent to the capillary. These changes are typical of those observed with the severe myxedema, although this capillary was selected to illustrate clearly the reduplication and lamination. Original magnification, × 7,500.
Changes in capillaries in myxedema

Measurements of these membranes is illustrated in figure 4.

Skeletal Muscle Capillaries

Findings not unlike those in the myocardium were observed in the basement membranes of skeletal muscle capillaries. Myxedema-involved basement membranes of skeletal muscle capillaries were thickened and more tortuous than those of controls. Lamination of the membranes, like that observed in myxedematous myocardium, was essentially absent. Perivascular fibrosis, however, was much more extensive in the majority of skeletal muscle specimens studied. The scattergram showing measurements of the skeletal muscle basement membranes is shown in figure 5.

Plots of widths of normal basement membranes of both myocardial and skeletal muscle capillaries indicated structures which were rather constant in width. Little variability in the thickness of basement membranes between different capillaries of the same dog were also exhibited. In addition, the "average" basement membrane widths of different dogs, indicated by the inscribed horizontal lines, did not exhibit a great deal of variability.

Plots of the myxedematous capillary basement membrane widths, however, showed a great deal of variability. The basement membrane widths varied within each capillary, between capillaries of the same dog, and to a significant degree between each of the diseased dogs. The most significant observation was the increased thickening of the myxedematous membranes over the normals. Myocardial and skeletal muscle tissues from an animal, which received similar treatment to induce hypothyroidism but did not develop clinical evidence of myxedema, did not appear abnormal nor was thickening of basement membrane observed.

The average widths of basement membranes of all dogs studied for quantitative changes are shown in table 1. From these data it was determined that myxedematous dogs exhibit a significant increase in their capillary basement membrane widths over the control dogs in both myocardial ($P < 0.05$) and skeletal muscle ($P < 0.01$), as well as change in the structural organization. From these studies it was also clear that skeletal muscle capillary basement membranes were thicker.

![Scheme of Capillary Basement Membrane Change in Myxedema](http://circ.ahajournals.org/)

Figure 3

*Schematic appearance of myocardial capillaries of normal and myxedematous dogs under the electron microscope.*

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Myocardial Capillary Basement Membranes in Normal and Myxedematous Dogs

Figure 4

Representative scattergram of widths of myocardial capillary basement membranes in normal and myxedematous dogs. Each dot represents a single measurement of the membrane width. Horizontally inscribed lines represent average widths of basement membranes.

Discussion

Myxedema causes widespread alterations throughout the cardiovascular system involving even the ultrastructure. There appears to be an increased perivascular fibrosis, increased tortuosity, and a two- to threefold increase in the thickness of dog capillary basement membranes in this disease. It is very likely that similar capillary changes occur in patients with myxedema, and such changes have been suggested as a cause of increased capillary permeability. In contrast to the concept that much of the cardiovascular changes are reversible in the treatment of myxedema, these observations suggest that considerable focal fibrosis can result in a sort of noninflammatory repair. Certainly, considerable focal fibrosis occurs in the myocardium in this disease. Other microcellular modifications, such as mitochondrial disruption, loss of cristae, myelin figures, and accumulation of intracellular lipid inclusions, were also observed in each diseased heart studied.

Although the precise nature of the chemical changes in the myxedematous material is unknown, other studies have suggested an involvement of the carbohydrate-protein macromolecules, such as the acid mucopolysaccharides and glycoproteins. Further morphologic and chemical studies on the cardiovascular connective tissue involvement in this and other metabolic and endocrine diseases are needed. Because cardiovascular connective tissue is so diffuse and becomes involved in many metabolic disorders, intimate knowledge of its biochemical and morphologic alterations would be desirable.
CHANGES IN CAPILLARIES IN MYXEDEMA

Peripheral Muscle Capillary Basement Membranes
in Normal and Myxedematous Dogs

Figure 5
Representative scattergram of widths of skeletal muscle capillary basement membranes in normal and myxedematous dogs. Horizontally inscribed lines represent average widths of basement membranes.

in disease is essential in providing a rational method for altering the metabolism during treatment and prevention of persistent anatomic changes.

Table 1
Basement Membranes of Heart and Skeletal Muscle Capillaries (Ten Each) from Normal and Myxedematous Dogs

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Mean range from myocardium (Å)</th>
<th>Mean range from skeletal muscle (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>933</td>
<td>818-1076</td>
</tr>
<tr>
<td>2</td>
<td>1209</td>
<td>1084-1320</td>
</tr>
<tr>
<td>3</td>
<td>1440</td>
<td>1160-1840</td>
</tr>
<tr>
<td>4</td>
<td>1280</td>
<td>836-1573</td>
</tr>
<tr>
<td>Myxedematous dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2996</td>
<td>1902-4084</td>
</tr>
<tr>
<td>6</td>
<td>1649</td>
<td>800-2596</td>
</tr>
<tr>
<td>7</td>
<td>2724</td>
<td>1556-5080</td>
</tr>
</tbody>
</table>

*Tissue sample was lost due to inadequate preservation.
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