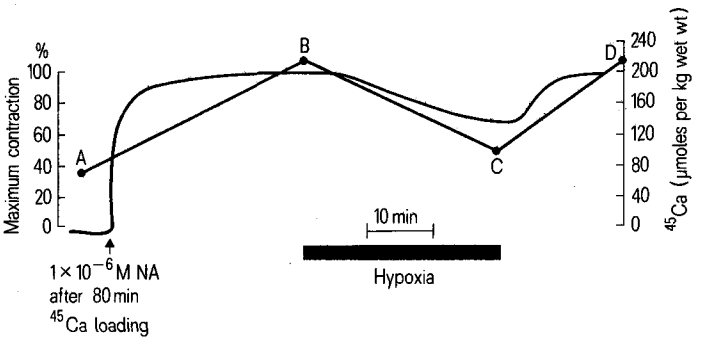

Figure 4. Relationship between ^{45}Ca uptake and typical mechanical activity of NA-stimulated rabbit aortic strips exposed to normoxic and hypoxic conditions. Each point is a mean of 7 determinations, n = 2.

for unlabelled Ca^{++}, rather than a real uptake. This possibility was however ruled out in experiments employing a preliminary loading period of 80 min. This duration was considered to be sufficient to allow complete labelling of all exchangeable Ca^{++}[17]. Using this procedure, NA-stimulated uptake was still observed to be less in hypoxia.

In this study, a net increase in ^{45}Ca uptake was observed on NA addition, as reported by other workers[18,19]. Hypoxic relaxation was associated with reduced uptake (fig. 4, C); this effect was, however, reversed by reoxygenation (fig. 4, D). Although no efflux studies were carried out, the reduced uptake in hypoxia (fig. 4, C) may be taken to reflect a loss of Ca^{++} accumulated during normoxic NA stimulation.

In conclusion, these results suggest that hypoxia depresses NA-induced contraction of rabbit aortic smooth muscle by interfering with Ca^{++} uptake.

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Silver labeling of vascular basement membranes in streptozotocin diabetic mice

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Summary. Diabetic and nondiabetic control mice were given silver by ingestion. The glomerular basement membrane was labeled with silver granules and the labeling was marked in the diabetic animals. The retinal capillaries failed to incorporate silver both in normal and diabetic animals.

The microangiopathy of diabetes mellitus affects the capillaries throughout the body. A prominent morphological change of the disease is the thickening of the basement membranes of the capillary walls[2]. The turn-over of these basement membranes has previously been investigated by silver labeling in vivo[3-6]. The aim of the present report was to compare the silver labeling of the vascular basement membranes of the renal glomeruli and the retinal capillaries in diabetic and nondiabetic mice.

Material and methods. 8 lean mice of the obese-hyperglycemic strain (gene symbol *ob*) were used[7]. At the age of 2 months, 4 of the animals were made diabetic by a single i.v. injection of streptozotocin (120 mg/kg b.wt). 4 control mice were treated with saline only. During the next 2 months all the mice were given free access to pelleted food and drinking water containing 0.25% silver nitrate. The animals were killed by decapitation and the retinas and sections from the kidneys were fixed for 30 min at 4 °C by immersion in a mixture of 2% glutaraldehyde and 1% formaldehyde in 0.15 M sodium cacodylate buffer at pH 7.4. The tissues were osmified, dehydrated and plastic embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

At the time of sacrifice, the serum glucose level of each animal was determined by a glucose oxidase method[8].

Results. The average serum glucose level was significantly higher ($p < 0.001$) in the diabetic mice (20.3 ± 1.2 mM) than in the controls (8.9 ± 0.9 mM). The diabetic animals were also lighter (28.3 ± 0.8 g) than the normal controls (30.5 ± 0.9 g).

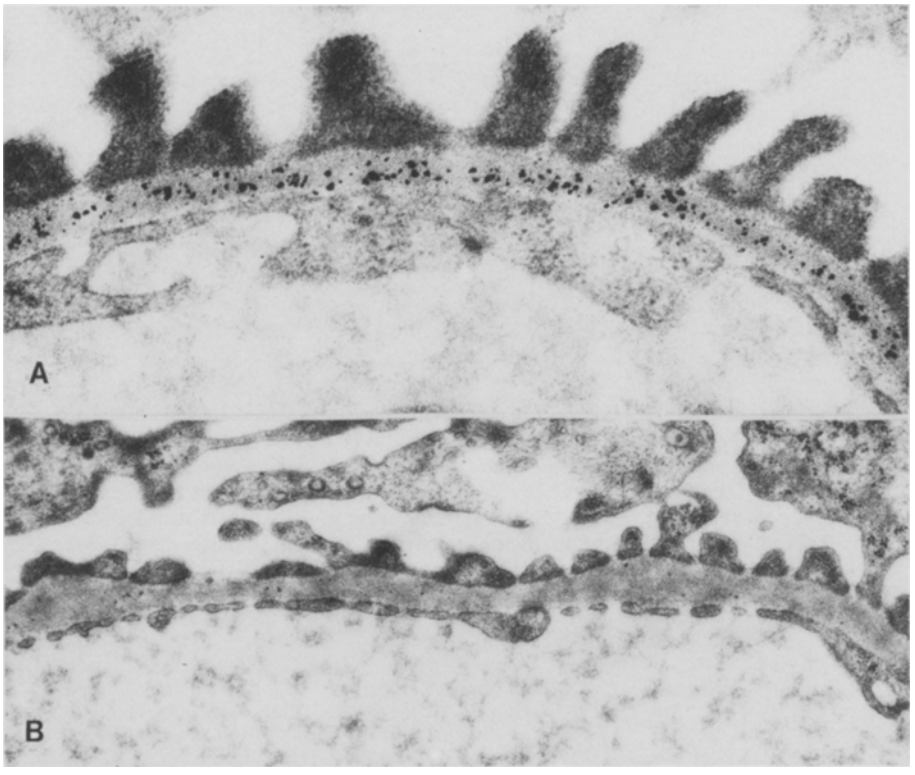

Figure 1. Electron micrographs of the glomerular basement membrane in a diabetic (A) and a nondiabetic mouse (B). The basement membrane displays silver granules in both cases but the granules in the diabetic mouse (A) are larger and more numerous than those of the control animal (B). A, × 40,000; B, × 19,270.

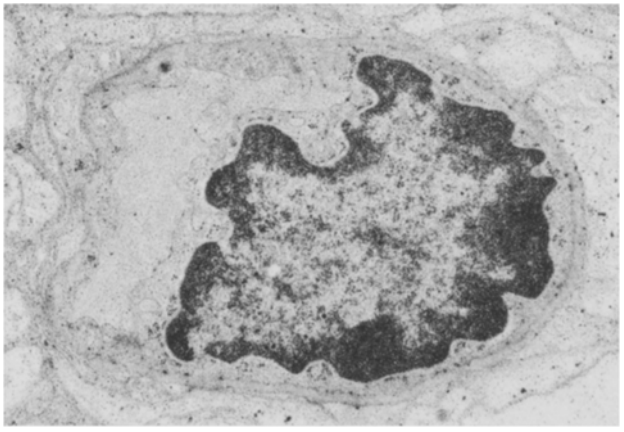

Figure 2. Electron micrograph of a retinal capillary in a diabetic mouse. The basement membrane shows no silver labeling. × 13,500.

The renal glomeruli of the diabeteic animals showed a marked deposition of silver granules throughout the basement membrane (fig. 1A). These basement membranes did not show any focal or diffuse thickening as compared to those of the control animals. The glomerular basement membrane of the control animals was also labeled with silver granules but these granules were fewer, considerably smaller and not lumped together as in the diabetic mice (fig. 1B).

The basement membranes of the retinal capillaries showed no labeling with silver granules in the diabetic (fig. 2) or in the control animals.

Discussion. In vivo, labeling of vascular basement membranes with silver granules was introduced by Kurtz and Feldman[3] for studies on the formation and turnover of the glomerular basement membrane. A marked granular labeling was found in the normal rat and the labeling was particularly intense in animals with chemical nephrosis or experimental glomerulonephritis[3–6]. In the present study, the glomerular basement membrane of the normal mice showed weak but constant labeling with fine silver granules. An intense labeling, with more coarse granules, was found in the diabetic animals. Such a marked labeling has also been found in the diabetic rat[10]. The differences in labeling between the normal and the diabetic mouse may be due to a difference in the chemical composition of the basement membrane[9,10]. The high rate of glomerular filtration in the diabetic animals may also be of importance as it exposes the basement membrane to increased amounts of silver.

The consistent lack of silver labeling of the basement membrane of the retinal capillaries was unexpected. It shows that the basement membrane of the renal glomerulus and the retinal capillary may differ both in the normal and in the diabetic mouse. It also shows that silver labeling as a method for studies on the capillary basement membrane has a limited value in some regions of the vascular bed.

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