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VASCULAR CASTING AND SCANNING ELECTRON MICROSCOPY IN DIABETES

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Abstract

Scanning electron microscopic (SEM) studies of the vascular casts of human eyes removed at autopsy from subjects with a long-standing history of Diabetes Type I were performed. Changes in the choroidal vasculature include: Venal focal dilations and narrowings, increased tortuosity, hypercellularity, increased formation of vascular loops and microaneurysms in choriocapillaries and formation of sinus-like structures between choroidal lobules. This study strongly supports earlier light microscopic and transmission electron microscopy observations on changes in the choroid. The retinal vascular changes shown by SEM are microaneurysm formation, drop-out of capillaries and neovascularization. Changes in the choroid, especially in diabetic choriocapillaries can be suspected to be a supporting factor in diabetic retinopathy by a decrease of oxygenation of the outer layers of the retina.

Key Words: Vascular casts, scanning electron microscopy, choroid, diabetes, microaneurysms

Introduction

Diabetes as a leading disease in human blindness is well recognized and literature regarding this topic is voluminous (3-8,12,17,18,21-23). Most of these reports concern changes in the diabetic retina, which are easily recognized by clinical studies (ophthalmoscopy, fluorescein angiography). Changes such as microaneurysm formation (closing vascular loops), drop-out of capillaries, neovascularization and retinal detachment are common in long-standing diabetes and different hypotheses have been developed to explain them (7,8,14-17,19,21,22). However, questions remain as to why, in many cases, changes in the retina occur and sometimes remain focal, when diabetes is a generalized vascular disease. Also, few studies have been done on changes in the human diabetic choroid (10,23). Even the choroid is well described elsewhere (20).

The present study was performed by using vascular casts and scanning electron microscopy (SEM). Many investigators have applied SEM techniques to elucidate complex anatomic relationships in the vascular of specific organ systems in animals (1,2,11,13,18). A similar injection technique has previously been applied to the study of the cerebral (2), gastrointestinal (2), renal (1), tracheal (13) and ocular vascular system in animals (18). However, this study represents the first application of our modified SEM technique (9) to systematic study of the human ocular vascular casts in health and disease.

Materials and Methods

Twenty eyes and the contents of the orbit from patients with long-standing Diabetes Type I were removed "en block" using a posterior approach at autopsy (2-12 h post mortem) Similarly, ten eyes from subjects with no known history of ocular or systemic vascular disease

were removed at autopsy and studied as the control group. A soft plastic catheter (16-22 GA Angiocatheter R) was inserted into the internal carotid artery, rather than the ophthalmic artery, to collect information from the vasculature along the entire length of the optic nerve and the globe. The vasculature was perfused with an anti-coagulant, heparinized saline (15 units/ml), to remove as many blood clots as possible. The plastic mixture used consisted of Batson 17 monomer base (25 ml), Batson B promoter (7.5 ml), Batson C catalyst (0.5 ml) and Sevitron (12 ml), and is suitable for capillary perfusion because of its low viscosity (approximately 20 centripoises). A fresh preparation of the cooled plastic mixture was gently manually injected (to avoid artifacts which usually occur when an electric pump is used). A volume of 7 ml (over 2-1/2 min) was injected until the material was observed to flow freely from the ophthalmic veins. The use of manual injection makes it possible to maintain and adjust the pressure when the resistance of the vessels increases. If this occurs, decreased injection pressure allows the plastic material to flow more smoothly and more gently through the capillary bed. The Batson mixture remains for several minutes unpolymerized inside the vessels so injection can be continued until the vessels are completely filled. The specimen was then immersed in warm water (50° C) for 4h prior to tissue digestion. Subsequently, the specimen was digested with 40% potassium hydroxide for 24 h ; following careful rinsing in a special sieve, the specimen was immersed in a 10% trypsin solution for three days. The specimen was washed daily in the same sieve with tap water and reimmersed in a fresh 10% trypsin solution. After the tissue had dissolved the vascular cast was inserted into a large glass container and dried with anhydrous calcium sulfate (Drierite) for four days. The whole vascular cast was mounted on a SEM stub with conductive silver paste and coated with gold-palladium to a thickness of 10nm (SEM coating Unit E5100). Low power photomicrographs of the intact vascular casts of the distal optic nerve and globe were taken at an accelerating voltage of 5 or 10 kV.

Next, the specimen was frozen (the Batson mixture is very brittle if not frozen) and dissected carefully while viewed through an operating microscope. Carefully labelled pieces of the choroid and retina were separately mounted on the stub and recoated with gold-palladium. All areas were examined by SEM from anterior (retinal) and posterior (scleral) views. The Diabetic vascular casts and the control group were studied separately and it was attempted to take photomicrographs from corresponding areas.

Results

Figures 1-4 show images of normal human choroid.

The anterior (Figures 1, 2, 3) and posterior view (Figure 4) present the choriocapillaries from the equatorial and midperipheral areas. In the equatorial area regular "lobular" patterns can be seen. In this area, regularly dilated structures were found between neighboring lobuli (Figure 2). Many of these structures present some annular constrictions suggesting the possibility of sphincters in the equatorial and midperipheral areas.

As shown in Figure 4, the posterior view of the venular drainage of the choriocapillaries presents multiple venules opening into a relatively small area, which facilitates venous outflow. A dense "lobular" choriocapillary network is present in this equatorial area.

Compared to the SEM image of the normal choroidal vasculature, one can recognize many changes that occur in the diabetic choroid. A dramatic increase in vessel tortuosity, dilations, outpouchings, looping and microaneurysm formation and finally, narrowing and drop-out of capillaries can be seen (Figures 5, 6 and 7).

The regular dilated structures which were observed between the normal lobuli in the equatorial area in long-standing diabetes change into sinus-like structures (Figure 8).

All of these changes were present on the venous side of the choroidal vasculature. In the retinal vasculature from diabetics, significant drop-out of capillaries, microaneurysms of varying stages of development, neovascular networks, and arteriovenous crossing phenomena were observed (Figure 9).

Discussion

Despite all objections to injection studies, coated vascular casts are the only presently known technique that allows studies in SEM of the three-dimensional aspects of vascular integration and continuity, and shows an overall view of large areas and details as well as focal vascular changes at high magnification. Vascular casts present replicas of blood stream and vessel walls seen "from inside", so that endothelial cells nucleus are seen as indentations and the vascular wall itself is digested. Also, occluded vessels will not be filled by injected plastic; on the other hand, some vessels which "in vivo", were eliminated from the blood flow because of vascular

constrictions can be filled post-mortem by plastic. Sometimes the injection pressure causes artifacts; however, with the Batson mixture this occurs rarely and can generally be avoided by appropriate adjustment of the manual injection pressure.

The retinal vascular pathology, which is clinically easy to observe, is well known to physicians.

Figure 9 shows three-dimensional images of drop-out capillaries, microaneurysm and arterio-venous crossings with filling defects, suspected to be replicas of plaques attached to the vein endothelium. If this is true, the relationship of the artery and vein in crossing can be important with regard to the development of bleeding or thrombi in the vein with all its clinical consequences. The most interesting of our findings are the vascular changes in the choroid in long-standing diabetes including dilations and narrowing, capillary drop-out, and microaneurysms formation. The observed significant narrowing of the vessels supports recent published findings by Hidayatt and Fine(10).In present study, all observed vascular choroidal changes in diabetes were recorded at the equatorial and mid-peripheral areas, and generally they were localized peripherally to those found in the retinal vessels. These findings have not been previously reported and require future investigations including regular histopathological sections, electron microscopy and SEM vascular casts studies.

The present findings in the choroidal vasculature in diabetes may contribute to additional points of view on diabetic changes. Since the outer retinal layers up to 130 μm (approximately) (16) are oxygenated and nourished from the choriocapillaries, changes in the choroid can induce hypoxia in these important and highly metabolic parts of the retina. In hypoxic tissue, karyolysis and karyorhexis in retinal capillary peritocytes can occur and result in the disappearance of peritocytes as well as capillary drop-out. Thickening of the basement membrane alteration of the aldose reductase inhibitor in the retinal capillaries in diabetes can also play a role in the disappearance of peritocytes. Next, prolonged retinal tissue oxygenation imbalance, vascular stretching of the endothelial cells and maybe release of the angiogenic factor causes outpouchings, microaneurysm formation and neovascularization. We speculate that most or all of these changes could be stopped by photocoagulation which increases oxygen tension in the retina by shifting oxygen back from the choroid and thus decreases retinal tissue oxygen demand by destroying part of the retinal cells, especially the mitochondria which are the major consumers of oxygen. In cases where after photocoagulation for some reasons retinal tissue oxygenation imbalance persist, next re-neovascularization can occur. All these speculations are very preliminary and need to be evaluated by future studies. However, the fact that diabetic choroid shows significant abnormalities suggests the possibility of choroidal participation in the diabetic vascular involvement of the eye. This topic was somewhat neglected in the past as stated by Hidayatt and Fine[10] and needs to be studied in the future.

Conclusion

The human ocular vascular cast study using SEM, showed significant abnormalities which occurred not only in the retina but also in the choroid of diabetics. The vascular changes which were found in the long-standing Diabetes Type I are consistent with vasodilation - oxygenation hypothesis (14, 19,22) of diabetic retinopathy and choroidopathy. These SEM studies of ocular vascular casts support light and transmission electron microscopy findings of choroidal vascular abnormalities (10).

Future studies on this topic which are undergoing now in our Department should bring reasonable explanation as to which factor(s) contribute to the focal changes in the choroid.

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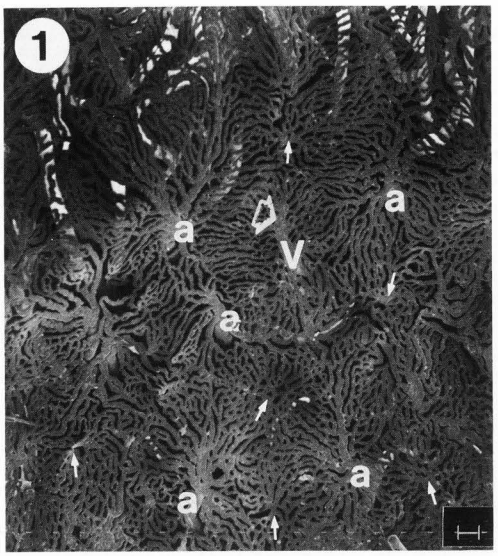
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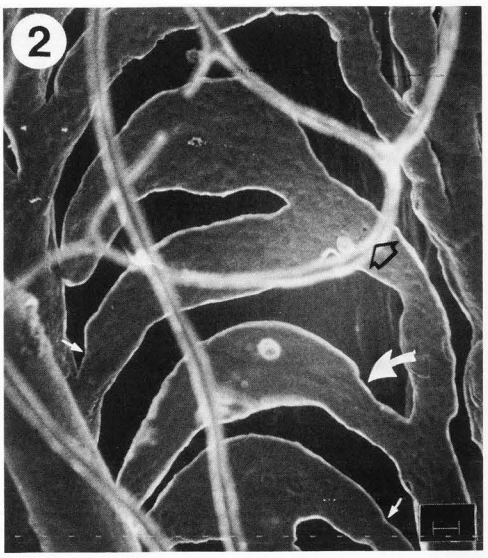
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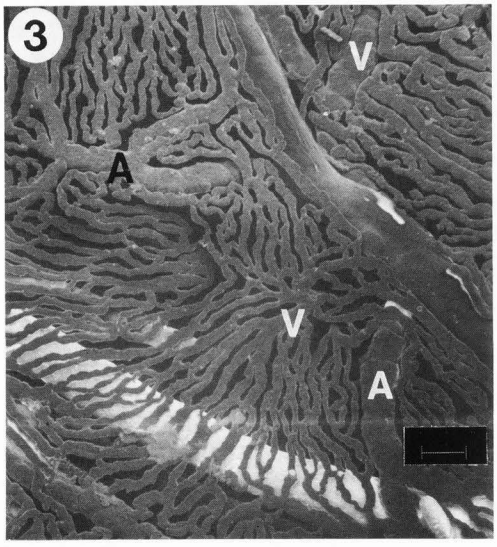
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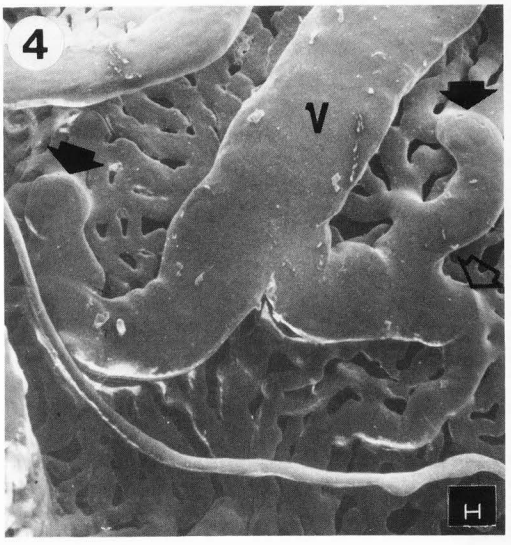
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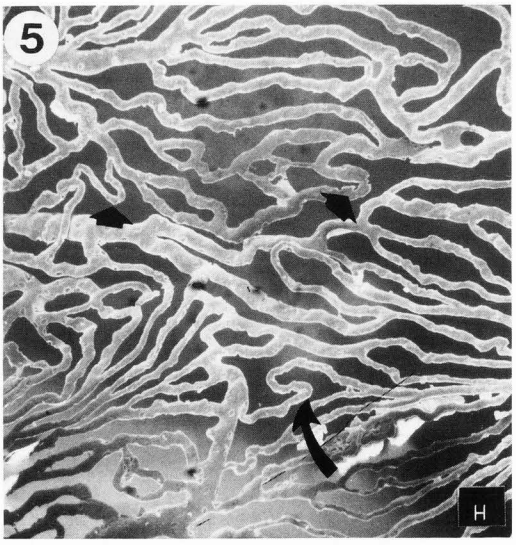
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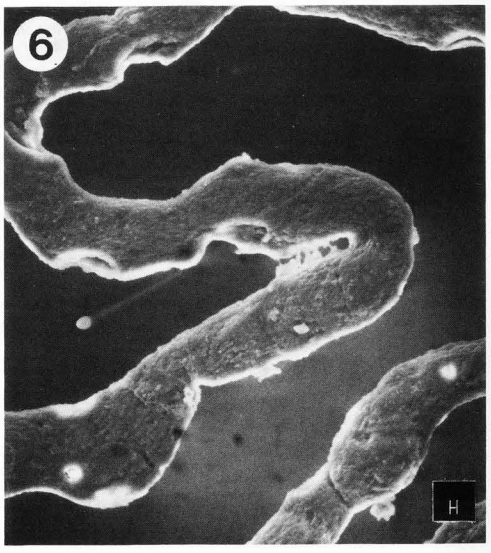
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Figure 1. SEM micrograph. 55 year old white male. Equatorial area. Anterior view. The lobular pattern of the choriocapillaries. Two main venular openings (V): "round" (solid arrow) and "in plane" of the choriocapillaries (open arrow) are present. Anteriolar openings (a) are mostly in plane of the choriocapillaries. Bar = 100 μm.

Figure 2. SEM micrograph. Equatorial and mid-peripheral area. Anterior view. Regular dilations between the lobuli with filling defects (solid arrows) which could represent constrictions on its borders. Retinal vessels out of focus (open arrow) are in front of the choriocapillaries. Bar = 10 μm.

Figure 3. SEM micrograph. 60 year old black female. Mid-peripheral choriocapillaries. Anterior view. Arterioles (A) and venules (V) are in one plane with choriocapillaries. Elongated pattern of the choriocapillaries. Bar = 100 μm.

Figure 4. SEM micrograph. Equatorial area. Posterior view. Collecting venule (V) with its round openings (solid arrows) and in the plane of the choriocapillaries (open arrow). Dramatic increase of venular diameter at short distance from the openings. Bar = 10 μm.

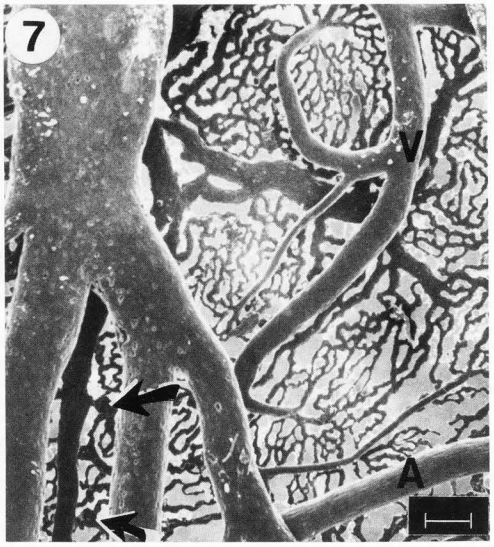
Figure 5. SEM micrograph. 68 year old white female with 20 year history of Diabetes Type I. Mid-periphery, anterior view. Narrowing of the capillaries, increase tortuosity, "looping" (arrows), outpouchings, hypercellularity and drop-out of choriocapillaries are seen. Bar = 10 μm.

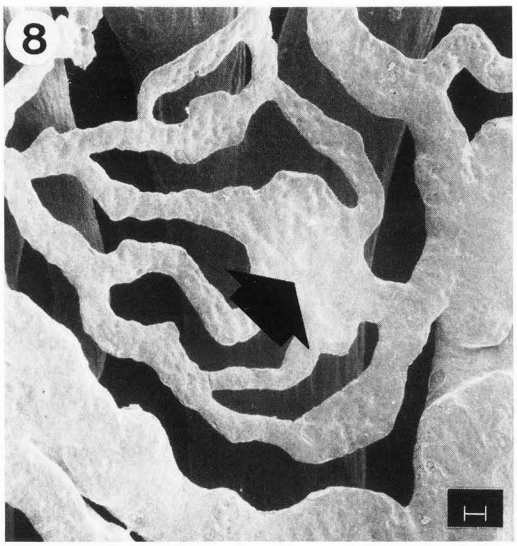
Figure 6. SEM micrograph. Same case as Figure 5. Mid-periphery. Anterior view. Higher magnification view of the choriocapillaries looping. Bar = 1 μm.

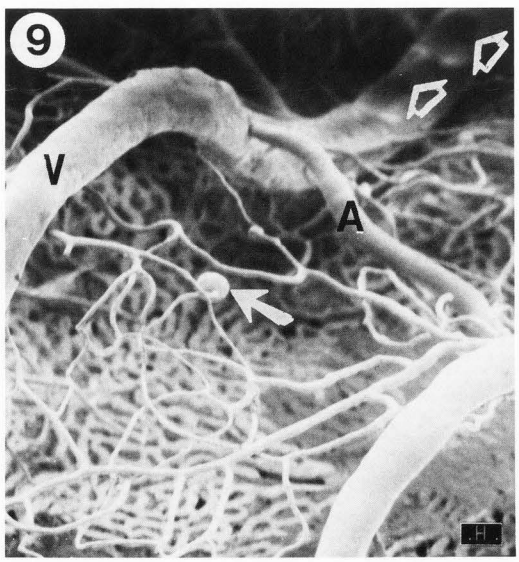
Figure 7. SEM micrograph. 62 year old black female with Diabetes Type I of 25 year duration. Equatorial area. Posterior view. Some drop-out of choriocapillaries from the lobuli, tortuosity, focal dilations, narrowing and outpouchings on venous side. Microaneurysms (arrows). Collecting venule (V), Arteiole (A). Bar = 100 μm.

Figure 8. SEM micrograph. 60 year old white male with Diabetes Type I of 28 year duration. Equatorial area. Anterior view. Sinus-like structure between the lobuli (arrow). Bar = 10 μm.

Figure 9. SEM micrograph. 56 year old black female with diabetes and hypertension of 22 year duration. Posterior pole. Anterior view. Changes in the retinal vessels arterio-venal crossing. Artery (A), Vein (V). Filling defects (open arrows) probably represent replicas of the plaques attached to the vein's endothelial side. Drop-out of the retinal capillaries. Microaneurysm (solid arrow). Bar = 10 μm.







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Editor's Note: All of the reviewers' concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.