Chorioretinal vascular occlusions with latex microspheres (a long-term study). Part II

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The results of a long-term study (3 to 9 months) of chorioretinal vascular occlusions resulting from injection of latex microspheres of known sizes are reported. Isolated occlusions of small retinal vessels (capillaries and precapillary arterioles) resulted in no histologic changes in the retina. Occlusion of large retinal vessels (arterioles) caused atrophic changes in the inner retinal layers (ganglion and bipolar cells). Flat preparations of the retina revealed no evidence of neovascularization or formation of new anastomotic vessels. Multiple occlusions of the choriocapillaris created atrophic changes in the external retinal layers (receptor cells and their nuclei). Occlusions of large choroidal vessels resulted in diffuse atrophy of all overlying retinal layers. Such occlusions also caused migration of pigment into the retina and formation of giant retinal cysts.

In a previous publication, the authors reported the first successful experimental attempt at isolated chorioretinal vascular occlusions of known size. That study, as well as later reports using similar techniques, dealt only with the acute and subacute changes following chorioretinal vascular occlusions. The purpose of this study was the investigation of long-term (three to nine months) lesions resulting from such occlusions. In addition, lesions resulting from occlusions of larger vessels are also reported.

Materials and methods

The same technique of injecting microspheres of known size into the retinal vessels of mongrel dogs was carried out as reported previously. The lesions were studied by ophthalmoscopic observations, fundus photography, and histologic preparations with serial section and flat preparation techniques. In the previous study, two groups of spheres 7 to 14 or 12 to 28 μ in size were used. In this study, these sizes, plus another group 25 to 50 μ in size, were utilized in separate experiments. A total of 20 dogs (40 eyes) surviving three to nine months after injection was studied.

Results

There was no essential difference in the lesions whether the animals survived three or nine months. The lesions found were more related to the size of the vessel occluded (arteriole versus capillary) and to the location of the occluded vessels (retinal versus choroidal).
Retinal vascular occlusions. In those areas of the retina in which only retinal vessels were occluded, two findings were evident:

1. The isolated occlusion of small retinal vessels (capillaries and precapillary arterioles) produced no detectable long-term histologic changes (Fig. 1).

2. Occlusion of a large retinal vessel (arteriole) resulted in atrophy of the inner retinal layers (ganglion and bipolar cells) in the area supplied by this occluded vessel (Fig. 2). These lesions had no apparent ophthalmoscopic counterpart. Flat retinal preparations of these atrophic areas showed nonpatent capillary strands which had lost both their mural and endothelial cells (Fig. 3, A). In no instance was there any neovascularization or evidence of attempts by remaining viable vasculature to form new anastomotic channels (Fig. 3, B).

Choroidal vascular occlusions. When choroidal vessels were occluded, the findings were as follows:

1. In occlusions involving only the choriocapillaris (whether the three or nine month eye), the histologic changes found were atrophy of the outer retinal layers (receptor cells and their nuclei) (Fig. 4A). Ophthalmoscopically, these lesions appeared as diffuse mottlings deep to the retinal vessels with ill-defined borders (Fig. 4, B and C).

2. In occlusions involving only large choroidal vessels, three salient changes were noted. (1) The immediate overlying retina showed complete atrophy of all layers. The retina was replaced by a thin gliotic membrane which was usually firmly adherent to the underlying choroid (Fig. 5, A and B).
Fig. 4A. Multiple occlusions of choriocapillaris by microspheres of 6 to 14 μ 8 months after injection. Note atrophy of outer retinal layers (receptor cells and their nuclei). (Hematoxylin and eosin.)

Fig. 4B. Ophthalmoscopic appearance of normal fundus in dog. C. Ophthalmoscopic appearance of lesions caused by multiple occlusions of the choriocapillaris with microspheres of 6 to 14 μ 6 months after injection. Note diffuse mottled lesions of retina lying deep to overlying retinal vessels.

B). Ophthalmoscopically, these lesions appeared as large punched-out areas deep to the retinal vessels with discrete, well-defined borders (Fig. 5C). (2) Around the periphery of the retinal atrophy, there was migration of pigment throughout all retinal layers (Fig. 6, A). In this zone, between normal and atrophic retina, the retina contained clumps of pigment debris and the underlying pigment epithelium showed a serrated inner border (Fig. 6, B). This migration of pigment was never found in areas of complete retinal atrophy. (3) In one eye with occlusion of the large choroidal vessel, the overlying atrophic retina extended to the ora serrata. In the center
Fig. 6. A, Histologic section showing migration of pigment in the periphery of an atrophic retinal lesion caused by occlusion of large choroidal vessels with microspheres of 25 to 50 μm 3 months after injection. B, Occlusion of large choroidal vessels by microspheres of 25 to 50 μm 3 months after injection. Note atrophic retina with underlying pigment epithelium showing a serrated inner border.

of this lesion, adjacent to the ora, the atrophic retina split into two layers, forming a large retinal cyst (Fig. 7, A, B, and C). In no other areas of this eye or the opposite undisturbed eye could such a large cyst be found on gross inspection or in serial sections.

Discussion

As noted in the previous report, isolated occlusions of small retinal vessels did not result in any histologic defect even though followed for nine months. Occlusion of large retinal vessels in the present study resulted in localized atrophic changes in the inner retinal layers. These lesions, containing atrophic nerve fibers and ganglion cells, may be the long-term result that in a more acute phase is represented as cytoid bodies. That the inner retinal layers were primarily involved is consistent with known concepts of the retinal blood supply. The fact that the flat preparations showed no evidence of neovascularization or formation of new anastomotic channels is contrary to the findings of other observers. The explanation of such a discrepancy is not well understood, but may represent a difference either in species or in ages of the animals.

Multiple occlusions of the choriocapillaris caused atrophic changes in the external
retinal layers. Thus the long-term effect showed no further pathologic changes over those produced by the more acute experiment. These findings again confirm the need of an intact choriocapillaris in order to maintain an adequate blood supply to the outer retinal layers.4, 6-10

However, the most interesting finding of the present study is the fact that ischemia from large choroidal vessels (if severe enough) results in diffuse atrophy of all overlying retinal layers. The fact that such changes were not found in our first study implies that several months are needed for such lesions to develop. Although complete retinal atrophy has been reported previously when the posterior ciliary arteries were ligated in the rabbit, this finding was open to interpretation, since the rabbit retina depends primarily on the choroidal vessels for its blood supply.11, 12 In addition, these studies did not distinguish between large and small choroidal vessels, nor did they rule out the effect of cutting ciliary nerves when the ciliary vessels were ligated. Of interest also is the fact that choroidal occlusion, without accompanying inflammatory response, can result in the migration of pigment into the retina just peripheral to the zone of complete atrophy.

These areas of complete retinal atrophy, surrounded by clumping of pigment, occurred only anterior to the equator. Thus the lesions created here by occlusion of large vessels in the choroid bear a striking histologic resemblance to lesions described as "paving stone degeneration" in human eyes.8, 10 And, although occlusion of the choriocapillaris has been suggested as the site of pathogenesis, this study provides the first experimental evidence that it is rather occlusion of large choroidal arterioles that leads to such changes.

The finding of a giant peripheral retinal cyst within an atrophic zone would seem to imply that occlusion of a large choroidal vessel may be a factor in its pathogenesis. Although peripheral cysts are frequently found in the aging dog's eye, the size and location of the cyst in the atrophic zone, as well as the lack of similar cystic changes in the same or fellow eye, argues against a coincidental occurrence.13

REFERENCES


Discussion

Paul Henkind. Once again the authors point up the value of using graded emboli to produce retinal and choroidal lesions. By using latex spheres they are able to cut cross-sections for histologic evaluation. Other material can be used, and we prefer to use glass emboli because of their easy visibility when they lodge in the retinal vascular tree. Goldor and Gay do not mention...
whether or not they are able to visualize latex particles in the living animal, and they have not reported any vascular flow changes following embolization. Their paper deals essentially with observations on the chronic effects of vascular occlusion.

Concerning the results and observations: The authors state that they were unable to find histologic changes in the retina after isolated occlusion of capillaries and precapillary arterioles. However, their Fig. 1, said to be free of pathology, appears to show granularity of the nerve fiber layer, absence of most ganglion cells, and thinning of the inner nuclear layer. It would be interesting to know whether these areas had ophthalmoscopically visible lesions at any time.

The lack of ophthalmoscopically visible changes in retinas with larger vascular occlusions and proved histologic lesions is puzzling. This may be accounted for at least partially by the use of indirect rather than direct ophthalmoscopy. Much more detail can be seen with the latter, and cats and pigs as well as dogs can be examined in this manner without recourse to anesthesia.

Neovascularization and anastomotic channels (collaterals) were not demonstrated in embolized retinas. The lack of neovascularization is understandable since it has never been demonstrated in mature animals but only in newborn cats with immature retinal vascularization. As regards the absence of collaterals, I can find little evidence that the authors can rule out such a possibility. Without in vivo dye studies or ink injected retinal preparations, it is difficult to state that such channels are absent. Furthermore, from the photographs the digest preparations appear somewhat overdigested. They also appear to depict an area near the retinal periphery (note the broad meshwork and sparsity of capillaries), a region where collaterals rarely develop.

Of major interest are the observations concerning the effects of choroidal occlusion on the overlying retina. They show, as one might expect, that localized occlusion of the choriocapillaris leads to degeneration of the outer portion of the adjacent retina. On the other hand, the occlusion of choroidal arteries seemed to cause a more extensive lesion that involved all retinal layers. We have noted the development of a clinical picture similar to that described by the authors within 11 days of embolization in the cat, but we have not studied these lesions in cross-section (see the accompanying Fig. 1). It is not clear from the paper how many of these lesions the authors produced and studied, and their illustrations are not completely in line with their assertion that there was total degeneration of all the overlying retinal layers (Fig. 6, B has a partially intact inner retinal structure; the serrated pigment epithelium is most likely artifact). Further they state that all lesions of complete retinal atrophy occurred only anterior to the equator, yet their clinical photograph of the condition (Fig. 5, C) seems to show an area at least partially posterior to the equator.

I should like to stress that arteries per se have little role in nourishing tissue, this is the province of capillaries and smaller caliber arterioles and venules. Blocking a choroidal artery leads to diminished or absent flow in a large segment of the choriocapillaris, this in spite of the fact that the choriocapillaris seems to be a continuous anastomotic network over the entire choroid.

Fig. 1. Fundus photograph of a cat taken 11 days after embolization with glass balls. The arrow heads point to areas which appear to be chorioretinal scars with sharply outlined borders. Hemorrhages and posterior polar retinal cysts are seen also.

Fig. 2A. India-inked specimen of the choriocapillar network in an adult cat. The arteries run as thick parallel lines above the capillaries. The venules have mainly been removed.
Blockage of choroidal arteries in the cat causes segmental defects in the capillary bed (see the accompanying Figs. 2A, 2B, and 2C).

If total degeneration of the retina does indeed occur following occlusion of a large area of the choriocapillaris it would imply that transneuronal degeneration must be occurring, since there is no evidence of degeneration secondary to inflammation. Before fully accepting the authors' findings I should like to be certain that retinal vessels were not embolized in the area of retinal atrophy. It would also be important to correlate the exact area of choroidal involvement with overlying retinal defects. This might be accomplished by using India ink preparations.

In conclusion I should like to thank the authors for allowing me to discuss their paper. I certainly feel that they should pursue the study of the effect of choroidal vascular disease upon the retina.

Reply

Andrew J. Gay, M.D. We would like to express our appreciation to Doctor Henkind for his critical discussion, and we shall attempt to answer his questions as completely as possible.

We have used latex microspheres primarily because of their availability through the courtesy of the Dow Chemical Company. However, the successful use of glass microspheres, as Doctor Henkind has done because of their ophthalmoscopic visibility, does not rule out employing latex particles, as they, too, are easily seen in vivo. Furthermore, the latex particles are easily sectioned, thereby permitting detailed histology; a contribution this paper makes that is not possible to obtain with the glass spheres.

The areas of sparse or single embolization of the retinal small vessels did not show any ophthalmoscopically visible changes. The lack of these ophthalmoscopic changes should not be surprising, in view of the normal histological appearance. However, the lack of a visible lesion when the large retinal vessels were occluded is more likely attributable to the long-term survival time (since lesions were visible earlier, as described in our previous paper) than to a discrepancy in resolution between the indirect and direct ophthalmoscope.

We agree with Doctor Henkind, and stated in the paper that the lack of neovascularization following occlusions is most likely attributable to our use of older, adult animals in contrast to his study of newborn kittens. However, we can only report the negative finding, and speculate about its causes.

The same argument applies to our not finding new anastomotic vessels developing in ischemic areas. I do not believe that in vivo dye studies are more conclusive than direct observation of ischemic areas by trypsin digestion. When one sees vessels carrying the dye in an area of previous ischemia, one can only speculate about its anastomotic characteristics, and whether it was...
previously there or not. We can only say that in digestion of these ischemic areas, months after the occlusion, no anastomotic channels or new-formed vessels were ever detected.

Our finding of complete retinal atrophy and peripheral pigment migration over an area of large vessel occlusion in the choroid was seen many times when the larger microspheres were used, and a long survival time was allowed. I believe Figure 5, A and B illustrate these findings very well. Doctor Henkind's reference to Figure 6, B as not illustrating complete retinal atrophy is quite true. However, as noted in the paper and figure legend, this animal survived only three months. Whether the shorter survival time or the smaller number of occluded vessels (as compared to Figure 5, A) was responsible for the lack of complete retinal atrophy, we cannot say. What is of importance is that such atrophy did occur, and was found repeatedly.

As to Doctor Henkind's concern about the retinal atrophy resulting from both retinal and choroidal occlusions in the same area, we can only repeat our statement in the paper that serial sections were done and carefully studied, and that, to the best of our ability, we could find no retinal vessel occlusion that we could logically associate with the area of retinal atrophy.

Again, may we thank Doctor Henkind for his very considered discussion.