Radial peripapillary capillaries

III. Their development in the cat

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In earlier papers (Henkind, 1967; Alterman and Henkind, 1968), we described the radial peripapillary capillaries (RPCs) in man and animals and their possible role in glaucomatous field defects. Recently, the entire subject of the radial capillaries in health and disease was examined in detail (Henkind, 1972; Shimizu, 1972). The development of the RPCs, however, has received no previous attention, and in this work we examine the growth and development of these vessels in the kitten.

Material and Methods

The retinæ of fourteen kittens ranging in age from less than 1 day to 8 weeks were Indian inked as previously described (Henkind, 1966). The enucleated globes from kittens less than 4 weeks of age were fixed in 5 per cent. neutral buffered formalin; older eyes were fixed in 10 per cent. neutral buffered formalin. After 2 to 5 days of fixation, the globes were washed overnight in running tap water and 25 of 28 eyes were coronally hemisected. The retinæ were dissected free, mounted on glass slides in Apathy's mounting medium, and examined by light microscopy. The left eyes from kittens aged 14, 21, and 28 days were processed for routine histological sectioning and sectioned at 9 μ . Sections were stained with haematoxylin and eosin, or haematoxylin and PAS, and studied by light microscopy.

All the specimens were examined independently by the three authors, and a quantitative judgment was made concerning the presence or absence of RPCs and their extent. Their relationship to underlying retinal vessels was also ascertained.

Results

Radial peripapillary capillaries were found in all specimens from kittens aged 25 days or more (Table). The 21-day specimen was considered to be the only poor preparation, and the absence of RPCs may have been spurious rather than real. None were seen in any specimen from kittens aged 18 days or younger. There was some correlation between the extent of the RPCs and the age of the kitten, especially in the older animals.

Not all regions of the embryonic retinal capillary bed matured at the same rate (Figs 1 and 2, overleaf) and the RPCs themselves showed no evidence of having an antecedent primitive meshwork.

Rather they appeared in a "mature" state (Fig. 3) without seemingly undergoing the remodelling process characteristic of the initial retinal vascular bed (Ashton, 1969; Wise, Dollery, and Henkind, 1971).

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FIG. 1 Photomicrograph of inked retinal whole mount, showing area of immature retinal vascular meshwork in 17-day-old kitten. $\times 112$

FIG. 2 Photomicrograph, showing area of more mature development of retinal vascular bed in same retina as Fig. 1. The deep capillary bed is out of focus and under-lies the superficial capillary bed. $\times 112$

FIG. 3 Photomicrograph, showing early presence of RPCs (arrows) in flat mount of retina from 25-day-old kitten. $\times 100$

Table Qualitative estimate of RPC presence

Age of kitten (days)	Observers			Note
	I	II	III	
less than 1	0	0	0	
7	0	0	0	
10	0	0	0	
14	0	0	0	Only right eye
17	0	0	0	
21	0	0	0	Only right eye
25	1+	2	2	
28	2+	2+	2	Only right eye
31	1+	1+	2	
35	1	1+	1+	
36	2	1	1+	
42	2	2	2+	
49	3	3	3	
56	3	3	3	

0 = no RPCs seen
1 = RPCs evident to a slight extent
2 = RPCs evident to a moderate extent
3 = RPCs fully developed when compared to mature

The deep capillary bed was first observed in the 17-day-old retina (Fig. 2) and it seemed to arise directly from the overlying initial retinal capillary bed. Like the RPCs, the deep capillary bed seemed to lack a primitive precursor phase and seemed to appear only as the initial primitive capillary meshwork approached maturity.

Communications were discerned between the RPCs and the optic nerve head capillaries, but the RPCs did not appear to derive from them. There were a few communications between adjacent RPCs, or between them and the underlying capillary plexus.

Histological sections (Fig. 4) of inked retinae confirmed the location of the three capillary layers demonstrated in the flat mounts (Fig. 5, *A, B, C*, overleaf).

FIG. 4 Photomicrograph of histological section of retina from 28-day-old kitten. Arrowhead shows RPC in nerve fibre layer; black arrow shows superficial capillary, inner plexiform layer; white bordered arrow shows deep capillary, outer plexiform layer. Indian ink and haematoxylin and eosin. $\times 330$

Discussion

Two observations made during this study require comment. First, the RPCs are a relatively late retinal vascular development in the cat, and secondly, their mode of development differs from that of the initial retinal capillary bed.

The initial vascular ingrowth into the foetal kitten retina has been demonstrated as early as the 45th day (Michaelson, 1954). The developmental process begins as an invasion of the retinal nerve fibre layer by a syncytium of primitive mesenchymal cells spreading centrifugally from the optic disc. Luminal formation, basement membrane production, and endothelial cell and intramural pericyte differentiation occur during the maturation process and these can all be studied by light and electron microscopy (Shakib and Oliveira, 1966).

Indian inking, as performed in the present experiment, only permits observation of the extent of luminal formation and the later phase of vascular remodelling. Using similar preparations, Michaelson (1954) demonstrated in the cat that the embryonic capillary network differed from the definitive (*i.e.* mature) network by having capillaries of greater diameter, smaller mesh size, and obvious luminal irregularity. By the first day after

FIG. 5

A. Photomicrograph of *intact* retinal whole mount from 35-day-old kitten, showing RPCs in focus. $\times 160$

B. Photomicrograph of *same* area, showing superficial capillaries in focus. $\times 160$

C. Photomicrograph of *same* area, showing deep capillaries in focus. $\times 160$

birth the capillaries around the disc have the appearance of the definitive network with narrower lumina and a broader meshwork. The first intimation we have noted of RPC presence was during the fourth post-natal week, by which time the initial network had achieved an almost mature appearance. These more superficially situated, elongated, straight channels with few intercommunications and of relatively constant diameter could be seen coursing in the superior and inferior temporal quadrants at the posterior pole. No irregular channels were seen at this location or level at an earlier stage of development. While this does not preclude the earlier presence of a primitive mesenchymal mesh or syncytium, we feel that the RPCs more likely develop from the underlying "superficial" retinal vascular bed, which by this time, is quite "mature".

The deeper retinal capillary network seems to develop in a similar fashion, for it too shows no early or "primitive" phase nor evidence of vascular remodelling. In our study, the deeper capillary network at the posterior pole was first seen in the 17-day-old retina. Similarly, Michaelson did not find evidence of a deeper capillary network in the 8-day-old kitten, even though the retina was vascularized almost to the periphery. He did, however, note a deeper capillary network in his 15-day-old preparation, and found that it was developing from the superficial net. If it is indeed true that the RPCs and the deeper capillary plexuses develop from a more mature system and not from an initial mesenchymal ingrowth, then we must look for the "factor(s)" causing such growth. Conceivably, such a factor would be different from that responsible for the earliest phase of retinal vascularization. If that were so, it would be interesting to know, for example, whether oxygen in high concentration would prevent the development of the RPCs and the deeper capillary bed as it does the "immature" retinal vessels. Presumably, some metabolic factor(s) related to retinal function induce the development of the RPCs and deeper capillary bed of the kitten retina. Only by a combined functional, biochemical, and ultrastructural approach are we likely to answer the question of what causes the RPCs to develop.

Summary

Indian-inked retinal flat mounts from kittens aged less than 1 day to 8 weeks were studied regarding the development of the radial peripapillary capillaries. RPCs were noted only in retinae of kittens more than 21 days old. They appeared to develop in a mature form in areas where the primitive vascular meshwork had already undergone maturation. A somewhat similar growth pattern of the deep retinal capillaries at the posterior pole was confirmed.

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