Development of the macular circulation

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The development of the primate retinal vasculature has been investigated in fetal monkeys (M. mulatta) during the latter half of gestation. Vascularization is found to be retarded in the region of the macula. The macula is found to become completely encircled by a network of primitive capillaries which then proliferate centripetally towards the presumptive fovea. These encircling vessels normally cease to proliferate before reaching the center of the fovea, and the resulting central avascular zone and centripetal pattern of vessels persist thereafter in the mature macula. The superficial peripapillary network of capillaries, like the outer vascular net, is found to be derived from the inner network of vessels.

Key words: retinal vessels, development, macula, fovea, fetus, primate.

At the macula of man and many other primates the retinal vasculature assumes a characteristic pattern. The thinness of the retina near the center of the macula is associated with local reduction of the vasculature to form a network of capillaries and small precapillary and post-capillary vessels which encircle a totally avascular zone, the center of the fovea. Arterioles and venules in the macula show a somewhat radial pattern about the fovea. Although the development of retinal vessels has been described a number of times in past years, the manner in which the macular vessel pattern arises has received little attention.

In order to investigate the development of the macular circulation, fetal eyes have been obtained by us at known durations of gestation from healthy rhesus monkeys, normal human fetuses of appropriate ages having proved to be not readily available. The results of the present study show that vascularization tends to be retarded locally in the region of the macula, and approaches the developing fovea as a progressively narrowing circle of vasoproliferation which normally fails to entirely cover the future site of the fovea.

Methods

Eyes were obtained from fetuses delivered by cesarian section of normal rhesus monkeys (Macaca mulatta) at precisely timed intervals of gestation. Such pregnancies were supervised and terminated in the course of other studies at the Wisconsin Regional Primate Research Center by Dr. George Kerr and co-workers. Through their kindness, eyes of 11 fetuses were available to us 75, 100, 125, 150, and 175 days after conception. Full-term gestation in rhesus monkeys is approximately 168 days; one animal was obtained at 175 days gestation, this being within a few hours after a normal vaginal delivery.
Table I. Retinal vascularization in rhesus monkeys

<table>
<thead>
<tr>
<th>Age of fetus</th>
<th>No. of fetuses</th>
<th>Observations</th>
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<tbody>
<tr>
<td>75 days</td>
<td>2</td>
<td>Vessels comprise a two-dimensional network in inner retina surrounding the optic disc; macula is avascular and not completely surrounded by vessels.</td>
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<tr>
<td>100 days</td>
<td>2</td>
<td>Macula is completely surrounded by vessels, leaving central 1 mm² avascular.</td>
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<tr>
<td>125 days</td>
<td>4</td>
<td>Foveal avascular zone approximates adult size (0.05 mm²) and inner retina thins at fovea; outer network and superficial peripapillary network of capillaries have appeared.</td>
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<tr>
<td>150 days</td>
<td>2</td>
<td>Avascularity and thinning of retina remain evident at fovea; retinal vascularization is nearly complete.</td>
</tr>
<tr>
<td>175 days</td>
<td>1</td>
<td>Immature &quot;chicken-wire&quot; pattern of capillaries has not yet completely disappeared.</td>
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Immediately upon delivery the fetuses were killed by decapitation or, in the case of a 175-day-old fetus, by overdosage with pentobarbital, and the eyes were removed and fixed in neutral formalin (10 per cent) or Heidenhain's Susa fluid. Formalin-fixed eyes were embedded for paraffin sectioning and staining or, alternatively, their retinas were digested with trypsin³ containing 0.2M sodium fluoride to inhibit occasional overdigestion of cell nuclei in vessels. Retinas from eyes fixed in Susa fluid, after conventional treatment with iodine and thiosulfate to remove mercury precipitates, were stained with a periodic acid—Schiff (PAS) reaction and were mounted flat, nerve fiber layer uppermost, on a glass slide. Background staining in whole mounts was inhibited by incubating retinas 1 hr. at room temperature in neutral 0.1M phosphate buffer containing 0.1M sodium fluoride (bacterial), after which the retinas were washed thoroughly in water and exposed to the PAS reagents. No reducing rinse was included in the PAS procedure. Background staining in whole mounts was inhibited by incubating retinas 1 hr. at room temperature in neutral 0.1M phosphate buffer containing 0.1M sodium fluoride (bacterial), after which the retinas were washed thoroughly in water and exposed to the PAS reagents. No reducing rinse was included in the PAS procedure. Since trypsin digestion, although revealing cytologic details of the vessels, often tends to disrupt the delicate proliferating capillaries (more so than mature vessels in which vascular basement membrane has developed), the following results have been derived chiefly from the stained whole mounts of retina.

Results

The development of the macular circulation is briefly outlined in Table I. The retinal vasculature of the youngest monkey fetuses studied (75 days gestation) is at an early stage of development and consists of a two-dimensional network of immature vessels located near the outer edge of the nerve fiber layer, and extending peripherad much farther into the superior and inferior quadrants than into the nasal and temporal. At this time the vascularized area thus presents a somewhat oval outline measuring about 8 by 2.5 mm. (vertical by horizontal), near the center of which is the optic nerve head. In the temporal quadrant the retina is found to be of greatest radius (8 mm.), whereas the radius of the vascularized area tends to be least in that quadrant (1 mm.), a circumstance which leaves the temporal retina disproportionately less vascularized than other quadrants. The principal arterial and venous vessels arise superiorly and inferiorly from the optic nerve head, and their branches tend to arch toward the superotemporal and inferotemporal retina, seemingly following the familiar arcuate pattern of nerve fibers there.

Between 75 and 100 days gestation the retina and its vasculature grow rapidly. The radius of the retina increases by roughly 50 per cent in all quadrants during this interval, and the growth of the vasculature is even more rapid, especially in the nasal and temporal quadrants where its radius increases fourfold to tenfold. The perimeter of the vasculature is now within 1 or 2 mm. of the ora serrata.

A remarkable modification of the vessel pattern is found to be emerging in the macular region of the 100 day fetus (Fig. 1). At the future site of the fovea, a large (1 mm²) area of retina is found to have remained avascular and is now surrounded on all sides by the immature vasculature. The delicate growing tips of the vessels encircling the presumptive fovea are seen to be directed centripetally, toward its center.

During the following 25 days, by 125 days gestation (Fig. 2), the vessel-free area at the fovea diminishes greatly (to about 0.05 mm²) due to continued cen-
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Fig. 1. Fetal macula, illustrating the centripetal proliferation of primitive capillaries towards the future site of the fovea. The central avascular zone is surrounded by budding capillaries and is about 1.8 x 1.1 mm. in size (the greater axis being naso-temporal). The retina has been incised and flattened for microscopy, and the inner limiting membrane, which in places has become folded, has been retained to assure that none of these delicate vessels have been artifactually lost during preparation. (T, Temporal; rhesus monkey, 100 days gestation; PAS-stained whole mount; magnification x52.)

tripetal proliferation of the converging capillaries. The center of the avascular zone, which is now approximately adult in area, contains no regressed vessels or other evidence that it might have become overgrown by the vasculature. A deep depression marking the center of the developing fovea has now appeared. Small arterioles and venules gradually have become differentiated from the primitive capillaries in the macular area, and accordingly appear also to converge upon the fovea.

Other major changes also have begun to appear in the vasculature at 125 days gestation. The previously two-dimensional network of vessels has now given rise to the outer network of capillary-sized vessels at the level of the inner nuclear layer, and to the superficial peripapillary network of capillaries near the inner surface of the nerve fiber layer. In the anterior half of the retina the primitive two-dimensional pattern is found to persist longer, but by 150 days gestation capillaries have penetrated into the inner nuclear layer throughout the retina. The single fetus examined at birth (175 days gestation) is found to have a vasculature which resembles that of adults except for the persistence of an immature “chicken-wire” pattern of the vessels in the peripheral retina. The area of the foveal avascular zone is found to be 0.04 to 0.05 mm.² by 150 to 175 days gestation, an area comparable to that observed (0.05 to 0.08 mm.²) in adult rhesus monkeys in this laboratory. The center of the zone appears never to have contained vessels.

Discussion

The absence of vessels from the adult fovea might be postulated as being due to
either of two developmental mechanisms. Embryonic vessels either fail to invade this region or, alternatively, they vascularize the region but subsequently atrophy and disappear from its center. In the mature eye, the foveal avascular zone occasionally is known to become pathologically enlarged through atrophy of the adjacent vessels, as occurs for example in senile macular degeneration or diabetic maculopathy. In the immature eye also, capillary atrophy or regression is a well-known phenomenon, and is an important means by which the primitive vasculature becomes remodeled into an adult pattern, including, for example, the process by which capillary-free zones develop specifically near arterial vessels. Some of the perifoveal vessels also undoubtedly atrophy in the course of normal development.

The absence of vessels from the normal adult fovea, however, is found in the present study to begin embryologically not as an expanding focus of vasoobliteration in the primitive vasculature, but instead largely through failure of the vessels to grow completely to the center of the macula. The macula is found to be invaded by blood vessels much more slowly than is the surrounding retina. The proliferation of the surrounding vessels tends to converge toward the center of the macula, and thus gives rise to the unique circulatory pattern at the posterior pole of the adult fundus. Since vessels were never found at the center of the developing fovea at any age, it seems unlikely that the fovea had at some time been completely overgrown by the vasculature.

The vasculature of the immature macula has been examined histologically also by Henkind and co-workers in three rhesus
monkey fetuses at 125, 145, and 150 days gestation. At 150 days gestation a capillary-free zone was observed at the fovea. However, shortly before, at 125 and 145 days gestation, the macular region was said to be fully vascularized and no foveal avascular zone was observed. It was concluded that the foveal avascular zone arises as a result of vessel atrophy. Their failure to observe an avascular zone earlier than 150 days is contrary to the present observations, but may be related to the relatively few animals in their study. Ink-filled vessels reported by them as occurring in the fovea may be perhaps near but not precisely at the center of the fovea, a misjudgment especially likely if the center were partly obscured by distortion of the tissue or leakage of ink from the newly-formed adjacent vessels. In the present study, the center of the macula has been found to be avascular in each of the six monkeys examined at 100 and 125 days gestation, as well as in the three older fetuses. Since Henkind and co-workers examined no fetuses of less than 125 days gestation, there would have been no opportunity for these workers to obtain data bearing upon the present findings that retinal vascularization is retarded in the region of the macula, and that a radial pattern of vessels develops there as a result of the ensuing growth of vessels towards its center.

The delayed growth of vessels into the macula coincides spatially with a retardation of neural differentiation which reportedly occurs there, differentiation being retarded in the macula between the third and eighth months of gestation in man. Retinal vascularization in this case, as in others, seemingly follows gradients of morphologic and presumably metabolic differentiation within the retina.

The retinal vasculature of a normal human fetus studied in this laboratory at 18 weeks gestation has proved to be nearly as advanced as that of the monkeys at 75 days gestation. This similarity is perhaps not surprising, since in either case the fetus had completed about 45 per cent of its gestation (in other words, 18 of 39 weeks for a human fetus seems roughly equivalent to 75 of 168 days for M. mulatta). Accordingly, a vessel pattern comparable to that of the 100 day monkey fetus might be expected to become apparent at 6 to 7 months gestation in man. The absence of any mention of macular vascularization in man by researchers in past years is perhaps attributable in part to shortcomings of their histologic methods, especially destruction of some immature retinas during trypsin digestion or inability to fill the vasculature uniformly without causing leakage artifacts when using intravascular injection media.

The superficial network of capillaries which occurs in the nerve fiber layer of the peripapillary retina consists characteristically of radially oriented capillaries which tend to branch less often than other retinal capillaries. Collapse of these vessels is suspected to contribute to the formation of Bjerrum's scotoma and early field defects of glaucoma. According to the present study, and a prior study of kittens, the superficial network develops appreciably later than the initial outgrowth of vessels into the retina from the optic nerve head. The superficial vessels evidently develop from the preceding inner network at about the time and place that the nerve fiber layer becomes increased in thickness, especially near the optic nerve head and where growing nerve fibers from the peripheral retina have been diverted into their arcuate pattern skirting the fovea.

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REFERENCES