Early Morphogenesis of Persistent Hyperplastic Tunica Vasculosa Lentis and Primary Vitreous

The Dog as an Ontogenetic Model

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Observations on (postnatal) persistent hyperplastic tunica vasculosa lentis/persistent hyperplastic primary vitreous (PHTVL/PHPV) in man and dog have been published previously. Up to the present, no evidence on the etiology of this entity was available. The hereditary occurrence of the disease in the Dobermann pinscher dog and the similarity of ocular development in mammals has provided a useful model in providing ontogenetic data. The present study deals with the early morphogenesis of PHTVL/PHPV, from day 25 to 44 post-coitum (D25–D44), in genetically affected dog fetuses. Normal beagle dog fetuses served as reference material, which has been described separately. At D30, the hyaloid system, including the tunica vasculosa lentis posterior, had developed further than in the reference fetuses. From that stage onward, a retrolental fibrovascular membrane developed. In some of the eyes of D37, posterior polar subcapsular cataracts and preretal glial proliferations were observed. Capsular anomalies and distortions of the lens shape as seen in clinical PHTVL/PHPV were not observed, and are believed to be secondary entities. Extrapolation of some of the obtained data from dog to man is possible by the use of comparable gestational time scales. The anterior form of (PHTVL/PHPV) in man probably develops its main features in the period of approximately 43 to 66 days of pregnancy. Recently, anti-angiogenetic properties of normal vitreous have been described. This, and the fact that overdevelopment and subsequent incomplete regression of the hyaloid system plays a major role in the pathogenesis of PHTVL/PHPV, gives rise to the hypothesis that a changed amount or effectiveness of such (humoral) factors is an important factor in the etiology of this disease.


The anterior form of persistent hyperplastic tunica vasculosa lentis/persistent hyperplastic primary vitreous (PHTVL/PHPV) is a congenital eye anomaly, in most cases leading to cataract. The occurrence of this entity has been described in man and in dogs.

In man, over 200 cases, mainly unilateral and supposedly nonhereditary, have been described under different names. The entity can be distinguished into an anterior and a posterior form.

The first description of PHPV in a dog was published by Grimes and Mullaney in 1969 and confirmed by other case reports as summarized by Stades. Only in recent years has the frequent occurrence of PHTVL/PHPV been described in specific dog breeds, at first in the Dobermann pinscher and lately also in the Staffordshire bull terrier. Clinical, surgical, pathological and genetic aspects of the disorder have been described for the Dobermann pinscher; in this breed a hereditary basis of the disease was demonstrated.

The close resemblance of the clinical manifestations in man and dog, as well as the frequent (inherited) occurrence of PHTVL/PHPV in the Dobermann pinscher breed provide a useful model for research on the ontogenesis. In addition, the high degree of similarity in development of the eye in different mammals allows extrapolation of some of the results of this study to PHTVL/PHPV of man.

The embryonic and neonatal development of the normal canine eye and the early morphogenesis of the lens, lens capsule, hyaloid system and vitreous in particular have been described. On the ontogenesis of PHTVL/PHPV only one preliminary communication has been published. The present study was undertaken to establish when and how the morphogen-
esis of the eye in genetically PHTVL/PHPV-affected dog fetuses becomes aberrant.

**Materials and Methods**

**Fetuses**

From matings of severely (grade 2–6) PHTVL/PHPV-affected Dobermann pinschers, 41 fetuses were collected at ages of 25, 28, 30, 33, 35, 37 and 44 days post-coitum (D25–44). The amounts per age are stated in Table 1. Sixty-eight of the obtained eyes were available for this study. The fetuses were collected as previously described. Seventeen fetuses

<table>
<thead>
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<th>D25</th>
<th>AD</th>
<th>Min</th>
<th>Max</th>
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<tbody>
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<td>(14)</td>
<td>0.284</td>
<td>0.275</td>
<td>0.294</td>
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<tr>
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<td>(4)</td>
<td>0.618</td>
<td>0.598</td>
</tr>
<tr>
<td>D30</td>
<td>(10)</td>
<td>0.747</td>
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<tr>
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<tr>
<td>D35</td>
<td>(4)</td>
<td>2.20</td>
<td>2.08</td>
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<tr>
<td>D37</td>
<td>(14)</td>
<td>2.56</td>
<td>2.37</td>
</tr>
<tr>
<td>D44</td>
<td>(12)</td>
<td>3.7</td>
<td>3.5</td>
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**Fig. 1.** D25; optic cups. Transverse sections; overall views. H & E; ×25. (LV = lens vesicle; HA = hyaloid artery; R = retina; SE = surface ectoderm). (A) The lens vesicle is still open. A clump of cells from the surface ectoderm (epitrichia) is present (arrow). (B) The lens vesicle is closed, but still attached to the surface ectoderm. Some loose epithelial cells are present (arrows). (C) The lens vesicle is closed and detached from the surface ectoderm. The annular vessel (AV) at the anterior edge of the optic cup is present.
from normal beagle parents, with ages up to D35, served as reference material. The results of that study have been published separately.\(^\text{16}\)

In order to minimize the variation of gestational ages, matings were carried out as soon as possible after ovulation, as determined by plasma progesterone measurements.\(^\text{16}\)

Animal care and treatment in this study were in compliance with the ARVO Resolution on the Use of Animals in Research.

### Histology

Following collection and death of the fetuses, the heads or the eyes alone were fixed in 4% buffered formaldehyde, processed and stained as described before.\(^\text{16}\) Because of damage to some of the D28 material, a few 1 μm sections of specimens fixed and embedded for electron microscopy were stained with the periodic acid-Schiff (PAS) reaction. The development of all eyes was examined by light microscopy.

The average diameters of the fetal eyes were measured according to a previously described method.\(^\text{16}\)

### Results

#### Optic Cup

Throughout this study, the eyes at all ages were round to oval, double-layered structures. The average diameter values of the eyes of D25 up to D44 showed a steady increase from 0.284 to 3.7 mm (Table 1).

#### Lens

At D25, the lens vesicles consisted of epithelial cells, and had a rounded to somewhat conical shape (Fig. 1a–c). In eight eyes the process of invagination, by which the lens vesicle is formed, had not yet been completed and the anterior central part of the lens vesicle was still open (Fig. 1a). In these eyes, a clump of epithelial cells was present at this location. In four eyes the lens vesicle had closed (Fig. 1c); in two of these it was still attached to the surface ectoderm (Fig. 1b). In most sections, the cavity of the closed lens

![Fig. 2. D30; equatorial region of the lens. Capillaries (arrows), connect the anterior and posterior portion of the tunica vasculosa lentis. (R = retina; PLF = primary lens fibers). PAS; ×40.](image)

![Fig. 3. D33; transverse section; overall view. A fibrovascular membrane (arrows) is present retrolentally. The peripheral detachment appears artificial (see also Fig. 8). Sections of hyaloid vasculature are not present within the vitreous (V). (L = lens; R = retina; N = optic nerve). H & E; ×4.](image)
vesicles contained some amorphous and cellular (epitrichial) material.

At D28, the primary lens fibers, formed by elongation of the posterior cells of the lens vesicle, had filled the posterior half of the lens vesicle.

At D30, the lens vesicles were almost obliterated by the primary lens fibers, thus leaving a flat cavity anteriorly (Fig. 2). From this developmental stage onwards the lens sphere was oval.

At D33 (Fig. 3) the flat cavity as at D30 was barely detectable in most specimens.

At D35 (Fig. 4) the lens spheres were obliterated.

At D37 posterior polar subcapsular cataracts were observed in two of the eyes (Fig. 5).

At D44, a small posterior polar subcapsular cataract was observed in one lens.

**Lens Capsule**

At D25, the anlage of the lens capsule was observed as a very thin basement membrane-like layer of faintly PAS-positive material, surrounding the lens vesicle. The histologic appearance of this layer was similar to that of the fibrillar contents of the primitive vitreous.

At D28, the lens capsule was observed as a faintly stained, straight and continuous structure (Fig. 6).

At D30, the posterior part of the capsule, as well as the equatorial region, where the vasculature of the tunica vasculosa lentis (TVL) was very well developed, PAS-positive and lying in close proximity to the capsule, was thicker than the anterior part.

From D33 onward, except in some eyes at D37 and D44, the capsule had a wrinkled appearance.

**Vascular System**

At D25 (Fig. 1a-c), a short and straight hyaloid artery was present in all eyes. No indication of a ramification of the hyaloid artery into vasa hyaloidea propria or the future tunica vasculosa lentis posterior was observed. The annular vessel at the distal edge of the optic cup was present.

In the D28 material, numerous cross-sections of vessels of the hyaloid-TVL system were observed.
TVL vasculature was present, positioned directly against the lens capsule (Fig. 6).

At D30 (Fig. 7), sections through vessels of the hyaloid-TVL system were more numerous than in the reference material. Predominantly in the central region, a proliferated layer of TVL vasculature was located against the posterior lens capsule, covering that region almost completely. The vascularization of the anterior side of the lens was similar to the normal controls. This vasculature was connected with the TVL posterior by capillaries in the equatorial region of the lens.

At D33 a distinct but thin retrolental fibrovascular layer, which characterizes PHTVL/PHPV postnatally, was present in all eyes (Figs. 3, 8). In these plaques, cross-sections of the TVL vasculature were present. At this stage, the central part of the plaque was locally attached to the posterior lens capsule (Fig. 3). At the periphery, the plaque was detached from the capsule. Still, capillaries and cellular components, including fibroblasts, were in contact with the capsule (Fig. 8). Cross-sections through the vasa hyaloidea propria in the vitreous space were less numerous than at D30 (Figs. 3, 7).
From D35 (Fig. 4) onward, a plaque, completely attached to the posterior lens capsule, was observed in all eyes. The central thickness of the plaques ranged from 29.0 to 45.1 (mean 37.9) μm. Except for the main trunk of the hyaloid artery, no vasa hyaloidea propria were present in the vitreous space.

At D37, the central thickness of the plaques was 45.1 to 119.1 (mean 73.4) μm. Many cross-sections of TVL and hyaloid vasculature were present within the plaques (Figs. 5, 9). Except for a straight main trunk of the hyaloid artery, no parts of the hyaloid system were found in the vitreous space (Fig. 10). Glial tissue was observed for the first time in the plaques, but no melanocytes were observed. At D44 (Fig. 11), the appearance of the anomalies was similar to that at D37 (Figs. 5, 9, 10). The central thickness of the plaques varied from 22.5 to 157.8 μm (mean: 95.4; median value: 106.3 μm). In 11 plaques varying numbers of melanocytes were present. The thinnest plaque did not contain any melanin-containing cells.

**Vitreous**

At D25 (Fig. 1a–c), the primitive vitreous contained some amorphous and fibrillar material but very few cellular components.

At D28, apart from the vascular elements as described above, the vitreous space contained cells with
the cytological characteristics of fibroblasts and hyalocytes (Fig. 6) amidst an amorphous and slightly fibrillar ground substance.

At D30 (Fig. 7), both the vascular and cellular components of the primitive vitreous of the affected fetuses had developed further than those described in the corresponding reference material. From D33 onward (Figs. 3, 4, 10, 11) the absence of vasa hyaloidea propria gave the vitreous space an “empty” image, i.e., a few loose cells within a randomly oriented, seemingly fibrillar matrix.

At D37, a proliferation of neuroglial tissue, intimately attached to and originating from the retina, was present in four eyes (Fig. 12a, b). These proliferations, followed by serial sections, protruded into the vitreous and reached the retrolental plaque in three of these eyes. The thickness of these strands varied substantially; the average size was about 50 μm. In these three eyes, as well as in six others, additional, very thin strands of neuroglia were present (Fig. 13a, b). These originated from the retrolental plaque, extended into the vitreous and connected with a larger, retina-derived proliferation (Fig. 12a, b), if such was present.

At D44 (Fig. 11) the vitreous showed a concentration of cells (fibroblasts, hyalocytes) in the proximity of the plaque.

**Retina and Pigment Epithelium**

Up to D35 the development of the neural retina and the pigment epithelium did not differ from that described in the reference material. Apart from the aforementioned glial tissue, a conical proliferation of glial tissue was present at the anlage of the optic disc in five eyes of D37.

In eight of the D44 eyes, the primitive optic disc contained a similarly shaped mass of glial tissue (Fig. 14).

**Discussion**

This study has demonstrated a first, marked difference in embryonic development of PHTVL/PHPV-
affected and normal canine eyes at D30. From this stage onward the (mesodermal) hyaloid-TVL vasculature is the main structure involved in the development of the anomaly. From D37 neuroglia, an ectodermal component, has also been found to participate.

Optic Cup

In the dog, microphthalmia is a less common feature of PHTVL/PHPV than in man. The measurements of the optic cup’s average diameter values (Table 1) did not differ significantly from those of the reference material. For a statistical evaluation of these values, larger numbers would be required.

Lens

As the eyes of D25 were from fetuses from the same litter, the differences in closure and separation of the lens vesicles must be due to slight differences of developmental stage.

Up to D35, the development of the lens was analogous to that described in the reference material. At D37 and D44, some posterior polar subcapsular cataracts were observed in the present material. The localization of these cataracts corresponds with the site of their onset in PHTVL/PHPV-affected patients. Abnormal lens shapes, such as lenticonus, microphakia or colobomas were not observed.

Lens Capsule

The morphological features of the posterior lens capsule were normal, although frequently wrinkled due to shrinkage, which was also the case in the reference material. Recently there has been some discussion as to whether the changes in the posterior lens capsule—as seen in postnatal PHTVL/PHPV—should be regarded as primary or secondary. Up to this moment, changes in the posterior lens capsule, varying from dissolution to capsular tears, have only been seen in severely affected patients. This suggests that the development of the frequently found lenticonus posterior either is a secondary event, or these matings failed to produce the most severe cases that were clinically found in man and dog.

Vascular System

Until D30, the development of the vascular structures of the optic cup matched that described in the reference material. From D30 onward the retrolental part of that vasculature, the hyaloid system (predominantly the vasa hyaloidea propria and the TVL posterior), showed signs of an increased development and subsequent incomplete regression. The peripheral detachment of the plaque at D33 appears to be an artifact due to the histologic processing (Fig. 8).

Vitreous has been reported to inhibit angiogen-
Impaired or defective anti-angiogenetical properties of the (secondary) vitreous might be the cause of the described overdevelopment of intravitreal vasculature in PHTVL/PHPV.

**Vitreous**

From D33 the vitreous space showed cross-sections through the main stem of the hyaloid artery only. Its ramifications had become enfolded in the retrorenal plaque. This situation gave the vitreous space its "empty" appearance, compared to the reference material of this age group.

The fibrillar appearance of the ground substance is probably due to coagulative artifacts due to fixation technique, as has been described previously. The fact that the D28 material does not show this coarsely-fibered structure supports this assumption, because it had been (differently) prepared for electron microscopic purposes.

**Glial Tissue Proliferations**

Reference eyes did not contain glial proliferations as were observed in the present material.

In his pathologic studies concerning human eyes with (PHTVL/PHPV), Manschot made special reference to preretinal glial proliferation as a pathologic characteristic of the entity. He described both larger and smaller, fibered and cellular preretinal proliferations in infant eyes with (PHTVL/PHPV). The fibers of many of these cells were observed to merge into the fibers of Mueller cells of the retina. Consequently, the probable neuroglial origin of these structures was postulated.

In the eyes described here, preretinal glial proliferations (Fig. 13a, b), mimicking those described by Manschot in man, were observed in nine eyes at D37 and D44. The finding of glial tissue in the plaques of these dog fetuses and in the plaques of (PHTVL/PHPV-affected) postnatal dogs and in man suggests...
suggests that this material derives from the glial proliferations described above.

Apart from the glial proliferations described above, in some eyes there was a small, conical proliferation of glial cells in the center of the presumptive optic disc (Fig. 14). A cone-shaped mass of cells of this kind in postnatal eyes is known as "primitive epithelial papilla" or "Bergmeister's papilla," and is a frequent finding in PHTVL/PHPV in man and dog.

Retina and Pigment Epithelium

The general development of the sensory retina and pigment epithelium in affected eyes was similar to that in the reference fetuses, except for the cases in which a neuroglial proliferation was present.

In the contemporary literature, the most current term for the described anomaly in man is "PHPV." However, other names have been used in the past, such as persistent hyperplastic tunica vasculosa lentis (PHTVL), pseudophakia fibrosa, membranous cataract leucocoria, pseudoglioma and cataracta congenita vasculosa. The differences in nomenclature suggest a lack of knowledge about the development of the anomalies.

Stades used PHTVL primarily, and PHPV secondarily, as names for the disease, as the anomaly appeared to be first of all a persistence and hyperplasia of the TVL posterior. Depending on whether or not the hyaloid system is considered a part of the primitive vitreous and according to the classical embryologic views, the name PHPV would suggest that the retrolental tissue proliferations which are pathognomonic for the condition are solely or mainly of ectodermal origin. However, the results of this and other studies indicate a primarily mesodermal origin, although neuroectodermal involvement (the presence of neuroglia) can be demonstrated in some cases. Thus the present study supports the name PHTVL/PHPV, as proposed by Stades, although a shorter name would be preferable for clinical use.

The results of this study give lead to the following hypothesis about the genesis of (PHTVL/)PHPV in man. The major visible embryological derangements in PHTVL/PHPV in the dog occur during the gestational period of D30 to D45. This developmental period extends from the obliteration of the lens vesicle until the first signs of regression of the hyaloid system. Extrapolating this to the human embryo and taking the difference in time of ocular development in pregnancy into account, it would appear that the anterior form of PHPV in man develops its main features in a gestational period of approximately 43 to 66 days of gravidity. Aguirre stated that extrapolation of ocular developmental events from man to dog could easily be erroneous, because in man the embryological development of the eye occurs relatively earlier in gestation than in dog. Therefore, the above-mentioned gestational period has been placed in comparable time scales for five mammal species, including man and dog. Thus, the risk of drawing erroneous conclusions is brought to an acceptable minimum.

One of the most remarkable changes observed in the studied fetuses was the overdevelopment of the hyaloid system and TVL. An increased growth promotion or decreased inhibition of intravitreal angiogenesis by humoral factor(s) is probable. For this reason further studies on the ontogenesis of PHTVL/PHPV in the Dobermann pinscher dog will involve the regulation of intravitreal angiogenesis, as well as ultrastructural aspects of the entity.

Key words: PHTVL/PHPV, eye, vitreous, morphogenesis, dog
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References