Ultrasonography of the Harderian gland in the rabbit, guinea pig, and chinchilla

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Abstract

**Objective** To evaluate the Harderian gland in rabbits, guinea pigs, and chinchillas using B-mode ultrasound and to determine normal size and changes in size and/or location in normal and diseased eyes and orbits by ultrasonographic measurements.

**Procedure** Normal Harderian glands were evaluated ultrasonographically in 20 rabbits, 10 guinea pigs, and eight chinchillas. The Harderian gland was measured ultrasonographically in horizontal and vertical planes. Normal Harderian gland sizes were then compared with sizes in 27 rabbits, 13 guinea pigs, and three chinchillas that had exophthalmos.

**Results** Harderian glands in normal rabbits were 0.69 ± 0.07 cm (mean value ± SD) horizontally and 1.33 ± 0.14 cm vertically. Harderian glands in normal guinea pigs were 0.58 ± 0.05 cm horizontally and 0.61 ± 0.10 vertically. In normal chinchillas, the Harderian glands were 0.53 ± 0.04 cm horizontally and 0.53 ± 0.03 cm vertically. Harderian glands were significantly larger in the vertical plane in rabbits with exophthalmos (P = 0.001) and in the horizontal plane in guinea pigs with exophthalmos (P = 0.018). Harderian glands of rabbits with exophthalmos were significantly larger in both diseased and healthy glands in both planes compared with those of normal rabbits. Guinea pigs and chinchillas with exophthalmos had larger Harderian glands bilaterally in only the vertical plane.

**Conclusions** Ultrasonography is a valuable diagnostic imaging technique to evaluate the Harderian gland in the rabbit, guinea pig, and chinchilla. Retrobulbar pathologic processes cause enlargement of the Harderian gland, which may be attributable to inflammation or possible obstruction of the excretory ducts.

**Key Words:** chinchilla, exophthalmos, guinea pig, Harderian gland, rabbit, ultrasonography

INTRODUCTION

The Harderian gland is an orbital gland found in some vertebrates and was first described in 1694 by Johann Jacob Harder in deer, giving the gland its name. The gland is also called the Glandula palpebrae tertiae profunda, associating it with the third eyelid in mammals. The gland is a tubuloalveolar gland with its secretory duct opening at the nictitating membrane in the posterior canthus. The main secretory substance of the mammalian Harderian gland is lipid. The Harderian gland has various functions including lubrication of the ocular surface and third eyelid, a site of immune response, a photoreceptive organ, a source of pheromones, thermoregulation, and a source of growth factors.

The Harderian gland of the rabbit is located infraorbitally between the eyeball and medial or ventral part of the orbit and is in contact with the nictitating membrane. It is divided into a white lobe, which is smaller and located more rostral, and a larger pink lobe in the ventral part of the orbit. Both lobes are divided by a thin band of connective tissue and converge into a single excretory duct which opens into the lower posterior part of the third eyelid near the conjunctival sac. The pink lobe consists of cuboidal cells and contains large lipid vacuoles, producing more lipids than the white lobe. The white lobe cells are columnar with smaller vacuoles.

The guinea pig’s Harderian gland is large and occupies a considerable part of the retrobulbar space, resting against the posterior part of the globe.
The glandular endpieces, maintaining their contour, and the excretory duct has not been possible. The third eyelid is rudimentary. The alveoli of the Harderian gland have wide lumina and produce lipids. Myoepithelial cells are attached to the glandular endpieces, maintaining their contour, and serving as their exoskeleton.

The Harderian gland of the chinchilla is oval, 0.9 cm in length, whitish in color, and nonlobulated. It is firmly attached to the posterior part of the globe and extends medially. The tubuli and alveoli are lined with cuboidal to columnar cells. The gland produces lipid and has small intra- and interglandular ducts. Clinical visualization of the excretory duct has not been possible. The third eyelid is rudimentary.

The purpose of this study was to determine, whether the Harderian gland is visible ultrasonographically and to establish normal size reference values for rabbits, guinea pigs, and chinchillas. These values were compared with those measured for the Harderian gland in animals with exophthalmos, to establish whether the Harderian gland becomes enlarged with orbital disease.

MATERIALS AND METHODS

Rabbit
Forty normal Harderian glands in 20 healthy, 4-month-old male rabbits with a mean body weight of 2–2.5 kg were examined ultrasonographically. Harderian glands of 27 rabbits that had unilateral or bilateral exophthalmos were also imaged. Exophthalmic rabbits were included when at least one Harderian gland was imaged. There were eight males (five neutered) and 19 females (two spayed). The mean age was 4.3 years (range 1.0–8 years).

Guinea pig
Normal Harderian glands of 10 healthy guinea pigs were imaged ultrasonographically. Mean age was 4.2 years (range 1.5–7.2 years). Six guinea pigs were male (two neutered) and four were intact females. Harderian glands in 13 guinea pigs with unilateral or bilateral exophthalmos were also imaged. Five guinea pigs were male (one neutered) and eight were female, with a mean age of 4.2 years (range 1.5–7.2 years).

Chinchilla
Harderian glands of eight chinchillas euthanized for reasons unrelated to this study were imaged ultrasonographically. Mean age was 6 months (range 5–7 months). The chinchilla heads with eyes and adnexa were fixed in 10% formaldehyde solution. Three chinchillas with exophthalmos (two females, one male, mean age of 5.4 years, range 0.1–8.5 years) were also imaged. Two chinchillas had bilateral exophthalmos.

Ultrasonography
Following application of a topical anesthetic, 0.4% oxybuprocain hydrochloride ophthalmonic solution (Novain®; Agepha, Söding, Austria) and manual restraint of the animals, the eyes and retrobulbar spaces of both normal and exophthalmic rabbits and guinea pigs were examined ultrasonographically. Chinchillas with exophthalmos were imaged the same way, while normal chinchillas were imaged directly. An HDI 5000 ultrasound unit (Philips, Bothell, WA, USA) with a 5–8 MHz small parts transducer or 15–7 MHz linear transducer was used for the examination of all animals, except for the normal rabbits, where a Logiqbook ultrasound unit (GE Healthcare, Buckinghamshire, UK) with a 5–8 MHz small parts ultrasound transducer was used. The ultrasound transducer was placed on the closed lids or directly on the cornea. The Harderian glands were imaged and measured three times in both a horizontal and vertical plane and then averaged. Results were confirmed by computed tomography, biopsy, surgery, pathology, or histology.

Statistical analysis
Ultrasonographic measurements of the Harderian glands of normal animals were made three times for the horizontal and the vertical plane of both sides and then averaged. A 95% confidence interval (CI) was determined for each plane in all normal and exophthalmic animals. The Harderian glands in animals with exophthalmos were also measured three times in each plane, and a mean value was established. A t-test was used to compare the size of the Harderian gland in animals with exophthalmos with the normal side in both planes. Means, ranges, and standard deviations (SD) are reported. Furthermore, the Harderian gland size in the exophthalmic and healthy side was each compared with normal measurements to determine whether they were within the CI both horizontally and vertically in each animal group. A P-value of <0.05 was considered significant.

RESULTS

Rabbit
Forty Harderian glands in normal rabbits were imaged ultrasonographically. The gland was located medial to the eyeball and extended toward posterior and ventral in the retrobulbar space. In the horizontal plane, the transducer was tilted slightly toward ventral, and in the vertical plane, the probe was directed medially. The gland was oval to elongated and appeared hypoechoic to hyperechoic with a coarse texture (Fig. 1a,b). Measurements of the normal gland were 0.69 ± 0.07 cm in the horizontal plane with a 95% CI of 0.66–0.72 cm and 1.33 ± 0.14 cm in the vertical plane with a 95% CI of 1.27–1.39 cm (Table 1).

The Harderian glands were imaged and measured in 27 exophthalmic rabbits. Five rabbits had bilateral
exophthalmos and 22 had unilateral exophthalmos. Twelve Harderian glands could not be visualized or measured ultrasonographically due to retrobulbar abscesses that obscured the gland, and one rabbit had only one eye. Of the remaining 41 Harderian glands, 20 were examined in conjunction with exophthalmos, and 21 were imaged in healthy orbits.

Causes of exophthalmos in the imaged orbits included retrobulbar abscessation in eight of 20 orbits, retrobulbar cellulitis or edema in nine of 20 orbits, unilateral lymphoma of the Harderian gland, unilateral glaucoma, and unilateral purulent, necrotic inflammation of the eyelids and adjacent tissues in one rabbit each. When comparing the Harderian gland of the exophthalmos side with the healthy side, there was significant enlargement in the vertical measurement (P = 0.001). The Harderian gland in both the diseased and the healthy orbit was significantly larger in both the horizontal and vertical values than the CI of the normal measurements (Table 2). Ultrasonographic images of enlarged Harderian glands showed a more nonuniform texture as well as a more heterogeneous echogenicity with the gland appearing more hyperechoic compared with the retrobulbar area. In the rabbit with lymphoma of the Harderian gland, the gland was more hypoechoic with hyperechoic nodular infiltrates. In the two cases of rabbits with thymoma, the Harderian gland was enlarged, more hyperechoic, and protruded rostral (Fig. 2a,b).

Guinea pig
In normal guinea pigs, 20 Harderian glands were imaged ultrasonographically. The gland was located posterior and slightly ventral to the eyeball, the ultrasound transducer was placed perpendicular to the eyeball in both the horizontal and vertical plane. The gland appeared round and seemed divided into two lobes in the horizontal plane. The gland was hyperechoic with a slightly coarse texture (Fig. 3a,b). Normal measurements of the Harderian gland were 0.58 ± 0.05 cm in the horizontal plane with a 95% CI of 0.55–0.61 cm and 0.61 ± 0.10 cm in the vertical plane with a 95% CI of 0.55–0.67 cm (Table 1).

Twenty-six Harderian glands were imaged in guinea pigs with exophthalmos (n = 13). Twelve guinea pigs had unilateral exophthalmos and one had bilateral exophthalmos. Fourteen Harderian glands were examined in conjunction with exophthalmos, and 12 were visualized in healthy orbits. Exophthalmos was found in conjunction with a retrobulbar abscess in five of 14 orbits, elongated roots of the cheek teeth in four cases, retrobulbar cellulitis in two orbits, and periorbital lipoma, prolapsed Harderian gland, and ameloblastoma in one orbit each.

Table 1. Measurements of the Harderian gland in normal animals

|        | n  | Mean value (cm) | SD | 95% Confidence interval |
|--------|----|----------------|----|-------------------------|
|        |    | Horizontal | Vertical | Horizontal | Vertical | Horizontal | Vertical |
| Rabbit | 20 | 0.69       | 1.33     | 0.07       | 0.14      | 0.66–0.72  | 1.27–1.39 |
| Guinea pig | 10 | 0.58       | 0.61     | 0.05       | 0.10      | 0.55–0.61  | 0.55–0.67 |
| Chinchilla | 8  | 0.53       | 0.53     | 0.04       | 0.03      | 0.49–0.57  | 0.51–0.54 |
Harderian glands in guinea pigs with exophthalmos were significantly larger in the horizontal plane than the contralateral healthy glands \((P = 0.018)\). Both the diseased and healthy Harderian glands in guinea pigs with exophthalmos were significantly larger vertically than in the normal guinea pigs (Table 2).

Table 2. Significant changes in Harderian gland size

|                      | Diseased vs. healthy eye in exophthalmic animals | Diseased vs. normal animals | Healthy vs. normal animals |
|----------------------|------------------------------------------------|----------------------------|---------------------------|
|                      | Horizontal | Vertical | Horizontal | Vertical | Horizontal | Vertical | Horizontal | Vertical |
| Rabbit               | No         | Yes      | Yes        | Yes      | Yes        | Yes      |
| Guinea pig           | Yes        | No       | No         | Yes      | No         | Yes      |
| Chinchilla           | No         | Yes      | No         | Yes      | No         | Yes      |

Harderian glands in guinea pigs with exophthalmos were significantly larger in the horizontal plane than the contralateral healthy glands \((P = 0.018)\). Both the diseased and healthy Harderian glands in guinea pigs with exophthalmos were significantly larger vertically than in the normal guinea pigs (Table 2).

The enlarged Harderian gland of the guinea pig had a coarser ultrasonographic appearance and was more

Figure 2. Enlarged Harderian gland in a rabbit with a thymoma. (a) Ultrasound image of an orbit in an exophthalmic rabbit in a horizontal view. The Harderian gland (HG) is enlarged and appears hyperechoic in the retrobulbar space; EB = eyeball. (b) Bilateral exophthalmos in a rabbit caused by a thymoma.

Figure 3. Normal Harderian gland in a guinea pig. (a) Ultrasound image of an orbit in a normal guinea pig in a horizontal view. The Harderian gland (HG) is located posterior to the eyeball (EB). The gland appears to be divided into two lobes. (b) Right eyeball and Harderian gland of a normal guinea pig. The Harderian gland is almost as large as the eyeball and envelops the globe ventrally.
hyperechoic than the normal gland. In the guinea pig with the prolapsed Harderian gland, the medial part of the gland appeared smaller and was displaced toward anterior (Fig. 4a,b).

**Chinchilla**

Harderian glands in normal chinchillas included eight heads with eyes and adnexa fixed in formaldehyde. The rounded Harderian gland was located posterior and medial to the eyeball. Ultrasonographically, the Harderian gland was hypoe- to hyperechoic and coarse (Fig. 5a,b). Normal horizontal measurements were 0.53 ± 0.04 cm in the horizontal plane with a 95% CI of 0.49–0.57 cm and 0.53 ± 0.03 cm in the vertical plane with a 95% CI of 0.51–0.54 cm (Table 1).

Six Harderian glands of five diseased and one healthy orbit in chinchillas with exophthalmos were imaged. One chinchilla had unilateral exophthalmos and two chinchillas had bilateral exophthalmos. Exophthalmos initially diagnosed in one Chinchilla was in fact buphthalmos attributable to secondary glaucoma associated with cataract and lens-induced uveitis. Another Chinchilla initially diagnosed with exophthalmos actually had bilateral buphthalmos associated with secondary glaucoma from posterior lens luxation. One chinchilla had a bilateral fractured mandible with bilateral elongated roots of the cheek teeth with exophthalmos.

Harderian glands in monocular exophthalmonic chinchillas were compared with Harderian glands in normal

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**Figure 4.** Prolapsed Harderian gland in a guinea pig. (a) Ultrasound image of an orbit in an exophthalmic guinea pig in a horizontal view. The medial part of the Harderian gland (HG) appears smaller (arrow) and is demarcated by a step-like hyperechoic line, which represents the gland margins; EB = eyeball. (b) Prolapsed Harderian gland ventral to the eye.

**Figure 5.** Normal Harderian gland in a chinchilla. (a) Ultrasound image of an orbit in a normal chinchilla in a horizontal view. The Harderian gland (HG) is located posterior and medial to the eyeball (EB). The gland has a coarse texture and is hypoechoic. (b) Eyeball and Harderian gland of a chinchilla from a medial view.
chinchillas. These glands were significantly larger than the normal Harderian glands of normal chinchillas in the vertical plane. Measurement of the Harderian gland in the healthy eye was above the CI of the normal gland in the vertical plane. These measurements were for orientation only as the number of chinchillas was too small for statistical analysis (Table 2).

Ultrasonographically, glands in chinchillas with exophthalmos were slightly more hyperechoic and heterogenous than normal (Fig. 6a–c).

**DISCUSSION**

**Rabbit**

The Harderian gland in the rabbit is one of four glands contributing to the precorneal tear film. The others are the lacrimal, intraorbital, and superficial gland of the third eyelid.22 The exact proportion of tear production by each gland has not been reported. The Harderian gland in the rabbit produces lipids, especially in the pink lobe.4,5,11,14 Due to this lipid production, the Harderian gland is difficult to visualize ultrasonographically and to differentiate it from retrobulbar fat. Knowledge of its special anatomy and size allows ultrasonographic imaging of the Harderian gland. The gland appears hypoechoic in the rabbit with the pink lobe slightly more hypoechoic than the white lobe. The echogenicity of fat depends on the number of fat-filled vacuoles in an area as well as their size. This phenomenon has been described with hypoechoic foci in hepatic lipidosis.23 The larger the fat vacuoles, the more hypoechoic the area appears due to fewer tissue interfaces. This would also explain, why the pink lobe with larger vacuoles is more hypoechoic than the white lobe.12–14 The white lobe has a higher content of lipid droplets which gives it its white color.24

Measurements of the normal rabbit Harderian gland in anatomic sections have been reported to be approximately 19 mm long and 4–6 mm thick.24 In our study, the mean length of the gland was 13 mm, the mean thickness was 6.9 mm. Differences in measurement values may be attributed in part to variation in age among rabbits of the study reported here. Additionally, morphological and biochemical property variations of Harderian glands exist and are influenced by endogenous factors (e.g., hormones) and exogenous factors (e.g., temperature, photoreception, and thermoregulation).5,13 The discrepancy in length may also be due to the fact that the gland has rounded margins and wraps around the medial and ventral portion of the eyeball.

Exophthalmos was caused by a retrobulbar abscess in a large number of rabbits reported here. Other pathologic processes included steatitis and purulent necrosis of the retrobulbar fatty tissue. In rats, inflammation of the lacrimal and salivary glands (sialodacryoadenitis) is associated with hypertrophy of the Harderian gland and is caused by a rat coronavirus.25 In the rabbit, primary inflammatory

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**Figure 6.** (a) Ultrasound image of the eyeball (EB) and retrobulbar space in a chinchilla with a fractured mandible and elongated roots of the cheek teeth in a horizontal view. The Harderian gland (HG) is enlarged, hyperechoic, and heterogenous with hypoechoic areas of edema. (b) Transverse computed tomographic images of the bilateral mandibular fracture (arrows) and elongated tooth roots (c) (arrows).
disease of the Harderian gland is not reported, but it appears to undergo a similar pathogenesis secondary to retrobulbar inflammation. One rabbit had lymphoma of the Harderian gland previously described.21 Tumors of the Harderian gland have been described in mice, with cystadenomas and adenocarcinomas occurring most frequently.26 Exophthalmos associated with a cranial mediastinal mass or thymoma is commonly bilateral. This is believed to be a paraneoplastic syndrome and occurs when venous drainage by the jugular veins is obstructed.22,27,28 In this study, two rabbits with thymoma and bilateral exophthalmos were found. There was retrobulbar edema, and all four Harderian glands were enlarged and appeared more hyperechoic.

Ultrasoundographic measurements of the Harderian gland in the diseased eye of the exophthalmic rabbits showed a significant enlargement compared with the gland in the normal group in both the horizontal and vertical planes. This is most probably caused by secondary inflammation from retrobulbar pathologies. Another hypothesis may be that the excretory and secretory ducts of the Harderian gland are filled with inflammatory cell infiltrates, causing obstruction. Obstruction of the Harderian gland excretory duct may also be caused by exophthalmos. The single excretory duct of the Harderian gland in the rabbit opens into the lower posterior part of the third eyelid near the conjunctival sac.5,11,12 When exophthalmos occurs from a retrobulbar lesion, the eyeball protrudes and applies pressure on the third eyelid, thereby obstructing the opening of the Harderian excretory duct.

The contralateral healthy Harderian gland was also enlarged significantly both in the vertical and horizontal planes when compared with the normal group. This is explained by the fact that the optic foramen in rabbits communicates with the opposite optic foramen through a 5-mm wide canal (optic canal).29,30 Whether or not inflammatory processes affecting one orbit may egress through the optic foramen to the contralateral side remains speculative. When comparing measurements of the diseased Harderian gland with the contralateral healthy side, significant changes in size were seen only in the vertical plane of the gland. Because the Harderian gland is located between the eyeball and the medial or ventral part of the orbit, there is more space to expand in a posterior direction.

Guinea pig

The Harderian gland in the guinea pig is large and fills most of the retrobulbar space. It is located posterior to the eyeball and is round in shape.5,6,16,17 The gland contours are smooth, and the surface is covered with endothelium of the orbital venous sinus.5 This may explain why the rounded structure was visualized ultrasonographically and easier to differentiate than in the rabbit.

There are no reported measurements of the normal Harderian gland, apart from stating that it has a volume approximately one half to two-thirds of the globe volume.17 Therefore, most of the retrobulbar space visualized ultrasonographically is the Harderian gland. The mean values are almost the same in the horizontal (0.58 mm) and vertical plane (0.61 mm), confirming almost the round shape.

Exophthalmos in the guinea pig was caused by retrobulbar abscesses, elongated tooth roots, and cellulitis. Inflammation of the retrobulbar area may have caused hypertrophy of the Harderian gland. One guinea pig had a prolapsed Harderian gland. The medial part of the Harderian gland was smaller and could only be identified when the transducer was moved ventrally. This suggests that the portion observed in the lower fornix is the medial portion and is indeed the Harderian gland. Periorbital lipoma in the guinea pig has not been described. The lipoma was imaged ultrasonographically medial and ventral to the eyeball and appeared larger and hyperechoic and was well demarcated. The Harderian gland was imaged and appeared enlarged.

Ultrasoundographic measurements of the Harderian gland in the diseased eye of exophthalmic guinea pigs differed substantially from normal guinea pigs in the vertical plane. The Harderian gland has both a dorsolateral as well as a medial attachment to the periorbita.17 This may limit the extent to which the Harderian gland can expand in the horizontal plane. There are discrepant reports on the opening of the excretory duct of the guinea pig which has been reported to open on the posterior16 or anterior5,6 surface of the rudimentary third eyelid. Obstruction of the excretory duct from exophthalmos is questionable. Hypertrophy of the Harderian gland may also be due to intra- and interlobular excretory ducts17 which may become obstructed by inflammation. The contralateral healthy Harderian gland also showed significant enlargement in the vertical plane when comparing it with normal guinea pigs. Similar to the rabbit, the optic foramina are connected by an optic canal and a chiasmatic sulcus (Fig. 7).

The diseased Harderian gland was significantly enlarged in the horizontal plane when comparing it with the healthy contralateral Harderian gland. Because the Harderian gland fills most of the retrobulbar space, enlargement can be found in both planes. We hypothesize that measurement data may have been biased by a systematic error if the round Harderian gland was measured incorrectly ultrasonographically. If the transducer was not completely perpendicular to the gland, the margins would be distorted. Furthermore, the inconsistency of the extent of Harderian gland enlargement in different planes when comparing it with normal guinea pigs (vertical or horizontal) may be due to incorrect measurements of the gland in the diseased side when the full extent of the gland was not visualized.

Chinchilla

Little information exists in references on pathologic processes affecting the chinchilla eye and orbit. In a recent publication, the glands of the eye were described.19 The Harderian gland is round and is approximately 9 mm
long. Ultrasonographically, the Harderian gland in normal chinchillas was smaller at 5.3 × 5.3 mm. One possible explanation for the discrepancy in size among groups may be dehydration and distortion of tissues attributable to tissue fixation for the normal group. Alternatively, ultrasonographic imaging may not have detected the full length of Harderian glands in Chinchillas, as the gland has a rounded form. The glands appeared similar to those found in the guinea pig, although they were smaller and were located slightly more toward medial.

Exophthalmos was found in only three patients, of which two had bilateral exophthalmos or hydrophthalmos. Cataracts, lens luxations, and concurrent glaucoma caused buphthalmos in two patients. This is one of the more common ocular diseases found in chinchillas.\(^{22,31}\) Exophthalmos from enlargement of the Harderian gland may have been attributable to inflammation of orbital tissues associated with uveal inflammation. In both cases, the Harderian gland was significantly larger in the vertical plane compared with measurements in the normal group. Bilateral mandibular fractures and elongated roots of the cheek teeth caused bilateral retrobulbar swelling and exophthalmos in one chinchilla. These findings were confirmed on computed tomography. The Harderian gland was enlarged in both orbits. Only one Chinchilla had unilateral exophthalmos in the study reported here. The contralateral Harderian gland was larger in this Chinchilla. However, it was not possible to determine whether Harderian gland size of the normal contralateral side may be affected by contralateral exophthalmos because of proximity between the gland and the optic foramina, similar to anatomic parallels that exist in the rabbit and guinea pig. A higher number of chinchilla Harderian glands will have to be examined to make a valid statistical analysis of the changes in size.

**CONCLUSION**

Ultrasonography is a valuable diagnostic tool to image the Harderian gland in the rabbit, guinea pig, and chinchilla. Retrobulbar pathologic processes cause enlargement of the Harderian gland. This may be caused by secondary inflammation and edema of the Harderian gland or possible obstruction of the excretory ducts. Contralateral enlargement of the Harderian gland is potentially caused by inflammation passing from one orbit to the other through the optic foramen and the optic canal.

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