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Degeneration of Lens and Overgrowth of Harderian Glands in Hamsters Neonatally Injected with Parvovirus MVM-i[1] (41569)

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Abstract. Injection of newborn hamsters with 500 plaque-forming units or more of the parovirus MVM-i, produces an eye defect in a large percentage of the treated animals at maturity. The incidence is directly related to the amount of virus given and is independent of sex, size, or antibody levels to MVM-i. The abnormality is characterized by very small eyes with degenerate lens and adjacent retinal layers, and also by such extensive hypertrophy of the Harderian glands that they may encase the orbit and occlude the eye. On the basis of this latter finding and of previous work by other investigators, we suggest that the glands, whose function is unknown, may act as accessory photoreceptors.

A recent paper reported the inhibition of a carcinogen-induced tumor in hamsters by the parovirus, H-1 (1). As an adjunct to this experiment, another parvovirus, the mouse-derived MVM-i (2, 3) was tested for possible similar inhibitory capacity. A suggestive effect, not equal to that seen with H-1, was noted and will be described elsewhere. Of special interest, however, was the finding that hamsters of either sex that had received 500 pfu[2] of MVM-i at birth, the maximum dose given in our initial experiment, often developed an eye deformity characterized externally by small size and microscopically by degeneration of the lens and the immediately adjacent layers of the retina. Also, in direct relation to the degree of eye defect, there was an overgrowth of the Harderian glands such that they often completely covered and occluded the external orbit. The experiment was then repeated without administration of a carcinogen and with a greater dosage range. Again a similar eye abnormality was seen. Since this particular pathology of the eye has not been noted previously, and, in addition, the function of the Harderian glands, an organ common to all vertebrates except possibly the higher primates, has long been the object of much speculation, we are reporting our observations.

Materials and Methods. *Animals.* Pregnant outbred LAK Syrian hamsters (Lakeview Hamster Colony line) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. They were time bred to arrive at our laboratory on Day 4 or 5 after breeding and to give birth on the early morning of Day 16. Babies were counted in the forenoon and given a 0.05-ml subcutaneous injection of MVM-i parvovirus suspension in the late afternoon on the day of birth so that none would be born after injections were completed. Mothers were maintained on regular hamster chow with a daily spoonful of canned beef dog food which, we have found, prevents cannibalism of their babies.

Virus. MVM-i parvovirus, obtained through the courtesy of Dr. Solon Rhode, was originally a gift of Dr. Peter Tattersall in whose laboratory it was grown on murine lymphoma (EL-4) cells. In this laboratory it was cultured in 324K NB human cells (4, 5). After the number of pfu of virus in the cultured suspension was determined, six 10-fold dilutions were made in phosphate-buffered saline [0.02 *M* sodium phosphate (pH 7.4)–0.14 $NaCl_2$] such that 0.05 ml of suspension, the amount injected subcutaneously in the nape of the neck of each newborn hamster, contained virus in amounts varying from 0.5 to 50,000 pfu per injection. A seventh group of babies, the controls, were given PBS only.

Tissues and sera. Animals were etherized,

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[2] Abbreviations used: pfu, plaque-forming units; MVM-i, minute virus of mice; PBS, phosphate-buffered saline; E.D., eye defect.

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TABLE I. RELATIONSHIP BETWEEN DOSE OF MVM-i VIRUS GIVEN AND PERCENTAGE EYE DEFECTS

pfu of MVM-i injected at birth[a]	Total/E.D.[b]	Percentage E.D.
0.5	9/0	0
5	14/0	0
50	8/0	0
500	15/5	33
5,000	7/4	57
50,000	9/8	90
Controls (PBS only)	27/0	0

[a] Estimated on the basis of 10-fold dilutions of a suspension containing 50,000 pfu/0.05 ml.
[b] Number of hamsters/number with eye defects.

bled from the heart to obtain sera for the determination of antibody levels to MVM-i, and then killed by further etherization. Eyes were removed immediately thereafter according to the method of Wetterberg *et al.* (5) by using forceps placed deep in the optic cavity to pull the eye, with the base of the optic nerve and the overlying or adjacent Harderian glands, forward and out. They were then placed on small thin cardboards for orientation and fixed in 10% Formalin, processed and embedded in paraffin in the usual manner, and, after sectioning at 5 μm, stained with hematoxylin and eosin.

Results. Hamsters injected with the higher doses of MVM-i, i.e., receiving 500 pfu or more at birth, tended to be smaller than control animals. There were also a number of deaths in these litters especially among those injected with 5000 or 50,000 pfu. The eye abnormalities observed, however, were similar in all groups and were not related to host size or sex. On the other hand, there was a definite correlation between virus dosage and percentage of eye pathology (Table I). The greater amount of virus given at birth, the more E.D. were seen. In all cases except one (an animal receiving 500 pfu) both eyes were affected and in this animal there were microscopic changes in the lens of the apparently normal eye; also this particular hamster showed no overdevelopment of the Harderian glands. The dose of 500 pfu was probably relatively critical as babies of two litters injected in a separate test with 300 pfu (not recorded on Table I) appeared normal.

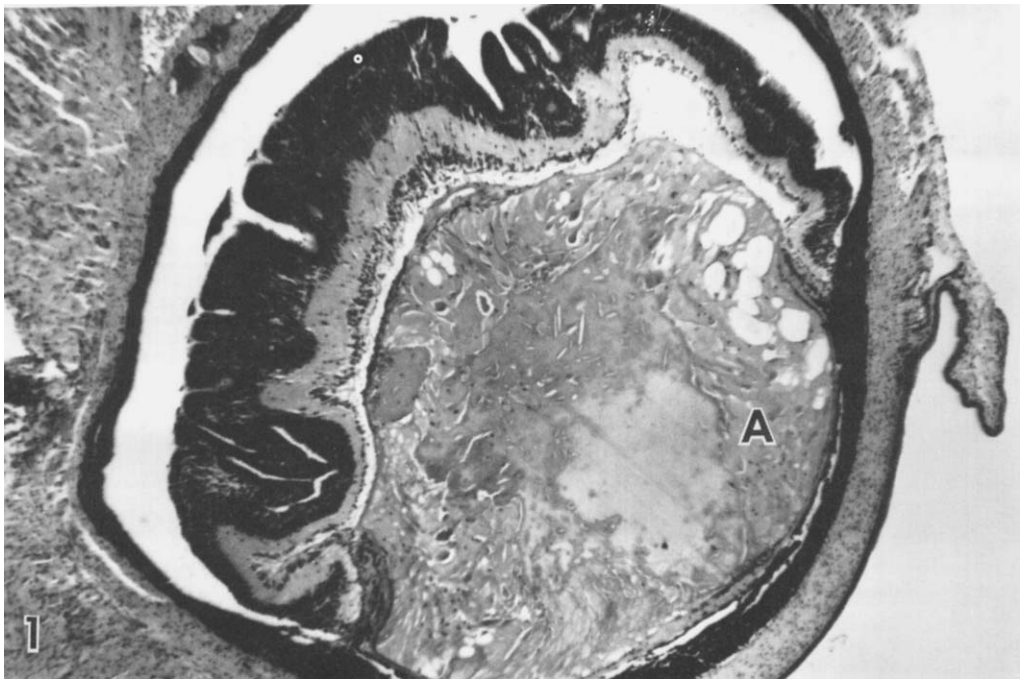

FIG. 1. Eye showing complete degeneration of lens (A). ×16.

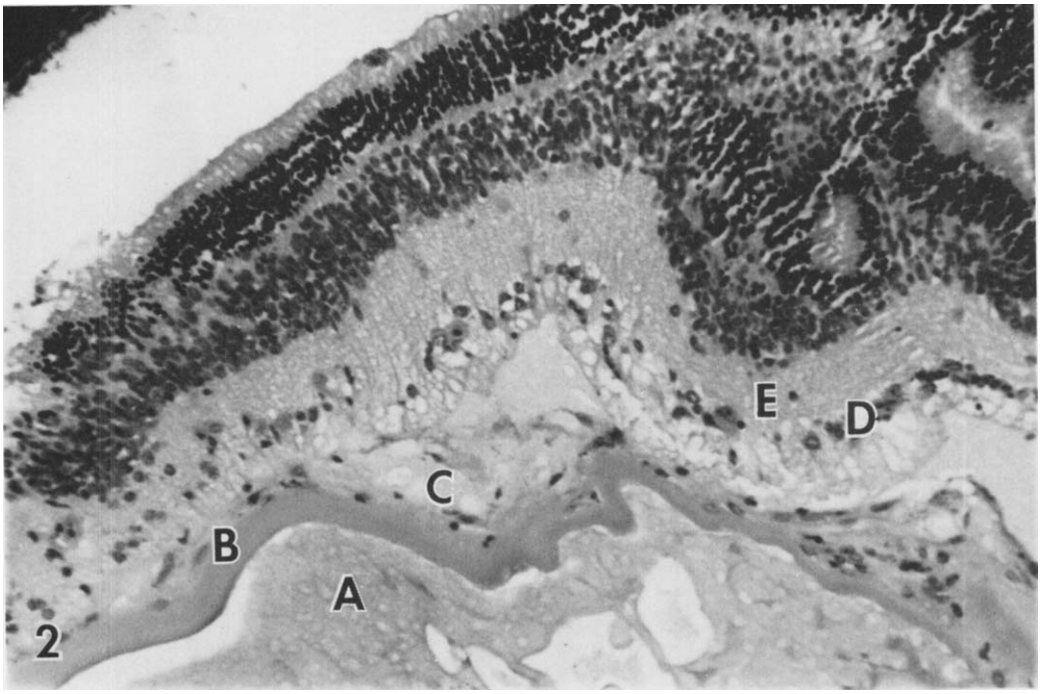

FIG. 2. Eye from another hamster showing edge of completely deteriorated lens (A), and degenerated adjacent retinal inner limiting membrane (B), optic nerve fibers (C), ganglion cells (D), and inner plexiform layer (E). Other layers of the retina appear normal. ×60.

Affected animals usually could be distinguished shortly after weaning (3 weeks) since their eyes remained small (i.e., about the size of a 14- to 20-day-old hamster or 1/3 the size of the normal adult eye). Subsequently, the Harderian glands gradually appeared in the nasal area of the orbit and grew out and over the eye, in some instances occluding it by the time the animals were 2 months old. Such afflicted hamsters tended to be harrassed by their more fortunate littermates but did well if separated and caged individually. All hamsters, both those with eye defects and their normal-appearing but injected siblings, as well as a group of control animals were bled and killed at 3–4 months of age. Hemagglutination-inhibition tests (6) for the presence of serum antibodies to the MVM-i virus, indicated that such antibodies were present and of similar levels in all the treated animals, with or without E.D., and absent in control hamsters. No differential organ pathology was observed visibly in the body tissues of the various groups. Since treated hamsters were generally smaller than control animals, a response to high doses of MVM-i similar to that recorded for hamsters after large doses of other parvoviruses such as H-1 and RV (7, 8), it follows that most of the hamsters with severe eye abnormalities were undersized. It is noteworthy, however, that several of the hamsters with E.D. were normal in size and weight while a few of the smallest animals had normal eyes. Therefore size, per se, is not directly associated with the E.D.

Histological examination of the abnormal eyes showed that in all instances the lens was primarily affected and degenerate (Figs. 1, 2). No vestige of the normal basal cuboidal layers or striations remained; usually there was only a vacuolated, amorphous, hyaline-like body present that was obviously nonfunctional. The adjacent retinal inner limiting membrane, optic nerve fibers, ganglion cells, inner plexiform layer, and, occasionally, the inner nuclear layer displayed various degrees of degeneration directly related to the extent of the lens pathology and to their proximity to this organ. As far as could be determined with the fixation and staining employed, the outer ret-

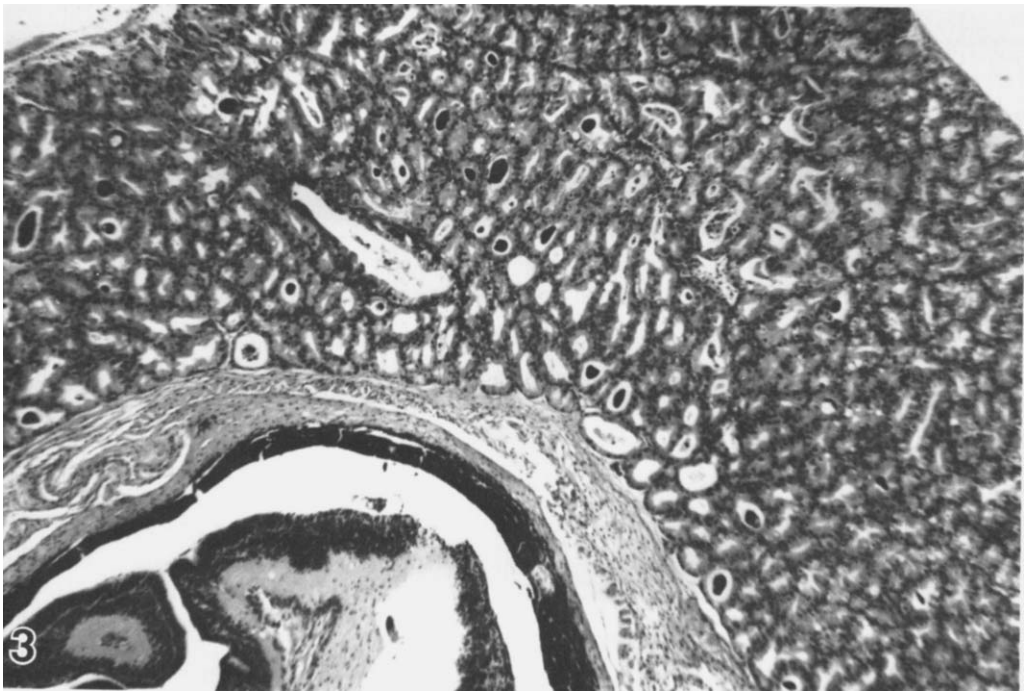

FIG. 3. Hypertrophied Harderian gland enveloping the orbit. The porphyrin content of the tubules indicates that this eye is from a female hamster. ×16.

inal layers appeared remarkably normal and intact, especially the outer plexiform layer, the outer nuclear layer, outer limiting membrane, the rods and cones, and the pigment layer. Where damage was great, small round cells in modest numbers were present, particularly in the inner plexiform layer. The choroid and sclera appeared normal. Except for the single instance already noted, the Harderian glands were always hypertrophied when an E.D. was present; occasionally they exhibited such massive overgrowth that they not only externally covered but totally encased the eyes (Fig. 3). In one animal the glands, while apparent in the inner canthus of both eyes, had confined their proliferation to the postorbital regions to such an extent that the small eyes were protruded outward. As documented previously and extensively by a number of investigators (9, 10, 11), these glands, though in this instance hypertrophied, were sexually dimorphic, those of females containing dark-staining porphyrin in the tubules and the glands of male hamsters, none. All cells of the hypertrophied Harderian glands of both sexes had large and numerous vacuoles, in contrast to comparable gland cells of normal hamsters where the cell vacuoles were smaller and more discrete in the female than they were in the male and in either case much less abundant than in affected hosts. Indeed a few of the Harderian glands in the animals with E.D. resembled a mass of vacuolated tissue with barely discernible glandular structure.

Discussion. Parvoviruses need dividing tissues for their proliferation (7, 12, 13) since their DNA replication requires specific host-cell functions, occurring in the late S-phase of cellular division (14). Therefore it is not surprising that the rapid cellular turnover in the developing eye might offer fertile ground for growth of MVM-i. Preliminary studies with MVM-i conjugated fluorescent antibodies and the eyes from 4- to 30-day-old hamsters given large amounts of this virus, have been suggestive but not conclusive to date so that no definite statement can be made about the presence of MVM-i in the affected eye regions. The specific relationship between the number of eye abnormalities and the concentration of virus given may reflect the immunosuppres-

sive nature of MVM-i (2, 3) in contrast to that of the prototype MVM (15); i.e., the greater the amount of virus present, the more any host resistance is overwhelmed. That the developing lens is a target organ for MVM-i seems apparent. Whether the degeneration seen in tissues adjacent to the lens indicates that they, too, offer a primary site for virus proliferation or whether their pathology is secondary to the lens deterioration cannot be determined at this time.

The Harderian gland overgrowth of the E.D. is most interesting. These glands have long been the subject of speculation. First described in 1694 by Harder (16) in deer, they have been found in almost all vertebrates except primates. Located in the inner canthus of the eye with a secretory duct near the third eyelid, they have been described generally as accessory lacrimal glands although the latter are found in the outer canthus. Sakai (17), in a recent extensive review, suggests that the mammalian Harderian glands originated phylogenetically from the lacrimal glands of ancestral mammals and that in aquatic mammals at the present time the function of these glands is to protect the eyes from sea water whereas in the rodents they may serve as a subaceous or pheromonal gland. Support for the latter function has been given by Payne (18) who indicated that fresh smears of the Harderian glands, especially those from females, were attractive to male hamsters. He further noted that the porphyrin content of the female Harderian gland alters during the estrous cycle and pregnancy (19) and that castration changes the male hamster gland to a female appearance (20). He concluded that some sexual function underlies the glands' presence.

Bucona and Nadahavukaren (21) have described the studies of a number of authors who have examined the fine structure of Harderian glands in various species. In their own detailed investigation of cellular components in Harderian glands of hamsters, they could come to no conclusion, on the basis of their observations, as to the function of these glands.

Of special interest to us has been work suggesting that the Harderian gland might act as a photoreceptor. In 1966 Zweig *et al.* (22) reported that blinded 12-day-old rats were receptive to light but that if hooded they failed to respond thereto. These investigators concluded that a nonretinal photoreceptor, influencing the pineal gland, existed somewhere in the head. Wetterberg *et al.* (5) extended these findings. He studied the circadian rhythm of pineal serotonin in rats and the influence of light thereon and found that removal of the Harderian glands abolished the response to light in blinded animals. He speculated that "since the visual elements of the mammalian retina face away from the incoming light but face toward the Harderian gland, the latter may act as a reflector and transducer of light information."

In the hamsters of this experiment we have been impressed with the definite correlation of lens defect and Harderian gland overgrowth and believe that our own observations may support the suggestion of Wetterberg *et al.* (5) that at least one of the functions of the Harderian glands is to act as a photoreceptor.

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