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Spontaneous Polar Anterior Subcapsular Lenticular Opacity in Sprague-Dawley Rats

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A spontaneous focal polar anterior subcapsular lenticular opacity characterized by focal epithelial proliferation was found in Charles River Sprague-Dawley rats from various breeding facilities around the world (France, Japan, and the United States). The incidence of this change slightly increased with age up to a maximal incidence of 9.8% in 28- to 35-week-old male rats (French source). Over that period, there was little change in the size of the opacity; however some rats that were examined over longer periods (more than 2 years of age) developed secondary anterior cortical changes, and rarely, histologic findings of pigmentation and/or mineralization. The lenticular change was present throughout the life of the animals and had no sex predilection; mode of inheritance was not investigated. Due to its small size, this lens opacity is more easily identified by use of slit lamp biomicroscopy than by use of indirect ophthalmoscopy, and serial sections of the eye aid in locating it for histologic evaluation.

Case Reports

Spontaneous ocular lesions are seen throughout the life of laboratory rats and many of them are similar to alterations induced by chemicals. These lesions, when detected, may complicate interpretation of ocular results in toxicity testing. A number of spontaneous lenticular changes have been reported in Sprague-Dawley (SD) rats in literature (1-6), as well as in other strains of rats (7-10). To the authors' knowledge, a polar anterior subcapsular lenticular change affecting principally the epithelium has not been reported in rats. We describe a focal polar anterior subcapsular lenticular opacity observed in Charles River SD rats from various breeding facilities, and discuss the pathogenesis of the lesion.

Data were collected from periodic routine ophthalmic examinations of untreated SD rats of both sexes used in a wide range of toxicity studies conducted from 1994 to 1999 at MSD-Chibret, Riom, France; in 1998 and 1999 at Merck Research Laboratories, West Point, Pa., and at Banyu Development Research Laboratories, Menuma, Japan. Data from Japan and the United States were obtained on Crl:CD(SD)IGS BR and data from France were obtained on Crl:CD(SD)BR and Crl:CD(SD)IGS BR rats. The animals (virus antibody free) were obtained as weanlings from Charles River France (Saint-Aubin-lès-Elbeuf, France), Charles River Laboratories (Raleigh, N.C.), and Charles River Japan Inc. (Tsukuba Breeding Center, Ibaraki Pref., Japan). The rats were free of viral (hantaviruses, pneumonia virus of mice, reovirus 3, Sendai, Kilham rat virus, rat coronavirus/sialodacryoadenitis virus, H1 and Theiler viruses), bacterial (Tyzzer's organism, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *P. multocida*, *Salmonella* sp., *Streptobacillus moniliformis*, β-hemolytic *Streptococcus* A, β-hemolytic *Streptococcus* G, and *Streptococcus pneumoniae*), and parasitic (ectoparasites, helminths, and pathogenic protozoa) pathogens.

Rats were examined at 5 weeks of age, and at later times (up to 35 weeks of age), if used as controls in different studies. Fifty female and 49 male Crl:CD(SD)BR rats that were not included in studies on the basis of pretest findings (including subcapsular lenticular opacities) were examined up to age 3 years in the French facility. All rats were housed individually in stainless-steel cages, fed a commercial diet (16 g/d for females and 22 g/d for males) and provided tap water ad libitum. Animal rooms were air-conditioned and maintained at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20% with a 12-h light cycle. Other husbandry conditions are listed in Table 1. In France and the United States, the animal facilities have animal care and use programs that were approved by AAALAC International and operated in accordance with current standards. The Japanese animal facilities conform with similar guidelines in that country. All study protocols were approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories (West Point, Pa.).

Eye examinations were performed, using indirect ophthalmoscopes of various brands, with interposition of a 28-diopter Nikon lens, and/or a hand-held slit lamp biomicroscope (Kowa Co., Ltd., Tokyo, Japan). Before examination, pupils were dilated by instillation of 0.5% tropicamide (Mydriaticum, MSD-Chibret, Paris, France) or 1% tropicamide (Mydriacyl, Alcon, Humacao, P. R.). Histologic examinations were performed on eyes from all control rats in toxicity studies and, in the French laboratory, on eyes from additional rats maintained up to approximately 3 years of age. For these examinations, rats were euthanized (exsanguination after CO_2 inhalation).

Eye specimens were fixed in neutral-buffered 10% formalin, processed in routine manner, embedded in paraffin, cut in 5-μm-thick sections, and stained with hematoxylin and eosin (H&E); some eyes were stained with periodic acid-Schiff (PAS) as well. Due to the small size of the lesion, serial sections were often necessary to retrieve the change seen during ophthalmologic evaluations.

The lesion consisted of a focal anterior subcapsular lenticular opacity (Figs. 1 and 2) located in the central portion of the lens. It was more easily detected by use of slit-lamp biomicroscopy than by use of indirect ophthalmoscopy. The incidence of this change within various age ranges in the 3 laboratories and the respective numbers of animals examined were as listed in Table

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Table 1. Husbandry conditions in rat facilities

	France	Japan	US
Lab chow	UAR A04C Certified Rodent Diet (UAR, Villemoisson-sur-Orge, France)	PMI Certified Rodent Diet #5002 (PMI Nutrition International, Brentwood, MO)	PMI Certified Rodent Diet #5002 (PMI Nutrition International, Brentwood, MO)
Light intensity*	350 lux	540 lux	500 lux
Air change	15 air changes/h	15 air changes/h	15 air changes/h

*At 1m above the floor, in the center of the room.

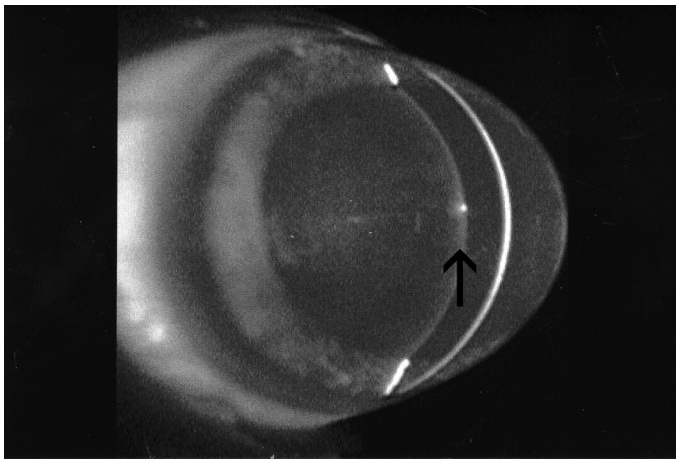

Figure 1. Discrete focal polar anterior subcapsular lenticular opacity (densitometric image; Nidek camera).

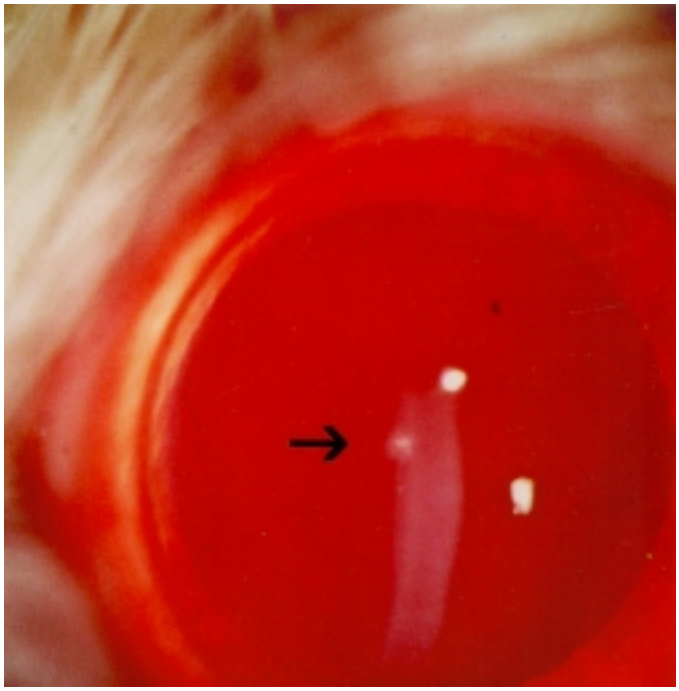

Figure 2. Discrete focal polar anterior subcapsular lenticular opacity (slit lamp biomicroscopy; magnification x 22).

2. Across the 3 laboratories, the incidence of the change ranged from 0 to 9.8%, but a conclusion cannot be drawn regarding possible age- or stock-related variations since the cumulative data were obtained from populations of different sizes and throughout different periods of examinations. However, the incidence slightly increased with age in the French rat population, with maximal incidence of 9.8% in the 28- to 35-week aged male rat population. This change was not related to other ocular abnormalities, had no sex predilection, and did not substantially progress in severity over this time.

Light microscopically, the lesion consisted of focal lens epithelial proliferation of different degrees of severity with, in some cases, extension toward the suture center, with slight hypertrophy of lens epithelial cells as well (Fig. 3). Rats kept up to approximately 3 years of age in the French laboratory had a 12% incidence of focal anterior subcapsular central lenticular opacity as weanlings. The incidence of the change increased with time (generally during the first year of observation) up to maximal incidence of 22% in males and 28% in females. As to the severity, during the second year of observation, some animals had an association of the focal anterior subcapsular central lenticular opacity with anterior cortical changes. Some anterior subcapsular central opacities progressed toward the anterior cortex or became associated with separate polar anterior cortical opacities. Most animals were euthanized during the third year of observation and had focal lens epithelial proliferation microscopically that was often associated with an accumulation of slightly eosinophilic basement membrane-like material, presumably produced by the proliferated cells (Fig. 4). This material, when characterized, had been shown to be PAS positive. In a few animals, the subcapsular changes contained foci of mineralization, and in rare instances, small amounts of a globular brownish pigment were present within the foci of epithelial proliferation. In some animals with more well-developed subcapsular changes, a slight disorganization of adjacent cortical fibers was present, although this was not readily distinguishable from artifacts often present in this area. It is not known whether this lesion is congenital or an early acquired one. Mode of inheritance of this change was not investigated.

Focal epithelial polar proliferation associated with marked cortical changes has been reported in rats submitted to ultraviolet-B irradiation (11), or given galactose (12) or cationic amphi - phili c drugs (13). Congenital or hereditary cataracts with epithelial and capsular proliferation together with abnormal lens fibers in SD rats (14, 15) and in other strains of rats (16, 17) have also been reported. In these animals, the principal ocular lesion appeared to involve the lens epithelium, which ultimately could account for the excess production of capsular material and, possibly, the changes in cortical lens fibers. However, the epithelial changes were always accompanied by marked changes in the cortical fibers, which was not the case for the spontaneous lens change described previously in the Charles River SD populations, where changes in the underlying anterior cortical fibers were generally absent, or only equivocal at an advanced age. There is no explanation for the location of the proliferation in the central area of the lens since, in normal rats, the active germinative zone is only present around the equator, and its activity decreases toward the axial zone. Focal epithelial polar proliferation

Table 2. Spontaneous focal polar anterior subcapsular lenticular opacities in Sprague-Dawley rat populations
Incidence (%) in males (M) and females (F)

	France (1994-1999)		Japan (1998-1999)		US (1998-1999)	
Age Range	Males	Females	Males	Females	Males	Female
3-6 weeks	3.0% (1475)	3.5% (1074)	1.2% (429)	1.2% (429)	-	-
7-12 weeks	2.4% (865)	2.6% (615)	5.6% (144)	3.6% (138)	0.9% (346)	0.6% (346)
14-17 wk	3.7% (459)	5.7% (435)	0% (150)	0% (149)	0.3% (329)	0.3% (339)
18-22 wk	1.5% (135)	2.7% (75)	3.3% (30)	0% (30)	0% (164)	0.6% (163)
28-35 wk	9.8 % (224)	4.6% (197)	2.8% (180)	3.9% (179)	1% (200)	0.5% (200)

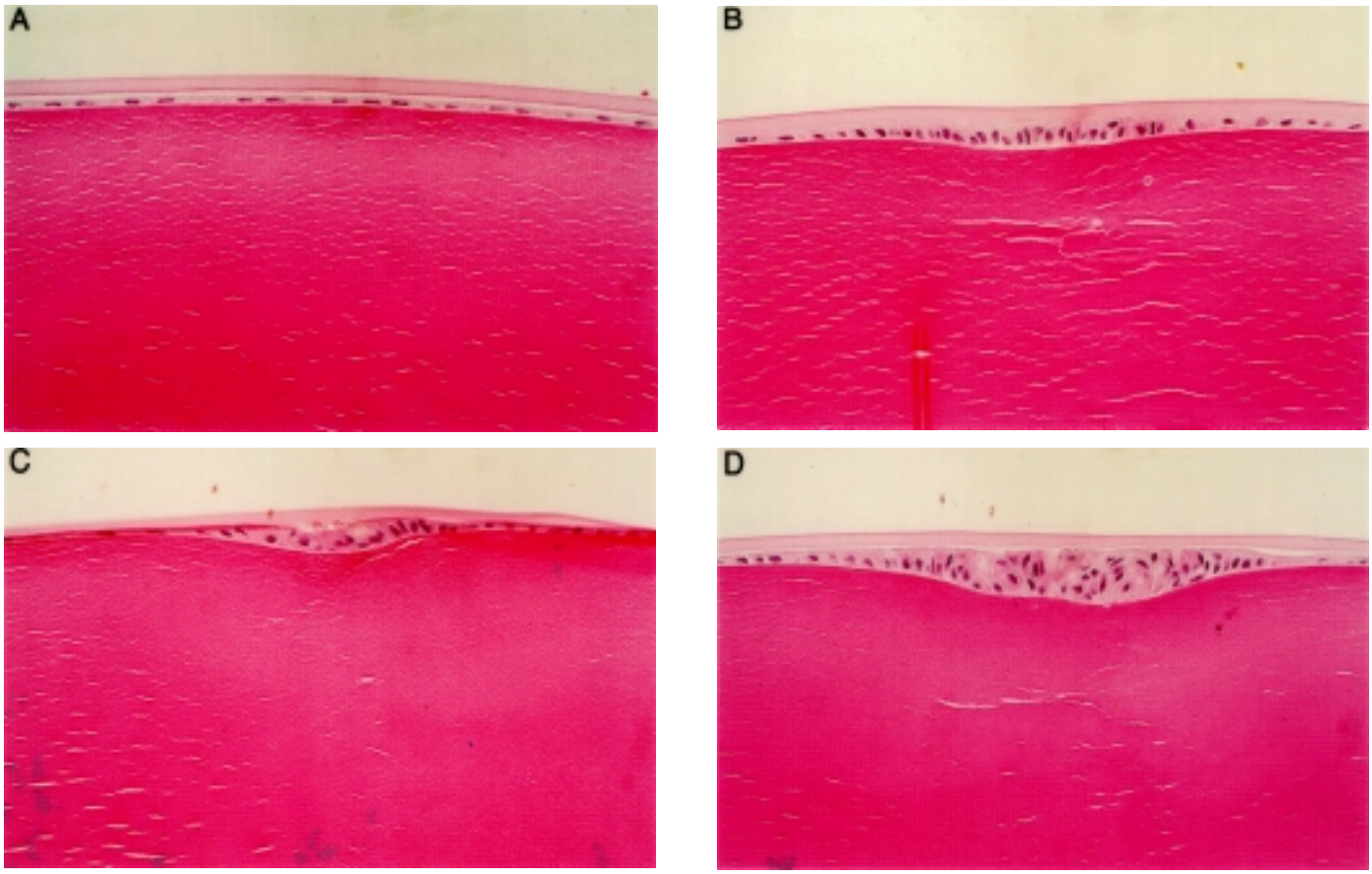

Figure 3. Photomicrographs of the polar anterior subcapsular area of the lens. Notice the slight to moderate epithelial proliferation associated with the clinically noted opacity, compared with the normal epithelium. H&E stain; magnification x 195.

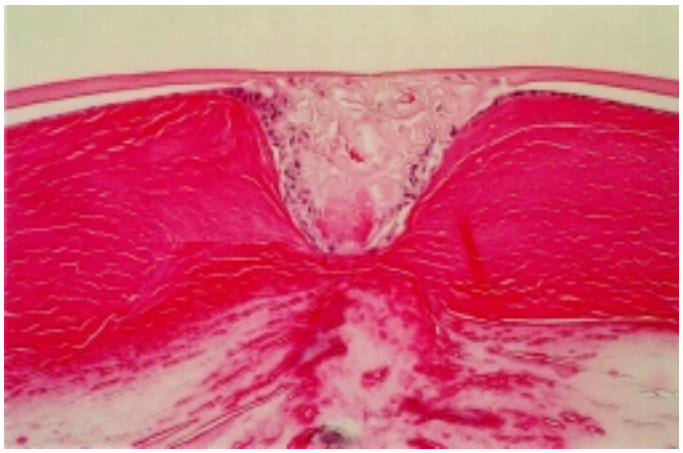

Figure 4. Photomicrograph of the lens opacity in aged rats. Notice epithelial proliferation and accumulation of eosinophilic material. H&E stain; magnification, x 88.

in humans has been reported, either as a congenital change or as the consequence of trauma, but always associated with degeneration of the underlying lens fibers (18). To the authors' knowledge, similar changes in other laboratory animal species have not been reported.

In conclusion, a spontaneous focal polar anterior subcapsular lenticular opacity was observed during ophthalmic examinations in Charles River Sprague-Dawley rats that corresponded histologically to a focal proliferation of the lens epithelium. This discrete lesion is different from the subcapsular lens changes described in rats of various strains after various physical or chemical treatments, or reported as hereditary or congenital cataracts. Once detected, the lesion is present throughout the life of the animals and does not substantially progress over time. The lesion has no sex predilection, but the pattern of inheritance has not been investigated. This change should be considered as a possible spontaneous ocular lesion when performing preclinical toxicity studies, and not mistaken for changes related to exposure to the agent under inves-

tigation. Due to its small size, this lens opacity is not as readily identified by use of indirect ophthalmoscopy as it is by use of slit lamp biomicroscopy, and serial sections of the eye aid in locating it for histologic evaluation.

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