bands III and IV. Also, the fractionation illustrated in the table shows a large increase in responsiveness of granulosa cells to FSH in cAMP synthesis in bands III and IV. The FSH was used since granulosa cells harvested from small porcine follicles are more responsive to FSH then LH in terms of cAMP accumulation[9].

Functional integrity of the granulosa cells obtained by the Percoll procedure was demonstrated by secretion of progesterone and binding of ^{125}I [hCG] to the cells incubated for 4 days in culture medium. The increase of about 7.4-fold in LH/hCG receptors was accompanied by a 3.5-fold increase in progesterone secretion by purified granulosa cells in band III in comparison with unfractionated cells. Granulosa cells used for a long-term incubation in culture need to be 3 times washed with culture medium[4]. Purification of granulosa cells on continuous Percoll gradients possesses several advantages. The Percoll gradient separates cells from cellular debris quickly and effectively (fig. 2). Percoll did not significantly change the osmolarity of the culture medium (310 mOs/kg H_2O in our experiment) and it can be prepared in sterile conditions prior to use. Even though the granulosa cells were found in all bands tested, band III contained the highest activity of cells with only small contamination by cellular fragments. This method of purification has considerable value for the investigation of many aspects of intracellular mechanisms of the maturation and differentiation of granulosa cells.

Production of cAMP (in 60-min incubation), progesterone secretion and ^{125}I[hCG] binding (in 4-day culture) to unfractionated and Percoll fractionated pig granulosa cells

	cAMP content (pmoles 10^{-6} cells) n=4		Progesterone secretion (ng 10^{-6} cells)	^{125}I[hCG] bound (cpm 10^6 cells)
	−FSH	+FSH		
Unfractionated cells	0.79±0.05	10.90±0.99	89.7± 7.5	956±137
Band I	2.25±0.20	2.65±0.10	87.2± 8.7	341±109
Band II	0.78±0.12	2.87±0.26	159.2±12.8	735±122
Band III	2.21±0.25	41.50±0.63	317.0±23.2*	7096±980*
Band IV	2.61±0.21	59.20±7.50	181.5±18.6	2680±187

The mean values ± SEM are expressed per number of viable cells.
*$p < 0.001$: compared to band II and Band IV.

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A new strain of rat with an inherited cataract

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Summary. A new rat strain has been developed, in which a spontaneous cataract occurs without exception at 3–4 months after birth and matures completely at 4–6 months of age, indicating that this rare strain possesses a maturity-onset cataract. In the present report, histological data are presented and discussed.

Although the occurrence of hereditary cataracts in mice has been reported[2–12], the application of these cataracts to the study of the etiology of human cataract has been restricted, because hereditary mouse cataracts occur in the embryonic or neonatal period and the available samples are very small. If hereditary cataracts occurred after birth in larger animals, this would be a more useful tool in studying the etiology of human cataracts.

In rats, hereditary cataracts have also been developed[13–19]. Among them are the cataracts that occur only after birth in Bourne-Grüneberg[14] and the Léonard-Maisin[16] strains. However, the cataract in the former strain is complicated by retinal degeneration and remnants of the hyaloid artery, while in the latter strain, the anterior polar cataract occurs spontaneously in the neonatal period, associated with epithelial hyperplasia and lens fiber degeneration in this area, perhaps through a mechanical stimulus of the cornea. In contrast to these strains, the strain now developed is characterized by the fact that cataract formation occurs definitely at 3–4 months after birth in every animal without any significant complication in the eye. The strain, which is tentatively termed the ICR cataractous rat, was developed by successive mating and selection.

Methods. The occurrence of cataract was examined biomicroscopically by using a slit lamp (Kowa), and the excised lenses were examined macroscopically. For histological

Results of back-cross experiment

Sex		♂		♀	
Cataract		+	−	+	−
P-generation*	Number	8(ICR)			8(JCL)
F_1(ICR×JCL)	Number	0	46	0	50
F_2(P×F_1)	Number	15	46	17	42
	% Ratio	24.6%		34.8%	

*8 pairs of ICR(♂)×JCL(♀) were mated, resulting in 8 litters of F_1 offspring. Though crosses were made between ICR(♀) and JCL(♂), no cataractous offsprings (♂: 48, ♀: 51) were obtained.

examination, the lenses were fixed in 10% formalin neutralized with calcium carbonate, embedded in plastic, i.e. water soluble methacrylate[20,21] (Poliscience's JB-4), and the 2-µm-thick sections were stained with toluidine blue. For demonstration of calcium deposits, Kossa's method was used for the paraffin sections. The inheritance mode was examined by a back-cross experiment; male cataractous rats were outcrossed to JCL Wistar rats and the F_1 females were back-crossed to their fathers.

Results and discussion. Macroscopic and biomicroscopic observations of the lenses at various stages revealed that at 1 month of age, the transparency of the lens appeared to be fairly normal. At 2 months of age, a slight or very faint diffuse opacity was observed in the posterior subcapsular area, especially around the suture, due to extensive swelling of the lens fibers; however, the larger remaining lens area retained almost normal transparency (fig. 1a). This incipient condition continued until just before the rapid occurrence of a mature cataract (fig. 1b). At the earliest stage of the cataract onset (after 3 months of age), a dense opacity became noticeable in the posterior polar region; this opacity rapidly extended to the whole cortical and the superficial nuclear zone of the posterior, then to the perinuclear zone of the anterior, and finally to the whole nuclear region (figs 2a and 2b). Thus, at about 4 months of age, a mature opacity could be seen over the whole lens area, excluding the anterior superficial and equatorial regions of the cortex. Histological examination at 1 month of age revealed a fusiform, elongated or spherical swelling of the lens fibers in the cortex except for the equatorial region. This swelling of the lens was found to be homogeneous, granular, or hydropic in nature (fig. 3). No abnormal proliferation of the lens epithelium accompanied by focal fiber necrosis was found in the anterior polar region such as that seen in the Léonard-Maisin strain[19]. At 2 months of age, more progressive changes were observed, especially in the posterior cortex. A number of swollen fibers were seen in the nucleus.

At cataract onset (after 3 months of age) the degeneration process had become pronounced, especially in the whole posterior region, the anterior perinuclear region, and in the nucleus, the location of which appeared to have shifted to the posterior region (fig. 4). Characteristic changes in the nuclear fibers were dissociation, atrophy, confluence and disintegration as well as homogeneous or diffuse swelling; finally they appeared as many large or small amorphous

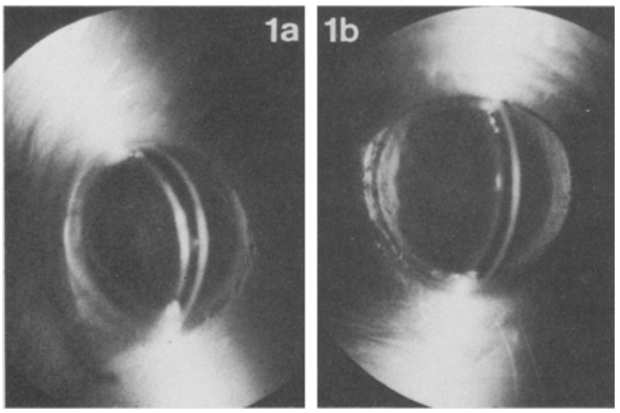

Figure 1. *a* Slit-lamp photograph of a 2-month-old rat of the ICR-strain, showing a localized opacity in the posterior subcapsular region. The remaining area shows almost no cloudiness. *b* Eye of a 3-month-old rat just before cataract occurrence. A very faint opacity in the posterior subcapsular region is observable. The anterior suture is also faintly seen.

Figure 3. Anterior cortical zone of a lens from an 1-month-old ICR rat. A distinct swelling of the fibers can be seen in this region (arrow), including the pre-equatorial zone. Scale: 50 µm.

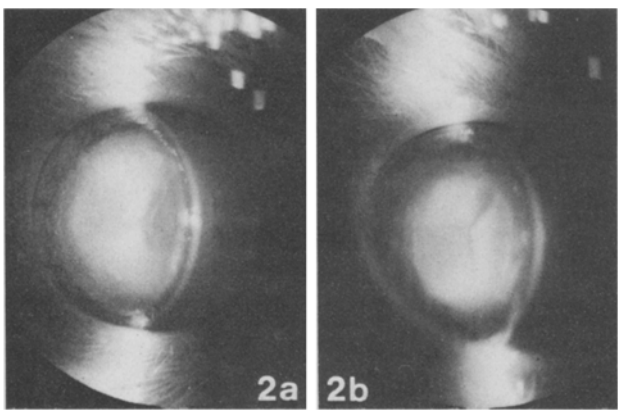

Figure 2. Eyes of a 3-month and 14-day-old rat immediately after cataract occurrence. *a* Anterior cortical region retains significant transparency. A tortuous suture can be seen on the anterior surface and a remarkable opacity in the posterior deep zone. *b* Retroillumination shows an extensive cloudiness in the posterior cortical region. The posterior suture is distinctly seen.

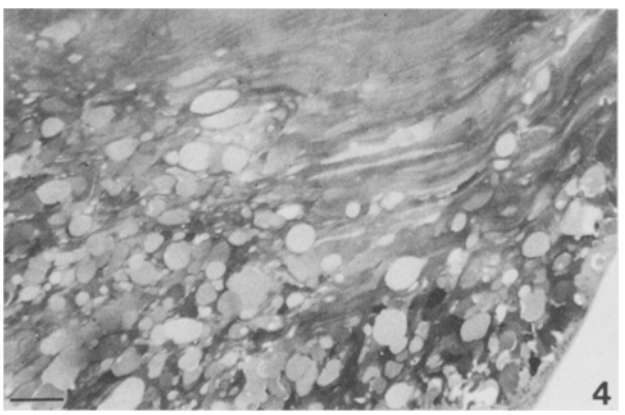

Figure 4. Posterior cortical zone of a lens from a 3-month-old ICR rat. A pronounced and crowded swelling of lens fibers is visible in this region. In the upper portion of this photograph, nuclear fibers can be seen showing distinct signs of degeneration such as dissociation, swelling, and atrophy. Scale: 50 µm.

structures, suggesting a liquifaction process. After complete maturation, calcium deposits became observable in the anterior outer region of the nucleus.

With regard to these morphological alterations of lens fibers, it may be speculated that the degeneration of protein in the lens occurs at a definite life stage either primarily or secondarily. The metabolic disorder responsible for the degeneration requires further investigation.

The mode of inheritance was examined by means of a back-cross experiment. The results are shown in the table. Although the percentage of cataract occurrence was higher in the female F_2 rats, the ICR-cataract is considered to be transmitted through recessive gene(s).

All these observations show that the strain now developed is unique among the various hereditary cataract strains so far reported, and this strain may be useful to elucidate the mechanism of cataract occurrence and as a model for human cataracts.

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Nucleolar organizer regions in *Biomphalaria* and *Bulinus* snails

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Summary. A method is described for the demonstration of nucleolar organizer regions (NORs) in freshwater snails and is applied to the study of one tetraploid and several diploid populations of *Bulinus*. We present evidence of dosage compensation with respect to the expression of NORs in that diploids and tetraploids both exhibit only 1 pair of NOR-bearing chromosomes.

Unexpected patterns of gene regulation may be important in determining the characteristics of hybrid and polyploid organisms. Motara and Rai[3] have demonstrated an unpredicted distribution of C-bands in hybrids of *Aedes aegypti* and *Ae. mascarensis*. Becak and colleagues have shown that total RNA (Becak and Goissis[4]), lactate dehydrogenase activity and hemoglobin content (Becak and Pueyo[5]) are similar in diploid and tetraploid frogs of the genus *Odontophrynus*. A reduction in the number of ribosomal cistrons may be responsible for this effect (Ruiz et al.[6]), although the evidence on this point is not clear. Tymowska and Fischberg[7] have reported only 1 chromosome pair bearing a secondary constriction in both diploid *Xenopus laevis* and the apparent autotetraploid *X. bunyonensis*. We may refer to a mechanism, apparently operating in these cases, and resulting in the production of quantitatively equivalent gene product from differing numbers of alleles, as dosage compensation[8]. Such a mechanism may not operate, however, in the hexaploid *X. ruwenzoriensis*, in which 3 pairs of NOR-bearing chromosomes are found[7].

The snail genus *Bulinus* comprises a naturally occurring polyploid series which we have shown to be of hybrid origin[9]. We have been studying *Bulinus* and the closely related genus *Biomphalaria* from a cytogenetic point of view, with particular emphasis on problems of chromosome evolution and gene regulation in polyploids. We report here our findings concerning the distribution and behavior of nucleolar organizer regions (NORs) in these genera.

In that cytological staining to reveal NORs[10–13] provides an assay for the activity of specific genes[14,15], the technique affords an opportunity to study directly the genetic effects of polyploidy and hybridization (see Brown[16], for a review of similar applications in plants and mammals). With this in mind we have applied the technique of Howell and Black[13] to the study of NORs in the snail genera *Biomphalaria* and *Bulinus*.

Snail stocks are briefly described in the data tables. Details, descriptions and maintenance regimes may be found in Goldman et al.[17].

G-banding of chromosomes was accomplished with air-dried preparations according to the ASG procedure found in Goldman et al.[17]. Ag-NOR banding to reveal nucleolar organizer regions was done on unstained slides which had been incubated in a dry oven at 40 °C for 1–3 days after air drying. Slides were stained according to the method of Howell and Black[13] keeping the staining mixture on the slide on a slide warmer for approximately 90 sec. Slides were rinsed, air-dried briefly, oven dried at 40 °C overnight,