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*.

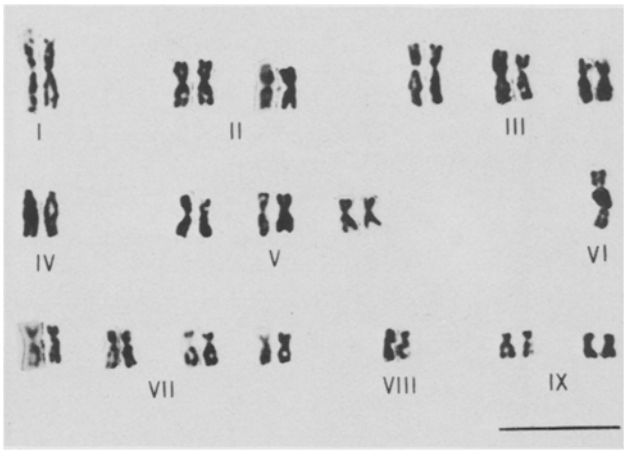
Snail stocks are briefly described in the data tables. Details, descriptions and maintenance regimes may be found in Goldman et al.[17].

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,

Table 1. Association of lightly staining arms and response to silver staining in Ag-NOR studies

Species	2n Chromosome No.	Cells scored	LSA[a] pairs present	Percent LSA pairs Ag-positive[b]	LSA Pairs associated No.	Percent	Number of additional Ag-positive regions 1	2
Bulinus tropicus	36	3	3	100	1	33	0	1
Bulinus natalensis	36	5	5	100	1	20	1	0
Bulinus truncatus/Egypt	72	4	2	100	0	0	0	1
Biomphalaria glabrata/NIH 6-4-1	36	12	12	100	4	33	0	1
Biomphalaria glabrata/NIH 10R2	36	11	10	100	8	80	1	1
Biomphalaria glabrata/Brazil	36	7	6	100	3	50	0	0
Biomphalaria glabrata/ Dominican Republic	36	1	1	100	0	0	0	0

[a]Stands for 'lightly-staining arm'; [b]staining darkly by the silver-staining technique of Howell and Black[13]. See text for further explanation.



Karotype of *Biomphalaria glabrata* NIH strain 6-4-1, treated to show NORs in group VI. Bar is 5 µm.

Table 2. Association of lightly-staining arms in cells not stained for Ag-NORs

Species	Association of LSAs LSA[a] pairs observed	No.	Percent	Total cells
Bulinus tropicus	25	7	28	35
Bulinus sp. (2n = 36)/ Mazoe Dam	5	0	0	8
Bulinus natalensis	9	1	11	18
Bulinus truncatus/Egypt	36	7	19	48
Bulinus truncatus/Ethiopia	2	0	0	2
Biomphalaria glabrata/ NIH 6-4-1	7	6	86	7
Biomphalaria glabrata/NIH 10R2	9	8	78	9
Biomphalaria glabrata/Brazil	8	3	38	10
Biomphalaria glabrata/ Dominican Republic	12	4	33	15
Biomphalaria straminea	2	1	50	2

[a]LSA stands for 'lightly-staining arm'.

then counterstained for 14 sec with 1:50 Gurr's improved R66 Giemsa in Gurr's buffer, pH 6.8. NOR-banded slides were photographed with black and white film, but the most satisfactory reproduction of the slides was obtained by photography with Kodak tungsten Ektachrome ASA 160 color slide film without a filter.

We reported for *Bulinus tropicus* and *Bulinus truncatus*[9] that the entire lightly staining arm (LSA) of the group VI chromosome pair stains positively as an Ag-NOR when slides were treated according to the procedure of Howell and Black[13]. The procedure has been repeated on 4 strains of *Biomphalaria glabrata;* an example of the karyotype from an NOR-stained metaphase cell of *Biomphalaria glabrata*/6-4-1 is shown in the figure. In every case, it was clear that the short arm of the LSA pair stained positively. There was some evidence for other pairs of chromosomes which showed positive staining as well defined terminal dots in some but not all of the preparations. Table 1 shows that this occurs in from 0 to 50% of cells scored. For the present, we consider only the LSA short-arm regions to be positive staining NORs.

Miller et al.[14] showed that positive staining Ag-NORs represent nucleolar organizer regions which were actively synthesizing ribosomal RNA during the previous interphase. King[18] has pointed out that not all Ag-positive regions are NORs. Nucleoli in interphase tend to fuse, and this fusion is often preserved into the following metaphase as an association among those chromosomes which gave rise to the nucleoli (i.e., the NOR chromosomes). Therefore the association or close apposition of the LSA pairs in metaphase cells (see tables 1 and 2) suggests NOR activity. The tables indicate a frequency of association varying from 0–33% in *Bulinus* and 33–86% in *Biomphalaria.* There was no clear relationship between the intensity of staining and the degree of association in particular cells. In the figure, showing the Ag-NOR stained karyotype of *Biomphalaria glabrata*/6-4-1, the group VI chromosomes were clearly associated and the positive staining regions fused.

The tetraploid *Bulinus truncatus,* like the diploid *Bulinus* species, shows only 1 clearly active pair of NORs – the LSA chromosome pair. This is consistent with the appearance of only 1 pair of LSA chromosomes in the tetraploid. Additional silver-positive regions were observed in a minority of cells, but this included both diploid and tetraploid cells. The degree of LSA association, which we take to be an indication of activity or size of the NOR region, was found to be less in the tetraploid than in the diploid. This observation is also consistent with our conclusion that the number or activity of NORs is not increased in tetraploid as compared with diploid snails, i.e., that dosage compensation does occur in tetraploid *Bulinus.*

In their study of polyploid *Odontophrynus,* Ruiz et al.[6] found that many tetraploid individuals had 4 or more Ag-positive regions, and that artificially produced hexaploids had NORs on all 6 homologous chromosomes. Intrapopulation polymorphism for the number of NORs was also encountered.

It is clear that extreme caution is required in interpretation of NOR data, because there is strong evidence that NOR activity is influenced by tissue derivation (Hsu et al.[19]) and

by laboratory conditions (Sasaki and Makino[20]; Palmer and Funderburk[21]; Bruere and McLaren[22]; Beck and Mahan[23]). On the basis of the data presently available, the number of active NORs in *Bulinus* appears to be independent of the number of genomes present.

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Continued in vitro and in vivo release of an antitumor drug from albumin microspheres[1]

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Summary. Heated albumin microspheres with an average diameter of 45 ± 8 μm and containing mitomycin C, released, in vitro, about 20% of this antibiotic over a 3-day period. VX-2 tumors were implanted into the hind leg of rabbits and the drug-containing microspheres were injected into the femoral artery of these animals. High levels of the drug were maintained for several hours in the tumor and growth of the tumor was inhibited considerably, compared to findings in control rabbits given the conventional mitomycin C. Half the number of the rabbits treated with our new method are alive with no evidence of tumor.

Antitumor drugs are often prescribed to treat a malignancy when surgery is not feasible. When the tumor thrives on a blood supply from an end-artery, these anti-tumor drugs are either infused or perfused, in order to obtain high local concentrations of the drugs with relatively few side effects. Methods describing the local application of drugs to treat tumors have been reported[4–8]. To provide a depot for the accumulation of the anti-tumor drug at the tumor-occupied site, we evaluated the depot effect of albumin microspheres containing mitomycin C (MMC) embolized in arteries on VX-2 tumors implanted into the hind leg of rabbits.

Materials and methods. Preparation of albumin microspheres. Bovine serum albumin microspheres containing MMC (Kyowa Hakko, Co., Ltd, Tokyo) were prepared by a modification of the method of Scheffel et al.[9]. In brief, an aliquot of MMC-albumin aqueous solution was emulsified in cottonseed oil containing Span 85, solidified at 150–170 °C and the solidified microspheres then immersed in ethyl ether to remove the oil. The drug concentrations were 8–10% and the average diameter 45 ± 8 μm, as shown in figure 1. Bovine serum albumin and Span 85 were purchased from Seikagaku Kogyo Co., Ltd (Tokyo) and Wako Pure Chemicals (Tokyo), respectively.

In vitro drug release. Drug release from microspheres was determined by a dynamic dialysis system with a cellulose tube (Visking Co., Chicago, Ill., USA). 100 mg of microspheres containing MMC were suspended in isotonic phosphate buffer (pH 7.2). To remove the MMC adhering to the microspheres, 10 min sonication and centrifugation at 50 × g were carried out; subsequently, the precipitate obtained was resuspended in 3.0 ml of isotonic phosphate buffer (pH 7.2) in a cellulose tube. The suspension in the cellulose tube was dialyzed at 37 °C against 47 ml of isotonic phosphate buffer. The inner (3.0 ml) and outer

Summary of experimental procedures (male albino rabbits)

Procedures performed	Control (n = 10)	Placebo microspheres (n = 10)	Conventional MMC (n = 20)	MMC microspheres (n = 25)
Drug administered	0.9% NaCl	Placebo microspheres (no MMC)	1.2 mg/kg of conventional MMC	MMC microspheres (1.2 mg/kg as MMC)
MMC levels in peripheral blood			Measured (3 animals)	Measured (3 animals)
MMC levels in VX-2 tumor tissues			Measured (10 animals)	Measured (12 animals)
Measurement of VX-2 tumor growth	yes	yes	yes (7 animals)	yes (10 animals)
Assessment of survival duration	yes	yes	yes (10 animals)	yes (10 animals)