site of the injected DMBA as was the case in rabbits and chickens. Transfer of living cells from the DMBA-tumours into chickens did not elicit any tumours. Experiments underway indicate that i.m. injection of RSV-SR into DMBA-treated rats, 4 weeks of age, induces tumours at the site of the virus injection which do not occur in untreated rats[10].

Zusammenfassung. Zwei bis drei Wochen alte Ratten wurden einer kombinierten Einwirkung von 7,12-Dimethylbenz(*a*)anthrazen und Rous-Sarkoma-Virus ausgesetzt. Das chemische Karzinogen wurde i.m., das Virus dagegen i.v., zugeführt. Viele der Ratten entwickelten 3–4 Monate später Fibrosarkomen und Zysten ohne topographische Beziehung zu dem lokal deponierten Karzinogen. In einem Fibrosarkom der Leber konnte der Rous-Sarkoma-Virus nachgewiesen werden. Virus allein war nicht imstande, Tumore und Zysten in gleichaltrigen Ratten hervorzurufen. Das chemische Karzinogen erzeugte Tumore nur am Platze der i.m. Injektion.

C. G. AHLSTRÖM and JULIET HEATON

Institute of Pathology, University of Lund (Sweden), 27 November 1967.

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PRO EXPERIMENTIS

A Method for Cytogenetic Study of Planarians

Investigation of chromosomes of planarians in mitosis and meiosis is of particular importance for the knowledge of the problem of polyploidy and its mechanism in animals. Up to the present, for the analysis of chromosomes, squash methods with dissolvable cover slips and acetic-orcein have been applied on gonads and cocoons by MELANDER[1–3]. BENAZZI[4] has combined the acetic-carmine squash method with colchicine as medium on the blastema.

We have modified the acetic-orcein method by pretreatment used in the culture of mammalian cells in vitro. By applying colcemide and hypotonicity it is possible to analyse the chromosomes of sexually mature animals more precisely. The modified method also facilitates the study of the mechanism of meiosis.

Methods. Two species of the endemic genus *Neodendrocoelum* Stank[5], *N. grande* and *N. maculatum*, were taken out of the well of the Lake of Ohrid. The tissues for chromosome analysis were the testes and ovaries of sexually mature animals. The neoblasts of the regenerative blastema in planarians show regional differences in chromosome sets[6]; this somatic tissue was not taken for analysis.

For the pretreatment, colcemide Ciba was employed. At the suggestion of Dr. Y. MELANDER low concentrations of colcemide was used in order to prevent the appearance of induced polyploidization. The concentration of 12 γ/ml gave the best results. This concentration was strong enough to give a contraction effect on the chromosomes but did not produce polyploidy. Colcemide was dissolved in well water in which the animals could survive the pretreatment. Two planarians at a time were placed in a Petri-dish containing 16 cm^3 of colcemide solution, and then transferred into a thermostat at a temperature of 15–16 °C. The planarians were kept in a thermostat for 4–5 h.

In consideration of the low osmotic pressure of the planarian tissues[7], distilled water was used for the hypotonic pretreatment. From the colcemide solution, a planarian was placed on a slide. Under a microscope (objective $\times$ 3) 2 lateral pieces from the testis and the ovary approximately 1 mm in length were dissected using a microscalpel. The 2 dissected pieces of gonads were put into a Petri-dish with distilled water and incubated at 20 °C for 30 min. It should be pointed out that the temperature for hypotonicity was a decisive factor both for the effect of hypotonicity and for the prevention of decomposition of tissues.

After the hypotonic treatment, the pieces of testis and ovary were transferred by a micropipette to 50% acetic acid. Fixation lasted for 4–7 min, and the fixative was changed twice.

The pieces of gonads were transferred, 1 by 1, from the fixative to a slide and macerated with the watchmaker's forceps into a milky cell suspension[8]. A few drops of 2% acetic-orcein in 60% acetic acid were added to the sus-

Table I. Chromosome number of *N. grande*

	Mitosis		No. of animals
	testes	ovaries	
No. of chromosomes	$2n = 32$	$2n = 32$	
No. of cells analysed	12	10	4

Table II. Chromosome number of *N. maculatum*

	Meiosis		No. of animals
	testes	ovaries	
No. of chromosomes	$n = 16$	$n = 16$	
No. of cells analysed	11	21	9

[1] Y. MELANDER, Hereditas *34*, 512 (1948).
[2] Y. MELANDER, Hereditas *36*, 19 (1950).
[3] Y. MELANDER, Hereditas *49*, 119 (1963).
[4] M. BENAZZI, Atti Accad. nac. Lincei, Rc. [8] *40*, 999 (1966).
[5] S. STANKOVIĆ, *The Balkan Lake Ohrid and Its Living World* (Mitgeverij Dr. W. Junk, Haag 1960), p. 357.
[6] M. BENAZZI, Chromosoma *19*, 14 (1966).
[7] P. RÖHLICH, Z. Zellforsch. mikrosk. Anat. *73*, 165 (1966).
[8] T. S. HAUSCHKA and V. V. BRUNST, Hereditas *52*, 345 (1965).

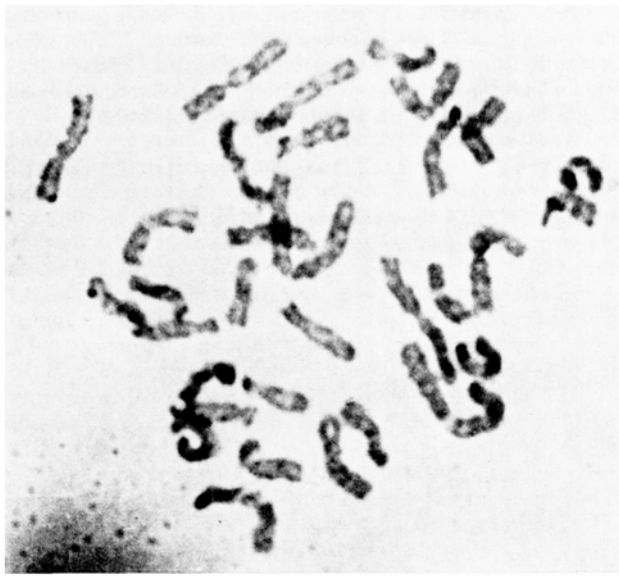
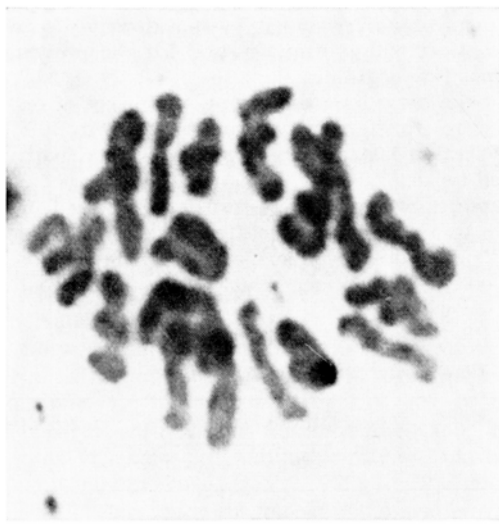
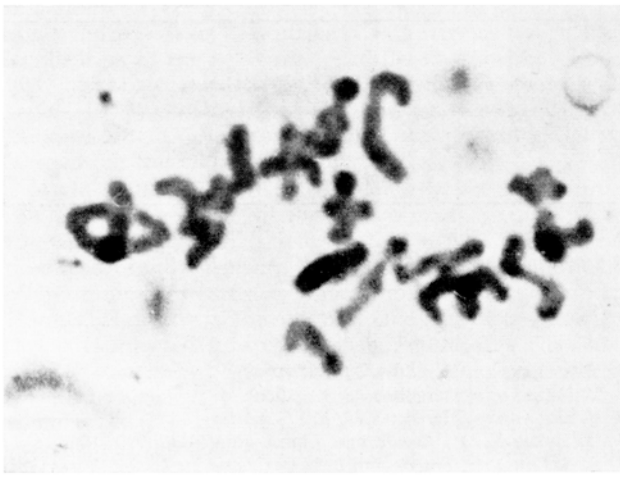


Fig. 1. Spermatogonial metaphase. *N. grande.*



a



b

Fig. 2. (a) Oocyte M I. *N. maculatum.* (b) Spermatocyte M I. *N. maculatum.*

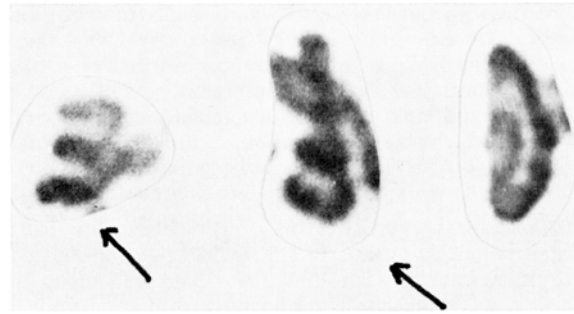


Fig. 3. Cut-out characteristic bivalents. Arrows indicate bivalents with loops.

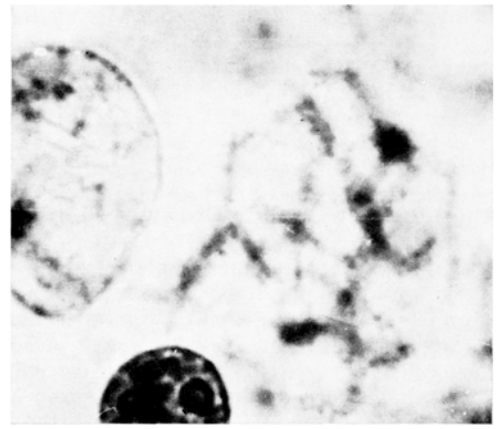


Fig. 4. Pachytene stage. *N. grande.*

pension and the specimens were squashed under the coverslip (22 × 40 mm) and then sealed with paraffin (melting temperature 58 °C).

Results. The effectiveness of the pretreatment can be judged from the good morphology of chromosomes and suitable spreading of metaphase figures (Figure 1). In the testis and ovary, bivalents with 1 or 2 chiasmata are most common at metaphase I (Figure 2). The most characteristic bivalents are shown separately (Figure 3). The chromosome number of *N. grande* and *N. maculatum* is $2n = 32$ (Tables I and II). The method also gives the possibility of analysing the meiotic prophase (Figure 4). The fact that the concentration of colcemide employed did not induce polyploidy, permits studies of experimental polyploidy[9].

Zusammenfassung. Eine neue Methode zur Analyse mitotischer und meiotischer Chromosomen in Hoden und Ovarien von Planarien wird beschrieben. Vorbehandlung der Tiere mit Colcemiden und destilliertem Wasser. Fixierte Gonadenfragmente wurden in Milchzellsuspension mazeriert und die Chromosomen mit Orceinsäure gefärbt und mit der Quetschtechnik untersucht.

D. M. Paunović and D. S. Rimsa

Division of Genetics, Institute for Biological Research, Belgrade (Yugoslavia), 27 October 1967.

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