

AUTORADIOGRAPHIC STUDY OF KINETICS OF SPERMATOGENESIS IN ASCARIS LUMBRICOIDES

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Abstract. The kinetics of meiosis and spermiogenesis in *Ascaris lumbricoides* in vitro was studied by keeping 12 mature males in nutrient medium. The spermatocytes were made radioactive by supplementing the medium with ^3H -thymidine for 2 h, following which they were transferred to fresh medium without ^3H -TdR. The nematodes were then killed for preparing histological and squash preparations of the testis. An analysis of the migration of "hot" spermatocytes till the formation of mature spermatozoa, in Kodak AR—10 autoradiograms, suggested that the duration of meiosis and spermiogenesis was a little more than 5.5 and 7.6 days, respectively. The total duration of these two events was about 13.1 days when radioactive spermatozoa were detected for the first time.

The duration of meiosis and spermiogenesis in *Ascaris lumbricoides* was estimated autoradiographically by monitoring progression of labelled spermatocytes from the onset of meiosis till the formation of fully formed spermatozoa. Twelve mature *Ascaris lumbricoides* were maintained in a culture medium composed of essential aminoacids and glucose (Rothstein and Nicholas 1969).

MATERIAL AND METHODS

^3H -thymidine (sp. act. 16 Ci/mM, Bhaba Atomic Research Centre, Trombay, India) was added to the medium in such a manner that the final concentration was 50 Ci per 100 ml of medium. Following a 4-hour incubation in "hot" medium, the nematodes were transferred to an isotope-free nutrient medium supplemented with unlabelled thymidine (Sigma Chemicals).

The nematodes, collected from man, were killed at convenient intervals covering a period from 8 hours (0.3 day) to 20.0 days post-labelling (d.p.l.). The testes were removed from each nematode. Both histological (Clermont and Trott 1969) and air-dried (Mukherjee and Ghosal 1969) slides were stained suitably and autoradiographed according to conventional techniques (Mukherjee et al. 1968, Ghosal et al. 1983). The most advanced stage of meiosis and spermiogenesis, detected as radioactive, was recorded in the testis of each animal (Table 1).

RESULTS AND DISCUSSION

As the majority of spermatogonia and spermatocytes (Fig. 1) were labelled in the first worm sacrificed as early as 8 h post labelling, it may be supposed that DNA synthesis occurred in these cells in their S period. Primary spermatocytes were labelled in the next 2 specimens (No. 2 and 3), whereas secondary spermatocytes (Fig. 2) were labelled in the 4th *A. lumbricoides* killed at day 5.0. As round early spermatids were detected to be radioactive (Fig. 3) for the first time at day 6.1, it may be deduced that between day 5 and day 6.1 after the administration of ^3H -TdR, meiosis was completed and spermiogenesis started. The duration of meiosis is 5—6 days. The late spermatids were found to be labelled (Fig. 4) on day 11.5 after labelling when this nematode was sacrificed. As mature spermatozoa were radioactive (Fig. 5) at day 13.1 after labelling, spermiogenesis appears to last at least for 7 days (6.1—13.1 days).

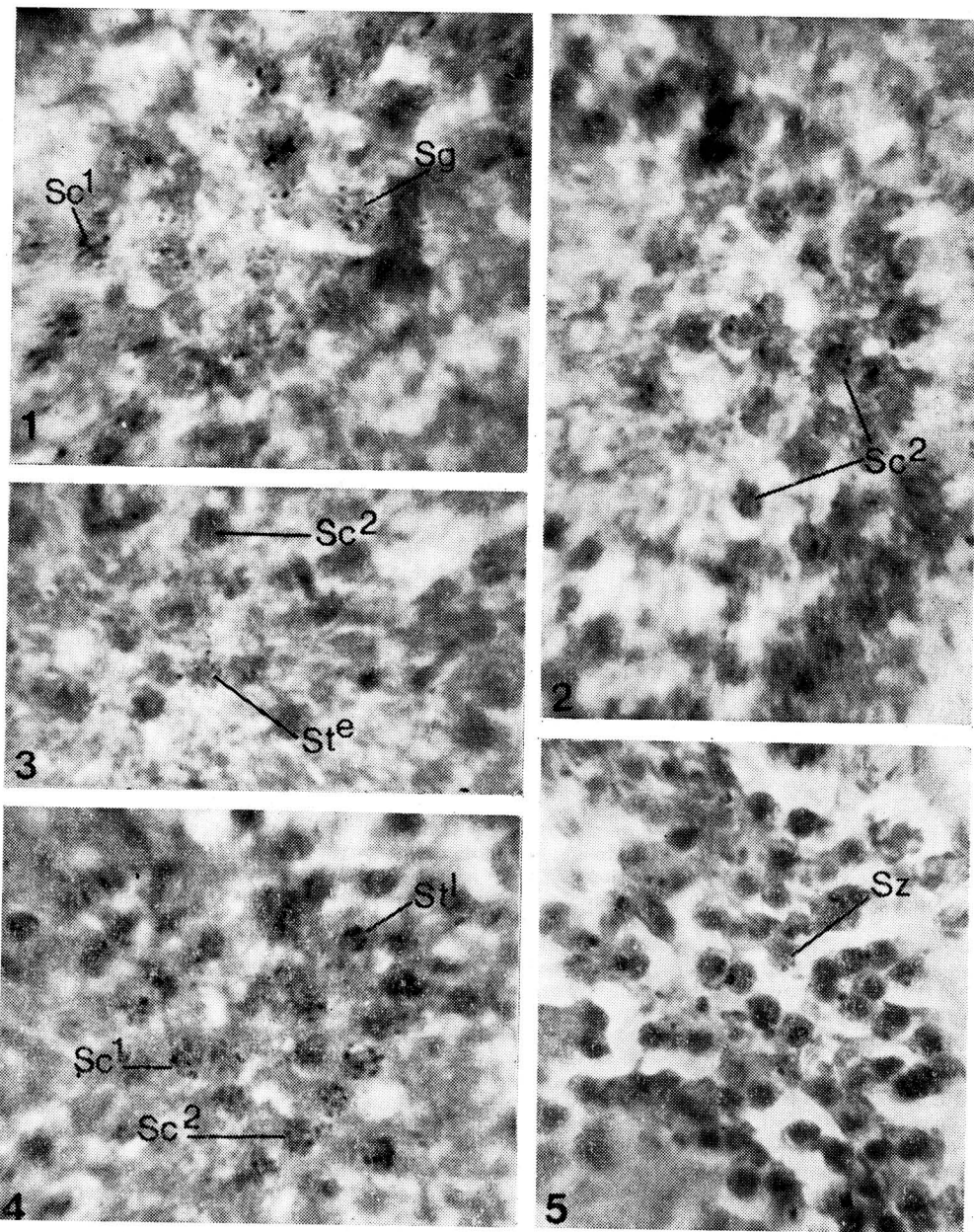


Fig. 1. Labelled spermatogonia (Sg) and early spermatocytes (Sc¹) were detected at day 0.3 after labelling ($\times 1.000$). Fig. 2. Radioactive secondary spermatocytes (Sc²) were scored at day 5 p.l. ($\times 1.000$). Fig. 3. Early spermatids (Ste) were detected as labelled for the first time at day 6 p.l., when a few secondary spermatocytes (Sc²) remained "hot" ($\times 1.000$). Fig. 4. Late spermatids, (Stl) were the most progressed labelled spermatogenetic elements at day 11.5 p.l. Radioactivity persisted in progenitor cellular entities such as the primary (Sc¹) and secondary (Sc²) spermatocytes ($\times 1.000$). Fig. 5. Fully formed labelled spermatozoa were detected at day 13.1 p.l. ($\times 1.000$).

Table 1. The most advanced stages of spermatogenesis detected to be labelled in the testis of *Ascaris lumbricoides* sacrificed at different days following the administration of ³H-thymidine in this in vitro medium

Serial No.	Days after labelling	Most advanced stage labelled
1	0.3	spermatogonia and spermatocytes (primary)
2	1.5	primary spermatocytes
3	3.0	primary spermatocytes
4	5.0	secondary spermatocytes
5	6.1	early spermatids
6	7.0	mild spermatids
7	9.1	mild spermatids
8	11.5	late spermatids
9	13.1	fully formed spermatozoa
10	15.0	the number of autoradiographic
11	17.3	silver grain was low and the grain size was much reduced
12	20.0	in the fully formed spermatozoa obtained from the testes of these specimens

It is supposed that the natural selection might have played a paramount role in stabilizing the duration of spermatogenesis in animals in relation to the nature of fertilization and ecological conditions. Its duration in *A. lumbricoides* is presumably related to the situation resulting from adaptations to the parasitic way of life.

Acknowledgements. We are grateful to Professor B. B. Mukherjee of McGill University and Dr. Somes Sanyal of Erasmus University Rotterdam for donating Kodak stripping film. Thanks are due to the Indian Council of Medical Research for providing Junior Research Fellowship to one of us (D. P. Mukhopadhyay).

АВТОРАДИОГРАФИЧЕСКОЕ ИЗУЧЕНИЕ КИНЕТИКИ СПЕРМАТОГЕНЕЗА У *ASCARIS LUMBRICOIDES*

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Резюме. Изучали кинетику мейоза и сперматогенеза у 12 половозрелых самцов *Ascaris lumbricoides*, которых держали в питательной среде. Для получения радиоактивных сперматоцитов в среду добавляли ³H-тимидин на 2 ч. После того нематоды переносили в свежую среду без ³H-TdR и использовали для приготовления гистологических и давленных препаратов из семенников. Анализом миграции „жарких“ сперматоцитов до образования зрелых сперматозоидов, наблюдаемой при помощи авторадииграфии (Kodak AR-10), показано, что продолжительность мейоза и сперматогенеза немного больше, чем 5,5 и 7,6 дней. Общее время мейоза и сперматогенеза — около 13,1 дней. В это время впервые появлялись радиоактивные сперматозоиды.

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Received 3 June 1984.

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FOLIA PARASITOLOGICA (PRAHA) 33: 64, 1986.

VIIIth State-wide dipterological seminar

The VIIIth State-wide dipterological seminar was held 24—27 September 1984 at České Budějovice. The Seminar was organized by the Parasitological and Entomological Institutes of the Czechoslovak Academy of Sciences in the premises of the South Bohemian Biological Centre, Czechoslovak Academy of Sciences. The state-wide dipterological seminars, which have been regularly held since 1969 at two-year intervals, indicate the present state of the research of Diptera, are instrumental in the exchange of information among Czechoslovak dipterologists and deal with measures to be taken in further development of this scientific field. At this seminar a special attention was paid to groups of Diptera important from parasitological and economic aspects.

The Seminar was attended by 45 workers of research institutes, universities and museums. A total of 41 papers were presented, one half concerning medical and veterinary dipterology (6 papers dealing with mosquitoes, 5 papers with blackflies, 2 papers with horseflies, 2 papers with warble flies and myiasis, one paper with midges and synanthropic flies respectively). One of the introductory papers was devoted to zoogeography of the family Culicidae in the Palaearctic region, other papers dealing with this family were concerned with the research of malaria in Slovakia, the occurrence of mosquitoes in some areas and their control using pyrethroid and bacteria *Bacillus thuringiensis*. Subsequent papers covered the research of blackflies in Slovakia and South Bohemia, of midges and horseflies in South and West Bohemia. The papers concerning synanthropic flies discussed the faunistic

research of the family Muscidae in Czechoslovakia. Also discussed were hypodermatosis of game animals in South Bohemia and some cases of myiasis in humans. Two scientific colour films on mosquitoes and warble flies were screened.

Other papers were devoted to the problems of systematics, faunistics and ecology of various Diptera, primarily families of economic importance. A special attention was paid to the problems of the use of Diptera as bioindicators and to the issues of environment, whose ever-increasing importance was stressed in a number of contributions presented. The results of the research revealed the disturbing state of natural environment, deteriorating due to thoughtless and insufficiently well organized economic human activities. The papers presented at the Seminar will be printed in a volume published by the South Bohemian Museum at České Budějovice.

Also discussed were some problems of further development of Czechoslovak dipterology, mainly the conclusions of the International Entomological Congress held in Hamburg last year in conjunction with the project of the compilation of a world catalogue of Diptera, the education of the young generation of biologists, the preparation of the Czech and Slovak nomenclature of Diptera and the preparation of the International Congress of Diptera to be held in 1986 in Hungary. The Seminar indicated the present state and development of research of Diptera in Czechoslovakia reflecting the medical and economic importance of this group in insects.

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