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RNA AND PROTEIN SYNTHESES DURING SPERMATOGENESIS OF *RHIPICEPHALUS SANGUINEUS* (ACARI-IXODIDAE)[1]

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ABSTRACT

Chung-Chia Huang, Yien-Shing Chow, Nin-Nin Chung and Lei-Chin Tina Yang (1976). *RNA and Protein Syntheses during Spermatogenesis of* Rhipicephalus sanguineus *(Acari-Ixodidae)*. Bull. Inst. Zool., Academia Sinica, **15**(1): 1-8. RNA and protein syntheses and meiosis in male germ cells of the brown dog tick, *Rhipicephalus sanguineus*, were studied. The spermatocyte possessed 20 autosomes and one X chromosome. Autoradiograms revealed that protein synthesis occurred persistently through two meiotic divisions. Residual activity of protein synthesis was found in early spermatids. Elongating spermatids gave no sign of protein synthesis. The RNA synthesis was restricted in the nucleus of premeiotic primary spermatocyte. No evidence of RNA synthesis was found during spermatogenesis and spermiogenesis.

A stock of brown dog tick, *Rhipicephalus sanguineus*, has been maintained in the Institute of Zoology, Academia Sinica since 1970. Papers concerning its biology and phenolic sex pheromone were published[4,5]. Nolte *et al.*[9] have obtained a phenolic compound, 2-methoxy-5-ethylphenol, as the gregarious pheromone of the locusts and have pointed out that four characteristics of gregarization could be used in the identification of the pheromone; colour change during various instars, chiasma frequency change, morphometric ratios and behavioral traits. Among these four characteristics, they also pointed out that chiasma frequency of the eight largest autosomes provided the most sensitive index of gregarization. In hard ticks, phenolic chemicals, such as 2, 6-dichlorophenol, 2, 6-ditert-butyl-*p*-cresol and *p*-cresol are also identified according to sex behavioral trait. Therefore, a chiasma frequency study is now proposed. The present communication deals with its normal cytogentics and autoradiographic studies on protien and RNA syntheses during spermatogenesis and spermiogenesis in order to have a better understanding of its chiasma difference.

MATERIALS AND METHODS

Partially fed male adults *Rhipicephalus sanguineus* (Latreille) were employed in this experiment. Temporary squashes of the testes were prepared for cytogenetic studies. The testes were dissected out in insect saline and stained in aceto-lacto-orcein (2%) overnight. Squashes were

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made in a drop of fresh stain. Photomicrographs were taken with phase contrast microscopy.

Tritiated uridine (sp. act. 20 C/mM) and leucine (sp. act. 40C/mM), which were purchased from New England Nuclear Corporation, were diluted separately with insect saline to the activity of 100 μc/ml. Each arachnid received 2 to 3 μl radioactive solution.

Fifteen minutes post-injection, the arachnids were dissected in Carnoy's fixative and fixed for 2–3 hours. Squashes were made in a drop of 45% acetic acid on a microscopic slide. The coverglass was removed according to the dry-ice technique. The squash preparations while in frozen state were transferred into 95% ethanol, hydrated through ethanol series into water. After a 20 min. wash in running tap water, the squashes were rinsed with distilled water and finally dried in the air.

The squashes of labeled specimens were singly dipped in Kodak NTB 2 Nuclear Tract Emulsion which was diluted with equal volume of double distilled water and kept in a 35°C water bath. The exposure time of emulsion coated slides lasted one to two weeks in light tight box at 5°C. The autoradiograms were developed in Kodak D19 at one to one dilution and fixed in Kodak Acid Fixer. Autoradiograms were stained with Giemsa, dehydrated and mounted in Permount. The observation was made with bright field microscopy.

RESULT AND DISCUSSION

A. Male Meiosis

The diploid complement of male brown dog tick consisted of twenty autosomes and one X chromosome. In premeiotic stage of primary spermatocyte, the chromatin was homogeneously distributed in the nucleus. The conspicuous nucleolus appeared as a dense solid mass in the nucleus (Fig. 1). There was one or two nucleoli per nucleus. The mass of two nucleoli was similar to that of single one.

During the early phase of first meiotic prophase, the nucleus became granular owing to the condensation of chromatin into chromomeres of different sizes (Fig. 2). The chromatin strung together to form leptotene chromosomes. The synapsis of homologous chromosomes was not readily visible as in Orthoptera. Nevertheless, the condensation and synapsis of chromosomes seemed orthordox, and the reduction of chromosome numbers through pairing of homologous

Plate I. Male meiosis of the brown dog tick, aceto-lacto-orcein stained, 600×. Fig. 1. Premeiotic primary spermatocyte, arrow indicates the nucleolus. Fig. 2. Primary spermatocyte, leptotene stage. Fig. 3. Primary spermatocyte, pachytene stage. Fig. 4. Primary spermatocyte, diplotene stage, arrow denotes an univalent X chromosome. Fig. 5. Primary spermatocyte, diakinesis, arrow denotes the X chromosome. Fig. 6. Premetaphase I, arrow denotes the X chromosome. Fig. 7. Metaphase I, arrow indicates the X chromosome. Fig. 8. Anaphase I, arrow indicates the X chromosome. Fig. 9. Prophase II. Fig. 10. Metaphase II, arrow indicates the X chromosome. Fig. 11. Anaphase II, arrow indicates the X chromosome. Fig. 12. Telophase II. Fig. 13. Early spermatid. Fig. 14. Oval shaped early spermatid. Fig. 15 & 16. Elongating spermatids.

Plate II. Incorporation of tritiated leucine (sp. act. 40C/mM) in male germ cells of the brown dog tick (1000×). Fig. 17. A labeled premeiotic primary spermatocyte. Fig. 18. A labeled prophase I. Fig. 19. A labeled metaphase I. Fig. 20. A labeled anaphase I. Fig. 21. A labeled interkinesis. Fig. 22. Labeled prophase II. Fig. 23. A labeled early anaphase II. Fig. 24. A labeled telophase II. Fig. 25. A lightly labeled oval-shaped spermatid. Fig. 26. Unlabeled elongated spermatids.

Plate III. Incorporation of tritiated uridine (sp. act. 20C/mM) in male germ cells of the brown dog tick (1000×). Fig. 27. Only the nucleus of the premeiotic primary spermatocyte was labeled. Fig. 28. A prophase I, diplotene. Fig. 29. A metaphase I. Fig. 30. An interkinetic secondary spermatocyte. Fig. 31. An early anaphase II. Fig. 32. A late anaphase II. Fig. 33. A Telophase II. Fig. 34. A oval-shaped spermatid. Fig. 35. Elongating spermatids.

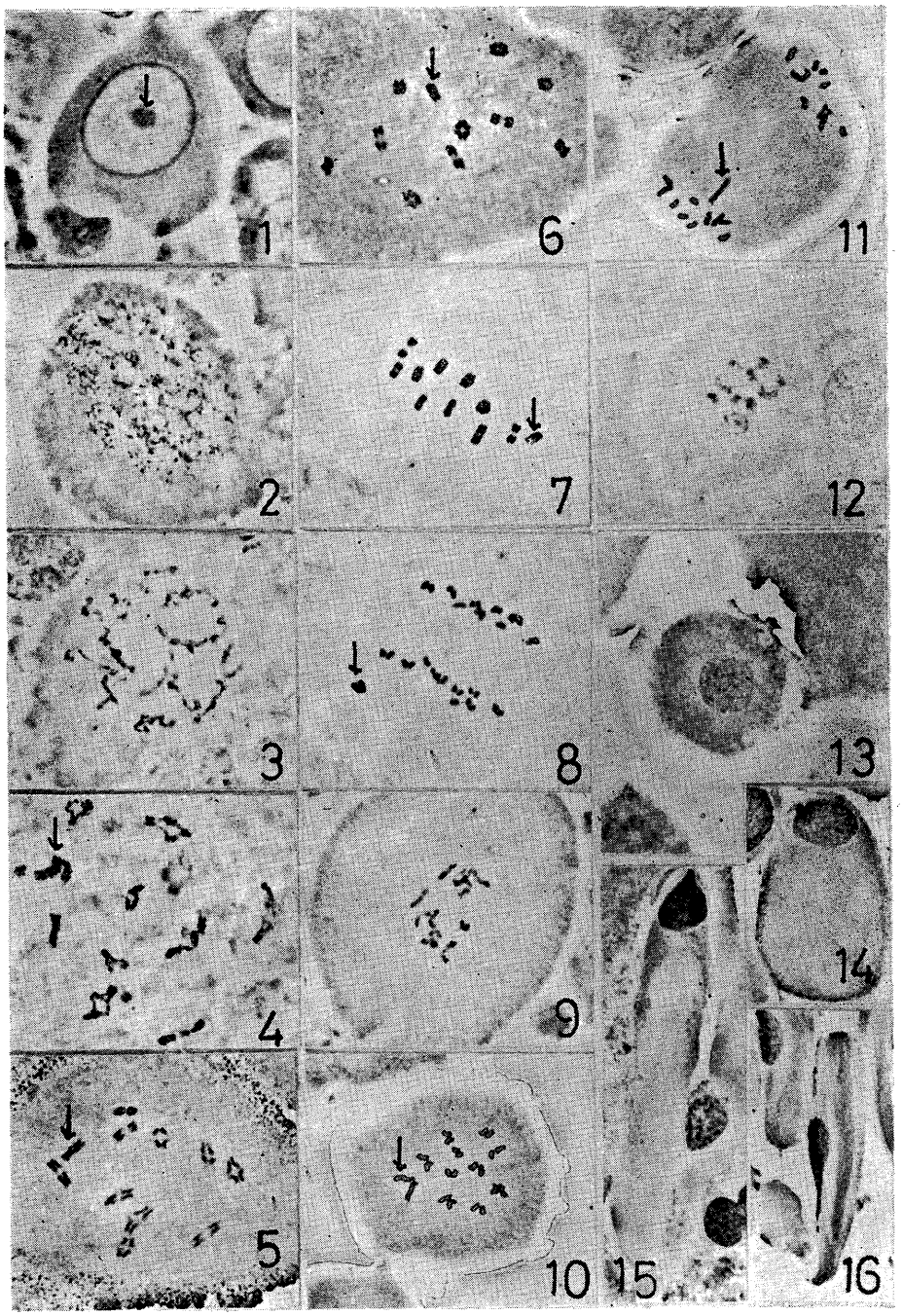

PLATE I

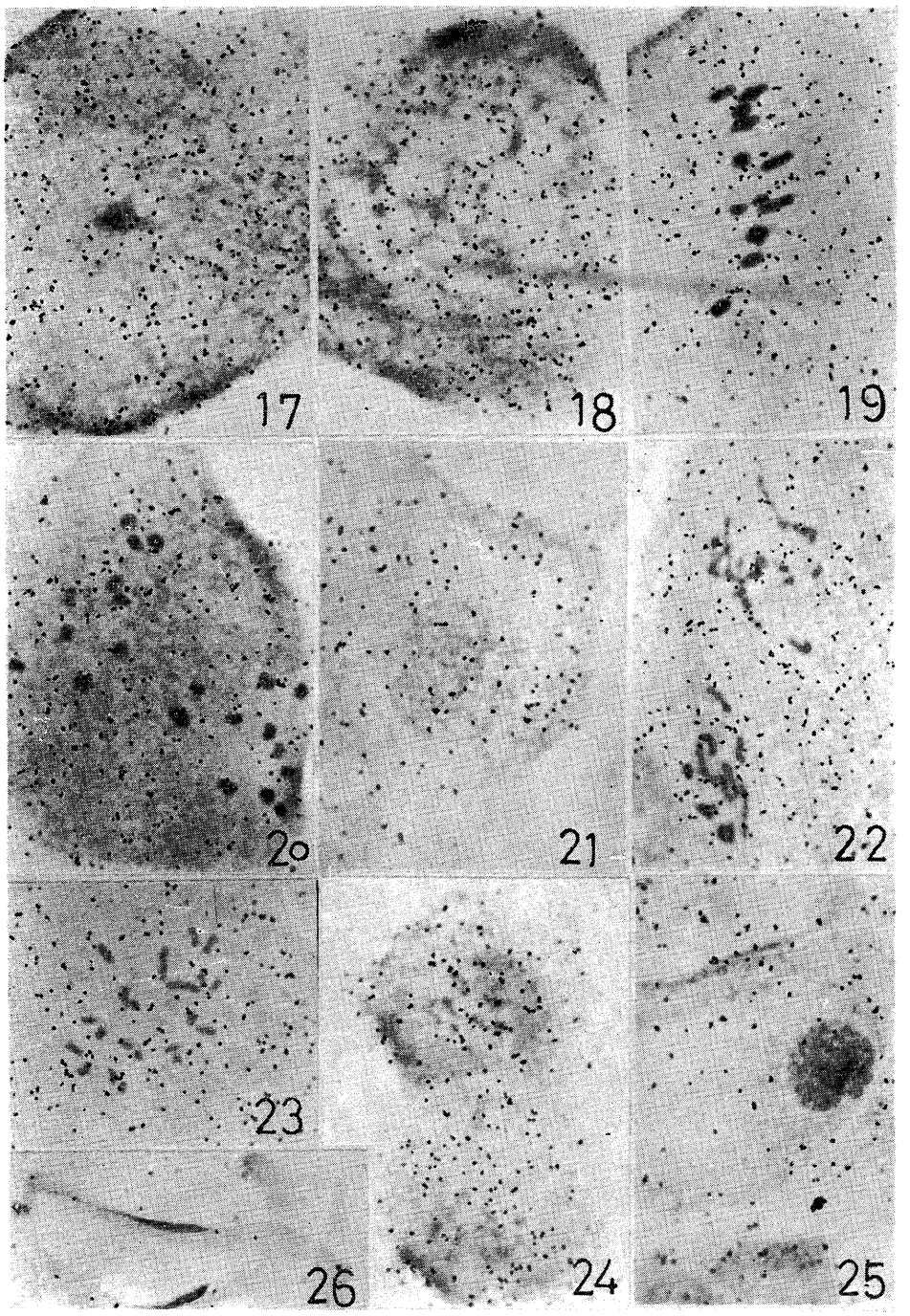

PLATE 2 10μ

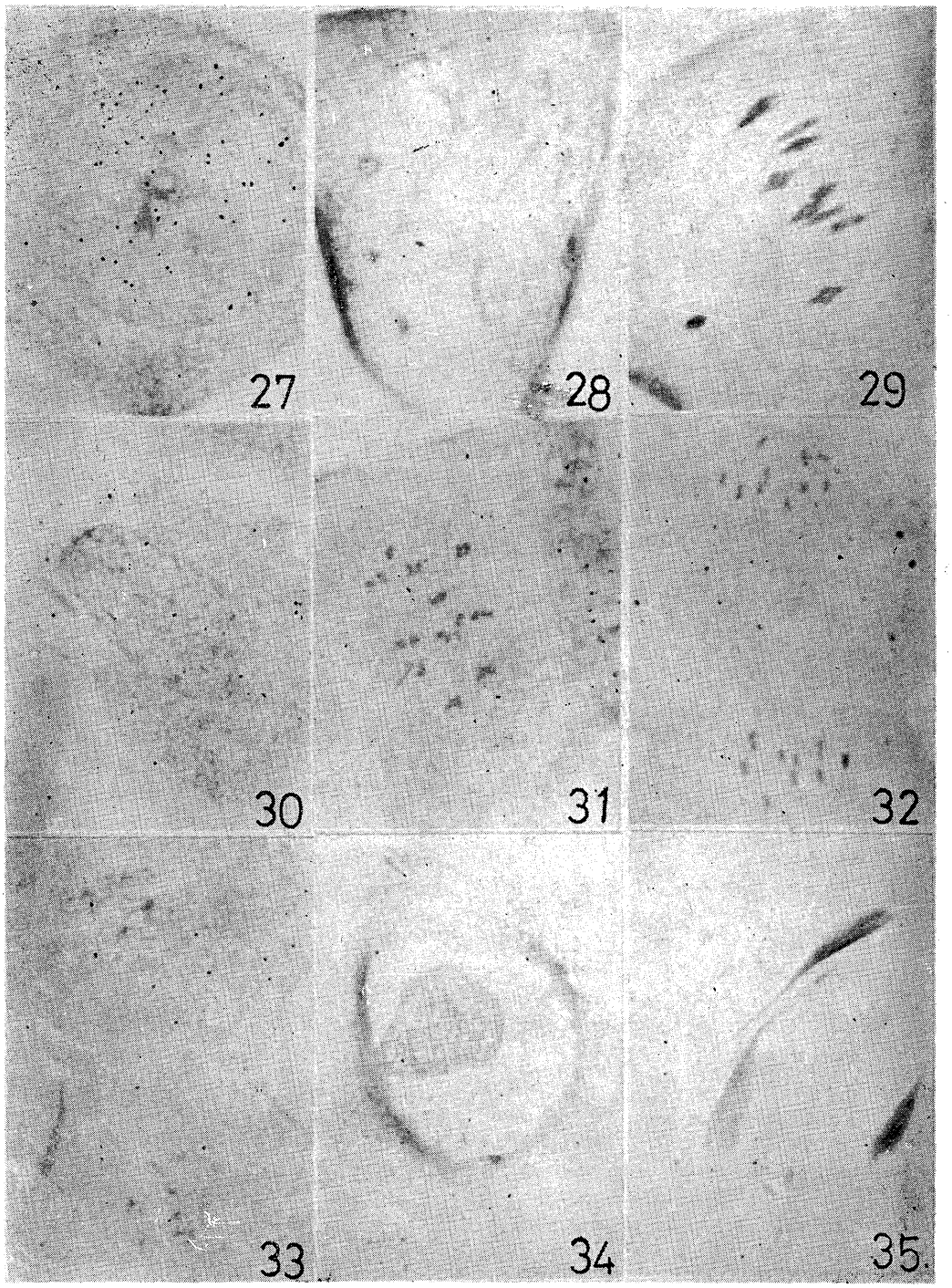

PLATE 3

chromosomes was evident as the prophase I proceeded (Fig. 3). In diplotene stage, ten bivalent autosomes and a single univalent sex chromosome (arrow) with similar level of chromatin condensation were revealed. The large X chromosome has a prominent primary constriction. Chiasma configurations were self-evident in diplotene chromosomes (Fig. 4). A termination of chiasmata was realized in diakinetic chromosomes (Fig. 5). There was at least one chiasma per bivalent[10], however, two chiasmata per bivalent were also formed in the diakinetic stage of prophase I. The maximun condensation of chromosome was at metaphase I (Fig. 6 and 7). The first meiotic division is reductional at least for the X chromosome (Arrow, Fig. 8). The precocious separation of sister chromatids during the first anaphase revealed ten and twelve V-shaped dyads. A secondary spermatocyte of prophase II is shown in Fig. 9. All the autosomes and X chromosome were telocentric or sub-telocentric. This evidence was supported by the observation of metaphase II chromosomes (Fig. 10). The sex chromosome (arrow) having a dot-shaped arm was the longest one in the complement. The second meiotic division was equational (Fig. 11). The maturation of spermatid (Fig. 12–16) was rather complicated. Since the spermiogenesis had been described in a electron microscopic study[3,12] by Brinton *et al.*, and Reger, no attempt was made to follow the sequence of this transformation in the present study.

Cytogenetics of ticks had been extensively studied in Asian Haemaphipsalis spp.[11] and Egypsian Hyalomma spp.[8]. Most of these species have twenty autosomes and one X chromosome in the males, and the sex chromosome was in general the longest one in chromosomal complement. With a few exceptions (i.e. *Haemaphipsalis hystricis*), the sex chromosome system was XX/XO type. The male was invariably heterogametic. Although the karyotype of the female is not yet determined, the sex chromosome system of the brown dog tick is assumed to follow the XX/XO type.

B. Incorporation of Tritiated Leucine in Male Germ Cell.

The incorporation of radioactive leucine in male germ cells is shown in Plate II. Primary spermatocytes of large premeiotic stage were active in protein synthesis (Fig. 17). Silver grains appeared to be evenly distributed in both cytoplasm and nucleus. Throughout the first reductional division, the primary spermatocyte remained active in the assimilation of tritium labeled leucine as suggested by the presence of silver grains on prophase I, (Fig. 18) metaphase I (Fig. 19) and anaphase I (Fig. 20).

All secondary spermatocytes in the interkinesis (Fig. 21) and second equational division were all heavily labeled. Cell divisions did not preclude protein synthesis (Figs. 22 & 23). Incorporation proceeded through the early phase of spermiogenesis (Figs. 24 & 25). However, protein synthesis drastically diminished in oval-shaped spermatid and completely paused in elongated spermatid in the male genital tract (Fig. 26).

No attempt was made to compare the relative incorporation rate of tritiated leucine in interkinetic and dividing spermatocytes. Comparisons could be done by analyzing the silver grain density of the cells concerned. Since the homogeneous distribution of the precursor in the injected arachnid and the identical pool of the protein precursor between the treated arachnids were not secure, precise quantitative studies would not be meaningful. However, judged from the available autoradiograms, the incorporation of tritiated leucine proceeded more or less equally throughout two meiotic divisions.

Quantitative studies of spermatocyte had been done in *Asellus* crustacean[1] Drosophila[2] and locust[6]. In *Asellus* the incorporation of tritiated lysine diminished in meiotic prophase and almost completely ceased during metaphase, while the incorporation of labeled amino acid significantly resumed in the early and elongating stages of spermatids. Cessations of the incorporation of labeled amino acid in the crustacean metaphase was different from the present study. In *Droso-*

phila and locust, the incorporation of labeled amino acid proceeded throughout the meiotic division as in the brown dog tick, however, the protein synthesis in the meiotic division of brown dog tick seemed to be earlier than the two aforementioned insects.

C. Incorporation of Tritiated Uridine in Male Germ Cell.

The uridine is a specific precursor of RNA synthesis *in vivo* and *in vitro*. In the testes of brown dog tick only the premeiotic primary spermatocyte appeared to assimilate the tritiated uridine; the distribution of silver grain was limited in the nucleus. The results are shown in Plate III (Figs. 27–35). These observations were quite different from those of locust[6]. In locust, the active incorporation of labeled uridine lasted till early prophase I and the incorporation decreased drastically from pachytene to diakinesis. No labelings were observed in metaphase I and anaphase I. Incorporations of labeled uridine was obviously found in interkinesis, prophase II and early spermatids.

We found in the experiment of the uridine incorporation 40 to 50 silver grains in each nucleus of the premeiotic primary spermatocyte, but only a few grains in the background of other stages. It seemed reasonable to conclude that RNA synthesis in male germ cells was restricted to premeiotic stage.

Differential incorporations of tritiated uridine in the heterochromatic X chromosome and the euchromatic autosomes were observed[6,7]. Heterochromatic X chromosome of condensed metaphase and anaphase was inactive in RNA synthesis. It was postulated that the condensation of chromatin could be responsible for the repression of RNA synthesis. In the brown dog tick, the study of differential activity in RNA synthesis between the X chromosome and autosomes was not yet made because the condensation of X chromosome was in phase with autosomes. Since the diffused chromosomes of early prophase and the interkinetic nuclei showed no silver grains, mechanisms other than chromatin condensation would play a role in the control of RNA synthesis in the brown dog tick.

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褐狗壁蝨精子成熟中核醣核酸及蛋白質之生合成

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分別用含氚的尿核苷和白胺酸研究褐狗壁蝨精子成熟中核醣核酸及蛋白質的生合成，並描述精子成熟的過程。初級精原細胞核具有二十個體染色體及一個性染色體。兩次精母細胞成熟分裂中有持續的蛋白質合成，精細胞蛻變的早期仍有合成之作用，但長形之精細胞則無聚合白胺酸之活性。初級精母細胞核在進入第一次減數分裂前，具有合成核醣核酸之活性，但在兩次成熟分裂過程中，以及精細胞蛻變中，均無聚合尿核苷之作用。