Extrachromosomal DNA and RNA-Synthesis in Oocytes of *Creophilus maxillosus* (Staphylinidae, Coleoptera, Polyphaga)[1]

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Summary. It has been found that the transcriptional activity of nuclear extra DNA in *Creophilus maxillosus* oocytes, as examined by autoradiography, increases parallel with its dispersion during the previtellogenic period of oocyte growth. The RNA, after being synthesized in the greatly enlarged oocyte nucleus, is subsequently transported into the cytoplasm. The oocyte chromosomes form a karyosphere and synthesize the RNA more weakly than other parts of the nucleus, which contain the extra DNA in a highly dispersed condition.

The occurrence of nuclear extrachromosomal DNA in female germ cells in insects has been known, so far, only in species with panoistic[2-6] or meroistic-polytrophic[7-13] ovaries. Recently, for the first time, the process of gene amplification has been described in oogenesis of Staphylinid *Creophilus maxillosus*, an insect with meroistic-telotrophic ovaries[14].

The activity of amplified DNA in RNA synthesis in previtellogenic stages of oogenesis in *Creophilus*, as suggested by a mass production of multiple nucleoli and enormous increase of the volume of germinal vesicle[14], has been fully confirmed by the autoradiographic investigations recently carried out in this Department. In these experiments, ^{3}H-uridine was injected into the body cavity of adult females, or dissected ovaries were incubated in vitro in a culture medium containing this labelled precursor.

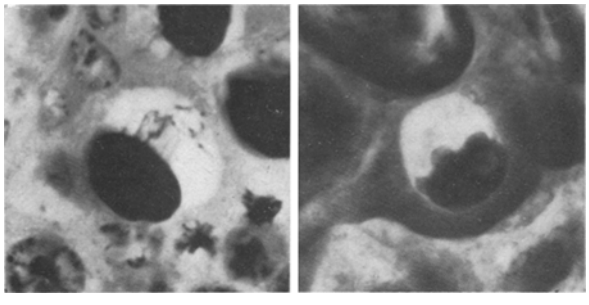
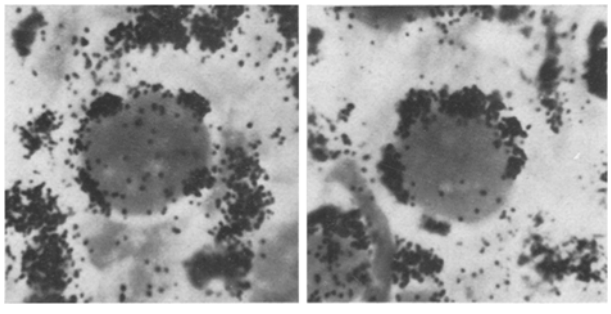


Fig. 1. Oocyte at pachytene. The compact extra-DNA body and faintly stained chromosomes protruding from it are clearly visible in the nucleus. Feulgen. ×1,000.

Fig. 2. Oocyte at pachytene. One side of the extra-DNA body, as in the nucleus in Figure 1, is in contact with the nuclear envelope; on the other side 3 nucleoli closely adherring to the surface of the body are present. In the part of the nucleus which is free of extra-DNA and nucleoli, the faintly stained chromosomes are also visible. Methyl green pyronin. ×1,000.



Figs. 3 and 4. Autoradiographs of 2 oocytes at pachytene. In each of the oocytes the extra-DNA body is the darkly stained round mass. The plane of section through the oocytes is such that the extra-DNA bodies occupy most of nuclei. The labelling of the oocyte nuclei occur mostly as distinct clusters of grains along the rim of the extra-DNA body. The arrangement of these clusters coincides with that of the small nucleoli situated on the surface of the extra-DNA body. Incubation with ^{3}H-uridine for 4 h. Methyl green pyronin. ×1,000.

In the nuclei of young oocytes which contain a compact, heterochromatic body of extra DNA (Figure 1), ^{3}H-uridine is incorporated, as a rule, on the surface of this body (Figures 3 and 4). From the inspection of autoradiographs and preparations stained with methyl green-pyronine, it is evident that the distribution of label in these nuclei corresponds exactly to that of the small nucleoli which are associated closely with the surface of the compact DNA body[14] (Figure 2).

Starting with the second period of oocyte growth, the DNA body begins to fragment and disperses gradually in the nuclear sap (Figure 5). As this process proceeds, the labelling which results from the incorporation of ^{3}H-uridine into newly synthesized RNA spreads all over the oocyte nucleus. The heavy label observed over the oocyte nucleus indicates also that the intensity of RNA synthesis increases considerably at that period of oogenesis (Figure 6). The process of intensive RNA synthesis in the germinal vesicle proceeds continuously, at least to late previtellogenic stages of the oocyte growth (Figure 7).

As has been pointed out in a previous paper[14], the chromosomes of previtellogenic oocytes of *Creophilus* form a more or less compact karyosphere. Such a karyosphere situated within a particular nuclear region free of the nucleolar material is shown on Figure 8. It is noteworthy that the labelling of the karyosphere in growing oocytes of *Creophilus* is much weaker than that of other parts of the nucleus (Figure 9) occupied by a finely granulated nucleolar material[14] (Figure 8). Moreover, one can see from the Figure 9 that the nuclear region adjacent to the karyosphere is nearly free of labelling.

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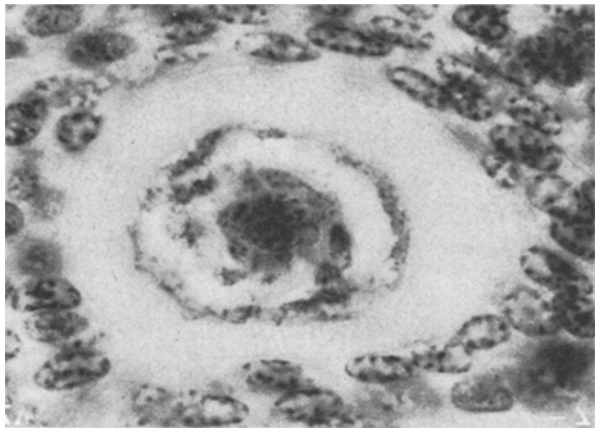

Fig. 5. An advanced stage of fragmentation and dispersion of the extra-DNA body at the beginning of the second period of the oocyte growth. Feulgen. × 1,000.

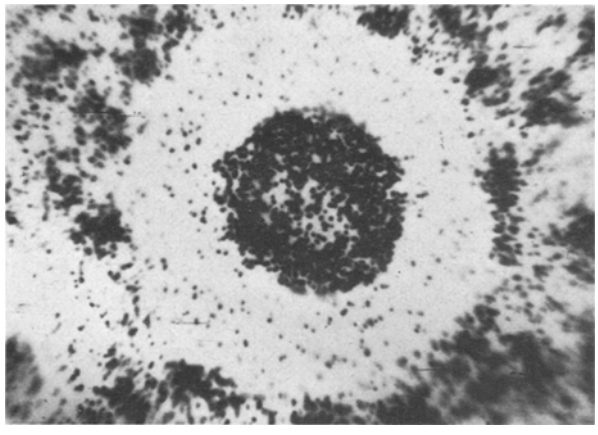

Fig. 6. Autoradiograph of an oocyte at the stage like that shown in Figure 5. The silver grains cover the whole nucleus, but the labelling over its central area containing the karyosphere and as yet unfragmented blocks of the extra-DNA is considerably weaker than over its remaining parts in which the extra-DNA occurs in a highly dispersed condition. As compared with the labelling of the germinal vesicle, the density of label over the ooplasm is very low. Incubation with ^{3}H-uridine for 4 h. × 1,000.

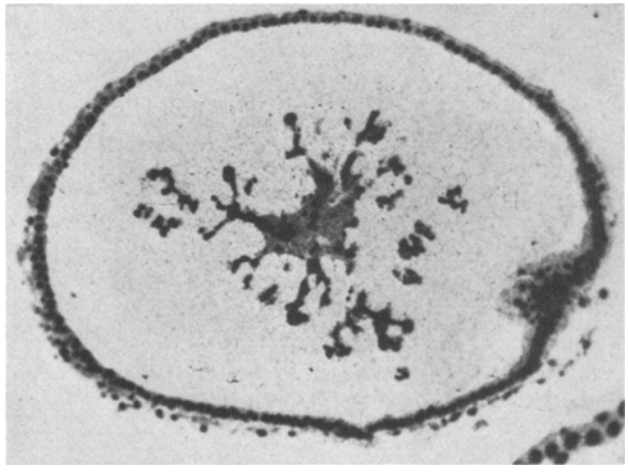

Fig. 7. Autoradiograph of a previtellogenic oocyte at the advanced stage of its growth. Note the haevy labelling of the unusually shaped oocyte nucleus. Incubation with ^{3}H-uridine for 4 h. × 100.

From the results presented above, it seems evident that the intensive synthesis of RNA in the oocyte nuclei of *Creophilus* is conditioned by the presence of extrachromosomal DNA. There is, also, no doubt that the fragmentation and dispersion of the extra-DNA body results in its activation in the process of RNA synthesis. The extremely high number of nucleoli produced in the nuclei of growing oocytes in *Creophilus*[14] seems to indicate that in this species, as in *Acheta*[15] and *Dytiscus*[16], the process leading to development of extrachromosomal DNA consists in the amplification of nucleolus organizers.

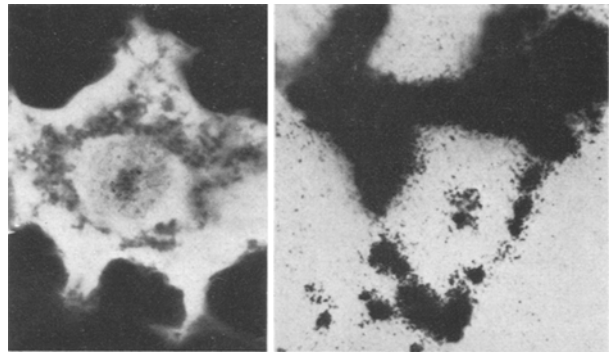

Fig. 8. A part of considerably enlarged and irregularly shaped oocyte nucleus containing the karyosphere. The karyosphere is seen to be situated within a capsule free of nucleolar material. The remaining regions of the germinal vesicle are penetrated by a network of nucleolar strands. Heidenhain's hematoxylin. × 500.

Fig. 9. Autoradiograph of the same part of a germinal vesicle shown in Figure 8, but at a slightly later stage of the oocyte growth. Note the relatively low density of label over the karyosphere surrounded by the karyosphere capsule which is nearly free of the label, and the solid black labelling over the remaining regions of the germinal vesicle in which the nucleolar material has accumulated. Incubation with ^{3}H-uridine for 15 h. × 500.

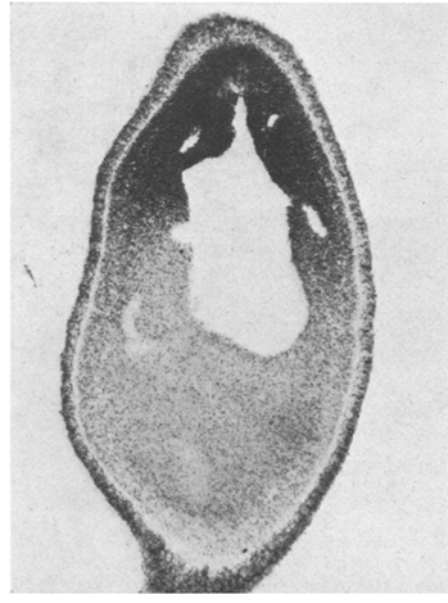

Fig. 10. Autoradiograph of an oocyte at late previtellogenesis. The germinal vesicle shows practically no labelling as compared with strong labelling of the cytoplasm. An anterio-posterior gradient of label distribution in ooplasm is clearly visible. Incubation with ^{3}H-uridine for 32 h. × 100.

Pattern of label distribution over the nuclei of late previtellogenic oocytes of *Creophilus* (Figure 9) is similar to that obtained in the case of oocytes of *Chrysopa perla* after ^{3}H-uridine administration[12]. In *Chrysopa* as in *Creophilus*, the oocyte nucleus activity in RNA-synthesis is to be ascribed, for the most part, to the extrachromosomal DNA, the chromosomes themselves being relatively inactive in this process. It seems, therefore, that in cases of gene amplification the rule of restricted RNA-synthesis in oocyte nuclei in the polytrophic and telotrophic ovary holds true so far as the oocyte chromosomes alone are taken into consideration.

It is invariably observed that, as the time of incubation is prolonged, the label over the germinal vesicle gradually disappears, and at the same time its density over the cytoplasm increases (Figure 10). It seems evident, therefore, that RNA after being synthesized in the nucleus is subsequently transported into the cytoplasm. Independently of this process, an anterio-posterior gradient of RNA concentration appears in the cytoplasm (Figure 10). This gradient, like in oocytes of other insects with meroistic-telotrophic ovaries[17–20], develops as a result of supplying the growing oocytes with RNA produced by trophocytes and transported to each of them by a trophic cord entering the oocyte at its anterior end. Detailed results of cytochemical studies on the extrachromosomal DNA in oogenesis of *Creophilus* will be published elsewhere.

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The Distribution of Endocrine-Like Cells in the Human Male and Female Urethral Epithelium

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Summary. Histochemical and electron microscopic techniques have been used to study the nature and distribution of fluorescent, endocrine-like cells in the urethra of the human male and female. The confinement of such cells to specific regions of the urethra is discussed in relation to the embryological development of this part of the urinary tract.

Histochemical and electron microscopic techniques have recently been used to demonstrate fluorescent, amine-containing, endocrine-like cells in the epithelium of the urethra in a variety of laboratory animals[1–3]. Similar cells have also been described in the human urethra although these electron microscopic observations were confined to biopsy samples obtained solely from male specimens[4]. In the present study, our purpose has been to establish the occurrence and distribution of these cells in the human urethra using material from both sexes. In addition, preliminary observations on the fine structure of such cells in the female have been sought using electron microscopy.

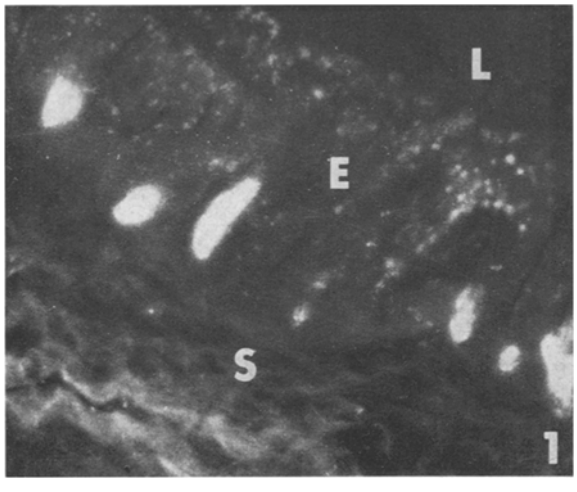

Fig. 1. Fluorescence photomicrograph of human female urethra. Numerous brightly-fluorescent cells occur at the base of the epithelium (E); urethral lumen (L); submucosa (S). ×600.

Materials and methods. Post-mortem tissue removed within 8 h of death occurring from non-urological causes was obtained from male and female specimens and processed for light and electron microscopy. The majority of samples were placed in iso-pentane cooled in liquid nitrogen, serially sectioned in a cryostat and processed for tissue catecholamines using a histochemical method[5]. In addition, small samples of female urethral epithelium were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin prior to thin sectioning for electron microscopy.

Results and discussion. Using fluorescence microscopy, large numbers of yellow brightly-fluorescing cells were observed in the proximal urethra of both male and female specimens (Figure 1). These cells were situated at the base of the urethral epithelium and appeared either elongated or flask-shaped, often with one or more processes extending towards the urethral lumen. They appeared randomly distributed around the circumference of the urethra, the majority occurring immediately below the internal meatus of the bladder. Similar cells were not observed in the bladder epithelium. In the female, fluorescent cells were numerous along the proximal

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