are oval or elongated with fine or thick axons and measure 16–30 µm and with oval nuclei 8–12 µm in diameter (Figure 2). The cytoplasm shows dense granular structure, full of mitochondria and Nissl bodies. The neurosecretory granules occur in aggregates in perinuclear as well as in the extreme periphery of the cells. The secretory substances, as well as the typical Nissl bodies, are stained with CHP and AF. It is probable that the basophilic substances may be identical with Nissl bodies. Many vacuoles containing conspicuous neurosecretory material appear in the peripheral region of the perikaryon (Figure 2). The neurosecretory granules are released from the cells via the axons or cell membrane. The neurosecretory granules can be traced far into the processes of the nerve cells. In the two large ganglia situated at both sides of the pharynx, numerous Gomori-positive granules may be also observed along the fibres. The vegetative ganglia which are very close to the pharynx and oesophagus contain great amounts of Gomori-positive secretion. The secreted granules can be detected within the nerve fibres. Thus it seems most likely that the vegetative ganglia also contain cells with secretory character and function. Further, accumulation of Gomori-positive material and position of neurosecretory cells in these ganglia suggest that the neurosecretory cells found in them may have some role to play in the digestive system.

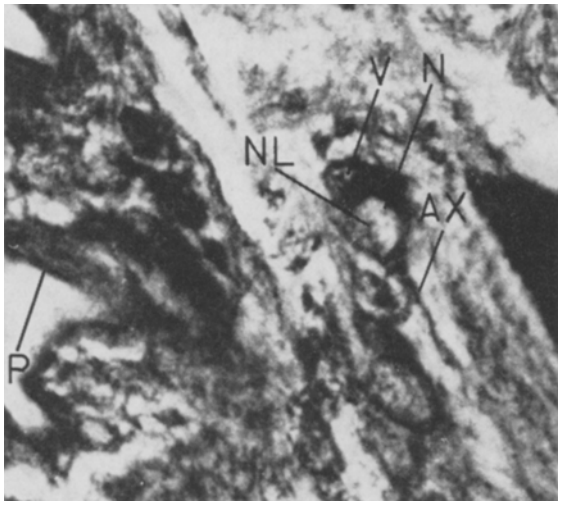


Fig. 2. Horizontal section through the vegetative ganglion showing the neurosecretory cells. AX, axon; NL, nucleolus; N, nucleus; P, pharynx; V, vacuole containing neurosecretory material. Bouin, AF, ×900.

Zusammenfassung. Neurosekretorische Zellen wurden in den vegetativen Ganglien von *Dendrobaena atheca* gefunden. Zahlreiche Gomori-positive Sekretgranulen konnten im Cytoplasma, in den peripheren Vakuolen und im Axon dieser Zellen nachgewiesen werden.

N. S. Gorgees and I. C. Baid

Department of Biology, College of Science, University of Mosul, Mosul (Iraq), 30 October 1974.

Gene Amplification and its Effect on the Structure and Function of the Oocyte Nucleus in the Whirligig Beetle *Gyrinus natator* (Gyrinidae, Coleoptera-Adephaga)*

In 1916, Hegner and Russell[1] in a short report described a chromatic extrachromosomal body in the germ-line cells of the Gyrinid, *Dineutes nigrior*, during the oocyte differentiation. According to these authors, this body, as regards its morphology, the time of its appearance in the female germ-line and its behaviour in differential mitoses which give rise to the oocyte and the group of nurse cells associated with it, closely corresponded to the so-called Giardina's body in Dytiscidae. Although whirligig beetles have not been studied cytologically since that time, nevertheless it seems evident to-day that the chromatic body described in *Dineutes* is the extrachro-

* This research was supported in part by funds from the Cytobiology Committe of the Polish Academy of Science.

[1] R. W. Hegner and C. P. Russell, Proc. natn. Acad. Sci. USA *2*, 356 (1916).

[2] J. G. Gall, Genetics *61* Suppl., 121 (1969).

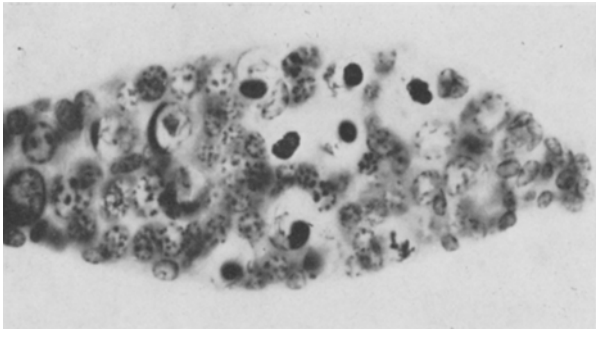


Fig. 1. Longitudinal section through the germarium of an ovariole. The anterior tip of the ovariole is to the right. Each oocyte nucleus contains a deeply stained body of extrachromosomal DNA which is situated in the middle of nucleus or is located excentrically under the nuclear membrane. Oocyte chromosomes are also clearly visible. Feulgen and light green. ×625.

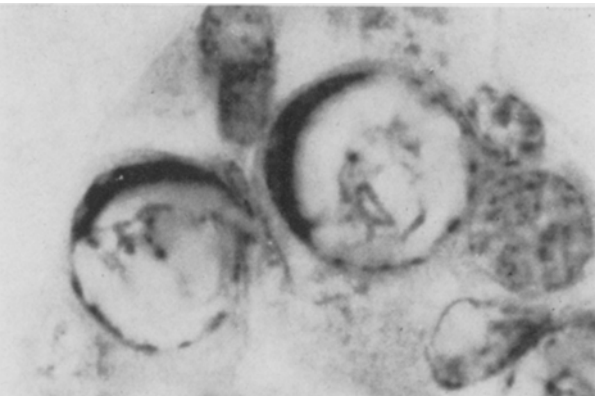
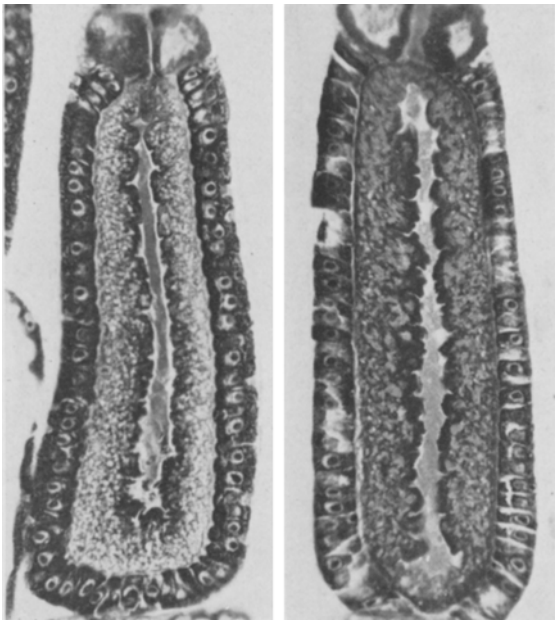


Fig. 2. Two early diplotene nuclei with conspicuous caps of extrachromosomal DNA. Diplotene chromosomes in the middle of the nuclei are clearly visible. Feulgen and light green. ×2050.



Figs. 3 and 4. Longitudinal sections through the egg follicles in late previtellogenesis, showing greatly elongated oocyte nuclei with many lateral processes. Nearly the whole nuclei are filled up with a huge mass of nucleoli which, in fixed preparations and at low magnification, appears as a homogenous substance rich in RNA. The clear space between the nucleolar material and the nuclear membrane in Figure 3 is an artifact resulting from a shrinkage of this material on fixation. Note the very strong basophilia of the layer of ooplasm surrounding the nucleus. Methyl green pyronin. ×240.

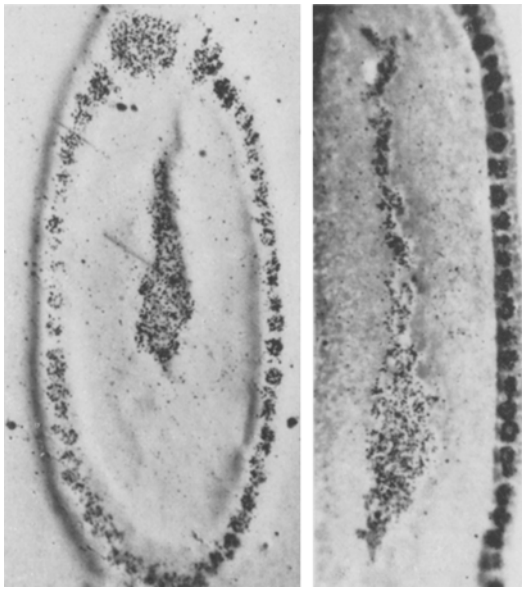


Fig. 5. Autoradiograph of an egg follicle in midprevitellogenesis. Labelling of nurse nucleus and oocyte nucleus is similar. Owing to the oblique section of the follicle, only a part of the oocyte nucleus is visible. Incubation with ^{3}H-uridine for 15 min. ×240.

Fig. 6. Autoradiograph of a portion of late previtellogenetic egg follicle in longitudinal section showing very long and heavily labelled oocyte nucleus. Heavy label over follicle cell nuclei is also visible. Incubation with ^{3}H-uridine for 15 min. ×220.

mosomal chromatin[2] accumulated in the oocyte nucleus as a result of a process known as gene amplification.

As the observations of HEGNER and RUSSELL were only concerned with the course of differential mitoses and very early stages of the oocyte growth, it seemed interesting to study the fate and role of extrachromosomal chromatin in the oogenesis of Gyrinidae. In this paper some data concerning modifications of oogenesis in the meroistic-polytrophic insects, as related apparently to the presence of extrachromosomal DNA, will be presented.

The adults of whirligig beetle, *Gyrinus natator*, have been used in this study. Although the course of differentiating mitoses has not been studied in this species, their number, like that of mitoses in *Dineutes* is clearly 3, because each oocyte is associated with the group of 7 nurse cells. The nucleus of each young oocyte occuring in the germarium differs from the nurse cell nuclei in that it also contains, in addition to the chromosomes, a compact Feulgen positive body (Figure 1). In pachytene oocytes, the extra DNA is a spherical structure, about 5 μm in diameter, situated in the middle of the nucleus. When the oocyte begins to grow, the DNA body migrates to one side of the nucleus, adhers to the nuclear membrane and assumes a cap-like form (Figure 2). Soon after that, the DNA body spreads over the whole inner surface of the nuclear membrane. This process is accompanied by its gradual dispersion, which results in the appearance in the nucleus of very small, Feulgen positive granules. Some of these granules adher to the inner surface of the nuclear membrane, and the others are evenly dispersed in the nuclear sap. As the fragmentation and dispersion of the extra DNA proceeds, considerable quantities of nucleolar material, most often in the form of large blocks of irregular shape, appear in the oocyte nucleus. At the later stages of the oocyte growth, the degree of dispersion of extra DNA becomes so high that it is impossible to detect it any further with the Feulegen method. While those changes in the structure of extra DNA are taking place, the oocyte nucleus gradually increases in size, but it still preserves its spherical shape. Up to that stage, the general course of nuclear processes in oocytes of *Gyrinus* resembles to a considerable extent the oogenesis in *Dytiscus* and other Dytiscid water beetles in which gene amplification takes place[3,4].

When the elongating growth of the oocyte begins, its nucleus is at first ellipsoidal and later assumes a cylindrical shape. During the whole period of further growth, along with the elongation of the oocyte, the nucleus is also elongated, so that in the middle previtellogenetic stages its length attains about 300 μm and it is only a little shorter than the whole oocyte (Figures 3 and 4). The average diameter of the nucleus at these stages does not, however, exceed about 16 μm. It may be calculated that the volume of such a nucleus in *Gyrinus* corresponds to the volume of a sphere about 50 μm in diameter; its surface, owing to extreme elongation, is however twice as large as the surface of such a sphere. In fact the volume, and particularly the surface of the oocyte nucleus in *Gyrinus* increases even more, as a result of production by the nucleus of numerous thin processes penetrating the ooplasm at a distance of 10 and more μm (Figures 3 and 4). Directly before the degeneration of nurse cells, the lenght of the oocyte in *Gyrinus* increases to 630 μm and its nucleus attains a length of up to 500 μm.

[3] E. URBANI and S. RUSSO-CAIA, Rc. Ist. Sci. Camerino *5*, 19 (1964).
[4] E. URBANI, Monitore zool. ital. *3*, 55 (1969).
[5] K. BIER, W. KUNZ und D. RIBBERT, Chromosoma *23*, 214 (1967).

During the growth of the nucleus, the nucleolar material is gradually fragmented into smaller and smaller granules and aggregates. In the advanced previtellogenetic stages, nearly the entire nucleus is evenly filled up with an almost homogenous substance rich in RNA (Figures 3 and 4), which is composed of very small granular nucleoli, similarly as it is in the nuclei of the growing oocytes of Dytiscidae in which the nucleolar extra DNA occurs[5]. Only a relatively small area of the nucleus remains free of the nucleolar material. In this area all the oocyte chromosomes are accumulated forming a compact karyosphere. This indicates that the behaviour of chromosomes in the oogenesis of Gyrinidae does not essentially differ from that of the chromosomes in nuclei of the growing oocytes in the majority of insects with polytrophic ovaries.

The very significant increase in the volume and surface of the oocyte nuclei in *Gyrinus* and the abundance of the nucleolar material on the one hand, and the condensed state of the oocyte chromosomes on the other hand, seems, to indicate a considerable transcriptional activity of the extra DNA contained in those nuclei. The autoradiographic examination carried out with the use of ^{3}H-uridine has fully confirmed this supposition. The results obtained have proved that during the whole period of previtellogenesis the oocyte nuclei are very active in RNA synthesis. The intensity of this synthesis seems to be of the same order as the intensity of RNA synthesis in the nurse cell nuclei (Figure 5). The labelling of the oocyte nuclei resulting from the specific incorporation of ^{3}H-uridine into newly synthesized RNA is evenly distributed all over them, in line with the distribution of the nucleolar material (Figure 6).

We did not succeed, so far, in demonstrating that the nucleolar RNA synthesized in the oocyte nuclei of *Gyrinus* is next transported through the nuclear membrane into the ooplasm. The fact that such a process, most probably very intensive, actually takes place throughout the whole previtellogenetic growth period of oogenesis, seems to be confirmed by the great RNA concentration in a thick layer of ooplasm adjacent to the nuclear membrane (Figures 3 and 4). The degree of basophilia of that layer of ooplasm begins to decrease only at the end of previtellogenesis, that is at the stage directly preceding the degeneration of nurse cells. It also seems that the contribution of nurse cells in supplying RNA to the ooplasm of growing oocyte is minor, at least in terms of quantity, in comparison to the role played by the oocyte nucleus in that process. The presented data are preliminary results of current investigations.

Zusammenfassung. Autoradiographische Untersuchungen an den Oozytenkernen des Taumelkäfers *Gyrinus natator* zeigen eine hohe RNA-Syntheseaktivität, kurz nachdem sich der extrachromosomale Chromatinkörper im Karyoplasma aufgelockert hat. Da die Oozytenchromosomen gleichzeitig in der Karyosphäre zusammengeballt sind, wird angenommen, dass die Transkriptionsaktivität an der extrachromosomalen DNA abläuft.

B. Matuszewski and P. Hoser

Department of Cytology, University of Warsaw, Krakowskie Przedmieście 26/28, P–00-927/1 Warszawa 64 (Poland),
17 September 1974.

Active Components of *Sargassum tortile* Effecting the Settlement of Swimming Larvae of *Coryne Uchidai*

Hydrozoa of Coelenterata are observed very often attached to various algae. This associative relationship between epiphytic hydrozoa and algae is formed by the sequence of settlement of hydrozoan larvae onto the associated algal thallus and then the growth of the hydrozoan colony thereon. It has been noticed biologically that most of epiphytic hydroid have their own preferred alga and that this particular association is established by the algal preference of the settling larvae. These observations suggest that an alga, to which the swimming larvae prefer to settle, might produce some specific chemical compound which induces the settling of swimming hydrozoan larvae.

It was found recently that the settling of the swimming larvae of *Coryne Uchidai*, a kind of hydrozoa, was clearly induced by adding the juice of *Sargassum tortile* (Japanese

Bioassay of synthetic I, II and III toward the larvae of *Coryne Uchidai*

Time (h)	12 ~ 24						48						72					
Stage[a]	m	cl	s	a	b	p	m	cl	s	a	b	p	m	cl	s	a	b	p
δ-Tocotrienol (I)[b]		6	2		2			7			1	2			7[d]			3
Control[c]	2	8						10						10				
Epoxide (II)[e]			3	7					5			5					5	5
Epoxide (II)[f]		3	4	3				2		5	1	2					7	3
Control[c]		5	5					5	5					2	3	5		
Dehydro epoxide (III)[g]		5	2	3					1	4		5				4		6
Control[h]	4	2	2				4	2	4				3	1	5	1		

[a] Abbreviation; m, swimming; cl, crawling; s, settling; a, attaching; b, formation of tentacle bud; and p, formation of polyps. [b] One drop (0.05 ml) of ethanol solution containing 15 mg of DL-(I) in 1 ml of ethanol was added to 10 larvae in 20 ml of sea water. The values show the number of larvae in different stages. [c] One drop of ethanol containing no material was added under same conditions. [d] All of the 7 larvae died accompanying cytolysis. [e] One drop of ethanol solution containing 30 mg of II was added to 10 larvae in 20 ml of sea water. [f] A quater of 1 drop of the above original solution was used. [g] One drop of ethanol solution containing 30 mg of epoxide (III) in 1 ml of EtOH was dropped on filter paper and the solvent was evaporated. The filter paper was put in 20 ml of sea water containing 10 larvae. [h] Filter paper without material was put in sea water.