Occurrence of a DNA Body in the Nervous Cells of a Fresh Water Snail (*Biomphalaria glabrata*)

In the oocytes of *Dytiscus*[1,2], *Tipula*[3,4] and *Pales*[5] a DNA body is present.

In *Tipula* the result of experiments of incorporation of labelled thymidine indicates that the DNA body synthesizes its DNA at a different time from chromosomes; after being synthesized in the oogonial interphase this body disintegrates at late diplotene releasing its DNA in the nucleus and cytoplasm[4]. The DNA body, so far as I know, has not been described in the somatic cells of vertebrates or invertebrates.

In the neurons the heterochromatic paranucleolar structures are the nucleolus associated chromatin and the irregular areas associated with the sex chromosomes[6].

Material and methods. The entire body of 30 specimens of the fresh water Planorbid snail *Biomphalaria glabrata* (Say, 1818), the South American intermediate host of *Schistosoma mansoni*, were fixed in Bouin, Sanfelice, Zenker and Fleming's fluids; 7 specimens were dissected in their hemolymph and the complex of the central ganglia was fixed in Carnoy or Zenker or Fleming fluid. The specimens were dehydrated in butanol and embedded in paraffin.

Serial sections (thickness 3–5 μm) were cut and these were stained by the ordinary Heidenhain method or with the Feulgen-light green method[7] or by the Toluidine blue method[8]; control sections were extracted with 10% perchlorid acid at 4°C for 18 h (for the removal of the RNA alone) or with 5% perchloric acid at 60°C for 30 min for the removal of both nucleic acids according to the directions given by PEARSE[7].

The nervous system of 1 specimen was dissected in CARRIKER fluid[9]; the ganglia were dissociated with tungsten wires and the living nerve cells were observed and photographed in phase contrast. All the pictures were taken with a Zeiss Photomicroscope using ORWO 15 DIN panchromatic film.

Results. In each of the 11 central ganglia[10,11] of *Biomphalaria*, there is a wide variability in cell shape

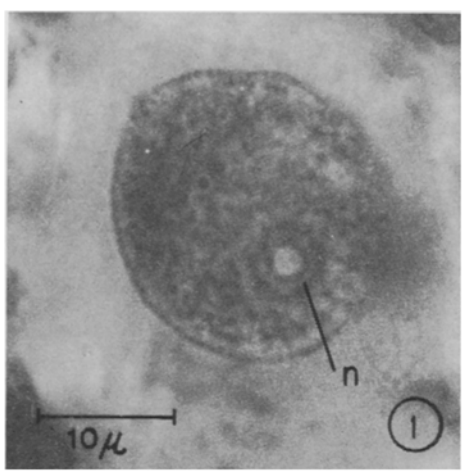

Fig. 1. A cell from the right cervical ganglion; the nucleolus (n) has the appearance of a ring. Feulgen-light green. ×2,000.

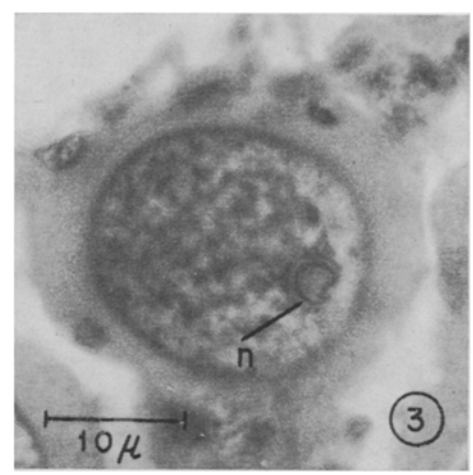

Fig. 3. The nucleolus (n) is cup-shaped. Feulgen-light green. ×2,000.

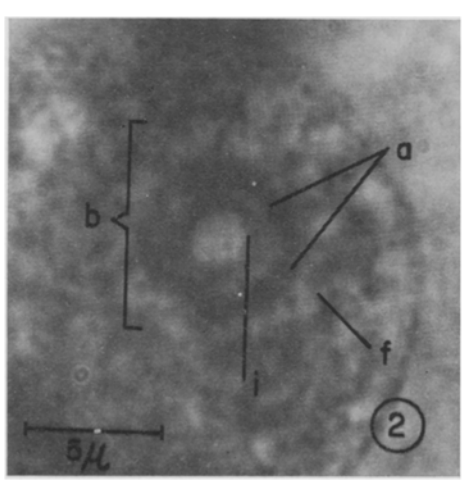

Fig. 2. Enlarged part of Figure 1; the nucleolus associated chromatin (a) lines the internal and external surfaces of the nucleolar ring in whose center is present a vacuolated inclusion (i); filaments (f) connect the nucleolus associated chromatin to the DNA body (b). Feulgen-light green. ×4,000.

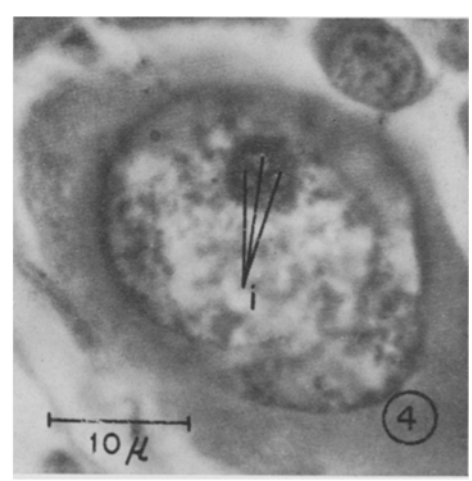

Fig. 4. Three refringent bodies (i) in the nucleolus. Feulgen-light green. ×2,000.

(spheroidal, ovoidal, piriform etc...) and cell size (from 530 μm^3 to 56,000 μm^3).

In the ganglia the largest cells are generally located in the periphery, the smaller in a more internal layer; nerve fibers run through the center of the ganglion. The small internal cells have 1 or 2 nucleoli, where the peripheral largest cells have a nucleolar number ranging from 1 to 20. In this last case the total nucleolar volume varies greatly and the nucleolar volume of a large cell can be less than in a small one; it was long ago described that the state of functional activity is a factor of importance in regulating the size of the nucleolus in neurons[12,13].

Some of the nucleoli are small (less than 1 μm) but 1 or 2 nucleoli are large (4 μm diameter). In Toluidine blue or Feulgen-light green preparation, these larger nucleoli appear like a ring (Figure 3) thus suggesting that the nucleolus has the shape of a cup. The internal and external surfaces of the cup are lined with Feulgen material, i.e. the nucleolus associated chromatin.

In the concavity of the cup there are one (Figures 1 and 2) or more (Figure 4) refringent bodies. A quite similar morphology of the nucleolus can also be clearly seen in the living cells (Figure 5).

In the Feulgen preparations the chromatin externally associated with the nucleolus is encircled, at a distance of 1.5–2 μm, by a ring of chromatin granules. The clear circular area between this ring and the chromatin externally associated with the nucleolus is crossed by tiny heterochromatic filaments. The same structure is present in the smaller more internal ganglion cells, but the number of granules in the ring is scanty.

Discussion. The pattern of the chromatin around the nucleolus recalls the DNA body of the oocytes of insects[1–5], because in this case also the nucleolus lies inside the body but we should stress that: 1. tiny filaments cross the clear area between the chromatin externally associated with the nucleolus and the DNA body which could indicate that each one of the granules of the DNA body belongs to the same part of the genonema bearing the nucleolus associated chromatin; 2. in the nervous cells, i.e. in highly specialized somatic cells, the largest peripheral cells of the ganglion have more granules of chromatin in the DNA body than the smaller, more internal ones; thus the increase in the cell size is accompanied by an increase of the DNA body. Then the behaviour of the DNA body of the nervous cells of *Biomphalaria* is different from that of the oogonia where the DNA body after being synthesized in the interphase is lost in the late diplotene.

It is possible to suppose that in the oocytes the influence of the cytoplasm on the genome takes a different pattern with the biochemical changes at the diplotene stage, whereas the constant pattern of the nucleocytoplasmic interrelationships in the differentiated cells induces as a consequence the permanence of the DNA body. The quantitative increase of activity could account for the enlargement of the DNA body: the paranucleolar irregular heterochromatic structure, associated with the *Y* chromosome in mammalian neurons, enlarges its area after stimulation and in other experimental conditions[6].

It is the goal of further experiments to see if the DNA body is under the control of endogenous or exogenous factors and if there is some type of correlation between the DNA body and the nucleolus associated chromatin for the production of the nucleolar material.

Résumé. Dans les cellules nerveuses de *B. glabrata* on observe un anneau de chromatine, «DNA body», qui entoure le nucléole; entre le «DNA body» et la chromatine attenante à la paroi externe du nucléole existe un anneau clair parcouru par de très fins filaments. Le «DNA body» des cellules nerveuses rapelle une formation similaire, mais transitoire, présente dans les oocytes d'autres Invertébrés.

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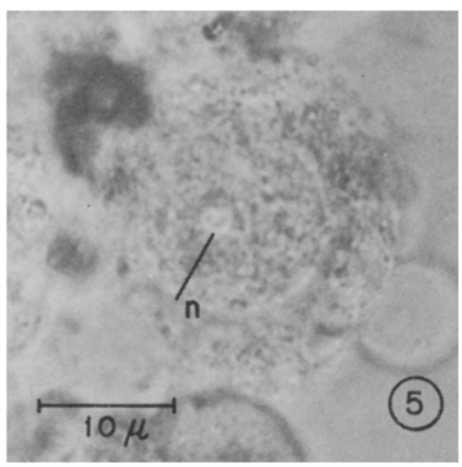

Fig. 5. A cup shaped nucleolus (n) in a living cervical ganglion cell. Phase contrast. UV Filter III. ×2,000.

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Interferon Production by Sendai Virus-Treated Hamster and Monkey Cells

By using SV40-transformed cells of Cl_2TSV_5 and RHaT lines[1], several generations of tumors produced in Syrian hamsters were studied[2]. Tumor cells were then fused with SV40-permissive monkey cells to study the rescuability of SV40; UV-light-inactivated Sendai virus (UV-SeV) was used as fusing agent. Since UV-SeV may induce interferon which may inhibit or reduce the rescuability of SV40 from fused cell cultures[3,4], a study was undertaken to test tumor and non-transformed cells for interferon production.

Experimental. Cl_2TSV_5 and RHaT cells, the SV40-permissive monkey cells (AGMK and BSC), the hamster primary kidney (HK) cell cultures, and the PK-15