

(Figures 1, 2 and 3). If the mean LI for left atrium myocytes of all rats killed at the end of the 2nd week attains  $1.60 \pm 0.59\%$ , which is 15 times more than control values ( $p < 0.05$ ), atria of several rats contained as many as 3–5% of labelled muscle nuclei; the sum of LI and MI

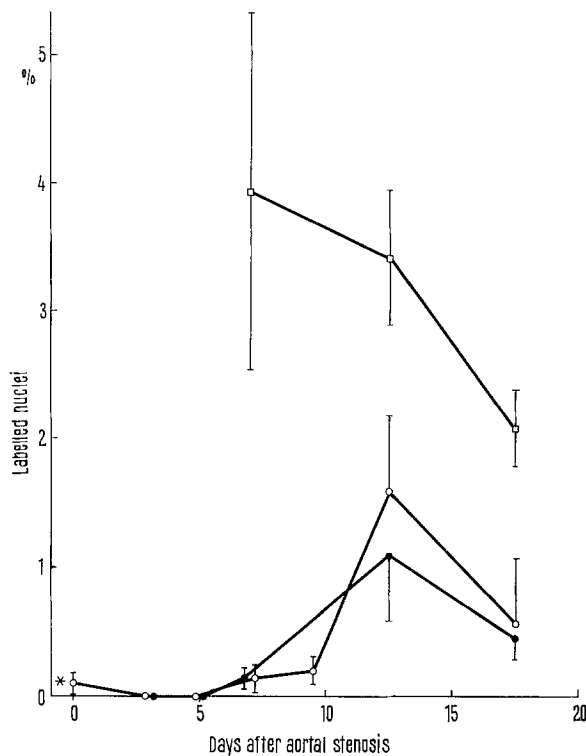


Fig. 3. Percentages of  $^3\text{H}$ -thymidine labelled myocyte nuclei in the left atrium, right atrium and in perinecrotic myocardium of the right atrium at different stages after aortal stenosis in rats. Designation of points and the number of animals as in Figure 1.

attains in some animals 4–7%. Repeated injections of  $^3\text{H}$ -thymidine result in labelling of 0.2, 2.1 and 5.8% of all myocytes in left atria of 3 rats, respectively. The degree of DNA synthesis activation was found to be similar both in left and right atrium myocytes (Figure 3), while mitoses were numerous only in the former ones. By the 3rd week, proliferation of auricular muscle nuclei is reduced clearly (Figures 1 and 3). As compared with the above data, DNA synthesis in the myocytes bordering the necrotic areas in atria was activated by 5–7 days earlier and 2–3 times more intensively (Figure 3).

Extreme individual variations of both LI and MI values are quite typical (Figures 1 and 3), indicating the inconstancy and/or partial synchronization of the reactive hyperplasia of highly differentiated heart muscle cells.

Thus both left ventricle hyperfunction and infarction<sup>6–8</sup> are followed by reactive hyperplasia of differentiated atrial myocytes which is somewhat belated as well as less intensive and regular in the bulk of hypertrophied heart auricular myocardium. It must be taken into account in the studies on the pathogenesis of different forms of heart failure.

**Выводы.** Гиперфункция и гипертрофия сердца при стенозе аорты сопровождаются у многих крыс активацией синтеза ДНК и митозов в миоцитах как левого, так и правого предсердий. Реактивная пролиферация максимальна к концу 2-й недели после коарктации аорты, когда  $1,60 \pm 0,59\%$  всех миоцитов левого предсердия синтезируют ДНК, а  $0,96 \pm 0,44\%$  – делятся митозом. Ещё более интенсивно размножаются миоциты вокруг некротических очагов в предсердиях. Напротив, в желудочках обнаружены лишь единичные меченые  $\text{H}^3$ -тимидином ядра.

P. P. RUMJANTSEV

Laboratory of Cell Morphology,  
Institute of Cytology of the Academy of Sciences  
of the USSR,  
Leningrad (USSR), 30 December 1969.

### Cilia in Axolotl Neurons (*Siredon mexicanum*)

The occurrence of cilia in neurons and glia of the mammalian nervous system described by del RIO-HORTEGA<sup>1</sup> as early as 1916 is now a well-established fact<sup>2–15</sup>, but few references are available about their existence in the nervous tissue of lower forms<sup>16, 17</sup>. The purpose of this note is to describe this organoid as seen in spinal cord neurons of the aquatic salamander *Siredon mexicanum* (Axolotl) and to suggest that the presence of cilia in nerve cells may be more widespread through the central nervous system in the various animal phyla than previously assumed.

**Materials and methods.** A series of 50 spinal cords either in situ or implanted for variable lengths of time into the dorsal fin of a host axolotl<sup>18</sup> were fixed by immersion in a variety of aldehyde mixtures, and post-fixed in osmium tetroxide, followed by aqueous saturated uranyl acetate and embedded in Epon. Ultra-thin sections were stained with lead acetate and studied under a Siemens I electron microscope. Thick (1  $\mu\text{m}$ ) sections of the same material were stained with 1%, pH 7.4, toluidine blue and observed under the optical microscope.

**Results.** A number of ciliated neurons were found in the spinal cords both in situ and in the implants. Identifica-

tion of the cells as neurons was made on the basis of their strong similarity to the mammalian neuron, location inside the cord and correlation with optical microscopy observations. Glial cells, on the other hand, are very similar to those described in the spinal cord of the newt<sup>19</sup> and no cilia have been found in these cells so far.

Most of the morphological features observed in the mammalian neural cilia were present here. Figure 1 illustrates a typical longitudinal section of a cilium in a neuron of an implant. The shaft has a diameter of 170 nm, slightly less at its origin, and is bounded by a ciliary sheet continuous with the cell membrane. In the cases where the centrioles remained in their original position in the vicinity of the nucleus, the cell membrane invaginates down to the level of the transition region of the cilium before reflecting on itself to cover the shaft and, as a result, a periciliary sulcus forms with the deepest portion dilated (Figure 1). With approximately the same frequency, however, cilia are found in which the basal body is located in the periphery of the cell so that no membrane invagination occurs (Figure 2). The microtubules inside the shaft have a diameter of about 20 nm and have attached to

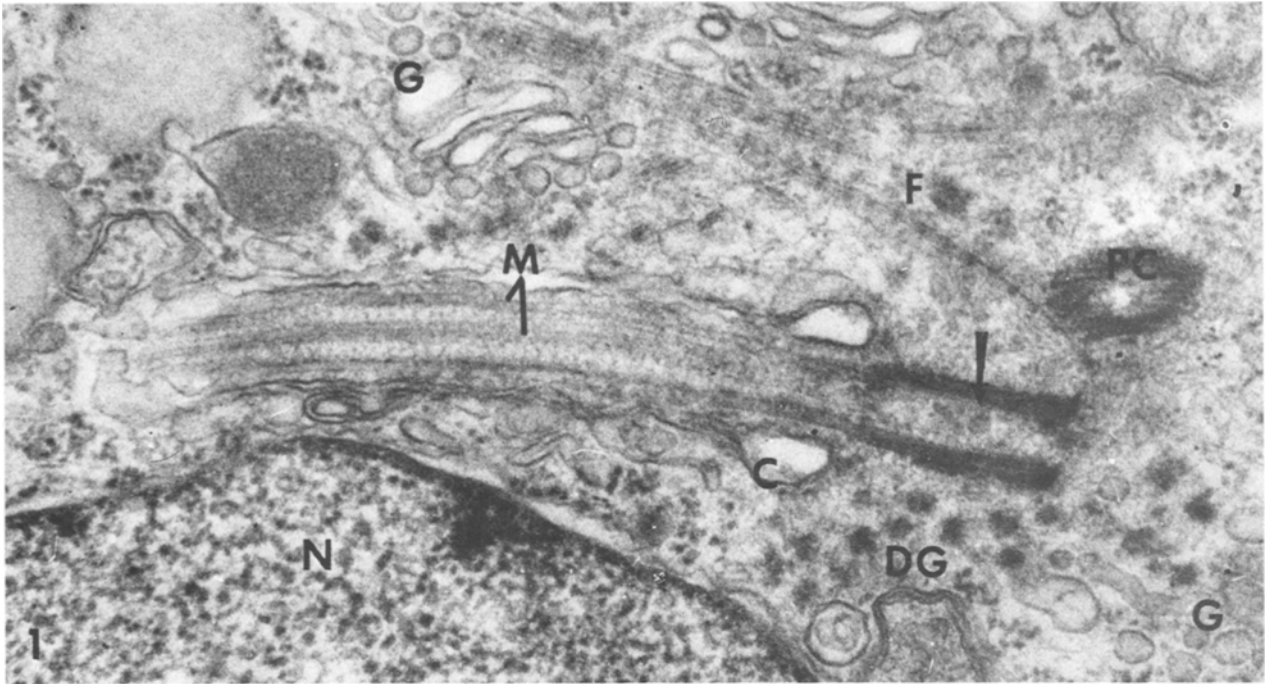


Fig. 1. Cilium in a neuron of a spinal cord implant. The centrioles are in the vicinity of the nucleus (N), a periciliary sulcus is formed as the cell membrane (C) infolds to the point where it reflects to cover the shaft. The ciliary microtubules (M) are interconnected by delicate transversal elements. Several Golgi apparatuses (G) and numerous dense granules (DG) surround the base of the cilium which contains a vesicle (arrow) of a size and appearance similar to the smaller Golgi vesicles. The proximal centriole (PC) is in a plane almost perpendicular to the basal body. (F) Cross-striated filament.  $\times 52,500$ .

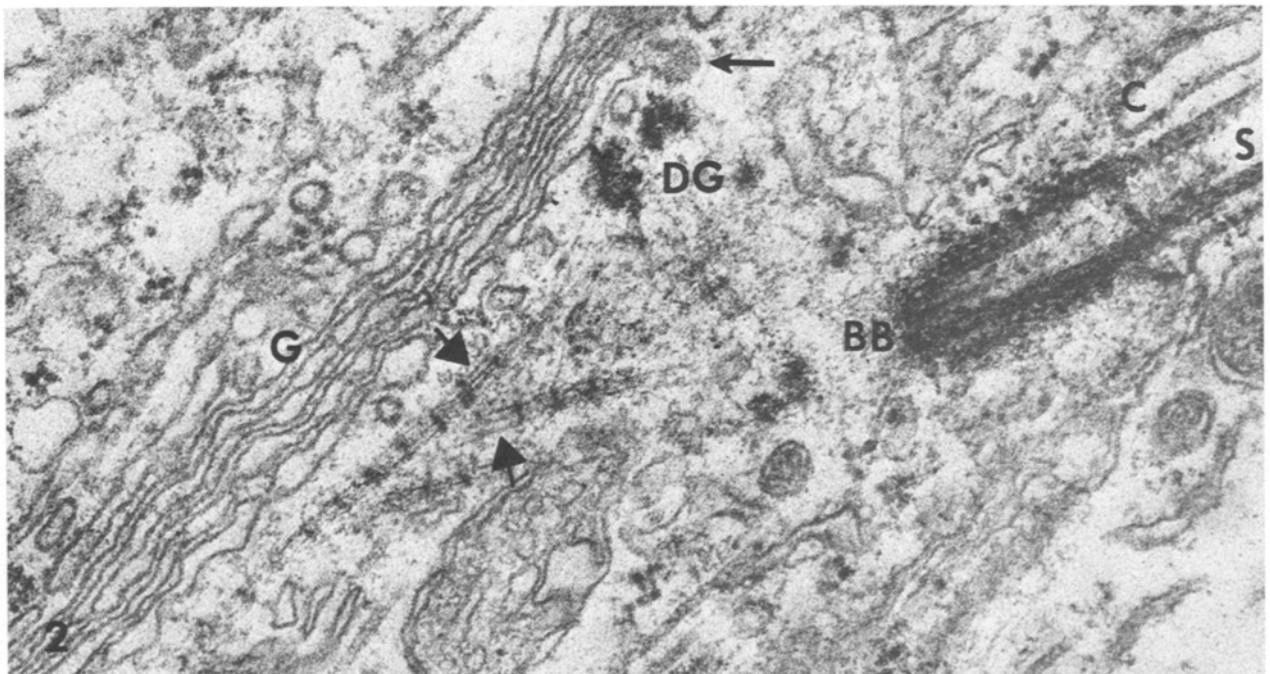


Fig. 2. Detail of the base of the cilium in a neuron of normal spinal cord. The basal body (BB) is immediately underneath the cell surface. The cell membrane (C) reflects on itself to cover the shaft (S). Striated filaments run parallel to a prominent Golgi apparatus (G). The longitudinal subunits forming the filaments are especially prominent at the points indicated by the thick arrows. Dense irregular granules (DG) are present between the basal body and the Golgi apparatus. The thin arrow points at a vesicle with dense content protruding from a Golgi cisternae.  $\times 75,000$ .

their surface side-arms of low electron density and blurred limits (Figure 1). The total length of the shaft has not been determined. However, the cilium in Figure 2 ran a trajectory of more than 2  $\mu\text{m}$  into the neuropile closely attached to a dendrite originating in the same cell. The proximal centriole is located next to the basal body or distal centriole and approximately  $90^\circ$  from it (Figure 1). The Golgi apparatus is also constantly associated with the base of the cilium. Often two or more of these structures can be counted in a single section, suggesting that actually a cluster of them encircles the basal body. A variable number of dense granular masses 70–80 nm in size and

irregular in profile are generally present in the area between the Golgi elements and the basal body (Figures 1 and 2). Striated filaments of indefinite longitude are constantly seen in association with the basal body. They are composed of longitudinally arranged subunits (Figure 2), about 7 nm thick, crossed at 50 nm intervals by bars of electron-dense material. Some cross-sections of cilia have been examined showing the 9+0 pattern of microtubules which is usual in neural cilia, and a single cilium of the 9+2 pattern was also observed. Due to the size of the neurons the chances for a section to intersect a cilium are low and it is easy to overlook a cross-section of cilium. Thus it is difficult to assess the percentage of ciliated neurons in the spinal cord.

**Discussion.** Functional questions related to the neural cilia remain open. One concerns the possible motility of these structures. Cilia exhibiting a 9+0 pattern are usually considered non-motile. However, there is increasing evidence<sup>20–23</sup> that the 9+0 pattern does normally exist in motile forms and this could be the case with the neural cilia. At any rate, the crucial question is what purpose, if any, is subserved by them. The opinions in the literature are divided. Sensory functions of an unspecified nature were attributed to the 9+0 cilia by several authors on the basis of Sjöstrand's<sup>24</sup> pioneer description of the highly modified cilium of retinal rods. However, 2 sources of evidence make this generalization untenable. First, the existence of 9+2 cilia in sensory receptors<sup>25–28</sup> indicates that lack of central fibers is not synonymous with sensory function. Second, 9+0 cilia do occur in the most diverse cell types of various organs<sup>5</sup> to which no sensory properties can be reasonably attributed.

The cilia being found in an increasing number of neural structures are always in association with the Golgi apparatus. Thus some metabolic relationship is suggested, perhaps the release under the form of dense granular bodies of substances concentrated by the Golgi cisternae and incorporated by the cilium. It is hoped that future experimental work will be able to answer some of these questions.

**Resumen.** Un estudio electro-microscópico de la médula espinal del axolote reveló la existencia de neuronas ciliadas, tanto en medulas normales como en segmentos medulares implantados por tiempos variables en la aleta dorsal de animales de la misma especie. La cilia que es única e implantada cerca de una dendrita se extiende en la neuropila por una longitud de varios  $\mu\text{m}$ .

M. P. DEL CERRO and R. S. SNIDER<sup>29</sup>

Center for Brain Research, University of Rochester,  
Rochester (New York 14627, USA), 5 January 1970.

- <sup>1</sup> P. DEL RIO-HORTEGA, Trab. Lab. Invest. biol. Univ. Madrid 14, 117 (1916).
- <sup>2</sup> H. A. DAHL, Z. Zellforsch. 60, 369 (1963).
- <sup>3</sup> M. A. GRILLO and S. L. PALAY, J. Cell Biol. 16, 430 (1963).
- <sup>4</sup> R. A. ALLEN, J. ultrastruct. Res. 12, 730 (1965).
- <sup>5</sup> A. R. CURRIE and D. H. WHEATLEY, Post-grad. med. J. 42, 403 (1966).
- <sup>6</sup> F. MOLLO, S. DALFORNO and M. G. CANESE, Boll. Soc. Biol. ital. Pat. 9, 261 (1966).
- <sup>7</sup> M. P. DEL CERRO and R. S. SNIDER, J. Microsc. 6, 515 (1967).
- <sup>8</sup> S. E. KORNGUTH, J. W. ANDERSON and G. SCOTT, J. comp. Neurol. 130, 1 (1967).
- <sup>9</sup> M. MILHAUD and G. D. PAPPAS, Comp. r. Acad. Sci., Paris 284, 474 (1967).
- <sup>10</sup> M. MILHAUD and G. D. PAPPAS, J. Cell Biol. 37, 599 (1968).
- <sup>11</sup> C. SOTELO and S. L. PALAY, J. Cell Biol. 36, 151 (1968).
- <sup>12</sup> K. D. BARRON and P. F. DOOLIN, J. Neuropath. exp. Neurol. 27, 401 (1968).
- <sup>13</sup> B. BERGER, Archs. Anat. microsc. Morph. exp. 58, 41 (1969).
- <sup>14</sup> H.-S. LIN and I. L. CHEN, Z. Zellforsch. 96, 186 (1969).
- <sup>15</sup> M. P. DEL CERRO and R. S. SNIDER, Anat. Rec. 165, 127 (1969).
- <sup>16</sup> J. TAXI, C. r. Soc. Biol. 155, 1860 (1961).
- <sup>17</sup> S. L. PALAY, Anat. Rec. 139, 262 (1961).
- <sup>18</sup> S. R. SNIDER, L. G. ABOOD and R. S. SNIDER, Expl. Brain Res. 6, 81 (1968).
- <sup>19</sup> C. SCHONBACH, J. comp. Neurol. 135, 93 (1969).
- <sup>20</sup> B. A. AFZELIUS, in *Electron Microscopy* (Ed. S. S. BREESE; Academic Press, New York 1962).
- <sup>21</sup> I. DESPORTES, Compt. r. Acad. Sci. Paris, Series D, 263, 517 (1966).
- <sup>22</sup> D. P. COSTELLO and H. M. COSTELLO, Biol. Bull. 135, 417 (1968).
- <sup>23</sup> D. P. COSTELLO, C. HENLEY and C. R. AULT, Science 163, 678 (1969).
- <sup>24</sup> F. S. SJÖSTRAND, J. Cell. comp. Physiol. 42, 15 (1953).
- <sup>25</sup> E. FAURÉ-FREMIET and C. ROUILLER, Comp. r. Acad. Sci., Paris 244, 2655 (1957).
- <sup>26</sup> E. FAURÉ-FREMIET, Q. Jl. microsc. Sci. 99, 123 (1958).
- <sup>27</sup> D. R. ROGGEN, D. J. RASKI and N. O. JONES, Science 152, 515 (1966).
- <sup>28</sup> E. K. MACRAE, Z. Zellforsch. 82, 479 (1967).
- <sup>29</sup> The authors acknowledge the excellent collaboration of Mrs. SUZANNE O'BRIEN and Mr. J. BRYKE. This work was supported in part by grants No. NS04592 and No. NS06827 from the National Institutes of Health.

## Toxicity of Deoxycholate at pH below 7.3 as a Potential Cancerostatic Property

Deoxycholic acid (DCA) is known to increase the permeability of cell membranes. In our experiments with decorporation of metals from yeast cells using DCA (details will be published elsewhere), we found a striking effect of pH at biological ionic strength and temperature (Figure 1). The decorporation of (labelled) cobalt takes place only below pH 7.3, and the onset of the biological activity is extraordinarily sharp. As any elimination of essential metals lowers the viability of cells, a study was

made on the over-all toxic activity of DCA in dependence on pH.

**Methods.** Commercial baker's yeast (production Kolín) was used; 100 mg (or less) of it was agitated in stoppered tubes with 8–20 ml of a solution containing a constant amount of DCA (production Spofa, CSSR) and of  $\text{KH}_2\text{PO}_4$ ; concentration of NaOH and NaCl were variable so that at any pH used the ionic strength remained constant 0.16. The temperature of  $37^\circ\text{C}$  was maintained,