# Effect of Stretching on Cell Proliferation in the Seminiferous Tubule of Guinea Pigs in Organotypic Culture

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Proliferation of the smooth muscle cells (SMC), the cells of the adventitia, and the endothelial and epithelial cells forming blood vessels and viscera play an important role in the pathogenesis of many diseases. In atherosclerosis proliferation of vascular SMC occurs in the plaque, and in hypertension polyploidization of SMC is enhanced [16]. There are various hypotheses on the causes of cell division: denudation of the vascular endothelium [11], the effect of growth factors [17], damage to the muscular layer [8,13], activation of tumor precursors [7], denervation [9,10], and an increase of functional loads [4]. In many cases cell proliferation proves to be a mechanism of general adaptation directed toward overcoming unfavorable conditions. Enhanced activity of the blood vessels as well as of the walls of the urinary bladder and uterus causes an increase of SMC proliferation necessary in order to compensate for the increased load. On the other hand, a decreased functional load reduces SMC proliferation in these organs [12]. The migration of cells of the seminiferous tubule promotes their proliferation under culturing conditions, and the subsequent reinnervation by the sympathetic ganglia suppresses SMC proliferation [9]. Any processes directed toward normalizing the functional activity of the tissues diminish SMC proliferation.

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It is of interest to study how the contractility of SMC affects their proliferation. The contractions of SMC are known to stop during the whole mitotic process [9,10]. It cannot be ruled out that both contractility and reinnervation reduce SMC proliferation.

This study was carried out under conditions of organotypic culturing. Contractility was stimulated by active intermittent stretching of explants from the seminiferous tubule of guinea pigs with the aid of a magnetic load or by means of passive stretching of circular segments by glass rods.

## MATERIALS AND METHODS

Male guinea pigs weighing 300-400 g were killed under ether anesthesia. The seminiferous tubules were extracted and dissected free from the connective tissue and blood vessels. For longitudinal stretching the prostatic portion of the organ was cut into longitudinal strips 4 mm long with a cross-section diameter of 0.75 mm. The strips were placed in a special device for periodic stretching of the muscle explants [6]. In this device, the strips were subjected to stretching under organotypic conditions with a frequency of 1 movement every 9 minutes by means of attached magnet loads and a magnet rotating under the culture dishes. During one cycle of stretching and subsequent relaxation, the length of a strip could change by as much as 0.05 mm.

For cross-stretching, plates made of borosilicate glass with perpendicular cone-shaped rods were

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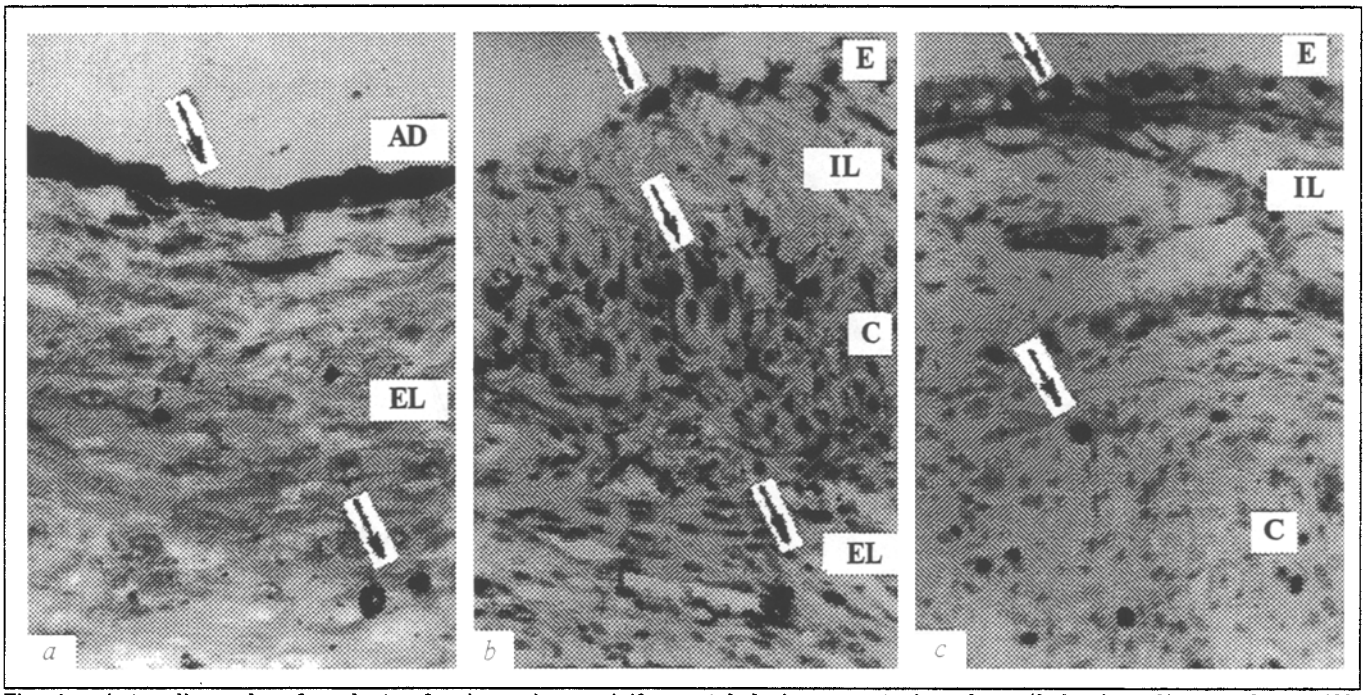


**Fig. 1.** Autoradiography of explants of guinea pigs seminiferous tubule in organotypic culture (3 days). *a, b*) controls; *a*:×400, *b*:×200; *c*) stretched, ×200. Staining: pyronine, methylene blue. AD: cells of adventitia; EL: external longitudinal layer of smooth muscle; C: circular layer; IL: internal longitudinal layer; E: epithelial cells. Arrows indicate cells with $^3$H–thymidine–labeled nuclei.

used. The prostatic portion of the seminiferous tubule was sectioned into circular segments 1 mm thick. The segments were put on the rods so that they dilated until signs of muscular tension appeared. The plates with the rods and the circular explants were placed in a plastic petri dish 40 mm in diameter, and culture medium was poured over it so that the explants were positioned at the interface of the medium with the gas mixture [2]. The Petri dish was placed in a miniexsiccator for culturing [5]. In both variants the culture medium consisted of DMEM medium, 10% fetal calf serum, 0.8 U/ml insulin, 10 mM HEPES, 0.85 g/liter $Na_2CO_3$, 100 U/ml penicillin, and 50 μg/ml streptomycin. The dishes and miniexsiccators were filled with a gas mixture containing 60% $O_2$, 35% $N_2$, and 5% $CO_2$, and hermetically closed. The device for stretching and the miniexsiccators were incubated at 37°C. The nutrient medium and the gas mixture were changed once every 3 days.

Every 3 days medium with $^3$H-thymidine in a final concentration of 0.2 MBq/ml (5 μCi/ml) was added to the culture dishes for a 24-hour incubation. Eight longitudinally-stretched and control explants, as well as eight cross-stretched and control explants were used for each time of measurement. After one day of incubation the cultures were treated for radioautography [3]. They were fixed in methanol-acetic acid (3:1), embedded in paraffin, and sections 5 mm thick were prepared. The preparations with deparaffinized sections were covered with an M-type nuclear photoemulsion, incubated for 3 weeks, and developed in a D-19 developer [1]. The preparations were stained with pyronine and methylene blue. The index of labeled nuclei, equal to the number of cells with labeled nuclei, was counted in the sections as a percentage of the total cell count [3]. The index of labeled nuclei was determined separately for the longitudinal and circular muscle layers, as well as in the cells of the adventitia and in the cells of mucous epithelium lining the cavity of the seminiferous tubule. The results were statistically processed.

## RESULTS

Cells labeled with $^3$H-thymidine were encountered in the cultured explants in all the tissues studied. The cells of the adventitia and the cells of the mucous epithelium were labeled most intensively. The 24-h incubation with $^3$H-thymidine probably overlaps the time of the mitotic cycle of these cells, so that chains of cells with labeled nuclei were encountered in the sections. The majority of SMC with labeled nuclei were arranged separately. Closely situated labeled cells which separated after the synthetic period were rarely encountered, although clusters of separate labeled cells were sometimes observed (Fig. 1).

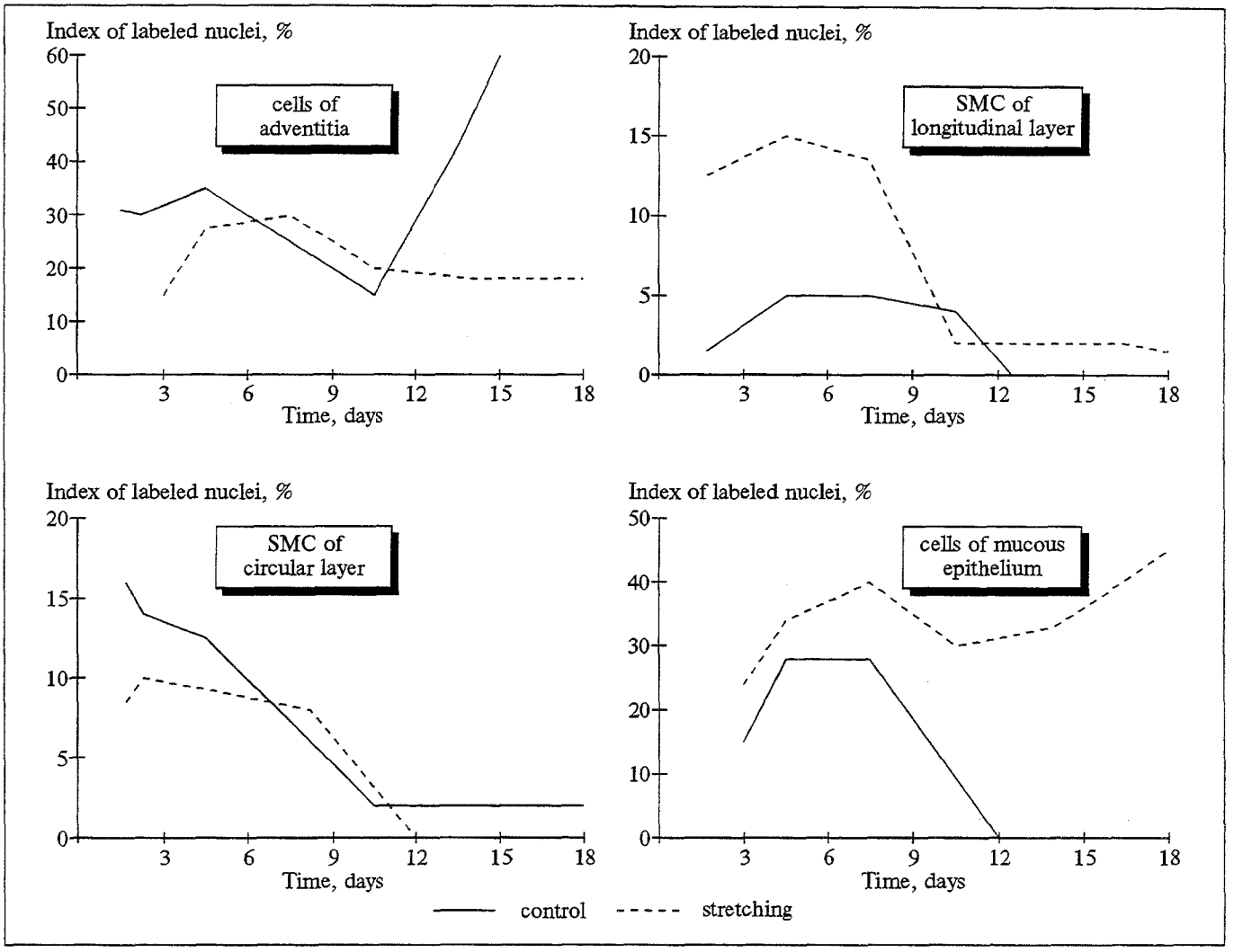


Fig. 2. Effect of periodic longitudinal stretching on cell proliferation of guinea pig seminiferous tubule in organotypic culture.

The transfer of tissues to culturing conditions is known to cause increased cell proliferation [9]. According to our data, in the control longitudinal explants an increased SMC proliferation, in the longitudinal and circular muscular layer alike, is observed over the first 6 days of culturing (Tables 1 and 2). During subsequent culturing the tissues adapt, and the index of labeled nuclei is tenfold reduced and is maintained on this basic level up to the 27th day of culturing. During the stimulation of contractility by periodic longitudinal stretching, the proliferation of SMC of the longitudinal muscular layer remains on the basal level, while in the circular layer the same increase of the in-

TABLE 1. Effect of Periodic Longitudinal Stretching on Index of Labeled Nuclei of Cells of Guinea Pig Seminiferous Tubule in Organotypic Culture ($M \pm m$)

| Time, days | Cells of adventitia | | SMC of longitudinal layer | | SMC of circular layer | | Epithelial cells | |
|---|---|---|---|---|---|---|---|---|
| | control | experiment | control | experiment | control | experiment | control | experiment |
| 1 | 32.28±2.7 | – | 12.67±1.04 | 1.08±0.24 | 15.41±0.92 | 8.89±0.83 | – | – |
| 2 | 28.22±2.36 | 18.41±3.54* | 13.52±2.35 | 3.57±0.49* | 11.11±1.39 | 12.66±1.34 | 17.53±1.91 | 21.58±6.77* |
| 6 | 40.01±11.29 | 37.6±4.46 | 15.11±4.71 | 6.02±1.19 | 12.33±5.06 | 10.44±1.26 | 36.58±4.59 | 45.2±3.01 |
| 9 | 14.2±4.94 | 25.07±3.13 | 1.34±0.29 | 3.5±1.03 | 2.01±0.33 | 5.34±0.72* | 19.63±3.09 | 35.34±3.98* |
| 12 | 18.3±2.94 | 12.53±1.8 | 0.89±0.15 | 0.97±0.87 | 0.18±0.06 | 0.25±0.18 | 2.67±1.58 | 25.48±4.96* |
| 15 | 62.5±3.59 | 15.7±1.57* | 2.33±0.25 | – | 1.86±0.18 | – | 4.16±1.6 | 34.6±5.0* |
| 18 | – | 19.0±1.9 | 0.27±0.06 | – | 0.41±0.05 | – | – | 43.8±6.33 |

Note. Here and in Table 2 an asterisk indicates reliable differences ($p<0.05$).

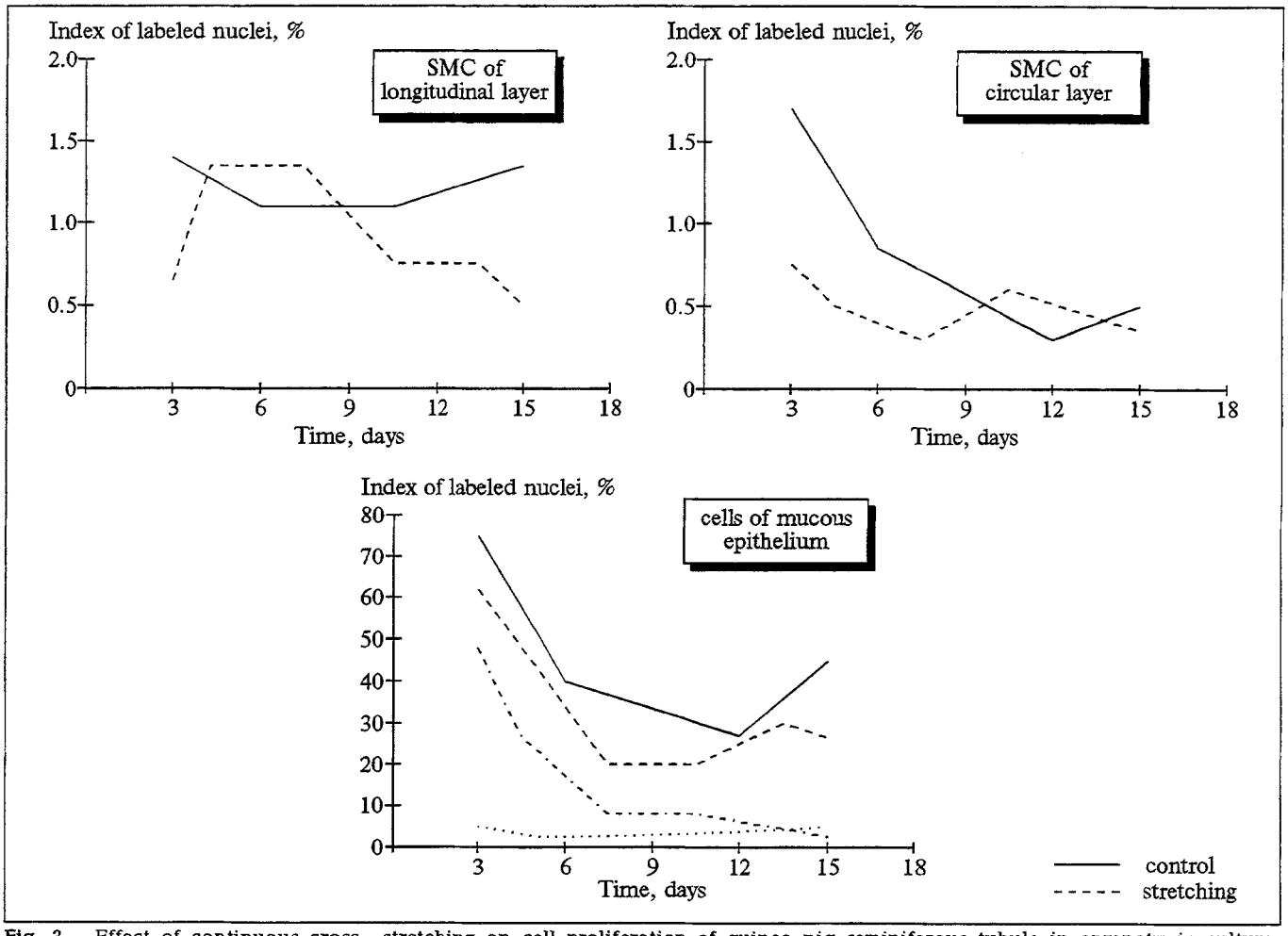


**Fig. 3.** Effect of continuous cross–stretching on cell proliferation of guinea pig seminiferous tubule in organotypic culture.

dex of labeled nuclei is observed as that in the control explants. Probably, stimulation of contractility does not per se allow the SMC of the longitudinal muscular layer to increase proliferation at the beginning of culturing. Since the circular muscular layer is less subject to stimulation when stretched lengthwise, an enhanced proliferation similar to that in the control explants is observed. Only after one day is a reliable decrease of proliferation during stretching noted; however, at the same time, the proliferation index is significantly increased ($p<0.05$) above the basal level (Fig. 2, *b*, *c*). The labeled SMC in the control and experimental explants were arranged randomly throughout the tissue, although local clusters of labeled cells were also noted. No increase of SMC proliferation was observed in the explants at the sites of tissue lesions and at the sites of greatest tension below the knot of the capron suture. Probably, an increase of SMC proliferation occurs spontaneously at the beginning of culturing, and the blocking of proliferation in the longitudinal muscular layer for periodic longitudinal stretching is a response to stimulation of contractility.

**TABLE 2.** Effect of Continuous Cross–Stretching on Index of Labeled Nuclei of Cells of Guinea Pig Seminiferous Tubule in Organotypic Culture ($M\pm m$)

| Time, days | Cells of adventitia | | SMC of longitudinal layer | | SMC of circular layer | | Epithelial cells | |
|---|---|---|---|---|---|---|---|---|
| | control | experiment | control | experiment | control | experiment | control | experiment |
| 3 | 73.23±3.39 | 61.85±3.31* | 1.42±0.31 | 0.68±0.14 | 1.64±0.23 | 0.77±0.1* | 4.74±5.5 | 47.57±13.77* |
| 6 | 40.29±1.98 | 33.9±3.25 | 1.22±0.22 | 2.19±0.26* | 0.91±0.27 | 0.18±0.03* | 3.15±2.87 | 5.87±1.53 |
| 9 | 8.75±0.57 | 4.63±0.68* | 1.02±0.13 | 0.62±0.06* | 0.17±0.3 | 0.63±0.07* | 1.55±0.23 | 8.36±0.64 |
| 12 | 26.68±3.47 | 34.2±5.61 | 1.16±0.16 | 1.2±0.22 | 0.33±0.24 | 0.57±0.15 | 2.86±0.92 | 5.9±1.15* |
| 15 | 44.6±6.37 | 26.88±5.68* | 1.3±0.18 | 0.63±0.06* | 0.48±0.17 | 0.41±0.09 | 4.16±1.6 | 3.45±1.66 |

During the first 6 days of culturing the proliferation of epithelial cells exceeds 2.5-3-fold that of the SMC, but then it decreases and after 12 days attains the level observed for SMC (Fig. 3, *d*). Longitudinal stretching causes a reliable increase of epithelial cell proliferation. At the end of culturing a boost of proliferation associated with stepped-up migration of epithelial cells into the growth zone is observed. The migration probably stimulates division of the neighboring cells [15].

At the beginning of culturing, the rate of proliferation of the adventitial cells is 3 times higher than SMC proliferation (Fig. 2, *a*). But on the 9th day the index of labeled nuclei drops threefold, and then, on day 15, sharply rises by a factor of 4.4. This rise may also occur due to the enhancement of cell migration into the growth zone. During the first few days and at the end of culturing, longitudinal stretching reliably reduces the proliferation of the adventitial cells. But in the growth zone the index of labeled nuclei of fibroblastlike cells (13.88±1.74) does not reliably differ from that of the cells which have migrated from the stretched explants (21.3±7.66). The cells of the mucous epithelium form portions of a monolayer in the growth zone. Labeled cells are observed at the boundary of the monolayer in the zone of solitary cells. SMC migrate into the growth zone at later times of culturing (days 24-27), when the explant loosens up, and the compact structure of the epithelial and adventitial layers is disintegrates. At the same time, SMC migration is not accompanied by enhanced cell proliferation at the boundary of the explant, as is observed for the epithelial cells.

At the beginning of culturing, no increase in SMC proliferation is observed in the control circular explants. The index of labeled nuclei corresponds to the basal level at all times of culturing. On the other hand, in the longitudinal layer the index of labeled nuclei is 11 times, and in the circular layer 15.46 times, lower than in the longitudinal explants. In the circular layer, a reliable increase of the index of labeled nuclei was noted only on the 3rd day as compared to later times (Fig. 3, *a*, *b*). SMC are known to possess the spontaneous activity [14]. In our cultures spontaneous activity was observed in the longitudinal explants on days 6-9. By days 15-18 the explants have maximally contracted and rolled into a ball. The circular explants have contracted as early as by day 3 of culturing, the longitudinal layer shortening so much that the ends of the circular layer open. The circular muscular layer, by contracting, has occluded the lumen of the tubule. It is possible that the spontaneous contractility in the circular explants is determined by the preservation of the normal architectonics of the tissues and prevents increased SMC proliferation at the beginning of culturing.

Passive stretching of the circular explants by means of inserted glass rods stimulates contractions of SMC from the circular muscular layer. On days 3-6 of culturing the index of labeled nuclei is reliably lower in the circular layer than in the control ($p<0.01$). However, tissue adaptation to stretching quickly occurs with this method of stretching, and after 6 days cell proliferation in the circular layer does not differ from that in the control (Table 2; Fig. 3, *a*). Cross-stretching of the circular explants weakly stimulates the contractions of SMC of the longitudinal muscular layer. Probably, for this reason the index of labeled nuclei of these cells does not reliably differ from the index of labeled nuclei of the circular explants in the control (Fig. 3, *b*).

At the beginning of culturing, the cells of the adventitia in the circular explants exhibit a high level of proliferation, which drops on the 9th day and then increases when cell migration is stepped up. Passive stretching causes a reliable, uniform reduction of the index of labeled nuclei for each time of measurement (Fig. 3, *c*).

Epithelial cells in the circular explants have a low index of labeled nuclei over the whole period of culturing, in contrast to the same cells of the longitudinal explants, where an increase of proliferation is primarily observed. Apparently, preservation of the normal architectonics of the epithelial cells blocks the rise of proliferation according to the closed-circuit principle. During the first days of culturing, passive stretching of the explants by glass rods increases the proliferation of epithelial cells tenfold. During further adaptation to stretching, the proliferation reverts to the control level (Fig. 3, c).

Thus, a disturbance of the normal architectonics and transfer to the conditions of organotypic culturing cause an increase of cell proliferation in the seminiferous tubule of guinea pigs. After 6-9 days, adaptation of the explants reduces proliferation to the basal level, with the exception of the cells of the adventitia, which increase their proliferation as they migrate into the growth zone. Stimulation of functional activity by longitudinal or cross-stretching prevents the primary increase in proliferation in the longitudinal and circular layers, respectively. Stretching reduces the proliferation of the adventitial cells, but increases that of the epithelial cells.

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# MORPHOLOGY AND PATHOMORFOLOGY

# Neuromorphological Basis of the Therapeutic Effect of Neurohumoral Agents of the Cerebrospinal Fluid

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**Key Words:** *sensorimotor cortex; cerebrospinal fluid; compensation of damage; horseradish peroxidase*

The possibility of plastic rearrangements of the descending tracts from the intact hemisphere is of great interest in the problem of restoring lost functions after unilateral damage to the brain [6]. Endogenous oligo- and polypeptides from the cerebrospinal fluid (CSF) have been found to take part in the pathogenesis of central motor disturbances [1] and in later compensatory processes [3]. Treating animals with brain damage with different combinations of these agents results in an accelerated restoration of impaired motor function [8]. Previous physiological and biochemical inves-

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