# MITOSES IN HYPERTROPHIED SMOOTH MUSCLE TISSUE OF THE RAT POSTERIOR VENA CAVA

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Injection of colchicine into rats revealed mitotically dividing leiomyocytes in the hypertrophied muscle tissue of the posterior vena cava of rats. The frequency of dividing cells under these conditions did not exceed 1:1000-1:10,000. Many of these smooth muscle cells were in prophase.

KEY WORDS: smooth muscle cells of veins; mitoses; colchicine; hypertrophy of smooth muscle tissue.

Smooth muscle cells in the walls of blood vessels are now no longer regarded as a stable, nonrenewing cell population [5]. However, the question of the presence and role of mitotic division of these cells in compensatory growth of smooth muscle tissue of arteries and veins have not been studied. Mitotically dividing leiomyocytes have been demonstrated in recent years in the damaged wall of arteries and in the hypertrophied muscle tissue of the ureters [7, 8]. The workers cited used a method of counting mitoses after injection of colchicine into the animals [1].

In the present investigation the presence of mitotically dividing muscle cells in hypertrophied muscle tissue of the posterior vena cava of rats was studied, also taking advantage of the mitostatic effect of colchicine.

## EXPERIMENTAL METHOD

Hypertrophy of the smooth muscle tissue of the posterior vena cava of noninbred rats weighing up to 260 g was induced by interfering with the outflow of blood from the posterior vena cava by a method published previously [3]. Colchicine was injected intraperitoneally in a dose of 1-1.5 $\mu$g/g body weight during the morning and evening (Table 1). The rats were decapitated 4, 6, and 8 h after injection of colchicine (Table 1).

Altogether the veins of 38 experimental (undergoing the operation) and 12 control animals were studied. Total preparations of the posterior vena cava, after fixation in Carnoy's fluid, were stained with gallocyanin for 48 h and then counterstained for 10-15 min with a 0.03% solution of cresyl violet. The number of mitoses was counted in the whole area of the preparation and then expressed per 100 mm$^2$ of its area.

## EXPERIMENTAL RESULTS

On examination of total preparations of the posterior vena cava in the control animals (12 rats) no mitotically dividing leiomyocytes could be found. In the muscle tissue of the veins of the experimental animals between 2 and 4.5 days after stenosis of the vein (27 observations) in all cases mitotically dividing smooth muscle cells were seen. However, these cells were very few in number.

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TABLE 1. Number of Mitotically Dividing Leiomyocytes per 100 mm$^2$ Area of Total Preparation of Wall of Rat Posterior Vena Cava

| Time after operation (in days) | No. animals | Time of day: when colchicine injected | Time of day: when animals killed | No. of mitoses | % prophases |
|---|---|---|---|---|---|
| I. 2—4 | 6 | 9 a.m. | 1 p.m. | 7,0±1,0 | 70 |
| II. 8 | 3 | 9 a.m. | 1 p.m. | 0 | — |
| III. Control | 3 | 9 a.m. | 1 p.m. | 0 | |
| | | | | $P_{I-III} < 0,01$ | |
| IV. 2 | 8 | 6 p.m. | Midnight and 2 a.m. | 15,0±1,4 | 32 |
| | | | | $P_{IV-VIII} < 0,01$ | |
| V 4(a) | 7 | 6 p.m. | Midnight | 26,0±1,9 | 65 |
| | | | | $P_{V-VIII} < 0,01$ | |
| VI. 4(b) | 6 | 6 p.m. | 2 a.m. | 80,0±3,7 | 51 |
| | | | | $P_{VI-VIII} < 0,01$ | |
| VII. 8 | 6 | 6 p.m. | Midnight and 2 a.m. | 4,0±0,8 | |
| | | | | $P_{VII-VIII} < 0,01$ | — |
| VIII. Control | 9 | 6 p.m. | Midnight and 2 a.m. | 0 | — |

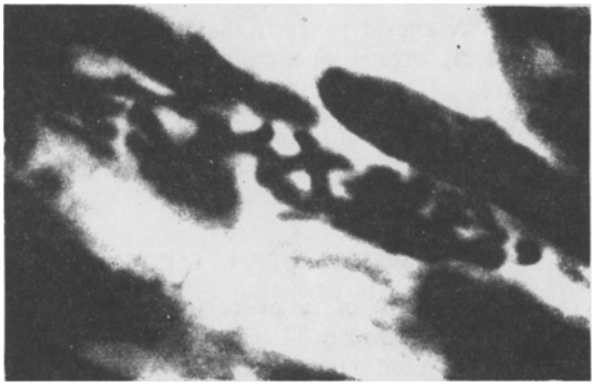

Fig. 1. Mitotically dividing smooth muscle cells in prophase stage in hypertrophied muscular coat of posterior vena cava of rat (fifth day after disturbance of venus drainage). Cresyl violet, 1350 ×, immersion.

The numbers of mitotically dividing leiomyocytes are given in Table 1. The standard deviation and standard error were calculated by equations for a Poisson distribution. This was because infrequent events obey this distribution [2, 6]. It is clear from Table 1 that mitotically dividing smooth muscle cells in the muscular coat of the veins of the experimental rats 2-5 days after interference with the drainage of blood were a significant and reliable event ($P < 0.01$).

The greatest number of mitotically dividing leiomyocytes was observed at night on the fourth and fifth days after interference with the blood drainage. However, even at this time the maximal concentration of these cells did not exceed 1:1000. For comparison it can be said that in a similar experiment the number of DNA-synthesizing leiomyocytes after a single injection of thymidine-H$^3$, with the animals sacrificed 1.5 h after the injection, was 70-80/1000, i.e., two orders of magnitude greater [4].

It is also clear from Table 1 that there is a significant difference between the number of mitotically dividing leiomyocytes depending on the time of injection of colchicine and the period before sacrifice of the animals. For instance, when the material was taken in the afternoon the number of dividing smooth muscle cells was several times smaller than at night ($P < 0.01$). In animals surviving 4.5 days after the operation and killed at night the number of mitoses was significantly higher if the time elapsing between the injection of colchicine and sacrifice was not 6 h, but 8 h ($P < 0.01$). This difference in the number of mitotically dividing leiomyocytes reflects changes in the intensity of proliferation depending on the time of day. Similar

diurnal rhythmic variations in proliferative activity of muscle cells have been observed during physiological growth also, judging from the intensity of DNA synthesis [5].

It was noted that most of the mitotically dividing leiomyocytes in the hypertrophied muscular coat of the rats' vein were in the prophase stage (Fig. 1). Besides smooth muscle cells in prophase and interphase, some leiomyocytes were seen in the metaphase stage of mitosis, together with cells with grossly swollen, hyperchromic, and agglutinated chromosomes (these were evidently dying).

The mitotically dividing leiomyocytes in prophase under these conditions could be easily distinguished from the other mitotically dividing cells, such as endothelial cells, by the shape of their nucleus. Meanwhile, muscle cells in metaphase of mitosis differed only slightly from mitotically dividing endothelial cells. The latter could be distinguished by their topography and shape: The cells were situated superficially compared with the muscle cells, and they had a widely spread and slightly basophilic cytoplasm. In addition, mitotically dividing endothelial cells were seen most frequently on the second day after the operation (up to 150-200 per $100mm^2$ of the preparation), whereas leiomyocytes were most numerous on the fourth to fifth day.

These results are interesting from several standpoints. Since mitotically dividing leiomyocytes were extremely few in number even in the period when they were most numerous, it can be concluded that the marked increase in mass of the muscle tissue, demonstrated by the writer previously [3], does not depend on the increase in the number of cells in the muscle coat.

The presence of many mitotically dividing smooth muscle cells in prophase is very interesting. The possibility cannot be ruled out that this is linked with the long duration of this phase of mitosis in leiomyocytes in the posterior vena cava of rats.

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