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# POLYPLOIDIZATION DELAY IN RAT HEPATOCYTES UNDER LIVER GROWTH INHIBITION PRODUCED BY HYPOKINESIA

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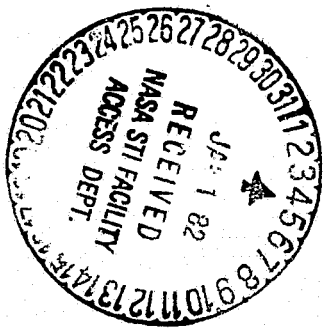
Translation of "Zaderzhka poliploidizatsii gepatotsitov krysy v usloviyakh tormozheniya rosta pecheni pri gipokinezii," Tsitologiya, Vol. 21, No. 4, 1979, pp 397-400.



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16. Abstract

Young rats, weighing 55-59 g, after being for 10 days in conditions of limited mobility, show a retardation of body growth as well as that of liver growth. The decrease in the rate of growth is accompanied by a reduction of cell proliferation and by delay in polyploidization of hepatocytes in the liver of experimental rats.

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# POLYPLOIDIZATION DELAY IN RAT HEPATOCYTES UNDER LIVER GROWTH INHIBITION PRODUCED BY HYPOKINESIA

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According to current concepts there is at the base of polyploi- /397* dy development in the liver a polyploidizing mitosis that induces the formation of cells with an enhanced number of chromosomes (see in the bibliography: Brodskiy and Urivayeva, 1977). Mitosis of the diploid hepatocyte, in the course of which there is no cell wall formation, starts off the first polyploid cells -- binuclear with diploid nuceli. The division of binuclear cells, which proceeds with the joining of paired metaphase plates, results in the formation of tetraploid hepatocytes, etc. We may consider it established, that all cell transformations occurring in the liver in the course of histogenesis (2n→2nx2→4n→4nx2→8n→8nx2...) result from sequential transition on the part of cells of different ploidity in the mitotic cycle. At the same time the transformations of cell types are stable and directed toward increasing cellular ploidity (Faktor, Urynayeva, 1975).

However these ideas are out of line with the data in a number of reports of observed reduction in cell ploidity under conditions where mitotic activity was repressed (Zaletayeva, 1963, 1965; Li, Kirillov, 1972, 1974; Kirillov, 1977). To explain the reduced number of polyploid cells and the increased amount of low-ploid cells there was hypothesized an amitotic division of nuclei and division of binuclear cells with the formation of mononuclear ones. Since these conclusions are important for assessing the mechanisms of hepatocyte polyploidization, we decided to study once more the effect of growth inhibition, using one of the experimental models of the authors. We studied the effect of growth inhibition in animals subjec-

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*Numbers in the margin indicate pagination in the foreign text.

ted to hypokinesia on the ratio of cells of different ploidity in the liver cells of the rat.

## Material and Method

Wistar male rats were used that weighed 56-59 g at the beginning of the experiment. To restrict their mobility they were placed in narrow plastic cases which allowed relative freedom of movement to the head, tail and extremities. Control and experimental animals were fed to excess and simultaneously.

16 young rats were divided into 3 groups: 7 as initial control,/398 5 put in the boxes, 4 as controls of growth. The experiment ended 10 days following the beginning of hypokinesia. Before hypokinesia began a part of each group were impregnated with $^{3}$H-thymidin administered every 5-9 hr over 3 days. The $^{3}$H-thymidin (specific activity 12 curies per millimole) was given IP in the amount of 0.7 microcuries per gram of weight. The animals were decapitated during the morning hours from 9 to 11 a.m. Body weight was noted and that of the perfused liver. Preparations of isolated cells were made for study (for methodology see Faktor, Uryvayeva, 1975). Following 30 min fixation in 96% alcohol the smears were stained by the Feulgen method: 15 min hydrolysis in 5 n HCl at 37°C and treatment with Schiff's reagent for 1 hr at room temperature. To obtain radioautographs we covered the smears with an M type emulsion (Gosniikhimfotoproyekt) and exposed them in darkness at 4° for a month. When the smears developed we identified nuclei of various ploidities on the basis of combined criteria: nuclei dimensions and intensity of stain in the Feulgen reaction (see Faktor, Uryvayeva, 1975). For this purpose we examined 500-1000 cells in the smear of each animal. The index for labeled cells was determined on the basis of a 1000-3000 cell count and expressed in percent.

## Results and Discussion

Under conditions of hypokinesia there is a considerable slow-down in body and liver growth (Table I). We know of the decrease in the

TABLE I. EFFECT OF HYPOKINESIA ON RAT BODY AND LIVER WEIGHT

| Experimental variant | Number of rats | wt of rat in g, $\bar{x} \pm s_{\bar{x}}$ | | Liver wt, g $\bar{x} \pm s_{\bar{x}}$ |
|---|---|---|---|---|
| | | start of experiment | end of experiment | |
| Initial control | 7 | 59.0±0.7 | --- | 3.8±0.2 |
| Hypokinesia, 10 days | 5 | 56.0±2.0 | 61.0±1.2 | 4.4±0.3 |
| Growing control | 4 | 58.0±2.5 | 84.0±3.3 | 5.9±0.4 |

TABLE II. RATIO OF CELLS OF DIFFERENT PLOIDITY IN NORMAL AND HYPOKINETIC RAT LIVER

| Experimental variant | Relative number of cells in respect to ploidity and different number of nuclei, in percent | | | | |
|---|---|---|---|---|---|
| | 2n | 2n×2 | 4n | 4n×2 | 8n |
| Initial control | 54.1 | 41.2 | 4.7 | 0 | 0 |
| | 61.2 | 33.5 | 5.1 | 0.2 | 0 |
| | 41.6 | 47.7 | 9.1 | 1.6 | 0 |
| | 44.1 | 44.2 | 10.5 | 1.2 | 0 |
| | 18.8 | 50.3 | 29.3 | 1.6 | 0 |
| | 58.1 | 38.8 | 2.9 | 0.2 | 0 |
| | 43.9 | 51.5 | 4.0 | 0.6 | 0 |
| $\bar{x} \pm s_{\bar{x}}$ | 46.0±5.8 | 43.9±2.7 | 9.4±3.8 | 0.8±0.2 | 0 |
| Hypokinesia, 10 days | 27.1 | 62.7 | 9.5 | 0.7 | 0 |
| | 19.6 | 59.9 | 18.8 | 1.7 | 0 |
| | 19.9 | 60.7 | 16.8 | 2.6 | 0 |
| | 27.4 | 64.6 | 6.9 | 1.1 | 0 |
| | 23.5 | 55.8 | 17.1 | 3.3 | 0.2 |
| $\bar{x} \pm s_{\bar{x}}$ | 23.5±1.9 | 60.7±1.7 | 13.8±2.6 | 1.9±0.5 | 0.04 |
| Growing control | 26.6 | 47.0 | 21.5 | 2.7 | 0.2 |
| | 14.7 | 40.8 | 38.9 | 5.4 | 0.3 |
| | 30.1 | 44.7 | 23.4 | 1.6 | 0.2 |
| | 30.5 | 48.4 | 19.4 | 1.7 | 0. |
| $\bar{x} \pm s_{\bar{x}}$ | 26.0±4.4 | 45.2±1.2 | 25.7±5.0 | 2.9±1.0 | 0.18±0.04 |

in absolute weight for the liver and a number of other internal organs in longterm hypokinesia (Kirillov, 1977). Animal growth lag was matched by a lag in liver cell polyploidization for experimental rats (Table II). This is particularly evident

TABLE III. TRANSFORMATION OF CELLS LABELED WITH $^{3}$H-THYMIDIN IN NORMAL RAT GROWTH AND UNDER CONDITIONS OF HYPOKINESIA

| Experimental variant | Rat | Relative number of cells of different ploidity and with different numbers of nuclei | | | | | | | | | | Index of labeled cells |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | nonlabeled cells | | | | | labeled cells | | | | | |
| | | 2n | 2n×2 | 4n | 4n×2 | 8n | 2n | 2n×2 | 4n | 4n×2 | 8n | 8n×2 | |
| Initial control | 1 | 19.1 | 51.6 | 28.1 | 1.2 | 0 | 9.4 | 28.1 | 51.8 | 10.1 | 0.6 | 0 | 3.3 |
| | 2 | 56.6 | 40.6 | 2.6 | 0.2 | 0 | 65.6 | 27.1 | 7.3 | 0 | 0 | 0 | 16.1 |
| | 3 | 45.4 | 51.4 | 2.5 | 0.6 | 0 | 34.6 | 47.2 | 16.7 | 1.5 | 0 | 0 | 8.3 |
| Hypokinesia, 10 days | 1 | 19.7 | 62.6 | 14.9 | 2.7 | 0.1 | 22.3 | 51.0 | 24.4 | 2.3 | 0 | 0 | 13.7 |
| | 2 | 26.6 | 67.2 | 5.6 | 0.6 | 0 | 24.5 | 49.6 | 23.0 | 2.9 | 0 | 0 | 8.7 |
| | 3 | 23.7 | 58.2 | 14.9 | 3.2 | 0 | 22.3 | 38.5 | 33.1 | 3.8 | 1.5 | 0.8 | 12.1 |
| Growing control | 1 | 33.1 | 47.4 | 18.2 | 1.1 | 0.2 | 22.6 | 37.6 | 37.1 | 2.7 | 0 | 0 | 27.7 |
| | 2 | 32.0 | 49.3 | 16.9 | 1.8 | 0 | 27.0 | 46.2 | 35.4 | 1.4 | 0 | 0 | 29.5 |





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in the case of cells with tetraploid nuclei - the 4n and the 4nx2 type. The number of diploid cells in hypokinesia dropped as sharply as in the growing control, while the number of binuclear hepatocytes with diploid nuclei increased. Similar relationships between cells of varying ploidity are typical of young growing animals (Carriere, 1969). During this period of ontogenesis the drop in the number of diploid cells goes hand in hand with tissue accumulation of binuclear cells with diploid nuclei and their subsequent entry into mitosis results in the appearance of tetraploid cells. In our experiments reduced intensity in liver growth showed itself in the accumulation, in tissue, of high-ploid cells of types 4n and 4nx2. This agrees well with literature data. Interruption or retardation of liver growth, as provoked by a whole range of experimental factors -- hypophysectomy, thyroidectomy (see the literature: Carriere, 1969), nonprotein diet (Nadal, Zajdela, 1966), prolongation of the lactation period (Wheatley, 1972) -- results in delayed polyploid development in the tissue. /399

Prior impregnation of young growing rats with $^{3}H$-thymidin made it possible for us to accumulate in the tissue a group of labeled cells and to follow their transformation under conditions of normalcy and of retarded growth due to hypokinesia. Among cells so labeled at the start of the experiment there was a notable increase in the proportion of tetraploid hepatocytes (Table III, initial control) indicating an intensive process of polydiploidization typical of the ontogenesis segment under study. At the close of the experiment all animals showed an increase in labeled cells, evidence of the continued growth of the organ during the experimental period. However, the controls showed a higher index of labeled cells (Table III, growing control). In both cases not only was there no decrease in the number of labeled tetraploid cells in the course of the experiment, but there was an actual increase (Table III). This indicates that in hypokinesia, despite decreased intensity of proliferation, a polyploidization process goes on, although in a smaller number of cells. The important point is that, both under conditions of restricted growth and under normal circumstances, cell transformation follows the scheme 2n→2nx2→4n→4nx2→..., i. e. it is oriented /400

toward increased cell ploidity (Tables II, III). We have previously pointed out (Brodskiy et al., 1969; Faktor, 1972; Faktor, Uryvayeva, 1975) the stability of cell transformation and the absence of division of high-diploid cells as the source of low-diploid cell formation.

In the assessment of a change in cell distribution on the basis of ploidity under different experimental conditions an important feature is comparison with the control, particularly if the experiments last a long time. For example, in experiments with chronic stress, lasting usually 10-20 days, the liver of control animals may show a real growth-related polyploidization of hepatocytes. Only when we compare the ratio between cells of varying ploidity in the liver of experimental animals with both the initial and the growing controls does it become clear that the development level of polyploidy in the experimental rats reflects not a reduction of the polyploid cells already formed but a lag in the growth-related polyploidization of the hepatocytes (Table II). At the present time there is no reason to assume that amitosis processes, as means of dividing high-ploid cells, have a role in any transformations of cellular types whatever that take place in the normal liver or under experimental conditions.

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