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# EFFECT OF REGENERATION OF THE SPLEEN UNDER DIFFERENT CONDITIONS ON ITS MEGAKARYOCYTES IN ALBINO RATS

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Megakaryocytes are found in the intact rat spleen in various forms: with a ring-shaped nucleus and as large multinuclear cells. The megakaryocytes divide by the formation of multipolar mitoses without subsequent cytotomy. During regeneration the number of megakaryocytes and their mitotic activity increased sharply. Gravitational overloading increased the number of megakaryocytes and their mitotic activity even more. Administration of splenic extract in addition to gravitational overloading reduced the number of megakaryocytes in the regenerating spleen but increased their mitotic activity. Under conditions of hypokinesia the number of megakaryocytes in the regenerating spleen fell sharply and no mitoses were observed in them.

KEY WORDS: megakaryocytes; regeneration of the spleen; gravitational overloading; hypokinesia.

There is only limited information in the literature on the morphological and functional characteristics of the megakaryocytes of the spleen. Some workers [2] state that in Werlhof's disease marked megakaryocytopoiesis is observed but thrombus formation is depressed. In another report [1] a case of megakaryocythemia is described in which the platelet count in the peripheral blood remained fairly high. Data has been obtained [8] on the role of the spleen, the organ controlling the content of thrombosthenin, which stimulates megakaryocytopoiesis. Information has also been published [5, 7] on the morphology of the megakaryocytes. No unequivocal solution has been found to the problem of the origin of the megakaryocytes.

Some workers consider that megakaryocytes arise as a result of fusion of several reticulum cells, and that lymphocytes and immature plasma cells are also involved in the process [6]. Other workers [9] have stated that the precursors of the megakaryocytes are large basophilic cells.

On the question of the method of division of the megakaryocytes it has hitherto been considered [3] that multipolar mitoses are characteristic of these cells and are more frequently found under experimental conditions. Information on the morphological characteristics of the splenic megakaryocytes under different experi-

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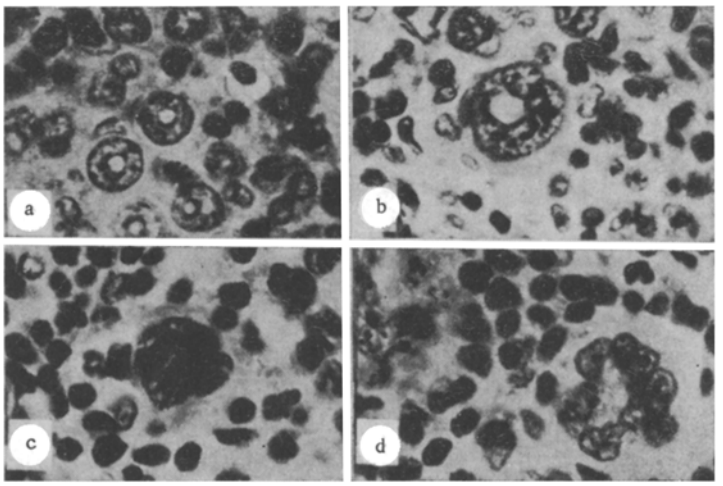


Fig. 1. Early stages of differentiation of splenic megakaryocytes: a) early form of megakaryocyte; b-d) appearance of separate segments. Ocular 12.5×, objective 63×. Hematoxylin–eosin.

mental conditions could not be found in the literature, other than the present writer's own preliminary observations [4].

Considering the limited quantity and contradictory character of the data on the splenic megakaryocytes it was decided to study these cells during regeneration of the spleen under different experimental conditions.

## EXPERIMENTAL METHOD

Experiments were carried out on 190 male albino rats weighing 230-250 g in three series of experiments. Half of the spleen was removed from all the experimental rats except the intact group.

In the experiments of series I the animals were exposed to repeated gravitational overloading with a force of 11g by spinning them on a centrifuge. In series II, after repeated gravitational overloading the animals were given an injection of splenic extract prepared as described by V. P. Filatov. In series III the rats were exposed to hypokinesia in special containers which restricted their movements. Animals undergoing splenectomy but other wise not exposed to any of the extremal factors, and also a group of intact animals served as the control.

The experimental and control rats were decapitated on the 3rd, 5th, 7th, 10th, 15th, 20th, and 30th days after removal of half of the spleen, six animals at each time. The material was processed by the usual histological method. [$^3$H]Thymidine was injected into all the animals 1 h before sacrifice.

## EXPERIMENTAL RESULTS

Most megakaryocytes in the intact rat spleen were located in the peripheral zone of the red pulp and were multinuclear cells measuring from 50 to 240 $\mu$ depending on the number of nuclei. During differentiation the megakaryocytes passed through several stages. The earliest forms were relatively small cells (under 30 $\mu$) with a ring-shaped nucleus and no evidence of fragmentation (Fig. 1a). The cells then grew in size and separate segments began to appear in the nucleus, which still remained ring-shaped (Fig. 1b, c). In the early stages the cytoplasm of the megakaryocytes showed weak basophilia. Later the number of nuclei increased; initially they remained ring-shaped (Fig. 1d), but later they were distributed uniformly throughout the cell (Fig. 2a), the cytoplasm of which became oxyphilic in character. In megakaryocytes which had completed their cycle of development the nuclei were pycnotic and displaced to the periphery of the cell (Fig. 2b, c); sometimes they were expelled, to leave an anuclear structure (Fig. 2d). These observations showed marked pyroninophilia of the megakaryocytes. After injection of [$^3$H]thymidine all the megakaryocytes remained unlabeled. According to [9], they become labeled only after 48-70 h.

As already stated, megakaryocytes characteristically have multipolar mitoses, as a result of which the chromosomes in the cells formed a compact mass with conical projections at its periphery (Fig. 2e, f). When the number of poles was large, as a rule, cytotomy did not take place and the cell remained multinuclear [3].

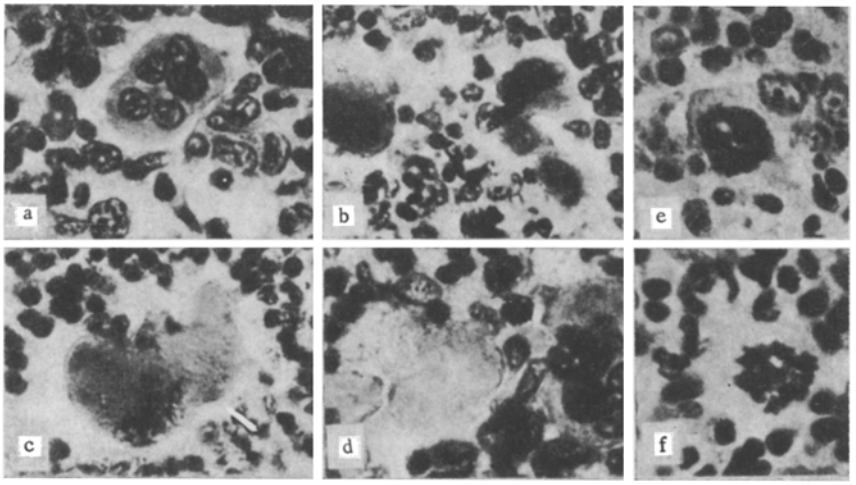


Fig. 2. Late stages of differentiation of splenic megakaryocytes: a) uniform distribution of nuclei throughout cell; b, c) pycnotic cells; d) anuclear structure; e, f) multipolar mitoses. Ocular 12.5×, objective 63×. Hematoxylin–eosin.

TABLE 1. Changes in Number of Megakaryocytes and in Their Mitotic Activity in Regenerating Spleen under Different Experimental Conditions

| Time after resection, days | Control group (removal of half of the spleen) | | Overloading | | Overloading and injection of splenic extract | | Hypokinesia |
|---|---|---|---|---|---|---|---|
| | number of megakaryocytes per field of vision (M ± m) | mitotic index, % | number of megakaryocytes per field of vision (M ± m) | mitotic index, % | number of megakaryocytes per field of vision (M ± m) | mitotic index, % | number of megakaryocytes per field of vision (M ± m) |
| 3 | 4,1±0,6 | 7,0 | 10,8±0,9 | 2,0 | 8,8±1,0 | 10,0 | 0,7±0,03 |
| 7 | 7,3±0,5 | 6,0 | 12,1±1,1 | 3,0 | 8,7±1,2 | 11,0 | 0,45±0,01 |
| 10 | 8,5±0,7 | 9,0 | 15,2±1,3 | 8,0 | 10,6±1,1 | 16,0 | 0,35±0,01 |
| 15 | 8,8±0,8 | 9,0 | 14,0±1,3 | 9,0 | 9,2±1,6 | 18,0 | 0,24±0,02 |
| 20 | 5,4±0,3 | 5,0 | 8,1±0,8 | 8,0 | 6,6±1,0 | 12,0 | 0,18±0,02 |
| 30 | 2,5±0,2 | 3,0 | 4.6±0,7 | 4,0 | 3,0±1,2 | 7,0 | 0,17±0,01 |
| Normal | 1,9±0,1 | 3,0 | | | | | |

To determine the index of mitotic activity megakaryocytes with multipolar mitoses were counted; the results are given in Table 1.

In the intact spleen the mitotic activity of the megakaryocytes did not exceed 3 ‰. The experimental conditions had an important effect on the number of megakaryocytes and on their mitotic activity (Table 1).

As already stated, the megakaryocytes in the spleen of the intact animals were distributed mainly at the periphery of the organ. In the experimental animals they were distributed throughout the organ. In rats on which resection of half of the spleen was performed, but without any other procedures (control) an increase was observed in the number of megakaryocytes per field of vision compared with normal (1.9 ± 0.1). The number of megakaryocytes varied from 4.1 ± 0.6 at the beginning of the experiment to 2.5 ± 0.2 when counted 1 month later, and the mitotic activity varied from 7 to 3 ‰. During overloading the number of megakaryocytes per field of vision increased considerably to 15.2 ± 1.3 or, in a few animals, to 21 per field of vision, with an increase in mitotic activity to 9 ‰. When the animals were stimulated against the background of gravitational overloading, the number of megakaryocytes per field of vision fell compared with the previous group, but their mitotic activity rose appreciably, to reach 18 ‰ on the 15th day.

Besides increased mitotic activity and an increase in the number of megakaryocytes, an increase also was observed in their size and in the number of multinuclear cells in the experimental animals compared with normal. Incidentally, the maximal number of megakaryocytes and the maximal increase in their mitotic activity were observed on the 10th–15th days both in the control group and in the groups exposed to overloading and subsequent stimulation.

Under the influence of hypokinesia a sharp increase was found in the number of megakaryocytes per field of vision -- from 0.17 ± 0.01 to 0.7 ± 0.03. Under these conditions not a single megakaryocyte in a state of mitosis could be found. The megakaryocytes were relatively small (100-120 $\mu$), their nuclei were shrunken, and their cytoplasm was brick red in color.

It can be concluded from the results of these investigations that megakaryocytes exist in the intact spleen of albino rats in various forms ranging from relatively small cells with a ring-shaped nucleus to large multinuclear cells measuring up to 240 $\mu$.

Division takes place by the formation of multipolar mitoses without subsequent cytotomy. During regeneration of the spleen the number of megakaryocytes rises. During gravitational overloading the number of megakaryocytes in the regenerating spleen is almost doubled and their mitotic activity is increased. Injection of a stimulator under these conditions reduces the number of megakaryocytes but increases their mitotic activity.

During hypokinesia the number of megakaryocytes in the regenerating spleen falls sharply and no mitoses are observed.

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