curve would be bound to change, but this did not happen. For comparison, the characteristics of a subpopulation of CFUs in which the class of stem cells with low self-renewal is shown and the different angle of slope of the curve of distribution of CFUs by self-renewal will be clearly apparent.

It is difficult to explain why these results differ from data in the literature [3, 4]. The line of mice may perhaps be relevant. In the investigations cited above mice of line BALB/c were used and daughter CFUs were recorded in 12-day colonies (in 11-day colonies in the present investigation). Whatever the case, the data given above do not settle the question of whether the mechanism of use of stem cells depends on their generation age.

LITERATURE CITED

1. O. A. Gurevich and I. L. Chertkov, Probl. Gematol., No. 9, 43 (1982).
2. I. L. Chertkov and A. Ya. Fridenshtein, The Cellular Basis of Hematopoiesis [in Russian], Moscow (1977).
3. M. Rosendaal, G. S. Hodgson, and T. R. Bradley, Nature, 264, 68 (1976).
4. M. Rosendaal, G. S. Hodgson, and T. R. Bradley, Cell Tissue Kinet., 12, 17 (1979).
5. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).

ULTRADIAN BIORHYTHMS IN MOUSE BONE MARROW

T. P. Ryabykh, N. I. Belyanchikova, and A. P. Suslov

UDC 612.419.014.2:612.6"414"

KEY WORDS: ultradian fluctuations; bone marrow; cytologic composition.

One of the most probable methods of spatiotemporal organization of differentiation processes and repopulation of the blood cells is through a single system of feedback manifested as biorhythms. Circadian fluctuations in the number of blood cells have been known for a long time [12]. Circadian fluctuations in the number of bone marrow karyocytes have been found in man and rats [4, 15]. A circadian biorhythm has been described for the blood erythropoietin level [17]. Meanwhile, biorhythms with a period of several hours (ultradian) are evidently more commensurate with the more rapid processes of new formation and migration of cells. However, the system of ultradian biorhythms has not yet been adequately studied. Ultradian fluctuations in blood hormone concentrations are known [13]. Ultradian fluctuations in the number of lymphocytes in the thymus, spleen, and lymph nodes were discovered recently [7]. Ultradian fluctuations in activity of cell mediators regulating migration and proliferation of lymphoid cells and of interferon in the culture fluid of normal and immune lymphocytes also have been found [8, 10].

The existence of ultradian fluctuations in the number of karyocytes in mouse bone marrow was established by the investigation described below and ultradian changes were found in the relative percentage and absolute number of cells belonging to the various branches of hematopoiesis.

EXPERIMENTAL METHOD

Mice of line AK (female) obtained from the "Stolbovaya" Nursery or bred by the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and selected for weight (±1 g), were kept four or five animals to a cage. Every hour during the experiment, mice from one cage were killed simultaneously by cervical dislocation and the femora were removed from them: the left — to count the number of karyocytes, the right — to prepare films. The distal epiphysis of each left femur was removed along the epiphyseal disk. The needle of a tuberculin

Department of Chemical Carcinogenesis and Laboratory of Immunochemistry and Diagnosis of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 103-106, September, 1983. Original article submited September 24, 1982.

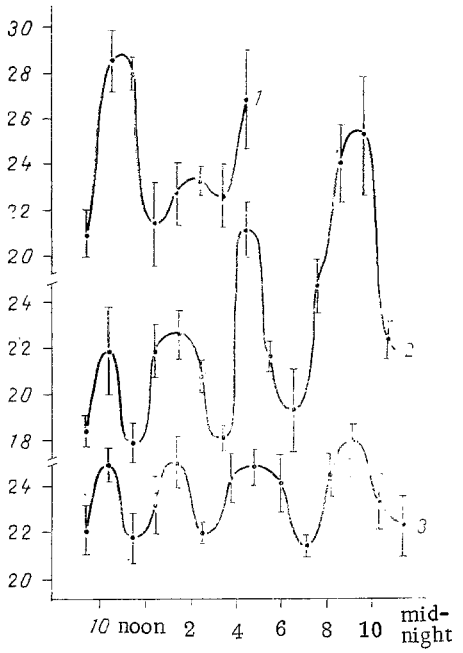

Fig. 1. Number of karyocytes isolated from femur of AKR mice as a function of clock time. Abscissa, clock time; ordinate, number of karyocytes per femur (in millions, M ± m); 1, 2, 3: Nos. of experiments. Mice aged 4 months were used in experiments 1 and 2, mice aged 1 month in experiment 3.

syringe was introduced into the medullary cavity from the proximal end. Cells were washed out of the medullary cavity a standard number of times into a test tube with 4% acetic acid in a volume of 2 ml. The total number of karyocytes was counted in a Goryaev's chamber. Bone marrow from the right femur was blown out into a drop of bovine serum and films prepared, which were fixed with methyl alcohol and stained by the Romanovsky–Giemsa method. Individual types of bone marrow cells and cells in mitosis were identified morphologically [1, 6]. In film 500 cells were counted. On the basis of the results the number of the corresponding types of cells per femur was counted. The significance of differences was determined by the Wilcoxon–Mann–Whitney U test [2]. Experimental data on the number of karyocytes and the relative percentage of individual cell forms in the bone marrow, shown in the form of time series, were analyzed by SM-3 computer using a program for evaluation of spectral power (worked out by A. L. Levshin, Institute of Earth Sciences, Academy of Sciences of the USSR) in order to determine the period of the changes of the parameter.

EXPERIMENTAL RESULTS

The total number of karyocytes isolated from the femur of AKR mice was found to vary periodically depending on the time of day (Fig. 1). Differences between maxima and minima of the number of karyocytes were significant ($P < 0.05$) and the fluctuations were considerable in amplitude (from 20 to 40% of the total number of karyocytes at the minimum), they had a stable period which was independent of the time of year, the age of the mice, and the place where they were reared. The periods of the oscillations, obtained by computer processing of the data, were 4 and 6 h. Significant fluctuations in the number of karyocytes with time also were observed in other lines of mice.

The study of the morphological compositon of the bone marrow of AKR mice isolated at different times of day revealed periodic fluctuations in the relative percentage of the different cell forms (Fig. 2). These fluctuations were significant ($P < 0.05$) and were characterized by a change in amplitude of 1.5-2 times, with a stable period peculiar to each cell form. In a given experiment periods of fluctuations of the relative percentage of lymphocytes and monocytes, calculated by computer, were 4 h (Fig. 2: 1, 2) and their fluctuations took place out of phase. The periods of fluctuations of the relative percentage of cells of the erythroid series and postmitotic granuocytes (juvenile, stab, and segmented polymorphonuclear granulocytes) were 6 h, and for cells of the mitotic group of granuloctyes (myeloblasts, promyelocytes, neutrophilic and eosinophilic myelocytes) and for cells actually in

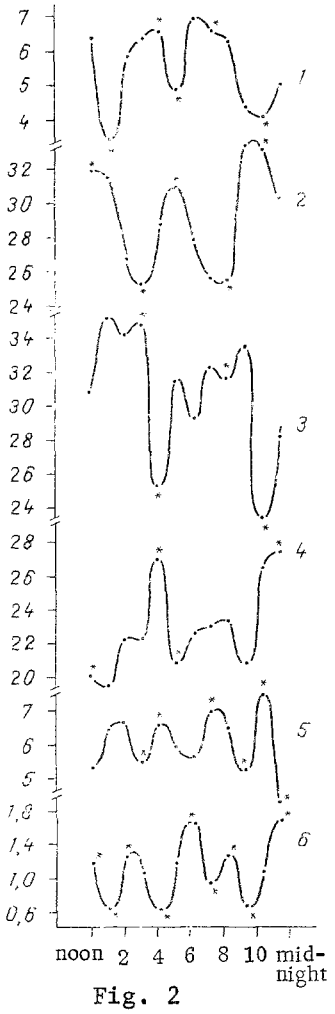

Fig. 2

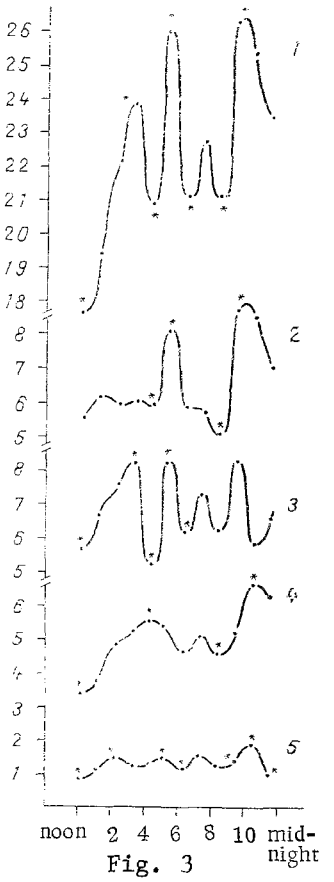

Fig. 3

Fig. 2. Percentage content of various cell forms in bone marrow of AKR mice depending on clock time. Abscissa, clock time; ordinate, number of cells of different branches of hematopoiesis and mitotically dividing cells (in %). 1) Monocytes; 2) lymphocytes; 3) erythron; 4) postmitotic granulocytes; 2) mitotic group of granulocytes; 6) mitoses. *) Maxima and minima of curves between which differences are signficant ($P < 0.05$).

Fig. 3. Changes in total number of karyocytes in bone marrow and absolute number of cells of different branches of hematopoiesis depending on clock time. Abscissa, clock time; ordinate, number of different cell forms (in millions per femur). 1) Total number of karyocytes; 2) lymphocytes; 3) erythron; 4) postmitotic granulocytes; 5) mitotic group of granulocytes. *) Maxima and minima of curves between which differences are significant ($P < 0.05$).

mitosis, two periods were found (6 and 3 h). The maximal percentage of cells of the erythroid series corresponded to the minimal percentage of the number of postmitotic granulocytes, and vice versa. A similar pattern also was observed for curves of the mitotic group of granulocytes and cells in mitosis. Significant changes in the percentage of the different cell forms were found in two further experiments, which were shorter in duration (7 h), so that the periods of the oscillations could not be determined.

Significant fluctuations ($P < 0.05$) also were found in the absolute number of cells belonging to the different branches of hematopoiesis (Fig. 3). Maximal values of the absolute number of the different cell forms (both mature and immature) coincided on the whole with

maxima of the number of karyocytes. Consequently, periodic fluctuations in the total number of bone marrow karyocytes cannot be explained by the selective accumulation of any one cell form. They are evidently determined by a complex combination of changes in the relative percentage of individual types of cells. Fluctuations in the total number of karyoctyes were probably also unconnected with differences in extractability of the cells from the bone-marrow sheath, for karyocytes (2-3 min), isolated by additional enzymic treatment of the crushed bone (0.3% trypsin solution and 0.03% solution of viakase, 10 min at 37°C) not only did not compensate the changes in the total number of karyocytes, but actually increased the amplitude of the fluctuations a little (the data are not given).

The number of karyocytes isolated by the standard method from bone marrow of intact mice thus varied with an ultradian biorhythm. The relative percentage of the number of different cell forms in the bone marrow, the number of dividing cells, and also the absolute number of cells of different branches of hematopoiesis also exhibited ultradian changes.

Lajtha et al. [14] who studied incorporation of ^{32}P and labeled [^{14}C]adenine in DNA by bone-marrow cells *in vitro*, postulated the existence of a circadian biorhythm for processes of cytogenetics. Later much information was obtained to confirm the existence of fluctuations of various cellular parameters, such as DNA and RNA synthesis, number of mitoses, glycogen accumulation, and so on, with a circadian rhythm [11]. Circadian fluctuations have been found in the number of karyocytes, DNA synthesis, and the number of mitoses in bone marrow [4, 14, 16]. On the other hand, changes are known in intracellular parameters such as synthesis of protein and polyamines taking place in cell cultures *in vitro*, with a circumhoral period [5]. All these data constitute grounds for understanding of cell differentiation processes as being structurally organized in time.

In accordance with these results it can be postulated that processes of division, differentiation, and migration of bone-marrow cells are organizaed in time in the form of a system of ultradian biorhythms. In this case division of cells, their transition from one differential compartment to another, migration in the space of the medullary cavity, and accumulation of blood cells in the medullary sinuses [9] — all these processes are coordinated with one another so that changing dynamic structure, repeated with a period of a few hours, is formed. Control of dynamic cytohomeostasis in the bone marrow may be effected by ultradian changes in blood hormone levels [13] and, in particular, hormones with a selective regulatory action on precursors of bone-marrow cells, such as erythropoietin [17], and also with ultradian flucuations of activity of soluble cellular mediators in the serum [8, 10]. The systemic regulatory action of hormones and mediators may be superposed on the regulatory action of local cytodifferentiation factors [9] through synchronization of circumoral fluctuations of intracellular parameters [5].

It must be pointed out that data on ultradian fluctuations in the number of karyocytes isolated from bone marrow and on changes in the cytologic composition in this organ under normal conditions with time must be taken into due consideration when experiments are undertaken in which these parameters are assessed.

LITERATURE CITED

1. A. I. Vorob'ev and Yu. I. Lorie, Textbook of Hematology [in Russian], Moscow (1979).
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametrical Statistical Criteria for Assessing Differences between Two Groups of Observations in Medical and Biological Research [in Russian], Moscow (1969).
3. T. S. Istamanova, V. A. Almazov, and S. V. Kanaev, Functional Morphology [in Russian], Leningrad (1973).
4. É. L. Neishtadt, M. A. Blank, V. A. Kochnev, et al., in: Current Problems in Experimental Chemotherapy of Tumors [in Russian], Chernogolovka (1980), Part 2, p. 162.
5. N. V. Nechaeva, K. N. Yarygin, V. I. Fateeva, et al., Byull. Éksp. Biol. Med., No. 8, 211 (1980).
6. M. P. Pokrovskaya, N. A. Kraskina, V. I. Levinson, et al., Zh. Mikrobiol., No. 3, 8 (1965).
7. A. P. Suslov and T. P. Ryabykh, Byull. Éksp. Biol. Med., No. 7, 85 (1981).
8. A. P. Suslov and D. D. Kharkevich, Byull. Éksp. Biol. Med., No. 4, 466 (1981).
9. A. Ya. Fridenshtein and E. A. Luriya, The Cellular Basis of the Hematopoietic Microenvironment [in Russian], Moscow (1980).
10. D. D. Kharkevich, A. P. Suslov, E. G. Slavina, et al., Immunologiya, No. 3, 85 (1981).

11. F. Halberg, E. Haus, and L. E. Scheving, in: Biomathematics and Cell Kinetics, Amsterdam (1978), p. 175.
12. F. Halberg and H. B. Visscher, Proc. Soc. Exp. Biol. (New York), 75, 846 (1950).
13. D. T. Krieger, in: Endocrine Rhythms, New York (1979), p. 123.
14. L. G. Lajtha, R. Oliver, and F. Ellis, Br. J. Cancer, 8, 367 (1954).
15. F. Levi, F. Halberg, M. Nesbit, et al., in: Neoplasms: Comparative Pathology of Growth in Animals, Plants, and Man, Baltimore (1981), p. 267.
16. A. M. Mauer, Blood, 26, 1 (1965).
17. E. Nir, P. Efrati, and D. Danon, Exp. Hematol., 4, 161 (1976).

DEREPRESSION OF HEPATOCYTE PROLIFERATION BY CHANGES IN RETICULO-ENDOTHELIAL SYSTEM FUNCTION

D. N. Mayanskii and V. I. Shcherbakov

UDC 612.35:612.6.03/]-06:612.429

KEY WORDS: Kupffer cell; proliferation; DNA synthesis; mitosis.

Hepatic stromal cells ensure an adequate microenvironment for hepatocyte proliferation [2]. The most reactive component of the stroma is the Kupffer cell (KC). In the early stages of stimulation a combination of mediators passes from it into the microenvironment: prostaglandins [9], lysosomal proteases and glycosidases, stimulators of lymphocytes and fibroblasts [5, 11, 13]. As a result of activation of KC mononuclear infiltration into the hepatic stroma takes place [6, 8].

It is not only the state of the hepatic stroma that depends on KC function. By changing the functional state of KC it is possible, a priori, to modify substantially the course of hepatocyte regeneration after partial resection of the liver [3, 4]. Preliminary stimulation of KC by the bacterial stimulator from *Serratia marcescens* (prodigiosan) 24 h before the operation created conditions for more rapid realization of the early stages of regeneration of the resected liver. Meanwhile, if the KC were loaded with colloidal iron 2 h before or 3 h after the operation, the course of reparative regeneration of the liver was delayed almost to half the control rate. This suggested that KC can become the source of factors inducing hepatocyte proliferation.

Further confirmation of this hypothesis was obtained in the present investigation. In experiments on mice and rats the effect of different agents causing primary modification of KC function on the DNA-synthesizing ability of the hepatocytes was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 240-280 g and on male (CBA × C57BL)F_1 mice weighing 18-22 g. Colloidal iron particles (brand R-100F) measuring 0.8-1.5 μ, in a dose of 100 mg, suspended in 5% isotonic starch solution, were injected into the femoral vein of some rats, and latex microspheres 0.2-0.5 in diameter, suspended in 0.85% NaCl, were injected into other rats in a dose of 10 mg. To stimulate KC, 2 mg zymosan, 10 μg prodigiosan, and 3 mg BCG in 0.5 ml 0.85% NaCl was injected into the caudal vein of the mice. The animals were killed 4, 8, 16, 24, 32, 48, and 72 h, 5, 7, 9, 12, 15, and 21 days, and 1 month later. [^{3}H]Thymidine was injected in a dose of 1 μCi/g body weight (specific activity 12.8 Ci/mmole). Liver sections stained with hematoxylin and eosin were coated with liquid photographic emulsion (Photographic Chemical Research Institute) and exposed in darkness at 4°C for 3 weeks. To determine the mitotic index (MI) 5000 hepatocytes were counted in liver sections stained with hematoxylin and eosin; to determine the index of labeled nuclei (ILN) of the hepatocytes and sinusoidal cells 3000 nuclei were counted in autoradiographs of liver sections. The numerical data were subjected to statistical analysis by Student's t test.

Laboratory of Pathophysiology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 9, pp. 106-108, September, 1983. Original article submitted December 17, 1982.

