cantly higher than the control (Table 3). However, the amplitude of noradrenalin and acrophase of the rhythms returned within normal limits.

During 3 days after alcohol consumption the hourly adrenalin and noradrenalin levels returned to their initial values (Table 1), and parameters (mesors, amplitudes, acrophases) of the circadian rhythms also were indistinguishable from the control (Table 3).

In a dose inducing an average degree of intoxication, alcohol thus modifies the circadian temporal organization of normal functioning of the sympathicoadrenal system. During the 1st day after alcohol intake a shift of acrophases and an increase in mesors and amplitudes of the circadian rhythms of adrenalin and noradrenalin excretion were observed. On the 2nd day partial normalization of the rhythm parameters took place, with restoration of acrophases first of all. Restoration of the normal circadian rhythm was complete in the course of the third 24-hourly cycle after alcoholization.

LITERATURE CITED

1. K. A. Bagrinovskii, N. V. Baginskaya, A. F. Bazhenova, et al., in: Cybernetic Approaches to Biology [in Russian], Novosibirsk (1973), p. 196.
2. G. N. Kassil', in: Physiology of the Autonomic Nervous System [in Russian], Leningrad (1981), p. 536.
3. V. P. Latenkov, in: Chronobiology and Chronopathology [in Russian], Moscow (1981), p. 146.
4. É. A. Matlina, V. N. Vasil'ev, and S. D. Galimov, Fiziol. Cheloveka, No. 6, 970 (1976).
5. V. V. Men'shikov, Lab. Delo, No. 4, 18 (1961).
6. R. V. Berezhnyi et al. (ed.), Textbook of Forensic Medicine of Poisoning [in Russian], Moscow (1980).
7. A. Reinberg, J. Clench, N. Aymard, et al., J. Physiol. (Paris), 70, 435 (1975).

CLONAL SUCCESSION OF HEMATOPOIETIC CELLS IN LONG-TERM BONE MARROW CULTURES

O. A. Gurevich, N. I. Drize, G. A. Udalov, and I. L. Chertkov

UDC 612.419.014.2:612.119:612.6

KEY WORDS: hematopoietic stem cell; proliferation; self-maintenance; bone marrow; long-term culture; chromosomal markers.

To study self-maintenance of hematopoietic stem cells (CFU-c) from long-term bone marrow cultures it was found that no regular changes in proliferative potential of the CFU-c take place in the course of culture. Self-maintenance fluctuates within very wide limits from week to week [1]. Since the number of CFU-c in the cultures is maintained at a relatively stable level, fluctuations of self-maintenance cannot be explained by selection of CFU-c with high or low proliferative potential during culture. It has accordingly been suggested that clonal expansion of hematopoietic cells takes place in cultures with succession of functioning clones. This hypothesis was tested in the investigation described below.

EXPERIMENTAL METHOD

CBA and CBAT6T6 mice of both sexes aged 8-12 weeks were used. The animals were irradiated with ^{137}Cs γ-rays on an IPK apparatus in a dose of 12 Gy, as described previously [1]. Self-maintenance of a CFU-c was characterized by the number of daughter CFU-c produced by it in irradiated mice during the formation of an 11-day splenic colony. Long-term bone marrow

Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 346-348, March, 1985. Original article submitted April 13, 1984.

0007-4888/85/9903-0348$09.50

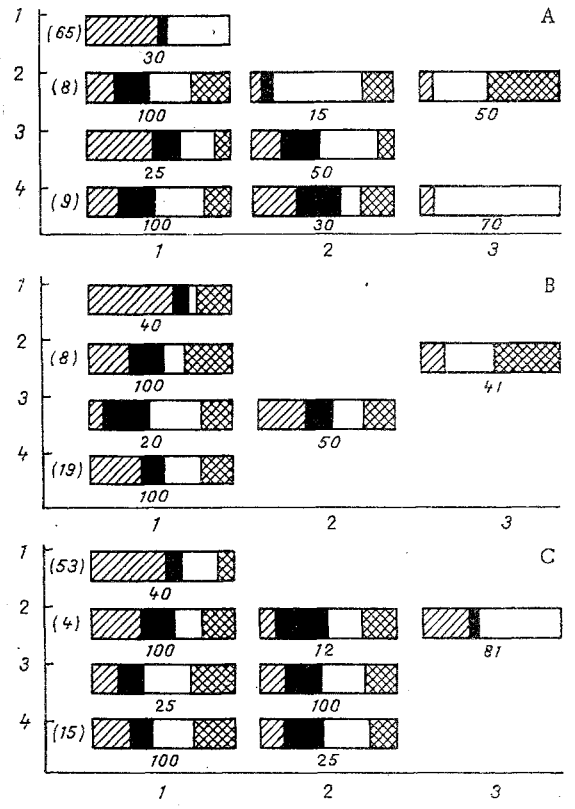

Fig. 1. Karyotype of hematopoietic cells and self-maintenance of CFU-c from long-term mouse bone marrow cultures. Abscissa: 1) cells of suspension of long-term cultures, 2) cells of multilinear colonies in methylcellulose, 3) cells of splenic colonies; ordinate: culture time (in weeks). A) Flask 1; B) flask 2; C) flask 3. Number of daughter CFU-c in 11-day splenic colony shown in parentheses. Numbers below diagrams give number of metaphases studied. Oblique shading, karyotype CBAT6T6/XX; black area of column, T6T6/XY; unshaded part, CBA/XX; cross-hatched, CBA/XY.

cultures were set up by the method in [4], as described in [3] with certain modifications. Fisher's medium with 20% horse serum, 10^{-6} M hydrocortisone sodium hemisuccinate, L-glutamine, and antibiotics was used. Bone marrow was flushed out of four femora of female CBA mice with 30 ml of complete nutrient medium into a plastic flask with a bottom measuring 75 cm^2. Every week half the medium was replaced. After 3 weeks, when a functioning layer of adherent cells had formed, the flasks were irradiated in a dose of 10 Gy and all the medium was replaced by fresh, in which hematopoietic cells (2×10^7 per flask) of four karyotypes were suspended: CBA male and female, CBAT6T6 male and female. An equal number of cells of each type was taken. Subsequently all the medium was replaced weekly. Nonadherent cells contained in it were used for karyologic analysis, both directly from the cultures and after injection into irradiated mice, with the formation of 11-day splenic colonies in the animals, or after culture for 11-13 days in a methylcellulose medium under conditions permitting colony development from polypotent precursors (CFU_{mix}) as described in [1]. Medium conditioned with mouse splenocytes, stimulated by pokeweed mitogen, and anemic mouse serum enriched with erythropoietin were used as stimulators of colony formation. Karyologic analysis was carried out by the C-bending method [1], so that both T6 and Y chromosomes could be easily identified.

EXPERIMENTAL RESULTS

The results obtained in three long-term cultures are illustrated in Fig. 1. With equal participation of all CFU-c introduced into the culture it must be expected that cells of any genotype would be equally represented, with an average 25% of each. However, the results differed significantly from this hypothesis. In all three cultures the number of cells of different origin changed quickly from week to week. For instance, after 1 week of culture,

dividing cells of the CBA/XY karyotype were absent in one flask (Fig. 1A), whereas after 2 weeks they accounted for 26% of the total, and after another week for 8%. In another culture (Fig. 1B) dividing cells of the CBAT6T6/XX karyotype accounted for 63, 29, 10, and 37% of the total respectively on the 1st, 2nd, 3rd, and 4th weeks of culture. These results cannot be explained by selective advantage of cells of one particular karyotype, first, because their ability to repopulate *in vivo* is identical [2] and, second, because fluctuations in cell composition of the cultures were not regular in character and cells of no one karyotype displaced others in the course of culture. On average for 4 weeks of culture the proportion of cells of any one karyotype present showed no tendency to rise or fall, and their overall contribution throughout the period of culture did not differ significantly from theoretical (25%). Consequently, clonal hematopoiesis takes place in culture with successive replacement of the clones taking part in it. This is in agreement with the sharp fluctuations in self-maintenance of CFU-c discovered previously and confirmed by the present investigation (Fig. 1).

The clonal succession process is evidently stochastic in nature, for characteristics of the cell suspension used, initially identical in karyotypic composition, changed rapidly and unequally in different flasks. The rate of these changes was high, which suggests that the functioning life of the clone in culture is comparable with the interval between investigations, i.e., measured in days (1-2 weeks). Meanwhile the presence of such sharp changes in composition of the proliferating cells in the same culture at different times of investigation is evidence that not very many clones function simultaneously. Preliminary calculations show that their number was not less than four and not more than a few score.

The karyotype of cells of multilinear colonies in methylcellulose culture correlated satisfactorily with the karyotype of dividing cells in long-term cultures used for explantation (Fig. 1, 2). This correlation shows that CFU_{mix} consisted mainly of more mature precursors, separated by fewer random events from cells dividing in culture (mainly myelocytes) than CFU-c. Conversely, correlation between the karyotype of CFU-c producing cells in irradiated recipients and dividing cells in long-term cultures was absent. Moreover CFU-c or, more exactly, splenic colonies formed by them, were represented by a smaller set of karyotypes than cells in cultures. In 11 of the 12 cultures studied cells of all four karyotypes were present, whereas in the splenic colonies, cells of not all karyotypes were represented in all of four cases studied (Fig. 1, 3), and in one investigation CFU-c of only one origin were in fact discovered (Fig. 1A, 4-week culture). It must be remembered that the spleen is a significantly larger territory of hematopoiesis than cells of the adherent layer of one flask, more especially because spleen cells from three to five mice were analyzed in each group. This shows that CFU-c in culture are represented not by all the possible karyotypes, although later hematopoietic cells are a mixture of all four karyotypes. The life span of the CFU-c among nonadherent cells in cultures is perhaps significantly shorter than that of the more mature cells, and this correlates with the 10-20-times lower concentration of CFU-c in the cell suspension of Dexter cultures compared with normal bone marrow. A less likely explanation is to assume that some clones bypass the CFU-c stage during expansion in culture, and that the choice of this bypassing method of differentiation is not accidental but is connected with the karyotype of the clone.

The results provide a weighty argument in support of the view that CFU-c are incapable of true self-maintenance, but they are members of transient cell populations which mature consecutively, starting from certain earlier clonogenic precursors, or pre-CFU cells.

LITERATURE CITED

1. I. L. Chertkov and O. A. Gurevich, The Hematopoietic Stem Cell and Its Microenvironment [in Russian], Moscow (1984).
2. I. L. Chertkov (J. L. Chertkov), O. A. Gurevich (O. A. Gurevitch), and G. A. Udalov, Exp. Hemat., 10, 90 (1982).
3. I. L. Chertkov (J. L. Chertkov), N. I. Drize (N. J. Drize), O. A. Gurevich (O. A. Gurevitch), et al., Exp. Hemat., 11, 231 (1983).
4. T. M. Dexter, T. D. Allen, and L. G. Lajtha, J. Cell. Physiol., 91, 335 (1977).