B. mesentericus was not statistically significant (P > 0.05). Meanwhile S. albus and S. faecalis caused marked stimulation of antibody formation (Table 1).

Similar results also were obtained with E. coli 055 (Table 2).

As regards complement formation the spore-bearing aerobes and S. albus were most active. For S. faecalis the difference from the germfree animals was not significant (Table 2).

The results indicate that the formation of natural immunity is dependent on the microbial factor. Individual representatives of the normal microflora were found to differ in their effect on the formation of antibodies and complement. An increase in the concentration of antibodies against E. coli in the absence of common antigens between E. coli and the microorganisms used for contamination is evidence of nonspecific stimulation of the corresponding clones of antibody-forming cells by individual representatives of the normal microflora.

LITERATURE CITED

1. V. N. Andreev, Lab. Delo, No. 9, 565 (1975).
2. V. I. Orlovskii and I. A. Bochkov, Zh. Mikrobiol., No. 8, 141 (1973).
3. G. I. Podoprigora, "Organization of gnotobiotic experiments and the study of aseptic inflammation and phagocytosis in germfree guinea pigs," Candidate's Dissertation, Moscow (1970).
4. O. V. Chakhava, Gnotobiology [in Russian], Moscow (1972).
5. W. J. Herbert, Veterinary Immunology [Russian Translation], Moscow (1974), p. 17.
6. T. Luckey, Germfree Life and Gnotobiology, Academic Press, New York (1963).
7. J. Miler and G. Podoprigora, Folia Microbiol. (Prague), 18, 319 (1973).
8. M. Wagner, Ann. New York Acad. Sci., 78, 89 (1959).

KINETICS OF COLONY-FORMING ABILITY OF MOUSE BONE MARROW CELLS AFTER ADMINISTRATION OF HYDROCORTISONE

V. A. Kozlov and I. G. Tsyrlova

UDC 612.419:612.6].014.46:615.357.453

The dynamics of the colony-forming and migration capacity of polypotent hematopoietic stem cells in the bone marrow of (CBA x C57BL) F_1 mice was studied after injection of hydrocortisone. The relative number of hematopoietic stem cells in the bone marrow was higher than in the control on the 3rd day after hydrocortisone injection. This increase was maximal on the 5th day after the injection. On the 8th day the number of hematopoietic stem cells was down to normal again.

KEY WORDS: polypotent stem cells; proliferation; migration; hydrocortisone.

For a long time the immunodepressive effect of glucocorticoids was explained by their cytotoxic action on the immunocompetent cells of lymphoid tissue [11]. However, recent observations have shown that cells of the thymus medulla of birds [15] and mice [7,8,10] are resistant to the action of high doses of hydrocortisone (HC). Various workers have obtained evidence to show that injection of HC into mice increases the activity of the bone marrow cells in the graft versus host reaction [10] and in response to phytohemagglutinin in vitro [12]. They consider that these effects can be attributed to migration of cortisone-resistant cells of thymus

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1102-1104, September, 1976. Original article submitted March 23, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

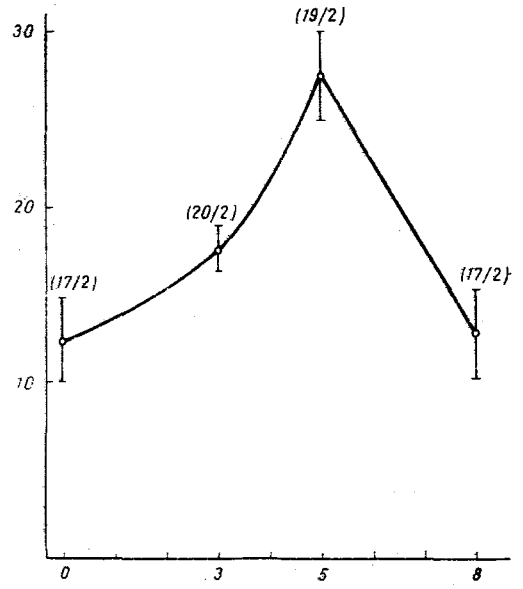

Fig. 1

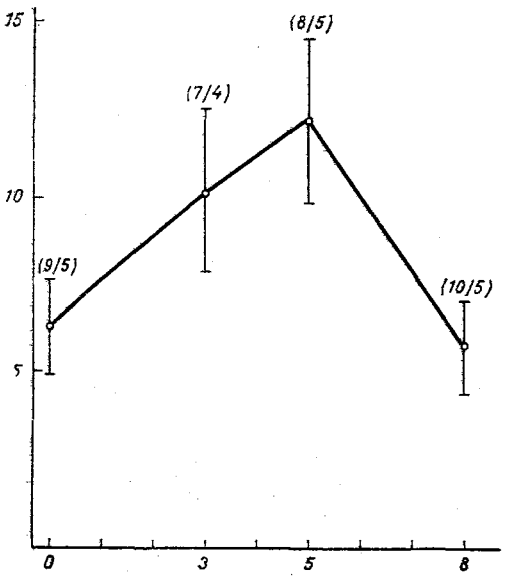

Fig. 2

Fig. 1. Relative number of CFU in bone marrow of (CBA x C57BL) F_1 mice at various times after single injection of 125 mg/kg hydrocortisone. In parentheses: numerator – number of animals; denominator – number of experiments. Ordinate) number of CFU/10^5 nucleated bone marrow cells; abscissa) time after injection of HC (days).

Fig. 2. Number of CFU forming endogenous splenic colonies of hematopoietic cells after their migration from bone marrow at different times after single injection of hydrocortisone into (CBA x C57BL) F_1 mice. Ordinate) number of endogenous CFU. Remainder of legend as in Fig. 1.

origin (T-cells) into the bone marrow under the influence of HC and their "sequestration" there for some time. The appearance of T cells in the bone marrow has been observed 6-9 h after stress [2].

Since thymus cells are known [3] to stimulate proliferation of polypotent hematopoietic stem cells (PHSC) the investigation described below was carried out to study the number of PHSC in bone marrow and to compare it with their ability to migrate at different times after injection of the hormone.

EXPERIMENTAL METHOD

(CBA × C57BL) F_1 mice were used. HC was injected intraperitoneally into the animals in a dose of 125 mg/kg 3,5, or 8 days before determination of PHSC in their bone marrow, the number of which was estimated from the number of colony-forming units units (CFU) by the method of exogenous splenic colonies of hematopoietic cells [14]. For this purpose, $0.1 \cdot 10^6$ bone marrow cells of the experimental animals were transplanted intravenously into lethally irradiated (850 R) syngeneic recipients. The effect of HC on migration of PHSC was studied by the method of Petrov and Khaitov [4]. HC in a dose of 125 mg/kg was injected into the mice 3, 5, and 8 days before irradiation (850 R), during which half of the leg was shielded by a plate made of lead (4 mm thick) and aluminum (1 mm thick). The animals were killed on the 9th day after irradiation and the number of endogenous colonies of hematopoietic cells in their spleens was counted.

The mice were irradiated on the RUD-200-20-3 apparatus (dose rate 50 R/min, voltage 180 kV, filter A1-3). The results were subjected to statistical analysis with the aid of Student's criterion.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Fig. 1. The graph shows the HC led to a significant increase in the number of CFU in the animals' bone marrow as early as on the 3rd day after hormone injection (17.6 ± 0.6 compared with 12.4 ± 1.2 in the control). The relative number of CFU in the bone marrow on the 5th day after injection of HC was 2.2 times higher than the values obtained in the control animals, whereas on the 8th day the colony-forming ability of the bone marrow cells had fallen again to the control

level. The maximal increase in colony-forming ability of the bone marrow cells was thus observed on the 5th day after injection of the hormone.

Since the number of endogenous colonies in the spleens of mice irradiated with half the leg shielded reflects the extent of PHSC migration from the bone marrow, it can be concluded from the results of these investigations (Fig. 2) that the ability of the PHSC to migrate was increased, although not significantly, by the 3rd day after injection of the hormone (from 6.3±1.1 to 10.3±1.8), on the 5th day it was twice as high as in the control animals, but on the 8th day the ability of the PHSC to migrate had fallen again to its initial level. The two processes studied thus followed a similar dynamics.

Evidence has recently been published that PHSC migration from bone marrow varies with the corticosteroid level [1,6]. Administration of hydrocortisone inhibits migration of CFU which has begun [5]. It thus seems likely that the increase in the number of CFU in the bone marrow and in their rate of migration into the spleen on the 5th day after injection of the hormone are due to the accumulation of CFU in the bone marrow as a result of the depression of their migration immediately after injection of HC. However, it is even more likely that the observed effect is based on the process of migration and "sequestration" of T-cells in the bone marrow described in the literature [9,13]. T-cells accumulating in the bone marrow under the influence of the hormone evidently stimulate the proliferative activity of the PHSC, which in turn leads to an increase in their number in the bone marrow. In all probability this process is accompanied by increased migration of PHSC from the bone marrow. This hypothesis is evidently confirmed by the experimental results shown in Figs. 1 and 2: The curve of the change of PHSC migration completely repeats the curve of the change in the number of PHSC in the bone marrow after injection of HC.

LITERATURE CITED

1. G. I. Bezin, R. M. Khaitov, R. V. Petrov, et al., Radiobiologiya, No. 2, 193 (1975).
2. Yu. I. Zimin and R. M. Khaitov, Byull. Éksp. Biol. Med., No. 12, 68 (1975).
3. V. A. Kozlov, S. M. Kolesnikova, and V. A. Trufakin, Dokl. Akad. Nauk SSSR, 216, 483 (1974).
4. R. V. Petrov and R. M. Khaitov, Radiobiologiya, No. 1, 69 (1972).
5. R. V. Petrov, R. M. Khaitov, and S. M. Rachkov, Byull. Éksp. Biol. Med., No. 11, 63 (1975).
6. R. M. Khaitov, R. V. Petrov, B. B. Moroz, et al., Radiobiologiya, No. 4, 516 (1974).
7. H. Blomgren and B. Anderson, Exp. Cell Res., 57, 185 (1969).
8. H. Blomgren and B. Anderson, Cell. Immunol., 1, 545 (1970).
9. J. J. Cohen, J. Immunol., 108, 841 (1972).
10. J. J. Cohen, M. Fischbach, and H. Claman, J. Immunol., 105, 76 (1970).
11. T. F. Dougherty, Physiol. Rev., 32, 379 (1952).
12. M. A. Levine and H. Claman, Science, 167, 1515 (1970).
13. J. W. Moorhead and H. Claman, Cell. Immunol., 5, 74 (1972).
14. J. E. Till and E. A. McCulloch, Radiat, Res., 14, 23 (1961).
15. N. L. Warner, Austral. J. Exp. Biol. Med. Sci., 42, 401 (1964).