of the transmembrane zinc transport systems. Zinc cations then diffuse into the enterocytes where they form complexes with the specific vitamin A-dependent transport protein of the cytosol. Among the intracellular compartments, a leading role in the maintenance of zinc homeostasis under physiological conditions was probably played by the SER, which is located mainly close to the absorbing surface of the enterocyte, along the path of flow of cations entering the cell. To explain the vector flow of exogenous zinc cations in the direction of the basolateral membranes, it must be assumed that the latter contain a special ATPase, pumping zinc out of the cells and activated by the above-mentioned protein (or by vitamin A directly), by analogy with mechanism by which vitamin D controls the transepithelial transport of Ca^{++} [10].

LITERATURE CITED

1. N. I. Berzin' and V. K. Bauman, Biochemistry of Absorption of Nutrients in Animals [in Russian], Riga (1982), pp. 68-76.
2. N. I. Berzin', V. K. Bauman, and A. R. Val'dman, Dokl. Akad. Nauk SSSR, 268, 239 (1983).
3. N. I. Berzin', V. K. Bauman, and G. Yu. Smirnova, Physiological Absorption Processes in Animals [in Russian], Riga, pp. 34-43.
4. N. I. Berzin', Scientific Principles of Vitamin Feeding of Live Stock [in Russian], Riga (1987), pp. 43-45.
5. N. I. Berzin', Vest. Skh. Nauki, No. 1, 106 (1988).
6. G. Geyer, Electron Histochemistry [Russian translation], Moscow (1974).
7. A. M. Ugolev, D. R. Zhigure, and J. J. Nurks, Fiziol. Zh. SSSR, 56, No. 11, 1638 (1970).
8. D. L. Antonson, A. J. Barak, and J. A. Vanderhoof, J. Nutr., 109, 142 (1979).
9. N. I. Berzin' and V. K. Bauman, Br. J. Nutr., 57, 255 (1987).
10. W. L. Davis, R. G. Jones, G. R. Farmer, et al., Anat. Rec., 219, 384 (1987).
11. J. M. Emes and D. Arthur, J. Nutr., 109, 142 (1979).
12. T. J. Peters, G. Sharp, D. Stiel, and J. Wilkes, Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract, New York (1984), pp. 181-187.
13. D. Sklan, O. Halevy, and S. Donoghue, Int. J. Vitam. Nutr. Res., 57, 11 (1987).
14. A. Suprasekt, T. Fujioka, and K. Yamada, Histochemistry, 86, 491 (1987).

USE OF ACRIDINE ORANGE TO ASSESS LYMPHOCYTE MIGRATION IN VIVO

M. V. Popugailo, A. M. Nalivaiko, A. A. Sharavara, and S. R. Ryabinin

UDC 616.155.32-033-076.1

KEY WORDS: migration; lymphocytes; transplantation; acridine orange; fluorescence

In the study of the mechanisms of repair processes, attention is increasingly being paid to lymphoid tissue. It has been shown that lymphocytes accumulate in intensively regenerating tissues as a result of their migration from lymphoid organs [1, 2, 5]. However, many mechanisms of this process remain unexplained, probably due to the absence of adequate methods of investigation.

Methods of studying lymphocyte migration based on the use of transplantation of donor's cells, labeled in vivo with radioactive isotopes, followed by autoradiography of squash preparations of organs, are known [8]. They have several disadvantages: the radioactive label is incorporated only by actively proliferating cells, and accordingly under physiological conditions not more than 20% of labeled lymphocytes can be obtained, or in the case of stimulation by phytohemagglutinin, not more than 40%. These cells possess the lowest level of functional activity, since they are less highly differentiated, and as a result the true picture of the distribution of the donor's lymphocytes is distorted. Also a radiological laboratory is required for work with isotopes such as ^{51}Cr, by means of which a substantially greater percentage of cells can be labeled [7].

Departments of Pathological Physiology and Histology, Sverdlovsk Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR, G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 40-42, July, 1989. Original article submitted June 22, 1988.

0007-4888/89/0007-0949$12.50

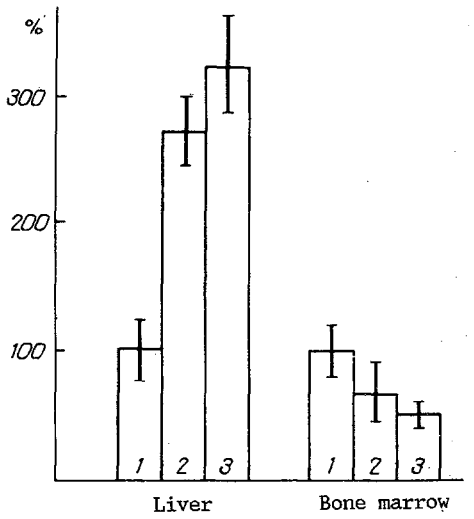

Fig. 1. Accumulation of transplanted AO-labeled lymphocytes in recipients' liver and bone marrow. 1) Transplantation of lymphocytes from intact donors into intact recipients (control, taken as 100%); 2) from donors after PHE into intact recipients; 3) from intact donors into recipients undergoing PHE.

A simpler method of estimating lymphocyte migration is proposed, in which acridine orange (AO) is used as the fluorochrome [3].

EXPERIMENTAL METHOD

Migration was studied on a model with transplantation of lymphocytes from donor animals into recipients. A suspension of donor's lymphocytes (6×10^7/ml) was incubated for 30 min in a solution of AO (1:40,000) at 4°C in darkness [6]. The cells were then washed off by passing the suspension through a column with activated charcoal or by centrifugation for 10 min at 450g, and then resuspended in medium 199. The two methods gave similar results. The number of nucleated cells was counted in the resulting suspension of cells stained with the fluorochrome. If not less than 95% of living lymphocytes was present in it, as shown by the trypan blue test, the suspension was injected intravenously into recipients, which were decapitated 1.5 h later. This interval was chosen because this was long enough for the donor's cells to be distributed throughout the vascular system and to be retained in various organs [1]. Analysis of the lymphocyte distribution 3 and 5 h after transplantation showed no changes in accumulation of the injected cells in the recipient's organs. An interval of more than 5 h was not studied, because of data on transmission by lymphocytes of part of their cellular substance to cells of a regenerating organ [4]. This modifies the pattern of fluorescence and makes analysis of the results more difficult.

The recipient's organs for testing were minced in physiological saline, the number of nucleated cells in the resulting suspension was determined, and films were prepared for examination under a luminescence microscope, coupled with the KF-1 phase-contrast system. The ratio between the number of cells labeled with AO and fluorescent in UV light and the total number of nucleated cells per field of vision, counted in visible light, was determined in the films. The number of labeled cells present in the organ was then calculated as a percentage of the total number of injected lymphocytes.

This method was used to study the characteristics of migration of thymus cells after partial hepatectomy (PHE). Experiments were carried out on 41 Wistar rats weighing 150-170 g. PHE was performed under general ether anesthesia by the method in [9]. The time interval from PHE to transplantation of the recipient's cells was 17 h [1]. Altogether three series of experiments were carried out with transplantation of AO-labeled cells: I) from intact donors into intact recipients (control); II) from donors after PHE into intact recipients, III) from intact donors into recipients undergoing PHE. The number of labeled cells was counted in the recipients' liver and bone marrow (Fig. 1).

If thymocytes from donors undergoing PHE were transplanted, 2.5 times more cells were retained in the liver of intact recipients than in the control ($p < 0.01$). After transplantation of intact thymocytes into recipients undergoing PHE, 3 times more cells were retained in the liver of those recipients than in the control ($p < 0.01$). Under these circumstances migration into the bone marrow was reduced to some extent.

It can be postulated on the basis of these data that, first, thymocytes of donors after PHE acquire the property of migrating selectively into the liver of intact recipients, and second, that accumulation of intact thymocytes in the liver of recipients after PHE is evidence of a local reaction of the damaged organ, leading to retention of lymphocytes in it.

The method described thus gives a quantitative estimate of the distribution of a donor's lymphocytes in the recipient's body, for up to 100% of cells in the injected suspension were labeled irrespective of the subpopulation to which they belonged, the degree of their differentiation, or the phase of the cell cycle. Likewise AO does not cause changes in the membrane of the lymphocytes [6], so that their functional properties are preserved.

The suggested method can be used in any laboratory equipped with a luminescence microscope or a luminescent source of the OI-18 type.

LITERATURE CITED

1. A. G. Babaeva, Immunologic Mechanisms of Regulation of Repair Processes [in Russian], Moscow (1972).
2. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, Stress and the Blood System [in Russian], Moscow (1983).
3. A. V. Zelenin, Interaction of Amino Derivatives of Acridine with Cells [in Russian], Moscow (1971).
4. S. I. Koval'chuk, Byull. Éksp. Biol. Med., No. 11, 114 (1965).
5. M. V. Popugailo, A. M. Nalivaiko, E. S. Tikhach, and V. V. Bazarnyi, Mechanisms of Emergency Regulation and Adaptation during Exposure to Extremal Factors [in Russian], Sverdlovsk (1984), pp. 20-25.
6. Z. A. Sokolova, Lab. Delo, No. 3, 140 (1985).
7. K. F. Fichtelius, Acta Pathol. Microbiol. Scand., 52, 11 (1962).
8. G. M. Higgins and R. M. Anderson, Arch. Pathol., 12, 186 (1931).
9. M. Hill and V. Drasil, Exp. Cell. Res., 21, 569 (1960).
10. M. Schlesinger, Z. Shlomai-Korzash, and E. Israel, Eur. J. Immunol., 3, 335 (1973).