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An Improved Diffusion Chamber (DC) for Culture of Hematopoietic Cells[1] (38983)

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Diffusion chambers (DC) implanted in the peritoneal cavity have had wide application in the study of diverse cell systems (1–8). In most cases the DCs used have been the Benestad (9) modification of the Algire (10) type chamber. This DC consists of a 13 mm diameter Lucite ring to which are attached 0.22 μm pore size Millipore[3] filters. Benestad has described the use of DC with Acropore[4] filters (9, 11).

Nuclepore[5] filters are composed of smooth polycarbonate through which are etched cylindrical uniform holes. A series of studies were conducted to compare the recovery of hematopoietic cells at various times after inoculation into chambers with Nuclepore or Millipore walls. In the first study the early (30–280 min) postinoculation recovery of normal human nucleated blood cells from chambers maintained in an *in vitro* environment was determined. The second study was designed to compare the growth of mouse bone marrow cells in Millipore and Nuclepore chambers for 1–13 days after implantation into the peritoneal cavity of X-irradiated mice.

Materials and methods. DCs were assembled by gluing either Millipore or Nuclepore filters to Lucite rings. Details of fabrication, leak testing, and heat sterilization of the DCs have been described previously (12, 13).

Peripheral blood was obtained from a healthy, hematologically normal volunteer. Nucleated cells were separated and the cell concentration was determined by the Boyum technique (14). To ensure sufficient cell harvest for accurate differential counts, 6.4–7.9 $\times$ 10^5 nucleated cells were inoculated in each DC and the filler hole in the lucite ring sealed with a nylon plug held in place by Millipore cement. Following filling, the chambers were kept in small vials (6 chambers/vial) containing medium 199 precooled and maintained at 4°. Cell recovery following *in vitro* incubation of DC was determined in 10 chambers harvested at 30, 60, 120, and 280 min postinoculation. The total number of cells in individual chambers was determined. The contents of all chambers at each sampling interval were pooled to prepare smears for differential counting.

Eight to 12-week old male mice of the Hale–Stoner–Brookhaven strain were used as bone marrow donors and chamber hosts. Bone marrow cell suspensions were obtained by grinding the femurs and tibiae of 4 donor mice in medium 199 precooled to 4° (15). The cell concentration was determined by counting in a hemocytometer. Each DC was filled with 0.1 ml of the cell suspension containing 23,300 cells and the filler hole in the lucite ring sealed. Two DCs, one Nuclepore and one Millipore, were implanted per mouse. Twenty-four hours previously the chamber hosts were irradiated with 700 rad whole body exposure at a dose rate of approximately 120 rad/min. Since this radiation exposure is lethal within 7–10 days (12), the DCs for 9 and 13-day cultures were reimplanted on Day 7 into newly irradiated recipient mice. Following 1, 3, 6, 9, and 13

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Informed consent was obtained from the human subject after the nature of the procedure had been fully explained.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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[3] Millipore—Millipore Corporation, Medford, MA.

[4] Acropore—Gelman Instrument Company, Ann Arbor, MI.

[5] Nuclepore—Nuclepore Corporation, Pleasanton, CA.

TABLE I. RECOVERY OF NUCLEATED HUMAN BLOOD CELLS FROM DIFFUSION CHAMBERS MAINTAINED *in vitro* AT 4° IN TISSUE CULTURE MEDIUM 199.

Minutes of *in vitro* incubation	Nuclepore (N)		Millipore (M)		
	Mean cell number ± SE	% of input	Mean cell number ± SE	% of input	N/M ratio
Inocula	785,700	100.0	641,000	100.0	1.00
30	282,901 ± 25,500	36.0	162,526 ± 14,400	25.3	1.42
60	242,256 ± 18,600	31.8	167,182 ± 82,800	26.1	1.22
120	318,586 ± 51,100	40.5	109,199 ± 18,600	17.0	2.38
280	320,868 ± 42,600	40.8	123,955 ± 22,500	19.3	2.11

TABLE II. RECOVERY OF MOUSE BONE MARROW CELLS FROM INTRAPERITONEALLY IMPLATED DIFFUSION CULTURE CHAMBERS.

Days after implantation	Nuclepore (N)		Millipore (M)		
	Mean cell number ± SE	% of input	Mean cell number ± SE	% of input	N/M ratio
Inocula	23,300	100.0	23,300	100.0	1.00
1	17,156 ± 4,082	73.5	3,323 ± 811	14.3	5.16
3	3,126 ± 671	13.4	31,541 ± 2,704	135.4	0.10
6	715,023 ± 43,985	3068.7	55,223 ± 10,352	237.0	12.95
9	1,367,173 ± 106,389	5867.7	160,615 ± 60,159	689.3	8.51
13	1,877,490 ± 375,409	8056.2	269,375 ± 90,112	1156.1	6.97

days of culture, 10 recipient mice were sacrificed at each sampling interval, the DCs were removed and placed in 0.5% pronase solution for 60 min, and the contents were removed for total cell count. Differential counts were made on pooled samples obtained at Days 6, 9, and 13, respectively.

Note that chamber fabrication with the Nuclepore filters was much easier than with the Millipore filters. However, the Nuclepore filter material showed considerable variation between different lots. The best results were obtained with the heavier grade material (CPI) which was used in the current studies.

Results. The mean cell count and standard error for the recovery of cells following *in vitro* incubation for periods of 30–280 min are shown in Table I. In all cases a higher cell recovery was obtained from the Nuclepore chambers. The ratio of Nuclepore to Millipore recovery ranged from 1.22 at 60 min to 2.38 at 120 min with a mean ratio for all time periods of 1.78. However, differential counts did not show any significant differences.

The mean cell count and standard error for *in vivo* cell recovery from intraperitoneally implanted DCs are shown in Table II. Following in initial drop in total cell number at Days 1 or 3 for Millipore and Nuclepore chambers respectively, rapid growth was observed in both the Nuclepore and Millipore chambers. On Days 6, 9 and 13, the cell growth in the Nuclepore chambers was significantly ($P < .01$) increased over that in the Millipore chambers. The ratios of Nuclepore to Millipore cell count on these 3 days were 12.95, 8.52, and 6.97, respectively, with a mean ratio of 9.48. The differential counts on the chamber contents from the *in vivo* study are shown in Fig. 1. No significant differences in differential count were observed between the Millipore values and the Nuclepore values ($P > .05$).

Discussion. The use of DCs with Nuclepore walls as compared to Millipore walls increased the recovery of nucleated, human peripheral blood cells following 30–280 min of *in vitro* incubations. Similarly, the growth of mouse bone marrow cells in intraperitoneally implanted DCs was better in Nuclepore chambers than in Millipore chambers. The improved total cell growth in Nuclepore DCs was not associated with any significant

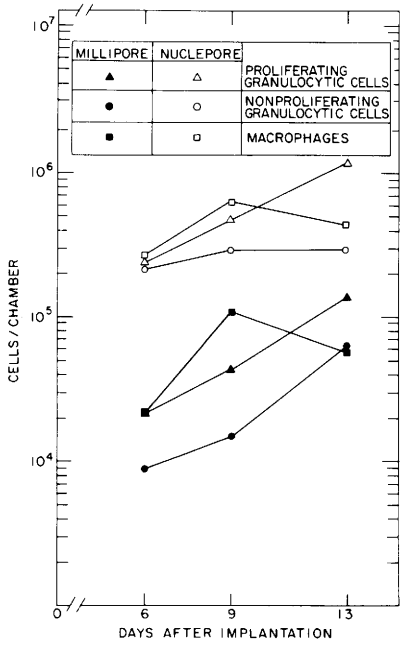

FIG. 1. Differential counts on mouse bone marrow cells cultured in intraperitoneally implanted diffusion chambers.

alterations in differential cell count. Whether this improved recovery is due to reduced cell loss and/or enhanced cellular proliferation remains to be determined. The cylindrical nature of the etched holes in the Nuclepore filters may permit a greater nutrient flow, which might account for the better cell growth as demonstrated in these studies.

Summary. A DC with Nuclepore filter wall has been described. Recovery of *in vitro* incubated human nucleated peripheral blood cells and *in vivo* growth of mouse bone marrow cells in intraperitoneally implanted DCs were improved when compared to the growth obtained in the more commonly used Millipore filter chambers.

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1. Hoelzer, D., Kurrie, E., Ertl, U., and Milewski, A., Eur. J. Cancer **10,** 579 (1974).
2. Niskanen, E., Tyler, W. S., Symann, M., Stohlman, Jr., F., and Howard, D., Blood **43,** 23 (1974).
3. Laissue, J., Chanana, A. D., Cronkite, E. P., Joel, D. D., and Pavelec, M., Blood, in press.
4. Chikkappa, G., Boecker, W. R., Borner, G., Carsten, A. L., Conkling, K., Cook, L., Cronkite, E. P., and Dunwoody, S., Proc. Soc. Exp. Biol. Med.**143,** 212 (1973).
5. Laerum, O. D., Grüneisen, A., and Rajewsky, M. F., Eur. J. Cancer **9,** 533 (1973).
6. Carsten, A. L., Bøyum, A., and Boecker, W., The Proceedings of a Workshop/Symposium on *in vitro* Culture of Hemopoietic Cells, Rijswijk, Netherlands, 1972.
7. Evgenjeva, T. P., Eur. J. Cancer **6,** 533 (1970).
8. Berman, G., and Newby, E. J., Nature (London) **213,** 900 (1967).
9. Benestad, H. B., Scand. J. Haematol. **7,** 279 (1970).
10. Algire, G., Weaver, J. M., and Prehn, R. T., J. Nat. Cancer Inst. **15,** 493 (1959).
11. Benestad, H. B., and Reikvan, Å., (in press) Experimental Hematology.
12. Bøyum, A., Carsten, A. L., Laerum, O. D., and Cronkite, E. P., Blood **40,** 174 (1972).
13. Bøyum, A., Boecker, W., Carsten, A. L., and Cronkite, E. P., Blood **40,** 163 (1972).
14. Bøyum, A., Scand. J. Clin. Lab. Invest. **21** (Suppl.), 97 (1968).
15. Stoner, R. D., and Bond, V. P., J. Immunol. **91,** 185 (1963).

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