through a hypodermic needle or trochar into inaccessible regions and the leads run to a transmitter to avoid the anesthetic problem (*3*).

The output of the circuit of Fig. 1 can be recorded directly by a magnetic tape recorder, so that a buoyant self-detaching recoverable unit can be used to handle the problem of an animal freely roaming the ocean (*5*). Circuits also exist that are suitable for relaying the internal signal by burst transmission each time the animal surfaces for a breath, though present technology demands that, in general, preprocessing of the information should be done so that essentially the result of the experiment is transmitted, rather than all of the detailed data. For example, in the case of heart-rate diving reflex, this means continuously energizing the external booster transmitter, which would be situated on the dorsal fin to minimize body turbulence, to radiate a frequency-modulated signal proportional to the recent derivative of instantaneous heart rate (*3*). Experiments on many species indicate that multiple transmitters can work simultaneously at different frequencies so that it is possible to record any of the physiological variables as a function of depth, velocity, or temperature, by merely making a separate simultaneous recording of this other variable.

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5. Electronic recording can be supplemented in the case of depth by attaching to the pointer of a pressure gauge a radioactive pellet, in which case degree of blackening on an adjacent film indicates percentage of time spent at different depths.
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Pluripotent Stem Cell Function of the Mouse Marrow "Lymphocyte"

Abstract. *Bone marrow from normal and polycythemic mice was filtered through glass wool columns to remove cells other than "lymphocytes." For a given number of nucleated cells, filtered marrow was more efficient than the original marrow in repopulating the spleen of an isogenic recipient previously exposed to lethal irradiation. The proliferative capacity of both the filtered and unfiltered marrow suspensions appeared to be a constant function of the number of small and medium "lymphocytes" present and not of any other cell type.*

Normal bone marrow contains stem cells which are capable of continued proliferation. These may be characterized by their ability (i) to regenerate the hemopoietic tissues of an animal exposed to sublethal irradiation (*1*), (ii) to repopulate, on transplantation, the depleted hemopoietic sites of recipients exposed to lethal irradiation (*2*), and (iii) to reestablish erythropoiesis after the disappearance of recognizable red cell precursors from the marrow of animals made polycythemic by erythrocyte transfusion (*3*). The morphological and functional characterization of the stem cells has long been the subject of controversy (*4*). Studies of the cellular composition of regenerating marrow and of marrow in young animals have suggested, however, that in guinea pigs (*1, 5*), rats (*6*), and mice (*7–9*) the hemopoietic competence of the marrow depends on its content of small, round, mononucleated cells rather than on its content of reticulum cells (*10*) or of granulocytoblastic and erythroblastic elements (*3, 8, 9*). The small round cells in question are lymphocytic in appearance (*1, 4–11*), and are capable of incorporating tritiated thymidine in the nucleus (*1, 8–11*) and of proliferating *in situ* (*1, 8, 10, 11*). Collectively, these findings constitute strong evidence for the concept that the marrow "lymphocyte" is a hemopoietic precursor cell. They do not exclude, however, the possibility that marrow "lymphocytes" may instead exercise some yet undefined trophic or other function that affects hemopoietic cell production by different cell types, although certain of the cited data are not easily reconciled with such an interpretation (*8, 11*).

In this report we describe an experiment in which the competence of mouse marrow "lymphocytes" to act as hemopoietic stem cells was tested directly. This was accomplished by using a glass-wool filtration procedure (*12*) for removing from a suspension of donor marrow cells most of the other elements (Table 1). The proliferative capacity of the cells in the filtrate was assayed by measuring their ability to promote hemopoietic repopulation in irradiated recipient mice. The results were consistent with the interpretation that marrow "lymphocytes" behaved as pluripotent stem cells.

Marrow from long bones of 12-week-old C3H/DiSn or (C3H/Anf × C57 BL)F_1 female mice was suspended in Tyrode's solution to give a concentration of 10^7 nucleated cells per milliliter. Aliquots of the marrow suspensions were filtered twice at room temperature through columns of tightly packed glass wool, prepared according to the description of Hildemann *et al.* (*12*). Original and filtered marrow-cell suspensions were diluted with chilled Tyrode's solution to the desired concentration (10^5 to 3×10^5 cells per milliliter), and 1- to 2-ml injections were given in the tail vein of isogenic recipient females (five to seven animals per group) exposed a few hours earlier to 900 r of 300 kv (peak) total-body x-irradiation.

Aliquots of the diluted cell suspensions were centrifuged for 10 minutes at 1800*g* in a refrigerated centrifuge, and the resulting pellet was smeared on glass slides with a sable brush for differential cell counts. The smears were stained with benzidine for hemoglobin and counter-stained with Wright-Giemsa stain to facilitate distinction between small erythroid cells and "lymphocytes." The total cell yield and the degree of lymphocyte enrichment were related to the compactness of the glass-wool columns; the more compact the column, the lower the yield of cells but the higher the percentage of "lymphocytes" in the filtrate (Table 1, experiment 1). When prospective donor mice were made poly-

Table 1. Percentage of different cell types in mouse marrow before and after filtration through glass wool.* Total numbers of cells counted shown in parentheses.

Cell type	Experiment 1. C3H/DiSn donors			Experiment 2. F_1 hybrid donors		Experiment 3. F_1 hybrid polycythemic donors	
	Unfiltered (300)	Filtered† (500)	Filtered‡ (1000)	Unfiltered (2500)	Filtered (1500)	Unfiltered (500)	Filtered (500)
Blast cells	0.7	0	0	1.0	0.9	0	0
Myeloblasts–promyelocytes	4.0	2.0	0	5.4	2.3	8.0	0.2
Myelocytes	2.0	1.4	0	3.3	0.7	3.6	0.2
Metamyelocytes	9.3	9.0	0.3	10.1	6.6	13.2	1.2
Granulocytes	33.7	42.4	34.9	28.4	25.7	52.8	57.8
Proerythroblasts–erythroblasts	21.7	3.2	0	13.3	2.6	0	0
Normoblasts	8.0	9.0	10.9	7.5	5.7	0	0
Histiocytes–monocytes–reticulum cells	0.6	0	0	0.8	0.1	0	0
Large lymphocytes	1.0	0	0	0.5	0.1	0.6	0
Medium-sized lymphocytes	2.6	2.4	0.8	6.6	6.0	5.4	0.6
Small lymphocytes	16.3	30.6	53.1	23.1	49.1	16.6	40.0

* Cells damaged beyond identification and megakaryocytes were not counted. Intact megakaryocytes were not found in filtered marrow. † Filtered through loosely packed glass-wool columns. ‡ Filtered through tightly packed glass-wool columns.

cythemic by the procedure of DeGowin *et al.* (*3*), their marrow was essentially devoid of identifiable erythroid cells and the cellular composition was further simplified on filtration (Table 1, experiment 3). No attempt was made to determine the viability of the filtered cells by use of dye exclusion techniques; however, microscopic examination did not reveal excessive injury, except in megakaryocytes. The nuclear chromatin was distributed somewhat more finely in some "lymphocytes" than in others, and lymphocyte nuclei sometimes showed indentation marks, resembling the nuclei of small transitional cells of the guinea pig (*1, 5*). These differences, however, did not permit clear-cut differentiation of types among the medium and small "lymphocytes." The passage of marrow granulocytes through the glass-wool columns in all our experiments contrasted sharply with the opposite behavior of mouse peripheral blood granulocytes reported by Hildemann *et al.* (*12*). Whether this reflects differences in the properties of our glass-wool columns or differences between the marrow granulocytes and the blood granulocytes remains to be determined.

The proliferative capacity of the marrow was estimated by injecting known numbers of either the unfiltered or the filtered cells into irradiated recipients. Proliferation of the injected cells was judged in terms of DNA synthesis in the recipient spleen by measuring the uptake of 5-iodo-2′-deoxyuridine-I^{125} (I^{125}-UdR)—a specific DNA precursor and thymidine analogue—in the spleen 5 days after injection of the marrow. Under the conditions used, the splenic incorporation of the radioactive DNA precursor, expressed as the percentage of the total radioactivity available for incorporation, is linearly related to the dose of donor marrow from 0.5 to 10 $\times$ 10^5 transplanted cells (*9*). Recipients of grafted marrow were given 1 μc of I^{125}-UdR (specific activity 1.13 mc/mg) intraperitoneally (*13*). To reduce competition by endogenous thymidilate for incorporation into DNA, the mice were also given 10^{-7} mole of 5-fluoro-2′-deoxyuridine (*14*) 1 hour before injection of the labeled DNA precursor. Each mouse was killed 17 hours later, its spleen was removed, and the incorporated I^{125} radioactivity was measured by means of crystal scintillation counting. The proliferative capacity of the marrow cells, as judged by their ability to promote DNA synthesis in the recipient spleen, increased on filtration (Table 2, results for all nucleated cells). This observation indicates that the filtered marrow contained a larger proportion of cells capable of sustained proliferation than did the original marrow in spite of losing several types of blastic elements.

If a single type of cell in the marrow was entirely responsible for the observed proliferation in the spleen, the uptake of I^{125}-UdR per unit number of such cells should be the same in the original and in the filtered marrow. However, only types of cells that increased in frequency on filtration could logically be considered as responsible for the increased overall proliferative potency of the filtered marrow. The respective uptake values normalized to

Table 2. Proliferative capacity of filtered and unfiltered mouse bone marrow in relation to various cell types. Uptake values for spleens of mice injected with marrow are given as the percentage of the total I^{125}-UdR radioactivity administered, above the percentage retained in spleens of irradiated control animals not injected with marrow; 95-percent confidence limits are given in parentheses.

Cell type	Mean uptake of I^{125}-UdR in recipient spleens per 10^5 injected cells						
	Experiment 1. C3H/DiSn donors			Experiment 2. F_1 hybrid donors		Experiment 3. F_1 hybrid polycythemic donors	
	Unfiltered	Filtered*	Filtered†	Unfiltered	Filtered	Unfiltered	Filtered
All nucleated cells	0.07 (0.06–0.08)	0.12 (0.09–0.16)	0.18 (0.16–0.21)	0.15 (0.11–0.20)	0.27 (0.25–0.28)	0.15 (0.11–0.20)	0.27 (0.21–0.35)
Small and medium lymphocytes‡	0.38 ± 0.04	0.37 ± 0.05	0.34 ± 0.02	0.51 ± 0.05	0.49 ± 0.01	0.68 ± 0.08	0.67 ± 0.07
Normoblasts‡	0.89 ± 0.18	1.35 ± 0.24	1.69 ± 0.18	2.00 ± 0.24	4.70 ± 0.50		
Granulocytes‡	0.21 ± 0.02	0.29 ± 0.03	0.53 ± 0.04	0.53 ± 0.05	1.04 ± 0.05	0.28 ± 0.03	0.47 ± 0.05

* Filtered through loosely packed glass-wool columns. † Filtered through tightly packed glass-wool columns. ‡ Standard errors of the mean uptake values for each of the individual cell types were calculated from the data in Table 1, taking into account also the variance of the percentages of the individual cell types.

each of the types of cells more prevalent in filtered marrow (lymphocytes, normoblasts, and granulocytes, Table 2) indicate that only small and medium "lymphocytes" promoted the same amount of I^{125}-UdR incorporation in recipients of unfiltered and of filtered marrow. The I^{125}-UdR uptake values for other cell types (for example, myeloblasts, metamyelocytes, and erythroblasts) were not computed although such cells were present in small amounts in the filtered marrow, since neither in this experiment nor in earlier studies (*9*) has the number of such cells in the marrow been found to parallel its proliferative capability.

Recipients of filtered marrow were also observed for periods longer than 5 days to establish whether or not such marrow could afford long-term survival to mice which had been exposed to lethal radiation and whether or not the regenerated hemopoietic tissue would contain all of the known classes of mature hemic cells. A detailed account of the latter study will be given elsewhere; however, it can be stated that a large proportion of the irradiated recipients infused with minimal numbers ($< 10^5$) of filtered marrow cells survived more than 30 days, and that after 10 days their spleens contained hemopoietic nodules with megakaryocytes, mature granulocytes, and granulocytoblastic and erythroblastic elements.

It seems reasonable to infer from these and earlier data (*9*) that the marrow "lymphocyte" of the mouse is capable of continued proliferation and is probably a pluripotent stem cell. Since, however, the transplanted "lymphocytes" of these experiments were not labeled with a marker recognizable in the descendent hemic cells, an alternative interpretation involving trophic or stimulating functions of the donor "lymphocytes" on host hemopoiesis cannot be conclusively excluded. Nevertheless, correspondingly good regeneration of functional hemopoietic tissue in comparably irradiated mice has been induced after irradiation only by the successful transplantation of competent hemopoietic stem cells (*2*). The properties of this marrow "lymphocyte" appear to differ markedly from those of the lymph and of the lymph node–lymphocyte in several ways, for example, hemopoietic competence and distribution among myelopoietic and lymphopoietic sites of recipients exposed to lethal irradiation (*9, 15*), duration of life span (*11*), and labeling with tritiated thymidine (*1, 8–12*). The marrow "lymphocytes" may not be a homogeneous population, however, and all of them may not be endowed with stem cell activity, as has been suggested (*4*). The transplanted mouse marrow "lymphocyte" responsible for repopulation of recipient spleens may, for example, be equivalent to the "primitive free cell" of Sabin (*16*).

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Serologic Typing of Human Lymphocytes with Immune Serum Obtained after Homografting

Abstract. *Serums obtained from paired humans following reciprocal fourth-set skin homografts, or from a prolonged series of reciprocal intradermal injections of leukocytes, displayed potent cytotoxic activity against lymphocytes of certain individuals and no activity against lymphocytes of other persons. Study of one family suggested that lymphocyte reactivity may be transmitted according to simple Mendelian genetics. It is probable that this reactivity reflects and can be used to identify specific histocompatibility antigens in man.*

It was previously shown that antibodies reactive with human white blood cells develop after skin-homografting in the human (*1, 2*). Reactivity in both human and guinea pig serums after grafting was readily demonstrable with homologous lymphocytes and poorly or not at all with neutrophils (*2, 3*). Antibodies reactive with lymphocytes of the donor strain have also been found in a number of other species after homografting, including mice and chickens (*4*). It has therefore been our view that the lymphocyte may be the "key" cell to use in developing typing methods for human transplantation antigens (*5*). Our earlier investigations with human serums were accomplished by means of a technique in which a purified, I^{131}-labeled antibody to globulin was used. Because of its complexity, this technique has not been found practical for large-scale typing experiments, but has been useful for identifying antibody-containing serums after homografting and for selecting certain donors whose leukocytes reacted strongly with these serums. By using highly purified lymphocyte suspensions, we have now found a simple method for demonstrating in selected human serums, obtained after homografting, po-