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11. RNA and protein syntheses were measured in duplicate cultures (35-mm dishes) at each point by adding 0.25 ml of Eagle's medium containing [5-^{3}H]uridine (250 μc/ml; 21 c/mmole, Schwarz/Mann) and L-[carboxyl-^{14}C]leucine (5 μc/ml; 50 μc/mmole, Schwarz/Mann) to the 1 ml of maintenance medium already present in the dish. After mixing, the cells were incubated at 37°C for 10.25 minutes, rapidly washed ten times with cold (4°C) PBS and solubilized in 1 ml of 1 percent sodium dodecyl sulfate. One milliliter of 20 percent cold trichloroacetic acid (TCA) was then added and the mixture was kept at 4°C for at least 0.5 hour. Samples (10 μl) in duplicate were spotted on Whatman GF/C glass fiber filters and dried, and the radioactivity was counted in a liquid scintillation spectrometer. The remaining sample was filtered through Whatman GF/C filters, and the precipitate was washed thrice with 5 percent cold TCA and twice with ethanol. The radioactivity on the dried filters was measured as above. Radioactivity in the acid-precipitable fraction was corrected for the inhibition of cellular uridine uptake in order to determine the rate of RNA synthesis. It is assumed that there was no alteration in the processing of the precursors after transport into cells.
12. Fifty-four 14-day-old cultures (35-mm dishes) were divided into three groups of 18 each. One group was treated with DRB at varying concentrations in 1 ml of maintenance medium and cellular processes (*11*) assayed after 0.5 hour of the treatment. The other two groups were treated with Eagle's medium containing both poly(I·C) and the appropriate DRB concentration for 1 hour and washed four times with warm PBS; the incubation was continued to 5 hours in maintenance medium that contained DRB. At this time one group was used to assay macromolecular synthesis (*11*) while the other was washed four times with warm PBS and incubated for a further 19 hours. At this time the supernatants were harvested and titrated for interferon.
13. The extent of inhibition of RNA synthesis was normalized with respect to the effect at 60 μ*M* DRB. The increase in interferon titer over the DRB-free control was expressed as a percentage of the maximum increase seen in this experiment at 40 μ*M* DRB.
14. The shutoff curve of interferon production after removal of DRB at 5 hours is based, in part, on data from a second experiment, which are not shown.
15. We thank A. F. Wagner of the Merck Sharp & Dohme Research Laboratories, Rahway, N.J., for a gift of DRB. This investigation was supported in part by research grant AI-03445 and contract NO1-AI-02169 from the U.S. Public Health Service.

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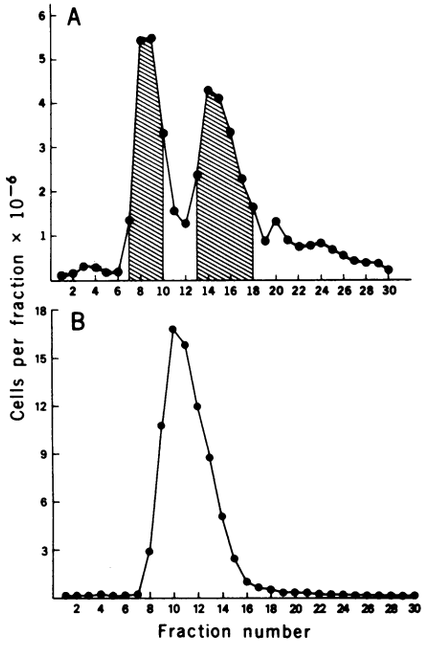
Hemopoietic Stem Cells in Human Peripheral Blood

Abstract. *A population of lymphocytes, separable from the great majority by virtue of their larger size and their failure to exhibit the rosetting characteristics of thymus-dependent lymphocytes and bursa-equivalent cells, possess true pluripotentiality. On culture in vivo they proliferate and differentiate into erythrocytic, granulocytic, and megakaryocytic progeny. This may be the first clear demonstration of the primitive progenitor blood cell in man.*

An operational definition of the hemopoietic stem cell includes two major criteria: the capacity for potentially unlimited self-renewal and the capability of differentiation into all types of mature blood cells. Experiments on small animals have provided convincing evidence that there is indeed a cell that has these two functions. A mononuclear cell, generally indistinguishable, by light microscopy, from a lymphocyte, has appeared to be the most likely candidate (*1*). Unfortunately, definitive investigative techniques, such as the spleen colony assay (*2*) and total body labeling with tritiated thymidine (*3*), are inapplicable to humans, for technical or ethical reasons. Culture methods in vitro, whether in liquid or semisolid media, preferentially support the growth of progenitor cells committed to differentiation in the granulocyte-macrophage pathway. These cells, which represent some of the early progeny of the true pluripotent stem cell, are demonstrable in normal human peripheral blood (*4*) and have been localized within nonadherent mononuclear cells (*5*). Culture in vivo, in Millipore diffusion chambers implanted intraperitoneally in lethally irradiated mice, supports the growth of unseparated normal human bone marrow and peripheral blood (*6*) and of mononuclear cell concentrates (*7*). By contrast with in vitro methods, this system appears to provide an environment conducive to stem cell proliferation and differentiation (*8*). Our study concerns the growth, in diffusion chambers, of subpopulations of mononuclear cells harvested from the peripheral blood of normal adult volunteers.



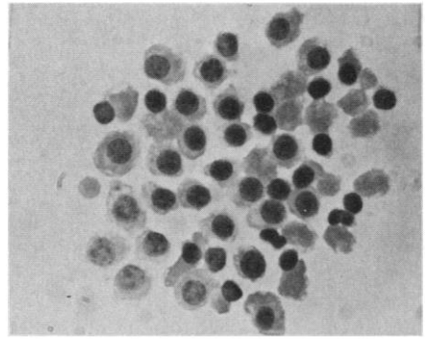


Fig. 1 (left). (A). Separation of non-T mononuclear cells by velocity sedimentation. Increasing fraction number reflects increasing sedimentation velocity and so increasing cell size. The slower peak of smaller cells consists of pure lymphocytes; the second peak is a monocyte concentrate. (B) (lower left). Separation, by velocity sedimentation, of mononuclear cells that fail to form EAC′ rosettes. Fig. 2 (right). Cluster of benzidene-positive late normoblasts harvested at 14 days from Millipore diffusion chamber culture of human hemopoietic stem cell concentrate.

Heparinized whole blood was subjected to isopycnic sedimentation (*9*), and the separated mononuclear cells were depleted of thymus-dependent lymphocytes (T cells) by rosette formation with neuraminidase-treated sheep erythrocytes (N-SRBC) (*10*). When cultured, with or without their accompanying rosettes, the T cells failed to transform. That this was not due simply to inviability was shown by repeating the cultures with immune lymphocytes in the presence of the appropriate antigen (tetanus toxoid or tuberculoprotein). Under these circumstances blastogenesis was marked.

The cells that failed to form these rosettes (non-T cells) were further separated by velocity sedimentation at 1 g in a sucrose gradient (*11*), a procedure that separates cells more on the basis of differences in size than of differences in density (*12*). Two populations of cells were obtained (Fig. 1A): one of pure small lymphocytes (non-T) and the other, a monocyte concentrate of mean composition of 82.7 percent monocytes, 9.9 percent lymphocytes, and 7.4 percent basophils. Culture of the non-T lymphocytes, alone and in combination with cells from the monocyte concentrate irradiated to 2500 roentgens, yielded only lymphocytes, although there was evidence of spontaneous blastogenesis. In contrast, when the monocyte concentrate, containing about 10 percent large lymphocytes, was subjected to culture in vivo, megakaryocytes and all forms of granulocyte precursors were produced on every occasion, and sometimes benzidene-positive normoblasts were obtained (Fig. 2).

In an effort to remove the monocytes, mononuclear cell concentrates were incubated with a complex of SRBC's (E) labeled with rabbit antibody to SRBC's (A) [the 7*S* immunoglobulin G (IgG) frac-

tion] and a nonhemolyzing amount of mouse complement (C′). This complex (EAC′) causes rosette formation (*13*) not only with lymphocytes carrying surface membrane immunoglobulin (bursa equivalent or B cells), but with monocytes [by virtue of their surface receptor sites for IgG and complement (*14*)] and with basophils, which carry surface receptors for complement (*15*). The EAC′ rosettes consistently failed to grow on culture, that is, the growth chambers contained only lymphocytes, macrophages, and basophils when examined at periods of up to 4 weeks. Cells that failed to form EAC′ rosettes constituted a single population on velocity sedimentation analysis (Fig. 1B); this population consisted of more than 95 percent lymphocytes, and demonstrated stem cell "activity" on culture.

Mononuclear cells were tested for rosette formation, first with N-SRBC and then with EAC′. Neither rosette product grew in the culture system, but the residual cells, which had failed to form rosettes by either technique, regularly exhibited true pluripotentiality. We believe that this study is the first unequivocal identification of the human hemopoietic stem cell.

RONALD D. BARR
JACQUELINE WHANG-PENG
Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014
SEYMOUR PERRY
National Institutes of Health, Bethesda, Maryland

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16. We thank E. Lee, A. Brooks, and T. Knutsen for technical assistance.

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Ultrasensitive Chemosensory Responses by a Protozoan to Epinephrine and Other Neurochemicals

Abstract. *A behavioral assay was developed based on differential tendency of a protozoan to attach to an agar gel containing the test substance. The heterotrophic marine dinoflagellate* Crypthecodinium (Gyrodinium) cohnii *responded negatively (less tendency to attach) to epinephrine at concentrations above* 5×10^{-15}M *and to norepinephrine at concentrations above* 5×10^{-9}M. *Response to choline as choline* H_2 *citrate, choline bitartrate, and choline chloride was negative above* 10^{-7}M, *but response to the choline analog carbachol was positive (greater tendency to attach) in the range* 5×10^{-6} *to* 5×10^{-4}M. *Other responses to neurochemicals at comparable concentrations were: dopa, betaine, and glycine—positive; L-glutamic acid, tryptophan, putrescine, and taurine—negative. Serotonin was inert, responses to tyrosine and γ-aminobutyric acid were variable, and phenylalanine* (6×10^{-3}M) *and 5-hydroxytryptophan* (5×10^{-4}M) *were negative only at the highest concentrations tested.*

Recent work indicates the importance of chemosensory responses in the ecology of aquatic microorganisms (*1*). We have developed a method for detecting a settling-and-attaching response by the heterotrophic marine dinoflagellate *Crypthecodinium (Gyrodinium) cohnii* to chemicals impregnating an agar gel (*2*). This free-swimming saprophytic species often appears in samples of rotting *Fucus* or other algae (*3*); it grows well on agar slants (*4*) and tends to attach to an agar surface in biphasic culture. This may denote an adaptation for finding and colonizing favorable substrates in nature, and so differential responses to chemicals incorporated in the gel may have an ecological interpretation. Elsewhere (*2*) we report sensitivities to chemicals likely to be present in the vicinity of rotting seaweeds. In an effort to learn the mechanism, or mechanisms, of these responses we have investigated the effects of chemicals that seem unlikely to occur naturally as ecological cues but which are known to be involved in the chemosensitivity of metazoan cells. Here we report extremely sensitive behavioral responses to epinephrine and other neurochemicals and analogs.

Table 1. Concentrations of neurochemicals at which responses occurred.

Chemical	Active molar range
Positive response (greater tendency to embed in experimental gel)	
Dopa	5×10^{-7} to 5×10^{-4}
Betaine	8.5×10^{-6} to 8.5×10^{-5}
Glycine	10^{-6} to 10^{-5}
Carbachol	5×10^{-7} to 5×10^{-4}
Negative response (less tendency to embed in experimental gel)	
Epinephrine	5×10^{-14} to 5×10^{-8}
Norepinephrine	5×10^{-9} to 5×10^{-6}
Choline H_2 citrate	3×10^{-7} to 3×10^{-3}
Choline bitartrate	2×10^{-7} to 2×10^{-3}
Choline chloride	7×10^{-7} to 7×10^{-3}
L-Glutamic acid	7×10^{-8} to 7×10^{-4}
Tryptophan	5×10^{-7} to 5×10^{-4}
Putrescine 2HCl	6×10^{-7} to 6×10^{-3}
Taurine	8×10^{-7} to 8×10^{-3}
5-Hydroxy-tryptophan	5×10^{-4}
Phenylalanine	6×10^{-3}
Inert (no response detected)	
Serotonin	6×10^{-7} to 6×10^{-3}

In the assay a suspension of organisms in salt solution (*5*) was poured as a fluid overlay onto agar gel in a divided petri dish. Control and experimental gels were made with salt solution and the experimental gel had the test substance added; the two gels were in different partitions of the same petri dish. After standing for 3 hours the suspension was poured off and the agar surface was gently rinsed with distilled water and stained with Lugol's iodine. The densities of embedded organisms remaining in control and experimental gels were compared by counting cells in 40 random microscope fields from each gel and by using Wilcoxon's nonparametric test (*6*). We adopted nonparametric methods because it was clear in all our data that the embedded cells have a clumped distribution with variance of the counts an order of magnitude greater than the mean (*7*). This suggests that the cells may be attracted to each other, as well as to the agar. (Some evidence which we have of such pheromonal activity will be presented elsewhere.) Dead or immobile cells do not embed in the agar (*2*).

By this method we found negative response (smaller density of embedded cells in the experimental partition) to epinephrine at concentrations of 5×10^{-14} to $5 \times 10^{-8}M$. At $5 \times 10^{-15}M$ the effect occurred in some but not all experiments, and at lower concentrations it was not detected. Norepinephrine HCl was negative at 5×10^{-9} to $5 \times 10^{-6}M$. These results and those with other neurochemicals are given in Table 1. Serotonin (6×10^{-7} to $6 \times 10^{-3}M$) was inert (no detected response). Tyrosine and γ-aminobutyric acid are clearly active but experiments to date are somewhat variable. The response to