phate in the effluent by controlling the character of the input. Concern has been expressed that heavy enrichment with nitrate may cause alga problems in waters with adequate natural supplies of phosphorus (*5*). While this might be true in some regions for geochemical reasons, it appears that Lake Washington in its unpolluted condition has more than enough nitrogen relative to phosphorus, and phosphorus is the dominating limiting element. Lake Washington probably represents a large class of lakes in which phosphorus is the dominating element (*6*).

An indication of the relative importance of these two elements can be seen by measuring their decreasing concentrations during the growth of phytoplankton in the spring (Fig. 2). In 1933, when the lake was less polluted, a small concentration of nitrate was left over when phosphate was nearly exhausted. In strong contrast, after many years of enrichment with sewage effluent rich in phosphorus, an excess of phosphate occurred when nitrate was exhausted in 1962; this excess phosphate was almost as much as the winter maximum of 1933. During diverison of sewage, the condition returned to resemble that of 1933 but continued to change, and in 1968 and 1969 a large excess of nitrate occurred. This kind of analysis should be generally useful in other lakes that have a winter maximum and spring decrease in nutrient concentration. It is obvious that the fact that phosphorus is in excess in a lake does not mean that control of phosphorus will be ineffective as long as it can be brought to low concentrations. Possibly in some regions excess phosphorus demonstrated this way can be regarded as evidence that the lake has been affected by sewage effluent and its quantity used as a measure of the magnitude of the effect.

Thus, Lake Washington has responded promptly and sensitively to changes in its nutrient income. While the changes during increase are not as well documented by direct limnological data as those during decrease, they are recorded by paleolimnological evidence (*7*). On the basis of the present data and existing knowledge, it seems valid to predict that noticeable improvements can be made in similar lakes even by partial limitation of phosphorus. Total elimination of phosphorus is impractical, of course. The worst sources are the most concentrated ones. The popular system of calculating total loadings on an areal basis (kilograms per hectare) without accounting for concentration can be misleading when dealing with lakes that receive large volumes of dilute drainage. The annual total of phosphorus can be very large, but if it is dispersed in a large volume of water it cannot generate as dense concentrations of algae as can the same total in much more concentrated sewage effluents and some kinds of agricultural drainage. Diversion of sewage from Lake Washington reduced the phosphorus income by only about half, but the sewage effluent was nearly 200 times as concentrated in phosphate as the influent streams (*2*), and the effect has been great.

W. T. EDMONDSON
Department of Zoology, University of Washington, Seattle 98105

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Bone Marrow Colonies: Stimulation in vitro by Supernatant from Incubated Human Blood Cells

Abstract. *A substance which stimulates growth of granulocytic and mononuclear cell colonies from mouse and human bone marrow was produced by incubated human blood cells. It is resistant to heat and freezing and is not dialyzable. Intact irradiated and unirradiated cells had very little activity, and sonically disrupted cells had no activity. The addition of plasma or sonically disrupted cells to the cell supernatant decreased its activity.*

Bone marrow cells of animals (*1*) and man (*2–4*) can give rise to granulocytic and mononuclear cell colonies when grown in vitro in a soft gel medium with proper stimulation. A substance which stimulates the growth of colonies from murine marrow has been demonstrated in various cell feeder layers (*1*), certain serums (*5*), urine (*6*), and conditioned media obtained from various tissues (*7*, *8*). Feeder layers of kidney tubules (*4*), peripheral blood cells (*2*), and urine (*3*) are capable of stimulating colony growth from human bone marrow. The fact that a feeder layer of peripheral white blood cells will stimulate growth of marrow cells prompted our search for a substance which was either produced by or stored within circulating white blood cells.

Blood was collected from healthy adults with no known illnesses and prevented from coagulating with heparin. After sedimentation, the cell-rich plasma containing erythrocytes and leukocytes in a ratio of approximately 1 : 1 was incubated in McCoy's 5A medium containing fetal calf serum. The concentration of peripheral leukocytes was 2×10^6 to 4×10^6 cells per milliliter of medium. After 10 to 14 days of incubation at 37°C in 6.5 percent CO_2, cells were removed by centrifugation at 2000*g*, and the supernatant was passed through a filter with a pore size of 0.20 μm (Nalge Company, Rochester, New York). The ability of the supernatant to stimulate colony growth was tested by an adaptation of the soft-agar technique for cloning cells in vitro (*1*, *9*). Methylcellulose (Dow Methocel), as described by Ichikawa *et al.* (*10*) and modified by Worton, McCulloch, and Till (*8*), was used in place of agar. Bone marrow cells were removed from the marrow of 6- to 8-week-old F_1 mice from a mating of C57 BL females and DBA males. The cells were dispersed in CMRL-1066 and counted electronically (Coulter Electronics, Hialeah, Florida). A known number of cells was suspended in 1.4 percent methylcellulose containing tissue culture medium (CMRL-1066) plus 10 percent horse serum; for each 1 ml of mixture, 0.1 ml of the test substance was added. One milliliter of the mixture was then plated in 35 by 10 mm petri dishes (Falcon Plastics, Los Angeles, California) and incubated at 37°C in 6.5 percent CO_2. After 7 days, the number of colonies of cells growing within

Table 1. Ability of supernatant from human blood cells to stimulate growth of bone marrow colonies in vitro. All substances are fractions of the same blood sample. The cells and plasma are from freshly obtained blood and the supernatant is from incubated cells. The supernatant was tested unmodified, after heating at 56°C for 60 minutes, after freezing at −20°C, or after dialysis for 72 hours in Visking tubing against phosphate-buffered saline at 4°C. The number of colonies is expressed as colonies per 100,000 marrow cells. Values represent the mean and standard error of five to ten plates.

Substance	Volume/plate (ml)	Colonies (No.)
Intact blood cells	0.1 (10^6 cells)	0
Sonically disrupted cells	.1 (10^6 cells)	0
Irradiated cells	.1 (10^6 cells)	0
Plasma	.1	0
Supernatant	.1	74 ± 3.3
Heated supernatant	.1	68 ± 2.0
Frozen supernatant	.1	65 ± 2.8
Dialyzed supernatant	.1	68 ± 3.1

the gel was counted by means of an inverted microscope. A colony was defined as a group of more than 50 cells. Intact cells, sonically disrupted cells, or irradiated cells (1000 roentgen) which were not incubated, as well as plasma, were also tested for their colony-stimulating ability (Table 1).

Considerable stimulating activity was present in the supernatant 10 to 14 days after incubation, with nearly all colonies containing 500 to 1500 cells (Table 1). Intact, sonically disrupted, or x-irradiated cells or plasma had no colony-stimulating activity by 7 days. Petri dishes containing intact irradiated or unirradiated leukocytes contained several collections of cells, but none exceeded 50 cells per group.

In two separate experiments, sonically disrupted cells, irradiated cells, or plasma were combined with the supernatant. The number of colonies was reduced by 40 percent by the sonically disrupted and irradiated cells and to a greater extent by the plasma. Irradiated and unirradiated cells washed in McCoy's 5A medium prior to plating had a mild degree of stimulating activity at 7 days, whereas sonically disrupted cells had no activity. The addition of washed irradiated and unirradiated cells to the supernatant resulted in a slight enhancement of colony cell growth. However, the addition of washed sonically disrupted cells resulted in a 40 percent decrease. These observations suggest that both plasma and sonically disrupted cells may be inhibitory to colony cell growth.

Morphologically, colonies contained predominantly granulocytic cells during the first 2 or 3 days and by day 7 were nearly all mononuclear in appearance.

The presence of a colony-stimulating factor in the supernatant of incubated blood cells and only minimum activity in the intact irradiated and unirradiated cells suggests that the substance is not stored within the cells but is produced and released during incubation. Plasma and sonically disrupted cells appear to be inhibitory to colony cell growth, since both reduced the stimulating effect of the leukocyte supernatant.

A study of some of the properties (Table 1) of the stimulating substance reveals that it is not affected by heat or freezing, and is not dialyzable. These properties are in part similar to those described for substances derived from urine (*6*) and other conditioned media (*7*).

Preliminary studies with human bone marrow indicate that colony growth of cells from normal as well as leukemic individuals is stimulated by this substance. Supernatants from incubated leukemic cells also possess colony-stimulating activity.

The finding that the substance produced by incubated human leukocytes will stimulate the growth of granulocytic and mononuclear cell colonies from human or murine bone marrow suggests that this may represent a regulator of granulocytic and mononuclear cell growth. Since some of the properties are similar to those of the substance described in urine (*6*), it seems quite likely that it is produced by leukocytes and excreted in the urine. This substance may serve an important function in regulating granulocytic and mononuclear cell growth in normal individuals as well as in individuals with diseases of the hematopoietic system.

PAUL A. CHERVENICK
DANE R. BOGGS
University of Pittsburgh School of Medicine, Division of Hematology, Pittsburgh, Pennsylvania 15213

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RNA and DNA Puffs in Polytene Chromosomes of Rhynchosciara: Inhibition by Extirpation of Prothorax

Abstract. *In the giant polytene chromosomes, gene amplification is made visible by formation of DNA puffs, and gene transcription is made visible by formation of RNA puffs. Ligation of the anterior portion of the larva at the end of the fourth larval instar inhibited the formation of the DNA puffs that normally develop at this stage and caused regression of RNA puffs.*

Synchronous development of sibling larvae of *Rhynchosciara angelae* permits a detailed analysis of the sequential pattern of puffs which occur during development (*1*). During the first three larval instars numerous and specific chromosomal puffs occur, but in general these puffs are small (*2*).

At the prepupal stage, that is, in the late fourth instar, when the larvae gather together to form a colonial cocoon, a new series of chromosomal puffing ensues, and the puffs enlarge greatly (*1, 2*). Principal among these puffs are the DNA puffs where, besides an enhanced RNA synthesis, large amounts of DNA accumulate in specific regions of the giant chromosomes (*1–4*).

Inhibition of puffs in cells under different experimental and pathological conditions has been observed by a number of investigators (*5*). We shall show here that the DNA puffing is inhibited by ligation of the front part of the larvae just behind the brain. This is consistent with the interpretation that an interruption of the flow of brain hormone from the neurosecretory cells to the prothoracic gland will block