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Analytical Review of Structure and Regulation of Hemopoiesis

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The development of knowledge on the structure of hemopoiesis and its regulation can be divided into four broad areas: 1) descriptive morphology. 2) kinetics of cell proliferation, 3) regulation of rates of cell proliferation through interaction of molecular regulators and their cell surface receptors, and 4) clinical applications. Descriptive morphology commenced in the last century. Hallmarks were the observation of mitoses in the bone marrow and the appreciation that red cells and white cells were being produced in the marrow, implying a birth rate within the marrow and death of the cells somewhere outside of the marrow (1).

Ehrlich, Maximow, Ferratta, Naegeli and many others in Europe described the cell lineages in intimate detail. Their studies are covered in Downey's Handbook of Hematology (1).

The notion of two levels of stem cells, one pluripotent, operating during growth and regeneration from injury, and multiple monopotent stem cells that maintain steady state cell production in the adult was conceived by Maximow (2).

Descriptive morphology revolutionized hematologic diagnoses, codifying them into a series of clearly distinct diseases, allowing correlations between diagnosis and prognosis which, with more modern classifications and techniques, are of great value in selecting the appropriate therapy.

Descriptive morphology is and will remain a very important element in the armamentarium of hematologists in the diagnosis and treatment of disease.

Metchnikoff (1) described the neutrophil and macrophage and elucidated much about their function. He never dreamed that the macrophage with its insatiable appetite for bacteria would become a central, perhaps the most important cellular actor, in regulating the rates of hemopoietic cell production.

Quantitative aspects of the kinetics of hemopoietic cell proliferation were initiated by two remarkable women scientists, Winifred Ashby and Florence Sabin. In 1921, Ashby (3), using differential red cell agglutination, determined that the mean lifespan of the human red cell was 120 days. Her work was not appreciated. Sabin (4) studied granulocytes in the peripheral blood and deduced that 6% died per hour, giving a mean lifespan of 8 hours. She described in detail the conditions of steady state cell renewal and also was not appreciated.

All tissues have a circulation. A unique observation was made by Fliedner et al.(5), showing that the bone marrow had a dual circulation. The major circulation to the bone marrow is from the nutrient artery. There is a second circulation from periosteum through cortical bone to the hemopoietic marrow perhaps to assure circulation to hematopoietic tissue in the event of destruction of the nutrient artery by fractures, or are regulatory factors being delivered from the periosteum to the hemopoietic marrow that influence hematopoiesis. Calvo (6) demonstrated myelinated and non-myelineated fibers in the bone marrow. Does the unique circulation and innervation of the bone marrow play a role in regulation of hemopoiesis?

Many techniques were exploited for study of details of the kinetics of cellular proliferation. When tritiated thymidine was synthesized in 1956 at Brookhaven by Hughes, it was rapidly exploited in the study of chromosomal replication (7) and cell proliferation in general and particularly in the study of hemopoietic cell proliferation in mammals (8) and then applied in human disease (9,10).

From a large series of studies, Cronkite et al. (10), Fliedner et al. (11,12), Killman et al. (13-15), and Bond et al. (16), enumerating the fraction of cells of different morphologic stages that are in DNA synthesis, the DNA synthesis time, the number of mitoses in each stage, and the time it takes labeled cells to flow from the myelocyte through the bone marrow into the peripheral blood, this deterministic model was designed by Cronkite and Vincent (17). It shows a mitosis at myeloblast, promyelocyte, two successive mitoses in the myelocytic stage and a minimum of about three hours from the terminal myelocyte mitosis to appearance of labeled cells in the metamyelocyte compartment. Two things were deduced from this model: 1) As was known, there must be a large pool of stored granulocytes available in the bone marrow to be dumped into the peripheral blood upon demand. 2) Normal transit time of about 100 hours from the myelocyte to the peripheral blood might be reduced substantially. 3) If a mechanism existed to tack on an additional mitosis at the myelocyte level, the production rate could be doubled and cells made available for use in the periphery more quickly than if the whole system were regulated at the stem cell level. Of course, if input were increased from the stem cell, it would further increase the rate of production. The decrease in the emergence time from the myelocyte to the appearance of granlocytes in the peripheral blood was first demonstrated in man by Fliedner et al. (11, 12).

They showed the emergence of labeled granulocytes from the bone marrow in diverse clinical states. The normal emergence time was decreased from about six days to little over two days in a patient with lobar pneumonia. Thus, one of the predictions of a shorter transit time had been confirmed earlier in the presence of a lobar pneumonia.

Cronkite and Vincent (17) postulated that in the presence of infection there would be an additional mitosis at the myelocyte level to increase production of cells and make them available more rapidly than if the production rate was regulated solely at the stem cell level. This postulate could not be demonstrated in man since one would not know when to perform bone marrows after development of infection, and one would promptly treat with antibiotics. We, therefore, decided to study the influence of acute inflammation on granulopoiesis in the dog. This was accomplished by the injection of turpentine into the uterus which induces a violent inflammatory reaction with granulocytosis and invasion of granulocytes in massive numbers into the inflammatory area (18). The results are shown in Figure 1. The ratio of myelocytic mitoses to erythrocytic mitoses prior to the induction of sterile inflammation over a period of 96 hours is shown by the lower line of the top panel and the changes in this ratio after the induction of sterile inflammation are shown by the upper line. Note it rapidly rises to a peak 3 times normal and then slowly subsides, but is still modestly elevated at 96 hours after the initiation of sterile inflammation. At the bottom of the slide, on the left side, is shown the fraction of different cytologic types that are labeled with tritiated thymidine administered one hour or 24 hours after the initiation of inflammation. The top line shows the effect on labeling when inflammation precedes by 24 hours the administration of

tritiated thymidine. M3 and M4 are the myelocytic compartment. The ^{3}HTdR labeling index in animals prior to induction of inflammation is 20%. By 24 hours after the initiation of inflammation, the index is 40%. Note also that M5, the metamyelocyte, has now entered the dividing pool of cells. The bottom of the left side shows the appearance of labeled M6 and M7 banded and segmented neutrophils in the bone marrow. The transit time from the myelocyte to these cells is markedly accelerated indicating that the genes for all of the enzymes required for maturation of the granulocyte are switched on with rapid transcription of messenger RNA, its processing and translation into the proteins for which the genes were encoded. The right hand side shows the mean grain count of these cell types, which is proportionate to the rate of DNA synthesis. In the normal steady state, the mean grain count is 8 in the myelocytic compartment, 24 hours after the initiation of inflammation it is greater than 20. Note also the mean grain count in the control period and one hour after initiation of inflammation is zero in the metamyelocyte M5 has risen rapidly from 0 time to 16 when inflammation was initiated 24 hours prior to the administration of tritiated thymidine showing that the metamyelocyte has now become part of the dividing pool of cells.

The emergence time from myelocyte to granulocyte in blood in the normal steady state and after inflammation is shown in Figure 2. The line with open squares shows emergence time in steady state. When inflammation is initiated 24 hours prior to the administration of tritiated thymidine, the emergence time is decreased to nearly 8 hours, line with solid circles from the steady state of 40 hours. These studies clearly show that inflammation induced in tissue far away from the bone marrow results in:

1) Recruitment of a larger fraction of myelocytic cells into DNA synthesis

2) Increases the rate of DNA synthesis

3) Increases the number of mitoses, thus increasing the production rate at myelocyte level

4) Increases the rate of messenger transcription and translation so that a process involving synthesis of dozens of enzymes with their packaging into specific granules is shortened from the order of 40 hours to 8 hours. Unfortunately, these studies were completed before assays for regulators were available in the dog.

From what is known today based on in vitro culture, one can assume that G-CSF, GM-CSF, IL-3, and IL-1 play crucial roles in the in vivo acceleration of granulocyte production. Whether one or more of these molecular regulators are produced in the inflammatory area or other unidentified molecules home on the bone marrow from inflammatory zones and induce the intramedullary production of these known molecular regulators has not yet been established. The first event in providing an increased number of granulocytes at an inflammatory site is the rapid mobilization of blood granulocytes and their exit from the blood resulting in neutropenia (Figure 3). The second event is the mobilization of stored granulocytes from the bone marrow. This is said to be due to production of an alpha-1 glycoprotein called leukocyte-inducing factor (LIF) (19). Whether this material is an entity separate from one of the established molecular regulators is not clear. Chikkappa et al. (20) produced evidence suggesting that LIF and CSF are separate entities. Is it conceivable that a decrease in population density of mature granulocytic cells in the bone marrow reduces a source of lactoferrin thus reducing an inhibitory effect on production of CSF by macrophages as believed by Broxmeyer et al. (21) and allowing granulopoiesis to proceed more rapidly? Another query is does inflammation stimulate neural influences on marrow circulation altering CO_2 and/or oxygen tension that may

have an effect on constitutive production of intramedullary molecular regulators? Evidence that O_2 tension regulates *in vitro* production of molecular regulators has been presented by Rich et al. (22,23). More *in vivo* work is required to elucidate mechanisms by which hemopoietic cell proliferation is in fact controlled.

There are three crucial events in regulating production of blood cells. First is control of self-renewal of the pluripotent stem cell; second, differentiation of stem cells into the diverse cell lineages; and third, amplification of the lineages to provide for the physiopathologic needs of the organism.

Figure 4 schematically illustrates the decisions that the stem cell is required to make: 1) self-renewal, 2) commitment. A large amount of work done by many individuals (24-26) leads one to believe that there is an age structure of stem cells based on the number of mitoses through which a stem cell has gone. To the far left there is a G_o population small in number with a very high mitotic capacity. As one moves to the right, the cells accumulate a greater mitotic history and to maintain the steady state the number produced by mitosis must be equaled by differentiation. Birth rate is equal to the number in DNA synthesis over the time for DNA synthesis divided by two, and the K_{out} or the flux-out is the birth rate divided by two. It has been shown that the spleen colony forming units which produce 14 day colonies have a very small fraction in DNA synthesis, whereas those that produce 7-day colonies have a high fraction in DNA synthesis (27).

There are five models for stem cell renewal and differentiation. They are:

1) Stochastic of Till et al. (28)

2) Hemopoietic inductive microenvironment of Trentin (29)

3) Stem cell competition of Van Zant and Goldwasser (30)

4) Erythropoiesis, an obligatory sltep of Johnson (31)

5) Progressive and stochastic restriction in differentiation potentials of stem cells of Korn et al. (32) and Ogawa et al. (33).

The hemopoietic-inductive microenvironment was based on different cytologic type colonies in different anatomic sites. This original attractive notion is less likely now since nearly all early spleen colonies are erythroid, probably formed by early BFU-E, have few if any CFU-S, and disappear as shown by Magli et al. (34), although, as we, in unpublished work, and Priestly and Wolf (35) have shown, the total number of colonies remains essentially constant from the 8th through 12th day after injection of bone marrow in the fatally irradiated mouse although colonies appear and disappear with time.

The model with progressive and stochastic restriction in the differentiation potentials of stem cells appears to fit more experimental evidence than the other models. The elegant research of Nakahata et al. (36) has provided much of the experimental basis to support this concept which succinctly states the following. When a stem cell differentiates, losing essentially all of its self-renewal capability, it is capable of expressing the entire genetic capability and thus can produce any of the cell lineages. This has been documented by <u>in vitro</u> culture of mixed colonies (36-39). This is then followed by progressive restriction of the genetic potentialities. The precursors successively divide, progressing from pluripotential to oligopotential to monopotent progenitors. Even though evidence strongly supports this notion of stochastic differentiation and progressive restriction of lineage potential, it does not appeal to me as a clinician since it cannot be manipulated to benefit the patient and IL-3 does put stem cells into cycle.

This stochastic model with progressive genetic restriction of lineage potential suggests that the only way by which blood cell production rate can can be altered is by increasing or decreasing the amplification of the monopotent precursor and its progeny. Earlier, it was shown that this is the case in both man and the dog in respect to accelerating granulocytopoiesis. The in vivo studies presented earlier showed that DNA synthesis rate was increased, production rate of the cells, and maturation was accelerated. Current in vitro culture research indicates that the stroma probably plays a central role in this modulation. The Dexter long-term bone marrow culture vividly demonstrated that there is no proliferation of the stem cells or production of monopotent progenitors and their progeny unless an adherent cell layer is present, consisting of fibroblasts, adiposites, macrophages, and epithelial-like cells. Thus, stem cell self-renewal, differentiation and amplification of monopotent progenitors require products of the adherent layer. Thus one assumes that the stromal cells modulate production rate in vivo.

Figure 5 is a schematic presentation of hemopoiesis. In the center there is a square representing the hemopoietic stem cell with a small central area for the pre-CFU-S or the G_0 stem cell. This is surrounded by pluripotent cells that proliferate and differentiate into the cell lines indicated. If differentiation is totally stochastic not controlled, on an average the same number of cells would be expected to differentiate into each of the cell lines depicted. Thus, since there is a marked difference in the number of red cells, granulocytes, megakaryocytes, B- and pre-T-cells that are being produced, the regulation of proliferation rate is most likely at the monopotent level. However, population size of the stem cell pool must be considered since Boggs and Boggs (40) showed that during regeneration the stem

pool repletes itself up until about 10% of its normal size before differentiation is allowed. The molecular control that shifts stem cell self-renewal from a probability of 1.0 during repletion to a probability of 0.5 in the steady state is not clear.

The Marrow Stroma:

The non-trilineal cells of the bone marrow are adventitial reticular, endothelial, fat, B and T-cells, and macrophages. All of these cells based on in vitro studies may play a role in regulating hematopoiesis. The macrophage may occupy a central role in regulation of hempoiesis. The first observation suggesting that it may have a central role was made by Bessis (41) who showed that erythropoietic islands have a macrophage in the center. He termed this the "nurse cell".

The macrophage is known to produce over 100 molecules from superoxide mass 32 to fibronectin mass 440,000 (42). This rich array of products, the abundance of macrophages in all tissues, and motility and responsivity suggests that they may modulate almost all aspects of cell proliferation, immunity, inflammation, phagocytosis, chemotaxis, and cell-killing. Metchnikoff never dreamed that the macrophage had any function other than an insatiable appetite for bacteria.

There have been many studies on in vitro culture of marrow fibroblasts (43,44). Apparently, the first demonstration that a stromal cell line (H-1) isolated from a murine long-term bone marrow produced colony stimulating activity is that of Harigaya et al. (45). Garnett et al. (46) demonstrated that the H-1 cell line is a derivative of an adventitial reticular cell. The properties of the H-1 line in stimulating and inhibiting hemopoiesis were shown by Garnett et al. (47).

Many investigators are studying the production of molecular regulators from diverse stromal cell lines that have been isolated from normal, diseased, and irradiated bone marrow. Figure 6 presents my present concept of the role of stromal cells in regulating hemopoiesis. In the center is shown the macrophage that produces GM-CSF at 2 to 3.5% oxygen concentration in tissue culture (22,23). It also produces IL-3 at a 5% concentration of oxygen and erythropoietin at 3.5%. Upon stimulation, it also produces IL-1 which in turn activates T-cells to produce GM-CSF (48) and interferons (49) which in turn impinge upon erythropoiesis and granulo-macrophage production. IL-1 also has been shown to stimulate the production of GM-CSF by fibroblast, adventitial reticular cells, and endothelium (48-51). Factors that regulate erythropoiesis, megakaryocytopoiesis and platelet production are omitted. Figure 6 illustrates the tangled network of factors that are produced in one cell and impinge upon other cells, either inhibiting or stimulating cell production rates. The preceding is based on in vitro cell culture techniques. Needless to say, all cells have the same genetic information except in those where there have been chromosomal deletions. In long-term tissue culture and in malignant tumors in vivo, there may be an inappropriate switching on of genes encoded for diverse hemopoietic regulators. In short-term cultures, the production of molecular regulators may have more relevance to in vivo control.

Radiation Effects on Bone Marrow

Macrophages, T-cells, B-cells are drastically reduced after irradiation leaving endothelial, adventitial reticular, fat, and smooth muscle cells, all of which are relatively radioresistant. If the radiosensitive cells occupy a central role in molecular regulation as in vitro culture would imply, then the

recovery of hemopoiesis requires that these cell populations be restored by spontaneous regeneration or be transplanted. Mulder and Visser (52) have shown that the transplantation of 1-10 separated stem cell results in a high 30-day survival of fatally irradiated mice, suggesting that accessory cells are not needed in the transplanted marrow. It is clear that only a very few pluripotent stem cells are required to repopulate the bone marrow of stem cells and the differentiated lineages. Under these circumstances, what are the factors that suppress differentiation, allowing the stem cell pool to replete itself to about 80% of normal with a higher fraction in DNA synthesis but never back to normal? An important question with clinical implications is when an animal is protected by a single or a few stem cells, will there be a reduction in life span due to early marrow failure?

Recombinant Hemopoietic Molecular Regulators

Recombinant DNA techniques have made available large amounts of diverse human and murine hemopoietic molecular regulators. There is great enthusiasm and optimism in respect to the probable clinical value of these agents in management of radiation and chemotherapeutically induced anemia, granulopenia, thrombopenia, anemia of renal failure, and other blood dyscrasias. Adamson (53) and Cotes et al. (54) have clearly shown that human recombinant erythropoietin is of great benefit in the treatment of anemia of renal failure. Motoyoshi et al. (55) showed that purified GM-CSF decreased the degree of granulopenia in patients undergoing chemotherapy for cancer. Animal studies (56-58) have shown that G-CSF produces a granulocytosis and accelerates regeneration of granulopoiesis in the granulopenic irradiated mouse. Neta et al. (59,60) have shown that IL-1 is a potent protector against radiation-induced mortality when administered 20 hours prior to fatal irradiation. Administration at 4 hours or 48 hours before irradiation is of

cancer. Animal studies by Metcalf, Broxmeyer, Moore and others have shown that GM-CSF produces a granulocytosis and accelerates regeneration of granulopoiesis in the granulopenic irradiated mouse. IL-1 is a potent protector against radiation-induced mortality when administered 20 hours prior to fatal irradiation. Administration at 4 hours or 48 hours before irradiation is of no benefit. Does the IL-1 shift stem cells into a more radioresistant phase or increase the tissue concentration of reduced glutathionine, superoxide dismutase or other enzymes that may scavenge free radicals, superoxide and peroxide reducing indirect effects of ionizing radiation?

If injured pluripotent stem cells would benefit by rest, one should be cautious in administering IL-3 or other agents that may force stem cells into cycle and perhaps fix a neoplastic transformation before DNA repair has taken place. If G-CSF, GM-CSF, and erythropoietin operate only on the monopotent progenitors, they ought to be beneficial by increasing amplification of the few monopotent progenitors present, producing more functional cells that are required. Perhaps exogenous IL-1 would be even more beneficial by inducing endothelial and fibroblastic cell production of GM-CSF in the marrow where it is needed. It is conceivable that there are situations in which the hemopoietic molecular regulators may be harmful. For example, in individuals who have received radiation and/or chemotherapy by agents known to be leukemogenic, will the administration of the molecular regulators force cells into mitosis before genetic repair takes place and fix these genetic lesions in DNA that may lead to an earlier onset of leukemia or a greater incidence? Another situation, fortunately, rarely seen today, is illustrated on the next slide.

benefit (59). Does the IL-1 shift stem cells into a more radioresistant phase or increase the tissue concentration of reduced glutathionine, superoxide dismutase or other enzymes that may scavenge free radicals, superoxide and peroxide reducing indirect effects of ionizing radiation?

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Summary and Conclusions

1. In vivo studies have demonstrated the sequence of events that take place when a granulocytosis is induced by sterile inflammation.

2. In vitro studies have identified a spectrum of factors that stimulate or inhibit hemopoiesis in tissue cultures. The genes for these factors have been cloned and recombinant molecules are available.

3. The in vivo role of these molecular regulators in modulating hemopoiesis and granulopoiesis in particular remains to be demonstrated. Which one(s) are resonsible for:

a) Inducing release of marrow stores of granulocytes,

b) Accelerating maturation of non-dividing granulocytic precursors

c) Increasing number of mitoses at the myelocyte level

d) Expanding GM-CFU-C population

e) Regulating stem cell input into differentiated lines by in vivo techniques for study of hemopoietic cell kinetics are available to answer these questions.

4. Hemopoietic molecular regulators will find important roles in the therapeutic armamentarium in combatting the harmful effects of pan and selective cytopenias. At the same time, exuberance over possession of these potent molecules should be tempered by the possibility of harmful effects since one does not know the physiological concentrations for controlling the steady state or accelerating hemopoiesis upon demand.

5. It is conceivable that a plethora of GM-CSF might induce a more rapid transition of a myelodysplastic state or chronic granulocytic leukemia into an acute leukemia.

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FIGURE CAPTIONS

Figure 1: Illustrates the effect of turpentine induced sterile inflammation upon granulocytopoiesis in bone marrow as function of time after inflammation and injection of ^{3}HTdR

a) Top panel - ratio myelocytic to erythrocytic mitoses in bone marrow
b) Left panel 1) fraction of myelocytes (M3+M4) labeled by ^{3}HTdR
2) fraction of metamyelocytes (M5) labeled by ^{3}HTdR
3) Fraction of band and segmented granulocytes (M6+M7)
c) Right panel mean silver grain count overlying the cells described for left panel

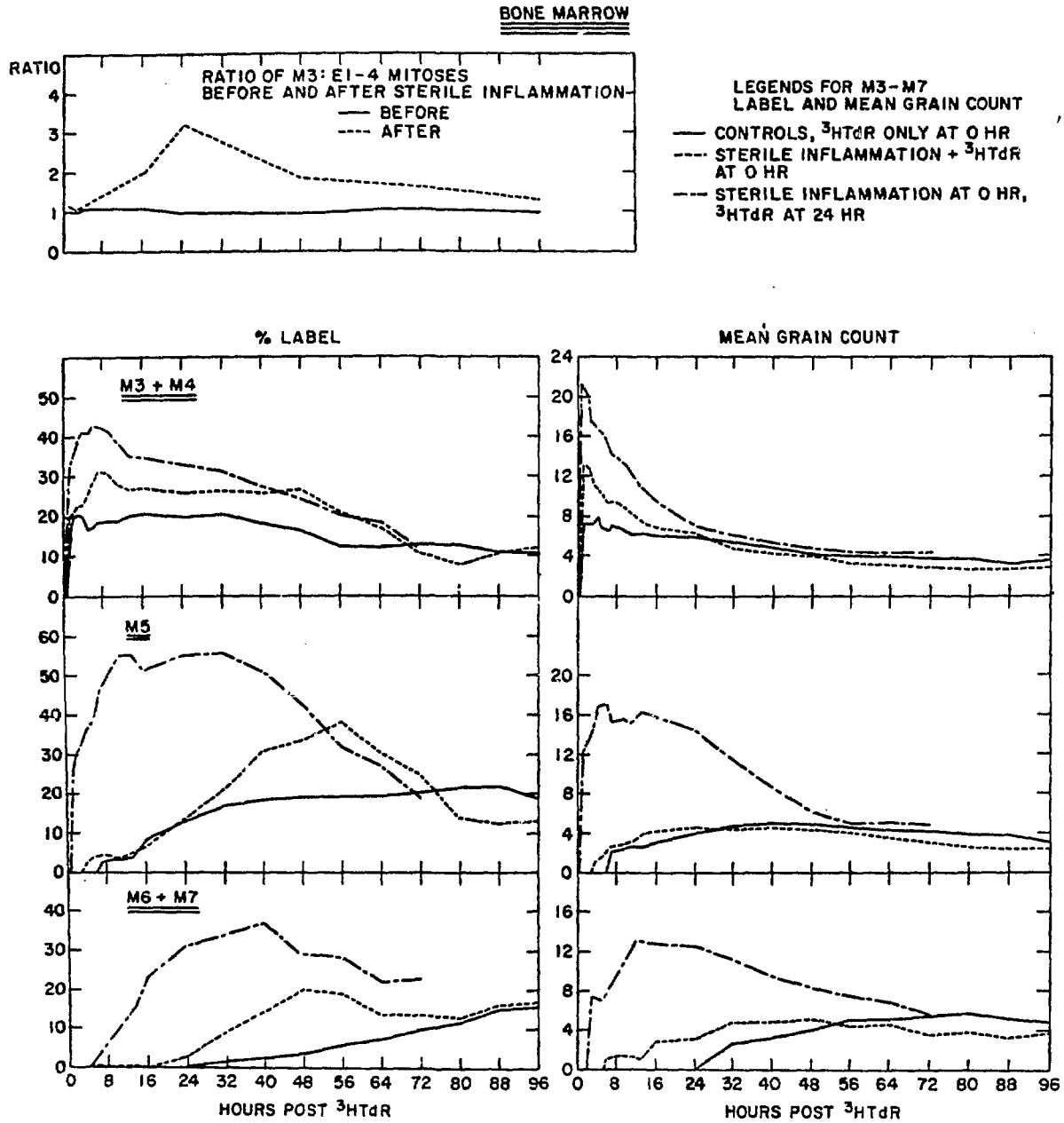
Figure 2: Illustrates the time from labeling myelocytes in the bone marrow until the appearance of labeled granulocytes in the peripheral blood (emergence time). In this case the mean grain count overlying the blood granulocytes is shown.

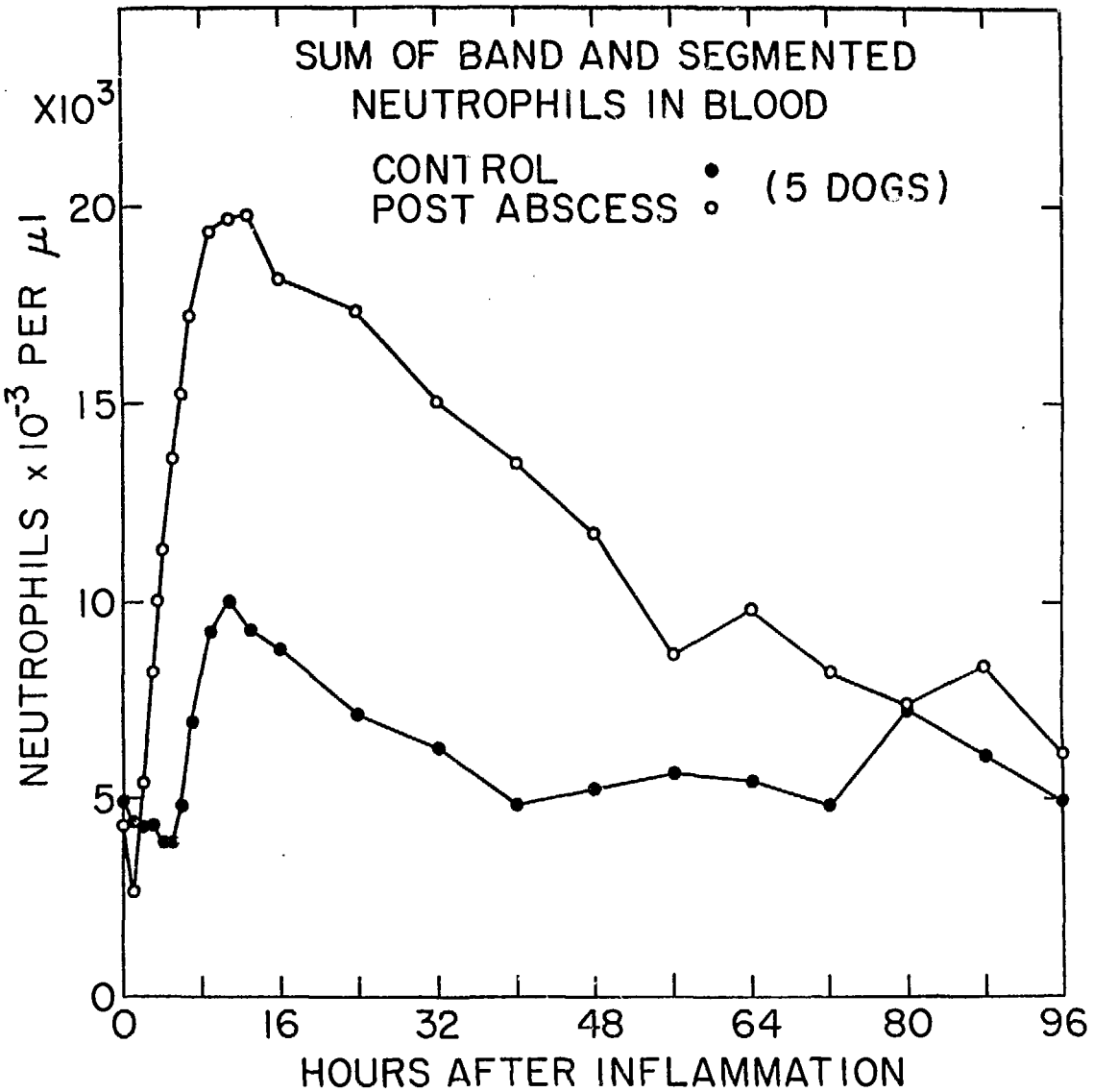
Figure 3: Peripheral granulocytosis produced by repeated bone marrow aspirations and the greater granulocytosis produced by intrauterine sterile turpentine inflammation in the dog. In both cases note the initial granulopenia induced by loss of circulating granulocytes into tissue.

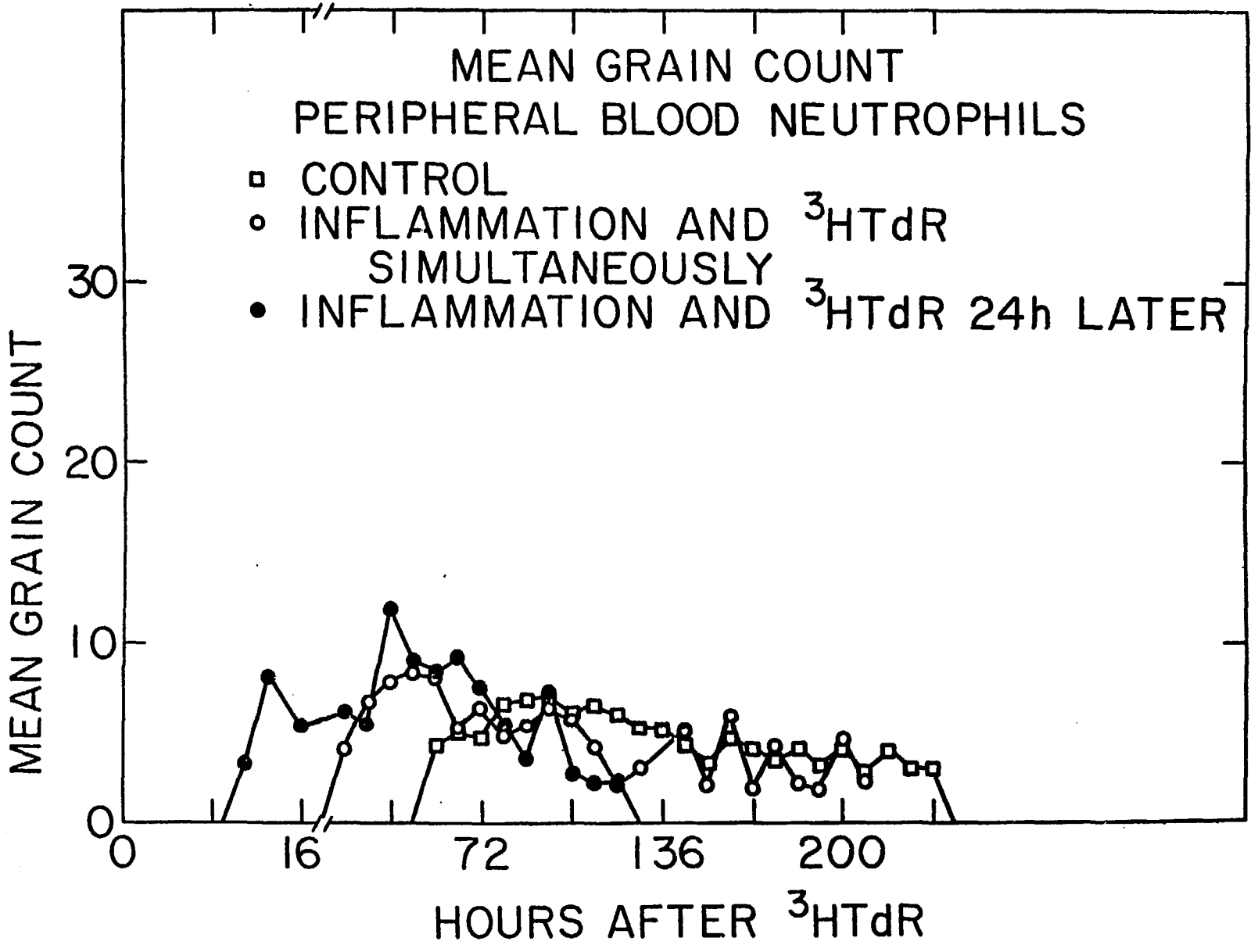
Figure 4: Schematic presentation of the stem cell pool showing decisions, age structure, birth and differentiation rates in the steady state.

Figure 5: Schematic presentation hemopoiesis showing the differentiation from the dividing stem cell pool into 6 cell lineages. Eosinophils and basophils are omitted.

Figure 6: Schematic presentation of the relationships among stromal cells, macrophages, production of hemopoietic molecular regulators suggesting a key role for the macrophage its production of IL-1 that starts a cascade of production of other regulators by other cells of the stroma. This scheme is synthesized on basis of *in vitro* culture studies and not yet shown to be operative *in vivo*.


BONE MARROW
RATIO
RATIO OF M3: E1-4 MITOSES
BEFORE AND AFTER STERILE INFLAMMATION
BEFORE
AFTER
LEGENDS FOR M3-M7
LABEL AND MEAN GRAIN COUNT
CONTROLS, 3HTdR ONLY AT 0 HR
STERILE INFLAMMATION + 3HTdR AT 0 HR
STERILE INFLAMMATION AT 0 HR, 3HTdR AT 24 HR
% LABEL
MEAN GRAIN COUNT
M3 + M4
M5
M6 + M7
HOURS POST 3HTdR
HOURS POST 3HTdR


SUM OF BAND AND SEGMENTED
NEUTROPHILS IN BLOOD
CONTROL •
POST ABSCESS ○
(5 DOGS)
X10³
NEUTROPHILS x 10⁻³ PER μl
20
15
10
5
0
0
16
32
48
64
80
96
HOURS AFTER INFLAMMATION

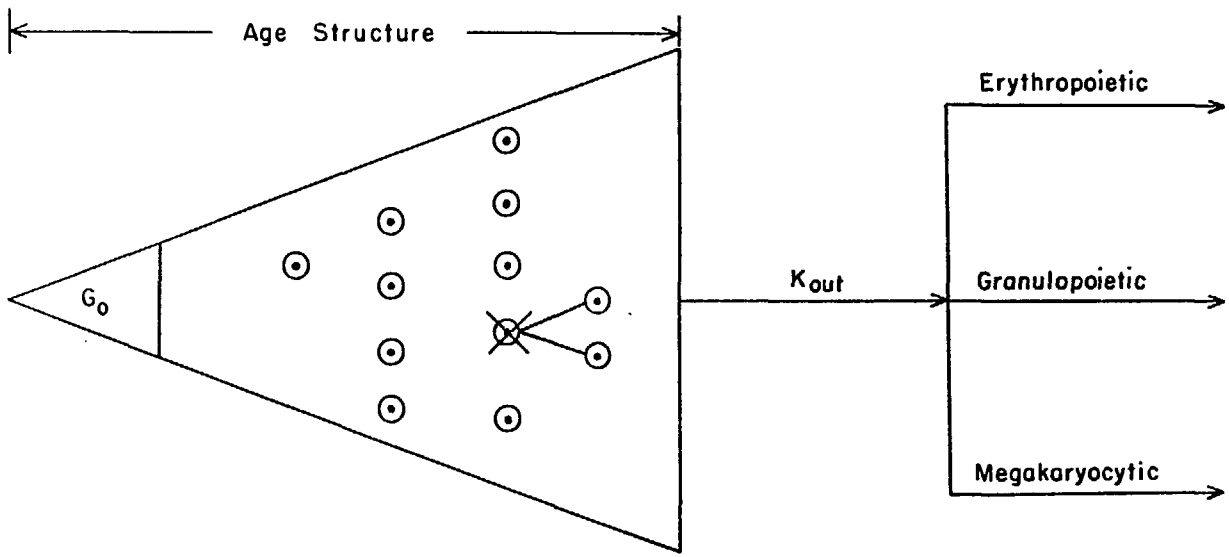

MEAN GRAIN COUNT
PERIPHERAL BLOOD NEUTROPHILS
CONTROL
INFLAMMATION AND 3HTdR SIMULTANEOUSLY
INFLAMMATION AND 3HTdR 24h LATER
MEAN GRAIN COUNT
0
10
20
30
0
16
72
136
200
HOURS AFTER 3HTdR

HEMOPOIETIC STEM CELL

Decisions

1. Self — renewal
2. Commitment

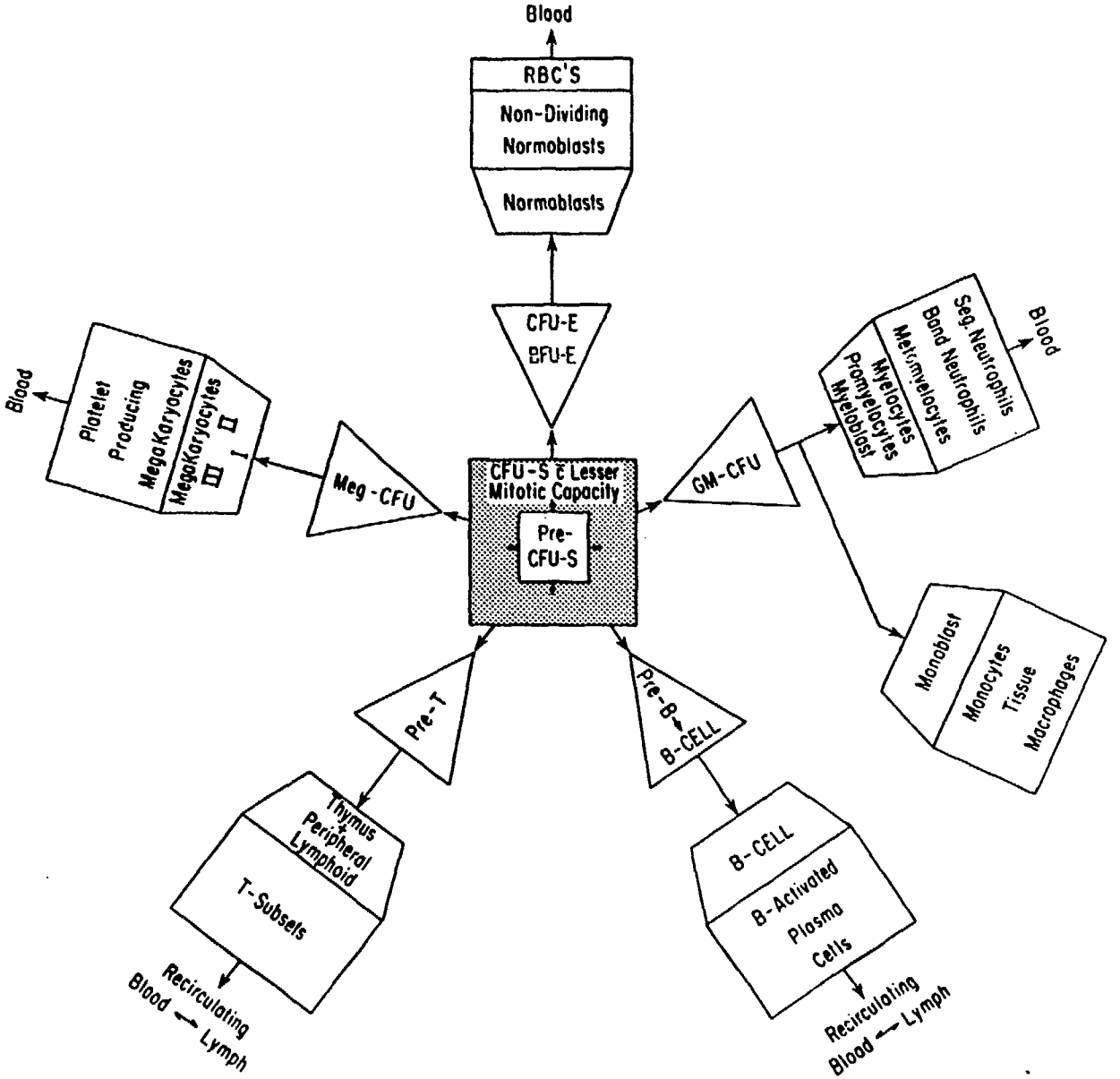
Pathways

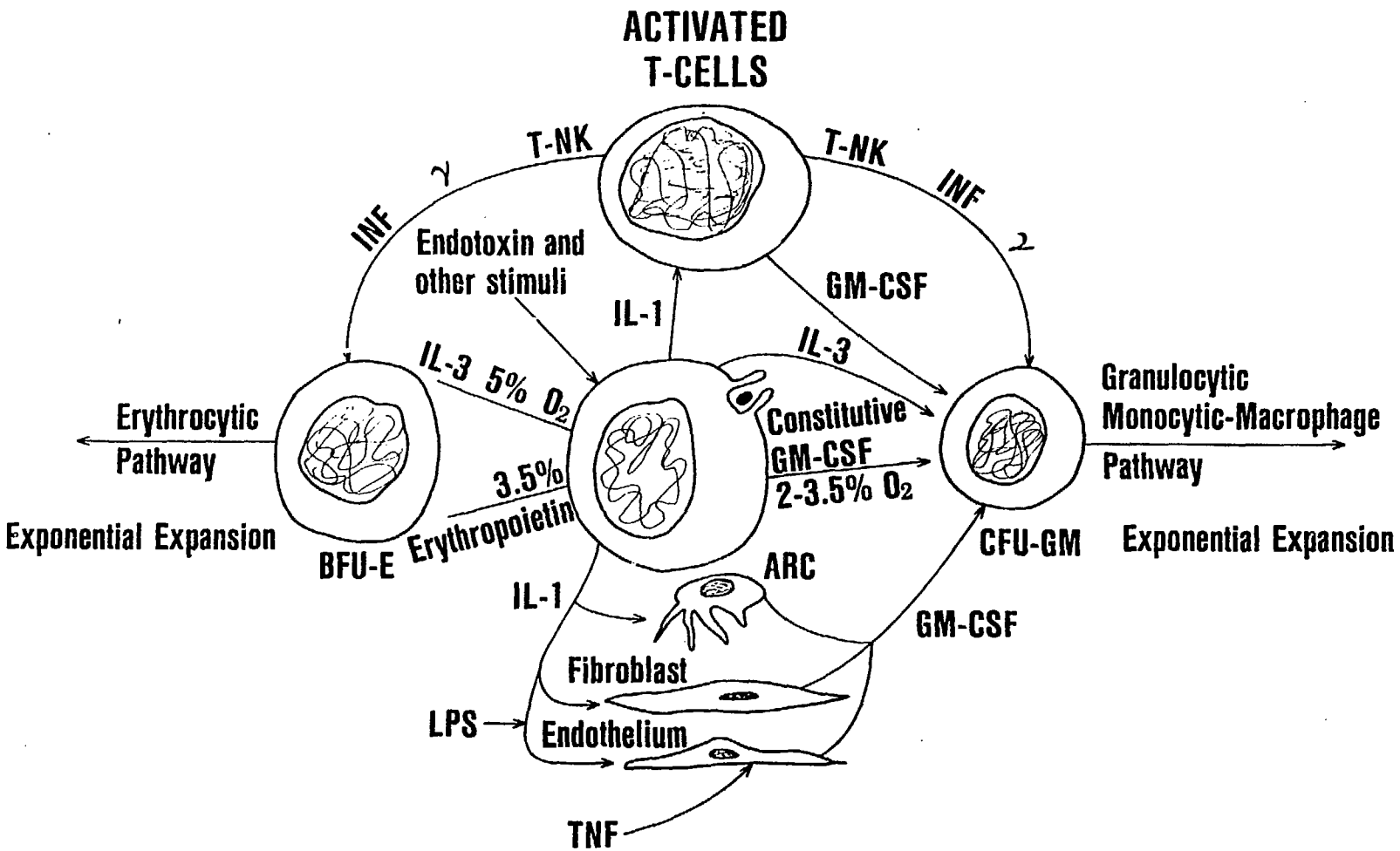


$$K_B = \frac{N_S}{T_S} \times 2$$

$$K_{out} = \frac{K_B}{2}$$

Steady State


Blood
RBC'S
Non-Dividing Normoblasts
Normoblasts
CFU-E
BFU-E
CFU-S & Lesser Mitotic Capacity
Pre-CFU-S
Blood
Platelet Producing MegaKaryocytes
MegaKaryocytes III II I
Meg-CFU
GM-CFU
Blood
Seg. Neutrophils
Band Neutrophils
Metamyelocytes
Myelocytes
Promyelocytes
Myeloblast
Monoblast
Monocytes
Tissue Macrophages
Pre-T
Pre-B
B-CELL
Thymus + Peripheral Lymphoid
T-Subsets
Recirculating Blood ⟷ Lymph
B-CELL
B-Activated Plasma Cells
Recirculating Blood ⟷ Lymph


ACTIVATED
T-CELLS
T-NK
T-NK
INF γ
INF γ
Endotoxin and
other stimuli
IL-1
GM-CSF
IL-3
IL-3 5% O2
Erythrocytic
Pathway
Exponential Expansion
BFU-E
3.5%
Erythropoietin
Constitutive
GM-CSF
2-3.5% O2
Granulocytic
Monocytic-Macrophage
Pathway
CFU-GM
Exponential Expansion
ARC
IL-1
GM-CSF
Fibroblast
LPS
Endothelium
TNF