1971

Granulocyte kinetics in cats

Keith William Prasse
Iowa State University

Granulocyte kinetics in cats

by

Keith William Prasse

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1971

TABLE OF CONTENTS

INTRODUCTION

Neutrophilic granulocytes comprise one of the cell systems in the body which undergo continual renewal. These granulocytes are produced by the bone marrow from which they are released into peripheral blood circulation. Granulocytes are also constantly leaving the circulation by diapedesis through small blood vessel walls into tissue in which they die. Sabin et al. (1925) stated, "The fact that there is a fairly constant number of white cells in a cubic millimeter of blood, and that there are variations of this number, characteristic of different types of disease, has become common medical knowledge since the methods for counting blood cells were introduced. There is indeed nothing in the entire animal economy more amazing than the fact that from hour to hour and from day to day, the number of white cells remains so remarkably constant. It is wholly obvious that the mechanism involved in the maintenance of this remarkable condition must be one of great importance."

It has long been known that neutrophilic granulocytes, which enter injured tissues in massive numbers, comprise one of the various natural body defense mechanisms. The kinetic mechanisms whereby circulating granulocyte numbers and concentration are maintained in health and are altered in disease have been a subject of research for many years. It has been shown in man and other animals that granulocytes are distributed in various pools throughout the body (Athens et al., 1961b,

Gaylor et al., 1969; Raab et al., 1964). Total blood granulocyte pool (TBGP) is comprised of two subpools, the circulating granulocyte pool (CGP) and the marginal granulocyte pool (MGP); the latter includes those neutrophils which are sequestered in capillary beds throughout the body. The CGP contains those neutrophils which are circulating, and which are counted in routine total leukocyte counts on peripheral blood. The two pools, CGP and MGP, vary in relative size and proportion depending on the physiologic or pathologic state of the body. The movement of neutrophilic granulocytes from bone marrow into peripheral blood and finally into tissue is normally under steady-state control or balance. Bone marrow production and maturation of neutrophilic granulocytes, i.e., granulopoiesis, are also in steady state balance under normal conditions. Therefore, the changes in granulocyte numbers observed in routine hematologic examination in disease, i.e., changes in CGP, are brought about by alteration of neutrophil kinetics. That is, change in size or proportion in CGP and MGP, increase or decrease in the rate of movement into tissue and increase or decrease in the rate of granulopoiesis may all contribute to variations which the clinical hematologist seeks to interpret.

The objectives of this research were to determine parameters of neutrophilic granulocyte kinetics in cats. TBGP, CGP, MGP, half-life of neutrophils in peripheral blood and granulocyte turnover rate, i.e., the number of neutrophils

replaced in peripheral blood per unit time, were determined with the first of two experimental approaches. These parameters were determined by *in vitro* labeling of granulocytes with tritiated diisopropylfluorophosphate, infusion of the labeled cells into cats and determination of specific activity of cells reisolated from the cats at selected times during a 24-hour period. These parameters were determined in hematologically normal cats, in cats with experimentally induced blood dyscrasias and in cats with naturally occurring blood dyscrasias.

Neutrophil maturation time and mean transit time of each morphologic compartment of granulopoiesis were estimated with the second experimental approach. These parameters were determined by *in vivo* pulse labeling of granulocyte precursors with tritiated thymidine and subsequent serial bone marrow and blood sampling for 5 days. Microautoradiographs were prepared from the bone marrow and blood smears. Data relating the progress of the label through the catenated compartments of granulocyte precursors in the bone marrow and the appearance of labeled neutrophils in the blood, versus time, were compared with that obtained with a hypothetical model of granulopoiesis. The comparison, with the aid of an analog computer, led to an estimation of the kinetic parameters of granulopoiesis. These parameters were estimated in hematologically normal cats and in cats with an experimentally induced and naturally occurring blood dyscrasias.

Cats are being used to great advantage in research on leukemia (Jarrett et al., 1969; Owen, 1969) which demonstrates their potential as models for human blood dyscrasias. Furthermore, the blood dyscrasia in feline infectious enteritis, characterized by neutropenia, requires clarification as exemplified by the finding that neutropenia does not occur in germ-free cats given the panleukopenia virus (Johnson et al., 1967). The parameters of neutrophil kinetics in cats were determined in this research. The basic knowledge to be gained and technological approaches developed in studying neutrophil kinetics in cats should lead to better understanding of underlying pathogenesis of blood dyscrasias in all species.

REVIEW OF LITERATURE

The neutrophilic granulocyte is known to play an important role in the natural defenses of the body. The dynamic aspects of the life span and function of this cell have stimulated much interest and research in the last 50 or 60 years. Among the areas of study have been the production of granulocytes in the bone marrow, the characteristics of the granulocyte sojourn in peripheral blood, and the nature of the function of granulocytes in injured tissue. The kinetic aspects of the tissue phase of the granulocyte life span have not been clearly defined except for the suggestion that, once having left the circulation and entered either normal or injured tissue, granulocytes do not return to the blood (Bierman et al., 1952; Moxley et al., 1965; Walker et al., 1964; Mauer et al., 1959). Neutrophil function and other aspects of the tissue phase will not be considered further. The kinetic parameters from bone marrow production to disappearance from circulating blood have been studied in detail and will be covered in this review.

Kinetics of Granulopoiesis

Cell population kinetics

The term population is used to refer to any group of cells of one or more types which can be defined as to its extent in space and time (Cleaver, 1967). The tissues of the

body are composed of many populations of cells with differing life spans and capacities for repopulation. Leblond et al. (1959) attempted a simple classification of the tissues of the rat on the basis of two parameters: the extent to which cells of a tissue incorporate tritiated thymidine (^{3}H-Tdr) and the rate at which labeled cells are lost from tissues. One category of cell populations which was distinguished from their studies was called a renewing cell population. They were characterized by maintenance of steady size in the adult animal whereby the rate of cell gain equaled the rate of cell loss with time. This, then, is referred to as the steady state.

The means whereby precursor cell generations maintain the steady state have been the subject of extensive research. The average duration from completion of one mitosis to completion of the following mitosis is referred to as cell cycle or generation time (Killmann et al., 1963). Cleaver (1967) has reviewed the research which has led to current knowledge of the stages through which cells progress during proliferation. The cell cycle has been represented as having four distinct stages, G_1, S, G_2, and M, which occur in that order. M and S refer to mitosis and deoxyribonucleic acid (DNA) synthesis respectively, whereas G_1 consists of a pre-DNA synthesis stage and G_2 consists of a pre-mitotic stage. Following a cell cycle, daughter cells may enter the G_1 stage for a second cycle or mature to a nonproliferative cell type.

Duration of the cell cycle of a population in tissue culture may conveniently be studied by exposing the cells to ^{3}H-Tdr. If a brief labeling period is used only the cells which are in the S stage incorporate ^{3}H-Tdr and these are clearly distinguished as labeled cells in autoradiographs. Analysis of data obtained with these techniques has resulted in estimation of the duration of the cell cycle and its stages. Cells from many tissues have been studied and in general, there has been close similarity in the durations of the S, G_2 and M stages, in contrast to wide variation in G_1 durations (Cleaver, 1967). Not only does the duration of G_1 vary considerably from one cell type to another, but within one particular system it is the stage in which most of the variation between individual cells occurs (Sisken and Moraska, 1965).

The duration of the S stage, the most frequently measured cell cycle stage, has been estimated for granulocyte precursors in some species. This time in normal man was reported to be about 13 to 14 hours (Stryckmans et al., 1966) and similar values were found in human chronic myeloid leukemia cells (Vincent et al., 1969). Maloney et al. (1962) reported the S stage for canine myelocytes to be about 7 hours. However, Lala[1], who estimated the average duration of each stage of the generative cycle in canine granulocyte precursors, reported that G_1 = 2.5 hours, S = 5.5 hours, G_2 = 1 hour, and M = 1 hour

---

[1]The original publication not found.

with a cell cycle time of 10 hours (Cleaver, 1967). In like manner Lobue et al. (1970) reported durations of myelocyte cell cycle stages in rats of various ages; they were: S = 6.4, M = 0.4 and total cycle time = 10.6 hours in 2 to 3 year olds.

^{3}H-Tdr, which was introduced by Verly et al. (1958) and Taylor et al. (1957) as a specific label for DNA, has provided a means not only for the detailed analysis of cell cycle durations, but for kinetic studies of cell populations in animal tissues as well. With this aid it has been possible to label cells that are synthesizing DNA which permits study of their division and their subsequent growth, maturation, function and death with great precision. These phases of neutrophilic granulocyte life have been studied in detail.

The bone marrow contains a number of distinct renewal systems, each of which is concerned with the production of one of the cell types found in peripheral blood. Neutrophilic granulopoiesis comprises one of these systems in which all stages of growth and maturation, as well as those of the other marrow cell renewal systems, are found side by side in the bone marrow. Consequently cell structure is the main indication of the progressive maturation of cells through the various compartments of the cell renewal system (Cleaver, 1967).

The granulocyte renewal system

The morphologic characteristics of each compartment of granulopoiesis, i.e., myeloblast, progranulocyte, neutrophilic

myelocyte, neutrophilic metamyelocyte, band neutrophil and segmented neutrophil are well described (Schalm, 1965; Wintrobe, 1961). Each cytologically defined compartment in the proliferating pool of cells, i.e., myeloblast, progranulocyte, and myelocyte, contains one or more cell generations. That is, it is possible for cells to complete one or more cell cycles before resultant daughter cells are recognizable as members of the following compartment (Killmann et al., 1963). After cells proceed through several generative cycles, cessation of division eventually occurs and the cells enter the nonproliferating pool, i.e., metamyelocyte, band and segmented neutrophil. While the ability to divide terminates, maturation continues throughout the remaining morphologic compartments of granulopoiesis.

Extensive research has been conducted on the kinetics of the granulocyte renewal system in order to gain insight into the mechanisms whereby neutrophilia, neutropenia, myeloid leukemia and other dyscrasias associated with the system may be better understood. Therefore recent studies on the kinetic aspects of the proliferating pool and the nonproliferating pool of granulocyte precursors will be reviewed in greater depth.

The proliferating compartments of granulopoiesis

A generally accepted view is that a stem cell pool exists, the cytologic identity of which is still obscure. In some

manner, agents act on cells in this pool to induce the cells into various differential pathways, one of which is neutrophilic granulopoiesis (Cronkite, 1969). The stem cell pool is believed to be a self-perpetuating compartment (Cronkite and Vincent, 1970; Osgood, 1970; Athens, 1969), i.e., division of a stem cell results in one stem cell and one differentiated daughter cell. Osgood (1970) has calculated that a stem cell divides once every 96 hours (human) while others have shown that the fraction of stem cells preparing to divide at any given time, i.e., the fraction in DNA synthesis, is small under steady state conditions (Bruce and Meeker, 1965; Valeriote et al., 1966). Yet, as stated by Cronkite and Vincent (1970), any proliferating system clearly requires a capacity for self-maintenance as well as for production.

Myeloblasts and progranulocytes are generally believed to be the sequential intermediates between the stem cell and the neutrophilic myelocyte (Cronkite and Vincent, 1970; Cronkite and Fliedner, 1964). Cells in each of these compartments are believed to be morphologically identifiable through one cell cycle with resultant daughter cells being immediately, or nearly so, identified as members of sequential compartments (Killmann et al., 1963). Mean compartment transit time, which has been defined as the average time from entrance of a cell into a cytologic compartment until it or its progeny leaves the compartment (Killmann et al., 1963) has been estimated to be 24 hours for myeloblasts and about 47 hours for progranulo-

cytes in man (Cronkite and Fliedner, 1964). Canine myeloblast and progranulocyte transit times have been estimated to be 10 and 8 hours respectively (Patt and Maloney, 1964).

The majority of cells in the proliferating pool of granulocyte precursors is in the neutrophilic myelocyte compartment. Analysis of ^{3}H-Tdr autoradiography data and blood granulocyte radioactivity data following in vivo labeling with radiolabeled diisopropylfluorophosphate has led to the current concept that at least 3 sequential divisions occur in the myelocyte compartment with an approximate compartment transit time of 108 hours in man (Cronkite and Vincent, 1970; Athens, 1969). As cells proceed through the myelocyte compartment, cytoplasmic granules become more prominent, nuclei become smaller and more mature and ribonucleic acid synthesis declines. Cessation of division apparently occurs as the result of a poorly understood cytoplasmic-nuclear interaction and the cells enter the nonproliferating pool. It is believed, therefore, that maturation places a natural limitation on the transit time through the myelocyte compartment (Cronkite and Vincent, 1970; Cronkite and Fliedner, 1964).

Increased granulocyte production, although partially explainable by increased stem cell input, apparently must be accommodated in large part by the myelocyte compartment. Cronkite and Vincent (1970) have proposed that increased production could be explained by shortening the G_1 stage of the myelocyte cell cycle, which they have estimated to be 37 hours in normal

man, thereby allowing one, or even two additional divisions within the myelocyte compartment before cytoplasmic maturation products inhibit further division. They stated that two extra divisions would only require G_1 to be shortened to 9 hours and production from the myelocyte compartment could be expanded fourfold without an increased stem cell input.

Patt and Maloney (1964) observed an overproduction of myelocytes in normal dogs; the observation was based on the ratio of metamyelocyte influx to myelocyte efflux from ^{3}H-Tdr autoradiography data. This observation led them to suggest that granulocyte production may be "death-controlled", i.e., adjusted by variations in mortality of newly formed myelocytes. They concluded that an appropriate adjustment of the balance of "effective" and "ineffective" production of myelocytes could increase the net production of granulocytes by more than fivefold.

Cartwright et al. (1964) and Boggs (1967) postulated that myelocytes are to a great extent self-perpetuating, which assigns to myelocytes the role of functioning as a "semi-stem cell" compartment. They suggested that contribution from myeloblasts and progranulocytes would occur only if increased cell loss or increased peripheral demand for granulocytes occurred; input from these compartments would be insignificant in the normal steady state.

The nonproliferating compartments of granulopoiesis

Fliedner et al. (1964a) have studied the appearance of labeled granulocytes in the blood following in vivo labeling of granulocyte precursors with ^{3}H-Tdr in man. The average duration from the terminal myelocyte S stage, in which the earliest appearing granulocytes could have been labeled, to the initial appearance of labeled granulocytes in the blood was termed emergence time. They found it was between 96 and 144 hours in normal individuals, but was shortened to 48 hours in individuals with severe infection. Similar studies in normal dogs have shown emergence time to be between 72 and 96 hours (Patt and Maloney, 1959b) and in a later trial a mean time of 102 hours (Maloney and Patt, 1968).

The total transit time through the nonproliferating pool estimated by Cronkite and Vincent (1970) ranged from 130 hours with the use of the data of Donohue et al. (1958) to 152 hours. These estimates were similar to the emergence time determinations by Fliedner et al. (1964a) in normal individuals mentioned previously. Cronkite and Vincent (1970) proposed that the 48 hour emergence time Fliedner et al. (1964a) had observed in subjects with infection may represent a minimum obligatory transit time through the nonproliferating compartments. They concluded that this may represent the time necessary for maturation to the segmented granulocyte and thereby dictates the maximum flux of cells which could be sustained through the nonproliferating compartments. Furthermore, any demand for

a flux greater than this would necessarily be met by an expansion of the metamyelocyte and band compartments.

Patt and Maloney (1964) estimated compartment transit times for metamyelocytes and bands in dogs to be 20 and 26 hours respectively. The mean transit time for segmented granulocytes in the bone marrow of dogs was 50 hours (Maloney and Patt, 1968).

Evidence has been presented that there exists a post-mitotic granulocyte reserve of considerable magnitude in the bone marrow (Walker et al., 1962; Walker et al., 1961; Craddock et al., 1956; Osgood, 1954). Cronkite and Vincent (1970) have estimated that this reserve in man varies between 3.7 and 7.2 times the total blood granulocyte pool.

Little information is available about the chronology of events within the granulocyte reserve pool due to the constant problem of dilution of bone marrow samples with peripheral blood. However, Maloney and Patt (1968) observed, following in vivo labeling with ^{3}H-Tdr, that granulocytes bearing the heaviest label appeared first in the peripheral blood and were followed at successively later times by cells bearing progressively less label. They surmised that heavily labeled cells originated from terminal myelocyte divisions whereas lightly labeled cells originated from earlier granulopoietic generations; consequently, the label was diluted by subsequent mitoses. They concluded that granulocytes follow "first in - first out" order through the marrow reserve in the normal

steady state. Furthermore, the appearance of the labeled cells in the peripheral blood was observed to have a relationship linear with time which clearly indicated that release from the bone marrow is not a simple random process.

Blood Granulocyte Kinetics

The compartmentalization of blood granulocytes

Vejlens (1938) observed the margination of granulocytes in areas of the circulation where blood flow is relatively slow. This implied that when the axial stream of a large vessel is sampled only granulocytes in the freely flowing circulation are measured.

The advent of granulocyte-specific labeling with radioactive diisopropylfluorophosphate (DFP) (Athens et al., 1959a; Athens et al., 1959b) clearly established the compartmentalization of granulocytes in peripheral blood. When granulocytes labeled in vitro with $DF^{32}P$ were autotransfused, only about 50% of the labeled granulocytes could be accounted for in the circulation at completion of the infusion (Mauer et al., 1959; Athens et al., 1961b). This led to the suggestion that the pool in which the labeled cells were distributed was larger than the product of blood volume and the circulating granulocyte count. Athens and co-workers concluded that the total blood granulocyte pool (TBGP) consisted of two subpools, a circulating granulocyte pool (CGP) and an ill-defined marginal granulocyte pool (MGP), consisting of cells adherent to the

walls of blood vessels in various organs throughout the body.

Granulocyte turnover in the blood

The first attempt to quantify neutrophilic granulocyte turnover in blood was by Sabin et al. (1925). From leukocyte counts, differential leukocyte counts and supravital differential counts they surmised that a significant number of circulating neutrophils was continually dying in the circulation and being replaced by young, viable cells from storage reserves. They concluded that about one-fifth of the neutrophils die within the circulation in 24 hours.

Further elucidation of the life span of neutrophils was attempted with a variety of experimental approaches. In vitro culture of bone marrow cells (Osgood, 1937), cross circulation between normal and neutropenic animals (Lawrence et al., 1945; Van Dyke and Huff, 1951), transfusion of atabrine-tagged cells (White, 1954), and leukopheresis (Craddock et al., 1955; Patt et al., 1957b) are examples of techniques which have been used to characterize the sojourn of neutrophils in peripheral blood. The dissimilarity of techniques and species used in these studies led to reported values for neutrophil life span in circulating blood which varied from minutes (Van Dyke and Huff, 1951) to hours (Lawrence et al., 1945) and even to days (Kline and Cliffton, 1952).

Granulocyte-specific radio-labeling with DFP has provided a means whereby the average half-life of blood granulocytes

can be determined with accuracy. $DF^{32}P$-labeled cells disappeared from the circulation at an exponential rate which indicated that granulocytes leave the peripheral blood in random fashion (Mauer et al., 1960). The half-life ($T_{\frac{1}{2}}$) of labeled granulocytes in the blood, calculated from the slope of the exponential disappearance curve, was 6.6 hours. The suggestion that granulocytes are lost randomly from the blood has been substantiated experimentally (Athens et al., 1961b; Alexanian and Donohue, 1965; Galbraith et al., 1965).

In addition to this random loss, a small loss of granulocytes due to senescence, as presumed by Sabin et al. (1925) in their attempt to quantify neutrophil turnover, has been substantiated. Osgood (1937) demonstrated the fact that neutrophilic granulocytes are able to undergo an aging process and become pyknotic. Fliedner et al. (1964b), who observed the appearance of labeled cells in the blood following in vivo labeling of granulocyte precursors with ^{3}H-Tdr, found from 1.5 to 2.5 pyknotic neutrophils per 1000 in their preparations. Regardless of the emergence time of labeled granulocytes, labeled pyknotic cells always appeared 24 to 36 hours later. Furthermore, they observed labeled granulocytes in an extravascular site, the sputum, simultaneous with the appearance time in blood. They concluded that granulocytes are lost from the blood by at least two routes: (1) random emigration from the blood and (2) removal of pyknotic cells, presumably by the reticuloendothelial system.

The evidence appears to establish, therefore, that the transit of granulocytes through the blood is not an orderly flow according to age. However, the average half-life of blood granulocytes is about 6 to 7 hours. Cartwright et al. (1964) stated that "the short span of time which the granulocyte spends in the blood should not be confused with survival time or life span of the cell. . . . The granulocyte, which carries out most of its functions extravascularly is only in transit in the blood on its way to tissues. The short blood transit time is only a fraction of the life span of the cell."

Application of Blood Granulokinetic Studies

The experimental model used and normal values

Knowledge gained from blood granulokinetic studies by using the DFP labeling techniques has been extensively applied to interpret abnormalities in blood granulocyte profiles. In order to facilitate clarity in discussion of this knowledge, the experimental model used will be briefly outlined.

Radio-labeled DFP, which is added to a volume of blood, selectively labels neutrophils by binding irreversibly to an active serine group of a cytoplasmic enzyme (Athens et al., 1959b). The specific activity (SAInfG) and the number (NoInfG) of labeled granulocytes are determined. The blood containing the labeled granulocytes is then infused into the experimental subject; the time of infusion is referred to as zero time (t_o). Samples of blood are taken periodically during the following

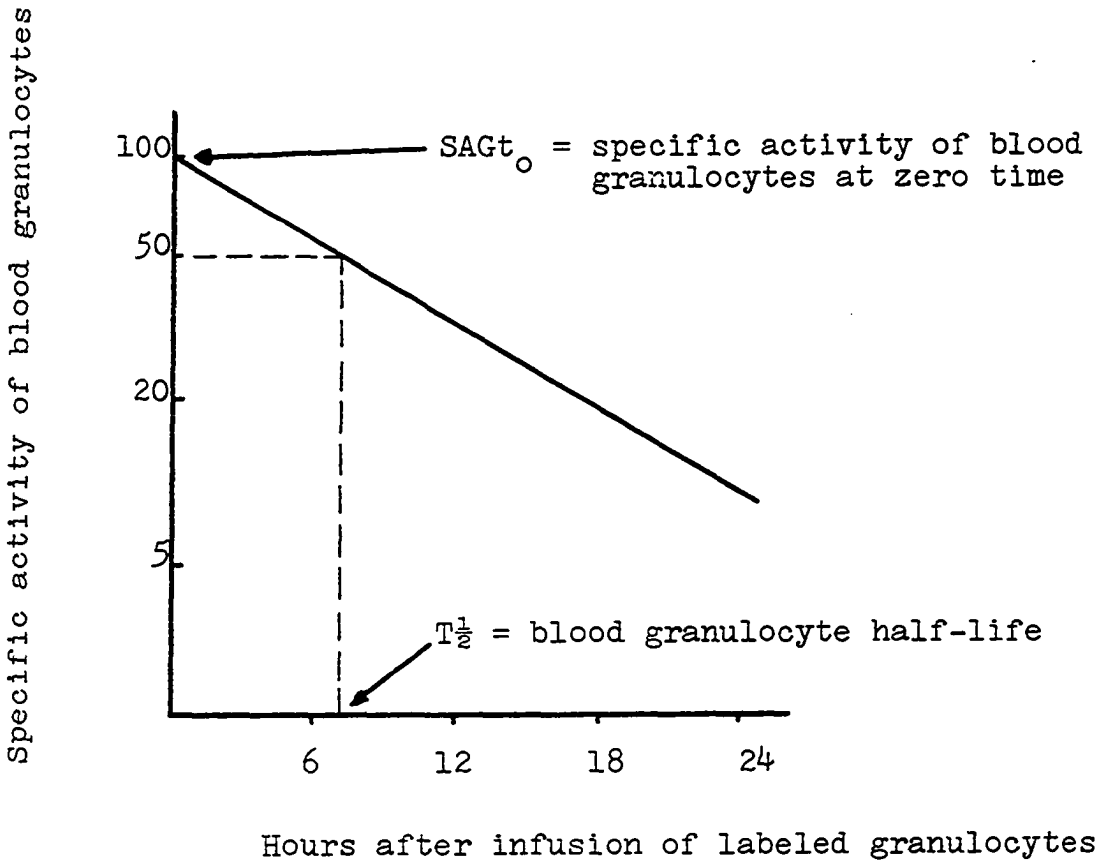


Figure 1. A model of the blood granulocyte disappearance curve obtained following infusion of radio-labeled DFP-tagged granulocytes

24 hours. Granulocyte specific activity, which is determined at each sample time, has been found to decrease exponentially; therefore, these values are plotted against time on semilogarithmic paper. Extrapolation of the plot back to the ordinate gives the specific activity of granulocytes at zero time ($SAGt_o$). The granulocyte disappearance curve is represented by this plot (Figure 1). The steepness of the slope of this curve is an indication of the rapidity with which labeled

granulocytes disappear from the blood. The formulae for pool sizes are:

$$TBGP = \frac{SAInfG \times NoInfG}{SAGt_o};$$

CGP = circulating granulocyte count at t_o x blood volume;

MGP = TBGP - CGP.

The $T_{\frac{1}{2}}$ of blood granulocytes, determined from the granulocyte disappearance curve, is that time at which one half of the $SAGt_o$ remains (Figure 1). The formula for granulocyte turnover rate (GTR) is:

$$GTR = TBGP \times \frac{0.693}{T_{\frac{1}{2}}}$$ (Cartwright et al., 1964).

Normal values of blood granulocyte kinetic parameters in man and dog reported by Athens and coworkers are summarized in Table 1.

Table 1. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), half life of blood granulocytes ($T_{\frac{1}{2}}$) and granulocyte turnover rate (GTR) in normal man and dog

	Man (Mean values)[a]	Dog (Mean values)[b]
TBGP x 10^7/Kg	70	102
CGP x 10^7/Kg	31	54
MGP x 10^7/Kg	39	48
$T_{\frac{1}{2}}$ (hours)	6.7	5.6
GTR x 10^7/Kg/Day	163	305

[a]Athens et al., 1959b.

[b]Raab et al., 1964.

Blood granulocyte kinetic parameters in altered states

Physiologic leukocytosis has been the subject of speculation for many years. Exercise, e.g., is a known cause of neutrophilia. Athens et al. (1961a, 1961b) studied the effects of exercise and epinephrine on granulocyte kinetics in subjects in which normal parameters had been determined previously. They found the expected increase in CGP to occur at the expense of the MGP while TBGP remained unchanged. GTR and $T\frac{1}{2}$ also remained normal in these subjects which indicated that no increase in the rate of release from marrow reserves occurred. The workers concluded that physiologic leukocytosis may be explained as a redistribution of cells from the MGP to the CGP.

Adrenal corticosteroids are also known to increase the circulating granulocyte count (Schalm, 1965; Wintrobe, 1961). Athens et al. (1961a) measured granulocyte kinetic parameters in 5 normal men. These individuals were then given a daily dose of 40 mg of prednisone orally for 11 days. Their circulating granulocyte counts increased by an average of 75% by the eighth day and then remained at this elevated value until the end of the trial. On the eighth day the kinetic parameters were measured again with the assumption that a new steady state existed in granulocyte kinetics. The CGP was increased an average 76%, the MGP was increased an average 53% and the TBGP was increased an average 67% in the five subjects. The $T\frac{1}{2}$ was prolonged and the combination of in-

creased TBGP and prolonged $T\frac{1}{2}$ resulted in a calculated GTR which was not significantly increased. In a review of this work, Cronkite and Vincent (1970) suggested that the steroid-induced increase in TBGP was apparently produced by an increase in marrow release rate (MRR). The MRR exceeded the egress of granulocytes from the circulation, resulting in a net rise in TBGP. They further surmised that the normal GTR indicated that the MRR must have returned to normal by the time the new steady state was reached. Maintenance of the increased TBGP was apparently accomplished by lengthening the sojourn of granulocytes in peripheral blood, i.e., a prolongation of $T\frac{1}{2}$.

The suggestion that corticosteroids increase the TBGP by increasing the MRR was confirmed by Bishop et al. (1968) who infused labeled granulocytes into 9 normal men and 5 to 7 hours later administered cortisol-phosphate intravenously. This produced a sharp rise in circulating granulocytes. During the period of this rapid rise there was a corresponding fall in granulocyte specific activity indicating that the blood granulocyte pool was being diluted by unlabeled cells being released from the bone marrow.

The finding that the sojourn of granulocytes in blood was prolonged in the new steady state induced by corticosteroids (Athens et al., 1961a) was examined by Bishop et al. (1968). They studied the cellular composition of inflammatory exudates in normal subjects with the "open window" technique

before and after steroid administration as a means of examining the effect of such steroids on the egress of granulocytes from the blood. In normal subjects not given steroids, exudative cells appeared within one hour after inflammation was initiated and reached maximum numbers after six hours; 3 hours after the inflammation was induced 98% of the cells in the exudate were granulocytes. In subjects given steroid the cellularity of exudates was greatly reduced if the initiation of inflammation was delayed until at least two hours after the injection of steroid. Therefore, it was concluded that corticosteroids induce an absolute granulocytosis by decreasing the rate of egress of cells out of the blood as well as by increasing the rate of release from the bone marrow.

Bacterial endotoxin is known to induce alteration of the blood leukocyte profile (Braude, 1964). Within a few minutes after the intravenous injection of a small amount of endotoxin in an experimental animal most of the leukocytes disappear from the blood stream. Then, approximately four hours after endotoxin injection, granulocytes suddenly flood the blood stream, raising the leukocyte count to an abnormally high level.

Athens et al. (1961a) used the radioactive labeling techniques described earlier to study the effects of endotoxin on granulocyte kinetics. Individuals in which control levels of the parameters had been previously determined were studied at the low point of their endotoxin-induced neutropenia. TBGP

was normal, but a shift of granulocytes from the CGP to the MGP had occurred. As the subsequent granulocytosis reached a peak, the pool sizes were measured again and a substantial increase in TBGP was noted. Furthermore, during the period of rising granulocyte count a sharp decline in the slope of the granulocyte disappearance curve was noted indicating that the labeled pool of cells in the blood was being diluted by unlabeled cells entering the blood from marrow reserves. On the basis of this, Athens (1969) postulated that the transient drop in granulocyte count which occurs after endotoxin administration is due to an intravascular shift of cells from the CGP to the MGP; then the marrow is stimulated in an unexplained manner to release large numbers of cells, which increases the blood pool size and, therefore, produces the neutrophilia observed after endotoxin administration.

Athens et al. (1965) studied granulocyte kinetics in 17 patients with subacute to chronic infections. TBGP values ranged from normal to six times normal mean for humans. The blood granulocyte count correlated with observed values for TBGP. The proportional distribution of cells between circulating and marginal pools varied considerably but was within the values encountered in normal subjects. The $T_{\frac{1}{2}}$ remained normal to moderately prolonged in all patients with infection. When TBGP was enlarged, GTR was also usually increased. Similar results were reported by Galbraith et al. (1965). Cronkite and Vincent (1970) state, " . . . in order to account

for the prolonged granulocyte half time despite manifest consumption of granulocytes at the site of infection, the conclusion is inescapable that generalized random loss must be curtailed in infection."

The effect of experimentally-induced infection on granulocyte kinetics in dogs was studied by Marsh et al. (1967). Infection was induced by intratracheal inoculation with Diplococcus pneumoniae. The parameters were measured during the early stage of infection, during the peak clinical stage and during the recovery stage. Early in the course of infection TBGP remained normal, but as granulocytosis began to develop an increased MGP was sometimes found to precede the rise in CGP. $T\frac{1}{2}$ was decreased early in infection. Therefore, early infections were characterized by a rapid egress of cells from the blood at a rate greater than the release rate from marrow reserves.

At the peak of the clinical course in these dogs, CGP and TBGP were significantly increased whereas the MGP was normal. $T\frac{1}{2}$ had also returned to normal. In the recovery stage of infection blood pool sizes were variable and the granulocyte disappearance curves were complex allowing a less precise approximation of $T\frac{1}{2}$. However, the $T\frac{1}{2}$ was found to be greatly prolonged indicating nearly complete cessation of marrow release during recovery from infection.

Response of the bone marrow to peripheral demand for granulocytes

The nature of the stimuli for release of marrow granulocytes and for increase in granulocyte production is unknown. However, a negative feedback mechanism has been proposed (Cronkite and Vincent, 1970; Morley and King-Smith, 1967; Osgood, 1957). Morley (1966) and Morley and King-Smith (1967) studied the peripheral blood granulocyte count of normal human individuals which contains periodic random variations about a mean value over many days. They suggested that a negative feedback controls the granulopoietic rate. As stated by Cronkite and Vincent (1970), the feedback proposal would be as follows: as a wave of granulocytes die, stem cell input would decrease; cell production would diminish; marrow release rate would go down; fewer cells would die in the periphery; there would be less of the hypothetical substance to inhibit stem cell input and granulopoiesis would then increase.

Cronkite and Vincent (1970) proposed that neutropenia is a sufficient stimulus to increase the marrow release rate, while an increased GTR would stimulate an increase in the marrow production rate. This concept was based on some of the work reviewed above and that of Craddock et al. (1955, 1956) who removed from the blood, over a period of a few hours, a number of granulocytes in excess of TBGP. They found that the granulocyte count began to rise following a lag phase of one or two hours; the subsequent granulocytosis reached twice

normal granulocyte values after 24 hours.

One of the characteristics of the granulocyte system is the promptness with which it responds to demand, and infection is a good example of this (considered earlier in this review). Although response at the stem cell level may begin at the very earliest stage of an infection, the effect of the response at this level in man will be delayed at least 5 days. Similarly, the effect of a response at the myelocyte level of production will also be delayed, although to a less extent than the effect of a stem cell response. Thus the responsibility for the earliest neutrophilic response must occur due to release of cells from the bone marrow reserve (Cronkite and Vincent, 1970).

MATERIALS AND METHODS

Experimental Animals, Housing and Surgical Procedures

Experimental animals

Cats used in this research were selected from those submitted by local residents or practicing veterinarians to the Department of Veterinary Pathology, Iowa State University, for experimental study or post mortem examination and from those unclaimed in the Ames, Iowa, animal pound. With the exception of those cats having known blood dyscrasias all cats selected were adults, tame and appreciative of handling; all cats were apparently healthy as indicated by normal appetites, rectal temperatures and lack of manifestations of clinical disease; data in hemograms were within the ranges of accepted normal values. Sex, breed and exact age were not criteria for selection. Blood granulocyte kinetic parameters were determined in 18 cats separated into 4 experimental groups. The kinetic aspects of granulopoiesis were evaluated in 9 cats separated into 2 experimental groups and 2 individual studies.

Housing

Prior to their use in radioisotopic experimental trials, in which only 1 or 2 cats were studied at a time, they were housed in animal quarters in the Department of Veterinary Pathology. Cats used in the blood granulocyte kinetic study

were subjected to surgical placement of an intravenous catheter after which they were moved to isolation quarters approved for radioisotope research. These facilities were located in the Department of Veterinary Microbiology and Preventive Medicine. Cats used in the study of the kinetics of granulopoiesis were moved directly to the radioisotope isolation housing facility without an intervening surgical procedure. All cats were given 2 or 3 days to acclimate to new surroundings before initiation of experimental studies.

Surgical procedures

A polyethylene catheter[1] was surgically placed in an external jugular vein anterior to the thoracic inlet and extended to the base of the heart. The catheter was used to facilitate collection of the numerous blood samples required in studying blood granulocyte kinetics. The cats were anesthetized with 1.1 mg/Kg of phencyclidine hydrochloride[2] injected intravenously. Routine aseptic surgical procedures were followed. Recovery from surgery was considered complete within 24 hours. Total and differential leukocyte counts were stable and normal 36 to 48 hours after surgery.

The sequence followed for collection of blood samples

---

[1]Intramedic PE20, I.D. = 0.015", O.D. = 0.043", Clay Adams, Inc., New York, New York.

[2]Sernylan, Parke, Davis and Co., Detroit, Michigan.

and maintenance of catheter patency was as follows: the catheter was charged with 1% heparin solution to prevent clotting; the catheter was aspirated with a syringe to remove the heparin and obtain a free flow of blood; a second syringe containing EDTA[1] anticoagulant was then used to obtain a sample; the blood was flushed from the catheter by injecting about 0.5 ml of physiologic saline solution (PSS); the catheter was recharged with 1% heparin, sealed and secured with a bandage.

During trials to study the kinetics of granulopoiesis, 10 sequential bone marrow samples were taken from each cat at selected intervals to be defined later. Each cat was given 0.0067 grains of atropine subcutaneously and placed in a large enclosed glass jar containing ether-soaked gauze pads. Following initial excitement the cat would begin to relax and was immediately removed from the ether jar for collection of the marrow sample. Recovery from the light anesthesia was apparent 5 to 10 minutes after the cat had been removed from the ether jar. This procedure was followed each time a sample was collected from a given cat.

The bone marrow samples were aspirated from the ilium or the proximal end of the femur. The techniques described by Schryver (1963) and Gilmore et al. (1964) were used for

---

[1] 1.5% sodium ethylenediaminetetraacetate in 0.7% NaCl solution.

collection of samples at these sites. Since 10 samples were collected from each cat, a specific rotation of sites was followed in all trials. The first sample was taken from the right ilium, the second from the left ilium, the third from the right femur, the fourth from the left femur, and then repeated in the same order until the 10 samples had been obtained. New needle positions were selected over iliac crests previously penetrated and a deeper penetration was made into femurs previously sampled in an attempt to avoid excessive blood dilution of marrow samples. Bone marrow, 0.1 to 0.2 ml/sample, was aspirated into a 20 ml syringe containing EDTA anticoagulant. Bone marrow smears were immediately made on glass slides and rapidly air-dried.

At the completion of each trial, cats were euthanatized with pentobarbital sodium. Those cats with known clinical diseases and blood dyscrasias were immediately necropsied. All cadavers were removed for disposal by the Radiological Services Group.

Radioisotope Procedures

All plastic- and glass-ware used in handling radioisotopes and waste liquids containing isotopes were packaged in plastic bags or glass bottles and picked up for disposal by the Radiological Services Group.

Blood volume determination

Blood volume was determined in cats with standard radioisotope dilution principles using radioiodinated (^{125}I) serum albumin (human)[1]. The following solutions were prepared:

(1) dose-standard, consisting of ^{125}I-albumin in PSS to contain approximately 1 microcurie radioactivity/ml.

(2) counting-standard, prepared by adding 0.2 ml of a 1:10 dilution of the dose-standard to 2 ml of blood.

(3) background sample, consisting of 2 ml of whole blood obtained from a donor cat unrelated to the trial.

One ml of the dose-standard was injected intravenously into the cat in which blood volume was to be determined. After a 15 minute interval (previously determined to be adequate time for complete mixing in the blood) a 2 ml blood sample was collected from the cat. Radioactivity in the background sample, the counting-standard and the cat sample was measured with a gamma counter.[2] Counts per minute (CPM)/ml of the dose-standard and CPM/ml of the cat sample were calculated. Total blood volume in the cat was determined by the following formula: total blood volume = CPM/ml dose-standard ÷ CPM/ml cat sample.

---

[1]Risa-125, Abbott Laboratories, North Chicago, Illinois.

[2]Picker Nuclear Omniscaler Autowell II, Picker Nuclear, White Plains, New York.

Blood granulocyte labeling procedure

Neutrophilic granulocytes were labeled in vitro with tritiated-diisopropylfluorophosphate (^{3}H-DFP)[1] using modifications of the procedure described by Alexanian and Donohue (1965). Some 15 to 20 ml of blood were withdrawn from a catheterized cat into a 35 ml siliconized plastic syringe containing from 1 to 2 ml of EDTA anticoagulant. ^{3}H-DFP was added to the blood in the syringe to give a final concentration of from 0.3 to 0.5 micrograms of DFP/ml of blood (specific activity was approximately 55 microcuries/ml of blood). The syringe containing the mixture was placed in a 37°C incubator and gently agitated for 45 minutes. At the end of this time 2 or 3 ml of blood were removed from the syringe for total and differential leukocyte counts and isolation of leukocytes for determination of granulocyte specific activity. The volume of blood remaining in the syringe was measured and then infused over a 10 to 15 minute period via the catheterized vein, into the cat from which the blood had originally been obtained.

One experiment was conducted to determine if additional granulocyte labeling would occur in vivo. Plasma was separated from cells following the routine labeling procedure and the specific activity was determined. Following this the plasma was infused into a cat. Leukocytes were isolated from this

---

[1]Diisopropyl-1-^{3}H fluorophosphate, specific activity = 1.0 curie per millimole, New England Nuclear, Boston, Massachusetts.

cat at various times after the plasma infusion and an attempt was made to detect radioactivity in the leukocyte isolates by scintillation counting.

The selectivity of ^{3}H-DFP for neutrophilic granulocytes was examined by autoradiographic procedures. Autoradiograms on smears of blood and leukocyte isolates from ^{3}H-DFP-treated blood were prepared and examined for labeling of cells other than neutrophils.

Isolation of ^{3}H-DFP labeled granulocytes

Leukocytes were isolated from 2 to 3 ml blood samples containing ^{3}H-DFP labeled granulocytes in preparation for determination of granulocyte specific activity. Leukocytes were isolated by using the techniques of erythrocyte sedimentation, hypotonic lysis of erythrocytes (Fallon et al., 1962) and differential centrifugation. They were obtained free of erythrocytes and reasonably free of platelets. The entire procedure was carried out at 0-4°C to maintain leukocyte viability.

The details of the leukocyte isolation procedure were as follows. The blood sample, collected in EDTA anticoagulant, was placed in a siliconized plastic tube for 15 to 20 minutes to allow time for sedimentation of erythrocytes. Occasionally, sedimentation did not occur and if so, the sample was centrifuged at 20 x g for 4 minutes. The plasma, which contained leukocytes, platelets and some erythrocytes, was transferred

to a second siliconized tube and centrifuged at about 150 x g for 5 minutes. The supernatant plasma was discarded and the sediment, which consisted of leukocytes and some contaminating erythrocytes and platelets, was resuspended in 1 ml of PSS; 3 ml of distilled water were added to create a hypotonic solution and the mixture was vigorously shaken for 30 to 45 seconds, depending upon the degree of erythrocyte contamination; 1 ml of 3.5% NaCl solution was rapidly added to restore isotonicity, and the mixture was centrifuged for 5 minutes at 130 x g. The supernatant was discarded and the sediment, which contained leukocytes, some erythrocytic debris and platelets, was resuspended in about 1 ml of normal cat plasma, which prevented clumping of cells. The mixture was centrifuged for 4 minutes at 20 x g during which the erythrocytic debris and many platelets remained in suspension; this step was repeated 2 more times to wash the leukocytes. After the final plasma wash, the leukocytes were resuspended in 1 ml of normal cat plasma from which total and differential leukocyte counts were made; the number of neutrophilic granulocytes in the isolate was calculated. The isolated leukocytes, which were separated from the plasma after centrifugation, were prepared for scintillation counting to determine granulocyte specific activity.

Determination of granulocyte specific activity

The specific activity of ^{3}H-DFP labeled granulocytes was determined by liquid scintillation counting using a Packard

Tri-carb liquid scintillation spectrometer.[1] Each leukocyte sample, in which the absolute granulocyte count was known, was prepared for scintillation counting by adding 1 ml of NCS[2] to solubilize the leukocytes; 18 ml of scintillation solution, consisting of 50 parts PPO[3] and 0.625 parts POPOP[3]/ liter of toluene, were added to the solubilized sample. The samples were counted for 50 minutes to achieve statistically significant gross counts. (Gross counts usually ranged from 5,000 to 20,000 counts/50 minutes/sample.) The external standard ratio and the net CPM were recorded for each leukocyte sample.

The counting efficiency for each leukocyte sample was interpolated from an efficiency calibration curve which had been prepared in the following way. Several scintillation standard solutions were prepared, each containing a known quantity of tritium radioactivity and decreasing amounts of hemoglobin, which was presumed to be a likely quenching agent in the leukocyte samples. The counting efficiency of each of these standards, calculated from the observed CPM and the expected CPM (known quantity of radioactivity), was plotted versus the observed external standard ratio to form the effi-

---

[1]Packard Instrument Co., Inc., Downers Grove, Illinois.

[2]Amersham Searle, Des Plaines, Illinois.

[3]PPO = 2-5 diphenyloxazole; POPOP - Para-bis /-2-(5 phenyl-ozazolyl)/ benzene, Amersham Searle, Des Plaines, Illinois.

ciency calibration curve. The counting efficiency for each leukocyte sample was obtained by fitting the external standard ratio for that sample to the efficiency calibration curve. Counting efficiencies for all leukocyte samples were near 30%.

A method was devised whereby CPM/million granulocytes could be obtained for each leukocyte sample, which had been prepared as described above. This method was similar, in principle, to that described by Deinard and Page (1970) for use in granulocyte kinetic studies. The CPM/million granulocytes/sample was calculated with the following formula: CPM/10^6 granulocytes = net CPM of the sample ÷ counting efficiency of the sample ÷ the absolute granulocyte count in the sample (to the nearest million granulocytes).

In vivo labeling of granulocyte precursors

Bone marrow granulocyte precursors in cats, in which the kinetics of granulopoiesis was evaluated, were labeled in vivo by intravenous injection of tritiated thymidine[1] (^{3}H-Tdr). These cats were given 0.3 microcuries (2 curies/millimole)/gram of body weight in two doses, 15 minutes apart.

Autoradiographic procedures

Blood and bone marrow smears from cats which had received ^{3}H-Tdr and smears of whole blood and leukocyte isolates, which

---

[1]Thymidine-methyl-^{3}H, specific activity = 2 curies/millimole, New England Nuclear, Boston, Massachusetts.

contained ^{3}H-DFP labeled granulocytes, were fixed in methyl alcohol for 5 minutes and air-dried. The smears were dipped in NTB-3[1] nuclear track emulsion diluted 1:2 with distilled water, dried in the air and packed in light proof boxes with a drying agent.

The boxes containing the emulsion-coated smears were stored for 7 or 8 weeks at -10°C to allow for measurable exposure of the emulsion. After exposure, the autoradiograms were developed for 2 minutes at 15°C in Dektol[1] diluted 1:2 in distilled water and fixed for 8 minutes. Autoradiograms were stained through the emulsion with Wright's blood stain (Schalm, 1965) and permanently mounted.

Hematological and Histological Procedures

In those blood samples in which no radioisotope was present, the leukocyte counts were determined with the aid of a Coulter electronic cell counter.[2] However, in those samples containing either ^{3}H-DFP or ^{3}H-Tdr labeled cells, leukocyte counts were made manually with disposable pipettes[3] containing diluent. Differential leukocyte counts were made on smears stained with Wright's blood stain.

---

[1]Eastman Kodak Co., Rochester, New York.

[2]Coulter Electronics, Inc., Hialeah, Florida.

[3]Unopette, Becton, Dickinson, and Co., Rutherford, New Jersey.

After all samples for radioisotopic experimentation were obtained, cats which had originally been submitted to the Department of Veterinary Pathology for examination were necropsied. Tissues were fixed in formalin, embedded in paraffin, sectioned at 6 microns and stained with Harris hematoxylin and eosin Y. One special stain, Gomori's reticulum stain, was also used.

PART I. BLOOD GRANULOCYTE KINETICS IN CATS

EXPERIMENTAL DESIGN

This study was designed to evaluate the kinetic parameters of blood neutrophilic granulocytes in cats. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), the half-life of blood granulocytes ($T_{\frac{1}{2}}$) and granulocyte turnover rate (GTR) were determined in 18 cats, which were divided into 4 groups.

The first group consisted of 5 clinically and hematologically normal cats. These will be referred to hereafter as normal cats.

The second group consisted of 3 cats in which circulating neutrophilia was induced by intravenous injection of 3 mg/Kg of bacterial endotoxin[1] approximately 10 hours before determination of kinetic parameters.

The third group consisted of cats in which hemorrhagic blood loss was simulated during the trials. The first of these cats, which was to have been considered normal, was accidentally heparinized and spontaneous hemorrhage occurred from the surgical wound and from the insertion point of the indwelling catheter. Hemorrhage continued from this cat for about 24 hours during which abnormal granulocyte kinetic parameters were determined. Subsequently, 6 additional cats were studied, in which hemorrhage was simulated by bleeding in excess of

---

[1] *Escherichia coli* endotoxin, serotype 055:B5, Difco Laboratories, Detroit, Michigan.

routine sample volumes during the trials. The excess amounts bled from these cats were recorded to estimate the degree of hemorrhagic blood loss in order to facilitate interpretation of results.

The fourth group consisted of 3 cats with naturally occurring blood dyscrasias. Of these cats, one was febrile and had a marked neutrophilic leukocytosis during the trial; a second cat, otherwise clinically normal, had a moderate neutrophilic leukocytosis for unexplained reasons; the third cat was neutropenic and had confirmed lymphosarcoma, diagnosed by surgical biopsy and histological examination.

The numbers and letters used to identify cats and their clinical status for all 18 cats are listed in Table 2. This identification will be used throughout the manuscript when data from individual cats are presented and discussed. Although sex was also recorded in Table 2, no correlations between sex and results of granulocyte kinetic parameters will be made due to insufficient numbers of cats in the various groups.

All trials were conducted according to the following sequence:

(1) Blood volume was measured between 6:00 and 10:00 o'clock in the evening.

(2) Blood was collected for labeling granulocytes with ^{3}H-DFP in the morning, usually starting between 5:00 and 7:30 o'clock, 8 to 10 hours after the blood

Table 2. Identification, sex and clinical status of cats in which blood granulocyte kinetics were studied

Cat	Sex[a]	Clinical status
12N	F	Normal
13N	M	Normal
14N	M	Normal
18N	M	Normal
26N	M	Normal
23E	M	Endotoxin-induced neutrophilia
24E	M	Endotoxin-induced neutrophilia
29E	M	Endotoxin-induced neutrophilia
15H	F	Spontaneous hemorrhage
19H	F	Experimental hemorrhage
21H	M	Experimental hemorrhage
27H	F	Experimental hemorrhage
28H	F	Experimental hemorrhage
32H	F	Experimental hemorrhage
33H	F	Experimental hemorrhage
16FL	M	Febrile; leukocytosis
17UL	F	Unexplained leukocytosis
20Ly	CM	Lymphosarcoma; neutropenia

[a]F = female, M = male, CM = castrated male.

volume procedure; samples of this blood were retained after labeling for total and differential leukocyte counts and for determination of granulocyte specific activity; the volume of blood which contained the labeled granulocytes was measured.

(3) A few drops of blood were removed from the cat to determine the circulating granulocyte count, which was used for calculation of the CGP; the blood containing the ^{3}H-DFP labeled granulocytes was then infused; completion of the infusion was referred to as zero time (t_o) or pool size determination time.

(4) Five blood samples were collected during the 24 hours following infusion of labeled granulocytes; granulocyte specific activity was determined at each sample time; the approximate times in all cats were 1.5, 3, 6, 12, and 24 hours after infusion.

(5) Each cat was euthanatized at the end of the sampling sequence.

The granulocyte specific activities, obtained following infusion of labeled granulocytes, were found to decrease with time at an exponential rate. These activities, versus time, were fitted with a line by exponential regression analysis using the statistical method of least squares. The intercept of the exponential regression line and the ordinate on a semi-logarithmic scale represented the specific activity of granulocytes at zero time. The $T_{\frac{1}{2}}$ of blood granulocytes was cal-

culated from the slope of the exponential regression lines; that is, $T_{\frac{1}{2}}$ was taken to be the time at which the specific activity of granulocytes at zero time was halved.

The data recorded for each cat are summarized:[1]

(1) Kilogram body weight (Kg).

(2) Blood volume, in ml/Kg (BV).

(3) Specific activity of granulocytes infused, in CPM/10^6G (SAInfG).

(4) Number of granulocytes infused (NoInfG).

(5) Specific activity of granulocytes at zero time, in CPM/10^6G ($SAGt_o$).

(6) Circulating granulocyte count at zero time, in G/ml (Gt_o).

(7) Slope of the exponential regression line (b); from this, $T_{\frac{1}{2}}$.

The formulae for pool sizes and GTR were:

(1) $TBGP = \frac{(SAInfG)(NoInfG)}{(SAGt_o)(Kg)}$; units were: granulocytes/Kg.

(2) $CGP = (Gt_o)(BV)$; units were: granulocytes/Kg.

(3) MGP = TBGP - CGP; units were: granulocytes/Kg.

(4) GTR = (24 hours) $\frac{0.693}{T_{\frac{1}{2}}}$ (TBGP); units were: granulocytes/Kg/day.

---

[1]These data are recorded for all 18 cats in Tables 15 to 18, Appendix.

RESULTS

Control Studies

Either *in vivo* labeling of granulocytes, or label on other than neutrophils would be detrimental to the logical interpretation of results. Therefore, the following experiments were conducted:

(1) Plasma was separated from all cells following the ^{3}H-DFP labeling procedure; the specific activity of the plasma was found to be 416,616 CPM/ml. Five ml of this plasma were infused into a cat and the remainder of the trial was conducted in a manner identical to that described for all cats. Subsequent leukocyte isolates from this cat did not contain detectable radioactivity, thus indicating that *in vivo* labeling was not occurring under the conditions of these studies.

(2) The specificity of ^{3}H-DFP for neutrophilic granulocytes was studied by preparing autoradiograms on leukocyte isolates and whole blood obtained from blood in which the labeling procedure had just been completed (Figures 2 and 3). Neutrophilic granulocytes were found to be heavily labeled whereas monocytes, lymphocytes, eosinophils, platelets, and erythrocytes were not labeled. The affinity of ^{3}H-DFP for neutrophils was indeed specific within the limits of these trials.

Figure 2. Autoradiogram of leukocyte isolate from blood in which ^{3}H-DFP labeling had just been completed. Note labeled neutrophilic granulocytes, and non-labeled platelets and eosinophilic granulocyte. x 1200

Figure 3. Autoradiogram of leukocyte isolate from blood in which ^{3}H-DFP labeling had just been completed. Note labeled neutrophilic granulocyte and non-labeled lymphocyte. x 1200

Blood Granulocyte Kinetics in Normal Cats

During the respective trials circulating leukocyte counts in all of the normal cats usually ranged between 10,000 and 16,000/cubic millimeter (cmm) of blood, which are accepted normal values (Schalm, 1965). Fluctuations of the count were usually observed within the 2 to 3 hour period after infusion of blood containing labeled granulocytes. The peak values, which exceeded 20,000 leukocytes/cmm of blood in 2 of the 5 cats, reflected changing levels of circulating neutrophils. The mean absolute circulating granulocyte count during trials in the 5 normal cats may be seen in Figure 4.

Granulocyte specific activity was observed to decrease at an exponential rate with time following infusion of ^{3}H-DFP labeled granulocytes in all cats studied. The specific activities of granulocytes isolated during the 24 hours following infusion and the fitted exponential regression line of these activities for cat 12N are illustrated on an arithmetic plot in Figure 5 and on a semilogarithmic plot in Figure 6. These plots illustrate the rate at which labeled granulocytes disappeared from the blood of cat 12N. Similar plots were produced from observed data in all normal cats.

The specific activity of granulocytes at zero time and the slope of the exponential regression line were determined for each normal cat and were subsequently used to calculate the blood granulocyte kinetic parameters. The range of granu-

Figure 4. The mean absolute circulating granulocyte counts in 5 normal cats before and after pool size determination time

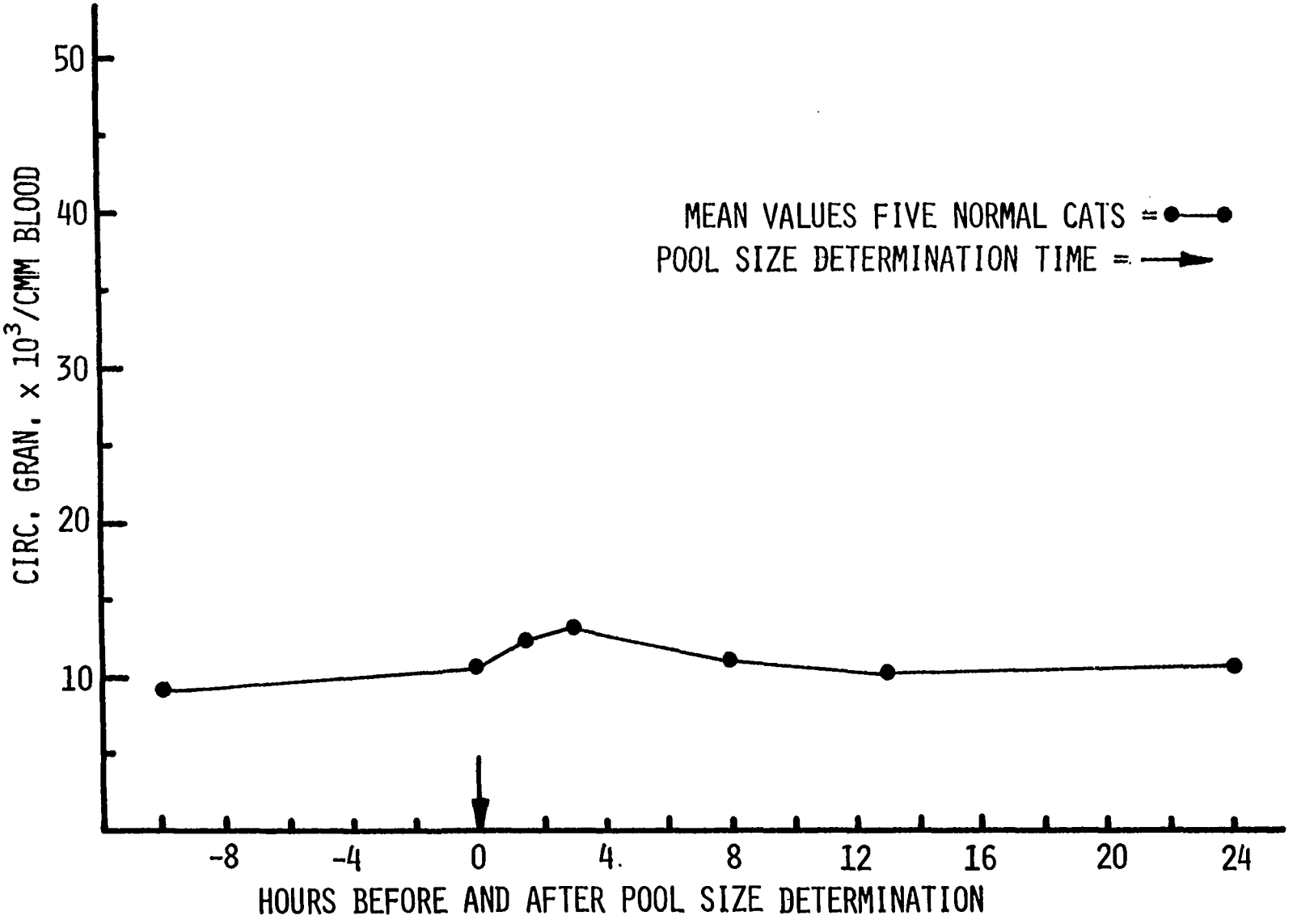

MEAN VALUES FIVE NORMAL CATS
POOL SIZE DETERMINATION TIME
CIRC. GRAN. x 10^3/CMM BLOOD
50
40
30
20
10
-8
-4
0
4.
8
12
16
20
24
HOURS BEFORE AND AFTER POOL SIZE DETERMINATION

Figure 5. The granulocyte disappearance curve determined for cat 12N illustrating the experimentally derived granulocyte specific activities following infusion of ^{3}H-DFP labeled cells and the calculated exponential regression line on an arithmetic scale

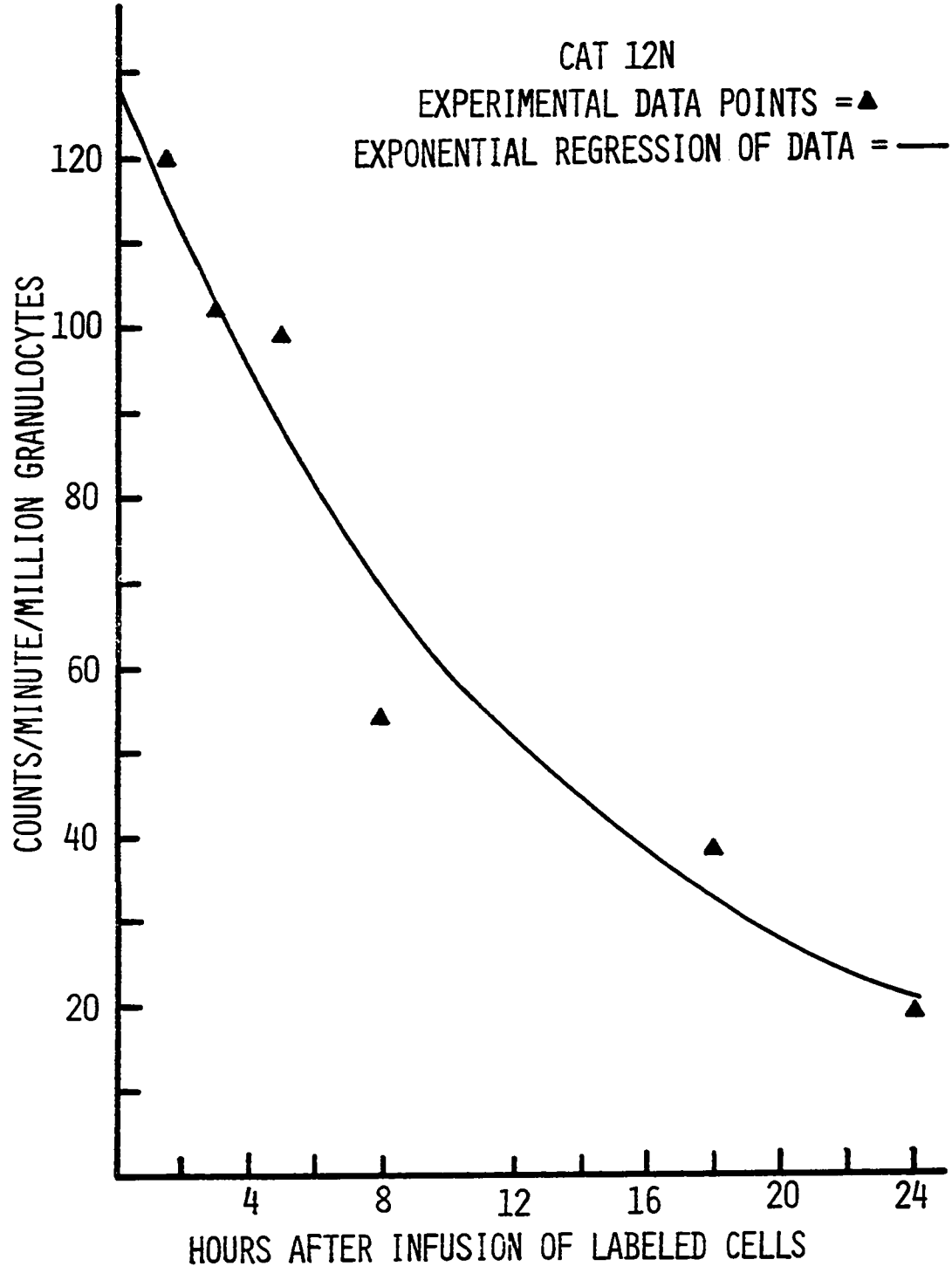
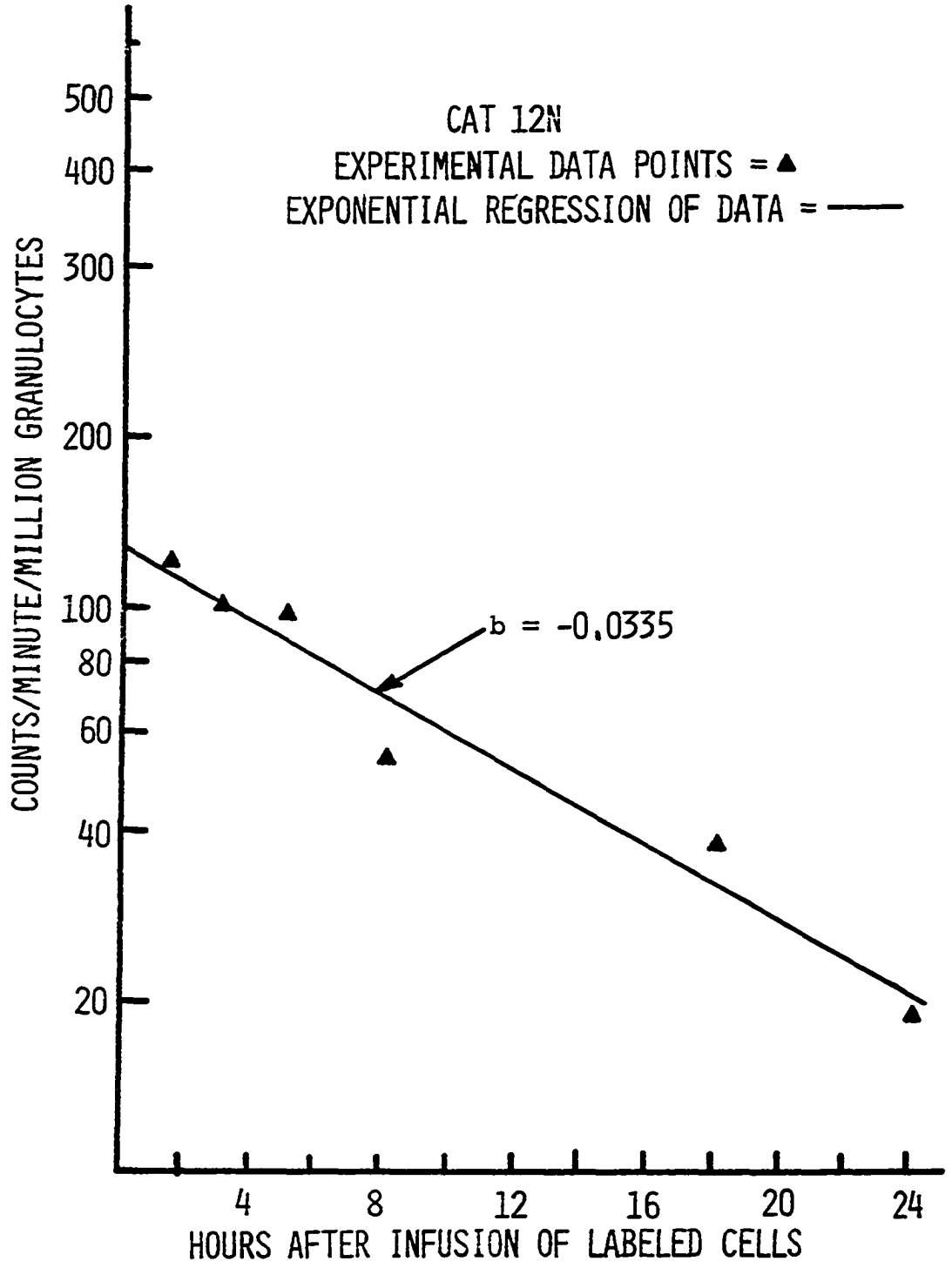

CAT 12N
EXPERIMENTAL DATA POINTS = ▲
EXPONENTIAL REGRESSION OF DATA = —
COUNTS/MINUTE/MILLION GRANULOCYTES
120
100
80
60
40
20
4
8
12
16
20
24
HOURS AFTER INFUSION OF LABELED CELLS

Figure 6. The granulocyte disappearance curve determined for cat 12N illustrating the experimentally derived granulocyte specific activities following infusion of ^{3}H-DFP labeled cells and the calculated exponential regression line on a semilogarithmic scale (b = slope)


CAT 12N
EXPERIMENTAL DATA POINTS = ▲
EXPONENTIAL REGRESSION OF DATA = ——
b = -0.0335
COUNTS/MINUTE/MILLION GRANULOCYTES
500
400
300
200
100
80
60
40
20
4
8
12
16
20
24
HOURS AFTER INFUSION OF LABELED CELLS

locyte disappearance curves for the normal cats may be seen in Figure 7 as the shaded area. The mean granulocyte disappearance curve and its slope, b, are shown also. The intercepts of the 5 exponential regression lines with the ordinate had considerably different absolute values for each cat. However, the intercept used in Figure 7 was arbitrarily chosen in order to allow illustration of the range of slopes.

The blood granulocyte kinetic parameters, TBGP, CGP, MGP, $T\frac{1}{2}$, GTR and the CGP:TBGP ratio, derived from the data shown in Table 15, Appendix, and the mean and standard deviation of the parameters for all normal cats are presented in Table 3. The CGP:TBGP ratios indicate the proportion of granulocytes in the peripheral blood which were circulating as opposed to remaining quiescent in the vascular bed.

Effect of Bacterial Endotoxin on Blood Granulocyte Kinetics in Cats

A systemic reaction occurred in cats following intravenous endotoxin administration, but the severity of the reaction varied independent of dosage. The reaction began within minutes after endotoxin administration and was characterized by vomiting and a tendency to withdraw to a motionless, inattentive sitting posture at the rear of the cage. Severe leukopenia caused by marked decrease in neutrophils and lymphocytes occurred within 1 or 2 hours.

The following 10 to 14 hour period was usually charac-

Figure 7. The range and mean slope (b) of granulocyte disappearance curves determined for 5 normal cats after infusion with ^{3}H-DFP labeled cells. Note that the intercept with the ordinate which was different in all cats was arbitrarily selected in order to demonstrate the range of slopes

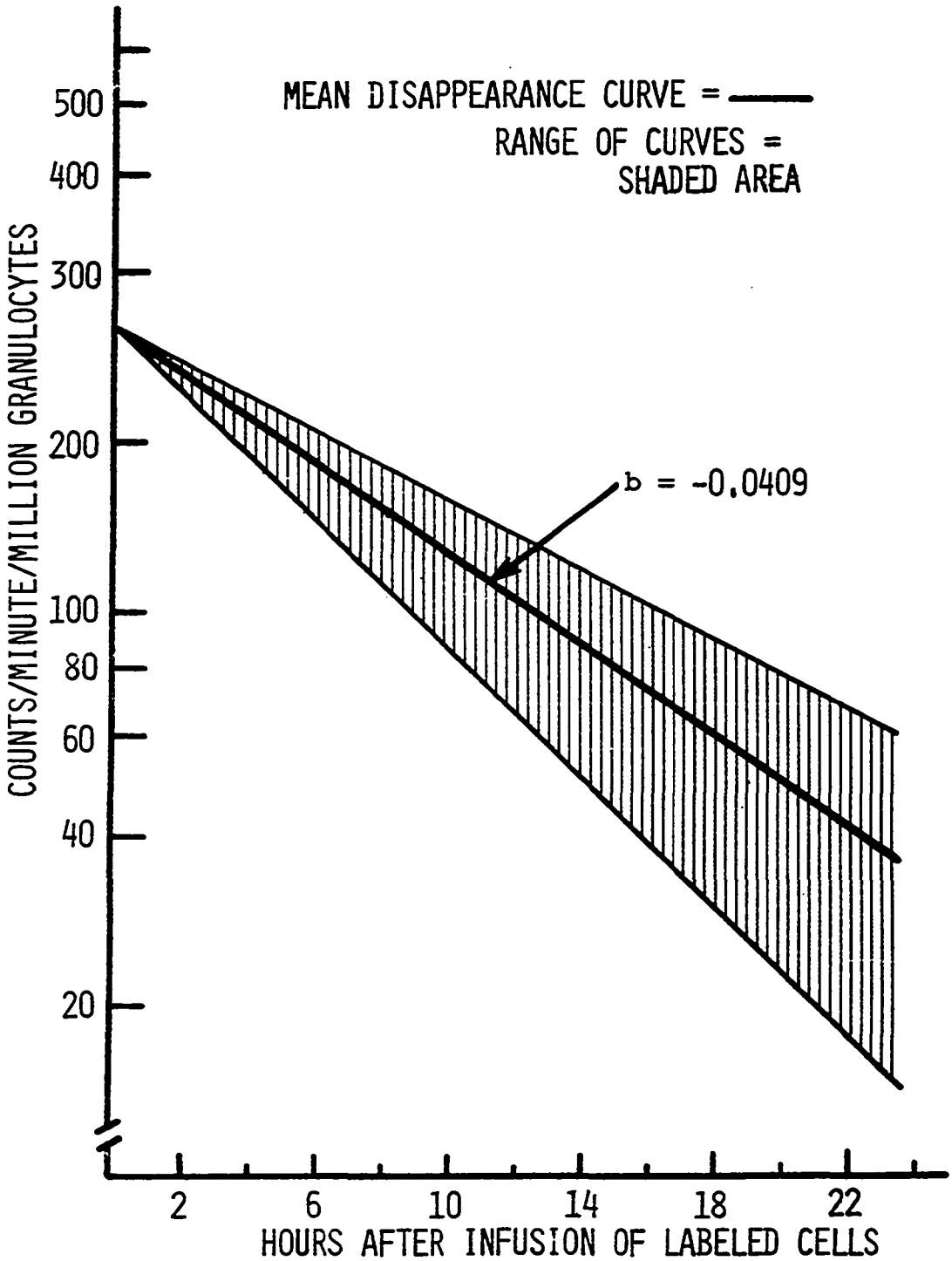

MEAN DISAPPEARANCE CURVE =
RANGE OF CURVES =
SHADED AREA
b = -0.0409
COUNTS/MINUTE/MILLION GRANULOCYTES
500
400
300
200
100
80
60
40
20
2
6
10
14
18
22
HOURS AFTER INFUSION OF LABELED CELLS

Table 3. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), CGP:TBGP ratio, half-life of blood granulocytes ($T_{\frac{1}{2}}$) and granulocyte turnover rate (GTR) in normal cats

Cat	TBGP ($\times 10^8$/Kg)	CGP ($\times 10^8$/Kg)	MGP ($\times 10^8$/Kg)	CGP:TBGP	$T_{\frac{1}{2}}$ (hours)	GTR ($\times 10^8$/Kg/Day)
12N	29.59	9.86	19.73	0.33	9.0	54.77
13N	42.30	10.65	31.65	0.25	9.6	73.49
14N	36.26	7.06	29.21	0.20	6.6	91.99
18N	19.06	6.99	12.07	0.37	5.2	61.06
26N	17.11	4.66	12.46	0.27	6.5	43.53
Mean	28.86	7.84	21.02	0.28	7.4	64.97
S.D.	10.84	2.42	9.15	0.07	1.8	18.58

terized by a rapidly increasing neutrophil count, elevation of rectal temperature, increased respiratory rate, and depression. The developing neutrophilia was usually characterized by a large increase in circulating immature neutrophils, although cat 23E did not show neutrophilia for an unexplained reason.

After 14 to 18 hours, clinical recovery began and was usually complete by 36 hours following endotoxin administra-

tion. If a significant neutrophilia had occurred it remained until the final hours of recovery. Several cats died from endotoxin administration and were discarded from the study.

Three cats, 23E, 24E, and 29E, survived the endotoxin-induced illness, providing the data base on which the granulocyte kinetic parameters were developed. The absolute circulating granulocyte counts in these cats, plotted before and after pool size determination time, are illustrated in Figure 8. The neutrophilic phase of the endotoxin reaction developed rapidly and became pronounced in cats 24E and 29E; cat 23E failed to develop a significant neutrophilia and had, in fact, a relatively low granulocyte count at the pool size determination time.

Granulocyte disappearance curves plotted for the 3 endotoxin-treated cats are shown in Figure 9. The range of slopes and mean slope of curves obtained in normal cats are superimposed on each graph in Figure 9 for comparison.

The calculated granulocyte kinetic parameters in cats 23E, 24E, and 29E, as well as the mean values for normal cats (Table 3) are presented in Table 4. Cat 23E, which remained neutropenic at pool size determination time, was found to have a normal TBGP with most of its blood granulocytes in the marginal pool. Cats 24E and 29E, which were neutrophilic at pool size determination time, each had an increased TBGP with both CGP and MGP increased in size; GTR, which is dependent on TBGP and $T\frac{1}{2}$, was increased in both of these cats because $T\frac{1}{2}$ remained

Figure 8. The absolute circulating granulocyte counts before and after pool size determination time in cats treated with bacterial endotoxin

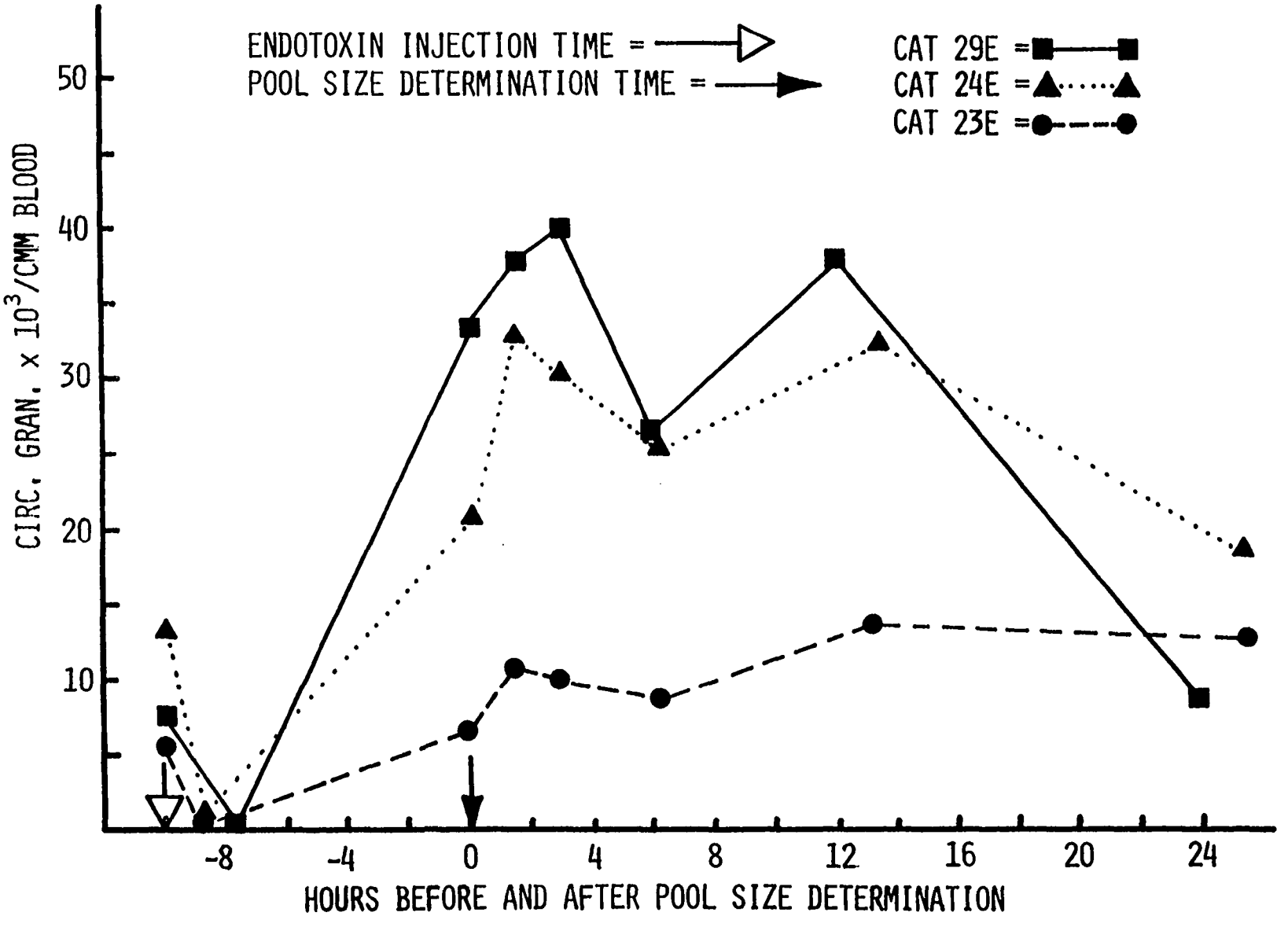

ENDOTOXIN INJECTION TIME =
POOL SIZE DETERMINATION TIME =
CAT 29E =
CAT 24E =
CAT 23E =
CIRC. GRAN. x 10^3/CMM BLOOD
50
40
30
20
10
-8
-4
0
4
8
12
16
20
24
HOURS BEFORE AND AFTER POOL SIZE DETERMINATION

Figure 9. Granulocyte disappearance curves determined for 3 cats treated with bacterial endotoxin 10 hours before pool size determination time. Comparison with mean and range of slopes determined in 5 normal cats

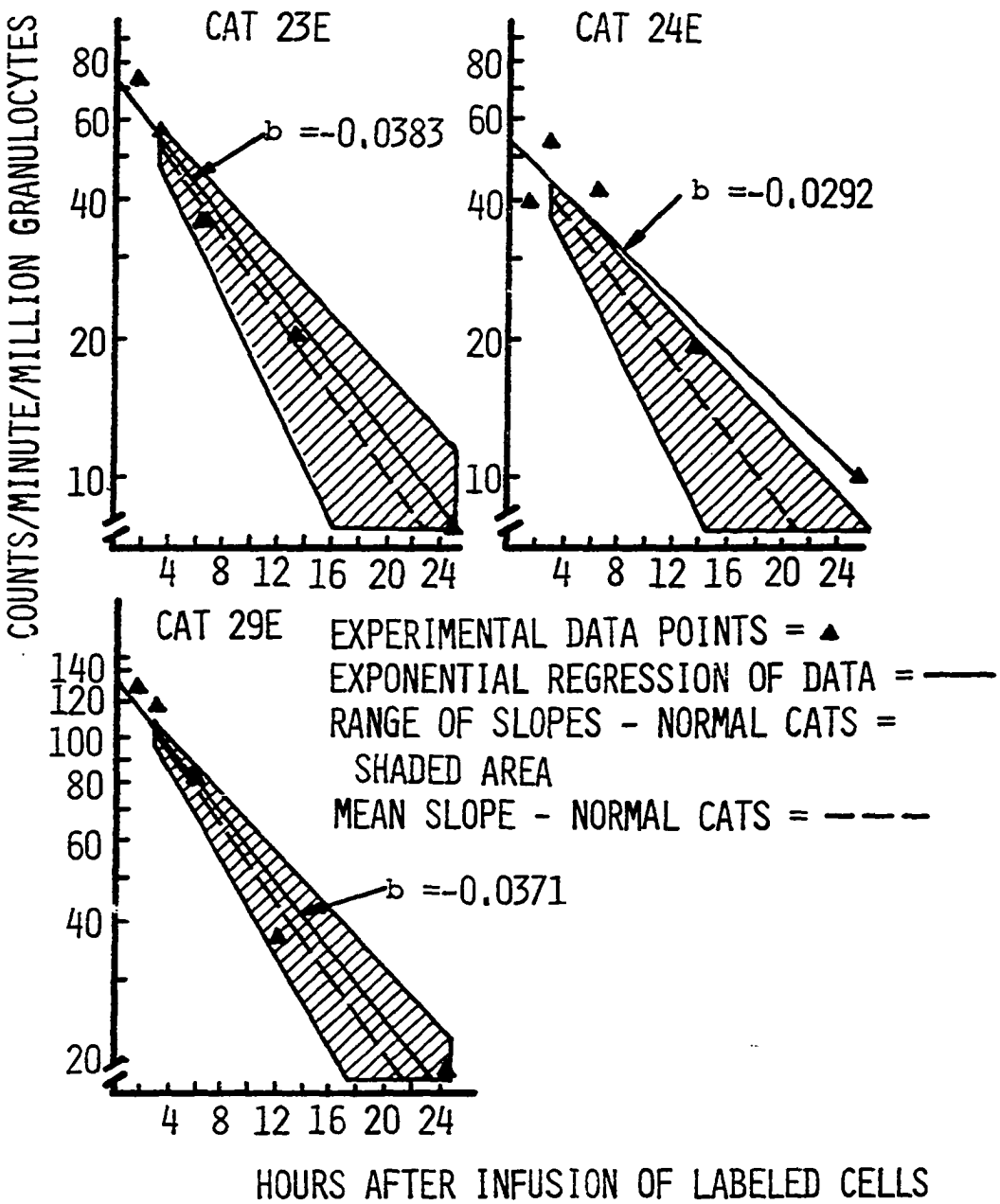

COUNTS/MINUTE/MILLION GRANULOCYTES
CAT 23E
b =-0.0383
CAT 24E
b =-0.0292
CAT 29E
b =-0.0371
EXPERIMENTAL DATA POINTS = ▲
EXPONENTIAL REGRESSION OF DATA = ——
RANGE OF SLOPES - NORMAL CATS = SHADED AREA
MEAN SLOPE - NORMAL CATS = — — —
HOURS AFTER INFUSION OF LABELED CELLS

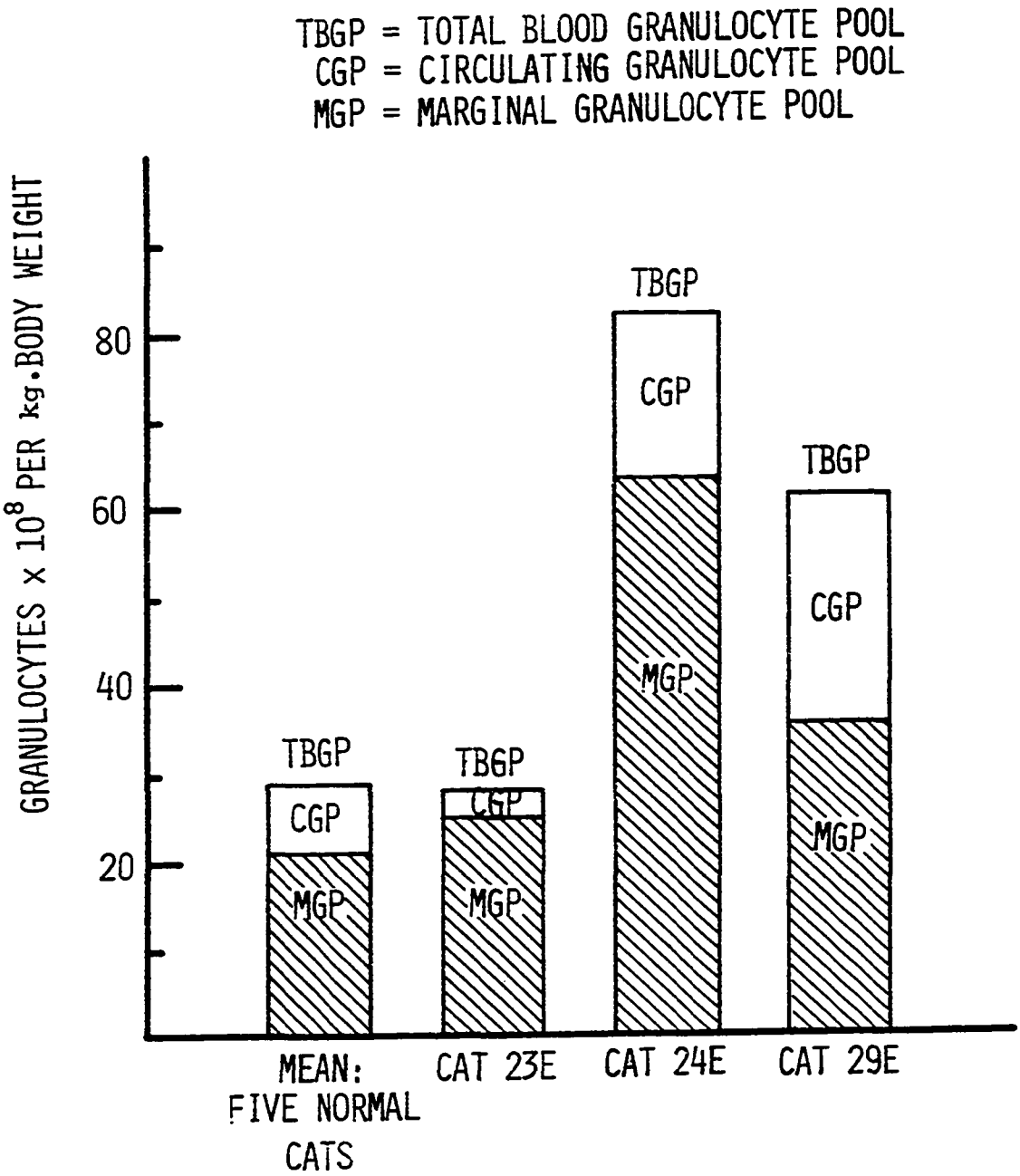
Table 4. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), CGP:TBGP ratio, half-life of blood granulocytes ($T\frac{1}{2}$) and granulocyte turnover rate (GTR) in 3 cats treated with bacterial endotoxin 10 hours before pool size determination time. Comparison with mean values in 5 normal cats

Cat	TBGP ($x10^8$/Kg)	CGP ($x10^8$/Kg)	MGP ($x10^8$/Kg)	CGP:TBGP	$T\frac{1}{2}$ (hours)	GTR ($x10^8$/Kg/Day)
23E	28.05	3.23	24.82	0.12	7.9	59.41
24E	81.92	18.49	63.43	0.23	10.3	131.95
29E	61.67	26.03	35.64	0.42	8.1	126.31
Mean: 5 normal cats[a]	28.86	7.84	21.02	0.28	7.4	64.97

[a]Data from Table 3.

normal or near normal, as could be seen from the slopes in Figure 9. The size of CGP and MGP, and thereby TBGP, for each endotoxin-treated cat is compared graphically to the mean pool size of normal cats in Figure 10. Data from which the kinetic parameters were calculated in cats 23E, 24E, and 29E may be seen in Table 16, Appendix.

Figure 10. Total, circulating, and marginal granulocyte pool sizes in cats treated with bacterial endotoxin 10 hours before pool size determination time. Comparison with mean values in 5 normal cats


TBGP = TOTAL BLOOD GRANULOCYTE POOL
CGP = CIRCULATING GRANULOCYTE POOL
MGP = MARGINAL GRANULOCYTE POOL
GRANULOCYTES x 10^8 PER kg. BODY WEIGHT
80
60
40
20
TBGP
CGP
MGP
MEAN: FIVE NORMAL CATS
CAT 23E
CAT 24E
CAT 29E

Effect of Hemorrhage on Blood Granulocyte Kinetics in Cats

The calculated blood volume of each cat before experimental hemorrhage began and the amount of blood removed from each cat in excess of routine sample volumes, before and after pool size determination time (t_o), are listed in Table 5. Cat 15H bled spontaneously due to accidental heparinization and no estimate of the amount of blood lost could be determined. The range of hematocrit values in the 7 cats were as follows: 24 to 36% at 10 hours before t_o, 23 to 30% at t_o, 20 to 29% at 8 hours after t_o and 14 to 25% at 25 hours after t_o. No clinical signs referable to the developing anemic states were observed in these cats.

Absolute circulating granulocyte counts before and after pool size determination in cats 15H, 19H, 21H, and 27H are shown in Figure 11 and those in cats 28H, 32H, and 33H may be seen in Figure 12. In the cat with spontaneous hemorrhage, 15H, and in cat 21H, neutrophilia developed most rapidly (Figure 11); neutrophil counts in cats 19H and 21H remained near normal throughout respective trials (Figure 11); neutrophilia developed more slowly in cats 28H, 32H, and 33H (Figure 12).

Granulocyte disappearance curves are presented for comparison with the mean slope and range of slopes of curves obtained in 5 normal cats; the curves for cats 15H, 19H, 21H, and 27H appear in Figure 13 and the curves for cats 28H, 32H,

Table 5. Blood volume (BV) in 7 cats in which the effects of experimental hemorrhage on blood granulocyte kinetics were studied. Amounts bled in excess of routine sample volumes before and after pool size determination time (t_o)

Cat	BV[a] (ml/Kg)	Bled before t_o (ml/Kg)	Bled after t_o (ml/Kg)
15H	63	?[b]	?[b]
19H	76	3.1	9.4
21H	73	6.1	13.5
27H	74	4.5	11.5
28H	63	4.5	10.4
32H	60	6.2	3.1
33H	57	6.5	4.6

[a]Blood volume determined before hemorrhage.

[b]Spontaneous hemorrhage, volumes could not be determined.

and 33H may be seen in Figure 14. The disappearance of labeled granulocytes was slower in cats 15H, 21H, 32H, and 33H as indicated by the decreased slopes. Also note that the curves for 19H, 27H, and 28H were within or slightly below the normal range.

A pattern characterized by an increased proportion of blood granulocytes in the marginal pool of cells, a prolonged

Figure 11. The absolute circulating granulocyte counts in cats 15H, 19H, 21H, and 27H during trials in which the effects of hemorrhage on blood granulocyte kinetics were studied. Bleeding began at -10 hours and was continued throughout each trial

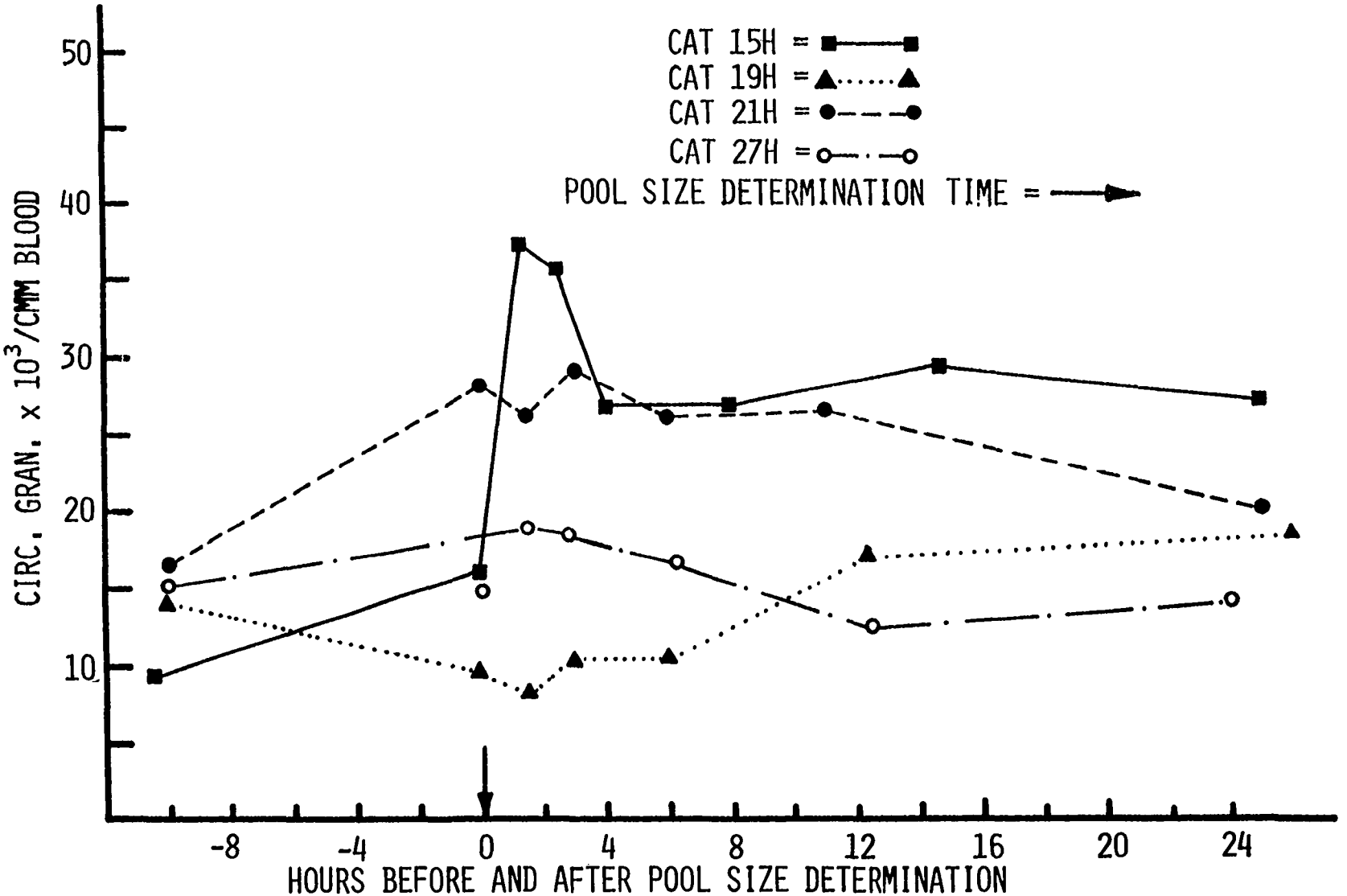

CAT 15H =
CAT 19H =
CAT 21H =
CAT 27H =
POOL SIZE DETERMINATION TIME =
CIRC. GRAN. x 10^3/CMM BLOOD
50
40
30
20
10
-8
-4
0
4
8
12
16
20
24
HOURS BEFORE AND AFTER POOL SIZE DETERMINATION

Figure 12. The absolute circulating granulocyte counts in cats 28H, 32H, and 33H during trials in which the effects of hemorrhage on blood granulocyte kinetics were studied. Bleeding began at -10 hours and was continued throughout each trial

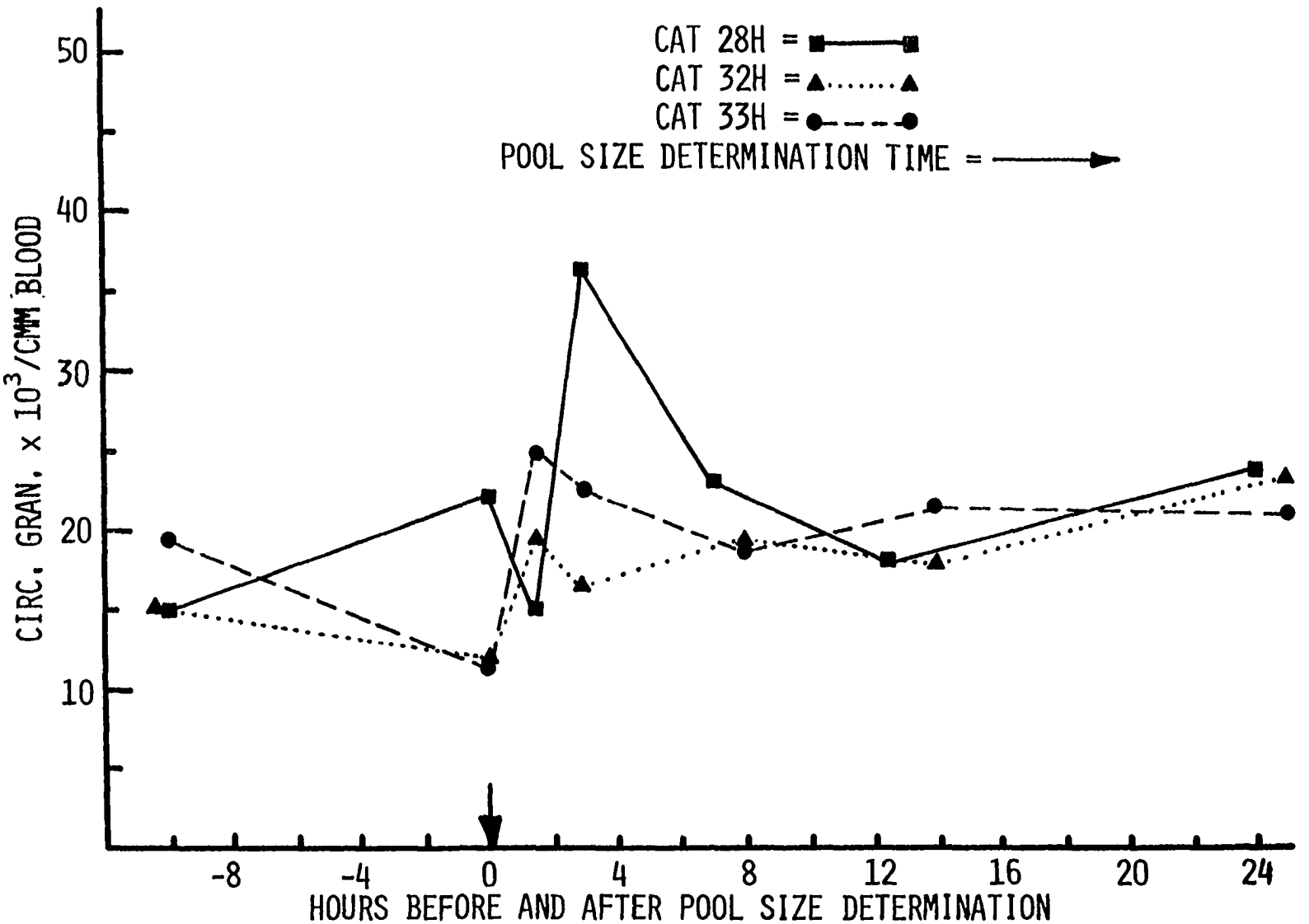

CAT 28H =
CAT 32H =
CAT 33H =
POOL SIZE DETERMINATION TIME =
CIRC. GRAN. x 10^3/CMM BLOOD
50
40
30
20
10
-8
-4
0
4
8
12
16
20
24
HOURS BEFORE AND AFTER POOL SIZE DETERMINATION

Figure 13. Granulocyte disappearance curves calculated for cats 15H, 19H, 21H, and 27H. Comparison with mean slope and range of slopes in 5 normal cats

Experimental data points = ▲
Exponential regression of data = ——
Range of slopes-normal cats = shaded area
Mean slope-normal cats = ---

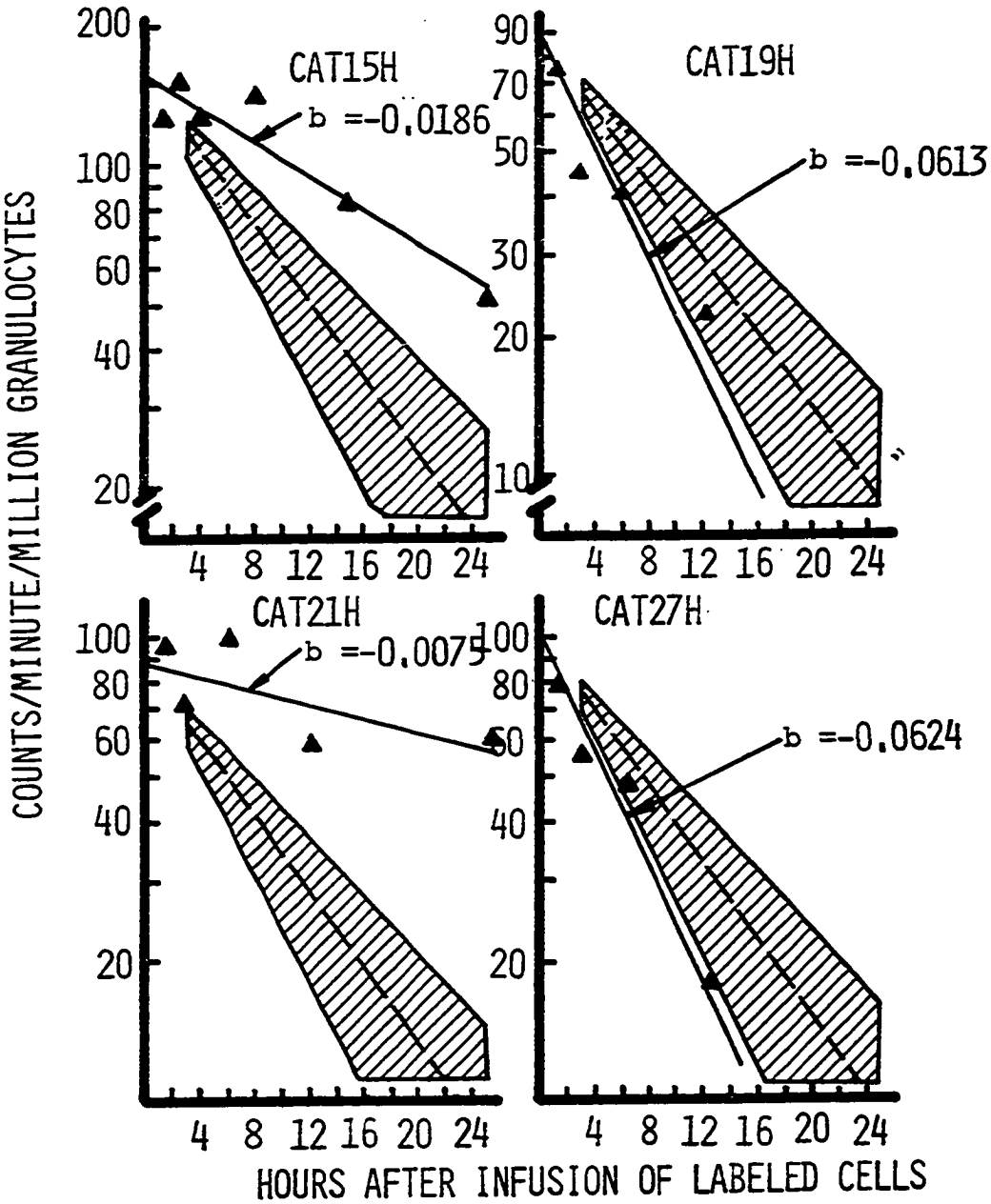
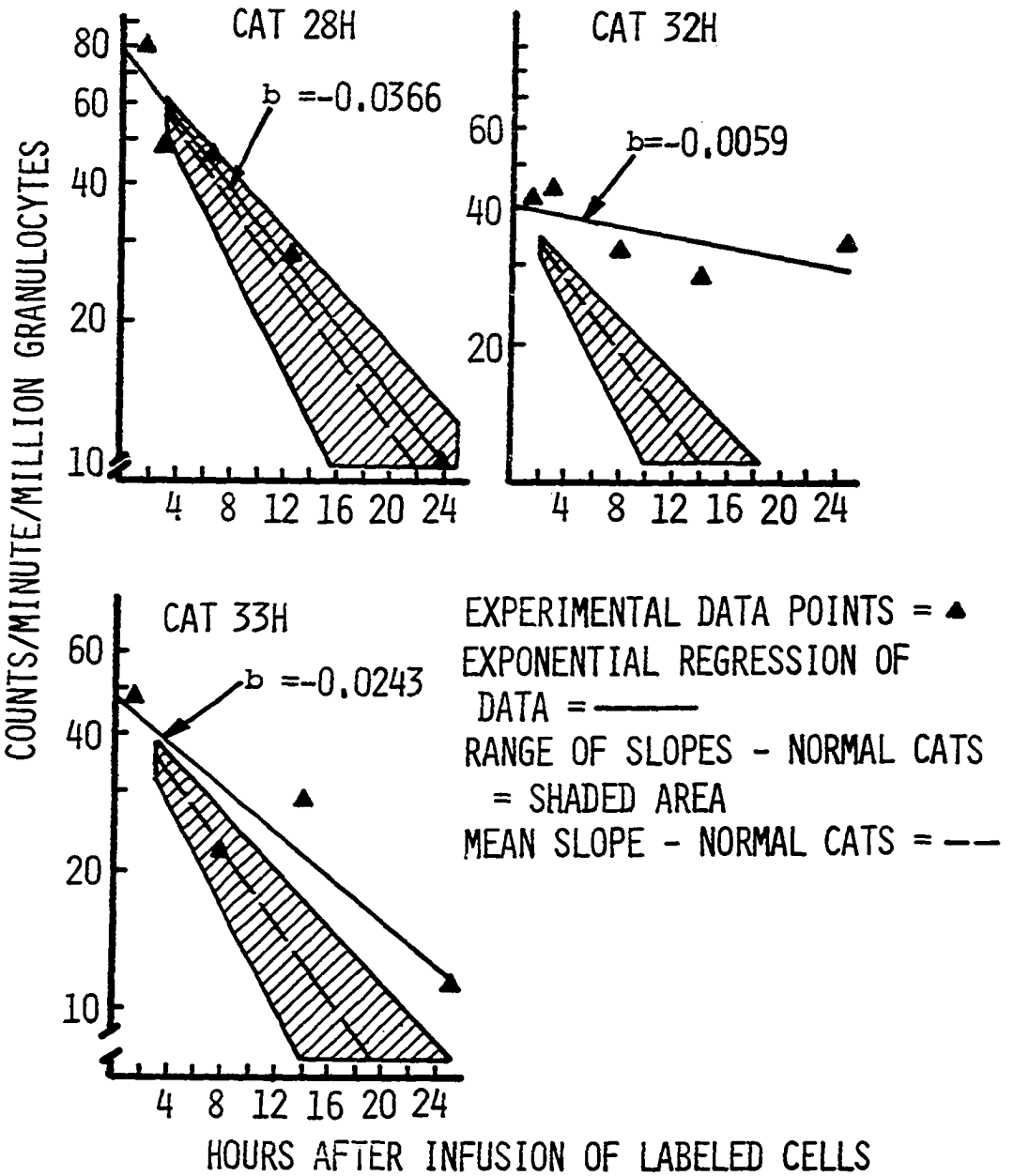

CAT15H
b =-0.0186
CAT19H
b =-0.0613
CAT21H
b =-0.0075
CAT27H
b =-0.0624
COUNTS/MINUTE/MILLION GRANULOCYTES
HOURS AFTER INFUSION OF LABELED CELLS
200
100
80
60
40
20
90
70
50
30
20
10
4 8 12 16 20 24

Figure 14. Granulocyte disappearance curves for cats 28H, 32H, and 33H. Comparison with mean slope and range of slopes in 5 normal cats


CAT 28H
b =-0.0366
CAT 32H
b=-0.0059
CAT 33H
b =-0.0243
COUNTS/MINUTE/MILLION GRANULOCYTES
80
60
40
20
10
4 8 12 16 20 24
EXPERIMENTAL DATA POINTS = ▲
EXPONENTIAL REGRESSION OF DATA = ———
RANGE OF SLOPES - NORMAL CATS = SHADED AREA
MEAN SLOPE - NORMAL CATS = ——
HOURS AFTER INFUSION OF LABELED CELLS

$T_{\frac{1}{2}}$ and a decreased GTR was observed in 4 cats: 15H (spontaneous hemorrhage), 21H, 32H, and 33H. TBGP was variable in these cats. Cats 21H, 32H, and 33H were those subjected to 6.1 to 6.5 ml/Kg experimental blood loss before t_o (Table 5). In contrast, cats 19H, 27H, and 28H, from which bleeding before t_o was of lower magnitude (Table 5), were found to have essentially normal granulocyte kinetic parameters. The calculated kinetic parameters for these contrasting groups of cats with experimental hemorrhagic blood loss and the mean values of the parameters in normal cats are listed in Table 6. Data from which the kinetic parameters were calculated for these cats may be seen in Table 17, Appendix. The differences between blood granulocyte pool sizes of normal cats (mean values) and cats 15H, 21H, 32H, and 33H are graphically illustrated in Figure 15.

Analysis of variance for $T_{\frac{1}{2}}$ and GTR between all normal cats (Table 3) and all cats which hemorrhaged (Table 6) revealed the following: $F = 5.20$ for $T_{\frac{1}{2}}$ which was significant at the 0.05 level; $F = 4.73$ for GTR which was significant at the 0.05 level. Subsequently the data for these 2 parameters were separated into that for normal cats, cats 15H, 21H, 32H, and 33H, and cats 19H, 27H, and 28H which were then compared with Duncan's multiple range test. Significant differences at the 0.05 level were found for both $T_{\frac{1}{2}}$ and GTR between normal cats 15H, 21H, 32H, and 33H, but not between normal cats and cats 19H, 27H, and 28H.

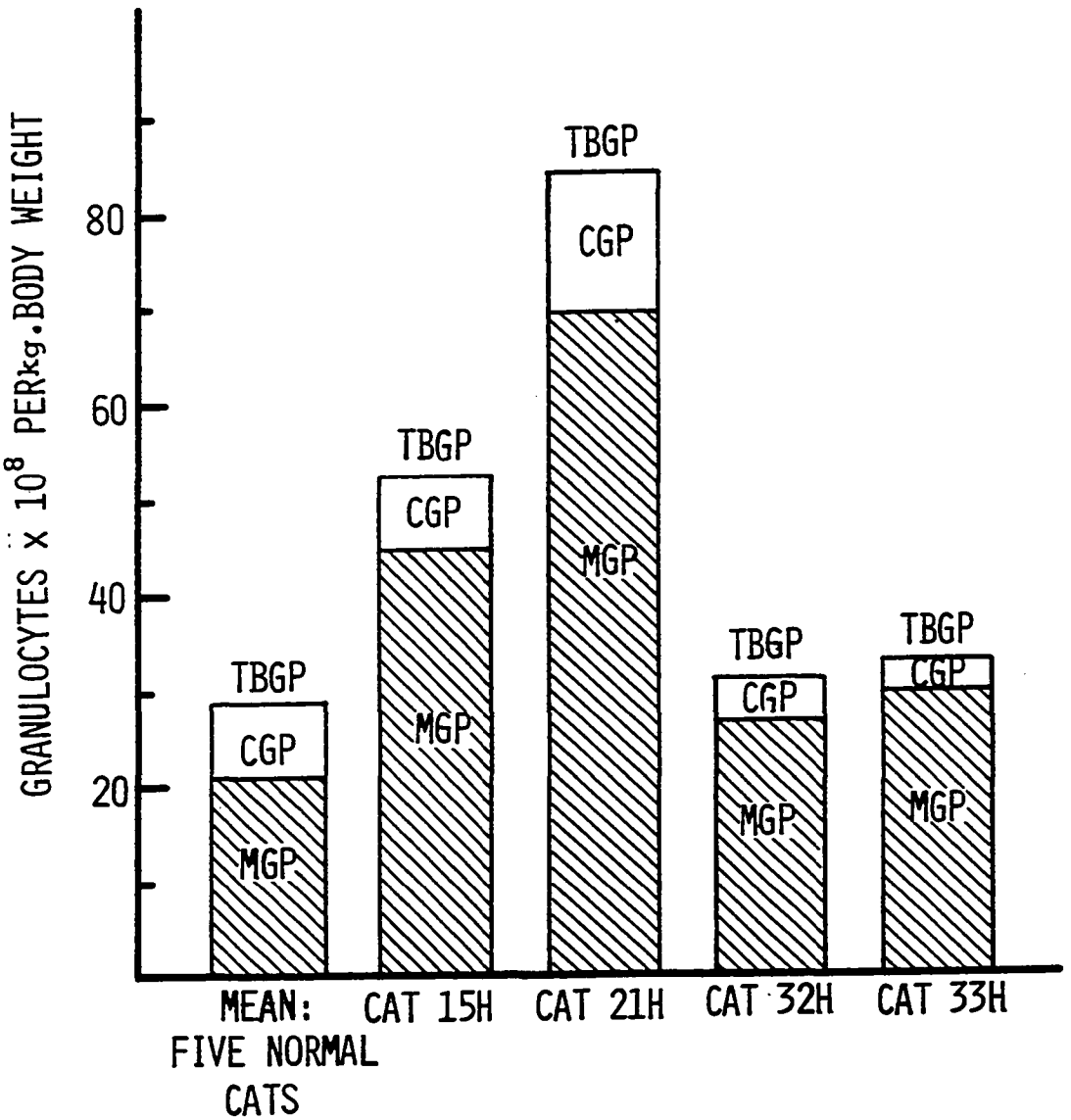
Table 6. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), CGP:TBGP ratio, half-life of blood granulocytes ($T_{\frac{1}{2}}$) and granulocyte turnover rate (GTR) in contrasting groups of cats in which the effects of hemorrhage on blood granulocyte kinetics were studied. Comparison with mean values in 5 normal cats

Cat	TBGP ($\times 10^8$/Kg)	CGP ($\times 10^8$/Kg)	MGP ($\times 10^8$/Kg)	CGP:TBGP	$T_{\frac{1}{2}}$ (hours)	GTR ($\times 10^8$/Kg/Day)
15H	52.27	7.50	44.78	0.14	16.2	53.72
21H	84.18	14.49	69.70	0.17	39.9	35.09
32H	31.46	4.92	26.54	0.16	59.1	10.25
33H	32.86	3.42	29.44	0.10	12.4	44.18
19H	20.47	6.14	14.33	0.30	4.9	69.30
27H	21.06	6.20	14.86	0.29	4.8	72.61
28H	32.74	9.55	23.18	0.29	8.2	66.27
Mean: 5 normal cats[a]	28.86	7.84	21.02	0.28	7.4	64.97

[a]Data from Table 3.

Figure 15. Total, circulating, and marginal granulocyte pool sizes in cats in which hemorrhage altered blood granulocyte kinetics. Compare with mean values in 5 normal cats

TBGP = TOTAL BLOOD GRANULOCYTE POOL
CGP = CIRCULATING GRANULOCYTE POOL
MGP = MARGINAL GRANULOCYTE



Blood Granulocyte Kinetics in Cats with Naturally Occurring Blood Dyscrasias

Absolute circulating granulocyte counts in cats 16FL, 17UL, and 20Ly before and after t_o are shown in Figure 16. There were differences in values of the granulocyte counts in these 3 cats. Cat 16FL was found to have a total leukocyte count of 39,700/cmm at 10 hours before t_o; the rectal temperature was 102°F at this time. By 13 hours after t_o the rectal temperature was 103.4°F, and the cat was observed to be depressed and anorectic. Cat 17UL, which was also observed to be neutrophilic during the trial, did not show clinical signs of illness. Cat 20Ly, which had confirmed lymphosarcoma, was neutropenic.

The granulocyte disappearance curves for these 3 cats are illustrated in Figure 17. The mean slope for normal cats and the slope of the curve for cat 16FL coincided. The slope of the curve for cat 17UL was moderately decreased and that for cat 20Ly was markedly decreased. It should be noted that the experimentally derived granulocyte specific activity data points in cat 20Ly were quite divergent (Figure 17). The leukocyte isolates from this cat were extremely low in granulocyte number due to the neutropenic state, which resulted in lower gross counts and greater error in scintillation counting.

The calculated blood granulocyte kinetic parameters for cats 16FL, 17UL, and 20Ly are listed in comparison with the

Figure 16. The absolute circulating granulocyte counts before and after pool size determination time in cats with naturally occurring blood dyscrasias

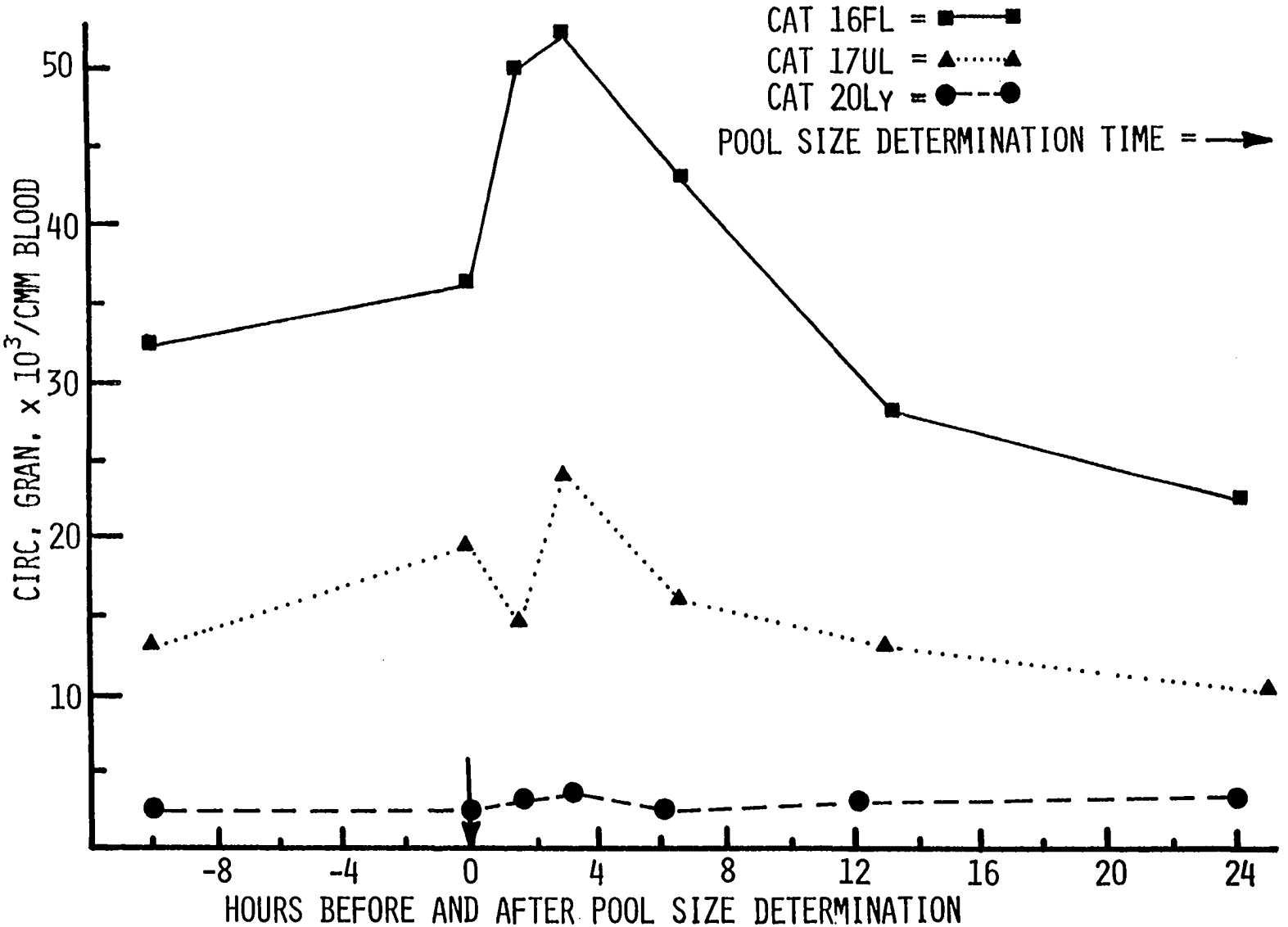
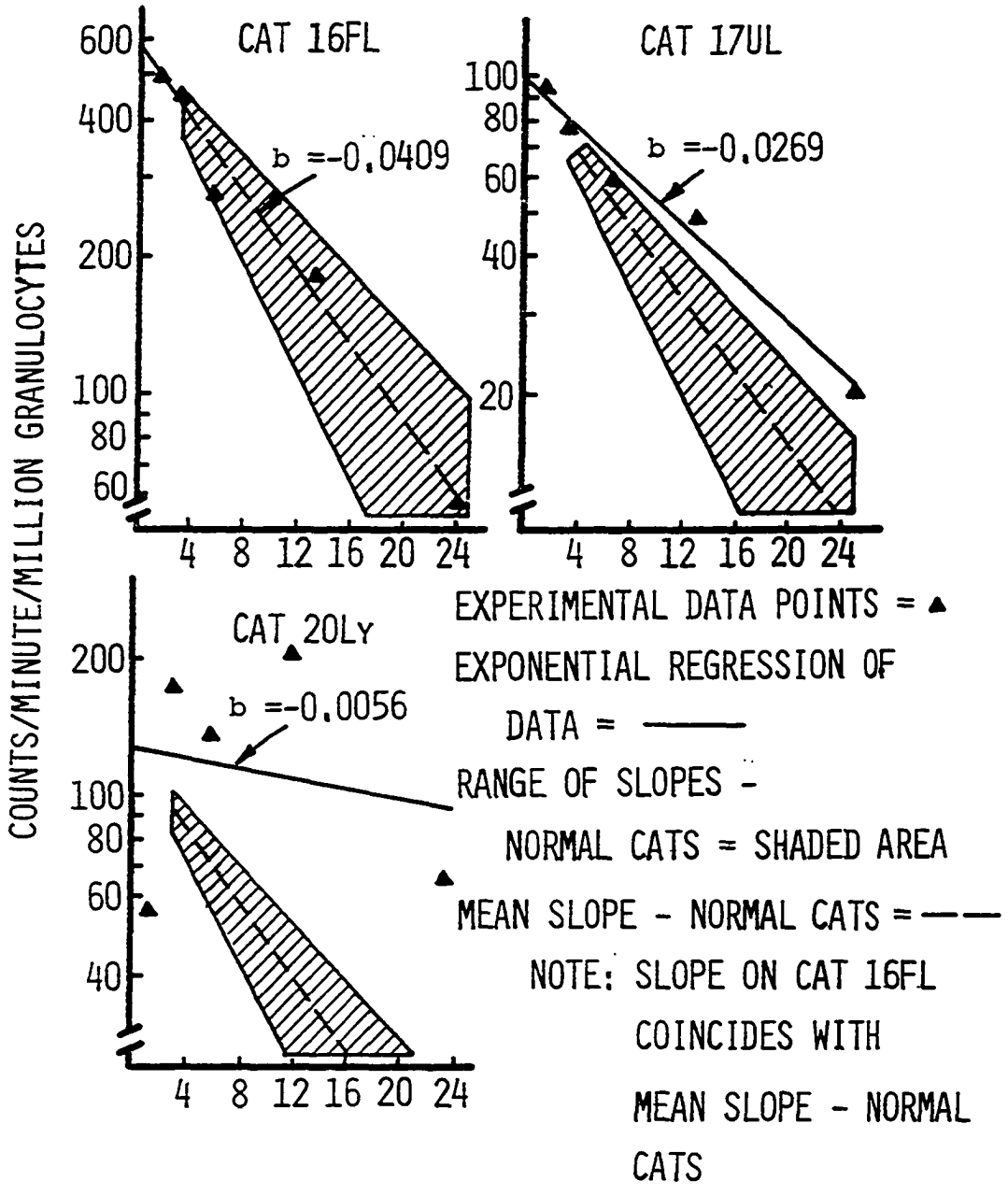

CAT 16FL =
CAT 17UL =
CAT 20LY =
POOL SIZE DETERMINATION TIME =
CIRC. GRAN. x 10^3/CMM BLOOD
50
40
30
20
10
-8
-4
0
4
8
12
16
20
24
HOURS BEFORE AND AFTER POOL SIZE DETERMINATION

Figure 17. Granulocyte disappearance curves in cats with naturally occurring blood dyscrasias. Comparison with mean slope and range of slopes in 5 normal cats


CAT 16FL
b =-0.0409
CAT 17UL
b =-0.0269
CAT 20LY
b =-0.0056
COUNTS/MINUTE/MILLION GRANULOCYTES
HOURS AFTER INFUSION OF LABELED CELLS
EXPERIMENTAL DATA POINTS = ▲
EXPONENTIAL REGRESSION OF DATA = ———
RANGE OF SLOPES - NORMAL CATS = SHADED AREA
MEAN SLOPE - NORMAL CATS = — —
NOTE: SLOPE ON CAT 16FL COINCIDES WITH MEAN SLOPE - NORMAL CATS

normal mean values in Table 7. Cat 16FL, which had a marked circulating neutrophilia during the trial (Figure 16) was found to have a TBGP within the normal range (Table 3); the greater proportion of granulocytes in the TBGP of this cat was present in the circulating pool of cells; with a normal TBGP and $T\frac{1}{2}$ the calculated GTR was also normal. Cat 17UL, in which neutrophilia was of lower magnitude than in Cat 16FL, (Figure 16), had a calculated CGP slightly above the range in normal cats (Table 3), and had an increased TBGP with increased proportion of cells in the MGP. Since $T\frac{1}{2}$ was near normal and the TBGP was increased, the calculated GTR was greatly increased. Cat 20Ly, which was neutropenic, had a markedly decreased TBGP, but the cells were distributed between CGP and MGP in normal proportion; note that $T\frac{1}{2}$ was greatly prolonged in this cat. Data from which the granulocyte kinetic parameters were determined in cats 16FL, 17UL, and 20Ly may be seen in Table 18, Appendix. The pool sizes for these 3 cats are graphically illustrated in comparison with mean values for pool sizes in normal cats in Figure 18.

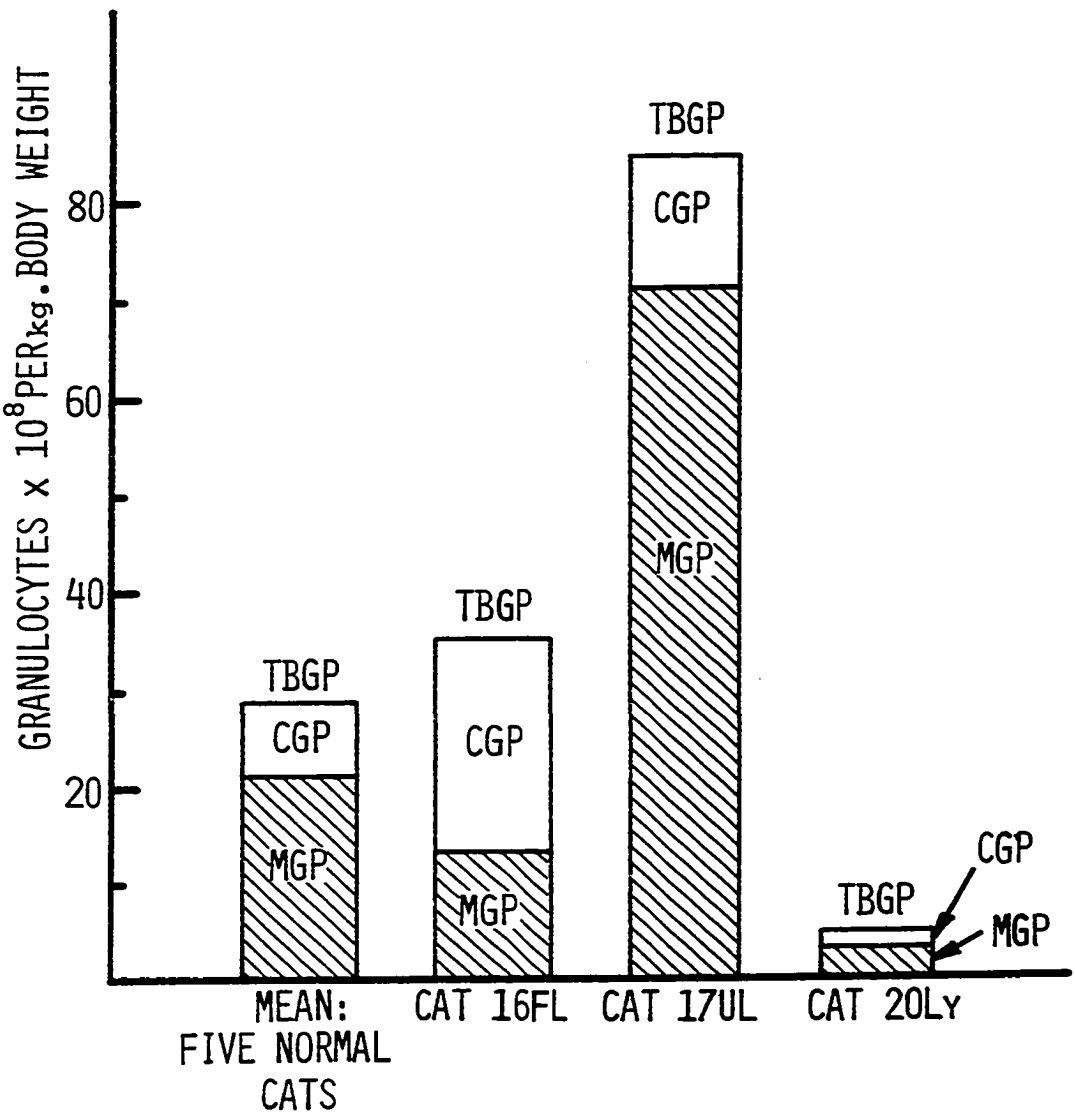
Table 7. Total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), CGP:TBGP ratio, half-life of blood granulocytes ($T_{\frac{1}{2}}$) and granulocyte turnover rate (GTR) in 3 cats with naturally occurring blood dyscrasias. Comparison with mean values in 5 normal cats

Cat	TBGP ($x10^8$/Kg)	CGP ($x10^8$/Kg)	MGP ($x10^8$/Kg)	CGP:TBGP	$T_{\frac{1}{2}}$ (hours)	GTR ($x10^8$/Kg/Day)
16FL	37.63	24.28	13.35	0.65	7.4	84.98
17UL	84.78	13.40	71.38	0.10	11.2	125.80
20Ly	4.50	1.32	3.17	0.29	53.7	1.39
Mean: 5 normal cats[a]	28.86	7.84	21.02	0.28	7.4	64.97

[a]Data from Table 3.

Figure 18. Total, circulating, and marginal granulocyte pool sizes in cats with naturally occurring blood dyscrasias. Comparison with mean values in 5 normal cats

TBGP = TOTAL BLOOD GRANULOCYTE POOL
CGP = CIRCULATING GRANULOCYTE POOL
MGP = MARGINAL GRANULOCYTE POOL



DISCUSSION

The findings in cats with respect to the intravascular distribution of neutrophilic granulocytes are similar to those reported for man and dog (Athens et al., 1961a; Athens et al., 1961b; Alexanian and Donohue, 1965; Raab et al., 1964). However, a major difference, apparently characteristic for the species, is the large MGP in cats. About 30% of the labeled granulocytes could be accounted for in the circulating blood of cats following infusion (Table 3, CGP:TBGP ratio). This indicates that a substantial number of cells in the TBGP resides in the marginal pool. This contrasts with findings in man and dog, for which CGP:TBGP ratios are closer to 1:2 (see Table 8 for comparison of TBGP, CGP, and MGP in man, dog and cat).

The species differences for CGP, the product of the circulating granulocyte count and the blood volume, may be explained by the unequal granulocyte counts used to calculate the pool size in the studies (Table 8). The mean circulating granulocyte count for the normal cat group was about 10,000/cmm (Figure 4) which was within the accepted normal range for cats. The mean count given by Alexanian and Donohue (1965) for man was 4,450/cmm; that for dogs was 7,350/cmm (Raab et al., 1964). These values differ by an order of magnitude similar to that for the CGP between species listed in Table 8. Thus, the apparent CGP values do not reflect a fundamental species dif-

Table 8. Comparison of total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP) and marginal granulocyte pool (MGP) in man, dog, and cat

	TBGP $x10^8$/Kg	CGP $x10^8$/Kg	MGP $x10^8$/Kg
Man[a]	6.53	3.07	3.46
Man[b]	6.80	2.90	3.90
Dog[c]	10.20	5.40	4.80
Cat[d]	28.86	7.84	21.02

[a]Mean, 45 subjects, Athens et al., 1961b.

[b]Mean, 12 subjects, Alexanian and Donohue, 1965.

[c]Mean, 31 dogs, Raab et al., 1964.

[d]Mean, 5 cats, from Table 3.

ference in granulocyte kinetics.

The reason for the larger MGP in cats compared to that of dog and man is not apparent from the results in this study. Yet, the significance of its size may be of great importance in interpretation of hematological data. For example, neutrophilic leukocytosis, which may be a manifestation of physiological changes induced by fear, apprehension or struggling while being handled, is extremely common in cats (Schalm and Smith, 1963; Schalm, 1965). Athens et al. (1961b) have demonstrated that this type of neutrophilia in man is simply mobili-

zation of cells from the MGP to the CGP. Certainly, the large MGP observed in cats may provide an explanation for the relative ease with which physiologic leukocytosis occurs in this species.

The findings with respect to the intravascular half-life of granulocytes in cats are also similar to those in dog and man. The mean $T_{\frac{1}{2}}$ for the normal group (Table 3) is in close agreement with values reported by Athens et al. (1961a) for man and Raab et al. (1964) for dog. The exponential disappearance rate of labeled granulocytes, from which $T_{\frac{1}{2}}$ is calculated, indicates random loss of cells from the blood of cats, as in dog and man (Figures 5 and 6). This interpretation is based on the assumption that elution of label from infused cells does not occur. The assumption is supported by the fact that the DFP-cytoplasmic bond is irreversible, remaining intact for the life of the protein (Athens et al., 1959b). Furthermore, when the protein is degraded in the body, diisopropylphosphate (DIP) is released; DIP does not react with protein and is promptly excreted in the urine (Cohen and Warringa, 1954).

Thus it can be seen that the fundamental features of blood granulocyte kinetics in cats, i.e., compartmentalization and random loss of cells, parallel those in man and dog. Therefore, the results obtained for the normal group, as compared to the endotoxin-treated group or those with naturally occurring blood dyscrasias, may be logically evaluated to gain in-

sight into mechanisms which underlie neutrophilic or neutropenic responses in cats.

The differences among cats in the normal group for pool sizes, $T\frac{1}{2}$ and GTR (Table 3) may be explained by natural biological variation. Although some of the variation could have been due to physiologic leukocytosis, the fact that mean granulocyte counts remained within normal ranges during these trials (Figure 4) would indicate that this factor did not influence the results. In fact, most of the experimental animals were calm to the point of purring and actively seeking affection during the handling procedures.

The CGP:TBGP ratio for cat 16FL leads to the suggestion that an early kinetic change in acute illness in cats is mobilization of cells from the MGP to the CGP (Table 7, Figure 18), which causes the observed neutrophilia. Furthermore, an acute illness, perhaps inflammatory in nature, would be expected to result in an increased peripheral demand for neutrophils and result in an increased GTR. The GTR in this cat (Table 7), although near 1 standard deviation of the normal group mean (Table 3), was slightly elevated, suggesting an increased peripheral utilization of cells.

The observed shift of cells from the MGP to the CGP is typical for physiologic leukocytosis as described by Athens et al. (1961b). However, Schalm and Smith (1963) state that the tendency for physiologic leukocytosis to occur in cats is diminished in acute illness.

The above interpretation is in disagreement with the observations of Marsh et al. (1967). They have interpreted data from experimentally infected dogs in which an early increase in the MGP preceded an increase in the CGP. Margination is logically an essential step before diapedesis occurs. However, the compartmentalization model for blood granulocytes, i.e., TBGP composed of CGP and MGP, implies free exchange of cells between CGP and MGP, and may be interpreted to indicate that cells leave the circulation from either pool (Cronkite and Vincent, 1970). Although the primary alteration noted in cat 16FL was a shift in cells from the MGP to the CGP, perhaps the true initial response had been completed by the time labeling procedures were initiated. The true nature of this initial response in cats could be clarified in greater detail with experimental production of inflammation.

Neutrophilia in chronic infection is characterized by increased TBGP, increased GTR, and moderately prolonged $T\frac{1}{2}$ in man (Athens et al., 1965; Galbraith et al., 1965). The findings in cat 17UL were similar (Table 7, Figures 16, 17, and 18). The size of the TBGP is dependent on the balance between the rate of efflux from the blood and the bone marrow release rate. The decreased slope of the granulocyte disappearance curve (Figure 17, cat 17UL) implies a slowed rate of efflux from the blood, yet the GTR was greatly increased in this cat. Cronkite and Vincent (1970) suggest that in order to account for the prolonged $T\frac{1}{2}$ noted in infection one must conclude that

generalized random loss is curtailed. Since no outward signs of illness were observed, no conclusions can be made that an infection was in progress in this cat.

Athens et al. (1961a) studied the effects of endotoxin on blood granulocyte kinetics in man in order to establish a model for changes which may occur in infection. A similar approach was attempted in this study. However, interpretation of the results will be directed at the specific effects of endotoxin. Additional studies in infected cats will be necessary before corollary conclusions can be attempted.

The systemic reaction observed following bacterial endotoxin administration in cats was typical of that described for other experimental animals (Braude, 1964). The classical reaction is characterized by fever and shock resulting from a large, precipitous fall in blood pressure (Kuida et al., 1961).

The shift of cells from the CGP to the MGP in cat 23E (Table 4, Figures 8 and 10), in which labeling procedures were instituted before complete recovery from the neutropenic stage of the endotoxin reaction, implies that margination of circulating granulocytes had occurred. This shift in the neutropenic stage has been described for endotoxin-treated humans (Athens et al., 1961a). The reasons for the observed margination of cells are unknown, but it is possible that either circulatory changes in pulmonary vasculature or direct endotoxin effect on granulocytes may be involved.

The change in pulmonary vasculature may be coincidental with margination of granulocytes in the lungs resulting in the observed neutropenia. Kuida et al. (1961) ascribed the early precipitous hypotension in cats to pulmonary vascular constriction and acute right ventricular hypertension and failure, rather than splanchnic pooling of blood as described in other species. The pulmonary blood vessels are known to be a significant site for sequestration of granulocytes. Bierman et al. (1952) observed a marked decrease in granulocyte count on the arterial side of the lungs during forced inspiration, without coincident change in the count on the venous side; a reversal of these results was obtained during forced expiration. These findings illustrate the degree to which dynamic changes in the pulmonary circulation may influence the circulating granulocyte count in endotoxin-treated cats.

The existence of a direct effect of endotoxin on granulocytes is still somewhat problematical. Whether the cells are direct or indirect targets of an endotoxin action is still unknown. Carey et al. (1958) detected radioactivity in leukocytes immediately after intravenous administration of isotopically labeled endotoxin, but no definitive conclusions as to whether this was a direct effect were possible. Presumably, marginated granulocytes are destroyed and do not return to the CGP. This is substantiated by the normal granulocyte disappearance curve determined for cat 23E (Figure 9). If granulo-

cytes were returning to the CGP in this cat, it would have been reflected in this curve as a decreased slope.

Following the neutropenic stage of the endotoxin reaction, bone marrow is in some way stimulated to release large numbers of cells. The labeling procedures were initiated during this stage of the reaction in cats 24E and 29E (Figure 8). The observed increase in TBGP and GTR in these cats (Table 4, Figures 9 and 10) is similar to those described for the same stage in man (Athens et al., 1961a). Herion et al. (1965) and Cronkite and Vincent (1970) have proposed that neutropenia itself may be a sufficient stimulus for the increased release rate of bone marrow granulocyte reserves. However, King (1960) has demonstrated that material with properties of an endogenous pyrogen, extracted from rabbit leukocytes or obtained from serum 2 hours after injecting endotoxin, produces a prompt granulocytosis when given intravenously to rabbits. These findings indicate that a substance liberated from granulocytes themselves, perhaps as a consequence of massive granulocyte death following the margination event, may be in part responsible for the increased bone marrow release rate.

The suggestions offered above would explain the developing neutrophilia and the increased GTR observed in cats 24E and 29E (Table 4, Figure 8). However, neither neutropenia alone nor massive granulocyte death, which apparently occurs, is adequate to explain the lack of neutrophilic response in cat 23E (Figure 8). The apparent inconsistency cannot be clarified from eval-

uation of the results in this study.

Leukopenia, although most frequently associated with infectious feline enteritis in young cats, is commonly observed in feline patients of all ages (Schalm and Smith, 1963). Study of granulocyte kinetics in any neutropenic animal is difficult due to problems encountered in isolation of sufficient numbers of granulocytes for accurate scintillation counting, as illustrated by the divergent data for cat 20Ly (Figure 17). However, it can be seen that the GTR was extremely low in this cat (Table 7) indicating a diminished release of granulocytes from the bone marrow. Furthermore, the prolonged $T\frac{1}{2}$ (Table 7) was indicative of retention of granulocytes in the circulation. This suggests the existence of some mechanism whereby the body attempts to maintain a population of granulocytes in the blood in spite of diminished influx from the marrow. Another possibility is that the stress produced by systemic disease, lymphosarcoma in this case, results in an increased production of endogenous corticosteroids, which have been shown to decrease the rate of granulocyte efflux from the blood (Athens et al., 1961a; Bishop et al., 1968).

Athens (1969) reports observing 2 types of neutropenic human patients with respect to blood granulocyte kinetics. The first of these was characterized by depression of granulopoiesis, low circulating granulocyte count and TBGP, but the $T\frac{1}{2}$ was below normal. The second type was characterized by an increased GTR and an extremely short $T\frac{1}{2}$; the latter type

of neutropenia apparently results from rapid granulocyte utilization and destruction in suppurative disorders, in which the ability of the bone marrow to replace cells is exceeded. A prolonged $T_{\frac{1}{2}}$ has apparently not been observed in neutropenic human patients.

While the mechanisms involving neutrophilia and, in some instances, neutropenia, with respect to blood granulocyte kinetics, have received considerable attention, little is known regarding the effects of hemorrhage. Hemorrhage is known to result in an increased circulating granulocyte count in man (Wintrobe, 1961) and in animals (Schalm, 1965). Although all experimentally bled cats tended to develop neutrophilia during the respective experimental trials (Figures 11 and 12), the data indicate that a critical amount of blood loss, before t_o, was necessary to induce changes in granulocyte kinetic parameters. This critical level of blood loss was about 7 to 8% of the blood volume (calculated from the data in Table 5 and Table 17, Appendix).

Evaluation of the kinetic parameters for those cats bled sufficiently to induce changes (cats 15H, 21H, 32H, and 33H; Table 6, Figures 13, 14, and 15) leads to the following hypothesis. Changes in blood granulocyte kinetics may be a manifestation of significant loss of erythrocytes, which are of more immediate concern to life than are neutrophilic granulocytes. The prolonged $T_{\frac{1}{2}}$ and the decreased GTR, determined in these cats, may reflect a diminished demand for granulo-

cytes, which in turn would free the bone marrow for more intense erythropoiesis to meet the life-deterring loss of erythrocytes.

The work reported by Lawrence and Craddock (1968), who demonstrated suppression of granulopoiesis in guinea pigs following acute massive hemorrhage, supports this contention. They suggested suppression of granulopoiesis occurs because myeloid and erythroid tissues are in competition for a limited space. During periods of intense demand for erythrocytes, multipotential stem cells would be stimulated to differentiate toward erythropoiesis at the expense of granulopoiesis. Furthermore, their data led to the proposal that inhibition of granulocyte outflow from the bone marrow also occurred in blood depleted guinea pigs.

The stimulus for depression of bone marrow outflow, i.e., diminished influx to the blood, may be secondary to the reduction of granulocyte efflux from the blood. This is implied by the decreased slopes of granulocyte disappearance curves and longer half-lives (Table 6, Figures 13 and 14) for cats 15H, 21H, 32H, and 33H. The reduction in granulocyte efflux may have been induced by an adreno-cortical response to the stress of bleeding. Athens et al. (1961a) and Bishop et al. (1968) have demonstrated this effect of corticosteroids. However, they have also shown that corticosteroids increase the rate of influx of cells from the bone marrow, which contradicts the findings in these cats. The role of corticosteroids

in this mechanism might be clarified by repeating the kinetic studies in hemorrhaged, adrenalectomized cats.

Cats with decreased granulocyte turnover rates and prolonged granulocyte half-lives caused by experimental hemorrhage were also found to have an increased proportion of cells in the MGP; in some instances the MGP was actually increased in size (Table 6, Figure 15). This may be explained by consideration of the effects of hemorrhage on the blood circulatory system. According to Guyton (1961) about 10% of the blood volume can be lost with no appreciable effect, but further loss usually diminishes the cardiac output first and then the blood pressure; diminished cardiac output and blood pressure result in sluggish flow of blood through minute vessels. Vejlens (1938) demonstrated that when the velocity of blood flow is reduced, granulocytes tend to assume a marginal position, become adherent to vessel walls and are withdrawn from the axial stream. These findings provide a logical explanation for the observed margination of granulocytes in cats with 7 to 8% of the blood volume removed before pool size determination time.

Gaylor *et al.* (1969), who studied total marrow granulocyte numbers and band/segmented neutrophil ratios in blood after hemorrhage in mice, proposed a sequence of events which is at variance with the findings in this study. They proposed, without additional evidence, that hemorrhage leads to sudden changes in vascular tone, which causes demargination of granu-

locytes producing the observed circulating neutrophilia. However, the observed neutrophilias in cats in this study (Figures 11 and 12) were not due to demargination as can be seen from the MGP values (cats 15H, 21H, 32H, and 33H, Table 6). Apparently the decreased rate of efflux from the blood resulted in a slight increase in TBGP and CGP with time.

On the basis of these results, the suggested sequence of events governing the kinetic changes observed in hemorrhaged cats is:

(1) blood loss of 7 to 8% of the blood volume leads to changes in circulatory dynamics, which in turn causes margination of granulocytes;

(2) inhibition of the rate of egress from the blood occurs perhaps as a result of a stress-induced, adreno-cortical response, which in turn diminishes the need for granulocytes in the blood and GTR declines;

(3) with decreased peripheral loss of cells the bone marrow release rate diminishes, and this allows for more intense erythropoietic effort necessary to meet the demand for erythrocytes caused by blood loss.

Certain technical problems were successfully overcome in adaptation of the procedures of Athens and co-workers to this study of blood granulocyte kinetics in cats, e.g., (1) a quantitative recovery of granulocytes from small volumes of blood was accomplished and (2) an exact measurement of granulocyte specific activity provided for accuracy of results. This

approach substantiates the ideas of Deinard and Page (1970) who desired better methods for studying granulocyte kinetics of neutropenia in man. Thus, the successful adaptation of procedures to a smaller laboratory species, such as the cat, may lead to more exhaustive research on the intravascular survival and distribution of neutrophilic granulocytes.

PART II. KINETICS OF GRANULOPOIESIS IN CATS

EXPERIMENTAL DESIGN

The progress of ^{3}H-Tdr-labeled cells through the catenated compartments of granulopoiesis was studied in cats. This experimentation was conducted in 2 groups of cats, control and endotoxin-treated, and in 2 individual cats with naturally occurring blood dyscrasias. The observations on granulopoiesis in these cats were compared to results predicted by a hypothetical model of granulopoiesis. From this comparison transit times for cells within the various cytologic compartments were estimated.

Experimentation in Cats

The control goup was comprised of 4 clinically and hematologically normal cats. However, the stress produced by repeated light anesthesia necessary to facilitate collection of bone marrow samples, resulted in slight elevation of circulating granulocyte counts. Therefore, rather than "normal", the term "control" will be used to identify this group, since all cats studied were subject to this treatment.

Three cats were each treated with 3 mg/Kg of bacterial endotoxin[1] intravenously 30 minutes after injection of ^{3}H-Tdr. This treatment was intended to create a peripheral demand for granulocytes greater than that for the control group. These

---

[1] *Escherichia coli* endotoxin, serotype 055:B5, Difco Laboratories, Detroit, Michigan.

3 cats will be referred to as the endotoxin-treated group.

One cat with confirmed lymphosarcoma was studied and will be referred to as the lymphosarcoma cat.

Another individual cat was presented to the Department of Veterinary Pathology with the following history. The cat had been periodically depressed and anorectic during the previous year. During this time a mild, progressive neutropenia had developed. The neutropenic state was severe when the radioisotopic study of granulopoiesis was initiated. A myeloproliferative disease, characterized by myelofibrosis and hyperplasia of hematopoietic tissues, was diagnosed at completion of the study. This subject will be referred to as the myelofibrosis cat.

Bone marrow samples were collected from each cat 0.5, 2, 6, 12, 24, 36, 48, 72, 96, and 120 hours after in vivo labeling of granulocyte precursors with ^{3}H-Tdr. These sample times were similar to those selected by Patt and Maloney (1959b), who conducted an autoradiographic study of granulopoiesis in dogs. The shorter sampling intervals early in the experimentation provided for study of anticipated, rapid change in the number of labeled cells within the immature cytologic compartments of granulopoiesis. Blood samples were taken at each 12 hour interval through 120 hours after labeling to determine the emergence time of labeled granulocytes. Autoradiograms were prepared from all blood and bone marrow samples.

A differential granulocyte precursor count was conducted on each bone marrow autoradiogram. The cytologic compartments were assigned identification letters: A = myeloblasts plus progranulocytes, B = neutrophilic myelocytes, C = neutrophilic metamyelocytes, D = band neutrophils and E = segmented neutrophils. The numbers of myeloblasts and progranulocytes were combined because of their small number and difficulty in separating them cytologically in the autoradiograms. The number of segmented neutrophils encountered was increased and variable in the fifth through tenth bone marrow samplings from each cat. This apparently resulted from sample dilution with peripheral blood. Therefore, a method was devised, whereby the differential counts were corrected for blood dilution, and normalized numbers of labeled granulocyte precursors were mathematically derived for each sample time.

Calculation of normalized numbers of labeled granulocyte precursors and correction for blood dilution was as follows:

(1) A minimum of 250 cells in the A through D compartments was differentially counted at each sample time. The number of segmented neutrophils, E, was also counted, but not included in the above total, which was established to ensure uniformity of counts in the precursor compartments.

(2) The total number and the number of labeled cells in each compartment were tallied for each sample time and the percentage of labeled cells in each compartment, A through E, was calculated.

(3) The mean differential count, including compartments A through E, was determined from the first 4 samples, which were not adversely affected by blood dilution. The number of cells for each compartment in the mean differential count was multiplied by the respective percentage of labeled cells obtained for all 10 samples. The products were normalized numbers of labeled cells for compartments A through E for the 10 sample times.

The above procedure was conducted for each cat. The arithmetic mean and range of normalized numbers of labeled cells were determined for the control group and the endotoxin-treated group. The normalized numbers for the lymphosarcoma cat and the myelofibrosis cat were evaluated separately.

The 10 sequential blood smear autoradiograms for each cat were examined until a minimum of 150 segmented neutrophils had been encountered at each sample time. The sample time during which labeled segmented neutrophils were first encountered was taken to be the emergence time of segmented neutrophils in the cat's blood. In like manner the emergence time of band neutrophils was determined. Mean emergence times for band neutrophils and segmented neutrophils were calculated in the control group and the endotoxin-treated group. These appearance times were evaluated separately in the lymphosarcoma and myelofibrosis cat.

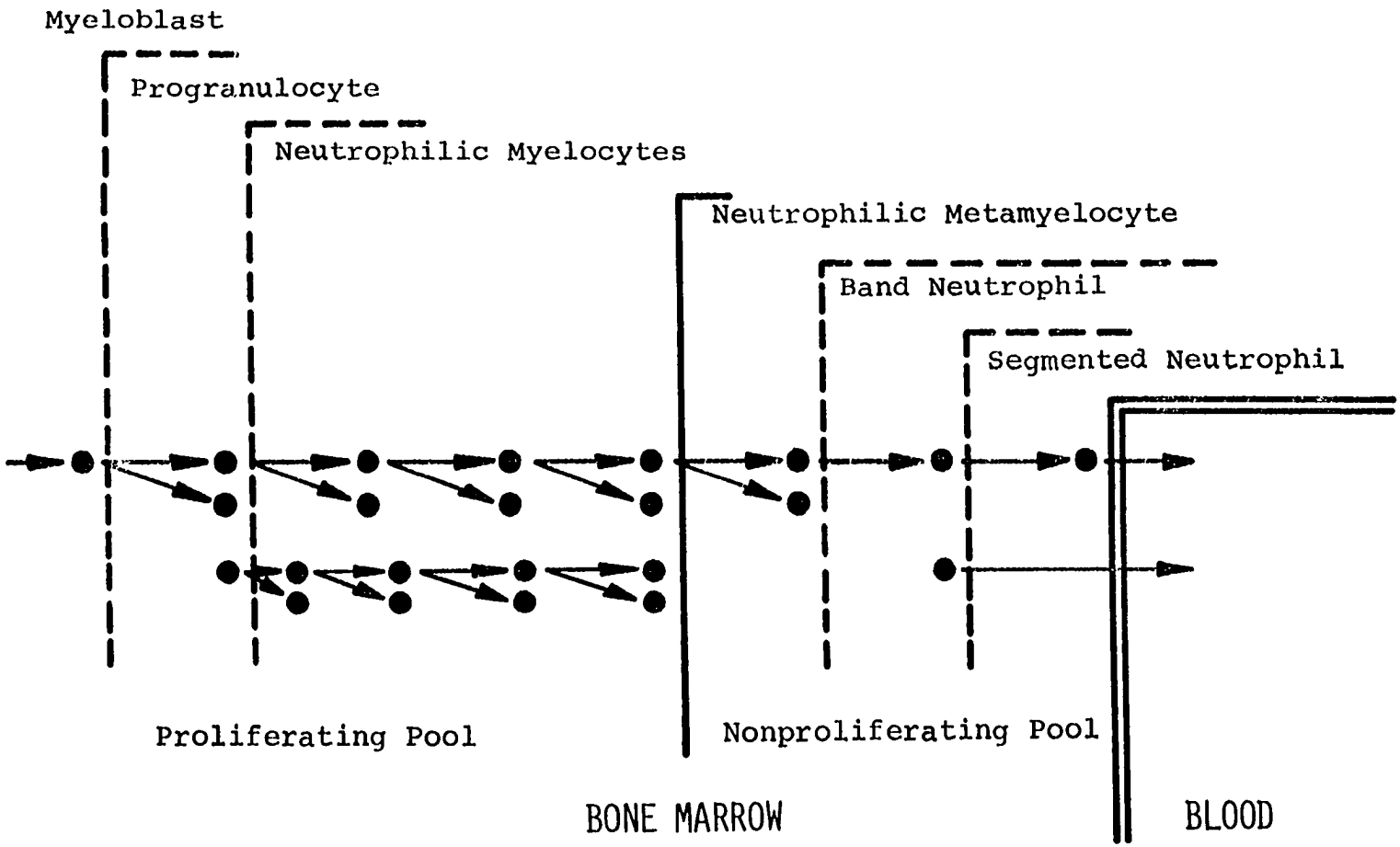
Description of the Hypothetical Model of Granulopoiesis

A model of granulopoiesis, similar to that described for man by Cronkite and Vincent (1970), was studied. The experimental observations on granulopoiesis in cats were compared to those predicted by the model which is illustrated schematically in Figure 19. Note that in the proliferating pool myeloblasts and progranulocytes are represented as having one generation each. Neutrophilic myelocytes have 3 generations. Furthermore, the scheme illustrates the possibility for shortening the first myelocyte generation, thereby permitting an additional mitosis before cells become members of the second myelocyte generation. The potential for cell production could in this way be increased twofold.

In the nonproliferating pool (Figure 19) cells are illustrated as maturing through 3 cytological compartments before being released to the blood. However, the arrow from a band neutrophil to the blood represents the capacity for release of immature cells if this is required by peripheral demand for granulocytes.

The descriptive terminology proposed by Killmann *et al.* (1963) with reference to division of cells will be used, i.e., daughter cells, which cytologically resemble mother cells, arise by homomorphogenic division and daughter cells, which resemble members of a following cytologic compartment, arise by heteromorphogenic division. In the latter situation the

Figure 19. Schematic representation of the hypothetical model of granulopoiesis. Note cytologic compartments are separated by dashed and solid lines; neutrophilic myelocytes have 3 generations, but the first generation time may shorten to allow for an additional division; arrow from a band neutrophil to the blood represents the capacity to release immature cells


Myeloblast
Progranulocyte
Neutrophilic Myelocytes
Neutrophilic Metamyelocyte
Band Neutrophil
Segmented Neutrophil
Proliferating Pool
Nonproliferating Pool
BONE MARROW
BLOOD

change in cytologic characteristics on which cell classification is based coincides in time with mitosis.

A mathematical representation of the granulopoietic model was based on the following assumptions:

(1) When ^{3}H-Tdr is available for a brief period of time only cells in DNA synthesis will be labeled. Therefore, cells in the DNA synthesis stage within the proliferating pool take up the label. Cells in the nonproliferating pool do not take up the label.

(2) Cells in the myeloblast and progranulocyte compartments and third-generation neutrophilic myelocytes leave their respective compartments by heteromorphogenic division. First- and second-generation myelocytes move respectively to second- and third-generations by homomorphogenic division.

(3) The number of labeled cells in each compartment at any time (t), after the initiation of the procedure by pulse labeling with ^{3}H-Tdr, can be related as follows:

   (a) The number of labeled myeloblasts (A_1) = the number originally labeled minus those leaving the compartment by heteromorphogenic division.

   (b) The number of labeled progranulocytes (A_2) = the number originally labeled, plus those arriving from heteromorphogenic division of A_1 cells minus those leaving the compartment by heteromorphogenic division.

(c) The number of labeled first-generation neutrophilic myelocytes (B_1) = the number originally labeled, plus those arriving by heteromorphogenic division of A_2 cells minus those leaving the first-generation by homomorphogenic division. The number may be enhanced by an additional mitosis within the first generation.

(d) The number of labeled second-generation neutrophilic myelocytes (B_2) = the number originally labeled, plus those arriving by homomorphogenic division of B_1 cells minus those leaving the second-generation by homomorphogenic division.

(e) The number of labeled third-generation neutrophilic myelocytes (B_3) = the number originally labeled, plus those arriving by homomorphogenic division of B_2 cells minus those leaving the compartment by heteromorphogenic division.

(f) The number of labeled neutrophilic metamyelocytes (C) = the number arriving from heteromorphogenic division of B_3 cells minus those leaving the compartment by maturation.

(g) The number of labeled band neutrophils (D) = the number arriving from maturing C cells, minus those leaving the compartment by maturation and minus those being released to peripheral blood.

(h) The number of labeled segmented neutrophils (E) =

the number arriving from maturing D cells minus those being released to peripheral blood.

(4) The above relations can be written in differential form over a time interval (dt), by assuming that the change in the number of labeled cells in any compartment is proportional to the number itself and to the rate at which cells enter the compartment by division or by maturation of progenitors. These first-order kinetic equations are as follows:

$\frac{dA_1}{dt} = -K_1A_1$, where A_1 at the time of labeling (t_o) = a number, A_1^o,

$\frac{dA_2}{dt} = 2K_1A_1-K_2A_2$, where $A_2\ (t_o)$ = a number, A_2^o,

$\frac{dB_1}{dt} = 2K_2A_2-K_3B_1(+R_1B_1)$, where $B_1(t_o)$ = a number, B_1^o,

$\frac{dB_2}{dt} = 2K_3B_1-K_4B_2$, where $B_2(t_o)$ = a number, B_2^o,

$\frac{dB_3}{dt} = 2K_4B_2-K_5B_3$, where $B_3(t_o)$ = a number, B_3^o,

$\frac{dC}{dt} = 2K_5B_3-K_6C$, where $C(t_o) = 0$,

$\frac{dD}{dt} = K_6C-K_7D-R_2D$, where $D(t_o) = 0$,

$\frac{dE}{dt} = K_7D-K_9E$, where $E(t_o) = 0$.

In these equations:

R_1 = the rate constant for the additional first-generation

neutrophilic myelocyte, hours $^{-1}$.

R_2 = the rate constant for release of band neutrophils to the blood, hours $^{-1}$.

K_i = the rate constant for the ith generation or compartment, hours $^{-1}$.

t = time, hours.

A, B, C, D and E = the number of labeled cells in their respective compartments of granulopoiesis.

In this model the rate constants, K_i, represent the inverse of the mean transit times of labeled cells in each compartment. These first-order kinetic equations are comparable to those used to describe chemical enzyme kinetics (e.g., White et al., 1968), radioactive decay and other first-order processes which occur in nature. They also form the basis for cell population analysis (Foerster, 1959).

The number of labeled cells, changing with time, in each compartment of granulopoiesis was recorded by solving the first-order kinetic equations with the aid of an analog computer.[1] Adjustment of the rate constants allowed for an attempted simulation of the experimental observations obtained in the autoradiographic study of granulopoiesis in cats. Once the best possible simulation was attained with the model, the

---

[1]Model TR-48, Electronics Associates Industries, Princeton, New Jersey.

rate constants were converted to estimates of the mean transit times of labeled cells through the various generations and compartments of granulopoiesis.

RESULTS

Experimental Observations

Autoradiograms of ^{3}H-Tdr labeled cells characteristic of each cytologic compartment of granulopoiesis are illustrated in Plate 1. Differentiation of these cells was based on the descriptions of granulopoietic precursors given by Schalm (1965). Cells in the myeloblast and progranulocyte compartments, which are differentiated primarily on nuclear characteristics, could not be separated. This difficulty was due to the unnatural staining qualities of cells in autoradiograms and to the presence of silver grains, which obscured nuclear characteristics of labeled cells. However, cytoplasmic characteristics allowed differentiation of these cells from neutrophilic myelocytes. Nuclear shape, cell size, and cytoplasmic characteristics were sufficient to differentiate cells of the other cytologic compartments.

Heavily labeled cells were easily identified. However, silver grains were counted over more lightly labeled cells; the cells were considered to be labeled if these counts exceeded that of a comparable area free of cells (background) on the autoradiogram.

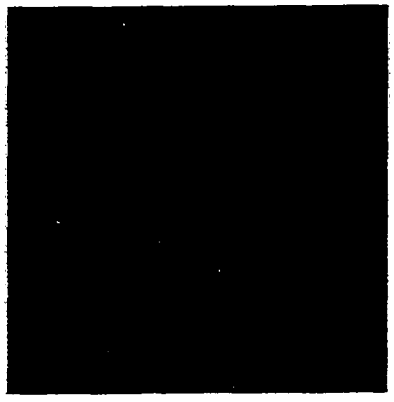
Blood granulocyte counts in all cats during the study

The mean, absolute, circulating granulocyte counts for the control and endotoxin-treated groups and the individual

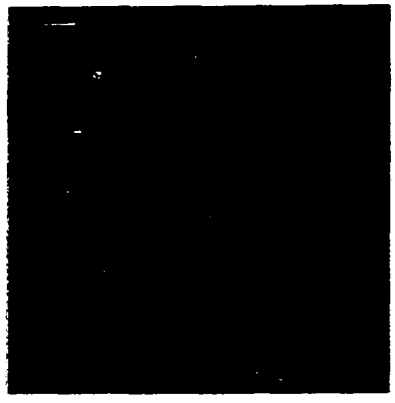
Plate 1. Autoradiograms of ^{3}H-Tdr labeled cells characteristic of each cytologic compartment of granulopoiesis:

A = myeloblast-progranulocyte
B = neutrophilic myelocyte
C = neutrophilic metamyelocyte
D = band neutrophil
E = segmented neutrophil

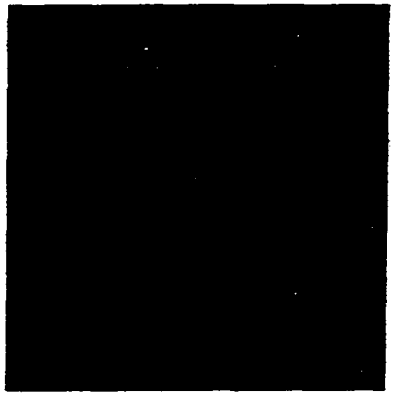
x1200



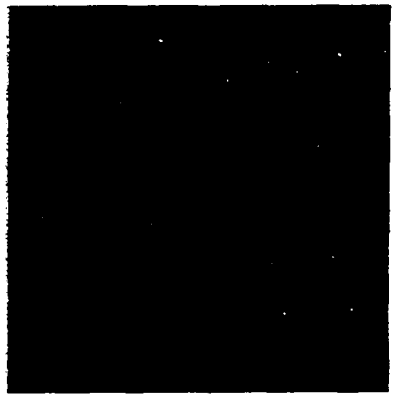
A



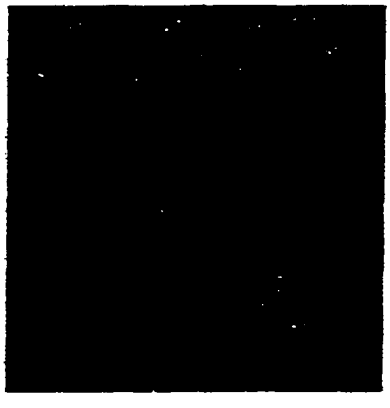
B



C



D



E

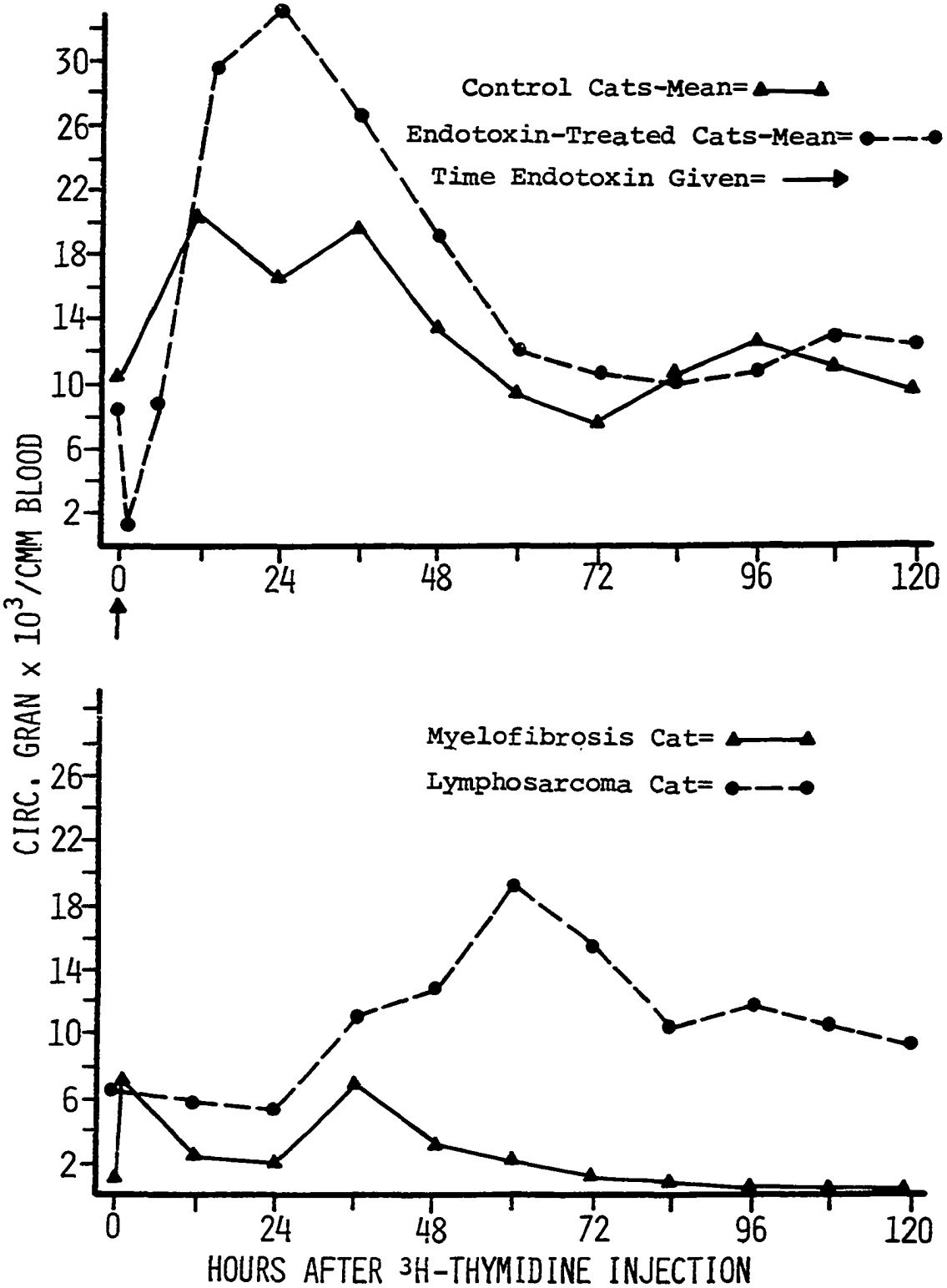
counts for the lymphosarcoma and myelofibrosis cats are presented in Figure 20. In the control group, granulocyte counts exceeded normal values from 2 through 50 hours after administration of ^{3}H-Tdr. During this time band neutrophils increased in number; band/segmented neutrophil ratios were about 1:4. Thereafter, the number of band neutrophils returned toward normal values; normal band/segmented neutrophil ratios in cats are about 1:75 (Schalm, 1965). Presumably, the neutrophilic response in the control cats occurred from stress induced by repeated handling during the bone marrow collection procedures.

The neutrophil response in endotoxin-treated cats (Figure 20) was similar to that observed in endotoxin-treated cats used in the blood granulocyte study; furthermore, the systemic reaction was also similar to that previously described. The band/segmented neutrophil ratios determined in these cats were about 1:10 at 0.5 hours, 1:2 at 2 hours, 1:7 at 12 hours, and 1:20 through 120 hours after ^{3}H-Tdr labeling. Therefore, the increased number of band neutrophils coincided with the observed rise in total granulocyte counts in these cats.

In the lymphosarcoma cat the granulocyte count exceeded normal values only at 60 hours after ^{3}H-Tdr injection (Figure 20), but band/segmented neutrophil ratios were about 1:10 for the duration of the study.

The myelofibrosis cat remained neutropenic throughout most of the study with granulocyte counts elevating to normal values at 2 sampling periods (Figure 20). However, band/

Figure 20. The mean, absolute, circulating granulocyte counts during the 120 hour - bone marrow sampling period following *in vivo* labeling with ^{3}H-Tdr in the control and endotoxin treated groups (Top); granulocyte counts for the lymphosarcoma and myelofibrosis cats (Bottom)


Control Cats-Mean=
Endotoxin-Treated Cats-Mean=
Time Endotoxin Given=
Myelofibrosis Cat=
Lymphosarcoma Cat=
CIRC. GRAN x 10^3/CMM BLOOD
HOURS AFTER ^{3}H-THYMIDINE INJECTION
30
26
22
18
14
10
6
2
0
24
48
72
96
120

segmented neutrophil ratios were consistently about 1:2, in addition, a few metamyelocytes and myelocytes were observed in the blood of this cat during the study.

Distribution of labeled cells in the bone marrow

Control group of cats The mean and range of normalized numbers of labeled cells determined at each sample time after ^{3}H-Tdr injection for the control group may be seen in Table 9. Casual observation of these numbers reveals the general progression of the label through the catenated compartments of granulopoiesis with time. Labeled myeloblasts and progranulocytes were few in number and declined until they were no longer observed at 24 hours. Labeled myelocytes were prevalent in the initial sample at 0.5 hours, and the number steadily declined with time until disappearing at 96 hours after ^{3}H-Tdr injection. Note that the number of labeled metamyelocytes was substantial at the first sampling, increased until 12 hours and decreased thereafter. As the number of labeled metamyelocytes declined, the number of labeled band neutrophils increased. However, a similar relationship between band and segmented neutrophils, although present, was less dramatic. The number of labeled segmented neutrophils was quite low, appearing at the time the peak number of band neutrophils was observed.

Endotoxin-treated group of cats The mean and range of normalized numbers of labeled cells determined at each

Table 9. Mean and range of normalized numbers of labeled myeloblasts-progranulocytes (A), neutrophilic myelocytes (B), neutrophilic metamyelocytes (C), band neutrophils (D), and segmented neutrophils (E) in 4 control cats following in vivo pulse labeling with ^{3}H-thymidine

		Hours after			
		0.5	2	6	12
A					
	Mean	2.5	1.6	0.7	0.3
	Range	1.8-3.9	1.0-1.9	0-1.1	0-0.8
B					
	Mean	15.1	15.6	11.3	11.9
	Range	13.1-17.4	9.2-23.9	4.3-17.9	8.0-15.4
C					
	Mean	8.8	14.4	12.9	17.1
	Range	6.6-13.4	9.0-18.8	12.3-13.6	11.7-19.9
D					
	Mean	0	0	1.4	5.5
	Range		0-4.0	0-4.0	2.5-7.4
E					
	Mean	0	0	0	0
	Range				

^{3}H-thymidine injection					
24	36	48	72	96	120
0	0	0	0	0	0
5.1 2.6-6.6	6.1 1.5-9.1	3.3 2.5-6.7	1.5 0-2.9	0	0
12.8 9.5-18.5	10.9 5.8-17.5	11.8 9.4-15.7	7.2 2.0-10.9	0.4 0-1.6	0.2 0-0.7
10.4 4.3-22.4	12.4 5.2-23.3	18.3 11.5-25.0	12.7 7.6-21.6	5.5 1.8-8.8	5.0 1.8-8.5
0	0	0.3 0-1.0	2.9 1.2-6.3	3.6 0.8-10.6	2.4 0.9-3.7

sample time for endotoxin-treated cats are presented in Table 10. The general progression of labeled cells through the cytologic compartments was similar to that observed for the control group, but subtle differences are notable. The numbers of labeled neutrophilic myelocytes and metamyelocytes decreased with time at faster rates than that observed for the control group. Furthermore, labeled band and segmented neutrophils each appeared earlier in the sampling sequence of endotoxin-treated cats.

Lymphosarcoma cat The normalized numbers of labeled cells in each cytologic compartment at each sample time in the lymphosarcoma cat are listed in Table 11. The most marked differences in the data from that of the control group were the delayed appearance and smaller number of band neutrophils in this cat. The appearance and disappearance of labeled cells in A, B, and C compartments were comparable to that of the control group.

Myelofibrosis cat Normalized numbers of labeled cells in each compartment of granulopoiesis at each sample time in the myelofibrosis cat may be seen in Table 12. In this cat, greater numbers of labeled cells were observed in the B, C, D, and E compartments. Labeled neutrophilic myelocytes remained prevalent throughout the study decreasing only at the 96 and 120 hour sampling times. Labeled neutrophilic metamyelocytes increased sharply in the first 24 hours, but unlike the control cats, the number did not decline as

Table 10. Mean and range of normalized numbers of labeled myeloblasts-progranulocytes (A), neutrophilic myelocytes (B), neutrophilic metamyelocytes (C), band neutrophils (D) and segmented neutrophils (E) in 3 endotoxin-treated cats following *in vivo* pulse labeling with ^{3}H-thymidine

	Hours after			
	0.5	2	6	12
A				
Mean	2.0	1.2	0.3	0
Range	0-3.4	0-2.4	0-1	
B				
Mean	9.2	9.8	8.4	8.3
Range	6.1-13.9	7.3-11.3	6.5-11.3	2.6-4.4
C				
Mean	3.9	13.7	10.4	19.5
Range	1.3-8.3	9.0-19.9	5.3-13.7	14.0-22.8
D				
Mean	0	0.9	1.1	9.6
Range		0-1.9	0-3.4	4.7-13.2
E				
Mean	0	0	0	0
Range				

^{3}H-thymidine injection					
24	36	48	72	96	120
0	0	0	0	0	0
3.5 2.6-4.4	2.1 0.6-3.6	1.1 0.8-1.4	0.4 0-1.2	0	0.2 0-0.7
12.7 10.8-14.6	8.9 8.2-9.7	4.6 3.8-5.4	2.9 1.6-3.6	0	0
23.6 16.5-30.8	13.5 9.8-17.2	10.4 8.2-12.7	9.6 3.2-14.3	1.9 0-3.4	1.2 1.1-1.3
0	0.3 0-0.6	0.8 0.6-1.1	2.3 1.3-4.2	1.0 0.5-1.8	2.1 1.0-4.0

Table 11. Normalized numbers of labeled myeloblasts-progranulocytes (A), neutrophilic myelocytes (B), neutrophilic metamyelocytes (C), band neutrophils (D) and segmented neutrophils (E) in the lymphosarcoma cat following *in vivo* pulse labeling with ^{3}H-thymidine

	Hours after ^{3}H-thymidine injection									
	0.5	2	6	12	24	36	48[a]	72	96	120
A	2.6	1.0	2.6	1.3	0	0		0	0	0
B	16.5	10.4	26.2	9.4	10.7	5.9		0	0	0
C	17.0	11.5	18.6	6.6	28.0	3.6		3.3	2.2	0
D	0	0	0	0	0	1.6		3.4	2.3	0.8
E	0	0	0	0	0	0		1.0	1.9	2.0

[a]No data available for the 48 hour sample due to technical problems.

Table 12. Normalized numbers of labeled myeloblasts-progranulocytes (A), neutrophilic myelocytes (B), neutrophilic metamyelocytes (C), band neutrophils (D), and segmented neutrophils (E) in the myelofibrosis cat following in vivo pulse labeling with ^{3}H-thymidine

	Hours after ^{3}H-thymidine injection									
	0.5	2[a]	6	12	24	36	48	72	96	120
A	3		2	0.7	0	0	0	0	0	0
B	22.5		22.3	23.7	29.4	23.5	14.1	20.8	11.7	2.5
C	10		10.2	18.6	25.3	19.3	26	36.2	26	28
D	0		0	0	5.8	4.4	12.5	30	8.5	15
E	0		0	0	0	0	9.2	30.8	4.8	0

[a]No data available for the 2 hour sample due to technical problems.

rapidly thereafter. The number of labeled band neutrophils increased sharply from 24 to 72 hours and then declined. Labeled segmented neutrophils increased dramatically from 48 to 72 hours and then the number decreased.

The greater numbers of labeled cells observed in the bone marrow autoradiograms of the myelofibrosis cat were indicative of granulocytic hyperplasia. Therefore, histological sections of midshaft femur marrow were prepared following necropsy for confirmation of the antemortem lesion diagnosis. The histological appearance of the bone marrow in this cat, compared to that of a clinically and hematologically normal cat of the same age, 5 years, may be seen in Plate 2. Pathologic alterations seen in the bone marrow of the myelofibrosis cat included erythrocytic, granulocytic and megakaryocytic hyperplasia, decrease in normal fat deposition and increase in intercellular proteinaceous substances and reticulin fibers. Some increase in fibrous connective tissue surrounding blood vessels was also noted. Other lesions in this cat were acute, septic, frontal sinusitis, splenic infarction, and extramedullary hematopoiesis in the spleen.

Emergence times of labeled band and segmented neutrophils in the blood

The emergence times of labeled band and segmented neutrophils in the blood for the control group (mean values), the endotoxin-treated group (mean values), the lymphosarcoma

Plate 2. Histological appearance of midshaft femur marrow in the myelofibrosis cat. Comparison with that from a clinically and hematologically normal cat

A = normal cat, hematoxylin and eosin Y stain
B = normal cat, Gomori's reticulum stain
C = myelofibrosis cat, hematoxylin and eosin Y stain
D = myelofibrosis cat, Gomori's reticulum stain

x200

A

B

C

D

cat and the myelofibrosis cat may be seen in Table 13. The appearance of labeled band neutrophils preceded that of segmented neutrophils in all subjects except the myelofibrosis cat. The emergence times in the latter cat and the endotoxin-treated cats were shortened as compared to the control group.

Comparison of Model Output and Experimental Observations

The number of labeled cells, changing with time, in each cytologic compartment of granulopoiesis was recorded by solving the first-order kinetic equations, which had been written to describe the hypothetical model. The model outputs for myeloblasts and progranulocytes were summed in order to simulate the experimental observations. In like manner, the model output for the myelocyte compartment was a summation of that for each myelocyte generation, which allowed for comparison with the experimental observations. This summation of information from the kinetic equations did not preclude calculation of individual transit times for myeloblasts, progranulocytes and each myelocyte generation.

The model output was recorded with time on plots which contained the experimentally derived normalized numbers of labeled cells. The various rate constants and the initial conditions, i.e., the probable numbers of initially labeled myeloblasts, progranulocytes and myelocytes, were adjusted until the best possible simulation of the experimental observations could be attained.

Table 13. Emergence times of labeled band and segmented neutrophils in the blood following *in vivo* pulse labeling with ^{3}H-thymidine for cats in the control group (mean values), the endotoxin-treated group (mean values), the lymphosarcoma cat and the myelofibrosis cat

	Emergence time (hours)	
	Band neutrophils	Segmented neutrophils
Control group	39	57
Endotoxin-treated group	20	28
Lymphosarcoma cat	48	60
Myelofibrosis cat	24	24

Plots, which resulted from the above procedure, were prepared for the control group of cats (Figure 21), the endotoxin-treated group of cats (Figure 22), the lymphosarcoma cat (Figure 23), and the myelofibrosis cat (Figure 24). The experimental data in these plots were those listed in Tables 9, 10, 11, and 12 for the respective groups and individual cats. The experimental mean and range of values for the control group and the endotoxin-treated group are represented as vertical bars at appropriate times in their respective plots.

Figure 21. Comparison of model output and experimental observations for the control group following *in vivo* pulse labeling with ^{3}H-thymidine. Lines labeled A, B, C, D and E = model output for respective cytologic compartments. Vertical bars = experimental mean and range of data for 4 cats in the control group

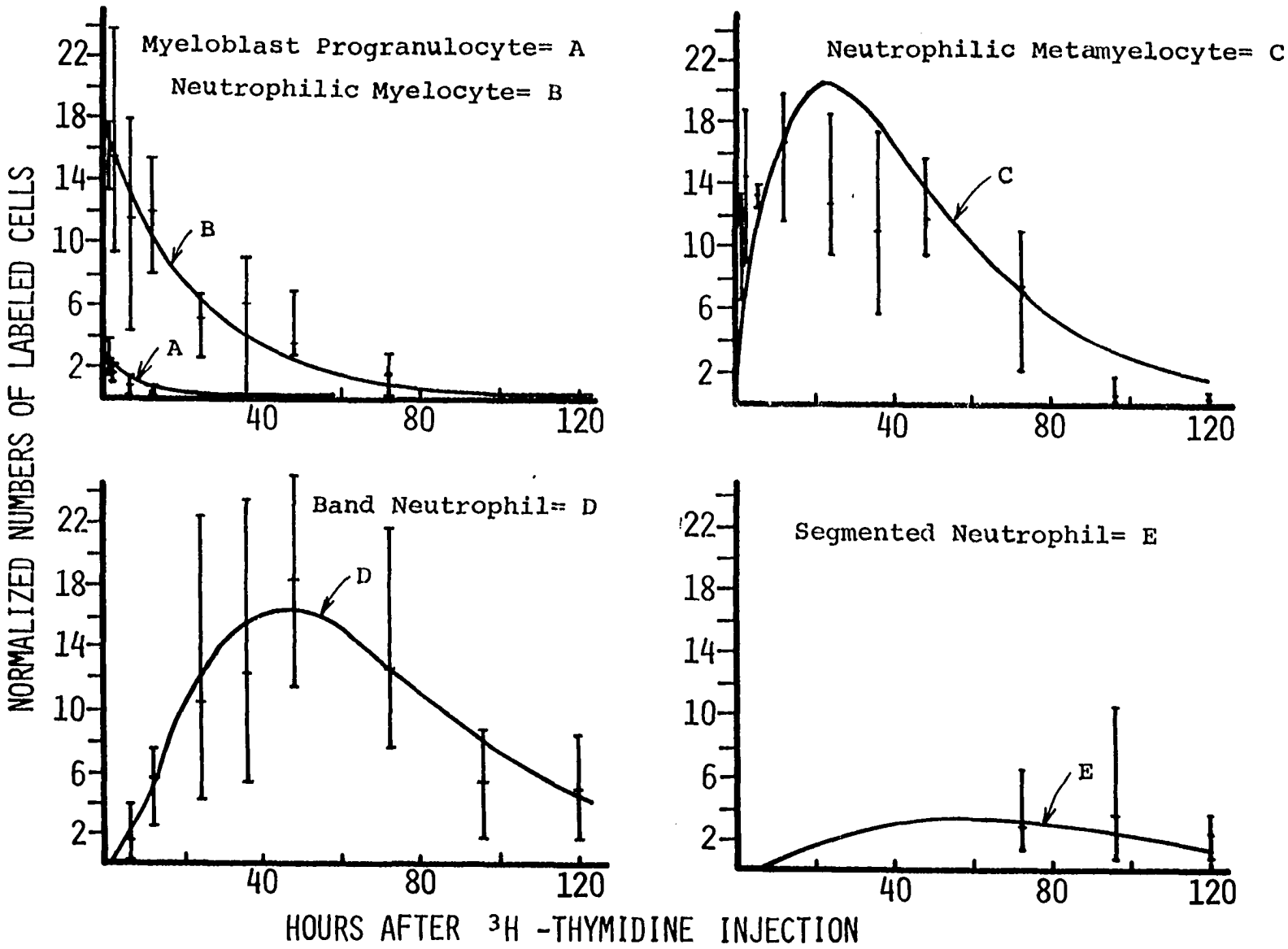

Myeloblast Progranulocyte= A
Neutrophilic Myelocyte= B
Neutrophilic Metamyelocyte= C
Band Neutrophil= D
Segmented Neutrophil= E
NORMALIZED NUMBERS OF LABELED CELLS
HOURS AFTER ³H -THYMIDINE INJECTION
22
18
14
10
6
2
40
80
120

Figure 22. Comparison of model output and experimental observations for the endotoxin-treated group following in vivo pulse labeling with ^{3}H-thymidine. Lines labeled A, B, C, D and E = model output for respective cytologic compartments. Vertical bars = experimental mean and range of data for 3 cats in the endotoxin-treated group

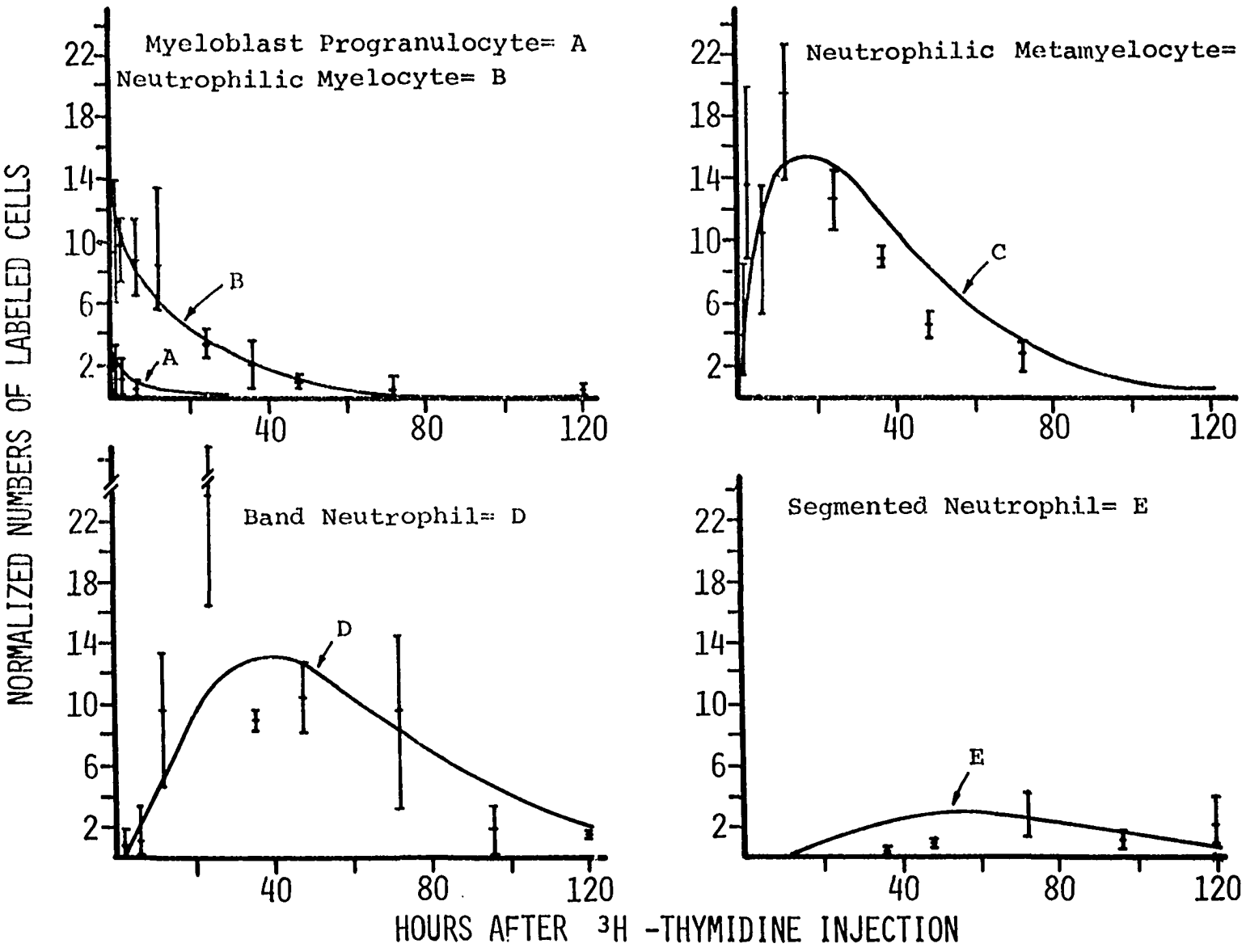

Myeloblast Progranulocyte= A
Neutrophilic Myelocyte= B
Neutrophilic Metamyelocyte= C
Band Neutrophil= D
Segmented Neutrophil= E
NORMALIZED NUMBERS OF LABELED CELLS
HOURS AFTER 3H -THYMIDINE INJECTION
22
18
14
10
6
2
40
80
120
A
B
C
D
E

Figure 23. Comparison of model output and experimental observations for the lymphosarcoma cat following *in vivo* pulse labeling with ^{3}H-thymidine. Lines labeled A, B, C, D and E = model output for respective cytologic compartments

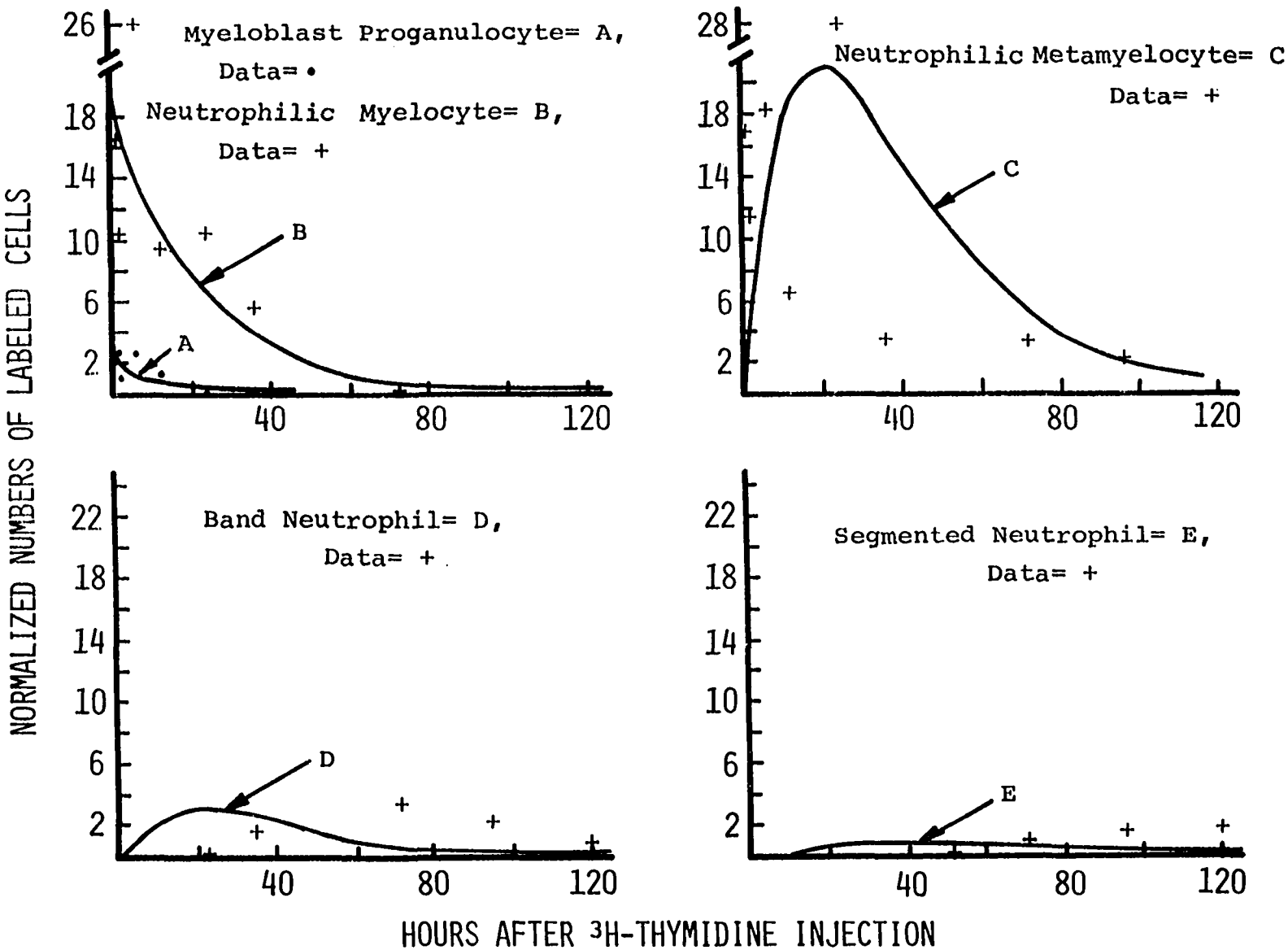
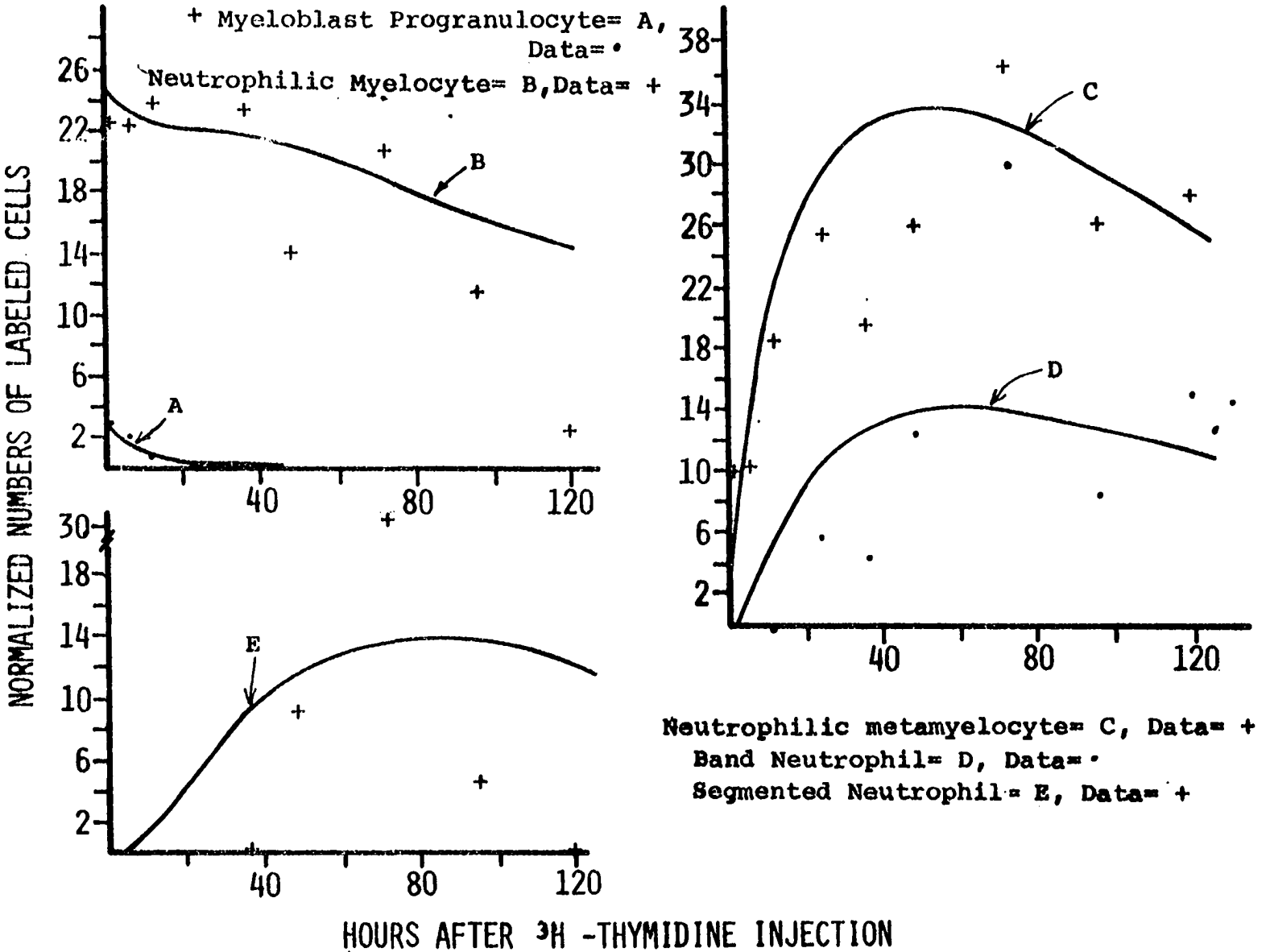

Myeloblast Proganulocyte= A,
Data= •
Neutrophilic Myelocyte= B,
Data= +
Neutrophilic Metamyelocyte= C
Data= +
Band Neutrophil= D,
Data= +
Segmented Neutrophil= E,
Data= +
NORMALIZED NUMBERS OF LABELED CELLS
HOURS AFTER 3H-THYMIDINE INJECTION
A
B
C
D
E
26
18
14
10
6
2
28
22
40
80
120

Figure 24. Comparison of model output and experimental observations for the myelofibrosis cat following *in vivo* pulse labeling with ^{3}H-thymidine. Lines labeled A, B, C, D and E = model output for respective cytologic compartments


+ Myeloblast Progranulocyte= A, Data= •
Neutrophilic Myelocyte= B, Data= +
Neutrophilic metamyelocyte= C, Data= +
Band Neutrophil= D, Data= •
Segmented Neutrophil= E, Data= +
NORMALIZED NUMBERS OF LABELED CELLS
HOURS AFTER ³H -THYMIDINE INJECTION
A
B
C
D
E
2
6
10
14
18
22
26
30
34
38
40
80
120

The final rate constants which resulted from the simulation of the experimental observations shown in Figures 21-24 were converted to estimates of the mean transit times of labeled cells through the various generations and compartments of granulopoiesis. The above values and the proliferating pool transit time, the nonproliferating pool transit time and the total marrow transit time are listed for the control and endotoxin groups of cats, the lymphosarcoma cat and the myelofibrosis cat in Table 14.

Note the similarity of total marrow transit times recorded in this table. The transit times require further comment. The population of myeloblasts, from which the myeloblast compartment transit time was estimated, was in its DNA synthesis stage when labeled. Therefore, the estimated transit time for this compartment is an average time from initial labeling until the cells leave the compartment by heteromorphogenic division into the progranulocyte compartment. Thus the estimated myeloblast transit times given in Table 14 should be shorter than the true compartment transit times.

The labeled cells which populated progranulocyte and myelocyte compartments were of 2 types: (1) those in their DNA synthesis stage when initially labeled, which contributed short cell cycle times to the average transit times and (2) those entering the compartments as labeled cells from precursor generations, which contributed complete cell cycle

Table 14. Estimated mean generation or compartment transit times, proliferating pool transit times, nonproliferating pool transit times and total transit times of labeled granulopoietic cells in the bone marrow of cats. Estimates based on rate constants for first-order kinetic equations used to simulate experimental observations for control cats, endotoxin-treated cats, the lymphosarcoma cat and the myelofibrosis cat. All values in hours

	Transit times: Control cats	
Myeloblast	15.2	
Progranulocyte	14.2	
1st generation myelocyte	12.0	
1st generation myelocyte[a]	0	
2nd generation myelocyte	10.0	
3rd generation myelocyte	8.9	
Total - proliferating pool		60.3
Metamyelocyte	20.5	
Band neutrophil	34.0	
Segmented neutrophil	7.1	
Total - nonproliferating pool		61.6
Total marrow transit time		121.9

[a]Additional 1st generation myelocyte as defined in model.

Transit times: Endotoxin-treated cats		Transit times: Lymphosarcoma cat		Transit times: Myelofibrosis cat	
15.2		15.2		15.2	
14.2		14.2		14.3	
10.0		12.0		10.1	
0		0		11.1	
11.4		10.0		11.4	
4.8		8.9		10.3	
	55.6		60.3		72.4
17.1		17.1		14.7	
40.8		41.0		14.6	
13.5		13.5		15.3	
	72.4		71.6		44.6
	128.0		131.9		117.0

times to the average transit times. Therefore, the estimated mean transit times for progranulocytes and first, second, and third generation myelocytes listed in Table 14 should also be shorter than the true transit times, but by a lesser magnitude than that for myeloblasts.

The mean compartment transit times estimated for metamyelocytes and band and segmented neutrophils are true estimates within the limits of the model. All labeled cells populating these compartments were labeled prior to entering the nonproliferating pool.

The rate of release of band neutrophils from the bone marrow to the blood (R_2 in the first-order kinetic equations) for each group or individual cat was: control group, R_2 = 0.15 hours $^{-1}$, endotoxin-treated group, R_2 = 0.293 hours $^{-1}$, lymphosarcoma cat, R_2 = 3.61 hours $^{-1}$ and myelofibrosis cat, R_2 = 0.937 hours $^{-1}$. These constants reflect the rate at which immature neutrophils were being released to the blood, i.e., band neutrophils were being released at a rapid rate from the bone marrow of the lymphosarcoma cat and at a much slower rate from the bone marrow of the cats in the control group.

DISCUSSION

The model of granulopoiesis (Figure 19) was adequate to describe the kinetic events of granulocyte production and maturation in cats exemplified in Figures 21, 22, 23, and 24. Simulation of the experimental observations, i.e., the change in numbers of labeled cells with time, may have been possible with other combinations of rate constant values. Constraints, both inherent and imposed on the model, were adopted in order to comply with current concepts of granulopoiesis.

One constraint, inherent in the model, was the number of generations in the proliferating pool. The myeloblast and progranulocyte compartments were limited to one generation each and the myelocyte compartment was defined as having 3 generations. A potential for increasing granulocyte production was considered to reside within the first myelocyte generation, in which an additional division could occur by shortening the generation time. These basic assumptions agree with current concepts of granulopoiesis (Cronkite and Vincent, 1970; Osgood, 1970; Athens, 1969) and inherently restrict the potentiality of the model.

The order of magnitude of the rate constant values was restricted in order to comply with existing knowledge of cell cycle and subcycle stage durations for granulocyte precursors. The duration of DNA synthesis, reported to be about 13 to 14

hours in man (Stryckmans et al., 1966; Vincent et al., 1969) and about 7 hours in the dog (Maloney et al., 1962), and the durations of G_2 and M stages were considered to be limiting factors with respect to the rate at which cells could progress from one generation to the next. Therefore only those combinations of rate constant values, which provided for realistic compartment transit times, as well as simulating the experimental data, were allowed in manipulation of the model.

Another constraint inherent in the model was the fact that the output from each first-order kinetic equation was influenced by a rate constant in the preceding equation. Changing a rate constant to produce a better simulation of results in a given compartment also produced a compensatory change in the output for a following (or a preceding) compartment. This effect naturally limits the potentiality of the model. However, the number of cells in a given compartment at any time is influenced by the rates at which cells enter or leave the compartment. The constraint on the model is unavoidable since this is a fundamental property of renewing cell populations.

Thus, the substantiality of this model is greatly enhanced by the fact that the kinetic aspects of granulopoiesis in differing physiopathologic states were adequately explained within the limits of the inherent and imposed constraints. Establishing the substantiality of the model of granulopoiesis in cats permitted more detailed interpretation of the kinetic

parameters derived in this study.

Small changes in rate constants and initial conditions, i.e., the numbers of initially labeled cells in the proliferating compartments, were sufficient to account for the differences in flux of granulopoietic cells through the bone marrow of cats with differing physiopathologic states. The total marrow transit times in these cats were quite similar (Table 14). This overall parameter represents the average transit time from myeloblasts preparing to divide, through the sequential compartments to the time when segmented neutrophils are released to the blood. The fact that this time parameter was within a narrow range in cats with dissimilar systemic states is a matter for conjecture.

The total marrow transit time may represent an obligatory maturation time for granulocytes in the bone marrow of cats. Maturation does occur in both proliferating and nonproliferating pools of the granulocyte renewal system. Cronkite and Vincent (1970) have proposed that a minimum obligatory transit time through the metamyelocyte and band neutrophil compartments may occur. They explained that an obligatory transit time dictates the maximum flux of cells which can be sustained through these compartments without increasing the size of the compartments. Furthermore, maturation is believed to place a natural limitation on the transit time through the myelocyte compartment (Cronkite and Vincent, 1970; Cronkite and Fliedner, 1964). Therefore an obligatory total marrow transit time

would limit the maximum flux of granulopoietic cells through the bone marrow unless the numbers of cells within the various compartments were caused to increase, or unless the relative distribution of cells among the compartments could change.

An implication of this suggestion is that, for the situations studied, a sustained, increased peripheral demand for cells must be accommodated by an expansion of the granulopoietic population and not by a decrease in the maturation time. This does not preclude the fact that a sudden, increased demand for cells in the periphery could be met by release of bone marrow granulocyte reserves, e.g., band neutrophils or even metamyelocytes. Further elucidation of the matter may be enhanced by evaluation of the kinetic parameters of granulopoiesis in cats with experimentally-induced, sustained, systemic or localized suppurative disease.

A satisfactory model of granulopoiesis must account for the early appearance and rapid rise in the number of labeled metamyelocytes (Tables 9, 10, 11, and 12). This phenomenon has been reported by others (Cronkite and Vincent, 1970; Patt and Maloney, 1964; Warner and Athens, 1964). Most workers propose models with serial divisions at the myelocyte level to account for this observed efflux into the nonproliferating pool.

Patt and Maloney (1964), who proposed a myelocyte compartment with 2 serial divisions in the dog, noted a production of proliferating cells which was greater than the flow

of cells into the metamyelocyte compartment. A similar phenomenon was not observed in this study. To the contrary, the observed early appearance and rapid rise in the number of metamyelocytes was difficult to simulate using the model with 3 serial myelocyte divisions. However, consideration of possible proportions and cell cycle times of the 3 myelocyte generations led to a successful simulation of the efflux into the metamyelocyte compartment.

The computer was programmed with the total number of myelocytes initially labeled. (The value was extrapolated from the observed number at 0.5 hours after ^{3}H-Tdr injection.) This number was then proportioned unequally among the 3 myelocyte generations, with the third generation receiving the majority and the first receiving the least of the cells which comprised the total. The corresponding rate constants for each myelocyte generation were adjusted to provide for a long cell cycle time in the first and progressively shorter times in the second and third myelocyte generations. This combination of initial conditions and rate constants results in an output which successfully simulated the early appearance and rapid rise of cell numbers into the metamyelocyte compartment (Figures 21, 22, 23, and 24).

One would naturally expect a larger number of labeled cells in successive myelocyte generations since the cells are dividing and hence multiplying as they progress through the chain. The result is an unequal proportioning in the three

generations. This effect is amplified by the progressively shorter transit times in the sequential myelocyte generations. The calculated transit times for each myelocyte generation tend to reflect the initial combination of conditions (Table 14) whereby the first myelocyte generation transit time was usually longer than that of the second or third generations.

The granulocyte renewal system is often subject to increased demand for cells and any kinetic model of granulopoiesis must account for this potential. Cronkite and Vincent (1970) have calculated that the G_1 stage of neutrophilic myelocytes in man is about 3 times longer than the DNA synthesis time. They propose that the G_1 stage may shorten to allow up to 2 extra divisions within the myelocyte compartment before maturation or maturation products inhibit further division.

In this study the model was defined with an additional division before cells mature beyond the level of first generation myelocytes (Figure 19). This could account for an increased production of cells. One way that this could occur would be a shortening of the G_1 stage of first generation myelocytes, thereby allowing an additional division within a similar total length of time. The model was not adequately tested with respect to this potential since the data from the control and endotoxin-treated groups and the lymphosarcoma cat could be simulated without the additional myelocyte division (Table 14). Although all of these cats had brief periods of neutrophilia (Figure 20) and immature cells were observed

in their blood, the model of granulopoiesis, excluding the additional myelocyte division, adequately accounted for their granulocyte needs (Figures 21, 22, and 23).

The additional myelocyte division was necessary to simulate the data for the myelofibrosis cat (Table 14). The clinical syndrome and histopathologic lesions (Plate 2) in this cat were similar to that described for myelosclerosis with myeloid metaplasia, a myeloproliferative disease in man (Rappaport, 1966). The disease in man is characterized by a progressive unrestrained proliferation of one or more cellular constituents of the bone marrow; occasionally individuals are leukopenic, which was the case in the myelofibrosis cat (Figure 20).

The developing sclerosis around blood vessels and the increase in intercellular substances in the bone marrow of this cat may have deterred the release of segmented neutrophils into the blood. Fliedner et al. (1964a) suggested this as a possibility to explain the decreased rate of appearance of ^{3}H-Tdr labeled granulocytes into the blood of a human patient with myelofibrosis. If the release of granulocytes was impeded in this cat, the estimated average transit time for marrow segmented neutrophils should have been prolonged. This time was twice that for the control cats (Table 14), but the total-nonproliferating pool transit time (Table 14) was shorter than that for the control group. A plausible explanation for this apparent inconsistency may be that intra-

marrow death of cells was occurring in the nonproliferating pool. However, no evidence to support this suggestion was found.

The results for the endotoxin-treated group require further comment. The proliferating pool transit time was shortened and that for the nonproliferating pool was lengthened, as compared to the estimates for control cats (Table 14). This implies that a single endotoxin injection may result in an increased mitotic rate, but only to replenish depleted bone marrow granulocyte reserves. The fact that endotoxin causes an increased rate of granulocyte release from the bone marrow was illustrated and discussed in Part I of this study. Herion et al. (1965) demonstrated in rabbits that endotoxin causes this release into the circulation independent of any effect on mitosis. They further noted an increase in marrow cellularity accompanying induction of tolerance by multiple injections of endotoxin, which occurred in face of a decrease in the postmitotic granulocyte pool. This indicated an increase in the number of cells in mitotic pool.

Presumably, the granulocyte reserve in the bone marrow of the endotoxin-treated cats was somewhat depleted during the period when the circulating granulocyte count was rising (Figure 20). Following this, when circulating counts returned toward normal, the movement and production of granulocytes in the bone marrow reflected an effort in restoration of the depleted reserve. Therefore, any increase in mitotic activity

of proliferating cells was probably an indirect effect from a single exposure to bacterial endotoxin.

The use of autoradiographic techniques and analysis of data with the model of granulopoiesis in cats treated with endotoxin could lead to further elucidation of the mechanisms whereby granulocyte production is increased or decreased. This could be accomplished by injecting endotoxin at varied times, both before and after in vivo labeling of granulocyte precursors.

The emergence times of labeled band or segmented neutrophils in the blood following in vivo pulse labeling with ^{3}H-Tdr is taken to be the time from the terminal myelocyte generation DNA synthesis stage until the cells appear in the blood. The values for these times, given in Table 13, are subject to error because of the rather long sampling intervals which were used (about 12 hours). However, several ideas with respect to movement of cells through the nonproliferating pool and subsequent release from the bone marrow may be suggested.

The emergence times of segmented neutrophils for the control group and the lymphosarcoma cat (Table 13) are in fair agreement with their respective estimated average transit times (Table 14). This indicates that after the final division, cells progress through the nonproliferating pool in an orderly manner, with release into the circulation being a function of time following entry into the pool. The fairly constant circulating granulocyte counts in these cats (Figure

20) would indicate that a steady state existed. Maloney and Patt (1968) have suggested that movement of cells through the nonproliferating pool follows "first in - first out" order and further, that the release of cells from the bone marrow is not a random process in the normal steady state. The findings in the control group and lymphosarcoma cat support their contention.

Comparison of the emergence times for labeled band neutrophils with transit times for the various compartments in the nonproliferating pool of all cats studied (Tables 13 and 14) reveals no apparent time relationships. This suggests that some band neutrophils are released at random from the bone marrow. Furthermore, the emergence times for segmented neutrophils and nonproliferating pool transit times of the endotoxin-treated group and the myelofibrosis cat do not agree. This implies random release of cells from the bone marrow in conditions characterized by a nonsteady state.

The model of granulopoiesis (Figure 19) was found to be adequate to describe the autoradiographic data for cats. However, the model may be further evaluated and additional kinetic aspects of granulopoiesis in cats might be elucidated with additional studies. The experimental production of a localized or systemic suppurative disorder, in which the peripheral demand for neutrophils would be sustained for several days, could provide a condition for testing the hypothetical model during sustained, intensified granulopoiesis. Further,

more frequent blood and bone marrow samplings in such a study may clarify the mechanisms whereby granulocytes move through the nonproliferating pool and are released to the blood.

CONCLUSION

Kinetic parameters can be used to describe the sojourn of neutrophilic granulocytes from their inception to their death. This experimentation has described the proliferation and maturation of granulocytes in the bone marrow, release of these cells from that site, and their compartmentalization and disappearance from the blood. A proposed model of granuloipoiesis was used to describe autoradiographic data with respect to the movement of cells through cytologic compartments of granulopoiesis. The distribution and half-life of blood granulocytes were determined following infusion of autologous, ^{3}H-DFP-labeled granulocytes.

The data indicate that production of granulocytes in the bone marrow of cats requires about 5 days for the physiopathologic states studied. The model of granulopoiesis, defined with at least 5 sequential divisions in the proliferating pool of cells, was adequate to account for the number of labeled granulocyte precursors, changing with time, in both proliferating and nonproliferating pools. Further, the data indicate that the total marrow transit time (about 5 days) may represent an obligatory maturation time for granulocyte production. One implication of this suggestion is that sustained, increased peripheral demand for granulocytes must be accommodated by an expansion of the granulopoietic population and not by a decrease in the maturation time. However, this

does not preclude the ability for release of immature cells from the granulocyte reserve to meet immediate peripheral demands.

The emergence time of labeled segmented neutrophils in the blood was similar to estimates of the nonproliferating pool transit time, about 60 to 70 hours, in situations characterized by steady-state conditions. This implies that the most mature cells are the first to be released under steady-state conditions. However, the data indicate that release from the bone marrow under nonsteady-state conditions may be a random process in cats.

In the peripheral blood, granulocytes were found to be distributed unevenly within the total blood granulocyte pool. Approximately 30% of the blood granulocytes are in the circulating pool in normal, steady-state conditions. Therefore the majority of blood granulocytes in cats reside in the marginal pool, which by comparison is much larger than that previously reported in dog and man. The distribution of cells between the circulating and marginal pool depends on the physiopathologic state. This was exemplified by the findings in experimentally-induced and naturally occurring blood dyscrasias in cats.

The disappearance of labeled granulocytes from the blood of cats occurred at an exponential rate which implies that granulocytes are lost randomly from the blood. The average half-life of the cells was estimated to be 7.4 hours in

hematologically normal cats, which is similar to that described for dog and man.

Thus the fundamental values of granulocyte kinetics in cats were established in this study. The proposed model of granulopoiesis, substantiated by successful simulation of experimental observations in cats with differing physiopathologic conditions, is suggested for continued application in the study of granulopoiesis in many diseases of cats. Further, the procedures for evaluating blood granulocyte kinetics in cats permitted interpretation of changes which occurred as a result of hemorrhagic blood loss. This illustrates the potential of the cat, a relatively small laboratory species, for research on the intravascular survival and distribution of neutrophilic granulocytes.

Toxemic conditions in cats are commonly characterized by defective maturation of granulocytes in which giant cells with bizarre nuclear shapes and abnormal cytoplasmic granules are observed (Schalm, 1965). This phenomenon could be studied with the aid of the proposed model of granulopoiesis to elucidate the mechanisms by which it occurs. The pathogenesis of infectious feline enteritis, with respect to the effect of the virus on granulopoiesis, is poorly understood and could be further elucidated by evaluating the kinetics of granulopoiesis during infection.

The procedures developed in this study for evaluation of granulocyte kinetics in cats could also be applied to

research on disease common to all species. Little is known about the effects of various environmental pollutants, e.g., lead, on granulocyte production, life span and function. These effects must be determined in laboratory animals. Certainly, the cat is a suitable laboratory species for this research.

SUMMARY

The kinetics of neutrophilic granulocytes were studied in 27 cats. Total, circulating, and marginal granulocyte pools, half-life of granulocytes and granulocyte turnover rate were evaluated in peripheral blood following infusion of granulocytes labeled with ^{3}H-diisopropylfluorophosphate. Autoradiographic data collected following *in vivo* pulse labeling of granulocyte precursors with ^{3}H-thymidine was interpreted with the aid of a hypothetical model of granulopoiesis. Transit times through the cytologic compartments of granulopoiesis were estimated.

The blood granulokinetic parameters were determined in 18 cats. These cats were separated into 4 groups which consisted of 5 clinically and hematologically normal cats, 3 cats treated with bacterial endotoxin, 7 cats with experimentally-induced hemorrhage and 3 cats with naturally occurring blood dyscrasias. Clinical and hematological features of the latter 3 cats were: (1) febrile with leukocytosis, (2) unexplained leukocytosis and (3) lymphosarcoma with neutropenia.

In the clinically and hematologically normal cats the mean total blood granulocyte pool was 28.86×10^{8} granulocytes per Kg of body weight. These granulocytes were unequally distributed with only about 30% of the cells accountable in the circulating pool. The mean half-life of blood granulo-

cytes was 7.4 hours and the mean granulocyte turnover rate was 64.97×10^8 granulocytes per Kg of body weight per day in the normal cats.

Bacterial endotoxin produced a neutropenia characterized by margination of circulating granulocytes and a normal total blood granulocyte pool within 1 to 2 hours after administration. Following the initial neutropenic phase, the circulating, and marginal pools increased and the granulocyte turnover rate increased.

The effects of hemorrhage on blood granulocyte kinetics were interpreted to be a manifestation of significant loss of erythrocytes, which are of more immediate concern to life than neutrophilic granulocytes. The granulokinetic changes were characterized by an increased marginal granulocyte pool, a prolonged half-life and a decreased granulocyte turnover rate. These changes were induced by removing 7 to 8% of the blood volume in excess of routine sample volumes during an 8 to 10 hour period before infusion of labeled granulocytes.

Evaluation of the blood granulocyte kinetic parameters in the 3 cats with naturally occurring blood dyscrasias did not give conclusive evidence with respect to the mechanisms which underly neutrophilic or neutropenic responses. However, the changes reflect the sensitivity of the procedures for further research on the intravascular survival and distribution of neutrophilic granulocytes in cats.

Autoradiographic analysis of granulopoiesis was conducted

on 4 clinically normal cats, 3 cats treated with bacterial endotoxin, 1 cat with lymphosarcoma and 1 cat with a myeloproliferative disease characterized by myelofibrosis and hyperplasia of hematopoietic tissues.

A hypothesized model of granulopoiesis was defined. Myeloblast and progranulocyte compartments were represented as being populated with cells having one generation in each compartment. The neutrophilic myelocyte compartment was defined as being populated with cells having 3 serial generations before entering the nonproliferative compartments; the majority of neutrophilic myelocytes were considered to be third generation myelocytes; cell cycle times were considered to be longest in first generation and shortest in third generation myelocytes. Neutrophilic metamyelocytes, band and segmented neutrophils completed the catenated compartments of granulopoiesis defined in the model.

First-order kinetic equations, based on the model of granulopoiesis, were solved with the aid of an analog computer in order to simulate data from the autoradiographic analyses. The model was adequate to describe the kinetics of granulopoiesis in the cats with differing physiopathologic conditions. Compartment transit times were estimated from the first-order kinetic rate constants. The range of total transit times, from the DNA synthesis stage of myeloblast cells to ultimate release of segmented neutrophils from the bone marrow was 117 to 131.9 hours for the physiopathologic

states encountered in the study. Emergence time of labeled segmented neutrophils in the blood was in agreement with estimated transit time through the nonproliferating pool in steady state conditions, which was interpreted to imply first in - first out order for cells leaving the bone marrow. In nonsteady state conditions the release of granulocytes from the bone marrow was interpreted to be a random process.

The successful simulation of data supports the contention that the proposed model of granulopoiesis is an adequate representation of the kinetic events of granulocyte production in cats.

LITERATURE CITED

Alexanian, R. and Donohue, D. M.
1965 Neutrophilic granulocyte kinetics in normal man. Journal of Applied Physiology 20:803-808.

Athens, J. W.
1969 Granulocyte kinetics in health and disease. National Cancer Institute Monograph 30:135-154.

Athens, J. W., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1959a A method for labeling leukocytes with radioactive diisopropylfluorophosphate (DFP^{32}). Annals of the New York Academy of Science 77:773-776.

Athens, J. W., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1959b Leukokinetic studies. I. A method for labeling leukocytes with diisopropylfluorophosphate (DFP^{32}). Blood 14:303-333.

Athens, J. W., Haab, O. P., Raab, S. O., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1961a Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. Journal of Clinical Investigation 40:989-995.

Athens, J. W., Raab, S. O., Haab, O. P., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1961b Leukokinetic studies. III. The distribution of granulocytes in the blood of normal subjects. Journal of Clinical Investigation 40:159-164.

Athens, J. W., Haab, O. P., Raab, S. O., Boggs, D. R., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1965 Leukokinetic studies. XI. Blood granulocyte kinetics in polycythemia vera, infection and myelofibrosis. Journal of Clinical Investigation 44:778-788.

Bierman, H. R., Kelly, K. H., Cordes, F. L., Petrakis, N. L., Kars, H. and Smpil, E. L.
1952 The influence of respiratory movements on the circulating leukocytes. Blood 7:533-544.

Bishop, C. R., Athens, J. W., Boggs, D. R., Warner, H. R., Cartwright, G. E. and Wintrobe, M. M.
1968 Leukokinetic studies. XIII. A non-steady state kinetic evaluation of the mechanism of cortisone-induced granulocytosis. Journal of Clinical Investigation 47:249-260.

Boggs, D. R.
1967 The kinetics of neutrophil leukocytes in health and disease. Seminars in Hematology 4:359-386.

Braude, A. I.
1964 Bacterial endotoxins. Scientific American 210: 36-45.

Bruce, W. R. and Meeker, B. E.
1965 Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony forming cells to tritiated thymidine. Journal of the National Cancer Institute 34:849-856.

Carey, F. J., Braude, A. J. and Zalesky, M.
1958 Studies with radioactive endotoxin. III. The effect of tolerance on the distribution of radioactivity after intravenous injection of Escherichia coli endotoxin labeled with Cr^{51}. Journal of Clinical Investigation 37:441-457.

Cartwright, G. E., Athens, J. W. and Wintrobe, M. M.
1964 Analytical review. The kinetics of granulopoiesis in normal man. Blood 24:780-803.

Cleaver, J. E.
1967 Thymidine metabolism and cell kinetics. Vol. 6 of Frontiers of Biology. New York, New York, J. Wiley and Sons.

Cohen, J. A. and Warringa, M. G. P. J.
1954 The fate of P^{32} labelled diisopropylfluorophosphate in the human body and its use as a labelling agent in the study of the turnover of blood plasma and red cells. Journal of Clinical Investigation 33: 459-467.

Craddock, C. G., Jr., Adams, W. S., Perry, S., Skoog, W. A. and Lawrence, J. S.
1955 Studies of leukopoiesis. The technique of leukopheresis and the response of myeloid tissue in normal and irradiated dogs. Journal of Laboratory and Clinical Medicine 45:881-905.

Craddock, C. G., Jr., Perry, S. and Lawrence, J. S.
1956 The dynamics of leukopoiesis and leukocytosis as studied by leukopheresis and isotope techniques. Journal of Clinical Investigation 35:285-296.

Cronkite, E. P.
1969 Kinetics of granulocytopoiesis. National Cancer Institute Monograph 30:51-62.

Cronkite, E. P. and Fliedner, T. M.
1964 Granulocytopoiesis. New England Journal of Medicine 270:1347-1352.

Cronkite, E. P. and Vincent, P. C.
1970 Granulopoiesis. In Myeloproliferative Disorders of Animals and Man. Proceedings of the 8th Annual Hanford Biology Symposium at Richland, Washington, May 20-23, 1968. U.S. Atomic Energy Commission Conf-680529:3-35.

Deinard, A. S. and Page, A. R.
1970 An improved method for performing neutrophil survival studies. Blood 36:98-110.

Donohue, D. M., Reiff, R. H., Hanson, M. L., Betson, Y. and Finch, C. A.
1958 Quantitative measurement of the erythrocytic and granulocytic cells of the marrow and blood. Journal of Clinical Investigation 37:1571-1576.

Fallon, H. J., Frei, E., Davidson, J. D., Trier, J. S. and Burk, D.
1962 Leukocyte preparations from human blood: Evaluation of their morphologic and metabolic state. Journal of Laboratory and Clinical Medicine 59: 779-791.

Fliedner, T. M., Cronkite, E. P., Killmann, S. A. and Bond, V. P.
1964a Granulocytopoiesis. II. Emergence and pattern of labeling of neutrophilic granulocytes in humans. Blood 24:683-700.

Fliedner, T. M., Cronkite, E. P. and Robertson, J. S.
1964b Granulocytopoiesis. I. Senescence and random loss of neutrophilic granulocytes in human beings. Blood 24:402-413.

Foerster, H. V.
1959 Some remarks on changing populations. In Stohlman, F., ed. The Kinetics of Cellular Proliferation. Pp. 382-407. New York, New York, Grune and Stratton.

Galbraith, P. R., Valberg, L. S. and Brown, M.
1965 Patterns of granulocyte kinetics in health, infection and in carcinoma. Blood 25:683-692.

Gaylor, M. S., Chervenick, P. A. and Boggs, D. R.
1969 Neutrophil kinetics after acute hemorrhage. Proceedings of the Society for Experimental Biology and Medicine 131:1332-1336.

Gilmore, C. E., Gilmore, V. H. and Jones, T. C.
1964 Bone marrow and peripheral blood of cats: Technique and normal values. Pathologia Veterinaria 1:18-40.

Guyton, A. C.
1961 Textbook of Medical Physiology. 2nd edition. Philadelphia, Pennsylvania, W. B. Saunders Co.

Herion, J. C., Walker, R. I., Herring, W. B. and Palmer, J. G.
1965 Effects of endotoxin and nitrogen mustard on leukocyte kinetics. Blood 25:522-540.

Jarrett, O., Laird, H. M., Jarrett, W. F. H. and Hay, D.
1969 Feline leukemia (lymphosarcoma) symposium. 2. Experimental studies in feline leukemia. Journal of Small Animal Practice 10:599-603.

Johnson, G. R., Koestner, A. and Rohovsky, M. W.
1967 Experimental feline infectious enteritis in the germfree cat. Pathologia Veterinaria 4:275-288.

Killmann, S., Cronkite, E. P., Fliedner, T. M., Bond, V. P. and Brecher, G.
1963 Mitotic indices of human bone marrow cells. II. The use of mitotic indices for estimation of time parameters of proliferation in serially connected multiplicative cellular compartments. Blood 21: 141-163.

King, M. K.
1960 Pathogenesis of fever: Effects of various endogenous pyrogens upon the level of circulating granulocytes in normal rabbits. Journal of Experimental Medicine 112:809-819.

Kline, D. L. and Cliffton, E. E.
1952 Life span of leukocytes in man. Journal of Applied Physiology 5:79-84.

Kuida, H., Gilbert, R. P., Hinshaw, L. B., Brunson, J. G. and Visscher, M. B.
1961 Species differences in effect of gram-negative endotoxin on circulation. American Journal of Physiology 200:1197-1202.

Lawrence, J. S. and Craddock, C. G.
1968 Stem cell competition: The response to antineutrophilic serum as affected by hemorrhage. Journal of Laboratory and Clinical Medicine 72:731-738.

Lawrence, J. S., Ervin, D. M. and Wetrich, R. M.
1945 Life cycle of white blood cells. The rate of disappearance of leukocytes from the peripheral blood of leukopenic cats. American Journal of Physiology 144:284-296.

Leblond, C. P., Messier, B. and Kopriwa, B.
1959 Thymidine-H^3 as a tool for the investigation of the renewal of cell populations. Laboratory Investigation 8:296-308.

Lobue, J., Monette, F. C., Gordon, A. S., Chan, P. and Alexander, P.
1970 Cell cycle analysis of erythroid and granulocyte precursors of rats of different ages. In Myeloproliferative Disorders of Animals and Man. Proceedings of the 8th Annual Hanford Biology Symposium at Richland, Washington, May 20-23, 1968. U.S. Atomic Energy Commission Conf-680529:84-102.

Maloney, M. A. and Patt, H. M.
1968 Granulocyte transit from bone marrow to blood. Blood 31:195-201.

Maloney, M. A., Patt, H. M. and Weber, C.
1962 Estimation of deoxyribonucleic acid synthesis period for myelocytes in dog bone marrow. Nature 193:134-135.

Marsh, J. C., Boggs, D. R., Cartwright, G. E. and Wintrobe, M. M.
1967 Neutrophil kinetics in acute infection. Journal of Clinical Investigation 46:1943-1953.

Mauer, A. M., Athens, J. W., Warner, H. R., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1959 An analysis of leukocyte radioactivity curves obtained with radioactive diisopropylfluorophosphate (DFP^{32}). In F. Stohlman, ed. The Kinetics of Cellular Proliferation. Pp. 231-241. New York, New York, Grune and Stratton.

Mauer, A. M., Athens, J. W., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1960 Leukokinetic studies. II. A method for labeling granulocytes in vitro with radioactive diisopropylfluorophosphate (DFP^{32}). Journal of Clinical Investigation 39:1481-1486.

Morley, A. A.
1966 A neutrophil cycle in healthy individuals. Lancet II (No. 7475) 1220-1222.

Morley, A. and King-Smith, E. A.
1967 A computer model for the mammalian (human) leukocyte. Engineering in Medicine and Biology Annual Conference Proceedings 20:19.9.

Moxley, J. H., Perry, S., Weiss, G. H. and Zelen, M.
1965 Return of leukocytes to the bone marrow in chronic myelogenous leukemia. Nature 208:1281-1282.

Osgood, E. E.
1937 Culture of human marrow. Length of life of the neutrophils, eosinophils and basophils of normal blood as determined by comparative cultures of blood and sternal marrow from healthy persons. Journal of the American Medical Association 109: 933-937.

Osgood, E. E.
1954 Number and distribution of human hemic cells. Blood 9:1141-1154.

Osgood, E. E.
1957 A unifying concept of the etiology of leukemias, lymphomas, and cancer. Journal of the National Cancer Institute 18:155-166.

Osgood, E. E.
1970 The leukokinetics of the granulocytic series in man: a historical review. In Myeloproliferative Disorders of Animals and Man. Proceedings of the 8th Annual Hanford Biology Symposium at Richland, Washington, May 20-23, 1968. U.S. Atomic Energy Commission Conf-680529:115-139.

Owen, L. N.
1969 Feline leukemia (lymphosarcoma) symposium. 3. Some comparative aspects of leukemia in man and animals with particular reference to lymphosarcoma. Journal of Small Animal Practice 10:605-613.

Patt, H. M. and Maloney, M. A.
1959a Kinetics of neutrophil balance. Conference on fundamental problems and technics of the study of the kinetics of cellular proliferation, Salt Lake City, Utah, January 19-21, 1959. In Stohlman, F., ed. The Kinetics of Cellular Proliferation. New York, New York, Grune and Stratton.

Patt, H. M. and Maloney, M. A.
1959b Patterns of neutrophilic leukocyte development and distribution. New York Academy of Science Annals 77:766-772.

Patt, H. M. and Maloney, M. A.
1964 A model of granulocyte kinetics. New York Academy of Science Annals 113:515-522.

Patt, H. M., Maloney, M. A., Jackson, E. M. and Straube, R. L.
1957a Neutrophil release from bone marrow. Federation Proceedings 16:98-99.

Patt, H. M., Maloney, M. A. and Jackson, E. M.
1957b Recovery of neutrophils after acute peripheral depletion. American Journal of Physiology 188: 585-592.

Raab, S. O., Athens, J. W., Haab, O. P., Boggs, D. R., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M.
1964 Granulokinetics in normal dogs. American Journal of Physiology 206:83-88.

Rappaport, H.
1966 Tumors of the hematopoietic system. In Atlas of Tumor Pathology. Section III. Fascicle 8. Washington, D.C., Armed Forces Institute of Pathology.

Sabin, F. R., Cunningham, R. S., Doan, C. A. and Kinwall, J. A.
1925 The normal rhythm of white blood cells. Johns Hopkins Hospital Bulletin 37:14-67.

Schalm, O. W.
1965 Veterinary hematology. 2nd ed. Philadelphia, Pennsylvania, Lea and Febiger.

Schalm, O. W. and Smith, R.
1963 Some unique aspects of feline hematology in disease. Small Animal Clinician 3:311-318.

Schryver, F.
1963 The bone marrow of the cat. American Journal of Veterinary Research 24:1012-1017.

Sisken, J. E. and Moraska, L.
1965 Intrapolation kinetics of the mitotic cycle. Journal of Cell Biology 25:179-189.

Stryckmans, P., Cronkite, E. P., Fache, J., Fliedner, T. M. and Ramos, J.
1966 Deoxyribonucleic acid synthesis time of erythropoietic and granulopoietic cells in human beings. Nature 211:717-720.

Taylor, J. H., Woods, P. S. and Hughes, W. L.
1957 The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium labeled thymidine. National Academy of Science Proceedings 43:122-128.

Valeriote, F. A., Bruce, W. R. and Meeker, B. E.
1966 Comparison of the sensitivity of normal hematopoietic transplanted lymphoma colony forming cells of mice to vinblastine administered in vivo. Journal of National Cancer Institute 36:21-27.

Van Dyke, D. C. and Huff, R. L.
1951 Life span of white blood cells as measured in irradiated parabiotic rats. American Journal of Physiology 165:341-347.

Vejlens, G.
1938 The distribution of leukocytes in the vascular system. Acta Pathologia et. Microbiologica Scandinavia, Suppl. 33.

Verly, W. G., Firket, H. and Hunebelle, G.
1958 Thymidine-H^3 in studies of DNA synthesis in tissue cultures. In Proceedings Second International Conference on Peaceful Uses of Atomic Energy. Vol. 25. P. 181. London, Her Majesty's Stat. Off.

Vincent, P. C., Cronkite, E. P., Greenberg, M. L., Kirsten, C., Schiffer, L. M. and Stryckmans, P. A.
1969 Leukocyte kinetics in chronic myeloid leukemia: I. DNA synthesis time in blood and marrow myelocytes. Blood 33:843-850.

Walker, R. I., Herion, J. C. and Palmer, J. G.
1961 Leukocyte labeling with inorganic radiophosphorus. American Journal of Physiology 201:1137-1141.

Walker, R. I., Herion, J. C. and Palmer, J. G.
1962 Leukocyte kinetics studied by nitrogen mustard induced alterations in DNA phosphorus labeling. Blood 20:72-81.

Walker, R. I., Herion, J. C., Herring, W. B. and Palmer, J. G.
1964 Leukocyte kinetics in hematologic disorders studied by DNA-phosphorus labeling. Blood 23:795-810.

Warner, H. R. and Athens, J. W.
1964 An analysis of granulocyte kinetics in blood and bone marrow. New York Academy of Science Annals 113:523-536.

White, A., Handler, P. and Smith, E. L.
1968 Principles of Biochemistry. 4th ed. New York, New York, McGraw Hill Book Co.

White, L. P.
1954 The intravascular life span of transfused leukocytes tagged with atabrine. Blood 9:73-82.

Wintrobe, M. M.
1961 Clinical Hematology. 5th ed. Philadelphia, Pennsylvania, Lea and Febiger.

ACKNOWLEDGMENTS

I would like to express appreciation to my major professor, Dr. F. K. Ramsey, and to my entire committee for their assistance and encouragement during the course of this study.

A special thank you is extended to Dr. M. L. Kaeberle, Veterinary Microbiology and Preventive Medicine, and Dr. R. C. Seagrave, Chemical Engineering and Biomedical Engineering. The counsel and guidance from these individuals was greatly appreciated.

Thanks are extended to Dr. R. L. Kemp for his constructive criticism during preparation of this manuscript.

Thanks are extended to Mrs. Shirley Hulse for assistance in typing this manuscript.

I am very grateful to my wife, Karen, and to my children, David and Gretchen, for their patience and devotion throughout the course of this endeavor.

APPENDIX

Table 15. Body weight (Kg), blood volume (BV), circulating granulocyte count at zero time (Gt_o), specific activity of infused granulocytes (SAInfG), number of granulocytes infused (NoInfG), specific activity of granulocytes at zero time ($SAGt_o$) and slope of granulocyte disappearance curve (b) for hematologically normal cats

Data	Cats				
	12N	13N	14N	18N	26N
Kg	2.13	4.54	2.42	3.58	2.27
BV(ml/Kg)	64	72	61	71	70
Gt_o($x10^6$/ml)	13.45	14.79	11.57	9.84	6.64
SAInfG(CPM/10^6G)	6675	10784	7839	7937	7622
NoInfG($x10^6$)	122.0	149.6	155.2	92.8	124.7
$SAGt_o$(CPM/10^6G)	129.2	84.0	138.6	55.6	244.8
b	-0.0335	-0.0315	-0.0459	-0.0580	-0.0461

Table 16. Body weight (Kg), blood volume (BV), circulating granulocyte count at zero time (Gt_o), specific activity of infused granulocytes (SAInfG), number of granulocytes infused (NoInfG), specific activity of granulocytes at zero time ($SAGt_o$) and slope of granulocyte disappearance curve (b) in cats treated with bacterial endotoxin 10 hours before pool size determination time

Data	23E	24E	29E
Kg	1.72	1.31	1.46
BV(ml/Kg)	74	87	77
Gt_o($x10^6$/ml)	4.37	21.25	33.81
SAInfG(CPM/10^6G)	5560	4946	5222
NoInfG($x10^6$)	61.2	116.9	232.1
$SAGt_o$(CPM/10^6G)	70.6	53.9	134.6
b	-0.0383	-0.0292	-0.0371

Table 17. Body weight (Kg), blood volume (BV), circulating granulocyte count at zero time (Gt_o), specific activity of infused granulocytes (SAInfG), number of granulocytes infused (NoInfG), specific activity of granulocytes at zero time ($SAGt_o$) and slope of granulocyte disappearance curve (b) in cats in which the effects of hemorrhage on blood granulocyte kinetics were studied

Data	Cats 15H	19H	21H	27H	28H	32H	33H
Kg	2.22	1.28	1.48	2.00	2.98	4.20	3.72
BV(ml/Kg)	63	76	73	74	63	60	57
$Gt_o(x10^6/ml)$	11.90	8.07	19.85	8.37	15.16	8.20	6.00
SAInfG(CPM/10^6G)	8573	5741	6619	4724	5392	5796	5730
NoInfG($x10^6$)	213.3	40.9	165.0	89.5	139.1	92.4	100.5
$SAGt_o$(CPM/10^6G)	157.6	89.6	87.7	100.4	76.9	40.5	47.1
b	-0.0186	-0.0613	-0.0075	-0.0624	-0.0366	-0.0059	-0.0243

Table 18. Body weight (Kg), blood volume (BV), circulating granulocyte count at zero time (Gt_o), specific activity of infused granulocytes (SAInfG), number of granulocytes infused (NoInfG), specific activity of granulocytes at zero time ($SAGt_o$) and slope of granulocyte disappearance curve (b) in cats with naturally occurring blood dyscrasias

Data	Cats 16FL	17UL	20Ly
Kg	1.77	1.65	3.58
BV(ml/Kg)	67	73	54
Gt_o($x10^6$/ml)	36.24	18.36	2.45
SAInfG(CPM/10^6G)	6588	8318	4114
NoInfG($x10^6$)	580.4	163.4	49.5
$SAGt_o$(CPM/10^6G)	574.1	97.2	126.4
b	-0.0409	-0.0269	-0.0056