is responsible. Evidence supporting this view will be reported later.

Summary. Collagenase activity was demonstrated by lysis of precipitated collagen gel. Such collagenase activity was found to occur in both viable gingival explants (14 of 41 cases) and in frozen and thawed specimens (14 of 46 cases). This collagenase activity appeared to be associated with the presence of inflammatory foci and the accumulation of gamma globulin in the tissue specimens in most of the cases examined. Scrapings of teeth adjacent to excised gingivae failed to yield lytic activity on collagen gel. On the basis of these findings, it is concluded that the observed collagenolytic activity is produced either by: 1) the inflamed gingival tissue itself, 2) the gingival tissue in response to microbial or other exogenous stimuli or 3) tissue plus microbial collagenases.

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The Different Effects of Vinblastine Sulfate and Nitrogen Mustard Upon Neutrophil Kinetics in the Dog.* (30973)

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It is generally assumed that an antitumor drug induces neutropenia simply by interrupting production of new neutrophils by its destructive effect upon the mitotic pool of neutrophil precursors in the bone marrow. Neutropenia should develop when the initially undamaged post-mitotic maturation and storage pool of the bone marrow [marrow granulocyte reserve, MGR(1)] is exhausted.

This hypothesis proved sufficient to explain observations of the effect of vinblastine sulfate (VLB) upon the morphology(2) and kinetics(3) of canine neutrophils. Within one day of administering 0.2 mg/kg of VLB, more than 90% of morphologically identifiable, potentially mitotic, neutrophil precursors had disappeared from the bone marrow. No direct effect of VLB upon either blood neutrophils or post-mitotic marrow neutrophils of the MGR was apparent. However, since production was interrupted, the size of the MGR gradually decreased as few new cells were added to it and cells continued to feed out into the blood. The rate at which cells entered the blood from the MGR remained normal until the compartment was exhausted. Therefore blood neutrophil concentration remained normal until the MGR was exhausted and then declined abruptly. The abrupt decline usually occurred on the fourth day after VLB, which suggests that the MGR of the dog normally contains enough cells to supply the blood for slightly less than 4 days. Independent measures of the size of this compartment in the dog, utilizing either radioactive diisopropylfluorophosphate (DFP^{32})(3), tritiated thymidine(4), radioactive phosphorus(5), or radioactive sulfate(6) as isotopic labels for neutrophils are in agreement with this figure.

Certain reports(7,8) suggest that nitrogen mustard (HN2) might have a qualitatively different effect upon neutrophils from VLB. After HN2 administration to the dog, the

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onset of neutropenia was reported to be earlier than after VLB, the nadir of neutrophil concentration to be reached later and recovery from neutropenia slower.

The present study was undertaken to determine if there is a difference in the effect of VLB and HN2 upon neutrophils in the dog and to delineate the nature of any such difference. To this end, normal dogs were given HN2 and the effect of HN2 upon the morphology of the blood and marrow and upon the blood granulocyte specific activity (BGSA) curve following intravenously administered DFP^{32} was compared to that of dogs given VLB(2,3).

Materials and methods. Dogs of either sex and weighing from 13 to 22 kg were given antihelminthic therapy and distemper vaccine and observed for 10 days before being considered healthy and therefore suitable subjects for the experiment. All studies were done in unanesthetized dogs.

Intravenously administered DFP^{32} labels myelocytes, but not myeloblasts and promyelocytes in the marrow mitotic pool, all neutrophils in the MGR and neutrophils in the blood(3). The subsequent curve of BGSA is divisible into 4 phases(3). Phase I, a 20% decline in BGSA during the first day, represents replacement of neutrophils in the blood at the time of DFP^{32} administration by cells from the MGR which have a slightly lower BGSA. Phase II is a relative plateau of BGSA which terminates when the progeny of the first myelocyte division after DFP^{32} administration begins to leave the blood. Thus, the duration of phases I and II (hereafter referred to as phase I+II) measures the time required for a myelocyte to divide and to traverse the MGR and the blood pool. Phase III is an exponential decline in BGSA, the slope of which is primarily dependent upon the generation time of the myelocyte population and variation in transit time through the MGR and the blood. Phase IV is a "tail" of low level BGSA representing reutilization of a metabolite of DFP^{32}-labeled enzymes.

All injections were given intravenously through a foreleg vein. Venous blood samples, 2 ml for leukocyte counts and 20 ml for determination of BGSA, were obtained by jugular puncture. Needle aspiration of bone marrow was from the sternum.

Results. Nine dogs were given 0.75 mg/kg of HN2, their neutrophils labeled one hour thereafter by intravenous administration of 4.5 mg of DFP^{32} and their subsequent neutrophil concentration and BGSA determined in daily blood samples. Results of these studies are summarized in the Table and representative individual studies are illustrated in Fig. 1.

In 5 of the 9 dogs, blood neutrophil concentration described a curve similar to that of certain previous reports of the effect of HN2 in the dog(7,8); that is, by the second day after HN2, neutrophil concentration had begun to decline and a steady decline persisted until a nadir of neutrophil concentration was reached at 7 to 10 days (Table I, Fig. 1-A). This type of response is referred to hereafter as "early" onset neutropenia. In these dogs, phase I+II of the BGSA curve did not differ in duration from that of normal, untreated dogs (Table I, Fig. 1-A).

In 4 of the 9 dogs, neutropenia developed in a qualitatively different fashion. In these dogs neutrophil concentration remained at or near normal levels for 2 to 3 days and then decreased precipitously reaching a nadir by 7 days (Table I, Fig. 1-B). The onset of neutropenia in these dogs was similar to that observed in VLB treated dogs(2) and like VLB-induced neutropenia(3) was associated with a significant ($P = <.05$) reduction in the duration of phase I+II of the BGSA curve (Table I, Fig. 1-B). This type of development of neutropenia has been termed "late" onset as opposed to the "early" onset neutropenia observed in the other 5 HN2 treated dogs.

Measures of other components of the BGSA curve (half-disappearance time in phase III, slope of phase II and IV and level of BGSA at onset of the various phases) did not differ in either group of HN2 treated dogs from VLB treated dogs(3).

There was no apparent difference in weight, control blood neutrophil concentration or control marrow morphology between HN2-treated

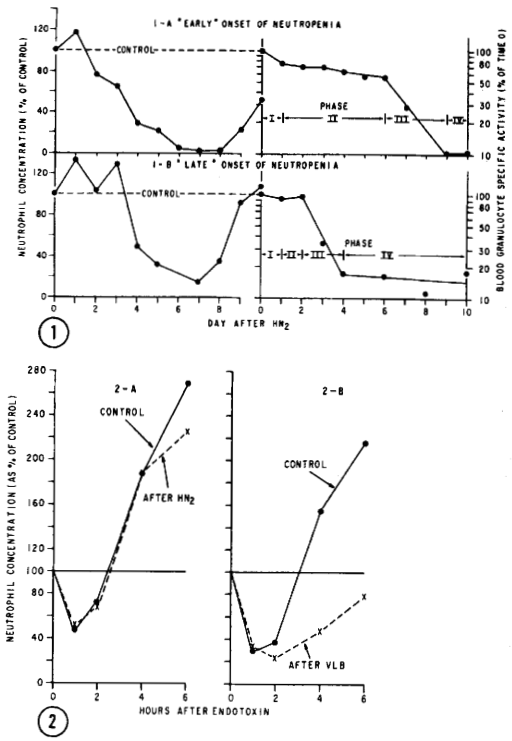

FIG. 1. Representative examples of the 2 types of blood neutrophil concentration and blood granulocyte specific activity (BGSA) curves observed after nitrogen mustard. In Fig. 1-A, results from a dog which developed "early" onset of neutropenia but in which a BGSA curve with a normal phase I + II (6 days) was observed are illustrated. In Fig. 1-B, the results from a dog which developed "late" onset neutropenia in association with a BGSA curve with a short (2 days) phase I + II are illustrated. Left half of each curve gives neutrophil concentration, plotted in proportion to the values in control animal, and right half describes the BGSA.

FIG. 2. Response of dogs to endotoxin given 2 days after nitrogen mustard (HN2) (2-A) or vinblastine sulfate (VLB) (2-B). The mean results in 6 dogs studied one week before (●-----●) and again 2 days after (×-----×) HN2 (Fig. 2-A) are compared to those obtained in 5 dogs studied one week before (●-----●) and 2 days after (×-----×) VLB (Fig. 2-B).

dogs with "early" or "late" onset neutropenia.

Differential cell counts of the marrow, 1 and 4 days after drug administration, were compared in VLB treated dogs and in HN2 treated dogs with "early" or "late" onset neutropenia. There was no detectable difference one day after drug administration in the 3 groups. Myelocytes were scarce in all, constituting but 1.4% of marrow neutrophils after VLB, 1.9% after HN2 with "late" onset neutropenia and 1.7% after HN2 with "early" onset neutropenia (control values were 13.6% in VLB group, 15.2% in HN2 group). Four days after drug administration, mitotic neutrophil precursors were returning rapidly to the marrow of VLB treated dogs(2) but were virtually absent in HN2 treated animals. Smears from the 4-day post-HN2 marrow aspirates were exceedingly hypocellular. In dogs given HN2 which developed "early" onset neutropenia, segmented neutrophils were seemingly more frequent in 4-day marrow aspirates than they were in dogs with "late" onset neutropenia.

To assess the availability of neutrophils in the MGR, the response to intravenously ad-

TABLE I. A Comparison of Blood Neutrophil Concentration and Blood Granulocyte Specific Activity (BGSA) Curves in Dogs Given Nitrogen Mustard (HN2) with Dogs Given Vinblastine Sulfate (VLB) and with Normal Dogs.

	Effect of drug on: Neutrophil concentration: Day of beginning decrease*	Neutrophil concentration: Day of nadir*	Duration of phase I + II of BGSA curve
Normal dogs (14) †			
Mean	—	—	4.8
Range	—	—	3.0–7.0
VLB treated dogs (8) †			
Mean	3.8	5.1	3.4
Range	3.0–5.0	4.0–7.0	1.0–4.5
HN2 treated dogs with:			
"Late" onset neutropenia (4)	3.0	7.0	3.0
	4.0	7.0	3.5
	4.0	7.0	2.0
	3.0	‡	2.5
Mean	3.5	7.0	2.8
"Early" onset neutropenia (5)	1.0	8.0	4.5
	2.0	9.0	5.0
	1.0	10.0	5.5
	2.0	8.0	6.0
	1.0	7.0§	4.5
Mean	1.4	8.4	5.1

* Day upon which neutrophil concentration decreased to at least 85% of control levels and failed to return to control levels for at least 5 days. Day of nadir represents lowest granulocyte concentration observed during the period of drug induced neutropenia.

† Data previously reported in reference 3.

‡ Died day 5.

§ Died day 10.

ministered endotoxin was compared in HN2 and VLB treated animals. One week before and 2 days after HN2, 6 dogs were injected with 1 μg of endotoxin ("Pyrexal," Wander Co.). This study was limited to animals in which "early" onset neutropenia was developing, that is, animals developing the pattern illustrated in Fig. 1-A. The post-HN2 neutrophilia induced by endotoxin was almost as brisk as in the control (Fig. 2-A) whereas in 5 dogs given endotoxin 2 days after VLB, neutrophilia failed to develop (Fig. 2B).

Discussion. Two qualitatively dissimilar defects in neutrophil kinetics were evident in different dogs given the same dose of HN2.

In certain animals a neutrophil concentration curve and a blood granulocyte specific activity (BGSA) curve similar to that observed after VLB(2,3) followed administration of HN2; that is, neutrophil concentration remained normal for 2 to 3 days and then decreased precipitously ("late" onset neutropenia) and phase I+II of the BGSA curve was shorter than normal. The kinetic interpretation for this sort of change would appear to be the same as that for VLB-treated dogs(2,3); namely, that after the mitotic pool is severely damaged by the drug, the post-mitotic marrow pool continues to supply cells to the blood at a normal rate until it is exhausted. Once it is exhausted the blood neutrophil concentration drops abruptly. The duration of phase I+II of the normal BGSA curve represents the time required for the myelocyte compartment to complete a generative cycle and for the progeny of that cycle to mature and traverse the marrow storage pool and the blood(3). The severe myelocyte damage induced with HN2 or VLB shortens phase 1+II by approximately one day by removing the myelocyte generative cycle from the curve. Acceleration of the blood granulocyte turnover rate will also shorten phase I+II but no change in turnover rate was demonstrable after VLB(3).

In other animals, neutropenia began to develop more quickly, a decline in neutrophil concentration being evident within 1 or 2 days after HN2 administration, but the rate of decline was more gradual ("early" onset neutropenia). In these animals phase I+II of the BGSA curve was of normal duration. A short phase I+II would be expected in these animals since myelocytes were virtually all destroyed and that portion of phase I+II representing a myelocyte generation time (approximately 1 day)(3) would be removed from the curve. Therefore the post-mitotic marrow granulocyte reserve (MGR) emptied at a slower than normal rate in HN2 treated dogs with "early" onset neutropenia and at a rate which was one-half that observed in dogs with "late" onset neutropenia. It would appear that the "early" onset neutropenia initially represents a failure of marrow release rate to keep abreast of blood egress rate despite the presence of appreciable numbers of neutrophils in the bone marrow.

The alternative hypothesis which could theoretically explain the "early" onset neutropenia after HN2 would be that HN2 did not produce an abrupt decrease in cell production but caused a gradually dwindling production which was accompanied by a marked increase in the rate of egress of cells from the blood. In this circumstance the MGR would be exhausted rapidly by an increased blood neutrophil turnover rate and the development of neutropenia would reflect dwindling production, primarily. Neither the isotopic labeling data nor the morphologic data from marrow examination will fit this explanation. Rapid exhaustion of the MGR should be reflected in a shortened phase I+II, for the acceleration in release rate from the MGR associated with administration of a single dose of endotoxin shortens phase I+II by one day(3). A 7-fold decrease in the mitotic to post-mitotic marrow neutrophil ratio was observed one day after HN2. If rapid exhaustion of the MGR was coupled with continuing mitotic production, this ratio should not have been reduced appreciably and might have been increased.

Endotoxin-induced neutrophilia is the result of an accelerated rate of release of neutrophils from the MGR to the blood(3,9). The failure of dogs to respond with neutrophilia to endotoxin injection 2 days after VLB is consistent with previously reported studies suggesting that marrow stores are virtually exhausted by this time(2). How-

ever, dogs which developed "early onset" neutropenia after HN2 developed neutrophilia when injected with endotoxin. This is compatible with the results of DFP^{32} labeling studies which suggested that these animals had appreciable marrow stores remaining at this time.

It would appear, therefore, that in certain dogs administration of HN2 decreases the rate at which mature neutrophils are released from the marrow despite the presence of cells in the marrow in numbers adequate to maintain a normal rate of output for some time. The normal response of such animals to endotoxin-stimulated marrow release suggests that the defect is not one of profound structural change like that, for example, produced by marrow hemorrhage after total body irradiation(10). Delayed maturation, as a theoretic effect of HN2, might contribute to a decreased rate of neutrophil release from marrow. The continuum of neutrophil maturation in the post-mitotic maturation and storage pool of the marrow can be divided arbitrarily into metamyelocyte, band and segmented neutrophils. Band and segmented neutrophils are readily released to the blood and constitute the readily available marrow reserve. Dogs recovering from VLB administration did not release newly formed metamyelocytes even though they were markedly neutropenic(2). Therefore a delay in metamyelocyte to band maturation could lead to delayed release. However, less than one-half of post-mitotic neutrophils are metamyelocytes(2), so delayed maturation in and of itself should not lead to a decrease in blood neutrophil concentration for 2 to 3 days. Decreased blood neutrophil concentration was evident by one day in 3 of 5 dogs with "early" onset neutropenia after HN2.

Recovery from HN2 induced neutropenia was uniformly slower than recovery from VLB induced neutropenia. Assuming that the rate of recovery of blood neutrophil concentration reflects recovery of the marrow mitotic pool, this suggests that marrow damage induced by HN2 is more profound (or at least of longer duration) that that induced by VLB. This assumption is supported by the observation that 4 days after drug administration, neutrophil precursors were easily demonstrable in the marrow after VLB but were scarce after HN2. However, other factors such as persistence of impaired release from marrow, different rates of peripheral utilization of newly formed neutrophils or different degrees of stimulus to marrow recovery could contribute to different rates of recovery of blood neutrophil concentration.

Zuelzer(11) and Krill, Smith and Mauer (12) reported studies in a patient with idiopathic neutropenia in whom it was suggested that neutropenia was the result of defective cell release from the bone marrow. Zuelzer (11) proposed the term "myelokathexis" (Kathexis = retention) for such a defect. While HN2 administration results in the development of neutropenia primarily through its damaging effect upon the mitotic pool, it would appear also to produce "myelokathexis," an observation which tends to confirm the existence of such a concept in neutrophil kinetics.

Summary. Dogs were given nitrogen mustard (HN2) and radioactive diisopropylfluorophosphate (DFP^{32}) and the type of neutropenia which developed, whether of "early" or "late" onset, was correlated with changes observed in the blood granulocyte specific activity (BGSA) curve. The duration of phase I+II of the BGSA curve measures the time required for the storage pool of the marrow to empty by releasing cells to the blood. In dogs developing "late" onset neutropenia there was an abnormally short phase I+II while in those developing "early" onset neutropenia phase I+II was normal. These results were compared with those reported previously in vinblastine sulfate treated dogs. It was concluded that dogs with "early" onset neutropenia after HN2 must have developed a defect leading to an abnormally low rate of output cells from the marrow granulocyte reserve (MGR) to the blood. Reduced output developed despite the presence of enough cells in the MGR to maintain a normal rate of output.

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Genetic Aspects of Resistance to Friend Leukemia Virus.* (30974)

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Resistance of inbred C57BL mice to Friend's leukemic agent has been documented in a number of studies(1,2,3). Analysis of F_2 and backcross progeny from RF $\times$ C57BL crosses suggested that resistance is a simple recessive character(4). In an attempt to differentiate susceptibility in the CFW and DBA2 strains from C57BL-resistance by standard mendelian methods, an alternate form of gene expression was suggested by our findings. These observations are described here.

Materials and methods. Mice. C57BL/Crgl and DBA2/Crgl mice were obtained from the Cancer Research Genetics Laboratory, University of California, Berkeley. Inbred CFW mice were obtained from the Lobund Laboratory, University of Notre Dame, Notre Dame, Ind. *Virus.* BALB/c-adapted Friend virus was obtained from Dr. W. Bostick, California College of Medicine, Los Angeles. It was specifically adapted to DBA and CFW mice by serial passage. After 7 passes in each respective strain, 2 pools of virus were prepared from spleen homogenates in the manner described by Fieldsteel *et al* (2). Virus titers of DBA-derived preparations were determined in DBA and CFW mice respectively as described by Friend(1). A similar reciprocal titration was made with CFW-derived virus. The results summarized in Table I indicate negligible differences. Passage history appeared to have little or none of the effects previously described(1,2). Stock virus for subsequent experiments consisted exclusively of DBA-derived preparations. *Inoculation and subsequent animal observations.* Cell-free preparations containing approximately 20 ID_{50} of virus were inoculated intraperitoneally in 35-40 day old test

TABLE I. Titration of Friend Virus* in CFW and DBA Mice.

Passage history	Assay strain	Titer per ml (log_{10})
DBA	DBA	3.48
DBA	CFW	3.56
CFW	CFW	3.43
CFW	DBA	3.35

* Virus prepared from spleen homogenates 21 days after infection: 0.5 ml was inoculated intraperitoneally into each of a group of 10 mice per decimal dilution.

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