# *Clinical and Pathologic Features of Cyclic Hematopoiesis in Grey Collie Dogs*

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**Clinical and pathologic features of cyclic hematopoiesis in 18 grey collie dogs, aged 10 to 113 weeks, were reviewed. The dogs were grouped according to weeks of age: 10–16 (I), 17–21 (II), 30–35 (III), and >52 (IV). Clinical illness occurring during each hematopoietic cycle was classified as none, mild, moderate, or severe, based on the neutrophil count, rectal temperature, clinical signs, and use of antimicrobial therapy. The dogs in Groups I, III, and IV had severe infectious episodes during one-fourth of all hematopoietic cycles; whereas the dogs in Group II had severe infections during two-thirds of cycles. However, during the cycle prior to death, all groups were similar, each having two-thirds of clinical syndromes classified as severe and one-third as mild. More dogs died during the neutropenic phase of the hematopoietic cycle than during the nonneutropenic phase. Pathologic findings showed distinct patterns in relation to age. Younger dogs showed evidence of acute infectious processes, especially in the lungs, gastrointestinal tract, and kidneys; whereas older dogs had chronic inflammatory changes in those organs. Amyloidosis was a prominent finding in dogs over 30 weeks of age. These findings indicate that predictable age-related changes in tissues of grey collie dogs impair various organ systems and thereby contribute to morbidity and mortality in older dogs. Consequently, future clinical and pathologic studies of grey collies should take into consideration the age of the dogs under study. (Am J Pathol 1983, 111:224–233)**

CYCLIC HEMATOPOIESIS of grey collie dogs is an autosomal recessive disease[1,2] characterized by periodic fluctuation in bone marrow production of hematopoietic cells.[3–5] It has been used as a model for the study of the regulation of blood cell production by the bone marrow[4,6–8] and for studies of the pathogenesis of secondary amyloidosis, a common sequela of the disease.[9–12] While clinical and pathologic features of the grey collie syndrome have been described,[10,13–15] characteristics and interrelationships of clinical events and pathologic changes as a function of age have not been well delineated. Cheville et al[9,10,14] reported the systemic disease in 5 dogs greater than 10 weeks of age, while Machado et al[12] described the distribution of amyloid and pathologic changes in lymphoid tissues in 18 dogs greater than 8 weeks of age. In this study of 18 grey collie dogs that died between 10 and 113 weeks of age, the pathologic changes were related to both the clinical history and the age of the dog.

## Materials and Methods

### Animals

Grey collie puppies were procured at 6–8 weeks of age from private breeders. Nine of the puppies were from 4 litters (3 litters of 2 dogs and 1 litter of 3 dogs); the remainder were the only surviving grey collie puppies from their litters. Commercially prepared distemper, hepatitis, and leptospirosis vaccine was administered parenterally without untoward effects. The dogs were housed in individual cages or runs in temperature-controlled rooms with the use of automatically controlled light and dark cycles and cared for according to procedures promulgated by the American Association for Accreditation of Laboratory Animal Care. Clinically healthy dogs were exercised together in runs, whereas febrile or ill dogs were isolated and treated. Empiric antibiotic therapy and fluid replacement were used at early signs of fever or dehydration. Hematologic studies were performed as described previously.[16,17] The oldest dog, aged 113 weeks, received 300–600 mg of lithium carbonate

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daily for 307 days for a total dosage of 153 g. Eighteen grey collie dogs, 10 males and 8 females, aged 10–113 weeks at death, were used in this study.

### Endotoxin Administration

Concentrated endotoxin (lipopolysaccharide W from *Salmonella typhosa* 0901, Lot 3124-25, Difco Laboratories, Detroit, Mich) was diluted to 500 μg/ml in normal saline and stored at −10 C. Dilutions of 50 μg/ml were stored not longer than 2 weeks before use, and highly diluted endotoxin doses (<1 μg/ml) were prepared immediately before injection. Endotoxin was administered intravenously once daily to 5 grey collies, starting at 0.0005 μg/kg per day, and the dosage was increased geometrically to 0.1 μg/kg per day. Subsequently, the dosage was increased arithmetically by 0.5 μg/kg per day, to a maximum dose of 5 μg/kg per day in 3 dogs and 30 μg/kg per day in 2 dogs. The dogs were treated for 40–170 days, and in 4 dogs treatment ended 0–3 months before death; whereas treatment in 1 dog ended 18 months before death.

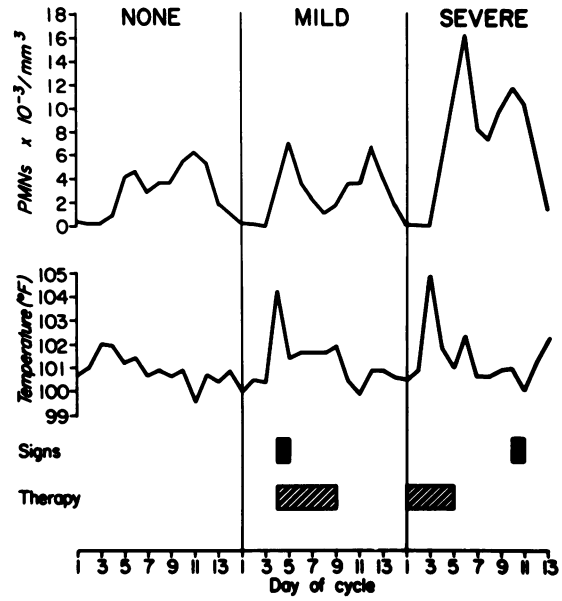


Figure 1 — Three consecutive cycles in grey collie IV-2 illustrating no, mild, and severe (moderate not shown) clinical infections by circulating neutrophil counts (*PMNs*), temperature, clinical signs (*signs*), and antimicrobial therapy (*therapy*).

### Clinical Histories

The neutrophil count, rectal temperature, and occurrence of clinical illness were used to characterize the clinical severity of infectious processes during hematopoietic cycles. The neutrophil count was used to delineate the hematopoietic cycle. The day the neutrophil count first fell below 1000/cu mm was designated as Cycle Day 1, and cycle days were numbered sequentially until the next occurrence of Cycle Day 1. The period of neutropenia usually persisted through Cycle Day 4 and was invariably followed by a sharp increase in neutrophils. A trough and second peak in neutrophils usually occurred before neutropenia recurred, and the periodicity of such cycles averaged 12 days. If the peak of the neutrophil count exceeded 10,000/cu mm, this was denoted as neutrophilia. A rise in rectal temperature ≥2.0 F above 102.5 F was designated as fever. Clinical signs and antimicrobial therapy were considered together in denoting morbidity, because it was found that signs of clinical illness were not always recorded, whereas chemotherapy was recorded, because it had to be administered over several days. Antimicrobials were administered when indicated and not prophylactically. Depending on the clinical syndrome, the following antibiotics were administered: hetacillin, ampicillin, methicillin, cephaloridine, gentamicin, and chloramphenicol. The severity of clinical disease in each cycle was classified as "none," "mild," "moderate," or "severe" on the basis of the presence or absence of these factors. "Severe" indicated the presence of neutrophilia, fever, and signs or therapy; "moderate" indicated neutrophilia with or without fever, signs, or therapy; "mild" indicated fever and/or signs or therapy; and "none" indicated the absence of neutrophilia, fever, and signs or therapy (Figure 1).

### Histopathology

Pathologic findings from 18 grey collies that died in the colony between 1975 and 1979 were reviewed. All dogs died of complications of cyclic hematopoiesis; none were killed. Necropsies were performed according to standard methods. Certain tissues such as brain and joint tissues were not examined routinely unless unusual clinical signs were present. Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. Congo red stain was used for confirmation of the presence of amyloid.

To validate comparison of findings between dogs, prior to histologic study we established a detailed grading system for assessing the extent and severity of pathologic changes and criteria for classification of inflammatory infiltrates. All tissue specimens were reviewed and graded by one individual (L.L.K.) without knowledge of the clinical history or age of the dog.

Table 1—Number of Grey Collie Dogs by Sex and Age at Death

| Group | Age (weeks) | Sex | |
|---|---|---|---|
| | | Male | Female |
| I | 10–16 | 3 | 4 |
| II | 17–21 | 3 | 2 |
| III | 30–35 | 3 | 1 |
| IV | >52 | 1 | 1 |

### Dog Groups

To facilitate analysis of the data, we divided the dogs into groups by age. Group I dogs (aged 10–16 wks) died within 2 months after arrival. Group II dogs (aged 17–21 wks) survived longer than 2 months but less than 6 months after arrival. The older dogs were divided into those surviving between 6 and 12 months (Group III) and those surviving more than 1 year (Group IV) (Table 1).

## Results

### Clinical Disease

The severity of clinical disease during all hematopoietic cycles as well as the cycle prior to death varied by age group (Table 2). In general, all of the dogs experienced mild or severe clinical infections during one-half and one-third of their cycles, respectively. The dogs in Group I exhibited severe infections in about one-fourth of cycles and mild infections in the remainder. The dogs in Group II had a marked shift in the severity of infections; infections were severe in about two-thirds of cycles, with about equal percentages of moderate, mild, and no infections. The dogs in Groups III and IV were similar in that they exhibited mild or severe clinical infections during one-half and one-quarter of their cycles, respectively; remaining cycles were characterized by moderate or no infections. Although the severity of clinical disease in the dogs differed when all the cycles were considered, especially for dogs in Group II, the characteristics of the cycle prior to death were similar for all groups. During the last cycle in all groups two-thirds of the dogs had severe clinical infections, whereas one-third of the dogs had mild infections. Although some of the dogs failed to exhibit infections during some cycles, there were no instances in which infection was absent during a dog's terminal cycle.

During the cycle prior to death, virtually all dogs were febrile (Table 3). Almost one-half were lethargic, and about one-quarter were anorectic. Enteric or respiratory disease occurred in about one-half of the dogs. Icterus was observed in 5 dogs in Groups II, III, and IV; however, only 2 of 4 dogs sampled (1 each in Groups II and III) had total bilirubin levels greater than 1.0 mg/dl. A variety of other signs were observed less frequently. Laboratory evaluation revealed that anemia (hematocrit <35%) was a consistent finding and occurred in virtually all dogs. Azotemia (urea nitrogen >60 mg/dl) was demonstrated in 2 dogs in Group III. While serial liver function tests were performed on only 4 dogs (1 each in Groups II and III and 2 in Group IV), all exhibited hyperglobulinemia, although total protein remained within the normal range. Three dogs evaluated during endotoxin treatment showed marked elevations of alkaline phosphatase, SGOT, and SGPT, which abated upon termination of treatment.

In general, there was no correlation between the age of the dogs and the number of days they were treated with either all antibiotics or nephrotoxic antibiotics (Table 4). However, in relation to time survived, the highest ratio of days treated occurred in the dogs in Group I. Successive groups exhibited declining ratios of days treated to days survived.

### Pathologic Findings

#### *Lungs*

The pattern of pulmonary inflammation varied according to the age of the dogs at death. Acute suppurative bronchopneumonia was observed only in dogs in Group I (Table 5) and was responsible for the death of 4 dogs (Table 6). Also, acute suppurative interstitial pneumonia occurred in the 3 other dogs in Group I. Although this lesion occurred diffusely

Table 2—Clinical Severity of Hematopoietic Cycles in Grey Collie Dogs

| Group | All cycles | | | |
|---|---|---|---|---|
| | Severe | Moderate | Mild | None |
| I | 29* | 0 | 65 | 6 |
| II | 63 | 9 | 12 | 16 |
| III | 27 | 21 | 46 | 6 |
| IV | 24 | 11 | 43 | 22 |
| Total | 33 | 13 | 40 | 14 |
| | Last cycle | | | |
| I | 60 | 0 | 40 | 0 |
| II | 60 | 20 | 20 | 0 |
| III | 75 | 0 | 25 | 0 |
| IV | 50 | 0 | 50 | 0 |
| Total | 63 | 6 | 31 | 0 |

* Percentage distribution by group.

throughout the lungs, it was mild to moderate in degree and did not appear to contribute to the death of these dogs. All of the dogs in Group II had acute suppurative interstitial pneumonia, (Figure 2), which accounted for the deaths of 3 dogs (Table 5 and 6). One death in Group II was attributed to a pulmonary abscess with associated chronic pleuritis. Dogs in Groups III and IV evidenced chronic interstitial pneumonia (Figure 3) that was usually diffuse but varied from mild to marked in degree. However, no deaths in these groups were directly attributable to pneumonia. Calcification of degenerating and thickened alveolar septa occurred in most dogs in Groups III and IV, with occasional mineralization of the basement membrane of bronchioles.

*Kidneys*

Inflammatory changes in the glomeruli were observed in all groups, but they occurred most often and with the greatest severity in dogs in Groups III and IV (Table 5). Glomerular inflammation in dogs in Group I was acute, mild and diffuse, whereas in Groups II, III, and IV it was chronic and more severe in nature. In the latter groups glomeruli were thickened, mesangial cells were increased, and thickening of the basement membrane was noted (Figure 4). Pyelonephritis, either acute or chronic, occurred in 3 dogs in Group I but was not observed in the other groups. Chronic interstitial nephritis occurred in 2 dogs in Group II and all dogs in Groups III and IV. Two deaths in Group III were attributed to renal failure (urea nitrogen >150 mg/dl) due to chronic interstitial nephritis (Table 6). Tubular nephrosis, mild to moderate in degree, occurred in all groups and exhibited no relationship with age. The death of 1 dog in Group I was associated with acute severe nephrosis characterized by marked interstitial edema and degeneration of tubular epithelium (Table 6).

Table 3—Frequency of Clinical Signs Observed in the Cycle Immediately Prior to Death in Grey Collie Dogs

| Clinical signs | Number | Percent |
|---|---|---|
| Fever | 17 | 94 |
| Lethargy | 8 | 44 |
| Anorexia | 5 | 28 |
| Enteric disease | 5 | 28 |
| Respiratory disease | 5 | 28 |
| Icterus | 5 | 28 |
| Dehydration | 2 | 11 |
| Lameness | 1 | 6 |
| Conjunctivitis | 1 | 6 |
| Abscesses (subcutaneous) | 1 | 6 |
| Melena | 1 | 6 |

Table 4—Duration of Administration of Antimicrobials to Grey Collie Dogs

| Group | Average number of days treated | |
|---|---|---|
| | All antibiotics | Nephrotoxic antibiotics* |
| I | 35 | 19 |
| II | 31 | 13 |
| III | 55 | 21 |
| IV | 68 | 23 |

* Cephaloridine, gentamicin, and methicillin.

Tubular calcification, mild to moderate in amount, occurred in 20–50% of dogs in Groups I, II, and III in conjunction with nephrosis. The oldest dog in Group IV had a mild degree of amyloid deposition in the interstitium of the renal cortex.

*Heart*

Valvular endocarditis was exhibited by 25–40% of dogs in Groups I, II, and III (Table 5). It was not observed in dogs in Group IV. The severity of valvular inflammation decreased with age. One death in Group I was attributed to valvular endocarditis with septicemia (Table 6). Three dogs in Groups II and III had chronic myocarditis, moderate in degree and focal or multifocal in distribution, associated with myocardial degeneration, necrosis, and calcification. One dog in Group III had myocardial degeneration, necrosis, and calcification without septic myocarditis.

*Gastrointestinal Tract*

The character of inflammation in the gastrointestinal tract also varied according to the age of the dogs at death. Acute, multifocal gastritis, moderate to severe in degree, occurred in 2 dogs in Group I (Table 5). Acute and chronic enteritis demonstrated an inverse relationship with age. Acute enteritis was observed in 40% of dogs in Groups I and II and not at all in dogs in Groups III and IV. On the other hand, chronic enteritis occurred in only 20% of dogs in Groups I and II but in 50–100% of dogs in Groups III and IV (Table 5). While the gross and microscopic distribution of intestinal inflammation remained relatively diffuse, the severity of inflammation in both acute and chronic enteritis increased with age. The death of 1 dog in Group II was attributed to ileocolic intussusception (Table 6).

Pancreatitis, both acute and chronic, occurred with increased frequency in dogs in Groups III and IV. In 1 dog in Group I it occurred in conjunction with acute peritonitis, which accounted for the death of the dog (Table 6). Calcification of the gastric

Table 5—Percentage Distribution of Histopathologic Findings by Organ System in Grey Collie Dogs

| Histopathologic findings | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Lungs | | | | |
| Acute bronchopneumonia | 57* | 0 | 0 | 0 |
| Acute interstitial pneumonia | 43 | 100 | 25 | 0 |
| Chronic interstitial pneumonia | 0 | 0 | 75 | 100 |
| Chronic pleuritis | 0 | 20 | 0 | 0 |
| Calcification | 0 | 0 | 75 | 50 |
| Kidneys | | | | |
| Mesangioproliferative glomerulonephritis | 43 | 20 | 75 | 100 |
| Acute/chronic pyelonephritis | 43 | 0 | 0 | 0 |
| Chronic interstitial nephritis | 0 | 40 | 100 | 100 |
| Nephrosis | 43 | 80 | 50 | 50 |
| Calcification (tubular) | 43 | 20 | 50 | 0 |
| Amyloidosis | 0 | 0 | 0 | 50 |
| Heart | | | | |
| Chronic valvular endocarditis | 29 | 40 | 25 | 0 |
| Chronic myocarditis | 0 | 20 | 50 | 0 |
| Myocardial degeneration/necrosis | 0 | 20 | 75 | 0 |
| Myocardial calcification | 0 | 20 | 75 | 0 |
| Gastrointestinal tract | | | | |
| Acute gastritis | 50† | 0 | 0 | 0 |
| Chronic gastritis | 0† | 0 | 25 | 0 |
| Acute enteritis | 40‡ | 40 | 0 | 0 |
| Chronic enteritis | 20‡ | 20 | 50 | 100 |
| Pancreatitis | 25† | 0 | 50 | 100¶ |
| Calcification (stomach) | 0† | 0 | 50 | 0 |
| Amyloidosis (small intestine) | 0‡ | 0 | 25 | 0 |
| Liver | | | | |
| Cholangitis | 29 | 0 | 0 | 0 |
| Fatty degeneration | 71 | 40 | 100 | 50 |
| Hepatic necrosis | 29 | 0 | 0 | 100 |
| Kupffer cell hyperplasia | 0 | 0 | 50 | 100 |
| Amyloidosis | 0 | 0 | 50 | 100 |
| Spleen | | | | |
| Acute/chronic splenitis | 66§ | 0 | 0 | 0 |
| Lymphoid depletion | 83§ | 100 | 100 | 100 |
| Histiocytosis | 100§ | 100 | 100 | 100 |
| Amyloidosis | 17§ | 40 | 100 | 100 |
| Lymph nodes | | | | |
| Lymphoid depletion | 100† | 100‖ | 25 | 0¶ |
| Histiocytosis | 100† | 100‖ | 100 | 100¶ |

* Number of dogs in group with finding/total number of dogs in group × 100.
† Four dogs.
‡ Five dogs.
§ Six dogs.
‖ Three dogs.
¶ One dog.

mucosa was observed in 2 dogs in Group III. In addition, 1 dog had multifocal mineralization of the subintimal region of the intestinal arterioles. One dog in Group III exhibited extensive amyloid deposition in the mucosal lamina propria of the small intestine.

*Liver*

Multifocal cholangitis, both acute and chronic, occurred in 2 dogs in Group I (Table 5). Fatty degeneration of hepatocytes occurred in all groups, but the distribution shifted from multifocal to diffuse and increased in severity with age. Centrolobular hepatic necrosis, mild to moderate, occurred in 2 dogs in Group I, while severe, diffuse hepatic necrosis occurred in all dogs in Group IV. The 4 oldest dogs exhibited concurrent Kupffer cell hyperplasia and severe amyloidosis (Table 5). Extensive amyloid deposition occurred in the space of Disse and was associated with marked atrophy of hepatic cord cells (Figure 5 and 6). The deaths of 4 dogs in Group III and IV were associated with severe hepatic amyloidosis (Table 6).

### *Spleen*

Splenitis, acute or chronic, occurred only in dogs in Group I (Table 5). Lymphoid depletion, manifested as a decrease in white pulp areas, occurred in virtually all dogs. While depletion was diffuse in all groups, the severity increased with age from moderate to marked. Histiocytic infiltration of the red pulp, of similar distribution and severity, was found in all dogs. However, histiocytic infiltration of the white pulp occurred with increased frequency in dogs in Groups III and IV. Amyloidosis was found in all groups but with increased frequency in Groups III and IV (Table 5). However, severe amyloidosis was observed in the spleen of a 14-week-old dog in Group I. In all dogs, regardless of age, the amount of amyloid deposition, both perifollicular and intrafollicular, was marked (Figure 7).

As part of the experimental protocol to investigate the mechanism of cyclic hematopoiesis, the 5 oldest dogs, aged 35–113 weeks, received from 2.3 to 9.3 mg of *S typhosa* endotoxin.[16] Whereas all the dogs treated had extensive amyloidosis, there was no apparent relationship between the amount of endotoxin administered and the severity or extent of amyloid deposition (Table 7).

### *Lymph Nodes*

The frequency of lymphoid depletion in lymph nodes declined with age. While the distribution of depletion remained diffuse within individual nodes, there was an increase in severity with age in dogs exhibiting this lesion. However, most dogs in Groups III and IV had normal-appearing lymphoid follicles (Table 5). Histiocytic infiltration of the paracortical and medullary sinus areas, of similar distribution and degree, was found in all dogs. Histiocytic infiltration

Table 6—Predominant Pathologic-Anatomic Syndrome Observed at Death in Grey Collie Dogs

| | Group | | | |
|---|---|---|---|---|
| Pathologic-anatomic diagnosis | I | II | III | IV |
| Acute bronchopneumonia | 4 | | | |
| Acute peritonitis | 1 | | | |
| Septicemia (valvular endocarditis) | 1 | | | |
| Acute renal tubular nephrosis | 1 | | | |
| Acute interstitial pneumonia | | 3 | | |
| Pulmonary abscess/pleuritis | | 1 | | |
| Intussusception | | 1 | | |
| Chronic nephritis | | | 2 | |
| Hepatic amyloidosis | | | 2* | 2† |

* One dog died 5 days after a barbiturate overdose.
† One dog died of lithium toxicity.

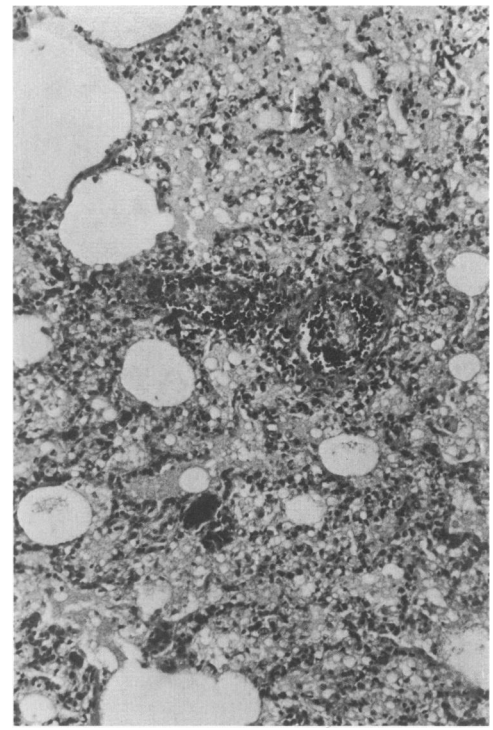

**Figure 2**—Acute suppurative interstitial pneumonia with extension of inflammation from a primary vasculitis to adjacent pulmonary parenchyma. (H&E, ×60)

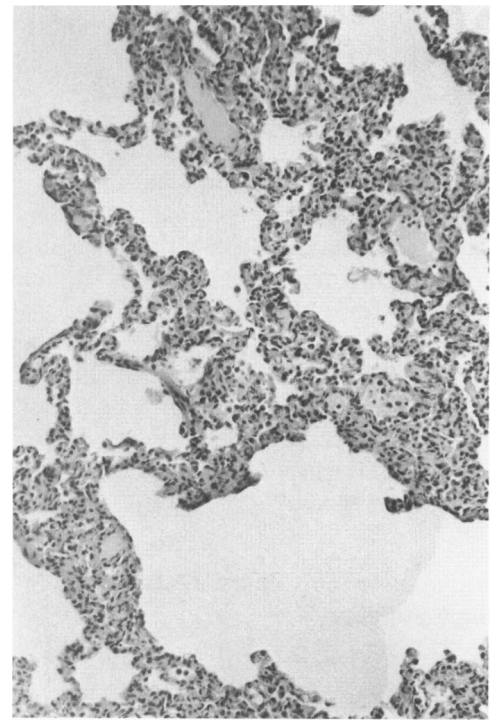

**Figure 3**—Chronic interstitial pneumonia with thickened alveolar walls due to proliferation of pneumocytes, infiltration of macrophages, and fibrinous debris. (H&E, ×60)

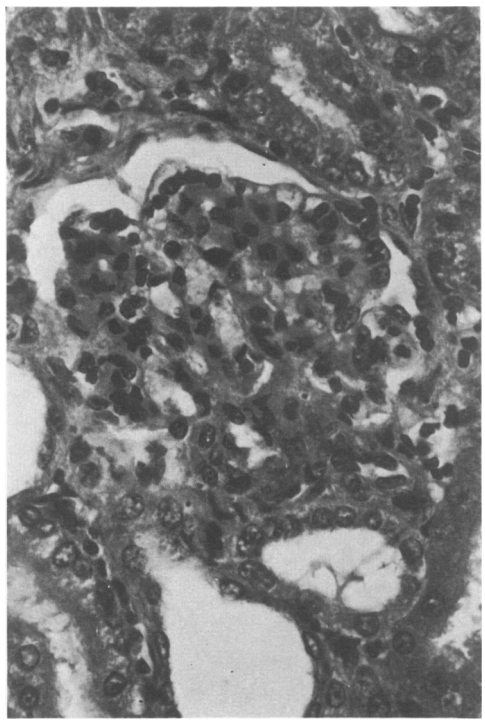

**Figure 4**—Mesangioproliferative glomerulonephritis with hypercellularity of the glomerulus involving mesangial, endothelial, and visceral epithelial cells. (H&E, ×200)

extended into the lymphoid follicles in 2 dogs in Group III. Amyloidosis was not observed.

*Other Organs*

The brains from 8 dogs representing each group were examined. Chronic meningitis, moderate in degree and multifocal in distribution, was observed in 4 brains from dogs in Groups I, II, and IV. Adrenal glands from 11 dogs were examined, and all were normal except that of the oldest dog, aged 113 weeks, which exhibited areas of focal cortical amyloid deposition and mineralization. The parathyroid and thryoid glands from 8 dogs were not remarkable. There was no difference in the occurrence of pathologic changes by sex.

## Correlations Between Clinical Features and Pathologic Findings

The death of 1 dog in Group I with acute renal tubular nephrosis was attributed to treatment with at least 2 nephrotoxic antibiotics for 5–10 days each during 3 of 4 cycles manifested between arrival and death. Except for this occurrence, there was no relationship between the pathologic processes and the nature and duration of treatment with antimicrobials. The day of the hematopoietic cycle on which each dog died was examined by group (Table 8). As expected, proportionately more dogs died during the days of neutropenia (Days 1–4) than during the days of more normal counts (Days 5–12). Examination of the pathologic syndromes associated with death (Table 6) in relation to the cycle day on which death occurred revealed that acute infectious deaths predominated during neutropenia (9 of 11), whereas noninfectious causes predominated when neutropenia had resolved (5 of 7) (Table 8). When the severity of clinical disease for the 2 cycles preceding each dog's death were examined, it was observed that Group I dogs had severe infectious processes in 3 of 10 cycles evaluated; whereas in Group III and IV dogs, severe infections were seen in 8 of 10 cycles evaluated. In Group I dogs, acute and presumably recent infection was only occasionally preceded by illness in previous cycles; whereas in older dogs, repeated infections in previous cycles suggested extensive prior exposure to inflammatory stimuli. Hence, the clinicopathologic findings in grey collies formed two relatively distinct patterns based on age (Table 9).

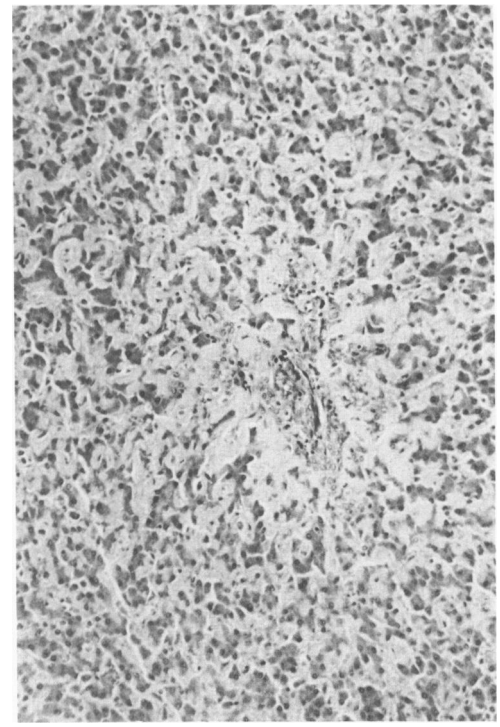

**Figure 5**—Severe hepatic amyloidosis with atrophy of hepatic cord cells. (H&E, ×60)

## Discussion

In our review of the clinical features and pathologic changes in grey collies with cyclic hematopoiesis, the predominant findings observed early (dogs aged 10–20 weeks) and late (dogs aged >30 weeks) were distinctly different. While some pathologic processes occurred at all ages, certain findings were unique to various age groups, whereas others appeared to be precursors for changes occurring later. Acute infectious processes manifested most prominently in the lungs, gastrointestinal tract, and kidneys early in life were supplanted by chronic inflammatory conditions later in life. For example, acute bronchopneumonia, of sufficient severity to cause death, occurred only in dogs in Group I. Acute interstitial pneumonia occurred with increased frequency in Groups I and II but declined in frequency and was supplanted by chronic interstitial pneumonia in Groups III and IV. Similar changes were observed in the kidneys and small intestine. This suggests that the effect of infectious insults is continual and cumulative; that is, the inflammatory process never completely resolves before the next inciting insult occurs. Comparison of the clinical histories of younger and older dogs further supports this concept. Also, it suggests that there may be a shift in the origin of infections from outside the body to some focus within the body. For example, bronchopneumonia, originating from exogenous microorganisms, is supplanted by interstitial pneumonia in older dogs, presumably derived from chronic inflammatory stimuli. The extent and severity of enteritis, particularly in the ileum of older dogs, suggests that this site may be a nidus for vascular seeding of microorganisms and their products. Thus, the portal of entry appears to shift from the lungs, with its exposure to exogenous agents, to the intestine or kidneys, as a source of endogenous agents.

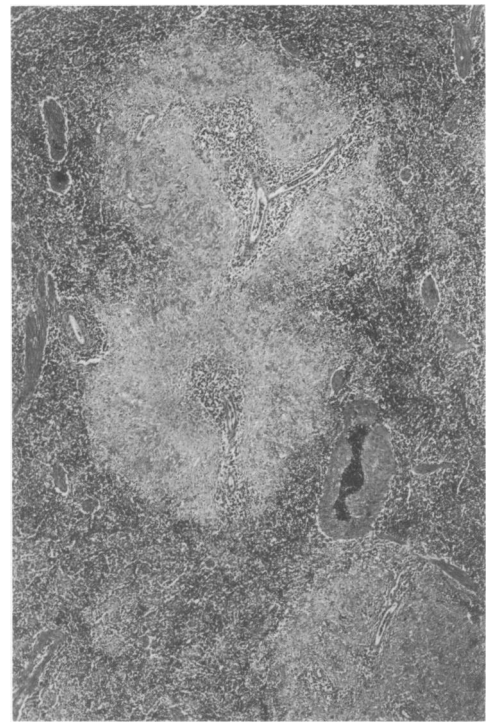

**Figure 7**—Perifollicular and intrafollicular deposition of amyloid in the spleen. Most of the amyloid is located within the cytoplasm of macrophages. (H&E, ×40)

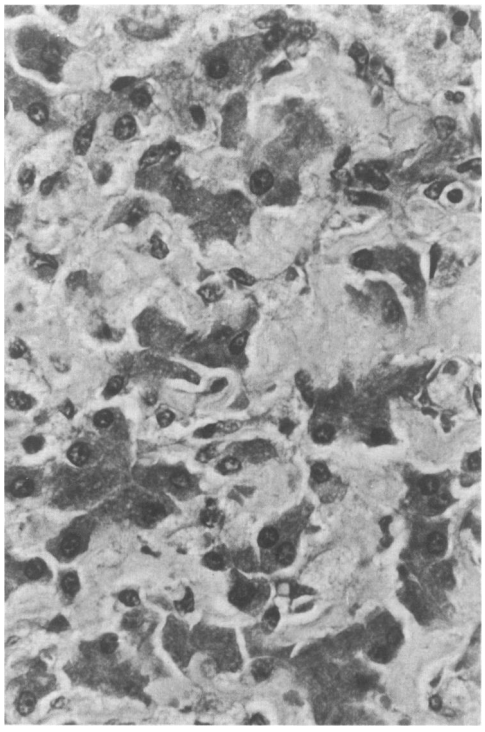

**Figure 6**—Amyloid deposition in the space of Disse and Kupffer cell hyperplasia. (H&E, ×350)

Amyloidosis was observed, in decreasing frequency, in the spleen (9 dogs), liver (4 dogs), kidneys (1 dog), small intestine (1 dog), and adrenal glands (1 dog). Amyloidosis of the kidneys, small intestine, and adrenal glands occurred in 1 dog with hepatic amyloidosis. The youngest dog to exhibit amyloidosis of the spleen was 14 weeks of age. However, amyloidosis of other organs occurred only in the 4 oldest dogs (aged 35–113 weeks). The administration of *S typhosa* endotoxin to 5 dogs aged 35–113 weeks did not appear to alter amyloid deposition. Hepatic amy-

Table 7—Endotoxin Administered and Amyloid Deposition in Grey Collie Dogs

| Dog | Age (weeks) | Endotoxin received (mg) | Amyloid deposition* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Spleen | Liver | Small intestine | Kidneys | Adrenals |
| III-3 | 35 | 2.3 | + + + | + + + | + + + | – | – |
| III-2 | 35 | 3.3 | + + + | NA | – | – | – |
| IV-2 | 113 | 5.7 | + + | + + + | – | + | + |
| III-4 | 35 | 7.1 | + + + | + + + | – | – | – |
| IV-1 | 57 | 9.3 | + + + | + + + | – | – | – |

* –, none; +, mild; + +, moderate; + + +, severe.
NA, tissue not available.

loidosis and Kupffer cell hyperplasia occurred concurrently in the 4 oldest dogs. Reticuloendothelial hyperplasia was a universal finding in the spleen and mesenteric lymph nodes, and it showed no relationship with age. Lymphoid depletion in the spleen occurred in virtually all dogs and exhibited no relationship with age. On the other hand, the frequency of lymphoid depletion in the lymph nodes decreased with age, and virtually all dogs in Groups III and IV exhibited normal lymphoid follicles in the cortex.

Chronic interstitial nephritis occurred commonly, and in 3 cases it was associated with clinical uremia. However, amyloidosis did not appear to play a major role in renal failure. Calcification was observed, with decreasing frequency, in the kidneys (7 dogs), heart (4 dogs), lungs (4 dogs), stomach (2 dogs), liver (2 dogs), spleen (1 dog), and adrenal glands (1 dog). Calcium deposits were observed initially in the renal tubules of dogs in Groups I, II, and III. In the dogs in Groups II and III it appeared in areas of myocardial degeneration and necrosis; and in the dogs in Groups III and IV it appeared in the interstitium of the lung, in conjunction with chronic interstitial pneumonia, and in the liver. Chronic renal disease in dogs in Groups III and IV may have contributed to secondary hyperparathyroidism, with the occurrence of both metastatic and dystrophic calcification. However, serial serum calcium levels obtained from several dogs were in the high range of normal, and all parathyroid glands examined were morphologically normal.

Table 8—Number of Deaths in Grey Collie Dogs by Hematopoietic Cycle Day

| Cycle day | Neutrophil count | Number of deaths | | | | |
|---|---|---|---|---|---|---|
| | | Group | | | | |
| | | I | II | III | IV | Total |
| 1-4 | Neutropenia | | | | | |
| | Acute infectious deaths | 6 | 1 | 1 | 1 | 9 |
| | All others | 0 | 0 | 1 | 1 | 2 |
| | Subtotal | 6 | 1 | 2 | 2 | 11 |
| 5-12 | Nonneutropenia | | | | | |
| | Acute infectious deaths | 0 | 2 | 0 | 0 | 2 |
| | All others | 1 | 2 | 2 | 0 | 5 |
| | Subtotal | 1 | 4 | 2 | 0 | 7 |

The pathologic features reported here are generally consistent with those reported by others.[9-15] Unlike the findings of Cheville et al,[10] bone necrosis was not a common finding; however, enteritis and amyloidosis occurred frequently. In general, the frequency and distribution of amyloidosis is in agreement with previous reports,[11,12] with the exception that renal amyloidosis was not a major feature in the dogs in this study. Reported studies did not indicate whether renal involvement with amyloid was as common as involvement of other organs and did not indicate the

Table 9—Relationship of Predominant Pathologic Findings and Age in Grey Collie Dogs

| Organ | Pathologic findings | |
|---|---|---|
| | Early (10-20 wks) | Late (>30 wks) |
| Lungs | Bronchopneumonia | |
| | Interstitial pneumonia (acute) | Interstitial pneumonia (chronic) |
| | | Calcification |
| Kidneys | Pyelonephritis | Interstitial nephritis |
| | | Mesangioproliferative glomerulonephritis |
| | Nephrosis | Nephrosis |
| | Calcification (tubular) | |
| Heart | Valvular endocarditis | |
| Gastrointestinal tract | Gastritis | |
| | Enteritis (acute) | Enteritis (chronic) |
| | | Pancreatitis |
| Liver | Fatty degeneration | Fatty degeneration |
| | | Hepatic necrosis |
| | | Kupffer cell hyperplasia |
| | | Amyloidosis |
| Spleen | Splenitis | |
| | Lymphoid depletion | Lymphoid depletion |
| | Histiocytosis | Histiocytosis |
| | | Amyloidosis |
| Lymph nodes | Lymphoid depletion | |
| | Histiocytosis | Histiocytosis |
| Cause of death | Acute infectious processes (primarily pneumonia) | Organ failure (liver and kidneys) |

concurrent presence of uremia. Most earlier reports described either clinical aspects of small numbers of cases[9,10,13-15] or emphasized pathologic changes in the one larger group studied.[12]

Using a systematic analysis of the clinical histories and a standardized pathologic grading system, we characterized age-related changes in grey collie dogs. The data suggest that the proper unit of study in these dogs is the hematopoietic cycle (averaging 12 days) and that clinical severity of illness in each cycle does predict pathologic findings, provided age-related changes in organ-system dysfunction are taken into account. The finding of extensive amyloidosis agrees with published reports, although the lack of specific renal involvement by amyloid suggests that an alternate explanation for renal failure in older dogs may be required. The clear delineation of organ failure in older dogs, as a major contributor to morbidity and mortality from inevitable infectious insults during neutropenia, suggests that future investigations with this animal model take into account the age of the dogs under study.

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