Cyclic Neutropenia in Grey Collie Dogs

By JOHN E. LUND, GEORGE A. PADGETT AND RICHARD L. ORR

Cyclic neutropenia is a rare hematologic disorder characterized by the chronic, cyclical recurrence of severe neutropenia which has previously been described only in humans. The neutropenia recurs approximately every 21 days and lasts from 3 to 10 days. The clinical manifestations of the disease are observed during the neutropenic phase of the cycle and include fever, malaise, oral ulcers, furunculosis and lymphadenitis. Page and Good reviewed the literature prior to 1957. Reimann recently reviewed 42 cases of the disease which he termed periodic myelodysplasia.

The purpose of this paper is to report the finding of a similar syndrome in grey (silver) collies.

Case Reports

Case 1

A grey, female, collie puppy, born March 19, 1965, was first observed at 8 weeks of age. She was 8 ounces lighter than the smallest of her litter mates but her previous history was uneventful. During the first month of observation, a severe neutropenia was noted on 3 occasions (Table 1). Simultaneous with the neutropenia, the puppy appeared depressed and was reluctant to stand or eat. Arthralgia of the carpal joints was determined by eliciting a sharp pain reflex with light pressure on the joints. The episodes were accompanied by high fever which exceeded 106 F. on several occasions. Since the neutropenia appeared to be exhibiting periodicity, a routine of daily blood counts was established. The neutropenia next occurred on July 5 and lasted until July 8 (Fig. 1). In addition to the signs observed previously, severe, bilateral keratitis developed along with shallow ulcers on the gingival mucosa near the base of the upper canine incisors.

Blood culture and intravenous injection of 5 cc. of her blood into a healthy dog both yielded negative results. Within 3 days after the neutrophils returned to the peripheral blood, all signs had abated and the keratitis and gingival ulcers had resolved. No treatment was administered.

Neutropenic cycles continued at an average interval of 10.6 days with a range of 8 to 12 days. The neutropenic phase lasted 3 to 4 days. A total of 21 cycles had been observed. During most cycles, medical treatment was not necessary. A lupus erythematosus test was performed on two occasions with negative results. Repeated direct Coombs' tests were also negative.
Table 1.—Hemograms for the First Month of Observation, Case 1

<table>
<thead>
<tr>
<th>Date</th>
<th>WBC, cells/mm³</th>
<th>Band neutrophils</th>
<th>Segmented neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophil</th>
<th>Unclassified</th>
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<tbody>
<tr>
<td>5/20/65</td>
<td>15,700</td>
<td>0</td>
<td>8,500</td>
<td>5,800</td>
<td>1,020</td>
<td>236</td>
<td>78</td>
<td>0</td>
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<tr>
<td>5/26/65</td>
<td>7,700</td>
<td>0</td>
<td>38</td>
<td>5,100</td>
<td>2,500</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5/27/65</td>
<td>14,800</td>
<td>148</td>
<td>6,440</td>
<td>3,550</td>
<td>4,510</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>6/7/65</td>
<td>10,200</td>
<td>0</td>
<td>2800</td>
<td>6,120</td>
<td>1,170</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6/11/65</td>
<td>19,900</td>
<td>99</td>
<td>13,820</td>
<td>3,980</td>
<td>1,000</td>
<td>102</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6/16/65</td>
<td>5,350</td>
<td>0</td>
<td>2,810</td>
<td>2,810</td>
<td>1,930</td>
<td>596</td>
<td>26</td>
<td>0</td>
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<tr>
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<td>9,550</td>
<td>0</td>
<td>4,300</td>
<td>4,300</td>
<td>1,720</td>
<td>304</td>
<td>143</td>
<td>152</td>
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<tr>
<td>6/25/65</td>
<td>7,600</td>
<td>0</td>
<td>2,280</td>
<td>2,280</td>
<td>4,256</td>
<td>304</td>
<td>143</td>
<td>152</td>
</tr>
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</table>

*The majority of the cells in the unclassified category had features of both neutrophils and monocytes. The nuclei were lobulated with a dense chromatin pattern. The cytoplasm had a slight gray tint, with faint granules.

Six bone marrow aspirations were collected from January 14 through January 24, 1966. Alternate left and right tibias were used on successive days. Samples were not taken closer than 2 cm from biopsy sites. Impression smears were stained with Giemsa's stain and 400 cells were counted on 2 slides. The results are shown in Table 2.

In order to compare the successive cell counts of each cell type, their values were compared to the marrow normoblast count obtained for each day. It was assumed that the number of normoblasts remained relatively constant since no fluctuation was observed in the PCV. The number of granulocytes was divided by the number of normoblasts. The values obtained are shown graphically in Figure 2.

The granulocyte-normoblast ratios for January 14, 16, and 18 were one order of magnitude larger than that of normal dogs, which indicates active granulocyte formation. This is also evident from the M:E ratios (Table 2) which are 3 to 20 times higher than normal. Starting on January 21, there was a rapid loss of segmented neutrophils from the bone marrow, which over a 4-day period was nearly logarithmic. The segmented and band forms were virtually absent on January 22. The number of segmented neutrophils remained extremely low for 3 days (Fig. 2). Their return to the marrow and blood was preceded by a sharp rise in the number of myeloblasts, promyelocytes, myelocytes, and metamyelocytes. An accumulation of promyelocytes was not observed in this series. It is, however, difficult to identify with confidence the promyelocyte in dogs because the granulation is not as striking as in man. In the absence of granulation, criteria used for identification were cell size, presence of nucleoli and basophilic cytoplasm as given by Patt and Maloney.31

The acute inflammatory response was studied during one cycle using the Rehuck skin window technic.32 The return of neutrophils to the blood following the previous neutropenic episode was considered as day 1 and the response was studied on days 4, 6, 8 and 10 of the cycle. The tenth day was during the neutropenic state. Total circulating leukocytes were 3250/mm³ with 65 segmented neutrophils per mm³.33 During the neutrophilic stage (days 4, 6 and 8), neutrophils migrated into the field at the end of the first hour and were present in large numbers by the end of the second hour. The first significant appearance of mononuclear cells occurred between hours 4 and 5 when lymphocytes accounted for 30 per cent of 500 cells and macrophages 5 per cent. At the end of the ninth hour, macrophages accounted for 60 per cent of 500 cells. On the tenth day, inflammatory cells were observed only after hours 3 and 4. The most cells counted were 44 on the entire coverslip after the third hour of which 29 were neutrophils, 10 were lymphocytes, and 5 were macrophages. The site was observed for a total of 11 hours. For the last 7 hours, only protein and erythrocytes were observed on the coverslips. Page and Good,1 in a similar study on a human patient, also noted a gross deficiency of an acute inflammatory response when the neutrophils were absent from the blood.
An 8-week-old female grey collie was obtained in August, 1965. The history indicated that late in July at the age of 6½ weeks the dog had an acute episode of vomition and diarrhea. Blood was observed in both the vomitus and feces.

A physical examination revealed a thin, smaller-than-normal puppy weighing 8 pounds. A large hematoma was present on the left thorax. The platelet count on admission was 134,000/mm³.

Daily blood examinations were begun immediately. Two episodes of neutropenia were observed separated by a period of 10 days. Each was of two days duration. The dog died one day following the second neutropenic episode. The leukocyte count had increased from 5,950/mm³ with 1 per cent band neutrophils and 1 per cent segmented neutrophils 2 days prior to death, to 59,100/mm³ with 5 per cent band neutrophils and 62 per cent segmented neutrophils on the day of death. Postmortem findings were acute suppurative bronchopneumonia, acute suppurative pleuritis and intussusception of the colon.

A 6-month-old male collie was first observed on April 26, 1966. The coat color was steel grey. The dog weighed 32 pounds and was 19 inches high at the shoulder. A normal colored male litter mate weighed 43 pounds and was 23 inches high. Five neutropenic episodes were observed during the first 42 days of observation. The cycles lasted an average of 11.7 days with a range of 10 to 13 days (Fig. 3).

During the first episode, the temperature did not rise above 103.6 F. Ulcers were observed
Fig. 2.—Upper chart illustrates the absolute neutrophil count from January 14 through January 26, 1966. The lower chart illustrates the variation in the cell to normoblast ratio over the same period.

on the gingival mucosa and arthralgia of the carpal joints was evident. Following the second neutropenic crisis, the right hind foot became swollen to approximately twice normal size. The temperature rose to 106.2 F. and the dog appeared very depressed. The neutrophilic response to the infection can be observed in Figure 3 (May 8-16). Intense inflammation of the foot was not observed until neutrophils were present in the blood. A similar but less severe swelling occurred following the third neutropenic episode. Antibiotic therapy was required to control the infection and was continued until the skin over the lesion was healed.

A direct Coombs' test on May 6 was negative.

Large immature lymphocytes were observed in the peripheral blood during and immediately following the neutropenic episodes.
Table 2.—Tibia! Bone Marrow Differential Counts, Case 1

<table>
<thead>
<tr>
<th></th>
<th>1/14/66</th>
<th>1/16/66</th>
<th>1/18/65</th>
<th>1/21/66</th>
<th>1/22/66</th>
<th>1/24/66</th>
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<td>Myeloblast</td>
<td>0.50</td>
<td>0.75</td>
<td>2.37</td>
<td>0.75</td>
<td>0.37</td>
<td>1.63</td>
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<tr>
<td>Promyelocyte</td>
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<td>2.37</td>
<td>3.00</td>
<td>3.13</td>
<td>4.50</td>
<td>10.37</td>
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<tr>
<td>N. Myelocyte</td>
<td>5.87</td>
<td>5.50</td>
<td>5.50</td>
<td>6.75</td>
<td>4.13</td>
<td>11.25</td>
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<td>N. Metamyelocyte</td>
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<td>8.00</td>
<td>9.33</td>
<td>6.75</td>
<td>3.25</td>
<td>8.63</td>
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<tr>
<td>N. Band</td>
<td>23.13</td>
<td>15.13</td>
<td>13.75</td>
<td>7.87</td>
<td>2.37</td>
<td>5.00</td>
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<td>N. Segmented</td>
<td>38.12</td>
<td>55.13</td>
<td>34.37</td>
<td>3.87</td>
<td>0.75</td>
<td>0.25</td>
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<tr>
<td>E. Myelocyte</td>
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<td>0.00</td>
<td>0.50</td>
<td>0.75</td>
<td>0.37</td>
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<td>E. Metamyelocyte</td>
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<tr>
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<tr>
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<td>0.75</td>
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<td>4.50</td>
<td>1.63</td>
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<td>Basophil</td>
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<td>0.00</td>
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<td>Lymphocyte</td>
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<td>11.63</td>
<td>9.50</td>
<td>7.87</td>
<td>7.37</td>
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<td>Monocyte</td>
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<td>0.63</td>
<td>0.00</td>
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<td>Histiocyte</td>
<td>0.25</td>
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<td>0.25</td>
<td>0.25</td>
<td>0.37</td>
<td>0.37</td>
</tr>
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<td>Plasma Cell</td>
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<td>0.50</td>
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<td>0.37</td>
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<tr>
<td>Reticuloh Cell</td>
<td>0.87</td>
<td>0.50</td>
<td>1.68</td>
<td>1.13</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>0.50</td>
<td>0.37</td>
<td>0.87</td>
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<td>1.37</td>
<td>0.37</td>
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<tr>
<td>Normoblasts</td>
<td>6.00</td>
<td>3.24</td>
<td>14.61</td>
<td>56.51</td>
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<td>M/E Ratio</td>
<td>12.75</td>
<td>24.10</td>
<td>3.98</td>
<td>0.50</td>
<td>0.22</td>
<td>0.74</td>
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</tbody>
</table>

DISCUSSION

Grey Coat Color in Collies

Collies with grey coat color, unless raised under close medical supervision, rarely live to the age of 1 year. Most grey puppies are weak at birth and do not survive the first week. Kennel owners who have attempted to raise them have observed frequent episodes of fever and cutaneous infection. We have observed a total of 6 grey collies, 3 of which are reported in this paper. Neutropenia was observed in isolated blood samples from the other 3 but their blood was not examined with enough frequency to determine if the neutropenia was cyclic. The dogs were troubled with frequent cutaneous infection and periods of high fever which necessitated frequent antibiotic therapy. Retrospect examination of their daily temperature revealed a definite cyclic tendency in the occurrence of elevated temperature. These findings have led us to believe that cyclic neutropenia is a hereditable disorder in the grey collie and points to the probability that cyclic neutropenia is present in all grey collies of the type described.

The grey coat color was reported to be inherited as a simple recessive factor by Ford. In a discussion of the “lethal effects” of the “grey gene” in collies, she stated that the condition was the result of the pleiotrophism of the recessive gene (d) for color dilution. The collies she described died either at birth or within the first months of life and were very susceptible to infection. Blood studies were not reported.

Pathogenic Mechanisms in Cyclic Neutropenia

The cause of cyclic neutropenia in man has not been found. Thompson studied the urinary excretion of estrogen and gonadotropins in a 25-year-old man with cyclic neutropenia and diabetes insipidus. He found a pattern similar
Fig. 3.—Illustrates the fluctuation in the total white cell count and number of neutrophils and monocytes of Case 3. A severe bacterial infection of the right hind foot was present during the period from May 8 through May 16.

to that seen in a normal menstruating female. The patient's fluctuations in hormone level were in phase with the neutropenia. Stephens and Laurence recorded the case of a woman who had several attacks of neutropenia which occurred simultaneously with the onset of menses. The neutropenia persisted after bilateral oophorectomy but remission occurred after medication was discontinued. It has been proposed that this case was due to aminopyrine sensitivity. Borne reported that a 3-year-old girl had small amounts of urinary estrogen at the onset of neutropenia but the hormone was not detectable between episodes. Reznikoff described an 18-year-old youth whose 17-keto-steroid excretion decreased at the onset of each neutropenic cycle. The estrogen and gonadotropin secretion was normal. Other investigators detected no fluctuations of hormone level related to the neutropenic episodes. The disease has also been observed in menstruating women in which no correlation was found between menses and the neutropenic episodes.

Several investigators have considered the disease to be a form of hyper-
splenism. Splenectomy, however, resulted only in a temporary alteration in the
degree of the neutropenia with moderate improvement in symptoms. It has
been observed\textsuperscript{15} that splenectomy is most effective in older patients, probably
because splenic enlargement may be found in older patients, whereas it is
never seen in the younger patients.

Attempts to demonstrate antileukocytic antibodies have not been fruit-
ful.\textsuperscript{1,11,17,18} In 2 cases\textsuperscript{1,10} serum and blood from neutropenic patients has
been injected into normal persons. No fluctuation in the recipients' peripheral
blood cells was observed.

Coventry\textsuperscript{19} suggested the disease was of congenital origin, since the majority
of the reported cases had their origin in infancy. Familial occurrence has been
reported in 3 cases and is suggestive in another case. Hahneman and Alt\textsuperscript{17}
reported a man who had suffered from the disease for 16 years and his
dughter whose symptoms started at the age of 3 months. Two father-daughter
cases were described by Gorlin.\textsuperscript{20} The duration of the disease in the father
was not established; however, it was presumed to have been present when
he was 18 years old. The daughter, 8 years old, had had gum infections and
mouth sores since 4 years of age. Videbaek\textsuperscript{18} added 2 cases, a father and his
2-year-old son. The disease had apparently begun at the age of 2 years in the
father. Cyclic neutropenia with simultaneous monocytosis was diagnosed when
he was 11 years old and was reported by Plum.\textsuperscript{21} The son was in good health
until the age of 10 months when his first tooth erupted. Since that time, he
experienced recurrent sore throats and stomatitis. The diagnosis of cyclic neu-
tropenia was established during his second year of life. Becker et al.\textsuperscript{14} described
a case in which a third child, a girl, became ill at 1 month of age when she
developed a cough and swelling about the nose. The child's condition became
progressively grave and she died on the sixth day of hospitalization at the age
of 7 weeks. A diagnosis of sepsis, gangrenous pyoderma and granulocytopenia
was established. It was the investigators' impression that the child had cyclic
neutropenia, based on the appearance of a small number of neutrophils in
the blood a day prior to death and on the changes observed in the bone mar-
row. Bone marrow aspiration prior to death revealed a decrease in the myeloid
cells. Postmortem examination showed them to be present in normal amounts
with progression into neutrophils.

Most investigators have explained the fluctuation in the peripheral neu-
trophil level as being a reflection of cyclic changes in the bone marrow. The
defect in the bone marrow has been referred to as a maturation defect which
takes place at the promyelocyte state,\textsuperscript{6,22,23} whereas others think that all cells
of the neutrophilic series are depressed.\textsuperscript{10,11,24,26}

Page and Good\textsuperscript{1} postulated the presence of two morphologically defin-
able defects which may have functioned in their patient: "(1) a periodic
failure of heteroplastic maturation of neutrophils from primitive reti-
culum cells; (2) defect in maturation at the promyelocyte stage, resulting
in accumulation of promyelocytes in the bone marrow, which becomes espe-
cially notable when heteroplastic maturation is proceeding normally."

Hormone excretion studies were not attempted on the dogs. However,
several factors indicate that the cyclic neutropenia is not related to the estrus cycle. One of the dogs (Case 3) was a male. In all cases the condition was observed within the first 6 months of life and none of the females was observed in estrus. Should the remaining female (Case 1) reach sexual maturity, estrus should occur only twice a year, the normal pattern for dogs. There would be no similarity between the estrus cycle and the neutropenic cycle.

Complete postmortem examinations have been performed on 8 grey collies ranging in age from 3 days to 9 months. In 3 discussed previously, occasional neutropenia was observed. The others were dogs that had died and were sent to us for examination. No splenic enlargement was noted, nor was any increase in phagocytosis of leukocytes observed in any of the dogs. The lymphoid tissue in the puppies ranging in age from 3 days to 2 weeks was normal in appearance. In 5, age range 2 to 9 months, all lymphoid tissue was extremely depleted.

The bone marrow observations in Case 1 were compatible with the hypotheses that the cyclic disease process originated in the bone marrow as an intermittent failure of maturation of the neutrophil series and that the cycles observed in the peripheral blood were secondary to the bone marrow changes. As illustrated in Table 2 and Figure 2, the decrease in bone marrow neutrophils preceded the blood neutropenia and all immature cells of the neutrophilic series were present in low numbers when compared to the normoblasts in the bone marrow.

We were not able to demonstrate an accumulation of promyelocytes in the bone marrow at any time in the cycle except just prior to the appearance of neutrophils in the peripheral blood in a bone marrow sample taken November 13 (Case 1). We interpreted this one finding as a chance sample taken at a time when the progression of maturation had reached the promyelocyte stage but had not yet progressed to the myelocyte stage. Neutrophils were absent from the blood on November 13 but were present in large numbers on November 14.

These observations indicate that the defect in maturation occurs at a very early point in the maturation process, possibly at the level of differentiation from the stem-cell and is reflected as a complete suppression of the neutrophilic series from the myeloblast to the mature neutrophil, or it indicates a lack of amplification in the myelocyte compartment.

**Similarities between the Human and Collie Disease**

The disease in these dogs had many similarities to the disease in man. A persisting cyclic neutropenia with regular, predictable cycles was observed. Fever, malaise, and oral and cutaneous infections were associated with the neutropenia but were absent between episodes. Arthralgia was present during the first 3 months of observation in Case 1 and was also observed in Case 3. This symptom was reported in 5 human cases. Large immature lymphocytes with intense basophilic cytoplasm were frequently observed during the neutropenic episodes and during the first few days after the return of the neutrophils to the peripheral blood. Rutledge observed similar cells...
in his patient. Monocytosis was observed in all 3 cases during the later portion of the neutropenic episode and for several days following it (Figs. 1 and 3). Monocytosis has been observed in a majority of the human cases.

There appear to be 2 basic disimilarities in the disease as observed in man and the dog. The cycle length in man averaged 21 days; in the dogs it was 10.6 days (Case 1), 10 days (Case 2), and 11.7 days (Case 3). The second variance was in the number of neutrophils present in the blood between the neutropenic episodes. In the affected people, the neutrophils were present in normal numbers or, more often, were below normal. In the dogs described, counts of 10,000 to 12,000 cells/mm³, consisting mainly of neutrophils, were frequently encountered on the first and second day following a neutropenic episode.

It is possible that this syndrome in dogs may represent a spontaneous disease which will be of value in the elucidation of the mechanism of cyclic neutropenia in man and which will also offer a good tool for the study of bone marrow kinetics.

SUMMARY

Cyclic neutropenia in a grey collie dog was first diagnosed at 10 weeks of age and has persisted 12 months. The average cycle length was 10.6 days with a range of 8 to 12 days. The condition was found in 2 other dogs of the type described and appears to be inherited along with a grey (silver) coat. The peripheral neutropenia results from a cyclic maturation arrest in the bone marrow at the level of differentiation from the stem cell.

SUMMARY IN INTERLINGUA

Neutropenia cyclic in un gris can collie esseva diagnosticate initialmente quando le animal habeva 10 septimanas de etate, e le condition ha persistite depot 12 menses. Le longor medie del cyclo esseva 10,6 dies con extremos de 8 a 12 dies. Le condition esseva constatate in 2 alte canes del typo descripte e pare esser hereditabile in association con un pellicia gris (argentee). Le neutropenia peripheric resulta ab un arresto cyclic de maturacion in le medulla ossee al nivello del differentiation ab le cellulas primordial.

REFERENCES

8. Jackson, H., and Merrill, D.: Agranulocytic angina associated with the men-
Cyclical neutropenia in Grey collie dogs

725, 1945.
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