EFFECTS OF TWO ISOLATES OF *EHRLICHIA CANIS* ON GERMAN SHEPHERD DOGS

By

DORIS FORD MCKOWN

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University of Missouri at Kansas City

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EFFECTS OF TWO ISOLATES ON EHRLICHIA CANIS

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Thesis Approved:

S. P. Ewing
Thesis Adviser

Helen E. Jordan

Ralph L. Buckner

N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Ehrlichia canis (Order Rickettsiales), the causative agent of canine ehrlichiosis, was first recognized at the Pasteur Institute in Algeria and was designated Rickettsia canis (Donatien and Lestoquard 1935). Subsequent reports related geographical distribution of the organism and described clinical manifestations of the disease it produced. The first report confirming the presence of E. canis in the Western Hemisphere was made in 1957 (Bool and Sutmöller) in the Netherlands Antilles. The organism was first recognized in the United States (Oklahoma) in 1962 by Ewing (1964a, 1964b) who reported that the parasite was found primarily in the cytoplasm of lymphocytes as an aggregate of organisms in a characteristic morula configuration. Under experimental conditions the organism was found to be quite pathogenic for young pups and in some instances produced fatal disease. Ewing and Buckner (1965a) reported clinical manifestations and hematologic changes associated with the 1962 Oklahoma strain of E. canis. Long-standing anemia of the normocytic-normochromic type was characteristic of the syndrome.

"A new canine disease syndrome" resulting in haemorrhages and death of dogs in Singapore was reported in 1967 (Wilkins et al., and Spence et al.). Tropical canine pancytopenia (TCP) was adopted as the name for this "new haemorrhagic disease," and E. canis was

reported to be the causative agent (Wilkins et al.,1967; Huxsoll et al., 1969; Seamer and Snape, 1970). Epistaxis was the most dramatic sign of this syndrome and fatalities were common in German Shepherd military dogs. Clinical and clinicopathologic findings in TCP were reported by Walker et al. (1970) and included severe anemia, leukopenia and thrombocytopenia.

Relatively non-pathogenic strains of E. canis were found in 1970 in Arkansas (Ewing et al.,1971) and in Oklahoma (Hayet et al., 1972). In both instances the organisms were found in neutrophils rather than in lymphocytes and produced a milder form of canine ehrlichiosis than did the Oklahoma isolate found in 1962. Pathogenesis of both the 1962 and 1970 Oklahoma isolates were studied by Hayat (1972) in dogs of mixed breeding. Severe anemia, leukopenia and thrombocytopenia were observed in pups experimentally infected with the 1962 isolate. Slight anemia, but neither leukopenia nor thrombocytopenia were observed in pups exposed to the 1970 isolate.

The purpose of the present investigation was to study the pathologic effects of the 1962 Oklahoma isolate and of the 1970 Oklahoma isolate in pure bred German Shepherd dogs. Specifically, the object was to investigate the cause of anemia and thrombocytopenia. This was accomplished by examination of bone marrow at intervals during the course of infection and by determination of the degree of disseminated intravascular coagulation.

CHAPTER II

REVIEW OF LITERATURE

Ehrlichia canis has been classified in the Order Rickettsiales and identified as the causative agent of a febrile, sometimes debilitating, disease of dogs. Ehrlichiosis is characterized by anemia and thrombocytopenia.

Rickettsia canis, as *Ehrlichia canis* was originally designated, was first recorded in dogs by Donatien and Lestoquard (1935) at the Pasteur Institute in Algeria. They found the parasite in circulating monocytes of the vertebrate host and determined that *Rhipicephalus sanguineus* served as the vector. Subsequently Danks (1937) and Carmichael (1939) reported the occurrence of *E. canis* in East Africa, and Neitz and Thomas (1938) found it in South Africa. Malbrant (1939), working in the French Congo, found rickettsial bodies resembling *Rickettsia* (*Ehrlichia*) *canis* in monocytes of the peripheral blood, lungs and liver of dogs. Pigoury and Bernard (1939), studying dogs in Beirut, also reported inclusion bodies in monocytes of the lung. In the same year Alexander and Mason isolated a Rickettsia from the peripheral blood of a dog in South Africa. Carmichael and Feinnes (1942) described canine rickettsiosis in Uganda, and Gillain (1942) reported it in the Belgian Congo. The latter author found rickettsiosis in association with *Babesia canis* infection and stated that *R. canis* alone did not cause serious disease. The disease was first

reported from India by Mudaliar (1944), who found R. canis organisms in both lymphocytes and monocytes of a dog which later died of the infection. He also incriminated R. sanguineus as the vector. Another report of concurrent R. canis and B. canis infections was published by Malherbe (1947) in the Pretoria district of Africa. Malherbe (1948) later reported further work dealing with methods of diagnosis, symptoms and therapy of rickettsiosis.

Twenty years after discovery of Rickettsia (Ehrlichia) canis in the Old World, Bool and Sutmőller (1957) described E. canis in blood smears from dogs in the Netherlands Antilles. They also found B. canis to be a complicating factor as had workers in the Old World. Further studies by Bool (1959) with this New World strain of E. canis confirmed many earlier findings of investigators in the Old World; and Ehrlichia, as first introduced by Moshkovskii (1945), was accepted as the generic name. Bool and Sutmőller also isolated the parasite from R. sanguineus confirming that this acarine was the vector in the New World. The continued occurrence of R. canis in other parts of the world was confirmed in India by Raghavachari and Reddy (1958) who found "darkly stained bodies" in monocytes of Alsatian and Golden Retriever dogs. The parasite was found to be morphologically indistinguishable from the South African strain of R. canis as verified by Henning who, in 1956, had published a review of the findings of the disease up to that time.

In the United States the first occurrence of E. canis was reported by Ewing (1964a, 1964b). He found dogs concurrently infected with E. canis and Babesia canis as had investigators elsewhere. Ewing and Buckner (1965a) studied the clinical manifestations of

uncomplicated E. canis infection as well as when combined with B. canis. Long-standing anemia was a notable feature of the E. canis infection.

Wilkins et al. (1967) reported "a new canine disease syndrome" resulting in death of a large number of military dogs in Singapore. Post mortem examination revealed haemorrhages in all organs of the body. Spence et al. (1967) reported that civilian and civil police dogs were also dying of the "new disease." The causative agent resulting in death of the dogs was not determined until two years later when Huxsoll et al. (1969) encountered a similar disease in Southeast Asia where military dogs were succumbing to a "haemorrhagic disease" similar to that described in the dogs in Singapore. Cytoplasmic inclusions were demonstrated in monocytes and were verified by Ewing (1971) to be Ehrlichia canis. Similar inclusions were also seen in mononuclear cells in lung, spleen and kidney impression smears of dogs that died of the disease. Petechial and ecchymotic haemorrhages were observed on numerous organs and in subcutaneous tissues. The most dramatic clinical sign of the disease was epistaxis, either unilateral or bilateral, with death often following in five to seven days.

Numerous reports of ehrlichiosis involving military dogs followed. The term tropical canine pancytopenia (TCP) was used by Seamer and Snape in 1970 to describe the haemorrhagic disease associated with E. canis. They recorded a decrease in circulating red and white cells and platelets, hence the name TCP. In the same year Walker et al. reported more detailed studies concerning an epizootic of TCP which began in 1968 in Vietnam. They recognized several phases of the disease beginning with an incubation period of approximately eleven

days followed by a febrile phase which was mild to severe. Based on physical examination, complete recovery was often thought to occur; in fact this apparent recovery actually marked the beginning of a sub-clinical phase which lasted for approximately three months. One of two terminal phases was then noted; either pancytopenia with epistaxis or severe pancytopenia without epistaxis. Dogs suffering from epistaxis died as early as two days or as late as three months after onset of bleeding. The dogs which exhibited no epistaxis, but were severely pancytopenic, were clinically asymptomatic until the terminal phase. German Shepherd and Labrador Retrievers were the breeds which most often exhibited epistaxis.

A correlation between a heavy infestation of ticks and epizootics of TCP suggested that at least one or more species of ticks were responsible for transmitting the disease (Wilkins et al., 1967; Walker et al., 1970). Controlled laboratory studies were in progress to determine if the tick was either a mechanical or biological vector of E. canis and what role it had in transmission of TCP, but no further references have been found.

Huxsoll et al. (1970b) reviewed the findings recognized in TCP and described the common occurrence of "morula-like" bodies in cytoplasm of monocytes. A series of papers dealing with different aspects of TCP in Southeast Asia have followed: Huxsoll et al. (1970a); Hildebrant et al. (1970); Nims et al. (1971); Burghen et al. (1971); Amyx et al. (1971); Jennings et al. (1971); and Huxsoll et al. (1972).

A case of pancytopenia was reported in Texas by Pierce (1971). E. canis morulae were found in lymphocytes and monocytes, but again Babesia canis was a complicating factor. A "new strain" of E. canis

was discovered in Arkansas by Ewing et al. (1971), and in Oklahoma by Hayat et al. (1972). Both reports dealt with the occurrence of E. canis in neutrophils rather than in lymphocytes or monocytes. These strains produced a milder form of ehrlichiosis than did the one isolated in Oklahoma in 1962.

Recent advances in diagnosis of E. canis have been reported by Carter et al. (1971), who demonstrated that immunofluorescence is a sensitive test for infectivity in dogs. By immunofluorescence they examined sera of dogs infected with E. canis from different areas of the world and "all appeared to be identical antigenically." Nyindo et al. (1971) cultivated E. canis in vitro; this advance may provide opportunity for further study of the organism and development of immunizing and diagnostic reagents.

No explanation of the cause of the anemia and thrombocytopenia which seem to characterize all E. canis infections has been advanced. Examination of the bone marrow during various stages of the infection could help to define the etiology of the anemia and thrombocytopenia.

Reports of bone marrow activity with E. canis infection are brief and conflicting. Malherbe (1948) found characteristic "elementary bodies" in cytoplasm of free histiocytic cells in the post mortem bone marrow. Upon observation at necropsy, Bool and Sutmöller (1957) described a hyperactive bone marrow and the presence of morulae in bone marrow cells. Panciera (1972) collected marrow 16, 17, 23, and 150, days postinfection. Hypercellularity was noted to result largely from hyperplasia of granulocytic components. Megakaryocytes were abundant in some specimens. Erythrocytic precursors were sparse in the specimens collected the 16th and 17th day post exposure but

were more normal in number in those collected later.

In another rickettsial infection, Rocky Mountain Spotted Fever (RMSF), the reports of bone marrow findings are more consistent. Atkin et al. (1965) reported hypercellular bone marrow with megakaryocytes present, but they were young with little evidence of platelet budding. Schaffner et al. (1965) observed a cellular bone marrow with increased myeloid elements and megakaryocytes in patients suffering from RMSF, and stated that "The bone marrow findings are consistent with the hypothesis that the thrombocytopenia results from platelet aggregation and thrombin formation rather than from direct toxic effect on the bone marrow."

Thrombocytopenia is an important finding in disseminated intravascular coagulation (DIC) and the most obvious cause of thrombocytopenia, according to Pitney (1971),is "aggregation and incorporation of platelets into platelet-fibrin microthrombi due to the presence of thrombin in the circulation." DIC implies activation of a coagulation process leading to utilization of platelets and clotting factors anywhere in the body. Hemorrhage may result from this excessive utilization (aggregation) of platelets and other coagulation factors, and the term "consumption coagulopathy" has been coined by Rodriguez-Erdmann (1965) to describe the phenomenon. Prothrombin time and partial thromboplastin time may become altered due to deficiency of clotting factors V and VIII in later stages of DIC. The intravascular fibrin clots are lysed in the microcirculation and fibrin/fibrinogen split products (FSP), i.e., fibrin/fibrinogen degradation products (FDP) appear in the blood.

The presence of FSP can be detected by immunological techniques

because fibrin split products retain antigenic determinants which react with antifibrinogen serum. Neutralization of diluted antifibrinogen antiserum by the split products results in inhibition of agglutination of fibrinogen-coated tanned formalinized erythrocytes.

Disseminated intravascular coagulation has been described in two rickettsial diseases of man; Rocky Mountain Spotted Fever (RMSF) and Scrub Typhus (Trigg, 1964; and Ognibene et al., 1971). Thrombocytopenia was noted in the two diseases and Trigg advanced the possibility of "consumption of platelets in capillary thrombosis." Thrombocytopenia has been reported in 40 to 60% of the cases of RMSF since the first reported case of thrombocytopenia by Phillips et al. (1960). Nida and Riley (1962); Atkin et al. (1965); Schaffner et al. (1965); Hazard et al. (1969); and Hand (1970), among others, have noted the frequency of thrombocytopenia and often intravascular thrombosis leading to platelet clumping in patients suffering from RMSF. Rubio et al. (1968) described 23 cases of RMSF complicated by thrombycytopenia and suggested that this may occur with significant frequency in other rickettsial diseases. Haynes et al. (1970) in their analysis of 78 children with RMSF, found DIC to be a complication and concluded that the occurrence of DIC was probably a much more frequent complication in RMSF than was recognized.

DIC has as yet not been described in rickettsial diseases of animals other than man, but thrombocytopenia has been noted in rickettsial infections of several animals. Gribble (1969) noticed a decrease in thrombocytes in equine ehrlichiosis, and Foster and Cameron (1968) reported thrombocytopenia in sheep associated with a tick-borne fever infection. Bobin et al. (1962) and Wilkins et al.

(1967) first reported a decrease in thrombocytes with canine ehrlichiosis. Huxsoll et al. (1970a), reporting on tropical canine pancytopenia, described a severe thrombocytopenia with that form of ehrlichiosis, and Pierce (1971) described thrombocytopenia in canine pancytopenia in Texas. Hayat (1972), in a comprehensive study of the 1962 Oklahoma isolate of E. canis, observed thrombocytopenia consistently in puppies experimentally infected with the parasite.

CHAPTER III

MATERIALS AND METHODS

Parasites Employed and Hosts Studied

Infectious Agents

Two strains of Ehrlichia canis were utilized in this study. One, designated the lymphocytic strain of E. canis (1962 Oklahoma isolate), was obtained from a Beagle dog maintained as a reservoir by the Department of Veterinary Parasitology and Public Health, Oklahoma State University. A second, designated the neutrophilic strain of E. canis (1970 Oklahoma isolate), was obtained from another Beagle dog also maintained as a reservoir by the same department. The effect produced by the two isolates was studied singly, in combination, and sequentially when the 1962 isolate was given as a second infection after clinical recovery following exposure to the 1970 isolate.

Experimental Animals

Three purebred German Shepherd dogs were used in this study; two full-siblings six months of age, and one a half-sibling eight months of age.

Housing

All dogs were housed in clean quarters which excluded other animals. Ticks were never found on any of the dogs in the kennel. Cockroaches of the family Blattidae and house flies (Musca domestica) were occasionally observed in the kennel. The canine control was kenneled in a cage similar to that of the infected dogs but was permitted access to an outside enclosed run during the day. The cages were cleaned and disinfected twice daily and a bedding of clean newspapers provided. Fresh food[1] and water were also provided twice daily.

Preconditioning

The dogs were vaccinated[2] during a preconditioning period to protect them against canine distemper, hepatitis, and leptospirosis. Parasitologic examinations by direct saline smear and by sodium nitrate flotation method revealed that the dogs were infected with Ancylostoma caninum and Giardia sp. They were treated successfully for ancylostomiasis by the administration of Task,[3] and the Giardia infection subsided spontaneously. A series of smears prepared from the blood of each dog was stained and examined, and all smears were observed to be free of blood parasites.

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[1]Purina Dog Chow; St. Louis, Missouri.

[2]Enduracell DH-L; Norden Laboratories; Lincoln, Nebraska.

[3]Task; Shell Oil Company; San Ramon, California.

Hematologic Procedures

Bleeding Procedures and Handling of the Blood

Bleeding from the Jugular Vein. Blood samples were drawn aseptically from the jugular vein using a syringe fitted to a 20 gauge needle. Hair was removed from the area of the jugular vein by clipping with an electric animal clipper (blade size 40). The dogs were restrained in a sitting position with head extended, and the venipuncture site sponged with 70% alcohol. The vein was located and held off with the thumb positioned just cranial to the thoracic inlet. The needle was inserted into the vein and the desired amount of blood was withdrawn, usually 2.5 ml. After removing the needle from the syringe the blood was transferred immediately to the appropriate tubes:

1. Fibrinogen Split Products. Two ml. of blood were added to a tube containing 2.0 mg. of soybean trypsin inhibitor;[4] the tube was inverted three times to ensure thorough mixing.

2. Hematologic Studies. Five-tenths (0.5) ml. was carefully placed in a silicone-coated tube which was immersed in an icewater bath maintained at approximately 0 C. Dilutions of this whole blood were made immediately with 1% ammonium oxalate solution; red blood cell (RBC) pipettes were used for a 1:100 dilution during the time when the platelet count was within normal limits. As platelet counts began dropping both 1:100 and 1:20 dilutions were made, the latter using a white blood cell (WBC) pipette. During the thrombycytopenic

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[4] Type I-S, Sigma Chemical Company; St. Louis, Missouri.

periods only 1:20 dilutions were made. A flat bottomed improved "Bright line" Neubauer hemacytometer and dark phase contrast microscope were used in counting as described in Brecher and Cronkite (1964). In addition to the number, the size and shape of the platelets were also recorded from observations on the counting chamber and on the stained smear.

Three capillary[5] tubes were also filled with blood from the silicone-coated tube. Two capillary tubes contained heparin which was mixed with the blood; the tubes were used to determine duplicate packed cell volumes (PCV). The third capillary tube contained no anticoagulant and the blood in it was used to make coverslip blood smears as described by Davidsohn and Wells (1963), for determination of parasitemia.

At approximately weekly intervals additional hematologic studies were performed. These included:

1. Hemoglobin determination
2. Total white count
3. Differential leukocyte count
    a. neutrophils
    b. lymphocytes
    c. monocytes
    d. eosinophils
    d. basophils

The procedures were performed according to the methods described

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[5]Micro Hematocrit, Scientific Products; Evanston, Illinois.

in Schalm (1965). The Klett colorimeter and cyanmethemoglobin[6] method were employed for hemoglobin determinations. Total white cell determinations were made using WBC pipettes and the improved "Bright line" Neubauer hemacytometer for the counting. For differential leukocyte counts coverslip blood smears were prepared. The percentage of each cell type in a differential leukocyte count of 100 white blood cells was calculated.

Bone Marrow Aspirations

Aspirations of bone marrow were obtained from each dog at approximately weekly intervals. The dogs were held off food 25 hours before the aspiration and were anesthetized using 2% Surital[7] at the rate of 8.0 mg. per pound of body weight. The marrow was obtained aseptically from the femoral medulary canal, alternating between left and right sides each week. On four occasions the marrow was obtained from the crest of the iliac when difficulty was encountered from repeated use of the femoral canal. The operative site was always clipped with an animal clipper, scrubbed with Phisohex[8] and sprayed with Betadine.[9] Yale B-D[10] bone-marrow needles were used; the preferred needle length for femoral canal aspiration was three and one-half inches, but

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[6]Hycel, Incorporated; Houston, Texas.

[7]Parke Davis & Company; Detroit, Michigan.

[8]Winthrop Laboratories; Division of Sterling Drug, Inc. New York, New York.

[9]The Purdue Ferderick Company, Yonkers, New York.

[10]Becton Dickinson, Inc., Rutherford, New Jersey.

needles one and one-fourth inches long were also used in either the femoral canal or iliac crest. After insertion of the bone-marrow needle into the cavity, the stylet was removed and approximately five-tenths ml. of marrow was withdrawn into a syringe containing five-tenths ml. of 10% EDTA solution. The diluted aspirate was placed immediately into a clean petri dish and smears of the marrow made on cover-slips. Marrow smears were stained with Wrights stain (buffer pH 6.8). The dogs were observed until they recovered from the anesthetic.

Differential Bone Marrow Counts

After the coverslip smears were stained, they were permanently fixed to microscope slides with Permount.[11] Differential bone marrow counts were determined by counting five hundred nucleated cells. The myeloid-erythroid (M:E) ratio was determined and the erythroid series further differentiated into the following morphologic categories:

1. Rubriblast
2. Prorubricyte
3. Rubricyte
4. Metarubricyte

Plasma cells, macrophages and reticulum cells (undifferentiated stem cells) were enumerated but not included in the count of five hundred. A careful screening of the smear was performed with special attention given to the general abundance of megakaryocytes. Fifty megakaryocytes were counted in each marrow smear and classified

[11]Fisher Chemical Company, Pittsburg, Pennsylvania.

morphologically into three groups (I, II, and III), as recommended by Feinendegen et al. (1962). The appearance of the enumerated megakaryocytes suggested there were successive stages of maturation also described by Craddock et al. (1955) as megakaryoblast, promegakaryocyte and megakaryocyte. Stage I was the classification which contained those megakaryocytes (megakaryoblast) with a dark basophilic cytoplasm and non-segmented nucleus occupying most of the cell. In stage II (promegakaryocyte) the nuclear-cytoplasmic ratio was lower and the foamy basophilic cytoplasm contained azurophilic granules. The nucleus was frequently lobulated. There was little evidence of platelet budding at this stage. Stage III (megakaryocyte) was the category assigned to all other megakaryocytes. The cytoplasm was profuse and irregular in outline with diffuse azurophilic granulation in the pale background. The nucleus was markedly lobulated and variable in staining intensity.

Lymphocytes, neutrophils and their precursers in the marrow smears were examined for the possible occurrence of morulae of E. canis.

Clinical Signs

Prior to exposure the dogs were examined and determined to be in good physical condition. The daily recording of body temperature was begun four days prior to exposure and continued throughout the study. After exposure both the principals and control were examined daily for signs of illness. Examinations for intestinal parasites were made periodically throughout the study.

Fibrinogen Split Products

The degree of disseminated intravascular coagulation was determined by modifying the procedure for detecting fibrinogen split products (FSP) described for use in human beings by Mertens et al. (1969). Whole dog blood in a nine to one dilution with sodium oxalate was centrifuged, and the erythrocytes were separated and washed four times with 0.9% NaCl. These cells were then formalinized with 3% formaldehyde in saline, and tanned using a 1:40,000 dilution of freshly prepared tannic acid, as described for human cells. A 4% suspension of the formalinized, tanned red blood cells were then coated with dog fibrinogen, incubated and washed in buffer as described by Mertens et al. (1969), then stored or used as needed.

Antidog-fibrinogen antiserum was prepared by modification of the method described by Haanen et al. (1970). Purified dog fibrinogen (Cohn fractionation) was thoroughly mixed with Complete Freunds Adjuvant,[12] and injected into a young rabbit. The antiserum was was harvested on the eleventh day following the second injection, by performing a heart puncture on the rabbit. The agglutination titer of the antifibrinogen serum was determined and the proper dilution for the performance of the assay was made.

The freshly drawn blood from each dog was mixed separately with two mg. aliquots of soybean trypsin inhibitor.[13] This blood was allowed to clot at room temperature and then the serum separated

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[12]Difco Laboratories, Detroit, Michigan.

[13]Ibid. p. 13

and stored at -20 C. At the time the serum was thawed, prior to performing the assay, thrombin[14] was added as recommended by Marder et al. (1970), to ensure complete clotting. The assay, with the above enumerated substitutions, was performed using the type U microtiter plate.[15] FSP is expressed in μg/ml. and is the product of the fibrinogen in control plasma and that of the inhibitor titer of control plasma divided by the inhibitor of test serum.

Detail of Experiment

Phase I

With completion of pre-exposure base line studies which included complete blood count (hemoglobin, packed cell volume, total and differential leukocyte counts), platelet count, FSP assay, bone marrow aspiration and fecal examinations, the dogs were exposed to *E. canis*. One dog was exposed to the 1962 Oklahoma isolate (lymphocytic strain) by intravenous injection of five ml. of heparinzed blood, freshly drawn from the reservoir host. The littermate was simultaneously exposed to the 1970 Oklahoma isolate (neutrophilic strain) by intravenous injection of five mls. of heparinized blood from the appropriate reservoir host. The control was not exposed to infectious blood. All dogs were bled from the jugular vein three times a week, from the beginning of the study. Procedures performed included: platelet count, packed cell volume, examination of Wrights stained smears of peripheral blood, and FSP assay. The complete blood count was performed weekly along

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[14] Parke David & Company; Detroit, Michigan.

[15] Flow Laboratories Incorporated; Rockville, Maryland.

with the aspiration of bone marrow. Rectal temperature and clinical signs were noted daily.

Phase II

The dog exposed to the 1970 Oklahoma isolate in Phase I was challenged 81 days later to a second strain of E. canis, the 1962 Oklahoma isolate. The control dog from Phase I again served as an unexposed control. The bleeding of each animal three times a week, with bone marrow aspirations and complete blood counts on a regular basis were performed as described in Phase I. Daily notation of rectal temperature and clinical signs were continued.

Phase III

The animal which served as a control in phases one and two was exposed to both the 1962 and 1970 Oklahoma isolates of E. canis by intravenous injection of five ml. of blood from the dog previously exposed to both isolates; the 1970 isolate 173 days earlier, and the 1962 isolate 92 days previously. Blood was drawn twice a week and the following procedures determined: platelet count, FSP assay, packed cell volume, and general parasitemia of each strain as noted on blood smears. Rectal temperature and clinical signs were recorded daily.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the three phases of this experiment will be discussed separately and discussion of these results will be interrelated with findings of other authors.

Phase I. Comparisons of *Ehrlichia canis* Infections in Littermate Dogs; One Exposed to the 1962 Oklahoma Isolate, the Other to the 1970 Oklahoma Isolate

Clinical Signs

Clinical signs attendant with infections resulting from the 1962 Oklahoma isolate of *E. canis* and from the 1970 Oklahoma isolate of *E. canis* have been described in dogs of mixed breeding (Ewing and Buckner, 1965a and Hayat, 1972). The clinical signs in German Shepherds infected with these isolates were found to be similar to the findings in the mixed breeds of dogs. Figure 1 shows the body temperature of the dog infected with the 1962 isolate, the dog infected with the 1970 isolate, and the unexposed control dog. The highest temperature of 105.8 F. that was recorded from the dog infected with the 1962 isolate occurred 14 days postexposure. After the peak there was a gradual drop in temperature but not to within

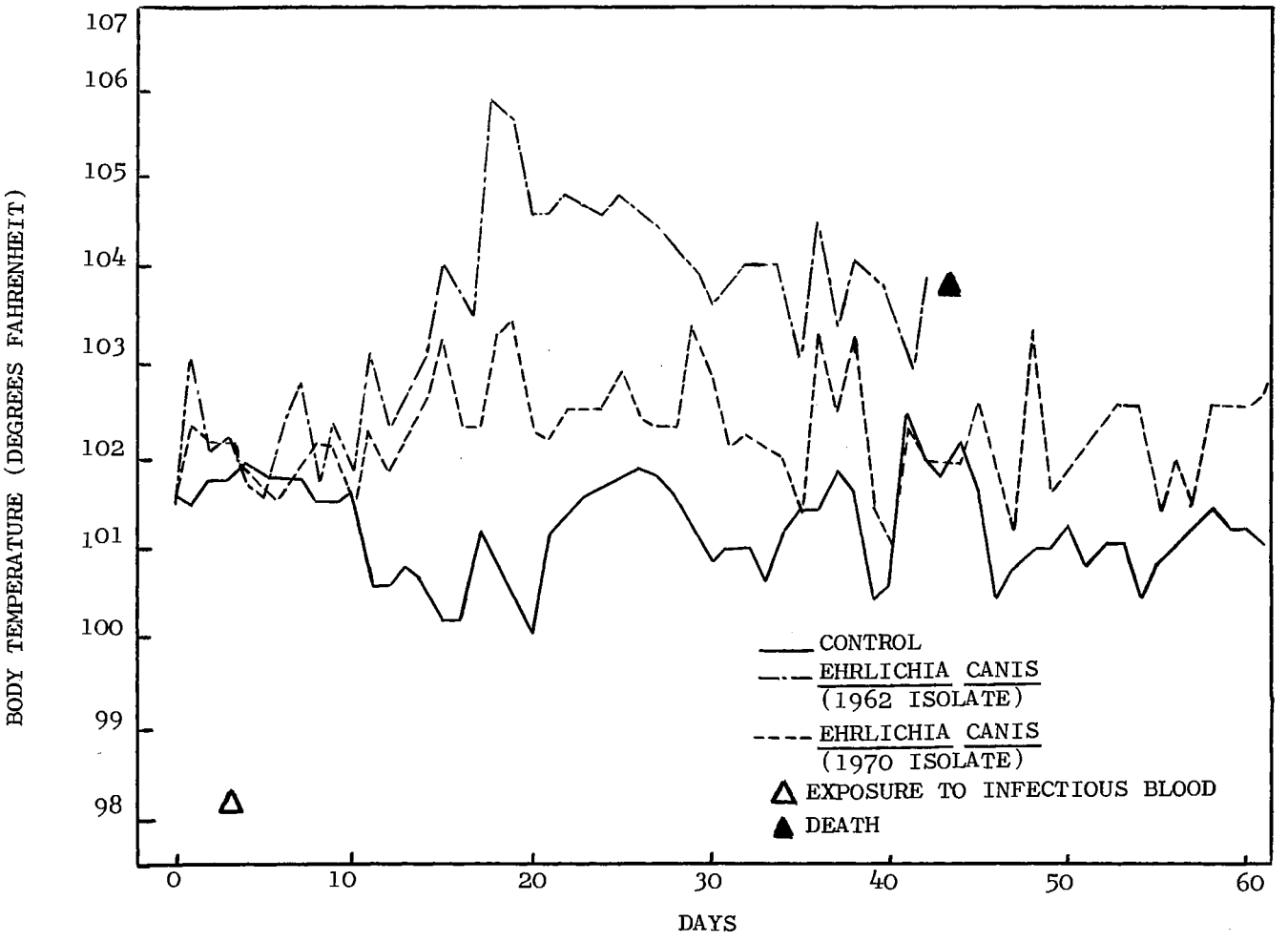

Figure 1. Body Temperature Fluctuations of Two Littermate Dogs and an Unexposed Control Dog; One Littermate Exposed to the 1962 Oklahoma Isolate of Ehrlichia canis, the Other Exposed to the 1970 Oklahoma Isolate of E. canis

normal limits before the accidental anesthetic death occurred 40 days postexposure. The peak body temperature of the dog infected with the 1970 isolate was 103.6 F. and occurred 15 days postexposure. There was then a gradual decline in temperature to within normal range by 40 days postexposure. Hayat (1972) observed a higher parasitemia on the blood smears of infected dogs on days when they had a higher body temperature. That general trend was not observed in this study. Exacerbations and remissions in body temperature occurred in the dog infected with the 1962 isolate, but not in the one exposed to the 1970 isolate.

Splenomegaly was detected by palpation in the dog infected with the 1962 isolate but not in the dog given the 1970 isolate. Loss of appetite with an accompanying loss of weight and decrease in activity occurred in both dogs but was much more apparent in the dog infected with the 1962 isolate. Bilateral mucopurulent ocular discharge and serous nasal discharge were also observed in both dogs. The dog infected with the 1962 isolate exhibited excess bleeding at the surgical site when bone marrow was collected, but when pressure was applied to the site with gauze squares, the bleeding ceased. Epistaxis was never observed in the dog exposed to the 1962 isolate, but two months after exposure the dog which was infected with the 1970 isolate was observed to experience transient nose bleed. Epistaxis was found to be the most dramatic sign of TCP (Huxsoll et al., 1969; Seamer and Snape, 1970; and Walker et al., 1970). The bleeding observed from the dog exposed to the 1970 isolate, on a single day, could not be described as either acute or chronic epistaxis as categorized by Walker et al. (1970). The significance of this single episode of

nose bleeding was probably negligible in light of the concurrent hematologic values.

Hematology

The hematological response in German Shepherds to the Oklahoma 1962 and 1970 isolates of E. canis was similar to that reported in mixed breeds (Ewing and Buckner, 1965a, and Hayat, 1972). A non-responding anemia occurred, and the PCV reached a low of 21%, 28 days postexposure in the dog infected with the 1962 isolate (Figure 2). Two days later the hemoglobin value was 7.1 gms. and the PCV still 21%. Subsequent specimens revealed the hemoglobin and PCV to be rising, but when accidental anesthetic death occurred 40 days post-exposure, they had not risen to pre-exposure levels. The lowest hemoglobin and PCV occurred in the dog infected with the 1970 isolate 22 days postexposure; they were 10.8 gms. and 29% respectively. They rose quite rapidly to pre-exposure levels.

A noticable decline in the number of circulating platelets was apparent as early as eight days postexposure with the 1962 isolate (Figure 3). The numbers continued to decline to a low of 8,500/cmm. one month after exposure. A slight increase then occurred and was followed by a decrease on the day of accidental death. The platelets observed during the thrombocytopenic period were nearly double the size of those observed before infection and had an increase of dentritic processes (Figure 4).

The dog infected with the 1970 isolate had a reduction in platelets beginning 18 days postexposure; the decline continued until 23 days postexposure (Figure 3), after which a gradual return to

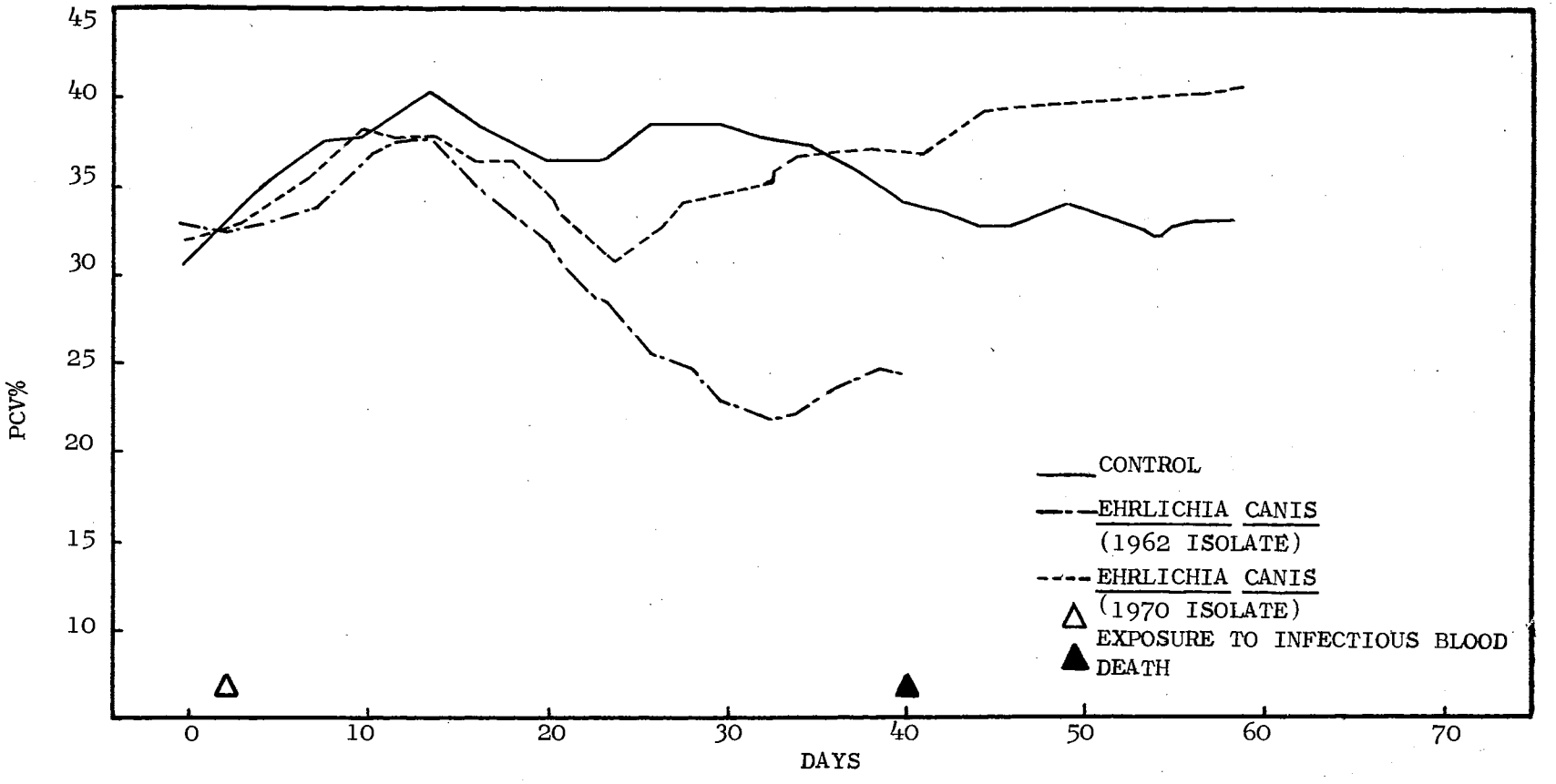

Figure 2. Moving Averages of the Packed Cell Volumes of Two Littermate Dogs; One Exposed to the 1962 Oklahoma Isolate of Ehrlichia canis, the Other Exposed to the 1970 Oklahoma Isolate of E. canis, and an Unexposed Control Dog

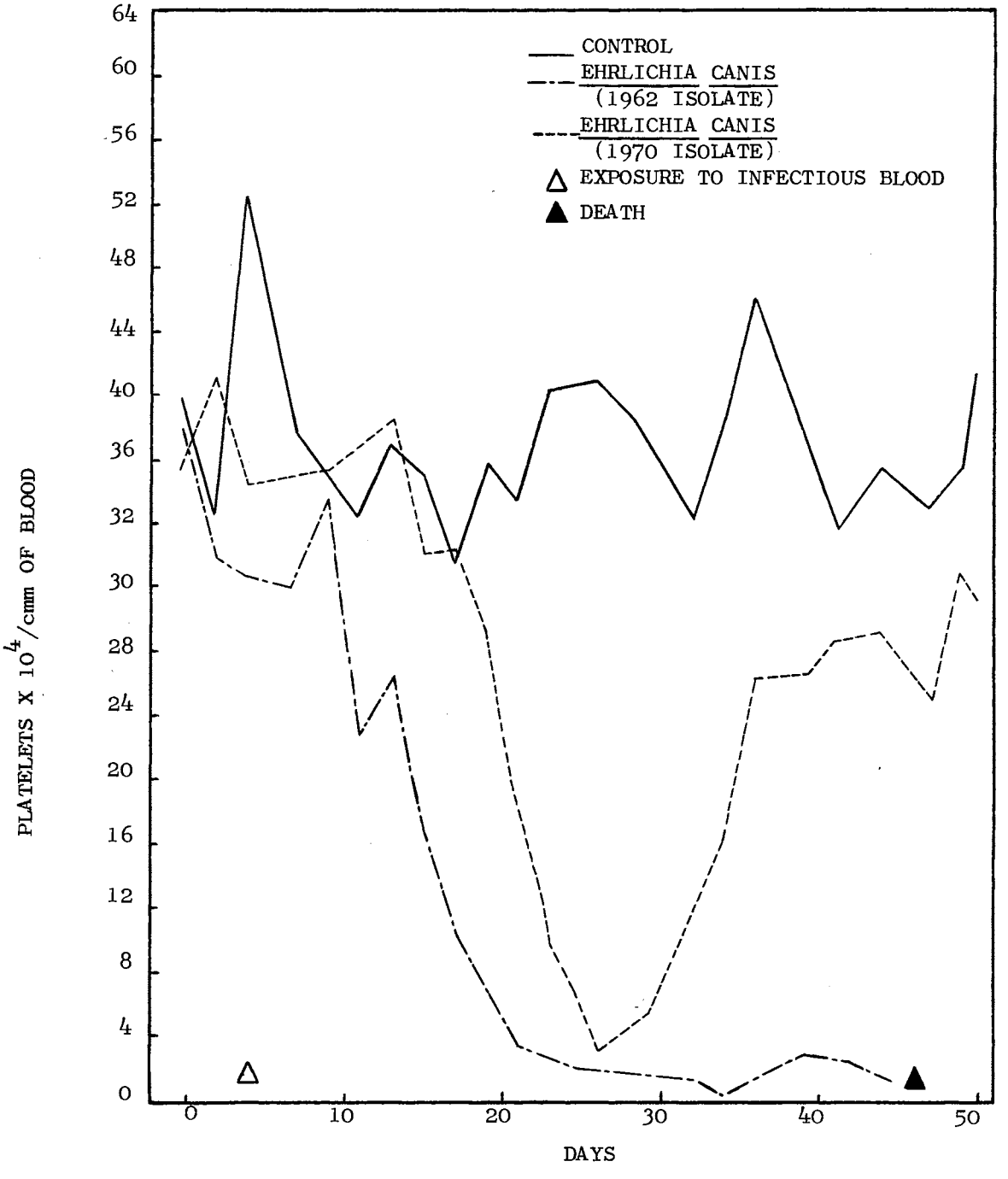

Figure 3. Platelet Counts of Two Infected Littermate Dogs and an Unexposed Control Dog; One Littermate Exposed to the 1962 Oklahoma Isolate of Ehrlichia canis, the Other Exposed to the 1970 Oklahoma Isolate of E. canis

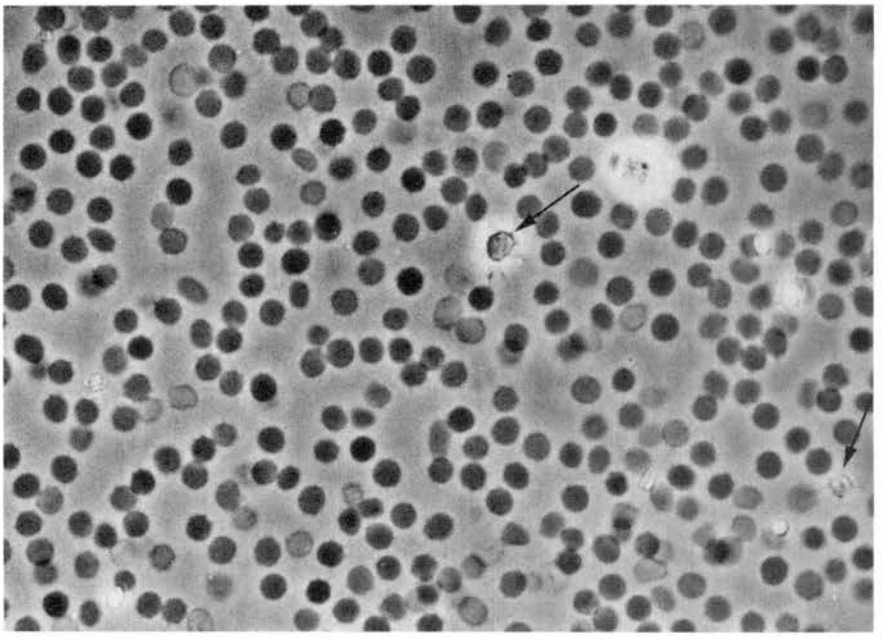

Figure 4. Platelets (1/20 dilution) from a Dog 25 Days after Exposure to Ehrlichia canis, 1962 Oklahoma Isolate. Notice Decreased Number and Large Size of Platelets as Compared with Figures 5 and 6

pre-exposure values occurred. A slight increase in size of platelets was noted (Figures 5 and 6), but not to near double in size as observed in the littermate infected with the 1962 isolate. These findings agree closely with those of Hayat (1972) working with the same isolates of E. canis, except that thrombocytopenia was much more profound in the present study.

The total white blood count (WBC) was determined weekly and slight leukopenia was found to occur in both dogs. Lymphopenia did not occur in the dog exposed to the 1962 isolate (lymphocytic strain), and neutropenia did not occur in the dog exposed to the 1970 isolate (neutrophilic strain), contrary to the findings of Hayat (1972). Morulae of the lymphocytic strain were observed only in lymphocytes (Figures 7 and 8) while those of the neutrophilic strain were observed in the neutrophils (Figure 9) and eosinophils of peripheral blood.

Bone Marrow

Dog Exposed to the 1962 Isolate. The meyloid:erythroid (M:E) ratio of the dog exposed to the 1962 isolate was normal before exposure (Table I). Three days after exposure a drastic decrease was noted in the M:E ratio. An exceptional amount of mitotic activity was also observed at this time. By the following week an increase in the M:E ratio occurred, but it was not within normal limits. A gradual decrease in the M:E ratio developed over the next two weeks. One month after exposure the M:E ratio was still below normal but an upward trend was again evident. The M:E ratio had not returned to normal when death occurred 40 days postexposure. Van Loon and Clark (1943) found the mean M:E ratio in bone marrow smears from the femur of normal

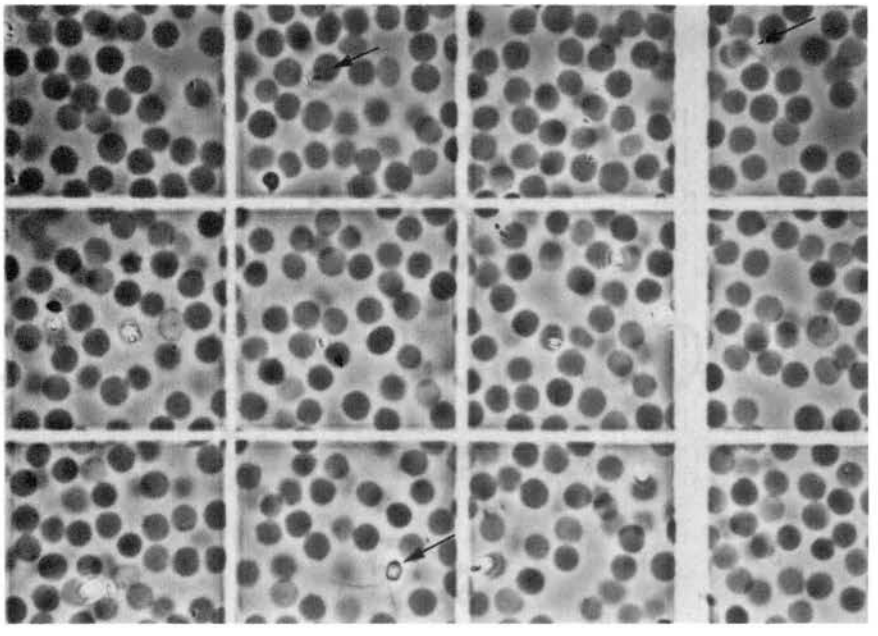

Figure 5. Platelets (1/20 dilution) from a Dog 25 Days after Exposure to Ehrlichia canis, 1970 Oklahoma Isolate. Notice Decrease in Number and Slight Increase in Size as Compared with Figure 6

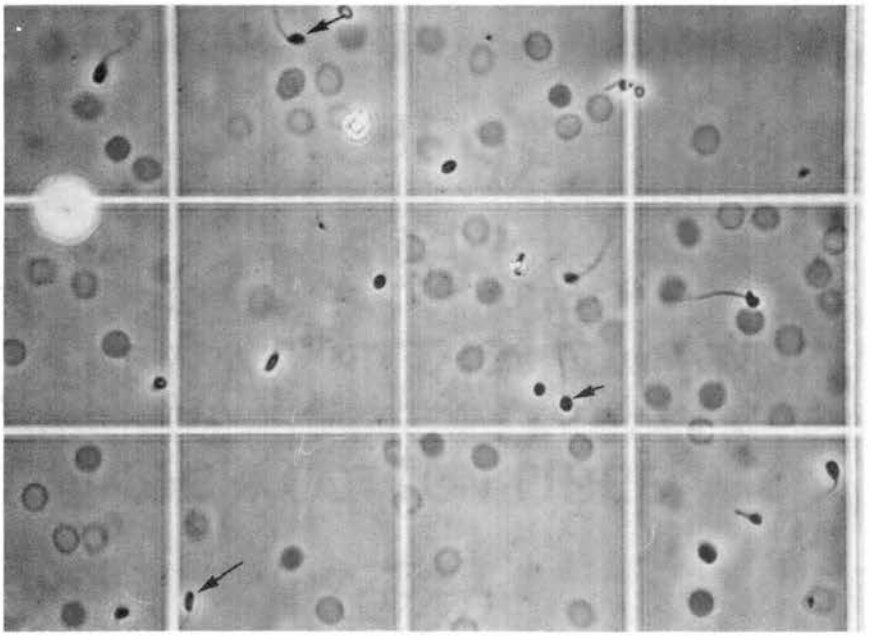

Figure 6. Platelets (1/100 dilution) from the Unexposed Control; Compare the Size of Platelets with Those in Figures 4 and 5

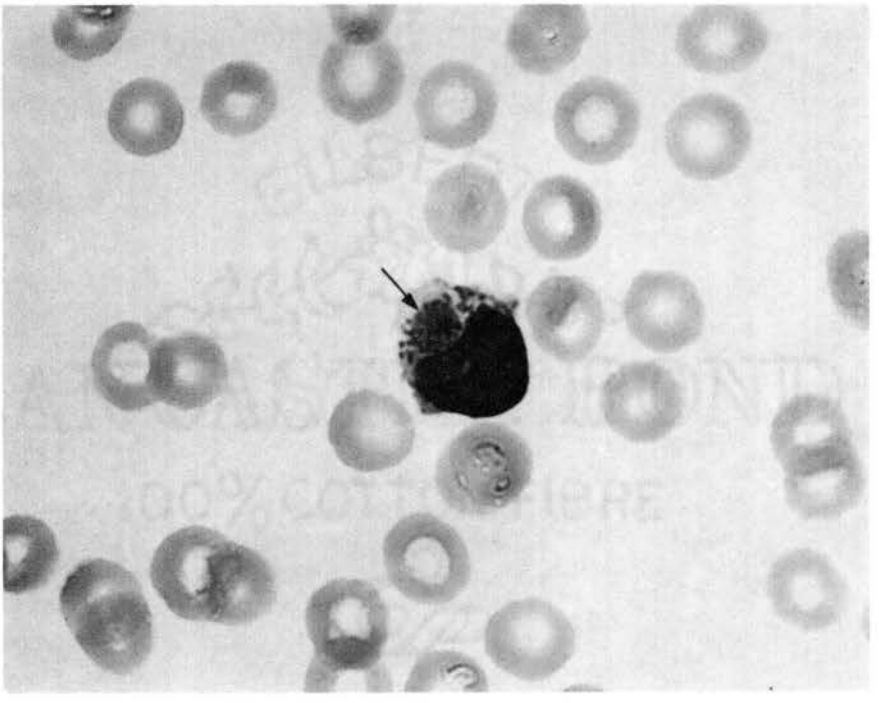

Figure 7. Lymphocyte Containing Morula of Ehrlichia canis, 1962 Oklahoma Isolate

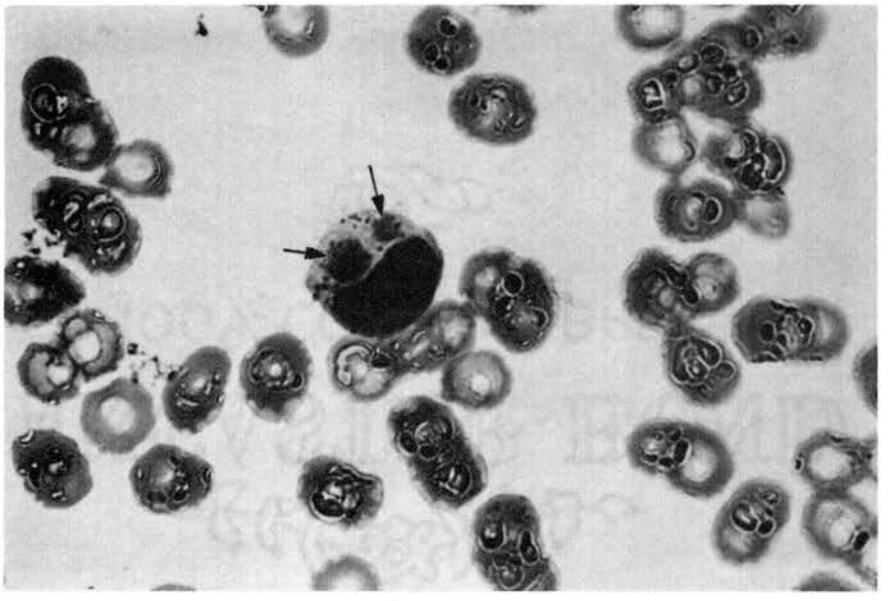

Figure 8. Lymphocyte Containing Two Morulae of Ehrlichia canis, 1962 Oklahoma Isolate. The Distortion of the Red Blood Cells is an Artifact of Staining

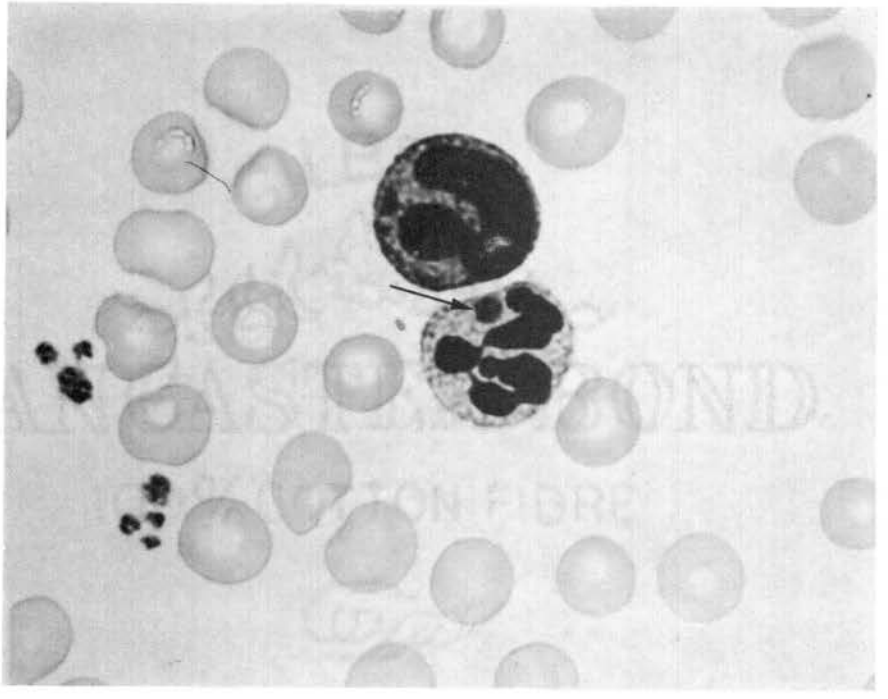

Figure 9. Neutrophil Containing Morula of Ehrlichia canis, 1970 Oklahoma Isolate

TABLE I

EVALUATION OF BONE MARROW FROM GERMAN SHEPHERD DOGS INFECTED WITH DIFFERENT STRAINS OF *EHRLICHIA CANIS*

Cell Type 1962 Isolate	Pre-exposure	Postexposure					
		3 Days	12 Days	19 Days	24 Days	31 Days	40 Days
Rubriblast	43	34	26	13	14	25	31
Prorubricyte	63	101	88	70	84	60	81
Rubricyte	97	226	159	175	226	194	148
Metarubricyte	22	76	17	33	43	60	70
Myeloid Cells	275	63	210	209	133	161	170
M:E Ratio	1.22:1	0.14:1	0.72:1	0.71:1	0.36:1 *E. canis* Morulae	0.47:1	0.51:1
Megakaryocytes							
Stage I	6	6	2	0	0	0	0
Stage II	14	16	20	3	11	2	7
Stage III	30	28	28	47	39	48	43
Cell Type 1970 Isolate							
Rubriblast	32	19	20	15	47	13	23
Prorubricyte	49	71	62	90	93	70	79
Rubricyte	136	154	89	90	193	209	150
Metarubricyte	39	46	28	35	26	65	74
Myeloid Cells	244	210	301	270	141	143	174
M:E Ratio	0.95:1	0.72:1	1.51:1 *E. canis* Morulae	1.17:1 *E. canis* Morulae	0.39:1 *E. canis* Morulae	0.40:1	0.53:1
Megakaryocytes							
Stage I	3	5	3	0	1	0	0
Stage II	19	8	4	7	5	11	5
Stage III	30	35	18	43	44	39	45
Cell Type Unexposed Control							
Rubriblast	30	22	20	11	18	13	
Prorubricyte	79	68	37	25	31	29	
Rubricyte	151	139	94	97	102	85	
Metarubricyte	49	60	15	28	46	38	
Myeloid Cells	191	211	334	339	303	335	
M:E Ratio	0.62:1	0.73:1	2.01:1	2.10:1	1.53:1	2.03:1	
Megakaryocytes							
Stage I	5	2	0	3	1	3	
Stage II	10	19	6	18	9	10	
Stage III	35	29	44	29	40	37	

dogs to be approximately 1:1; other investigators have reported 1.25:1 and 1.197:1 as normal findings in other long bones of this species.

Examination of the individual components of the erythroid series (Table I) revealed an increase in all stages beyond the rubriblast stage in the bone marrow following exposure; that finding coupled with the decreased M:E ratio indicated intensification of erythrogenesis. The rubricyte count was increased substantially and accounted for nearly half the cells found on the first examination of marrow after exposure. The rubricyte was the dominant cell of the erythroid series in subsequent marrow smears. Metarubricytes were not proportionately increased indicating the possibility of impediment of erythropoiesis by an arrest of red cell maturation at that level. Saifi and Vaughn (1944) reported similar findings after examining the bone marrow of human patients with an anemia associated with chronic infection.

Morulae of E. canis were observed in the bone marrow of the dog exposed to the 1962 isolate about three weeks postexposure (Table I). Lymphocytes and their precursors are not normally abundant in the bone marrow, and morulae were very scarce.

The bone marrow smears were also examined for the presence of megakaryocytes. Fifty megakaryocytes were enumerated in various stages of development (Table I). The first indication of possible impairment in platelet production was found in the bone marrow smears 12 days postexposure. As each megakaryocyte was counted the presence or absence of platelets was recorded. The smaller, younger stage III megakaryocytes were observed to have attached platelets; the larger

apparently older, stage III megakaryocytes had an abundance of cytoplasm, but no attached platelets. No stage I megakaryocytes (megakaryoblasts) were found in counts made 19 days, 24 days, 31 days, and 40 days postexposure. Stage III remained the dominant morphologic type, but the coloration was similar to that of a younger megakaryocyte. The cytoplasm was nearly homogeneous, purple to dark blue in coloration and difficult to distinguish from the multi-lobulated nuclei. Granulation was apparent in the cytoplasm of these large cells, and the multi-lobulation of the nucleus defined them as stage III. No platelet differentiation was observed in these cells. The coloration of stage III megakaryocytes was more normal in the last marrow smear taken before death of the dog, but production of platelets was still questionable.

Dog Exposed to 1970 Isolate. The dog infected with the 1970 Oklahoma isolate of E. canis was found to have a slightly decreased M:E ratio prior to exposure. Several days postexposure a further decrease in the M:E ratio was found (Table I), but the decrease was not so drastic as that which occurred in the dog given the 1962 isolate. The M:E ratio returned to within normal limits 12 days postexposure and then decreased to its lowest value about three weeks postexposure. This was concurrent with the low M:E ratio which occurred in the dog exposed to the 1962 isolate. A gradual increase occurred in subsequent weeks, but the ratio did not return to within normal limits.

Morulae of E. canis were found in the bone marrow smears taken for three successive weeks (Table I). One to four small morulae per cell were found, predominantly in mature segmented neutrophils, less

less often in unsegmented neutrophils and metamyelocytes (Figures 10, 11, and 12). A morula was also found in an immature eosinophil (Figure 13). Hayat (1972) observed morulae of the 1970 isolate in peripheral eosinophils. Several morulae observed on the marrow smear obtained 19 days postexposure appeared as a ring of elementary bodies with no organisms in the center (Figure 14). The relationship between this ring-like configuration of elementary bodies and the developmental cycle of E. canis postulated by Donatien and Lestoquard (1940) and more recently by Nyindo et al. (1971) is unknown.

The results obtained from counting and categorizing fifty megakaryocytes from marrow of the dog infected with the 1970 isolate were comparable to results obtained on the dog infected with the 1962 isolate (Table I). After a similar amount of time, few stage I megakaryocytes were found. Stage III dominated, but the coloration of the cytoplasm was often of a bluish tint. Some platelet budding was observed on these megakaryocytes.

Initial bone marrow examinations revealed depressed M:E ratios in the control dog. This may have resulted from malnutrition for when the dog's nutritional plane was improved during preconditioning the M:E ratio gradually returned to normal and in fact was increased.

Fibrinogen Split Products

To evaluate the amount of disseminated intravascular coagulation associated with E. canis infections, the hemagglutination-inhibition assay was utilized to determine fibrin/fibrinogen split products (FSP). The results of the FSP assays on the principals and control were, for the most part, within the normal range; i.e., what has been

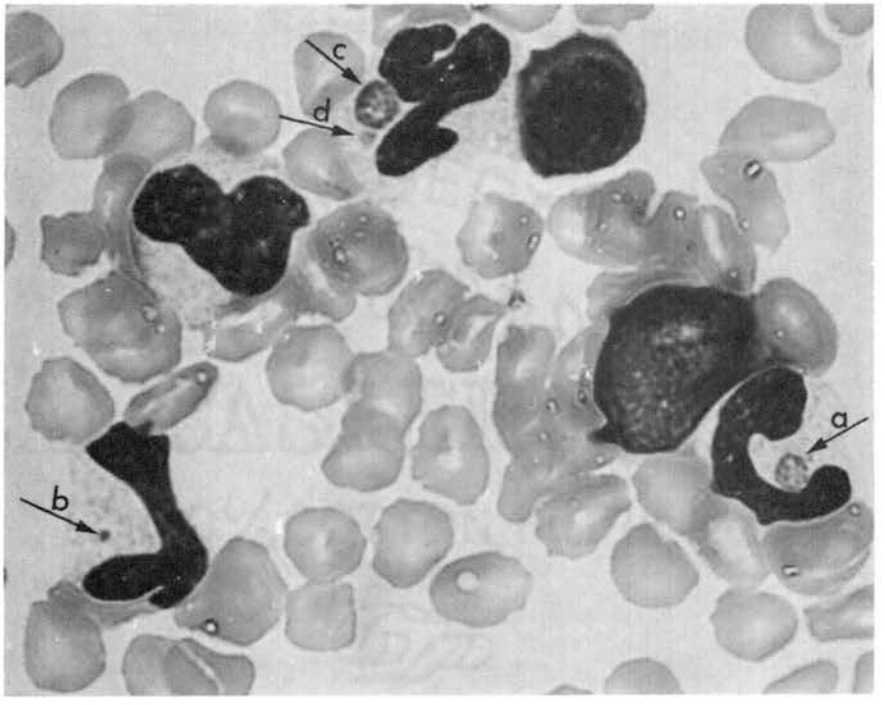

Figure 10. Bone Marrow. Unsegmented Neutrophils, One Containing One Morula (a) of Ehrlichia canis, and One Containing a Small Inclusion (b) Comparable to an Elementary Body Described by Donatien and Lestoquard, 1940. One Mature Neutrophil Containing Two Morulae of E. canis, 1970 Oklahoma Isolate; One Large (c) and one Small (d)

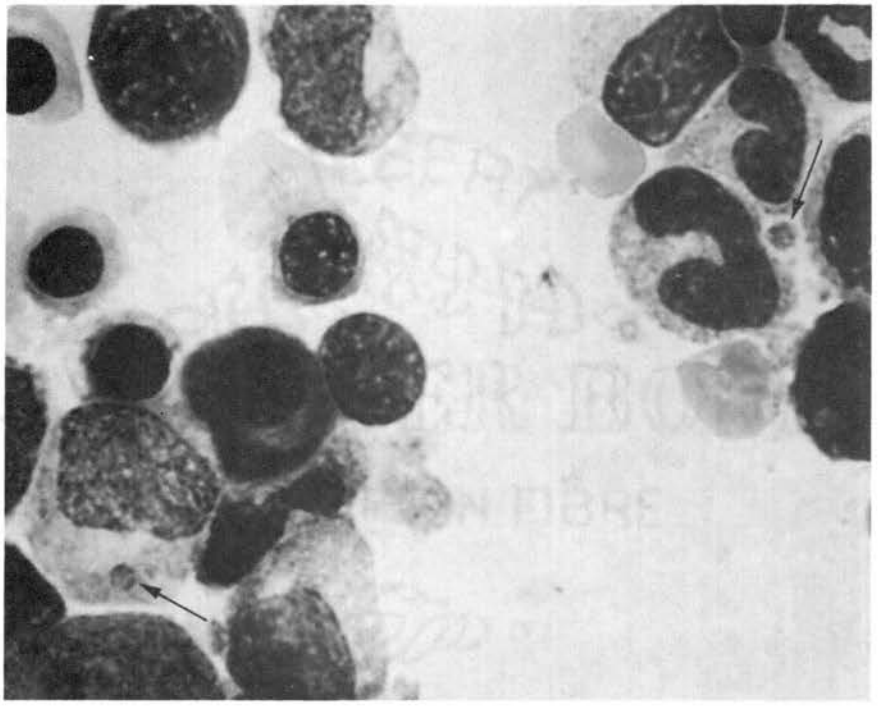

Figure 11. Bone Marrow. Unsegmented Neutrophil and Metamyelocyte Each Containing Morula of Ehrlichia canis, 1970 Oklahoma Isolate

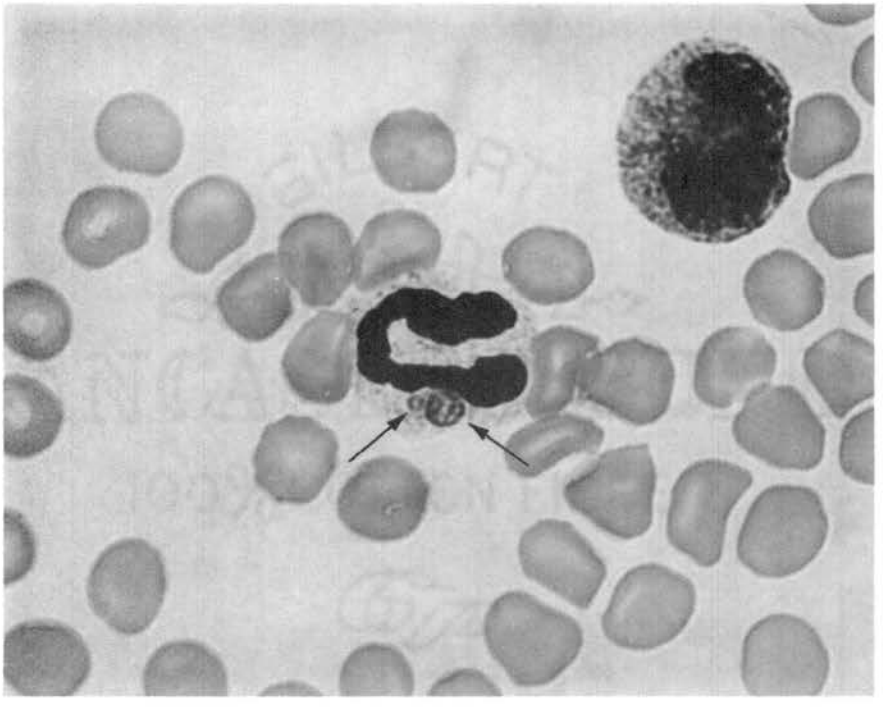

Figure 12. Bone Marrow. Young Neutrophil Two Morulae, One Quite Small, of Ehrlichia canis, 1970 Oklahoma Isolate

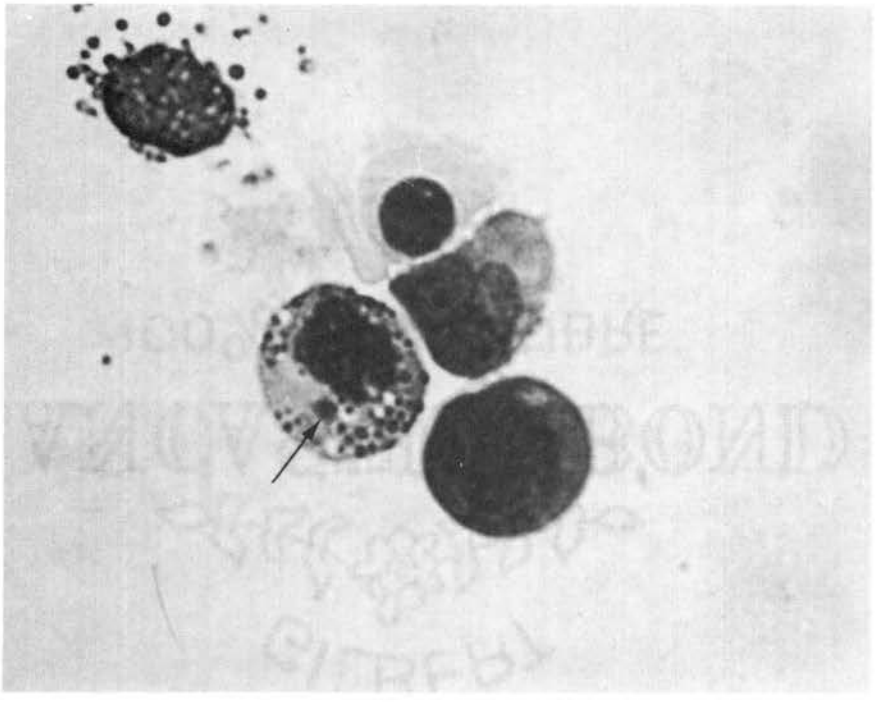

Figure 13. Bone Marrow. Immature Eosinophil Containing Morula of Ehrlichia canis, 1970 Oklahoma Isolate

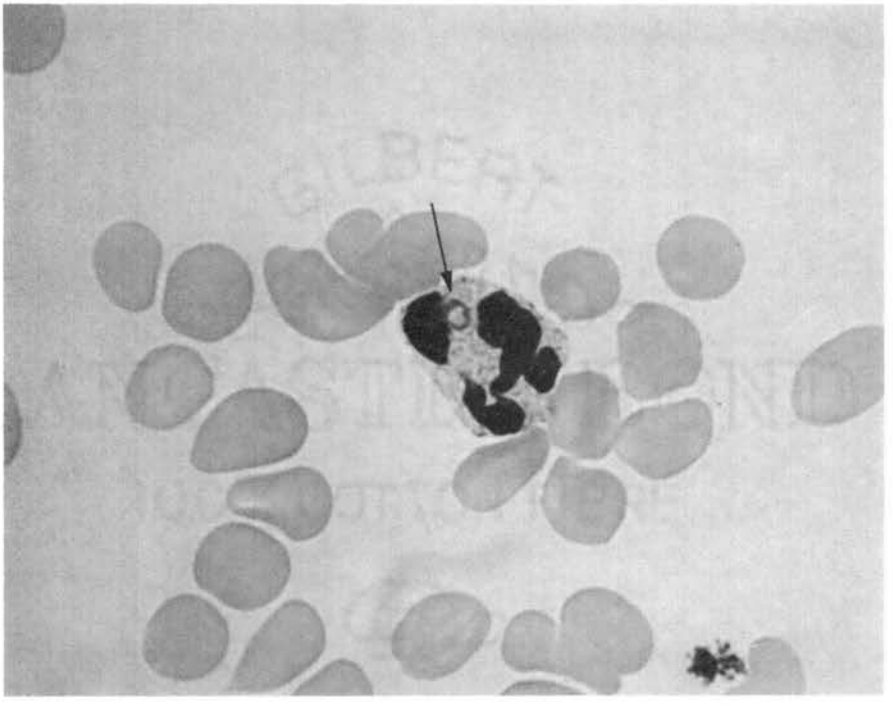

Figure 14. Bone Marrow. Neutrophil Containing Morula of Ehrlichia canis, 1970 Oklahoma Isolate. Notice that the Elementary Bodies Appear to be in a Ring Configuration with no Organisms in the Center

reported as normal for man, 1 to 6 µg/ml. (Mertens et al., 1969). The dog which was infected with the 1962 isolate exhibited 20 µg/ml. FSP in the specimen drawn 14 days postexposure. The serum was prepared for determination of circulating split products three times a week, so the level of FSP on the 13th or 15th day is not known. The high amount of FSP occurring on the 14th day postexposure does not correlate with the hematologic values at that time. The platelet count was 112,500 but continued to drop until 30 days postexposure when the fewest number of platelets were counted. One clinical sign was notable, however, on the 14th day postexposure; the body temperature was 105.8 F, the highest recorded during the infection.

The dog infected with the 1970 isolate of E. canis and the unexposed control exhibited normal amounts of circulating split products throughout the study. The result of the FSP assay on serum obtained from the dog infected with the 1970 isolate on the 14th day postexposure was 2.5 µg/ml.; the highest result obtained from this dog was 5 µg/ml., 32 days postexposure.

Phase II. Ehrlichia canis, 1962 Oklahoma Isolate Given During Convalescent Period--81 Days Following Exposure to 1970 Isolate

Clinical Signs

The dog that had been exposed to the 1970 isolate in the first phase of the experiment was challenged 81 days later by exposure to the 1962 isolate of E. canis. The clinical signs accompanying this second infection were similar in all respects to those exhibited by the

dog infected with the 1962 isolate in the first phase.

The temperature pattern was possibly more interesting and showed more classical exacerbations and remissions on roughly alternate days (Neitz and Thomas, 1938; Ewing and Buckner, 1965a and 1965b; Ewing, 1969; and Hayat, 1972). The initial high peak in body temperature, of 105.2 F. (Figure 15) occurred 14 days postexposure. Two later peaks of 106.2 F. were recorded 22 and 32 days postexposure. The body temperature then returned to normal range two months postexposure and remained there for a three month observation period. Epistaxis was not observed in the five months following exposure to the 1962 isolate.

Morulae were observed predominantly in lymphocytes, very rarely in neutrophils. Again no general trend was noted as to the occurrence of higher parasitemia on the days the dog exhibited the elevated temperature. The dog was euthanized 182 days after exposure to the 1962 isolate, i.e., 263 days after exposure to the 1970 isolate.

Hematology

During the convalescent period after infection with the 1970 isolate the PCV and platelet count had risen to pre-exposure levels. The both began to fall 13 days after exposure to the 1962 isolate (Figures 16 and 17). The low platelet count of 30,000/cmm occurred 24 days postexposure, and the low PCV of 21.5% occurred 34 days after exposure to the second strain of *E. canis*. It is interesting to note that the decrease in numbers of circulating platelets occurred later than in the dog infected with the 1962 isolate in the previous phase; and the lowest level occurred sooner, but was not as low. The PCV

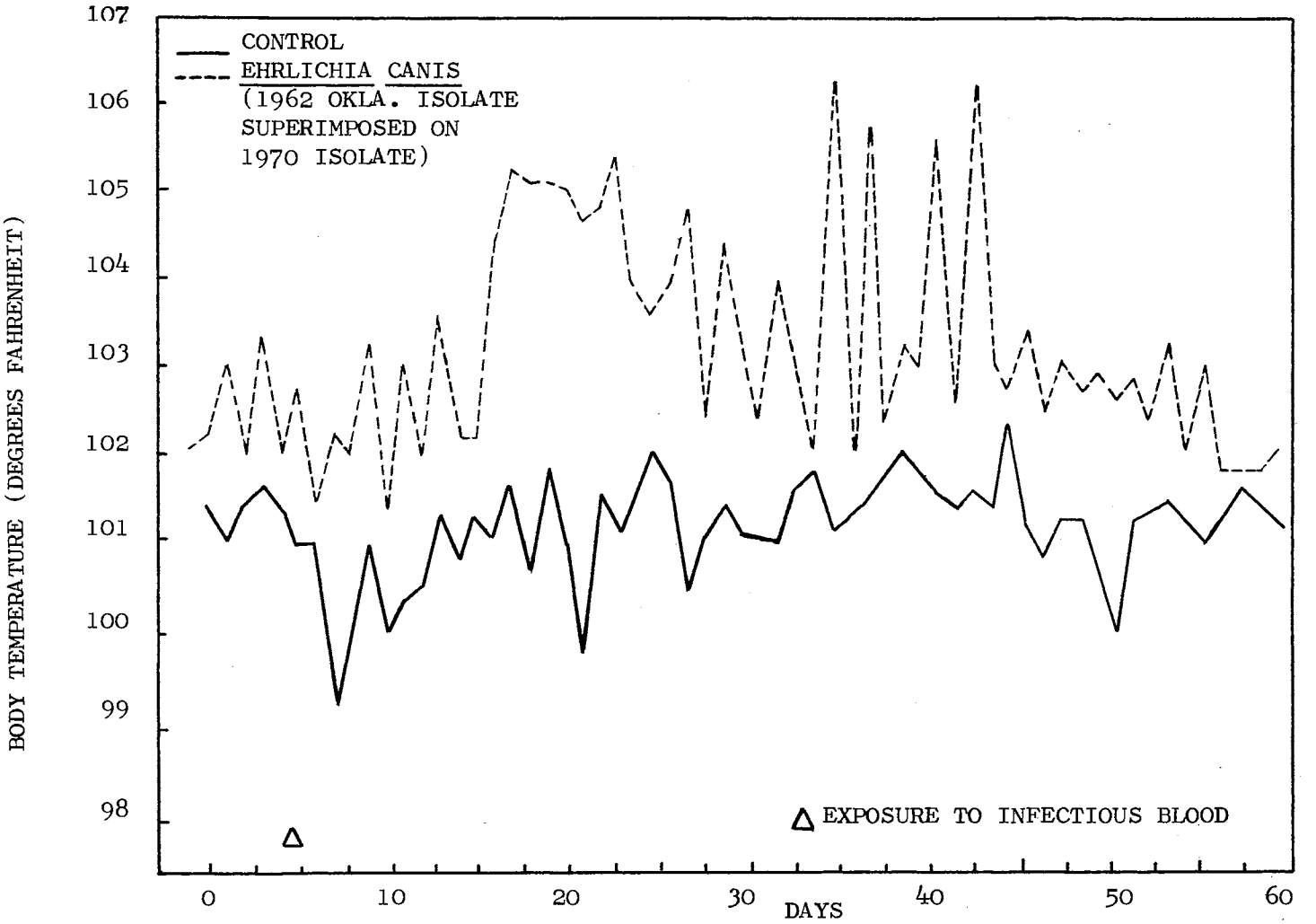

Figure 15. Body Temperature Fluctuations of Unexposed Control Dog and Dog Infected with the 1962 Oklahoma Isolate of Ehrlichia canis While Convalescing from Infection with the 1970 Isolate Administered 81 Days Previously

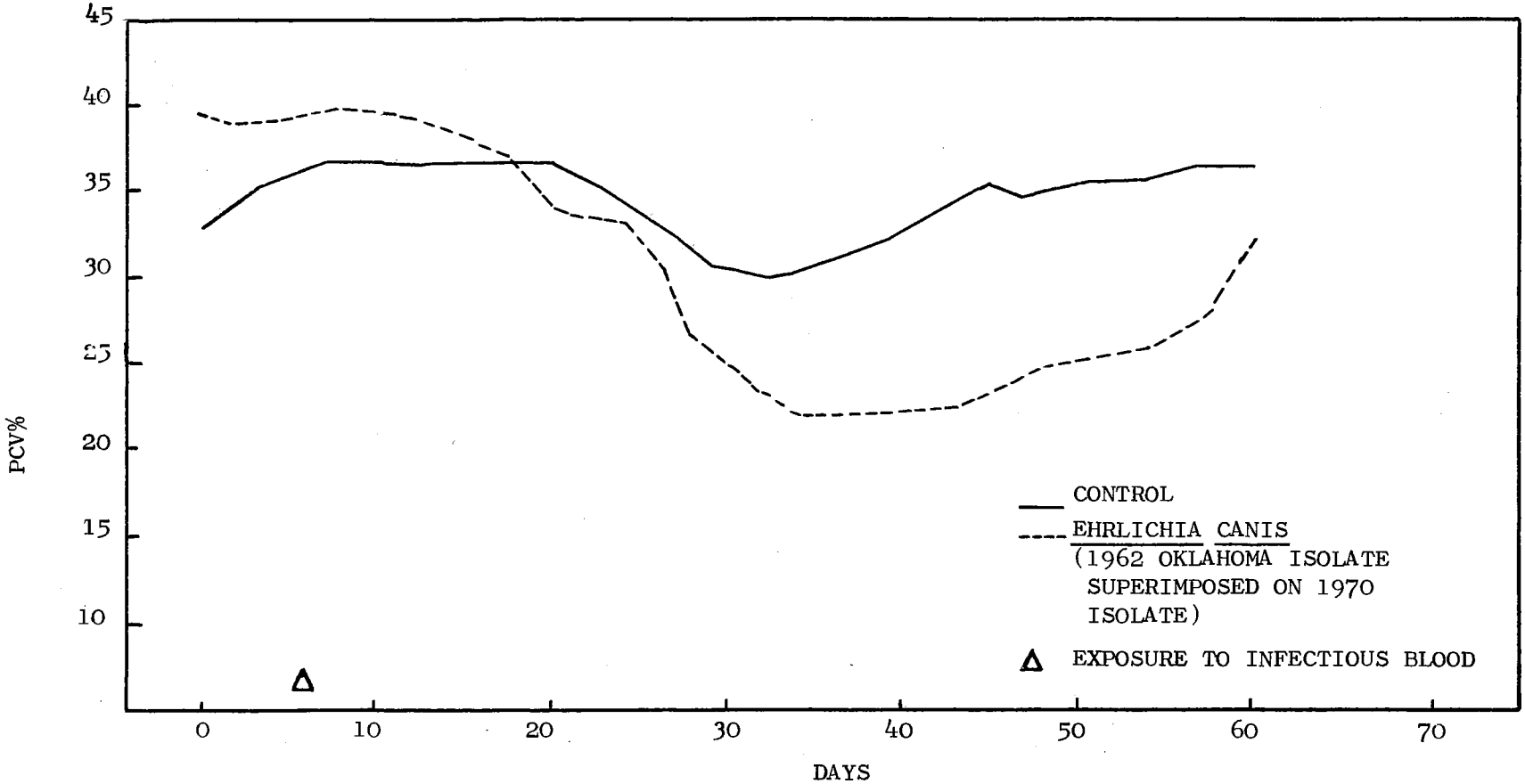

Figure 16. Moving Averages of the Packed Cell Volume of Unexposed Control Dog and Dog Infected With the 1962 Oklahoma Isolate of Ehrlichia canis while Convalescing from Infection With the 1970 Oklahoma Isolate Administered 81 Days Previously

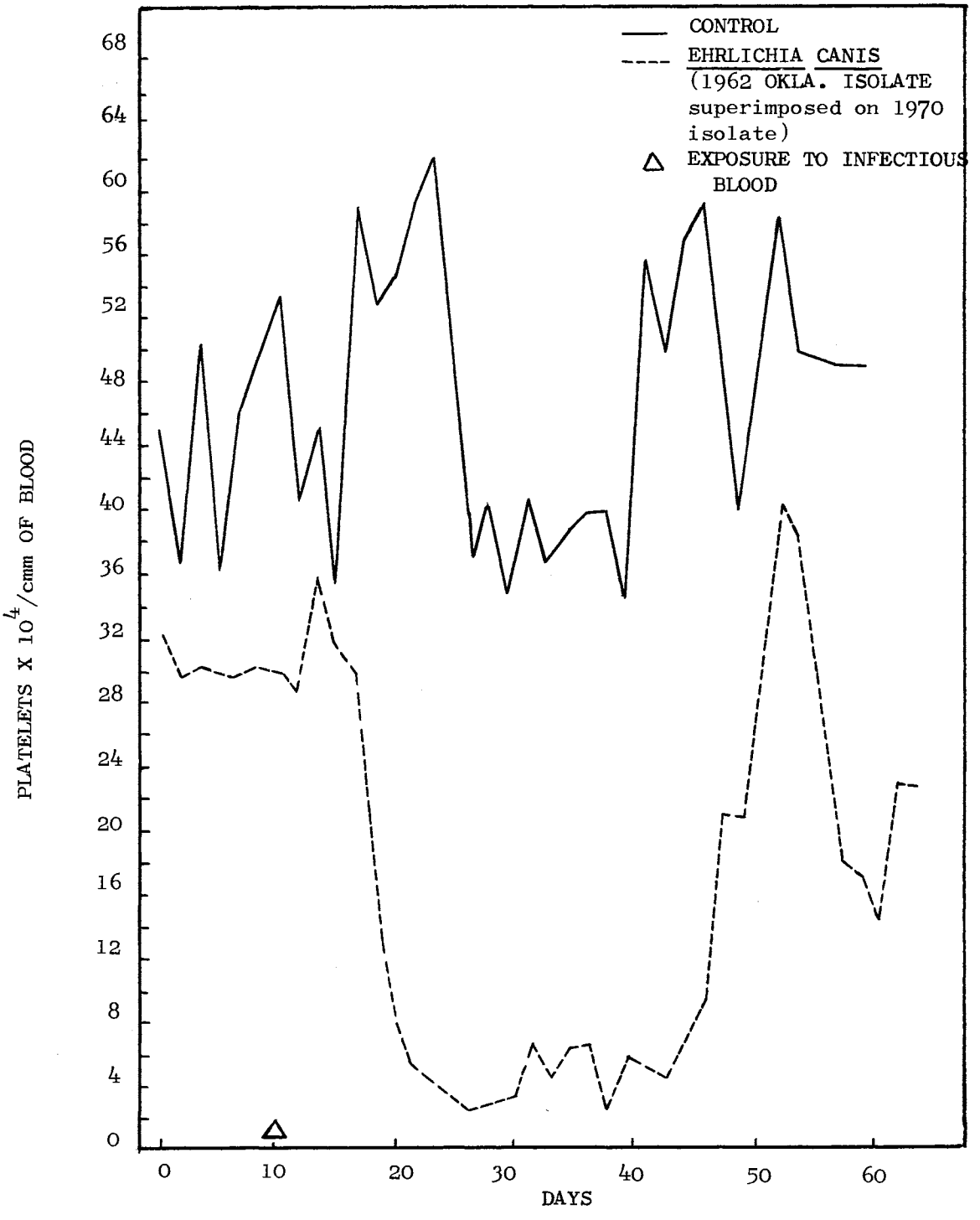

Figure 17. Platelet Counts of the Unexposed Control Dog and Dog Infected with the 1962 Oklahoma Isolate of Ehrlichia canis While Convalescing from Infection with the 1970 Isolate Administered 81 Days Previously

likewise dropped later, and also reached its lowest value later in the infection. The difference could be explained on the basis of individual difference in dogs, the increase in age, or could be the result of the body reacting to a second similar parasite, therefore less disruption to hemopoietic mechanisms. The platelet numbers very gradually increased and were within normal limits four months after exposure to 1962 isolate.

Bone Marrow

Before the M:E ratio of the dog infected with the 1970 isolate of E. canis in Phase I returned to normal, infection with the 1962 isolate was superimposed. The bone marrow was then examined at two-week intervals. As shown in Table II there was an initial decrease in the ratio 14 days postexposure, followed by an increase at 31 days and finally, another decrease 44 days postexposure. The M:E ratio was still below normal limits 62 days postexposure, but at no time was the ratio as low as that which occurred in both dogs in Phase I. It is not readily apparent why the second, more pathogenic, isolate of E. canis would have less effect on the bone marrow than did the first isolate. Several factors could influence these results, i.e., immune mechanisms, and/or age of animal. Impression smears of the bone marrow made post mortem, 182 days postexposure (1962 isolate), revealed an M:E ratio still slightly below normal.

Examination of the individual components of the erythroid series again revealed the rubricytic stage to be the most prevalent (Table II). The numbers of metarubricytes were lower than the numbers of prorubricytes in most specimens. The impediment of erythropoiesis

by arrest of red cell maturation at the metarubricyte level was again indicated, but was not nearly as pronounced as that observed after exposure to the first (1970) isolate of E. canis. The reason for the lesser effect on the cells of the bone marrow is not known.

TABLE II

EVALUATION OF THE BONE MARROW FROM A GERMAN SHEPHERD DOG INFECTED WITH THE 1962 ISOLATE OF EHRLICHIA CANIS WHILE CONVALESCING FROM INFECTION WITH THE 1970 ISOLATE ADMINISTERED 81 DAYS PREVIOUSLY

Cell Type	Pre-exposure (1962 isolate)	Postexposure 14 Days	31 Days	44 Days	62 Days	182 Days
Rubriblast	10	26	21	11	8	7
Prorubricyte	66	63	48	50	60	75
Rubricyte	146	123	160	218	193	123
Metarubricyte	44	74	32	17	22	53
Myeloid Cells	234	214	239	204	217	242
M:E Ratio	0.87:1	0.74:1	0.91:1	0.68:1	0.77:1	0.94:1
Megakaryocytes						
Stage I	1	0	1	0	0	
Stage II	4	8	4	0	4	
Stage III	45	42	45	50	46	

Among the megakaryocytes enumerated (Table II), stage I megakaryocytes were again rather sparse, and stage III megakaryocytes

predominated. There was evidence of platelet production 14 days post-exposure and in all subsequent specimens. The blue coloration of the cytoplasm that is characteristic of the younger megakaryocytes was again evident in the stage III megakaryocytes.

Fibrinogen Split Products

The FSP values of the dog challenged with a second isolate of E. canis, and those of the unexposed control dog were similar during all of Phase II. They ranged between 0.3 µg/ml. and 5 µg/ml.

Phase III. Ehrlichia canis 1962 and 1970 Oklahoma Isolates--Simultaneous Infection in the Same Dog

Clinical Signs

In the third phase, the dog which served as control for the two previous phases was challenged with blood from the dog in Phase II that had been infected with both the Oklahoma 1962 and 1970 isolates. The resultant clinical signs were similar to those described for the dog in Phase I infected with only the 1962 isolate. The initial peak in body temperature of 106.8 F. occurred on the 14th day postexposure which coincides exactly with the initial peak in the previous phases. Exacerbations and remissions in body temperature on roughly alternate days were also noted (Figure 18). High peaks in temperature persisted and 106.6 F. was recorded as late as one month postexposure. The body temperature did not return to normal before death occurred fifty days after exposure due to penumonia.

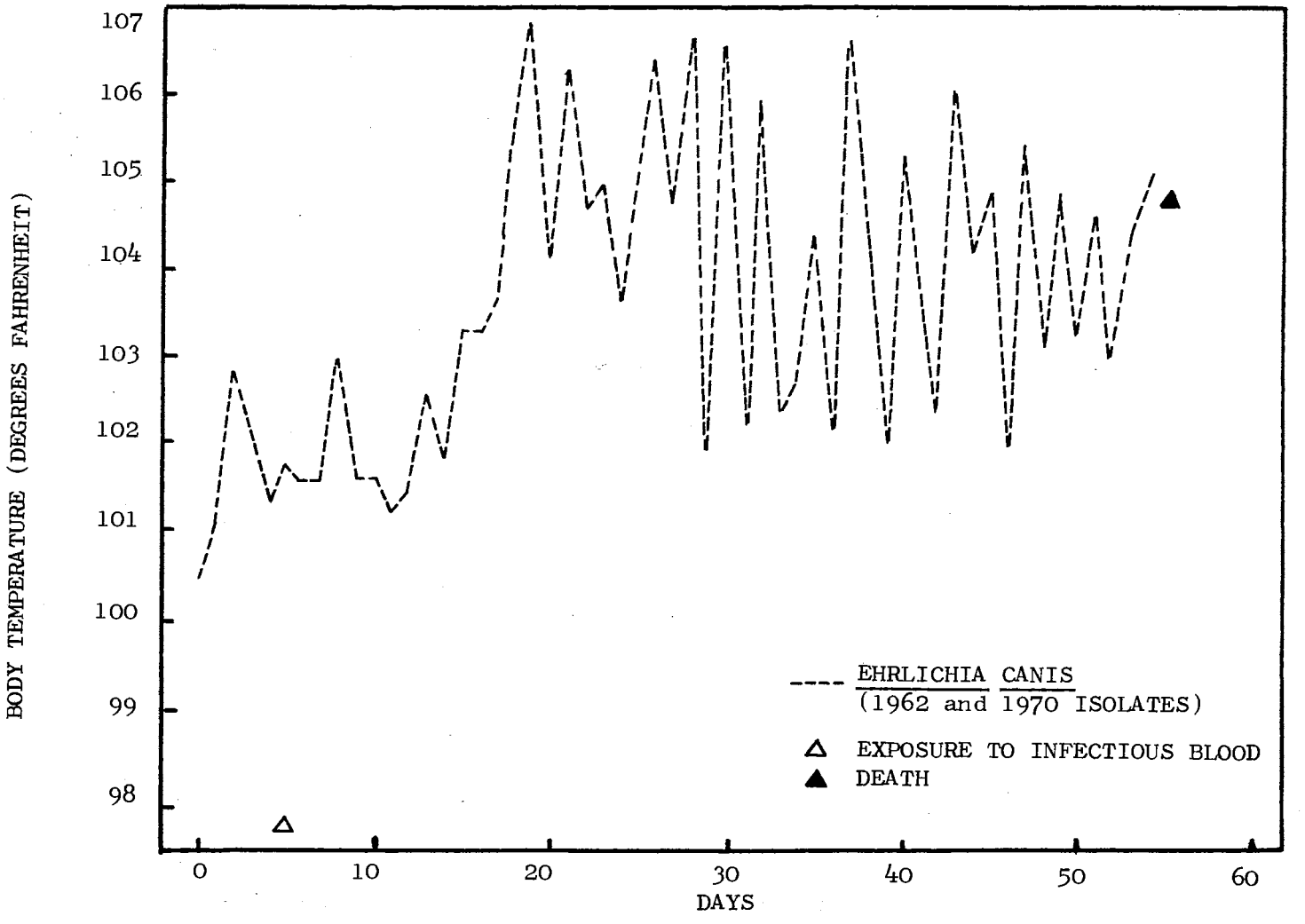

Figure 18. Body Temperature Fluctuations of the Dog Simultaneously Exposed to Both the 1962 and 1970 Oklahoma Isolates of Ehrlichia canis

Morulae of E. canis were observed in neutrophils, but more often in lymphocytes. Since smears were not examined daily it was not possible to observe whether there was any general trend toward higher parasitemia on days of higher body temperature.

Hematology

A decrease in PCV and platelet count values occurred 14 days postexposure (Figure 19). That general trend continued with a low platelet count of 45,000/cmm occurring 27 days postexposure. An upward trend in both the PCV and platelet count had begun when death occurred, but they were not to pre-exposure level.

Bone Marrow

Bone marrow studies were not conducted in this Phase of the experiment.

Fibrinogen Split Products

All FSP values were within the "normal range" with one notable exception. Sixteen days postexposure a value of 20 µg/ml. was observed. This level corresponds to the 20 µg/ml. obtained on the dog infected with the 1962 isolate, in Phase I, on the fourteenth day postexposure. No specimen was drawn on the fourteenth day from this dog in Phase III, and no specimen was drawn on the sixteenth day postexposure from the dog in Phase I, making further comparisons impossible. It is interesting to note that the values occurred at similar times in the infection of the dogs.

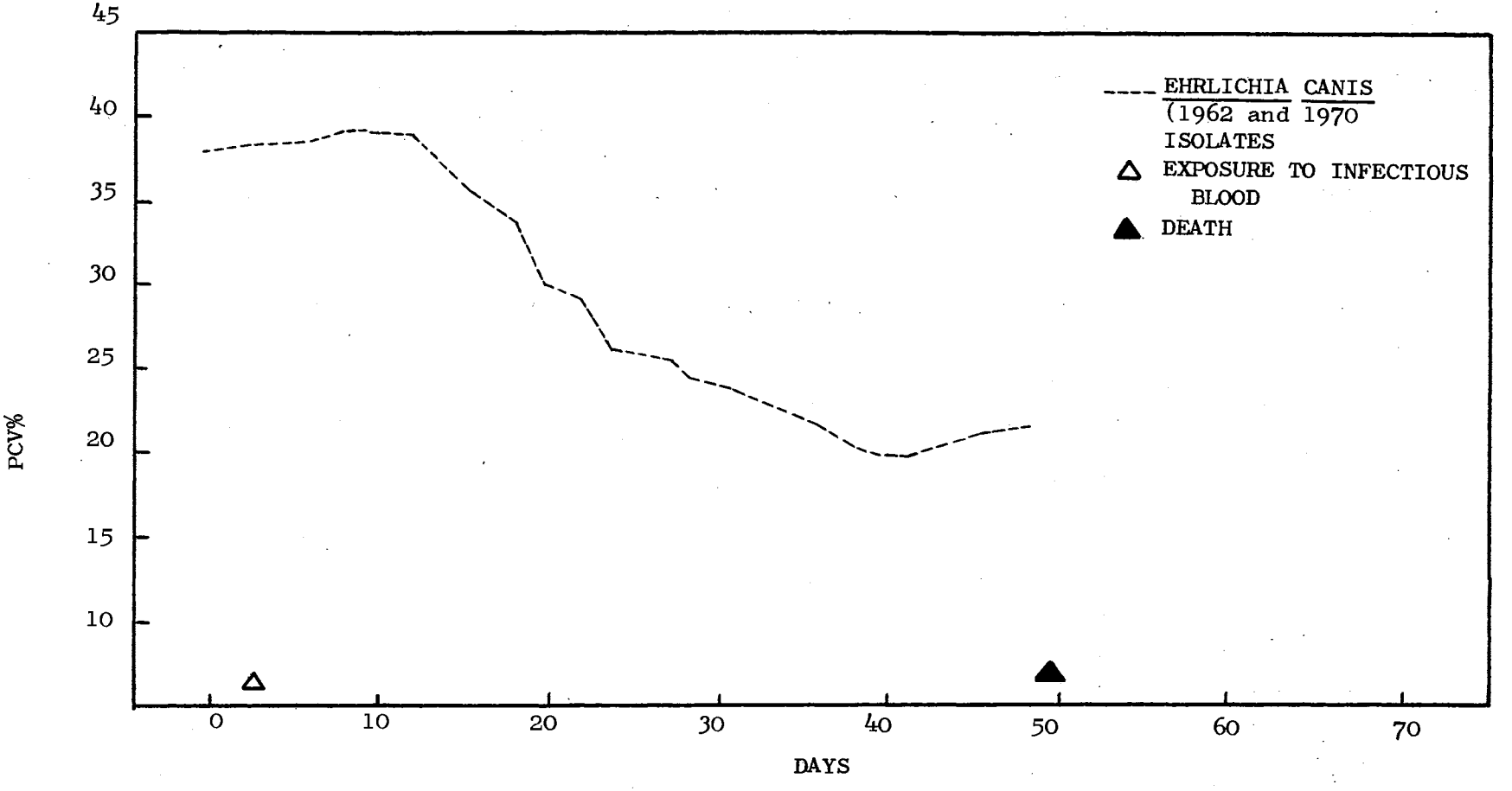

Figure 19. Moving Averages of the Packed Cell Volume of the Dog Simultaneously Exposed to Both the 1962 and 1970 Oklahoma Isolates of Ehrlichia canis

CHAPTER V

SUMMARY AND CONCLUSIONS

The effects of the 1962 and 1970 Oklahoma isolates of Ehrlichia canis were studied in young German Shepherd dogs. The pathogenesis of the isolates in experimental infections in that breed did not differ greatly from that in pups of mixed breeding as reported by Hayat (1972). The morula-type aggregates of the organisms in the 1962 Oklahoma isolate were observed both in peripheral and in bone marrow lymphocytes. Morulae of the 1970 Oklahoma isolate occurred in peripheral neutrophils and eosinophils. They were also found in metamyelocytes, eosinophils, unsegmented neutrophils and, most often, mature neutrophils in the bone marrow.

Clinical signs attendant with infection of the 1962 isolate were rather severe. When given in combination with the 1970 isolate the degree of severity did not seem to be altered from that observed with the infection of the 1962 isolate alone. Body temperature fluctuations were quite characteristic with exacerbations and remissions occurring on roughly alternate days and temperatures reaching as high as 106.8 F. Clinical signs in the German Shepherd infected with the 1970 isolate were mild. The highest temperature recorded was 103.6 F. and exacerbations and remissions in body temperature were not observed.

A common finding in E. canis infections has been long-standing

anemia and thrombocytopenia. Those findings were substantiated in the present study, especially in the case of the 1962 isolate. Although anemia and thrombocytopenia occurred after infection with the 1970 isolate, the persistence and degree of severity was less than that occurring with the 1962 isolate. Examination of bone marrow revealed an increase in the number of rubricytes without a corresponding increase in metarubricytes. This could indicate the level at which impediment of erythropoiesis occurs by an arrest of red cell maturation. There was a notable decrease in the M:E ratio at approximately the time the PCV and hemoglobin were at their lowest levels.

The origin of the severe thrombocytopenia caused by the 1962 isolate could not be explained fully by data gathered from examination of bone marrow. There were no young (Stage I) megakaryocytes observed in the marrow smears beginning two and one-half weeks post-exposure, to the end of the study. Older megakaryocytes (Stage III) were the predominant type, but upon closer examination very little platelet budding was observed. There was likewise a decrease in platelets following infection with the 1970 isolate. The population of megakaryocytes observed in the marrow smears was similar to that observed in the marrow of the dog exposed to the 1962 isolate except that some platelet budding was observed on the Stage III megakaryocytes.

An attempt was made to determine the origin of thrombocytopenia by examining the possibility that they were "consumed" in a process of disseminated intravascular coagulation (DIC). The results obtained do not give a full explanation. It is possible that DIC

occurred but that the resultant circulating split products were too minute to be detected by the adaptation of the hemagglutination-inhibition test for fibrin/fibrinogen split products used so successfully with man. The possibility of DIC occurring with infections of E. canis, especially the more serious form resulting in TCP, should be examined further.

The severity of the disease produced by the 1962 and 1970 Oklahoma isolates of E. canis in experimental infection in German Shepherd dogs is little different from experimental infection in dogs of mixed breeding. The 1970 isolate produced a mild disorder, but thrombocytopenia was evident together with slight anemia. The 1962 isolate produced a severe anemia and profound thrombocytopenia. Profuse epistaxis, as is known to occur in German Shepherds with E. canis infection in Southeast Asia, did not occur with either Oklahoma strain of E. canis. Both isolates produced a decreased M:E ratio and impediment of erythropoiesis, the latter possibly by arrest of red cell maturation at the metarubricyte stage. Morulae of E. canis of both isolates were found in the bone marrow. FSP were also found to occur with the 1962 isolate of E. canis. Follow-up studies of disseminated intravascular coagulation (DIC) and the resultant fibrin/fibrinogen split products should be performed, especially with a strain of Ehrlichia canis which produces tropical canine pancytopenia.

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VITA

Doris Ford McKown

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF TWO ISOLATES OF EHRLICHIA CANIS ON GERMAN SHEPHERD DOGS

Major Field: Veterinary Parasitology

Biographical:

Personal Data: Born in Omaha, Nebraska, December 4, 1943, the daughter of The Rev. and Mrs. Charles P. Ford.

Education: Graduated from Larned High School, Larned, Kansas, May, 1961. Attended Sterling College, Sterling, Kansas, Kansas State University, Manhattan, Kansas, and received Bachelor of Science degree in Medical Technology from University of Missouri at Kansas City, Kansas City, Missouri, June, 1965. Registered as a Medical Technologist of the American Society of Clinical Pathologists, December, 1965. Completed requirements for Master of Science degree at Oklahoma State University in May, 1973.

Professional Experience: Intern and Staff Medical Technologist, Menorah Medical Center, Kansas City, Missouri. Medical technologist at the following hospitals: Shawnee Mission Hospital, Shawnee Mission, Kansas, St. Mary's Hospital, Manhattan, Kansas, Mercy Medical Center, Chicago, Illinois. Research technologist, Hemophilic Dog Colony, Oklahoma State University, Stillwater, Oklahoma.

Professional Organizations: Medical Technologist of the American Society of Clinical Pathologists.