EXPERIMENTAL CANINE MALIGNANT LYMPHOMA: TRANSMISSION STUDIES AND ISOLATION OF A CANINE HERPESVIRUS

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ABSTRACT

EXPERIMENTAL CANINE MALIGNANT LYMPHOMA:
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A CANINE HERPESVIRUS

by Thomas John Kakuk

Canine malignant lymphoma (CML) was transmissible to the Beagle neonate in 2 serial passages, and a canine herpesvirus, designated as canine herpesvirus-Kakuk (CHV-K), that was pathogenic for the germfree Beagle neonate was isolated from a dog with CML. The clinical, hematologic, macroscopic, and microscopic findings of experimentally induced CML and a septicemia in Beagle neonates produced by CHV-K were described and discussed.

Two serial passages of CML were accomplished with suspensions of viable CML whole cells in 2 litters of Beagle neonates. Definite evidence of malignant lymphoma developed in 3 of 11 dogs inoculated with a single dose of a cell suspension by 53, 54, and 78 days postinoculation, respectively. Hematologic results indicated that leukemia was present in 1 dog and subleukemia was present in 2 dogs between 41 and 52 days. Two dogs were anemic, whereas all 3 dogs had thrombocytopenia. Two dogs with overwhelming CML were preirradiated with x rays, whereas 1 dog was not so irradiated. Successful transmission of CML without pretreatment of total body irradiation was considered noteworthy. Clinical and pathologic findings were similar to those reported for naturally occurring malignant lymphoma of dogs and cats. Organs and tissues

having neoplastic involvement included: lymph nodes, thymus, liver, lung, kidney, spleen, bone marrow, gastrointestinal tract, and muscle. Malignant lymphoma did not occur in the third serial passage, although the inoculated animals had enlargement of the superficial lymph nodes. Biopsies of these enlarged lymph nodes revealed lymphocytic hyperplasia.

The CHV-K was isolated from 18 germfree Beagle neonates inoculated with either cell suspensions prepared from a dog with CML or with extracts prepared from puppies with the septicemic disease. Two contact control puppies died from similar septicemic conditions, whereas the 3 non-contact control puppies remained healthy.

The production of a fatal septicemic disease in Beagle neonates inoculated with material prepared from a dog with CML and the isolation of the virus from the kidney of this dog indicated that the virus came from the donor dog.

A fatal septicemic disease occurred in colostrum-deprived, germfree Beagle neonates 6 to 16 days after inoculation with the virus. The fundamental histopathologic lesion was necrosis in the liver, lung, kidney, heart, skeletal muscle, pancreas, and adrenal gland. A few intranuclear basophilic and/or acidophilic inclusion bodies were seen in cells adjacent to the areas of necrosis. Splenomegaly and generalized lymphadenopathy were commonly seen which microscopically consisted of marked lymphocytic and reticular cell hyperplasia. The marked hyperplasia produced by this virus resembled neoplasia, although obvious neoplasms were not induced.

Results of cross-serum neutralization tests indicated that the virus was closely related, or possibly identical to, the canine herpesviruses isolated from young puppies (Carmichael's strain:F205V and Stewart's

strain:SL18HLV). However, this was the first report in which a CHV was isolated from a dog with malignant lymphoma, and also the first report concerning the isolation of a CHV from an adult dog which was pathogenic for puppies.

Results indicated that age at time of exposure is important in reproducing the disease in puppies. Newborn, germfree Beagles inoculated prior to 8 days of age were highly susceptible and nearly all died. One of 18 recipients has survived the infection. If puppies were not inoculated until after 8 days, there were clinical manifestations of the disease, but few died.

Factors of importance in establishing a diagnosis included: (1) age of the puppies; (2) clinical signs; (3) marked thrombocytopenia; and (4) pathologic findings. Pathologic changes in affected puppies were characteristic. Widespread hemorrhages were apparently related to the marked thrombocytopenia consistently observed in herpes-infected puppies. Necrotic and hemorrhagic lesions in the liver, lung, and kidney of dead puppies suggested this viral infection. Focal renal hemorrhages have not been reported in dogs infected with infectious canine hepatitis or distemper viruses. Occasional intranuclear inclusions were seen in recently infected cells adjacent to areas of necrosis. The virus caused characteristic CPE in 12 to 16 hours when grown in dog kidney cell or thymic cell tissue cultures.

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By

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Dedicated to my wife

Martha

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INTRODUCTION

Canine malignant lymphoma (CML) has attracted considerable attention because of its histopathologic similarity to Burkitt's lymphoma of man (Basherville et al., 1966; Bras et al., 1965; Lukes et al., 1966). Malignant lymphoma of the dog has been used by several investigators in transmission studies and in morphologic investigations at the ultrastructural level in an attempt to determine the etiologic agent and to compare or contrast the findings with those in leukemia of man.

Cellular transmission of lymphoma in domestic chickens and rodents has been accomplished with relative ease by inoculating cells into the same species. Many of the murine and avian strains of leukemia virus have been obtained from in vivo serial whole cell transmission studies (Eddy, 1964). Cellular transfer of lymphoma in domestic animals has, however, proved difficult. Recently, Moldovanu et al. (1966) reported cellular transfer of malignant lymphoma to x-irradiated mongrel puppies. The research described herein was undertaken in order to attempt to transmit CML to the Beagle neonate by inoculating cell suspensions and cell-free extracts prepared from dogs with spontaneous malignant lymphoma, and to attempt to isolate a causative agent(s).

In the foregoing report CML, which was found to be transmissible to the Beagle neonate in 2 serial passages, and a canine herpesvirus, designated as canine herpesvirus-Kakuk (CHV-K), was isolated from a dog with CML that was pathogenic for the germfree Beagle neonate. The clinical, hematologic, macroscopic, and microscopic findings of

experimentally induced CML and a septicemia in Beagle neonates produced by CHV-K are described herein.

REVIEW OF THE LITERATURE

Spontaneous Canine Malignant Lymphoma

History. Siedamgrotzky, in 1871, first reported lymphatic leukemia in a dog in which the lymph nodes and spleen were enlarged, and the ratio of white to red blood cells was 1 to 15. Soon after this, a number of authors described cases of malignant lymphoma, principally of the lymphatic type, in dogs (Bollinger, 1874; Cadiot, 1892; Stockmann, 1893; Olt, 1899). The literature on canine malignant lymphoma (CML) is voluminous and thus many references which are essentially case reports are omitted.

Definition. This is a highly fatal, malignant neoplasm characterized by the uncontrolled proliferation of neoplastic cells of the lymphoreticular system in almost any organ with a corresponding variety in clinical signs (Moulton, 1961; Smith, 1963).

Classification. The literature reports on malignant lymphoma in dogs were confusing because of the different names applied to the syndrome. The most common names used were malignant lymphoma, leukemia, lymphosarcoma, and leukosis, although many others, such as pseudo-Hodgkin's disease, aleukemic-leukemia, adenosarcoma, and reticulum cell sarcoma have been employed. Smith (1963) suggested that the term "leukemic neoplasia" be used as an inclusive term to cover: (1) those neoplastic diseases characterized by a great increase in the white blood cells, which was leukemia, (2) the few examples in which the disease is

recognized because of the presence of immature, anaplastic leukocytes in the blood stream, even though the total number of white blood cells were within or close to normal limits (subleukemia), and (3) those forms, the majority as far as the canine species was concerned, in which the disease is recognized because of tumorous masses of lymphocytic or reticular cells somewhere in the animal's body. However, Jarrett et al. (1966) favored the terms lymphosarcoma and/or leukemia. They thought lymphosarcoma was the most acceptable term because the basic pathologic process was a malignancy of lymphoid tissue. Yet the prefix would include the lymphocytic series but would not include by definition the reticulum cell or histiocytic cell sarcomas. Also, Jarrett et al. (1966) thought leukemia was an excellent descriptive term because it gave an indication of the prognosis; however, the aleukemic form is the most common type observed in the dog (Bloom and Meyer, 1945; Jarrett et al., 1966; Meier, 1957; Smith, 1963; Squire, 1964), and therefore, the term leukemia would not be descriptive.

In this study, the term malignant lymphoma was chosen because it best described the clinicopathologic picture in the dog. The term was coined by Gall and Mallory in 1942, who classified 618 cases of malignant lymphoma in man. This classification has been applied to malignant lymphoma in the dog by Bloom and Meyer (1945) and by Squire (1965), who did extensive cytologic studies on the disease. With this classification the neoplasms were divided into 4 main types: histiocytic, lymphocytic, plasmocytic, and Hodgkin's.

Clinical Findings

Incidence. According to Bloom and Meyer (1945), Meier (1957), and Moulton (1961) the incidence of malignant lymphoma in the dog population

varies between 0.1 and 0.3%. Recently, Dorn et al. (1966) did a population-at-risk survey in 2 counties in California over 2-1/2 years, and found that the average annual incidence was 0.024%. In contrast, Jarrett et al. (1966) reported a 1.3% incidence of CML in England, and Backgren (1965) reported an 0.013% incidence in Sweden.

Age. The peak incidence of CML was found to occur between 5 and 9 years (Bloom and Meyer, 1945; Dorn et al., 1967; Jarrett et al., 1966; Meier, 1957; Moulton, 1961; Van Pelt and Conner, 1968), with an over-all spread of 6 months to 15 years. The peak incidence was best shown by Priester (1967), who did an extensive statistical study involving 237 cases of CML in 3 regions of the United States: Midwest, South, and West Coast. The data clearly indicated that the peak incidence was between 4 and 9 years.

Sex. Some reports indicated a higher incidence of disease in male dogs (Bloom and Meyer, 1945; Irfan, 1961; Jennings, 1952; Priester [West Coast and Southern U.S.], 1967; Smith, 1963), whereas other reports indicated no sex differences (Dorn et al., 1967; Jarrett et al., 1966; Meier, 1957; Moulton, 1961; Priester [Midwest], 1967). One report indicated a higher incidence of this disease in females (Van Pelt and Conner, 1968).

Breed. Some investigators claimed that this neoplasm was more common in Scottish Terriers than could be accounted for by the population proportion of this breed (Bloom and Meyer, 1945; Mulligan, 1949; White, 1946). According to Moulton (1961), this claim was not confirmed in a large series of CML studied at the University of California. In Priester's

(1967) statistical studies on CML, he found that the relative risk was, in every instance, significantly higher for Boxers than for all other purebreds. He did, however, find a high risk of CML for English Pointers in the South but risks were considerably lower for other regions (7.5 compared to 1.1). Some reports indicated that the incidence of CML was higher in purebred than in crossbred dogs (Dorn et al., 1967; Priester, 1967). Priester claimed that the higher incidence could have been accounted for on a genetic basis and/or because purebred dogs were more commonly licensed and were probably given more medical attention.

Duration. In most instances, it was difficult to determine the exact duration because this was based on case histories provided by the owner. However, based on such information most reports indicated a duration ranging from 7 to 434 days, the mean being 99 days (Bloom and Meyer, 1945; Jarrett et al., 1966; Meier, 1957; Moulton, 1961). Jarrett et al. (1966) and Schalm (1966) had dogs with the disease that lived from 15 to 18 months after diagnosis.

Signs. Reports indicated that bilateral peripheral lymphadenopathy was the most common clinical sign (Bloom and Meyer, 1945; Meier, 1957; Moulton, 1961; Smith, 1963; Van Pelt and Conner, 1968). However, Jarrett et al. (1966), in their study of 122 cases, indicated that 50% of the dogs did not have peripheral lymphadenopathy; this was not in agreement with reports by other investigators, and thus this could be a unique finding of dogs with the neoplasm in England. Enlarged lymph nodes (3 to 10 times normal size) were smooth, painless, well defined, rather firm, relatively mobile, and rarely adherent to the overlying skin or adjacent tissues. It was reported that 40% of the spleens were

enlarged enough to be palpated, and about 20% of the tonsils were enlarged and protruded from their crypts. Splenic enlargements could be verified with radiographic examination.

Many clinical signs were nonspecific, such as inappetence, listlessness, depression, lethargy, vomiting, weight loss, and polydipsia. More characteristically due to lymphadenopathy, there usually was interference with function, including: dyspnea, gagging and choking, coughing and difficulty in swallowing, ascites, hydrothorax, and local or generalized edema.

Hematologic Findings. Reports on the hematologic findings of CML were rarely diagnostic unless possibly a subleukemic or leukemic blood picture was found. However, in the dog, as previously mentioned, malignant lymphoma was characteristically aleukemic. According to reports in the literature, anemia, neutrophilic leukocytosis and, at times, thrombocytopenia were most frequently seen in later stages of the disease (Bloom and Meyer, 1945; Irfan, 1961; Jennings, 1953; Meier, 1957; Schalm, 1966; Squire, 1964). It was suggested that anemia was due to bone marrow infiltration of neoplastic cells and to myelogenous hyperplasia (Bloom and Meyer, 1945; Irfan, 1961; Meier, 1957; Schalm, 1966). These investigators considered that marked absolute leukocytosis was attributed to tissue destruction, secondary infections, and toxicosis.

Pathologic Findings

Gross

Lymph nodes. Generalized involvement of both visceral and superficial lymph nodes was commonly reported with this neoplasm. According to most reports the visceral lymph nodes were affected as

frequently as the superficials, although one investigator (Jarrett et al., 1966), in a study of 122 cases of CML in England, claimed that either the visceral lymph nodes or superficial lymph nodes were enlarged, but not both. Bloom and Meyer (1945) and Cotchin (1954) indicated that the lymph nodes that were involved first were those of the throat and neck but mentioned that their location could make them more easily noticed. Lymph nodes involved in CML in order of frequency were: the mandibular, cervical, retropharyngeal, prescapular, mediastinal, mesenteries, sublumbar, axillary, inguinal, bronchial, tracheal, iliac, and popliteal (Moulton, 1961; Smith, 1963). Most of the literature reports indicated that the peripheral lymph nodes were frequently enlarged in dogs with malignant lymphoma, as compared to other domestic animals (Bloom and Meyer, 1945; Meier, 1957; Moulton, 1961; Smith, 1963; Squire, 1964).

The mesenteric and sublumbar lymph nodes were the largest of the visceral lymph nodes (Meier, 1957; Moulton, 1961; Smith, 1963; Squire, 1964). Often groups of lymph nodes coalesced (mesenteric, mediastinal) and formed enormous tumor masses. The cut surface was yellow, homogeneously gray, pink-gray or cream colored; usually moist, and had bulging cut surfaces. Some had necrotic centers, and others had hemorrhagic streaks. Demarcation between cortex and medulla was usually absent.

Spleen. Splenomegaly, either moderate or severe, was present in a majority of dogs. The follicles appeared more numerous and prominent due to enlargement. At times, follicles fused forming large protruding masses (Jarrett et al., 1966; Meier, 1957; Moulton, 1961; Smith, 1963).

Liver. Hepatomegaly was common and had either a diffuse mottled appearance or nodular masses protruding above the surface forming whitish-yellow tumorous masses (Meier, 1957; Moulton, 1961; Squire, 1964). Hepatic tissue was yellow and friable.

Other Organs. Grossly visible whitish-gray masses involved other organs, particularly the kidney and lung.

Microscopic. A review of the histopathologic changes was limited to the lymphocytic and reticulum cell types, since they were most commonly found (Dorn et al., 1967; Priester, 1967; Smith, 1963). However, as pointed out by Bloom and Meyer (1945), Meier (1957), and Squire (1965), classification of malignant lymphoma was best made by employing imprint techniques. Using this method, these investigators concluded that CML could be classified into 4 types: histiocytic (reticulum cell), lymphocytic (divided into prolymphocytic, lymphoblastic, and lymphocytic types), plasmocytic, and Hodgkin's.

Lymphocytic Type. According to literature reports, the lymphocytic type was 4 times more common than the reticulum cell type (Dorn et al., 1967; Priester, 1967; Smith, 1963).

In fresh imprints, the cell size ranged from 8 to 15 μ. Cells generally were round, but irregular shapes occurred. Basophilic cytoplasm, at times, was more abundant depending on the type of cell, and the nuclei were round or oval. Chromatin patterns were stippled or pachychromatic. Nucleoli were usually multiple and the number of mitotic figures varied; however, they were more numerous in the lymphoblastic and prolymphocytic types. In contrast, cells in paraffin sections were

round or oval, with round or irregular vesicular to hyperchromatic nuclei. The cytoplasm was more abundant in the lymphoblastic and prolymphocytic cell types, and stained slightly basophilic with occasional azurophilic granules.

Reticulum Cell Type. In imprints the cells were larger (15 to 25 u) than most lymphocytic cells. The cytoplasm was more abundant but less basophilic. The nuclei were large and most commonly irregular in shape. Chromatin existed as coarse, unevenly divided, violet particles which were distinct from the colorless parachromatic spaces. There was no clumping of the chromatin. Nuclei were pale blue spheres embedded in the chromatin-parachromatin network (Meier, 1957; Squire, 1965). In contrast, reticulum cells in paraffin sections were often larger and more pleomorphic than in the lymphocytic type. Cytoplasm was broad or indefinite and varied from slightly basophilic to acidophilic. Deposition of reticulum was often seen. The nuclei were large and frequently had folded, indented, or bizarre shapes. Nuclear membranes were distinct, chromatin was scarce, and nucleoli were evident and very large.

Some reports indicated that Hodgkin's disease did not occur in the dog (Feldman, 1932; Smith and Jones, 1966) and they classified it as an atypical reticulum cell sarcoma. Other reports indicated that Hodgkin's-like lesions did occur in the dog (Smith, 1963; Squire, 1965).

Organ Involvement. The frequency at which the organs were invaded by tumor cells based on histopathologic examination of dogs with malignant lymphoma has been reported (Dorn et al., 1967; Jarrett et al., 1966; Meier, 1957; Smith, 1963; Van Pelt and Conner, 1968). According to these reports, the lymph nodes, spleen, liver, kidney, lung, and bone marrow were most frequently affected. The architecture was disrupted

in most of the organs infiltrated; however, the lymph nodes were most severely affected. Extramedullary erythro- and myelopoiesis were often present, occurring particularly in the liver and spleen (Jarrett et al., 1966; Moulton, 1961; Meier, 1957; Squire, 1964). It was reported that this, along with myeloid hyperplasia in the bone marrow, contributed to the frequent neutrophilia (Meier, 1957; Squire, 1965; Schalm, 1966).

Diagnosis. Because of the wide range of clinical signs, a positive diagnosis of CML could be difficult. Since various infectious diseases caused enlargement of the lymph nodes in the dog, surgical biopsy with subsequent histopathologic examination provided the best means for a positive diagnosis (Jarrett et al., 1966; Meier, 1957; Moulton, 1961). In the absence of peripheral lymphadenopathy, exploratory laparotomy was the sole means of identifying the disease, and a biopsy of the neoplasm or lymph node permitted histopathologic verification (Jarrett et al., 1966). In patients with subleukemic or leukemic leukemia, the presence of anaplastic cells in the peripheral blood indicated a positive diagnosis (Schalm, 1966). Unfortunately, the neoplasm in the majority of dogs was extravascular (Moulton, 1961; Schalm, 1966; Meier, 1957; Squire, 1964).

Transplantable and Transmissible Canine Neoplasms

According to Shimkin (1955), Novinsky, a Russian veterinarian, was the first to successfully transplant tumors in animals. After 44 unsuccessful attempts, Novinsky, in 1877, transplanted a venereal sarcoma from an adult dog to a number of puppies. Thus, he is given credit for being "the father of transplantable tumors". Soon after this, Ellermann and Bang (1908) and Rous (1910) not only transmitted tumors with whole cells

but were also able to produce tumors with cell-free extracts from these tumors, thus opening the new field of viral oncology. Since this time, a number of viral agents have proved to be the cause of neoplasms in birds and mammals (Eddy, 1964).

Neoplasms known to be transplantable in the dog are presented in Table 1. It is seen that the papilloma (DeMonbreun and Goodpasture, 1932), venereal sarcoma (Karlson and Mann, 1952), anaplastic thyroid carcinoma (Allam et al., 1954, 1956), and mastocytoma (Lombard et al., 1963) have successfully been transmitted from dog to dog passage through 9 or more generations. In addition, there are a few reports of other canine tumors that were transplanted through 3 or less generations (Nielsen and Cole, 1961; Stewart et al., 1959). Of the transplantable tumors, the papilloma (DeMonbreun and Goodpasture, 1932; M'Fadyean and Hobday, 1898) and mastocytoma (Lombard et al., 1963) have been transmitted by cell-free filtrates.

Venereal Sarcoma. An extensive review of the literature concerning this tumor was given by DeMonbreun and Goodpasture in 1934. Numerous experiments have shown that the venereal sarcoma can only be transmitted with viable whole cells. In nature, the tumor is transmitted to the genitals by coitus or through wounds of the skin by contact. Experimentally, this tumor has been passed through 40 generations (Karlson and Mann, 1952) of dogs during a period of 17 years (Table 1). During passage, there was no change in the ability of the tumor to become established and no change in its histologic characteristics. The pathologic features of venereal sarcoma are well described by Moulton (1961) and Smith and Jones (1966).

Table 1. Canine neoplasms known to be transplantable and transmissible in the dog

Type of Neoplasm	Immuno-suppressants	Age inoculated (days)	Number injections	Passage generations	Latent[a] period (days)	Reference
Venereal sarcoma[b]	None	8-56	1	1	---	Novinsky, 1877 (Shimkin, 1955)
	None	puppies	1	40	21	Karlson & Mann, 1952
Papilloma[b,c]	None	42-365	1	2	28-42	M'Fadyean & Hobday, 1898
	None	puppies	1	10	30-33	DeMonbreun & Goodpasture, 1932
Chondro-osteo-sarcoma	None	puppies	1	2	21-25	McWhorter & Prime, 1919
Anaplastic thyroid carcinoma	150-500 r cortisone[d]	puppies	1	30	16	Allam et al., 1954, 1956
Ovarian adeno-carcinoma	600 r prednisolone	40	1	1	15	Nielsen & Cole, 1961
Mixed mammary tumor	600 r prednisolone	26-50	1	1	10-12	Nielsen & Cole, 1961
Osteosarcoma	500 r prednisolone	244	1	1	13	Nielsen & Cole, 1961
Mastocytoma[c]	None	neonate	1	9	14-150	Lombard et al., 1963
Lymphosarcoma	85.4-128 r	neonate	multiple	2	14-40	Moldovanu et al., 1966
Malignant lymphoma	60.8 r-1st none-2nd	neonate	1	2	50-80	Kakuk et al., 1968

[a]Injection to gross recognition.
[b]Venereal sarcoma and papilloma: once regression, immune to reinoculation.
[c]Papilloma and mastocytoma: only canine neoplasms transmitted with cell-free filtrates.
[d]Cortisone used only through 11th passage generation.

Oral Papilloma. This was the first tumor to be transmitted to the dog with cell-free extracts (M'Fadyean and Hobday, 1898), indicating a viral etiology. The incubation period of the experimental disease was between 28 and 42 days (Table 1). The virus multiplies only in the oral or pharyngeal mucosa of the dog, with no autotransplantation to extraoral sites (Moulton, 1961). Pathologic features of the oral papilloma are described by DeMonbreun and Goodpasture (1932).

Anaplastic Thyroid Carcinoma. Allam et al., in 1954 and 1956, serially transplanted a spontaneous canine thyroid carcinoma through 30 generations in puppies (Table 1). Immunosuppressants used were x-irradiation and cortisone. After the 11th serial passage, 9 passages were accomplished without pretreatment, with 61% of the inoculated dogs having tumors. In contrast, the x-irradiated group had a tumor incidence of 86%. Metastasis to the regional lymph nodes was more common in the irradiated group. Regression of tumor occurred frequently in both groups. Histopathologically, the tumors appeared similar through all serial passages.

Mast Cell Leukemia. Lombard et al., in 1963, serially transplanted to Beagle neonates a spontaneous mast cell leukemia through 9 generations using fresh tumor tissue in the first 7 and frozen tissue in the last 2 passages. Also, they were successful in transmitting mast cell leukemia with cell-free extracts through 3 generations. The latent period varied from 14 to 150 days, with a mean of 60 days. However, the latent period was longer for the first 3 passages as compared to subsequent passages. The histopathologic features of cellularly induced and cell-free induced tumors were similar. Widespread metastasis was common in

dogs with either whole cell or cell-free extract induced neoplasms. Hematologic examinations revealed large numbers of mast cells in the peripheral blood smears.

Malignant Lymphoma. Nielsen and Cole, in 1961, attempted homologous transplantation with 17 different canine neoplasms using high doses of x-irradiation and prednisolone as immunosuppressants. Of the 17 neoplasms attempted, 3 were successfully transplanted by 10 to 15 days postinoculation (Table 1). There were transplantation attempts with 9 reticulum cell sarcomas and 7 lymphomas. Therefore, they were unsuccessful in transferring canine malignant lymphoma by inoculation of cells into puppies.

In 1966, Moldovanu et al. reported transmission of malignant lymphoma to newborn mongrel puppies. Puppies were x-irradiated with 84.5 to 128 r of total body radiation immediately after birth. The puppies were inoculated with whole cells or cell-free filtrates subcutaneously in the nape of the neck and intramuscularly in the leg 10 to 24 hours after irradiation. Repeated inoculations were given at weekly intervals. Fresh cell suspensions were made from biopsy specimens obtained from dogs with spontaneous malignant lymphoma. After trypsinization, the trypan blue stained cells were counted. It was found that 65% of the cells remained viable. Cell filtrates were kept frozen at -80 C. during the interval between inoculations.

They found that most dogs had lymph node enlargement 26 to 58 days postinoculation. However, surgical biopsies of these nodes with subsequent histopathologic examination revealed lymphoid hyperplasia. Three dogs at 20, 26, and 40 days postinoculation had malignant lymphoma, although

they did not include any histopathologic verification of their findings. The second serial passage was based on a 14-day-old puppy that died of generalized malignant lymphoma 14 days postinoculation. Again, there was no histopathologic verification of malignant lymphoma presented. In the third passage attempt, all puppies died from a nondescript "intercurrent infection".

Herpesvirus Infection in the Dog

History. Carmichael and co-workers (1964) first described a fatal septicemic disease in infant puppies which they believed to be caused by a pleuropneumonia-like organism (PPLO). However, Stewart et al. (1965a) found that a virus was responsible for this hemorrhagic disease of puppies and that the virus belonged to the herpesvirus group based on morphologic characteristics. Later, Carmichael et al. (1965a) also found that the septicemic disease was caused by a herpes-like virus and that their tissue cultures were contaminated by PPLO.

Natural Disease. Carmichael and associates (1964 and 1965a) isolated a cytopathogenic agent with dog kidney cell (DKC) cultures from blood, lungs, livers, spleens, and kidneys of 3 Springer Spaniel puppies that died between 2 and 3 weeks of age. All the remainder of the litter of 12 also died. The only sign of illness had been continual crying that began 8 to 12 hours before death. The isolate was designated as strain F-205V, and was used for experimental studies. The aforementioned isolate was obtained from a New York kennel in 1961. Carmichael et al. (1964 and 1965a) obtained another isolate from puppies in Illinois in 1962, designated as strain A-1.

Stewart et al. (1965a) reported deaths and runted pups among 2 litters of "close to term" fetal pups obtained from apparently healthy bitches by cesarean section. In 1 litter, 2 of 8 pups were diseased. Three of 8 pups in the second litter were infected, as shown by virus isolation from their kidneys. Virus also was isolated from the kidneys of the apparently healthy littermate fetuses. This strain of virus has been designated as SL18HLV.

Distribution. Herpesvirus has been isolated from young puppies in 4 states and 1 District: New York, Illinois (Carmichael et al., 1964), Washington, D.C. (Stewart, 1965a), Georgia (Schwartz and Martin, 1966), and Michigan (Carter, personal communication, 1967). Recently, the virus has also been isolated from young puppies in the United Kingdom (Cornwell et al., 1966; Prydie et al., 1966). This apparently indicates that canine herpesvirus is widespread in the dog population.

Etiology and Properties of the Virus. The disease is caused by a herpesvirus which produces a cytopathic effect (CPE) in DKC cultures. Characterization and serological studies of the virus indicated that it was a new member of the herpesvirus group (Carmichael et al., 1965b; Spertzel et al., 1965). Electronmicroscopy studies by Strandberg and Carmichael (1965) indicated that virus particles in thin sections of dog kidney cells had an average diameter of 142 mμ. The particles contain a DNA core surrounded by 2 membranes. The protein coat was composed of 162 subunits, a characteristic shared by other herpesviruses. Carmichael and co-workers (1965b) found that the virus was inactivated by chloroform and ether, and was destroyed in less than 4 minutes at 56 C. Also, virus titers were maintained for months at -70 C. in virus stocks that

contained 10% serum. Infectivity was lost below pH 4.5 after 30 minutes. The virus was not related serologically to infectious canine hepatitis, distemper, infectious bovine rhinotracheitis, B-virus, equine rhinopneumonitis, avian laryngotracheitis, or herpes simplex viruses (Carmichael et al., 1965b; Spertzel et al., 1965; Stewart et al., 1965a).

Clinical Signs. Incubation period varies between 3 and 8 days in puppies inoculated by intranasal instillation or by intraperitoneal injection (Carmichael et al., 1965a). Route of inoculation and virus dose did not appear to be related to the time of onset of signs or severity of illness. To the present, the natural disease has been recognized only in puppies less than 1 month old. Fatal illness occurred in those less than 1 week of age, whereas puppies older than 2 weeks did not become manifestly ill following inoculation but developed neutralizing antibodies (Carmichael et al., 1964). Carmichael and associates found that signs in older dogs inoculated with virus were limited to mild rhinitis or vaginitis. Recently, Binn et al. (1967) recovered herpesviruses from 2 of 16 adult dogs with upper respiratory diseases by tissue culture means. Motohashi and Mosanari (1966) also reported isolating a herpesvirus from a "diseased adult dog"; however, they were unable to reproduce disease in young puppies with the agent.

Carmichael et al. (1964) and Stewart et al. (1965a) found that illness in puppies may start between the 5th and 18th days after birth. Principal signs included a soft yellowish-green, odorless stool, anorexia, labored breathing, abdominal pain, and crying. Carmichael and co-workers found that inoculated puppies generally appeared normal until 1 or 2 days before they died. After the onset of overt illness, they found death usually occurred between 24 and 48 hours.

Lesions. Pathologic findings have been described by Carmichael et al. (1965a) and Stewart et al. (1965a). They are characteristic: lesions in inoculated and naturally infected puppies consist of disseminated focal necrosis and hemorrhages. These lesions may be found in virtually all organs. Especially noteworthy changes occur in the kidneys, where subcapsular hemorrhages appear as bright red spots on a gray background of necrotic cortical tissue. The lungs are diffusely pneumonic. There is marked hyperemia, edema, and often there is froth in the air passages. Necrosis of alveolar walls with exudation of fibrinoid material in the alveolar spaces is a common finding. Some bronchial epithelial cells contain oval acidophilic intranuclear inclusion bodies. Focal necrosis and hemorrhages are also frequent in the liver, intestinal tract and adrenal glands. Spleens and lymph nodes are enlarged. Microscopic examination may reveal occasional cells in areas of necrosis with faintly acidophilic intranuclear inclusions. Such inclusions are not common. Inclusions are seen most commonly in sections of kidney, liver, and lung. Olander (1966) has reported encephalitis in puppies inoculated intracerebrally with canine herpesvirus.

Species Susceptibility. So far as is known, only dogs are susceptible, and fatal infections have been reported only in puppies less than 1 month of age. Carmichael et al. (1965) found that suckling and weanling mice, chick embryos, rabbits, ferrets, and cell cultures derived from a variety of species were refractory to infection. Mild rhinitis and vaginitis were the only signs of illness in older dogs inoculated with virus. There was no evidence that CHV is pathogenic for man.

Immunity. Only limited information was available concerning immunity to this viral disease. Limited serologic studies indicate that antibody to the virus is widely distributed in the dog population (Carmichael et al., 1965b; Spertzel et al., 1965). The author found that low neutralizing antibody titers developed in adult dogs inoculated with virus. The following findings on immunity were reported by Carmichael et al. (1965b): antibody levels reached maximal titers 4 to 5 weeks following intranasal or oral inoculation and gradually declined until they were no longer measurable after 6 months. They found that 2 bitches, whose naturally infected pups died, gave birth 1 year later to normal pups. Both bitches came from kennels in which the disease was known to be enzootic. Likewise, they found that when susceptible bitches were inoculated intravaginally with virus, they gave birth to puppies that all died within 2 weeks. Yet puppies from similarly inoculated bitches that had neutralizing antibody at the time of inoculation did not become ill. The puppies acquired maternal antibody and did not become ill following inoculation with virus at 1 week of age.

MATERIALS AND METHODS

Donor Dogs of Canine Malignant Lymphoma

Two dogs with spontaneous canine malignant lymphoma (10019 and 10074) were used as donors (Tables 2, 3 and 4). Since 1 of the dogs used had typical lesions of CML which are well documented in the literature and are cited in the review of the literature, a description of lesions will not be repeated. The other dog (10019) had the thymic type of malignant lymphoma, which is not commonly seen in the dog. The lesions for the donor dog with the thymic type of CML will be described herein.

The donor dog with thymic involvement was a 20-month-old, female, purebred Doberman Pinscher (10019, Table 2). Clinical history and signs included a sudden onset, dyspnea, anorexia, listlessness, and hydrothorax. Radiographs of the thoracic cavity revealed a tumorous mass. A hemogram indicated anemia and lowered platelet count, both of which have been common findings in over 65 spontaneous CML cases examined in this laboratory. In the thoracic cavity, there was approximately 200 ml. of fluid which contained numerous neoplastic lymphocytes. The tumor mass observed at necropsy was located bilaterally in the anterior half of the thoracic cavity. The tumor appeared to involve the thymus and anterior mediastinal lymph node and extended to the trachea and esophagus dorsally and the lungs posteriorly. Many of the superficial and deep body lymph nodes were enlarged and edematous. The liver and spleen were markedly enlarged.

Microscopically, the affected lymph nodes and anterior thoracic tumor mass consisted of a uniform distribution of neoplastic lymphocytes (Figure 1). The lymph node architecture was obliterated with proliferating lymphocytes. Neoplastic lymphoid cells were characterized by prominent, round to ovoid nuclei rich in chromatin, conspicuous cytoplasm, prominent nucleoli, and mitotic figures. Other organs affected with lymphocytic infiltration (Table 9) had a similar cell type as mentioned above with variable replacement of tissue parenchyma.

Experimental Animals

Experiment I. Conventionally raised purebred Beagle neonates of both sexes, varying in age from birth to 3 days, were used (Table 2). There were 3 serial passages of CML attempted, utilizing 3 litters of Beagle neonates (17 dogs). Using single doses of inoculum, 11 dogs were given viable whole cells and 4 were given cell-free extracts. Two were contact controls (Table 2). After weaning (5 weeks), they were transferred to isolation rooms operated under a barrier system and were maintained on a pathogen-free diet.[a]

Experiments II, III, IV, and V. Cesarean-derived, colostrum-deprived, germfree Beagle neonates of both sexes, varying in age from birth to 8 days, were used. Four litters of puppies were used in 4 transmission trials as outlined in Tables 3, 4 and 5. Experiment II involved the inoculation of 7 puppies with a fresh whole-cell suspension prepared

[a]Ralston Purina Co., Checkerboard Square, St. Louis, Mo.

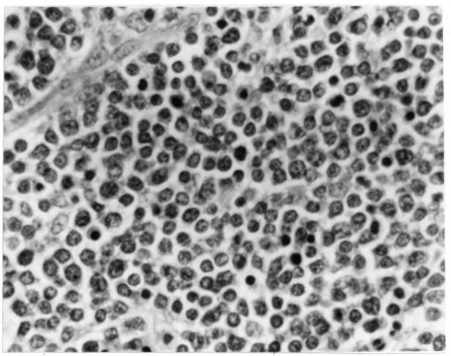

Figure 1. Iliac lymph node from initial donor dog (10019), a 20-month-old female Doberman Pinscher. Notice the monotonous uniformity of neoplastic lymphocytes. H & E stain. x 680.

Table 2. Experiment I. Serial passage of canine malignant lymphoma (CML) to 3 litters of Beagle neonates

Donor of CML	Passage generation	Recipients	Age Inoculated	Sex	Irradiated[a]	Tissues inoculated	Routes & amount[b]	Cell counts (cells/ml.)	Termination[c] (days)
10019	1	30030	Birth	F	Yes	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	54
		30031	Birth	M	Yes	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	
		30032	Birth	M	Yes	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	78
		30033	Birth	F	No	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	
		30034	Birth	F	No	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	
		30035	Birth	M	No	Thymus	IP 1.5 ml. SC 0.5 ml.	8.5×10^5	
30032	2	30086	3 days	M	No	Thymus	IP 1.5 ml. SC 0.5 ml.	9.2×10^5	
		30087	3 days	M	No	Thymic extract	IV 0.5 ml.		
		30088	3 days	F	No	Control			
		30089	3 days	M	No	Thymus	IP 1.5 ml. SC 0.5 ml.	9.2×10^5	

Table 2--continued

Donor of CML	Passage generation	Recipients	Age Inoculated	Sex	Irradiated[a]	Tissues inoculated	Routes & amount[b]	Cell counts (cells/ml.)	Termination[c] (days)
		30090	3 days	F	No	Thymic extract	IV 0.5 ml.		
30089	3	30142	2 days	M	Yes	Thymic extract	IV 3.5 ml.		
		30143	2 days	F	Yes	Thymic extract	IV 3.5 ml.		
		30144	2 days	M	Yes	Thymus & lymph node	IP 1.5 ml. SC 0.5 ml.	1.1×10^6	
		30145	2 days	F	No	Control			
		30146	2 days	M	No	Thymus & lymph node	IP 1.5 ml. SC 0.5 ml.	1.1×10^6	
		30147	2 days	M	No	Thymus & lymph node	IP 1.5 ml. SC 0.5 ml.	1.1×10^6	

[a]Total body irradiation: 140 Peak Kilovolts (PKV), 3 mm. Al (aluminum) HVL (half-value layer), 20 milliamperes (MA), 32 seconds (60.8r).

[b]IP - intraperitoneally, SC - subcutaneously, IV - intravenously

[c]Dogs 30030, 30032, and 30089 were killed; the other dogs are 2-1/2 years old and apparently healthy.

Table 3. Experiment II. Colostrum-deprived germfree Beagle neonates inoculated
prepared from lymph node from a dog with malignant

Table 3. Experiment II. Colostrum-deprived germfree Beagle neonates inoculated with a cell suspension prepared from lymph node from a dog with malignant lymphoma (ML)

Donor of ML	Recipients[a]	Age inoculated (days)	Sex	Material inoculated	Termination (days)
10074	30236	3	M	Popliteal lymph node	12 - killed
---	30237[b]	3	F	Contact control	10 - died
10074	30238	3	F	Popliteal lymph node	8 - died
10074	30241	3	M	Popliteal lymph node	13 - killed
10074	30242	3	F	Popliteal lymph node	8 - died
10074	30243	3	F	Popliteal lymph node	16 - killed
10074	30244	3	F	Popliteal lymph node	11 - died
10074	30245	3	M	Popliteal lymph node	12 - killed

[a]All inoculated recipients were given 2.5 ml. of inoculum, diluted 1:1 with physiologic saline by the following routes: 0.5 ml. intramuscularly, 0.5 ml. subcutaneously, and 1.5 ml. intraperitoneally.

[b]30237 - The contact control was inoculated with 2.5 ml. physiologic saline and had the same lesions as recipients of experimental inoculum.

Table 4. Experiment III. Colostrum-deprived germfree Beagle neonates inoculated with a stored cell suspension prepared from lymph node of a dog with malignant lymphoma (ML)

Donor of ML	Recipients[a]	Age inoculated (days)	Sex	Material inoculated[c]	Termination (days)
10074	30270	1	M	Popliteal lymph node	8 - killed
10074	30271	1	F	Popliteal lymph node	8 - killed
10074	30272	1	F	Popliteal lymph node	6 - killed
---	30273[b]	1	F	Non-contact control	255 - alive
10074	30276	1	M	Popliteal lymph node	7 - killed
---	30277[b]	1	M	Non-contact control	7 - killed

[a]All inoculated recipients were given 2.5 ml. inoculum, diluted 1:1 with physiologic saline by the following routes: 0.5 ml. intramuscularly, 0.5 ml. subcutaneously, and 1.5 ml. intraperitoneally.

[b]30273 and 30277 - Non-contact controls were reared in a separate isolator from recipients of experimental inoculum and were inoculated with 2.5 ml. of physiologic saline via the aforementioned routes.

[c]Material was stored at -70 C.

Table 5. Experiments IV and V. Colostrum-deprived gremfree Beagle neonates inoculated with cellular and cell-free extracts prepared from tissues of canine herpesvirus (CHV) infected dogs

Experiment	Donor of CHV	Recipients[a]	Age inoculated (days)	Sex	Material[d,e] inoculated	Termination (days)
IV	30242	30251	1	M	Cellular extract	295 - alive
	30242	30253	1	M	Cellular extract	7 - killed
	30241	30261	1	M	Cellular extract	6 - killed
	---	30262[b]	1	M	Contact control	10 - died
	30241	30263	1	M	Cellular extract	7 - killed
	30241	30265	1	F	Cellular extract	6 - died
V	30271	30246	8	M	Cell-free extract	171 - alive
	30245	30247	8	M	Cell-free extract	171 - alive
	30245	30248	8	M	Cell-free extract	6 - died
	30245	30249	8	M	Cell-free extract	171 - alive
	---	30250[c]	8	M	Non-contact control	171 - alive

[a]All recipients were given 2.5 ml. of inoculum, diluted 1:1 with physiologic saline by the following routes: 0.5 ml. intramuscularly, 0.5 ml. subcutaneously, and 1.5 ml. intraperitoneally.

[b]30262 - Contact control inoculated with 2.5 ml. physiologic saline and died from herpesvirus septicemia.

[c]30250 - Non-contact control inoculated with 2.5 ml. physiologic saline; remained healthy.

[d]Cellular extract prepared from homogenates of kidney, spleen, lymph node, and liver.

[e]Cell-free extract prepared from kidney and spleen.

from a dog with malignant lymphoma, and 1 was a contact control. Experiment III was a duplication of Experiment II, except that 2 puppies were used as non-contact controls and the inoculum for the 4 recipients was not a fresh cell preparation but was stored in a freezer[a] at -70 C. (Table 4). In Experiment IV, puppies were recipients of a cellular extract prepared from puppies in which canine herpesvirus-Kakuk (CHV-K) was isolated. For this experiment, 4, 1-day-old puppies were inoculated and 1 was used as a contact control (Table 5). In Experiment V, 4, 8-day-old puppies were inoculated with CHV-K, while 1 puppy was a non-contact control (Table 5). Each inoculated puppy was given 2.5 ml. of inoculum diluted 1:1 with physiologic saline by the following routes: 0.5 ml. intramuscularly, 0.5 ml. subcutaneously, and 1.5 ml. intraperitoneally. All control puppies (contact and non-contact) were injected with 2.5 ml. of physiologic saline via the aforementioned routes.

Rearing of Germfree Puppies

The germfree puppies were reared according to the methods described by Griesemer and Gibson (1963), which involves the feeding of a sterile diet[b] every 4 hours with a baby bottle. After weaning, they were transferred to an isolation room and maintained on a pathogen-free diet, as previously mentioned. Also, oral and fecal swabs were taken periodically to insure that a bacteria-free environment existed. No bacteria were isolated from puppies while maintained in germfree isolators.

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[a]Revco, Inc., Industrial Products Div., Deerfield, Mich.

[b]Varamel, Baker Laboratories, Inc., Cleveland 15, Ohio

Transmission Technic

Cell suspensions were prepared from thymus for the first and second serial passages and thymus and mesenteric lymph nodes for the third passage (Experiment I, Table 2). After mincing, the tissue was homogenized in a homogenizer[a] at a moderate speed with equal volumes of sterile physiologic saline solution at 5 C. Homogenization was done at time intervals which totaled a period of 1 hour. The cells were sedimented by centrifugation at 1000 rpm, resuspended in physiologic saline, and counted in a hemacytometer. Preparations of whole cells for other experiments (II and III) were carried out in a similar manner. Cell-free extracts were prepared according to the method of Moloney (1953). The amount of material inoculated, route, pretreatment, number of cells, and tissues inoculated are presented in Tables 2, 3, 4 and 5.

Tissue Culture Procedure

The tissue culture procedure described by Mitchell *et al.* (1967) was followed. This procedure employed the use of primary dog kidney cultures and canine thymus. The nutrient and maintenance media used, number of cells used per tissue culture bottle, and inoculation procedures are described elsewhere (Carmichael *et al.*, 1965b; Mitchell *et al.*, 1967; Spertzel *et al.*, 1965). Virus isolation and identification were done in dog kidney cell cultures as described by Carmichael *et al.* (1965b), Mitchell *et al.* (1967) and Spertzel *et al.* (1965).

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[a]VirTis Model-45, VirTis Research Equipment Co., Gardiner, N.Y.

Fecal Examination

Using a qualitative sugar concentration method (Benbrook and Sloss, 1961), fecal samples were examined at periodic intervals for parasitic ova. Those dogs infested with hookworms, ascarids, and Isospora bigemina were treated with disophenol,[a] piperazine citrate,[b] and nitrofurazone,[c] respectively, according to recommended dosages.

Hematology

Blood samples for hematologic procedures were taken by jugular venipuncture at various daily intervals as noted in Appendices 1, 2, 3, 4, 5, 6, 7, and 8. Blood was collected in vials containing 0.05 ml. of a 30% aqueous solution of tripotassium ethylenediametetraacetate monohydrate;[d] hemoglobin content (Hb.) was determined by the cyanmethemoglobin method; packed cell volume (PCV) was determined by using microhematocrit tubes. Total erythrocytic and leukocytic counts were made with an electronic counter.[e] Platelet counts were obtained with a hemacytometer.[f] Differential leukocytic counts were made by observing 100 cells in a blood smear stained with Wright's stain.[g]

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[a]D.N.P., American Cyanamid Co., Princeton, N.J.

[b]Pipcide, Haver-Lockhart Laboratories, Kansas City, Mo.

[c]Furadex, Eaton Laboratories, Div. of Norwich Pharmicals Co., Norwich, N.Y.

[d]B-D Vacutainer, Becton, Dickinson and Co., Rutherford, N.Y.

[e]Coulter Counter, Model B, Coulter Electronics, Hialeah, Fla.

[f]B-D Unopette Method, Becton, Dickinson and Co., Rutherford, N.J.

[g]National Aniline Division, Allied Chemical Corp., New York

Pathology

Gross Procedures. Lymph node specimens were obtained by surgical biopsy of enlarged lymph nodes where a diagnosis was warranted. The dogs that died and those that were tranquilized with promazine hydrochloride,[a] and subsequently exsanguinated by cardiac puncture, were subjected to a gross pathologic examination.

Microscopic Procedures. Representative specimens were selected and immediately fixed in 10% buffered formalin, Bouin's solution, and Zenker's solution. All specimens were paraffin[b] embedded, sectioned at 6 μ, and stained with Harris' hematoxylin and eosin Y (H & E). Selected specimens were subjected to special stains following the procedures as outlined in the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics (1960).

Ultrastructure

Electronmicroscopy Procedures. Specimens of spleen, liver, lung, lymph node, kidney, and thymus were prepared for ultrastructural studies as described by Strandberg and Carmichael (1965). This technic employs the use of glutaraldehyde-osmic acid fixation and uranyl-acetate, lead-hydroxide staining.

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[a] Sparine, Wyeth Laboratories, Inc., Philadelphia, Pa.

[b] Paraplast, Scientific Products, Evanston, Ill.

EXPERIMENTAL TRANSMISSION OF CANINE MALIGNANT LYMPHOMA

TO THE BEAGLE NEONATE

EXPERIMENT I

RESULTS

Canine malignant lymphoma developed in 3 of 11 Beagle neonates inoculated between 1 and 3 days of age with the cell suspensions at 54 and 78 days postinoculation in the first passage and 53 days in the second passage. Two of the 3 dogs with malignant lymphoma were pre-irradiated, whereas 1 dog was not irradiated. In no case was CML transmitted using cell-free extracts. Even though CML did not occur in the third serial passage, dogs inoculated with either a cell suspension or a cell-free extract had enlargement of the superficial lymph nodes. Surgical biopsies of these enlarged lymph nodes with subsequent histologic examination revealed lymphocytic hyperplasia but not malignant lymphoma.

Clinical Signs. Clinical signs included sudden onset with listlessness, extreme weakness, loss of weight, inappetence, diarrhea, dyspnea, and distention of the abdomen. Radiographs revealed an opaque mass in the anterior mediastinal region and a fluid line in the abdominal cavity. Paracentesis disclosed fluid in the peritoneal cavity which contained anaplastic lymphoid cells. Masses were palpable in the abdominal cavity. In Dogs 30030 and 30032, there were nodular masses in the abdominal and thoracic musculature and subcutaneous tissues. In Dog 30032, the super-

ficial lymph nodes were somewhat enlarged, whereas in Dogs 30030 and 30089, the superficial lymph nodes were normal in size. Enlarged lymph nodes were also observed in dogs inoculated with either cellular suspensions or cell-free extracts; however, malignant lymphoma did not develop in these dogs (Table 6).

Hematology. Results of hematologic examinations are given in Appendices 1, 2, 3 and 4 and Tables 7 and 8. It was found that 2 of the 3 dogs with malignant lymphoma had anemia, all 3 had a left shift, and all 3 had a markedly lowered platelet count (Tables 7 and 8), as compared to the control. One dog (30030) had an absolute lymphophilia (leukemia), and between 41 and 52 days, all 3 dogs had atypical neoplastic lymphocytic cells with retained nucleoli in the peripheral blood.

Pathology

Gross. Experimentally transmitted CML involved primarily the organs and lymph nodes of the thoracic and abdominal cavities. Typically, the thymus and mediastinal lymph nodes were so greatly enlarged that the heart was displaced posteriorly. In the abdominal cavity the liver was markedly enlarged, friable, and had discrete whitish nodules or diffuse mottling; the lymph nodes were extensively enlarged and had a homogeneous whitish color throughout (Figure 2); the pancreas had a soft texture and resembled lymphatic tissue; and the omentum was diffusely thickened (Figures 3 and 4). There was infiltration of grossly visible neoplastic tissue through the intercostal and flank muscles to the subcutaneous tissue of the body (Figure 5) and, in 1 dog, into the skin.

Table 6. Transmissibility of malignant lymphoma (ML) following serial passage of cell suspensions and cell-free extracts into Beagle neonates

Donor of ML	Passage[a] generations	Total no. of dogs	No. of animals with ML	Lymph node[b] enlargement
10019	1	6	2/6	4/6
30032	2	5	1/4	2/4
30089	3	6	0/5	5/5

[a]In passage generations 2 and 3, 1 puppy was set aside for uninoculated controls.

[b]There was transient lymphadenopathy in the dogs that did not have ML.

Table 7. Pre- and postinoculation sequential hemogram values taken from Appendices 1 and 2 on Dogs 30030 and 30032; representing the 1st serial passage of induced malignant lymphoma

Day	Corrected WBC/cmm. blood	Platelets /cmm.	Erythrocytic Series RBC x 10^6/cmm.	PCV (%)	Hb. (Gm.%)	MCV (cu.μ)	MCHC (%)	Absolute Lymphocytic Counts Monocytes	Lymphocytes	Neutrophils	Eosinophils
						Dog 30030					
0[a]	23,000	280,000	5.03	45.0	15.0	90	33	692	3,115	18,226	1,038
21[b]	15,756	463,000	3.93	34.5	10.9	88	31	867	7,642	7,090	158
41[c]	15,268	439,000	3.86	27.0	8.8	70	33	1,374	3,970	9,924	0
52	24,410	260,000	3.89	28.2	8.9	73	32	610	15,256	8,544	0
54	13,054	80,000	3.58	26.8	8.4	75	31	914	10,378	1,697	65
						Dog 30032					
0[a]	21,802	390,000	5.20	46.0	15.8	89	34	1,090	1,417	19,186	109
21[b]	12,019	290,000	3.59	34.0	10.8	95	32	961	4,988	5,950	120
41	12,727	320,000	3.99	30.5	10.0	76	33	764	4,391	7,318	254
52[c]	16,484	275,000	3.96	31.0	10.1	78	33	1,154	3,297	11,539	494
61	14,356	545,000	4.19	33.0	11.3	79	34	431	6,747	7,034	144
70	9,922	75,000	4.26	36.0	11.6	85	32	446	2,430	6,945	99
78	5,971	100,000	5.30	38.5	13.1	73	34	239	2,388	3,344	0

[a]Preinoculation blood sample.

[b]Both dogs had moderate Isospora bigimena infestations between 15 and 21 days.

[c]At 41 (30030) and 52 days (30032), respectively, there were atypical lymphocytic cells with retained nucleoli in the peripheral blood.

Table 8. Pre- and postinoculation sequential hemogram values taken from Appendices 3 and 4 on Dog 30089; representing the 2nd serial passage of induced malignant lymphoma and control dog 30088

	Corrected		Erythrocytic Series					Absolute Lymphocytic Counts			
Day	WBC/cmm. blood	Platelets /cmm.	RBC x 10^6/cmm.	PCV (%)	Hb. (Gm.%)	MCV (cu.μ)	MCHC (%)	Monocytes	Lymphocytes	Neutrophils	Eosinophils
					Dog 30089						
0[a]	25,250	285,000	4.57	45.0	15.1	99	34	1,515	5,934	15,781	2,020
21[b]	14,941	395,000	2.86	30.0	8.9	105	30	523	5,155	8,367	896
39[c]	14,256	460,000	3.93	29.0	9.4	71	32	713	1,782	11,761	0
49	9,285	157,000	2.54	20.5	6.6	81	32	279	1,857	7,103	46
53	6,070	126,000	2.31	16.5	4.5	71	27	0	3,703	2,337	30
					Control Dog 30088						
0[a]	14,224	220,000	4.28	44.0	14.6	103	33	284	5,050	6,614	2,276
21[b]	15,096	450,000	2.35	22.5	6.8	96	30	604	8,001	5,586	905
39	14,400	568,000	4.21	28.5	8.8	68	31	648	4,896	8,568	288
53	12,250	269,000	4.09	30.5	9.4	75	31	490	5,206	6,554	0
71	12,750	390,000	4.20	32.3	10.3	77	31	225	3,889	8,479	127
82	15,200	425,000	4.34	35.0	11.4	81	33	456	4,864	9,576	304

[a]Preinoculation blood sample.

[b]Both dogs had moderate hookworm infestations between 13 and 21 days.

[c]At 39 days postinoculation, there were atypical lymphocytic cells with retained nucleoli in the peripheral blood.

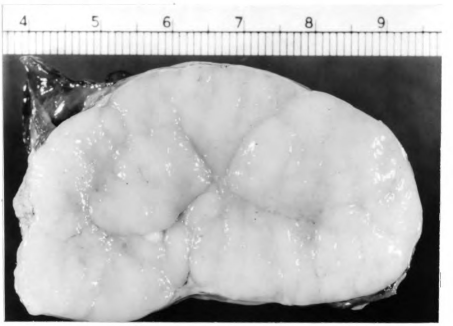


Figure 2. Mesenteric lymph node from Dog 30089. Notice the lack of demarcation between cortex and medulla, and the homogeneous whitish color.

Figure 3. First passage of cellularly induced malignant lymphoma in Dog 30032. At birth the dog was pretreated with 60.8 r total body irradiation. It was then inoculated with a cell suspension prepared from Donor 10019 (Figure 1). (A) Liver, notice the nodular areas which protrude above the surface and the enlarged hepatic lymph nodes. (B) Kidney and enlarged renal lymph node. (C) Markedly enlarged mesenteric lymph node. (D) Pancreas.

Figure 4. Dog 30089, representing the second serial passage of malignant lymphoma induced by inoculating a cell suspension prepared from 30032 (Figures 3 and 5). Subject was inoculated at 2 days of age without radiation pretreatment and was killed when 53 days old. Notice the enlarged liver (A) and the thickening of the central veins and portal triads (arrow). (B) Kidney and renal lymph node. (C) Mesenteric lymph node. (D) Portion of thymus. (E) Portion of omentum.

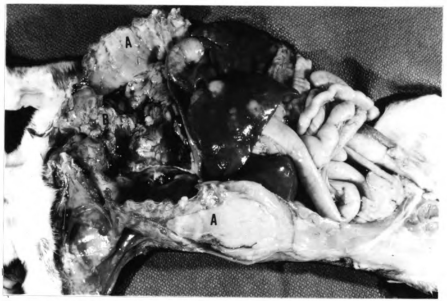


Figure 5. Dog 30032. Notice neoplastic infiltration into subcutaneous tissues and intercostal muscles (A) and the markedly enlarged thymus (B).

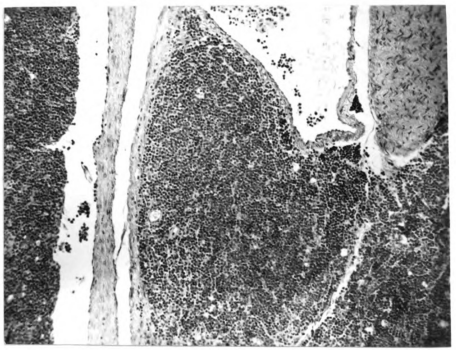


Figure 6. Mesenteric lymph node obtained from Dog 30089. Notice the invasion and proliferation of lymphocytic cells in the perinodal tissue. H & E stain. x 130.

Microscopic. The organ and tissue distributions of lymphocytic infiltration in the donor dog and the 3 experimentally induced malignant lymphoma dogs are presented and compared in Table 9. Although the superficial lymph nodes were not affected grossly in Dogs 30030 and 30089, they were affected microscopically. Superficial lymph nodes of Dogs 30030 and 30089, which did not appear enlarged grossly, had diffuse proliferation of lymphocytic cells that disrupted the architecture of the germinal centers, filled the sinusoids, and infiltrated the capsule. Enlarged superficial, thoracic, and visceral lymph nodes consisted of lymphocytic cells of monotonous uniformity (Figures 6 and 7). In many instances, the cells had invaded the nodal capsule resulting in complete obliteration of normal architecture (Figure 6). The nodes consisted of continuous sheets of neoplastic lymphocytes (Figure 7) without demarcation between cortex and medulla. Vessels, nerves, and surrounding perinodal fat were diffusely infiltrated with neoplastic lymphocytes. Lymphocytic infiltration in the capsular and perinodal areas was so profuse that circulation was disturbed to a point of thrombus formation. Lymphocytic cell populations invading the capsule and replacing normal histologic structures were substantial evidence of malignancy. Mitotic figures were observed in all parts of the node and atypical mitotic figures were often present.

Infiltrating neoplastic lymphocytes in the thymus were morphologically similar to those described for the lymph nodes. There was, however, such an extensive proliferation of neoplastic lymphocytes that Hassall's corpuscles (Figure 8) and interstitial connective tissue were invaded, resulting in architectural obliteration. In the liver the lymphocytic

Table 9. Organ and tissue distribution of lymphocytic infiltration demonstrated by histologic examination

Dog No.	Lymph nodes[a]				Thymus	Liver	Lung	Kidney	Spleen	Bone marrow	GI tract[b]	Other tissues[c]
	Head	Peripheral	Abdominal	Thoracic								
10019[d]	+	+	+	+	+	+	+	+	+	+	-	-
30030	+	+	+	+	+	+	+	+	+	+	+	+
30032	+	+	+	+	+	+	+	+	+	+	+	+
30089	+	+	+	+	+	+	+	+	+	+	+	+

[a]Head - mandibular, retropharyngeal, cervical
Peripheral - prescapular, axillary, popliteal, external iliac
Abdominal - hepatic, renal, mesenteric, sublumbar, internal iliac, iliocecocolic
Thoracic - mediastinal, bronchial, tracheal

[b]GI tract - stomach, intestine, pancreas, omentum, mesentery

[c]Other tissues - muscle, skin

[d]Donor of spontaneous malignant lymphoma

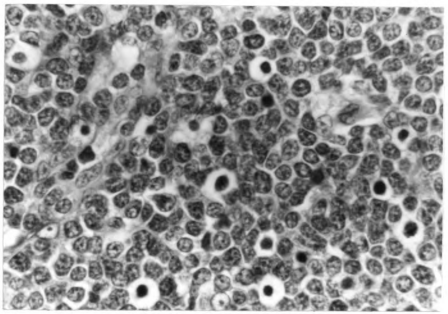


Figure 7. Higher magnification of Figure 4. Notice monotonous distribution of lymphocytic cells. H & E stain. x 680.

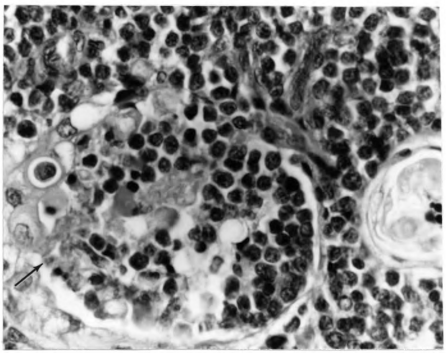


Figure 8. Thymus. Malignant lymphoma. Hassall's corpuscle (arrow) which was invaded by neoplastic lymphocytes. H & E stain. x 680.

proliferation was usually limited to the periportal areas (Figure 9) and, in some instances, there was destruction of hepatic tissue (Figure 10). Widespread infiltration was observed in the pancreas, which also resulted in extensive destruction of its parenchyma (Figure 11). Infiltration of lymphocytic cells in the kidney was confined to the cortex (Figure 12), particularly near the corticomedullary junction. The spleen was not markedly enlarged, yet the capsule and the sinusoids contained extremely anaplastic cells which resembled reticulum cells (Figure 13). Many intercostal muscle fibers were destroyed and replaced by infiltrating lymphocytic cells (Figure 14). Other organs and tissues were also infiltrated with neoplastic lymphocytes (Table 9).

DISCUSSION

This investigation demonstrated unequivocally that cellular induced malignant lymphoma is possible in the dog without pretreatment of total body radiation. In Experiment I, CML developed in 3 of 15 attempts in 53 to 78 days after inoculation using a single dose of inoculum. A previous report by Moldovanu et al. (1966) indicated that cell-induced CML can be accomplished by inoculating irradiated mongrel newborn puppies repeatedly.

Results of Experiment I indicated that lymphadenopathy was most frequent in dogs of the third serial passage, yet based on clinical examination and subsequent biopsied lymph node specimens, CML was not present.

In agreement with the results, Moldovanu et al. (1966) found that dogs inoculated with either cell-free extracts or CML cell suspensions developed lymphadenopathy. It is well known that, rather than being a pathognomonic indication of early CML, lymphadenopathy may be initiated

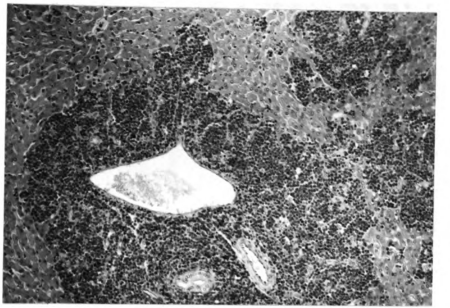


Figure 9. Liver from Dog 30089. Accumulation of neoplastic lymphocytic cells at portal triad. H & E stain. x 130.

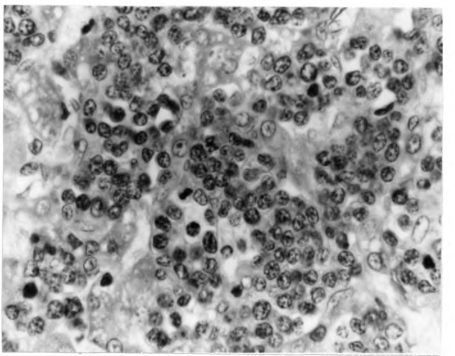


Figure 10. Liver from Dog 30032. Notice massive lymphocytic cell infiltration; only a few hepatic cells remain. H & E stain. x 680.

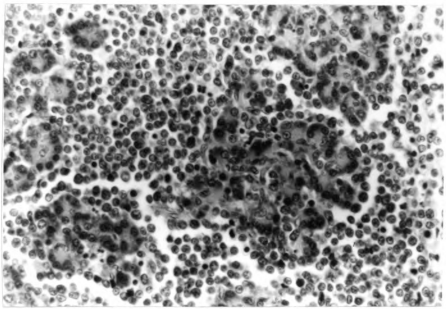


Figure 11. Pancreas. Malignant lymphoma. Overwhelming replacement of pancreatic parenchyma by neoplastic lymphocytes. H & E stain. x 375.

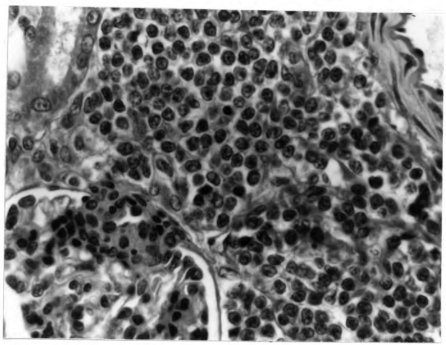


Figure 12. Massive accumulation of neoplastic lymphocytes in interstitial tissue of renal cortex. H & E stain. x 680.

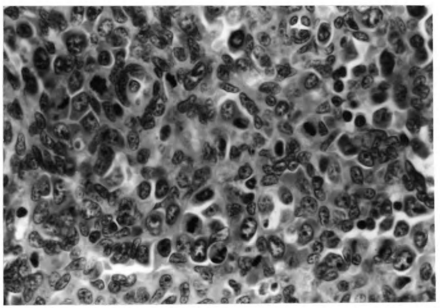


Figure 13. Spleen. Malignant lymphoma resembling the reticulum cell type. Notice the degree of undifferentiation. H & E stain. x 680.

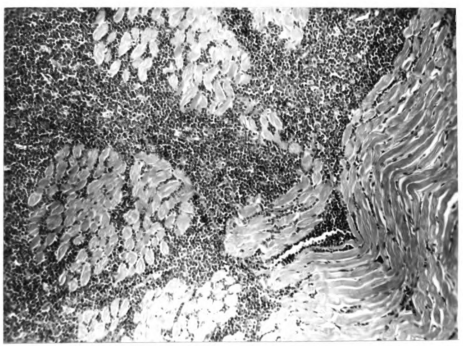


Figure 14. Section taken from intercostal muscles of Dog 30089. Invading lymphocytic cells have proliferated and replaced many muscle fibers. H & E stain. x 130.

by several infectious agents. Since Moldovanu et al. (1966) reported difficulty with "intercurrent" infection, it is possible that much of the lymph node enlargement they observed was related to an infectious agent. With the aid of a barrier system for housing dogs, distemper and infectious hepatitis have been controlled.

There are 2 possible explanations for the development of cellularly induced CML: (1) the cells inoculated could have multiplied and metastasized, and hence acted similarly to an in vivo tissue culture system, or (2) an agent could have been associated with the inoculated cells which, while multiplying, supported the replication of the agent, thus resulting in more cell transformation and multifocal malignant lymphoma. An argument against filterable agents having produced CML in these transmissions is that cell-free extracts failed to induce lymphoma, yet all 3 dogs with malignant lymphoma had numerous intracytoplasmic crystalline structures (Kakuk et al., 1968), which have been associated with Rous sarcoma virus induced tumors (Monroe et al., 1964; Rabotti et al., 1966), and in leukemia of man (Dmochowski et al., 1967). Crystals were more numerous in experimental CML than in spontaneous CML. Also, the latent period (50 to 80 days) which malignant lymphoma manifested in these transmissions, along with the inability to obtain localized growth at the inoculation site, may rule out simple transplantation as previously described (Marshak et al., 1967; Nielsen and Cole, 1961; Stewart et al., 1959).

Cell-free transmitted malignant lymphoma in the cat has been demonstrated (Jarrett et al., 1964b; Kawakami et al., 1967); also, recently the Cornell group (Richard et al., 1968, unpublished data) has induced ML in kittens inoculated with cell-free filtrates. Jarrett et al.

(1964a,b) has demonstrated, by electronmicroscopy, virus-like particles in the tissues of cats with lymphoma. Recently, Kawakami and co-workers (1967) identified C-type viral particles associated with cell-free transmitted ML of cats, whose density was similar to those associated with the mouse leukemias. Although cell-free extracts have not transmitted lymphoma in the dog, the presence of C-type virus-like particles in naturally occurring CML suggests a viral etiology (Chapman et al., 1967). It has been observed by electronmicroscopy that the number of viral particles is greater in feline malignant lymphoma than in CML and thus this might explain the relative ease in transmitting lymphoma to cats with cell-free extracts. Therefore, it is possible that inoculated cells could temporarily grow in the recipient, thus giving a viral agent a chance to mature and replicate, infect, and transform more cells, which then may result in a widespread malignancy. Temporary growth of the cells and replication of an agent could explain the transient lymphadenopathy noticed 25 to 45 days postinoculation. Biopsy specimens from dogs having transient enlargement of lymph nodes revealed lymphocytic hyperplasia but not lymphoma.

It has been reported that, in spontaneous CML, lymphadenopathy was most frequent in the superficial and mesenteric lymph nodes (Bloom and Meyer, 1945; Jarrett et al., 1966; Moulton, 1961; Smith, 1963). The results indicate, however, that lymph nodes that appear normal in size may contain neoplastic cells (Table 9). The 20-month-old donor dog (10019) had neoplastic involvement of the thymus, which has been a rare finding in spontaneous CML. This could be related to age since, in most dogs, lymphoma occurs between 4 and 9 years of age (Bloom and Meyer, 1945; Dorn et al., 1967; Meier, 1957; Moulton, 1961; Priester, 1967).

Results indicated that in transmitted CML the thymus, visceral organs, thoracic lymph nodes, and abdominal body organs were involved in all 3 dogs. Thus, the experimentally induced CML pathologically simulates spontaneous lymphoma of the cat, which is predominantly a visceral form with little or no peripheral lymphadenopathy (Holzworth and Nielsen, 1955; Nielsen and Holzworth, 1953). The thymic involvement in experimental lymphoma of the dog also resembles the thymic form described for the cat.

Hematologic results indicate that Dog 30030 had a leukemic leukemia, and Dogs 30032 and 30089 had a subleukemic leukemia based on the classification devised by Dameshek and Gunz (1958). However, in spontaneous CML, the disease is most commonly an aleukemic leukemia (Bloom and Meyer, 1945; Smith, 1963; Schalm, 1966) or, better described as an extravascular neoplasm. In agreement with literature reports on CML (Bloom and Meyer, 1945; Irfan, 1961; Schalm, 1966; Meier, 1957; Jennings, 1955) anemia, neutrophilic left shift, and lowered platelet counts are probably related to the massive infiltration of neoplastic lymphocytes into the bone marrow, necrosis, and myeloid hyperplasia.

Several investigators have reported close histopathologic similarity of spontaneous CML to that of Burkitt's lymphoma (Basherville et al., 1966; Bras et al., 1965; Lukes et al., 1966). This has been based on the histologic "starry-sky" pattern, a nonspecific phenomenon which has been observed in spontaneous malignant lymphoma of dogs (Basherville et al., 1966; Bras et al., 1965; Lukes et al., 1966; Smith, 1963), cattle (Smith, 1965) and cats (Squire, 1966).

A CANINE HERPESVIRUS ISOLATED FROM A DOG WITH CANINE MALIGNANT LYMPHOMA PATHOLOGIC FOR THE BEAGLE NEONATE

EXPERIMENTS II, III, IV, V

RESULTS

A herpes-like virus was isolated from Beagle neonates inoculated with popliteal lymph node material prepared from a dog with spontaneous malignant lymphoma. Because tissue culture and virus isolation results, clinical signs, hematologic findings and pathologic lesions were so similar in these 4 experiments, they are described together.

A total of 25 germfree puppies between 1 and 8 days old were used in 4 experiments (Tables 3, 4 and 5): 20 inoculated, 2 contact controls, and 3 non-contact controls. All dogs except 1 (30251, Table 5), inoculated between 1 and 3 days of age, died or were killed between 6 and 16 days postinoculation. Both contact control dogs died with herpetic septicemia, and the 3 non-contact controls remained healthy. Only 1 dog died (30048, Table 5) when inoculated after 8 days of age; the others had clinical manifestations of disease but survived.

Tissue Culture Findings

Herpes-infected Puppies. An agent cytopathogenic for primary dog kidney cultures was isolated from the lungs, livers, spleens, lymph nodes, and kidneys of dogs having the septicemic disease. The transmissible agent, based on serum neutralization tests, was designated as a strain of canine herpesvirus (Canine herpesvirus-Kakuk: CHV-K). Cytopathic

effects (CPE) were noticed 16 to 36 hours following preparation of the tissue cultures. The agent produced a focal type of CPE with rounding of the cells and swelling of the nuclei. The agent could be readily transmitted to dog kidney cell cultures or dog thymic tissue cultures but did not produce CPE or multiply in human embryo kidney.

Donor Dog 10074. In primary dog kidney cell cultures, CHV-K was isolated from a section of kidney stored at -70 C. Donor 10074 was the dog in which the prepared cell suspensions produced a septicemic disease in germfree Beagle neonates (Tables 3 and 4); thus, it was the origin of CHV-K. Histopathologic examination of the kidney from Dog 10074 revealed interstitial hemorrhage, focal glomerular damage, vacuolation of the tubules and infiltration of the renal pelvis and cortex with neoplastic lymphocytes (Figure 15).

Cross-Serum Neutralization Tests

The results of cross-serum neutralization tests between the CHV-K isolate and Carmichael's canine herpesvirus (F205V) are presented in Table 10. Canine herpesvirus-Kakuk did not neutralize antiserum against infectious bovine rhinotracheitis, pseudorabies, canine hepatitis, and canine distemper viruses.

Clinical Signs

Incubation period was 4 to 10 days. The first sign was anorexia; puppies rejected food 5 to 24 hours before death. This was followed by severe abdominal pain; even slight pressure applied to the abdomen initiated cries. Breathing was rapid and shallow, and many of the puppies had periods of severe gasping. During this pneumonic stage a serous

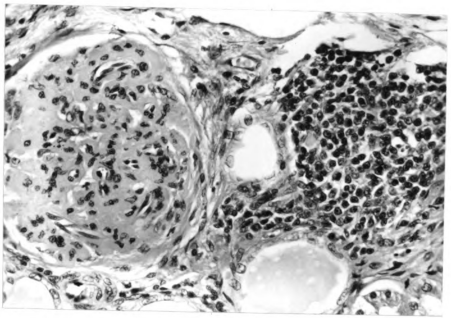


Figure 15. Donor Dog 10074. Notice hyalinization of the glomerulus and invasion of renal cortex by neoplastic lymphoid cells. H & E stain. x 680.

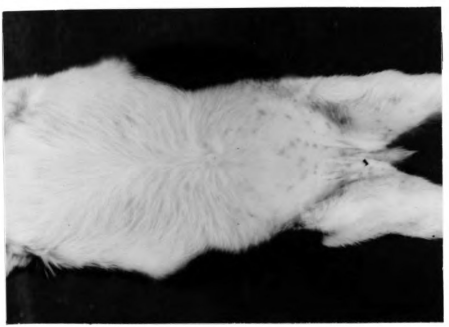


Figure 16. Herpetic erythematous rash in inguinal region of a 16-day-old puppy inoculated with CHV-K.

Table 10. Cross-serum neutralization tests between canine herpesvirus-Kakuk (CHV-K) and Carmichael's canine herpesvirus (F205V)

Virus	vs.	Antiserum[a]	Highest dilution of antiserum giving complete neutralization
CHV-K[b]		F205V	1:320
F205V[c]		F205V	1:160
CHV-K		CHV-K	1:80
F205V		CHV-K	1:80

[a]Antiserum against CHV-K was produced by hyperimmunizing an adult male dog with 10 intravenous injections of viable virus grown in primary dog kidney cells.

[b]CHV-K: canine herpesvirus-Kakuk

[c]F205V: canine herpesvirus-Carmichael, Cornell University

exudate came from the nose and, at times, was accompanied by epistaxis. Puppies then went through periods of extreme weakness, listlessness and, finally, death. Some puppies had an erythematous rash, principally in the skin of the inguinal regions, whereas others had accumulations of fluid in the ventral regions of the body. Elevated body temperatures were not detected; however, in moribund pups, body temperatures were below normal.

Microbiologic Examination

Portions of spleen, lung, liver, kidney, and lymph node were submitted for bacteriologic examination. Bacteria were not isolated.

Hematology

Experiments II, III, IV. Pre- and postinoculation sequential hemogram values in dogs inoculated between 1 and 3 days of age are presented in Appendices 5 and 6. Blood values in the appendices only include those dogs in which a blood sample was obtained before death or before the puppies were killed. It is seen that the platelet count drops markedly between 6 and 11 days postinoculation. This is better compared and contrasted in Table 11, which includes a summary of platelet counts in herpetic dogs and non-contact control dogs. It is seen that the mean platelet count was 70,400 (range: 24,000-178,000) for the herpetic dogs, as compared to 386,000 for non-contact control dogs (Table 11). Four of 10 dogs had decreases in total erythrocytic counts, and 3 of 10 had leukopenia (Appendix 5). The differential counts, packed cell volumes, hemoglobin contents, erythrocytic indices, and absolute leukocytic counts were not significantly altered (Appendices 5 and 6).

Table 11. Platelet counts taken from 11 herpes infected dogs and 3 non-contact control dogs from Appendices 5 and 6

Dog Number	Platelet counts/cmm.	
	Infected	Uninfected
30236	144,000	---
30241	178,000	---
30243	24,000	---
39245	32,000	---
30248	32,000	---
30250[a]	---	425,000
30270	62,000	---
30271	100,000	---
30272	57,000	---
30273[b]	---	362,000
30276	83,000	---
30277[c]	---	372,000
30262[d]	38,000	---
30263	24,000	---
	(Range) 24,000-178,000	362,000-425,000
	70,400 (Mean)	386,000 (Mean)

[a,b,c]Dogs 30250, 30273, and 30277 were non-contact controls

[d]Dog 30262 was a contact control with herpetic lesions

Experiment V. Preinoculation and subsequent sequential hemogram values (Days 0, 1, 4, 8, 10, 14, 22, 29, and 44) are presented in Appendices 7 and 8. Notice that 2 of 4 dogs (30246 and 30247) had left shifts between 4 and 10 days, as compared to Dogs 30049 and 30050 (Appendix 7). Dog 30048, that died, had marked erythropenia and neutropenia. Notice, too, the drop in platelet counts in inoculated dogs between 6 and 22 days as compared to the control (Table 12). Otherwise, the packed cell volume, hemoglobin content, erythrocytic indices, and absolute leukocytic counts were not significantly affected.

Pathology

Gross

Skin. Five of 18 puppies had an erythematous rash (Figure 16). Also, 7 of 18 herpetic pups had subcutaneous edema, particularly in the ventral regions. Several puppies had petechial and/or ecchymotic hemorrhages in the subcutis.

Nasopharynx and Trachea. Petechiae and ecchymoses were observed rather frequently throughout various areas of the nasopharynx. Both the nasopharynx and trachea commonly contained a frothy exudate.

Organs of the thoracic cavity. The lungs were edematous, had gray and red mottled areas, and a frothy fluid exuded from the bronchioles and bronchi when pressure was applied (Figures 17 and 18). Subpleural petechial, ecchymotic, and suffusive hemorrhages were commonly observed. Some lungs were rather firm, indicating consolidation, but they floated in 10% buffered formalin. Usually, hearts appeared pale, valves were

Table 12. Pre- and postinoculation sequential platelet counts taken from Appendices 7 and 8

Dog Number	Day	Platelet counts /cmm.
30246	0	300,000
	1	486,000
	4	229,000
	8	251,000
	10	175,000
	14	289,000
	22	300,000
	29	271,000
	44	313,000
30247	0	336,000
	1	489,000
	4	211,000
	8	205,000
	10	261,000
	14	290,000
	22	126,000
	29	300,000
	44	23,000
30248[a]	0	428,000
	1	378,000
	4	244,000
	6	32,000
30249	0	518,000
	1	275,000
	4	278,000
	8	115,000
	10	250,000
	14	57,000
	22	207,000
	29	220,000
	44	229,000
30250[b]	0	375,000
	1	549,000
	4	425,000
	8	420,000
	10	400,000
	14	300,000
	22	342,000
	29	414,000
	44	362,000

[a]Died 6 days postinoculation

[b]Uninoculated - non-contact control

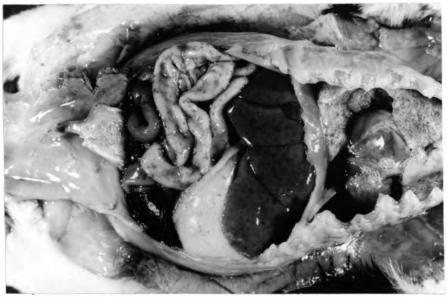


Figure 17. Dog 30270, inoculated with a cell suspension prepared from Donor 10074. Notice the lungs with a frothy exudate, enlarged mottled liver, splenomegaly, and petechiation of gastrointestinal tract. A canine herpesvirus was isolated from dog kidney tissue culture cells inoculated with extract prepared from affected tissue.

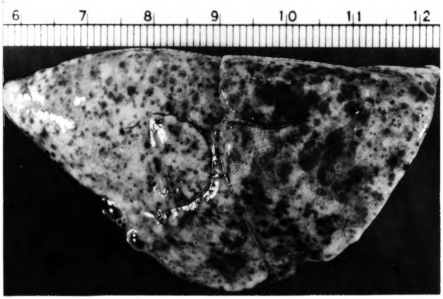


Figure 18. Lung from Dog 30243. Notice the petechial, ecchymotic and suffusive hemorrhages and the frothy exudate.

swollen, and frequently there were subendocardial and epicardial hemorrhages. Petechial hemorrhages and congestion were the most common macroscopic lesions in the thymus.

Organs of the abdominal cavity. Livers were enlarged, had numerous well-circumscribed small, red areas that measured between 1 and 3 mm. in diameter, and were yellowish (Figures 17 and 19). The kidney surfaces had a mottled appearance (Figure 20). Hemorrhagic areas were sharply demarcated from the adjacent pale portions. The cut surface revealed wedge-shaped to irregular areas of hyperemia and hemorrhage (Figure 21). The bases of the hemorrhages were located in the cortex and the apices extended into the medulla. There were a number of subserosal petechial and ecchymotic hemorrhages along the entire gastrointestinal tract in 10 of 18 pups. In 3 puppies, there was blood within the intestinal lumen. Feces had a richly mucoid appearance. The pancreas and adrenal glands appeared normal macroscopically. Spleens appeared enlarged and had a dark mahogany color. Upon cutting, the cut edges bulged.

Lymph nodes. All lymph nodes were enlarged; some were also hemorrhagic and hyperemic. Hemorrhages were, however, most commonly observed in the bronchial lymph nodes.

Skeletal muscles. Whitish streaks were commonly seen in muscles of the pelvic limb but were also found scattered in other muscles. In 4 puppies there were petechial and ecchymotic hemorrhages distributed throughout the skeletal muscles.

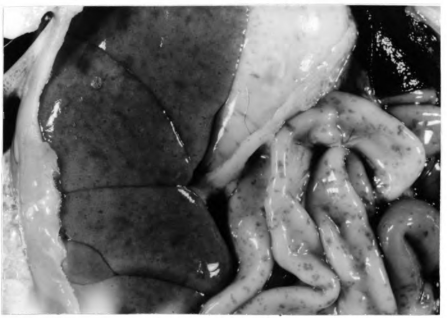


Figure 19. Higher magnification of Figure 17. Notice the focal areas of necrosis in the liver and the hemorrhages along the gastrointestinal tract.

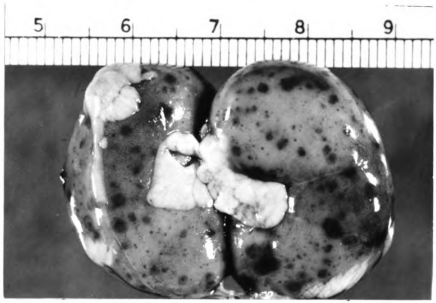


Figure 20. Focal hemorrhages in kidney of Dog 30242, that was inoculated with a cell suspension prepared from Donor 10074. Hemorrhagic areas represent necrotic foci packed with erythrocytes.

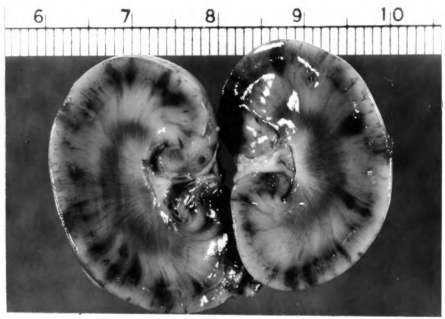


Figure 21. Sagittal section of kidney in Figure 20. Notice that the hemorrhages extended from the capsule to the corticomedullary junction.

Central nervous system. Hyperemia and hemorrhage were the only macroscopic lesions noticed. A few puppies had diffuse hemorrhages beneath the periosteum of the cranial bones.

Petechial and ecchymotic hemorrhages occurred in many other organs and tissues, but with less frequency than the aforementioned.

Microscopic. The most frequent histopathologic lesions were necrosis, hemorrhage, and congestion, as shown in Table 13. It was also noticed that the microscopic picture is characteristic of an acute septicemic infection. Marked lymphocytic and reticular cell hyperplasia were also prominent features.

Skin. The microscopic lesions were made up of clusters of vesicles containing an acidophilic fluid. There was hyperplasia, vesicle formation, and necrosis of the stratum germinativum (Figures 22 and 23). In the necrotic areas, there was infiltration of neutrophils. Marked fibroblastic hyperplasia was located in the corium which surrounded the vesicle formations. These hyperplastic areas resembled fibromatous growths (Figures 24 and 25).

Nasopharynx and trachea. Congestion, scattered hemorrhages, and hypersecretion of mucus were frequently observed in the nasopharynx. In addition, focal necrosis was seen in the nasal epithelium of a few puppies. The trachea contained an abundance of mucus.

Organs of the thoracic cavity. The most prominent lung lesion was focal necrosis. These necrotic areas were characterized by swelling of the alveolar walls, loss of structure, and accumulation of a fibrillar

Table 13. Percent incidence of histopathologic lesions in 18 canine herpesvirus infected dogs

Pathologic changes	Lung	Liver	Kidney	Heart	Skeletal muscle	Pancreas	Adrenal	GI tract	Spleen	Lymph nodes	Thymus	Skin[a]	Naso-pharynx	Brain
Necrosis	100	100	100	100	56	61	44	28	100	44	100	28	50	11
Hemorrhages	100	100	100	61	28	22	28	61	---	33	22	44	44	28
Hyperemia	100	100	100	56	22	---	67	100	100	72	67	28	72	61
Swelling	---	100	72	44	---	---	---	---	---	---	---	28	---	---
Vacuolation of cytoplasm	---	100	83	28	---	---	---	---	---	---	---	---	---	---
Phagocytosis	100	22	---	---	---	---	---	---	61	100	100	---	---	---
Edema	100	---	100	72	44	---	---	11	---	---	---	72	---	---
Reticular cell hyperplasia	28	---	---	---	---	---	---	---	100	---	---	---	---	---
Lymphocytic hyperplasia	22	56	---	---	---	---	---	---	---	83	---	---	---	---

[a]Marked fibroblastic hyperplasia in the dermis resembling fibromas

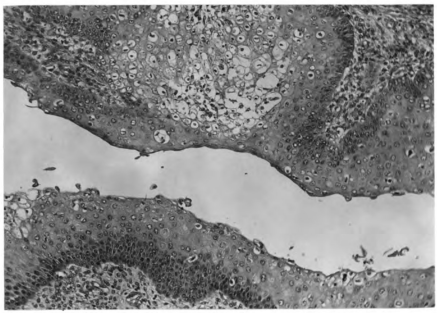


Figure 22. Skin section from Dog 30243. Notice the hyperplasia of prickle and basal cell layers with hydropic degeneration and necrosis of the prickle cell layer. H & E stain. x 140.

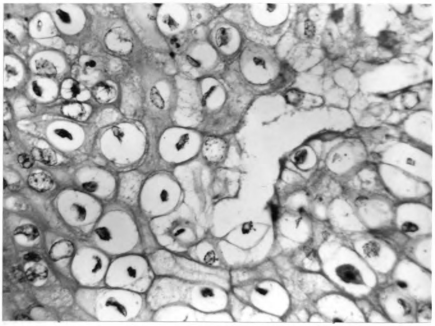


Figure 23. Higher magnification of an area in Figure 22. Notice the marked hydropic degeneration and necrosis of the prickle cell layer. H & E stain. x 350.

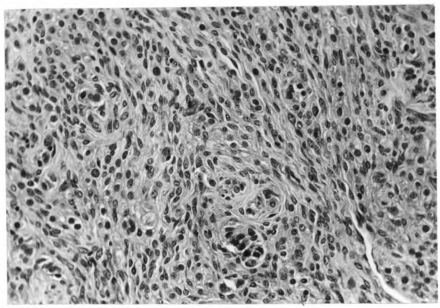


Figure 24. Fibroblastic proliferation resembling a "fibroma" in the corium produced by CHV-K. H & E stain. x 275.

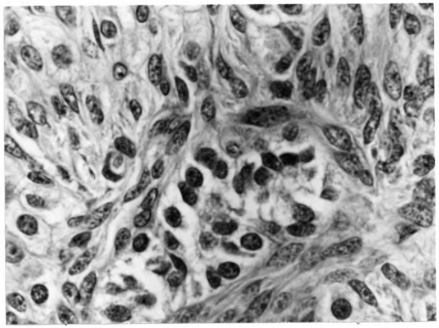


Figure 25. Higher magnification of Figure 24. H & E stain. x 680.

material which was acidophilic (Figures 26 and 27). The fibrillar material stained positive for fibrin, and the acidophilic fluid in the alveolar lumens was PAS positive. Acidophilic staining oval inclusions were seen in the nuclei of some alveolar cells. A lacy exudate within the alveolar spaces was common. Alveolar macrophages were numerous and often vacuolar. Erythrocytes were within the necrotic lesions as well as in the alveolar lumens. Five of the puppies had marked reticuloendothelial cell proliferations intermixed with lymphocytes and neutrophils (Figures 28 and 29).

All hearts had variable degrees of interstitial focal necrosis (Figures 30 and 31) and, less frequently, areas of edema, congestion and myolysis. Associated with these necrotic areas were numerous Anitschkow myocytes (Figure 31) and, sometimes, hemorrhages. Several puppies had subendocardial and pericardial hemorrhages.

Phagocytosis of thymic lymphocytes by macrophages giving the "starry-sky" pattern was the most frequent lesion in the thymus (Figure 32). Other lesions seen microscopically were necrosis, hemorrhage, and hyperemia.

Organs of the abdominal cavity. In the livers, focal areas of necrosis scattered haphazardly throughout the parenchyma were a common histopathologic lesion. Characteristic of these necrotic areas were loss of structure, collapse of the reticular framework, and extensive hepatocellular damage (Figures 33 and 34). In most instances, there was no inflammatory reaction around the necrotic foci; however, there was lymphocytic hyperplasia in the periportal areas in 6 dogs. At the periphery of the necrotic foci, hepatocytes occasionally had a single, large intranuclear inclusion body (Figures 34 and 35). Other hepatocyte nuclei contained smaller irregular acidophilic inclusions (Figure 34).

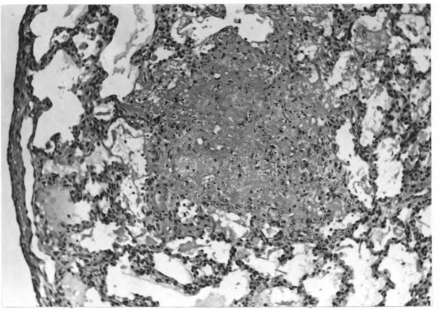


Figure 26. Lung section from puppy inoculated with CHV-K. Necrotic alveolar wall is dilated and contains an acidophilia fibrillar material. H & E stain. x 140.

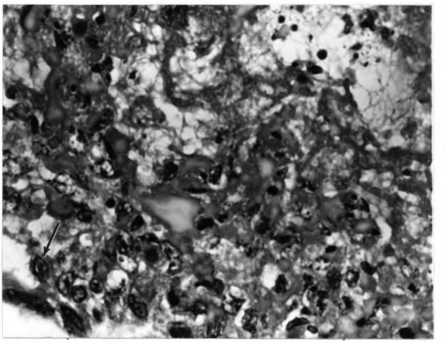


Figure 27. Higher magnification of Figure 26. Notice the intranuclear inclusion body (arrow). H & E stain. x 720.

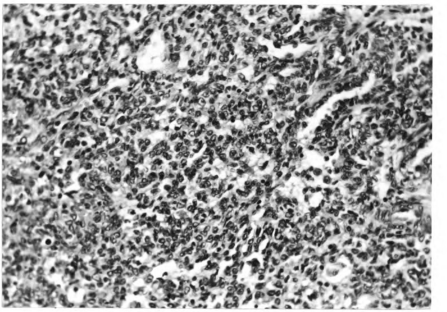


Figure 28. Section of lung taken from a puppy inoculated with CHV-K. Notice the marked reticular cell proliferation which obliterated pulmonary architecture. H & E stain. x 300.

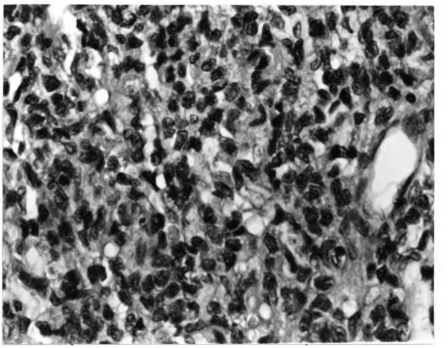


Figure 29. Higher magnification of Figure 28. H & E stain. x 660.

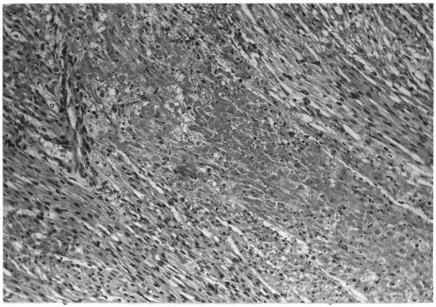


Figure 30. Focal necrosis of myocardium. Puppy was inoculated with CHV-K, and died 6 days postinoculation. H & E stain. x 142.

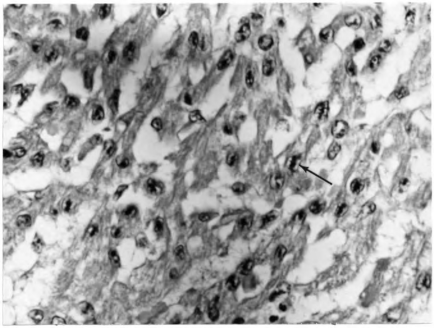


Figure 31. Higher magnification of area in Figure 30. Notice the numerous Anitschkow myocytes (arrow) adjacent to necrotic zone. H & E stain. x 540.

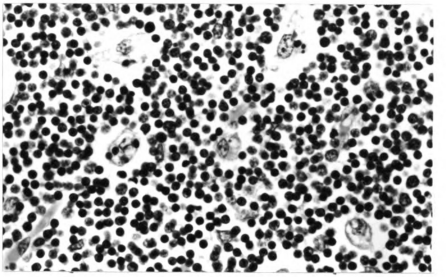


Figure 32. Marked phagocytosis in thymus of a canine herpesvirus infected puppy. Note "starry sky" pattern. H & E stain. x 600.

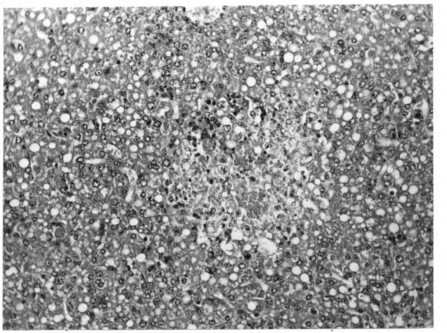


Figure 33. Focal herpetic necrosis in liver of CHV-K inoculated puppy. Note the vacuolar appearance of the surrounding hepatocytes and the obliteration of hepatic sinusoids. H & E stain. x 165.

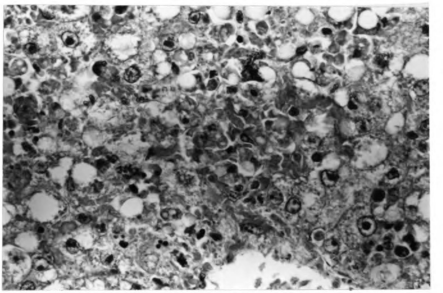


Figure 34. Higher magnification of Figure 33. Notice the oval to irregular shaped intranuclear inclusion bodies in hepatocytes. H & E stain. x 480.

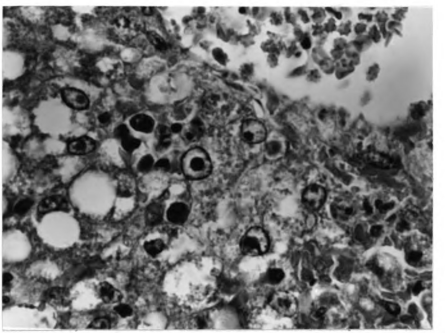


Figure 35. Higher magnification of Figure 34. H & E stain. x 800.

Inclusions were more numerous in some livers than in others; nevertheless, they could be found in most livers with considerable search. Hepatocytes surrounding the necrotic foci were markedly swollen (obliterating the sinusoids) and had vacuolated cytoplasm which did not contain fat.

Lesions in the kidney were usually limited to the cortex, involving both glomeruli and tubules (Figure 36). Glomeruli were markedly swollen, obliterating Bowman's space, and were frequently acidophilic and structureless. Hyaline-appearing material was PAS positive, although no fibrin could be demonstrated with Weigert's stain. Glomerular tufts often were hyalinized, and structural patterns were obliterated (Figure 37); however, there also were glomeruli with less damage (Figure 38). Intranuclear inclusion bodies were seen in the parietal epithelial cells of the glomeruli of most kidneys (Figure 39). They were usually oval, stained basophilic and the chromatin was marginated forming a rim around the nuclear membrane. Sometimes the inclusions were faintly acidophilic; however, they were usually basophilic. In no case were inclusions observed in the renal tubules. Leakage of blood appeared to occur in the vicinity of the capillaries. Erythrocytes thus were seen around glomeruli and adjacent tubules. Tubules had undergone various degrees of swelling, vacuolation and ischemic necrosis in the cortex (Figure 36). Hemorrhages were frequently located subcapsularly, around areas of necrosis, in interstitium of medulla and submucosa of the renal pelvis.

Subserosal, submucosal and mucosal hemorrhages and hyperemia were commonly seen in the gastrointestinal tract. Focal areas of necrosis were limited to the gastrointestinal crypts (Figure 40), which many times led to hemorrhage into the mucosa. Peyer's patches appeared hyperplastic with some of the lymphoid elements undergoing necrosis. Goblet cells

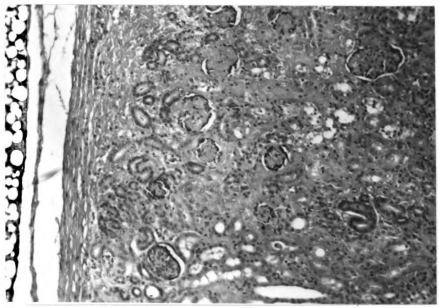


Figure 36. Subcapsular hemorrhage, damage of glomerular tufts, and tubular necrosis in renal cortex of CHV-K inoculated puppy. H & E stain. x 142.

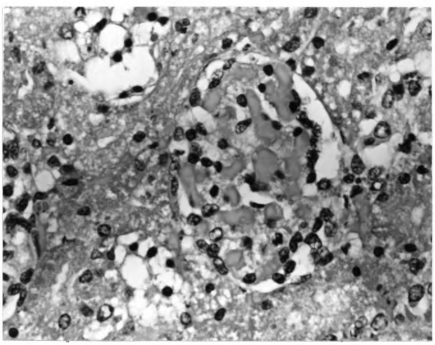


Figure 37. Cellular destruction and hyalinization of glomerular tuft taken from an infected puppy. Note tubular necrosis. H & E stain. x 620.

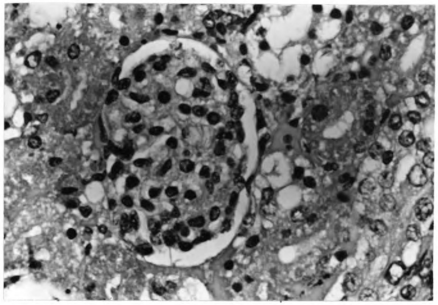


Figure 38. Glomerular tuft with less damage than Figure 37. H & E stain. x 620.

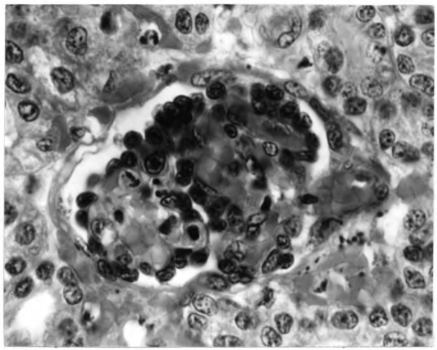


Figure 39. Intranuclear inclusion body in parietal epithelial cell of glomerular tuft. H & E stain. x 736.

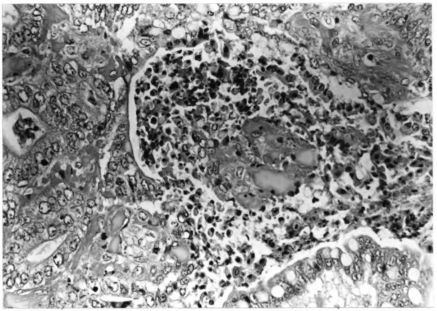


Figure 40. Notice focal necrotic area in duodenal crypt. Puppy was inoculated with CHV-K and died 6 days postinoculation. H & E stain. x 300.

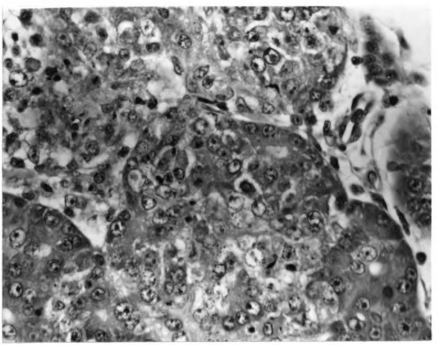


Figure 41. Focal necrotic area in pancreas of a puppy that was killed 7 days postinoculation. H & E stain. x 480.

appeared markedly dilated, resulting in hypersecretion of mucus, thus explaining the mucous-laden feces seen grossly.

The pancreas and adrenal glands had focal areas of necrosis (Figures 41 and 42) along with a few intranuclear inclusion bodies adjacent to and within these necrotic zones.

In the spleen the reticular cells had undergone diffuse hyperplasia, obliterating the normal histologic structures, as compared to the control, and thus there was no demarcation between Malpighian corpuscles and red pulp. The spleen contained numerous erythrocytes which perhaps gave it the dark mahogany color seen grossly. Numerous megakaryocytes had undergone necrosis. Pyknosis, karyorrhexis, karyolysis, and erythrophagocytosis were evident throughout the sections of spleens examined. The hyperplastic reticular cells appeared anaplastic (Figure 43) as compared to the control (Figure 44), yet the splenic capsule was not infiltrated.

Lymph nodes. Histopathologically there were 3 outstanding lesions: (1) diffuse lymphoid hyperplasia (Figures 45 and 46), (2) profuse hemorrhages, especially in the bronchial lymph nodes, and (3) marked karyorrhexis of lymphocytes in the lymph nodes of a few dogs. Occasional basophilic intranuclear inclusions were seen in lymphocytes (Figure 47).

Skeletal muscles. The white streaks seen grossly consisted of various degrees of Zenker's necrosis (Figures 48, 49, 50, and 51). In many dogs, there was marked destruction of the skeletal muscle fibers, while in others congestion, hemorrhage, and edema prevailed.

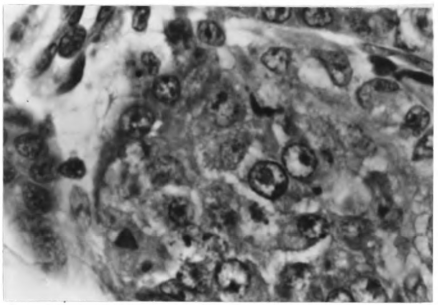


Figure 42. Higher magnification of Figure 41. Notice intranuclear inclusion body in pancreatic acinar cell. H & E stain. x 1200.

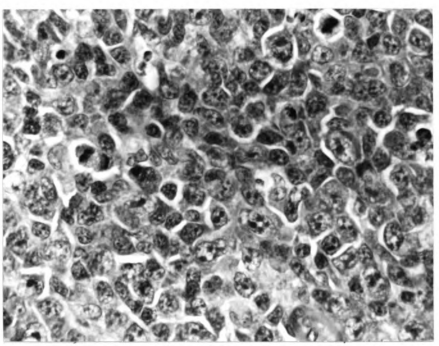


Figure 43. Section of spleen taken from a 12-day-old puppy inoculated as a newborn with a lymphocytic tumor preparation from Donor 10074. Notice the undifferentiated cells and mitotic figures. H & E stain. x 680.

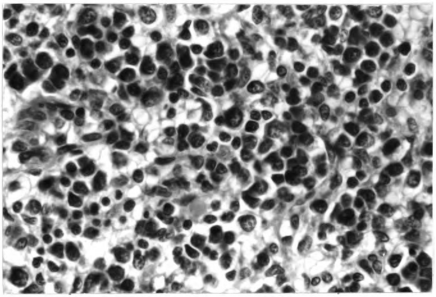


Figure 44. Section of spleen taken from non-contact control Puppy 30277. H & E stain. x 680.

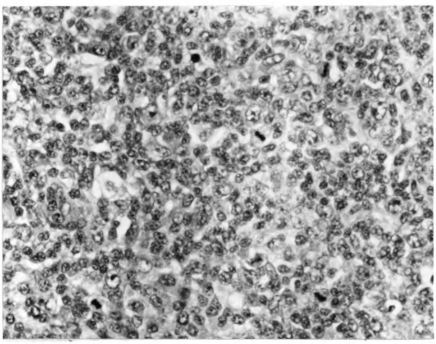


Figure 45. Section of mesenteric lymph node taken from Puppy 30241, that was inoculated with a cell suspension prepared from Donor 10074. Notice the marked lymphocytic hyperplasia and numerous mitotic figures. H & E stain. x 350.

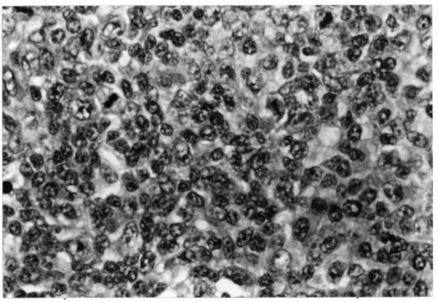


Figure 46. Higher magnification of Figure 45. H & E stain. x 580.

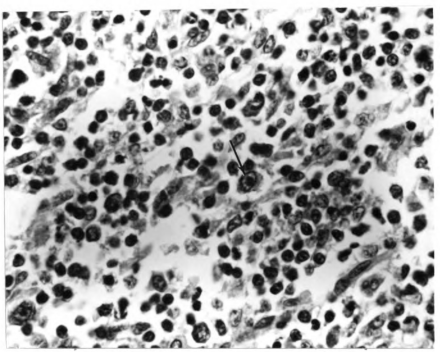


Figure 47. Section of mesenteric lymph node taken from a puppy inoculated with CHV-K. Notice lymphoid depletion, necrosis and intranuclear inclusion body (arrow). H & E stain. x 640.

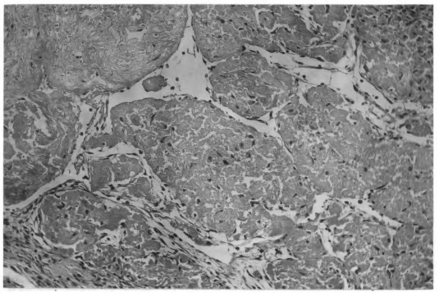


Figure 48. Section of severe skeletal muscle necrosis taken from a puppy inoculated with CHV-K. H & E stain. x 140.

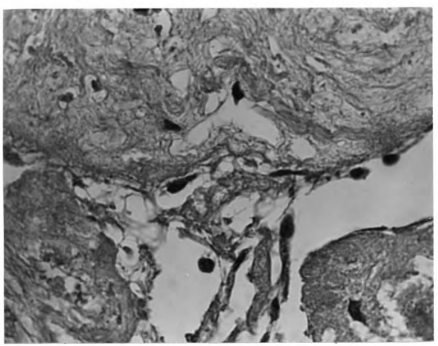


Figure 49. Higher magnification of area in Figure 48. H & E stain. x 700.

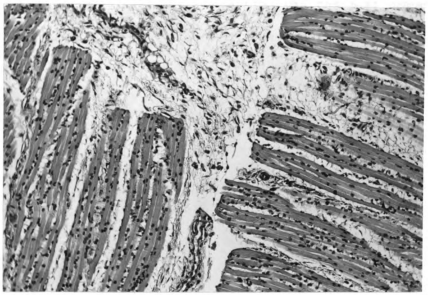


Figure 50. Section of muscle taken from a puppy inoculated with CHV-K. Notice edema and proliferation of sarcolemmal cells. H & E stain. x 140.

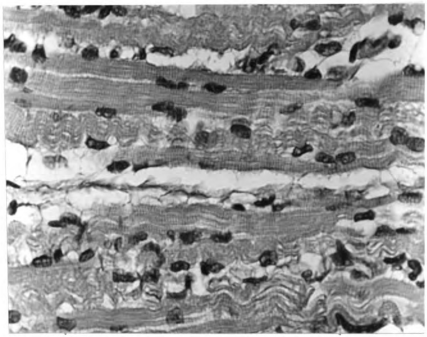


Figure 51. Higher magnification of area in Figure 50. Notice the loss of striations. H & E stain. x 700.

Central nervous system. Congestion was the most common histopathologic lesion, followed by focal capillary hemorrhage, neuronal degeneration, and glial cell accumulation (Figure 52).

Electronmicroscopy

Electronmicroscopic examination demonstrated herpes-like virus particles in affected tissues. Immature intranuclear viral particles (Figure 53) measured 100 mμ in diameter and were enveloped by the nuclear membrane upon release from the nucleus into the cytoplasm. Mature particles in the cytoplasm (Figure 54) which possessed outer envelopes were found in vacuoles and measured 175 mμ in diameter.

DISCUSSION

Cross-serum neutralization tests indicated that the virus isolated from a dog with CML (10074) is closely related to or possibly identical to the strain of canine herpesvirus (F205V) isolated by Carmichael et al. (1965a) from young puppies. Likewise, the strains of canine herpesvirus isolated from young puppies by Stewart et al. (1965a) and Spertzel et al. (1965) also appear immunologically related. This is the first report, so far as the writer knows, concerning the isolation of a herpes-like virus from a dog with malignant lymphoma. There may be 4 different views as to the role of this virus in a disease process, as follows: (1) the virus passed the placental barrier as previously reported (Stewart et al., 1965) and thus the puppies of Experiment II were infected in utero; (2) the virus is responsible for a septicemic disease in newborn puppies and possibly can subsequently lead to malignant lymphoma; (3) canine herpesvirus infection was an incidental infection

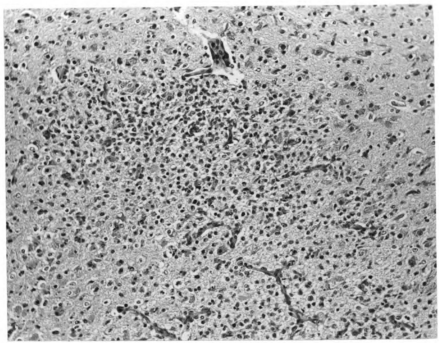


Figure 52. Section of cerebrum taken from a puppy inoculated with CHV-K. Notice glial cell accumulation. H & E stain. x 300.

Figure 53. Intranuclear immature herpes-like particles (arrow) in ultrathin section of lymph node. Glutaraldehyde osmic-acid fixation, uranyl-acetate lead-hydroxide stain. x 65,000.

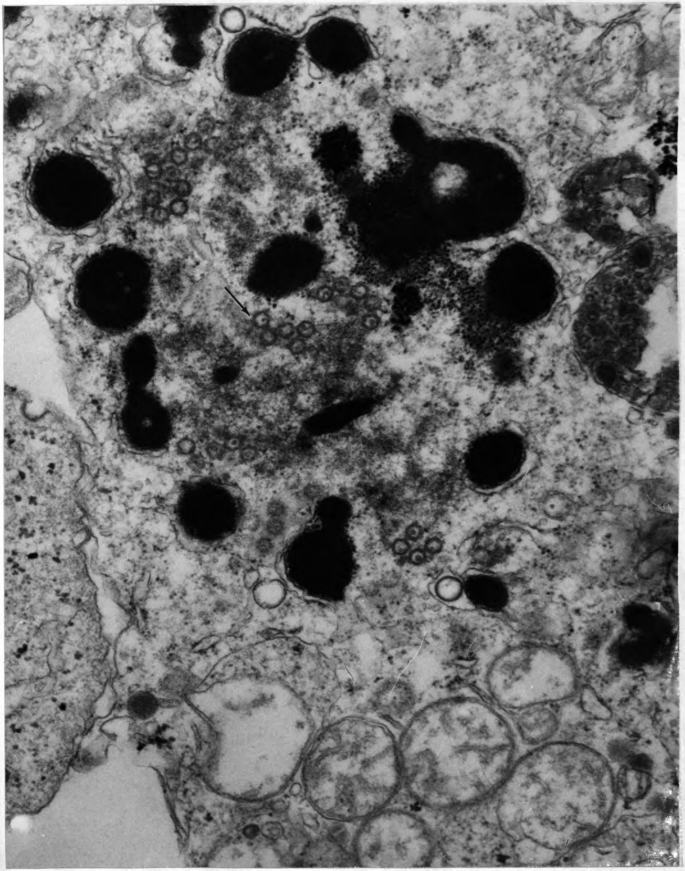
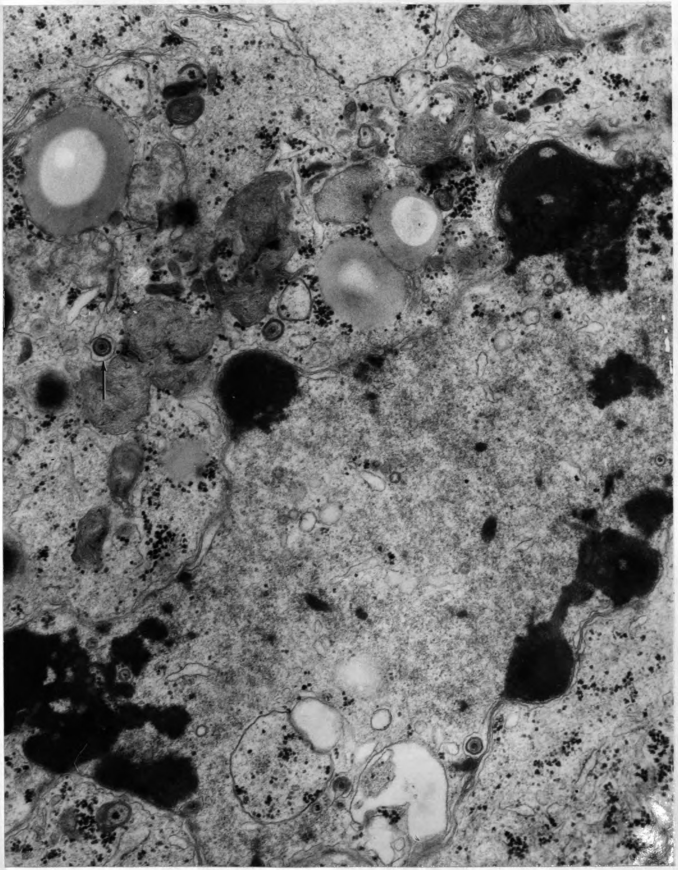


Figure 54. Mature, membrane associated particle (arrow) in cytoplasm of ultrathin lymph node section. Glutaraldehyde osmic-acid fixation, uranyl-acetate lead-hydroxide stain. x 50,000.



to malignant lymphoma in the donor dog; and (4) the virus, because of its latent potentialities, could trigger another agent(s) [virus(es)] to produce malignant lymphoma in dogs. These 4 views are discussed herein.

In this study, passage across the placental barrier appears not to be the source of virus. In Experiment II, all 7 of the inoculated puppies, as well as the contact control, had the septicemic lesions and thus, at this time, it was not known whether the virus was from the malignant lymphoma dog or from the bitch. Results of Experiment III, using non-contact controls, indicated that the origin of the canine herpesvirus was the dog with malignant lymphoma. The septicemic disease developed in all inoculated puppies while the isolated controls remained healthy, which indicated that the virus was from the original ML donor dog. This was further substantiated when the virus was isolated, by tissue culture means, from the frozen kidney of the dog with CML.

In agreement with Carmichael et al. (1964, 1965a) and Stewart et al. (1965a), the results indicate that the virus does produce a fatal septicemic disease in newborn puppies. In their experiments, conventionally raised puppies were used to reproduce the disease, whereas in this investigation germfree Beagle neonates were utilized. It was found that age at time of exposure is very important in reproducing the disease. Newborn, germfree Beagles inoculated prior to 8 days of age are very susceptible, the condition being almost 100% fatal 6 to 16 days postinoculation. According to Carmichael et al. and Stewart et al., the disease has not been seen in the field after puppies are 2 to 3 weeks old; thus, in agreement with results obtained in these experiments, susceptibility decreases with age. Only 1 recipient (30051) inoculated at 1 day of age and at this writing 295 days old has survived the infection.

If the puppies are not inoculated until after 8 days, there may be clinical and hematologic manifestations of the disease, but very few deaths occur. The abrupt resistance is possibly related to the temperature control mechanism which does not develop until the puppy is about 1 week old (Fox, 1966). One recipient (30048) died when inoculated after 8 days; the clinical signs, hematologic findings, and pathologic findings were identical to those found in puppies inoculated before 8 days. Littermates of this dog are presently 171 days old and appear healthy.

Transmission of canine herpesvirus infection appears to occur by contact as demonstrated by the results. Experimentally, Carmichael et al. (1965a) have shown, too, that conventionally raised puppies are susceptible when exposed to virus in aerosol chambers. Likewise, Carmichael and co-workers found that intravaginal inoculation of bitches with virus 1 to 2 weeks before whelping resulted in fatal infections of the newborn, yet illness was not observed in the bitches. It appears that the virus can cross the placenta, since infections have been seen in pups obtained by cesarean section (Stewart et al., 1965a).

Clinical signs were similar in germfree puppies and puppies raised conventionally by Carmichael et al. (1965a). However, emesis and greenish-yellow diarrhea observed by Carmichael and co-workers was not observed in these experiments. This may be due to secondary bacterial invaders in conventionally raised puppies. In germfree puppies anorexia was the first sign, followed by evidence of severe abdominal pain and finally dyspnea with severe gasping, and death 5 to 24 hours after rejection of food.

When comparing hematologic results of non-contact control dogs with those of inoculated and contact control dogs, it is seen that the latter had a markedly lowered platelet count. This may account for the widespread hemorrhage seen pathologically. Leukocyte counts were not significantly affected. Nevertheless, in germfree dogs inoculated with canine distemper virus, Gibson et al. (1965) observed a marked leukopenia. Thus it appears that the infectious agents alter the blood picture differently under germfree conditions.

Focal hemorrhages covering the lung and kidney surfaces are gross diagnostic features of herpes infection compared to canine distemper and infectious canine hepatitis (ICH). Hemorrhages can, however, involve any organ and tissue, and thus ICH must be included in a differential diagnosis. In addition, splenomegaly, hepatomegaly and lymphadenopathy are frequently observed in CHV infections.

The pathologic features of CHV infection in germfree puppies and conventionally reared puppies (Carmichael et al., 1965a) are similar. However, Gibson et al. (1965) found that the prominent lesions of canine distemper virus infections using germfree puppies are lymphoid depletion, reticular cell hyperplasia, and neuronal degeneration. In conventionally reared puppies, secondary bacterial invaders cause widespread lesions (DeMonbreun, 1937; Dunkin and Laidlaw, 1926), unlike those observed in germfree dogs. It is noticed, therefore, that canine distemper in its natural form is a synergistic interaction between virus and secondary bacterial invaders, whereas this does not seem to occur with CHV infections.

Histopathologically, 2 types of lesions were observed: (1) focal necrosis of the kidney, lung, liver, heart, skeletal muscle and other

organs, and (2) reticular cell and lymphocytic hyperplasia. The necrotic lesions in the lung and kidney are diagnostic histopathologic features of this disease. In the lung, the alveolar walls are dilated and filled with a fibrillar acidophilic staining material that stains positive for fibrin. This lesion is surrounded by necrotic septal cells, some of which contain oval to irregular acidophilic intranuclear inclusion bodies. In some cases inclusions are very difficult to find. It appears that the virus causes swelling of a number of glomeruli which is followed by ischemic necrosis of the tubules. This observation is based on the histopathologic finding that only tubules in the renal cortex are affected. Variable degrees of degeneration are seen (cloudy swelling, vacuolation, and necrosis), which apparently are related to the degree of pathologic alteration in the glomeruli. This observation is interesting since, if a dog recovers from herpesvirus infection, glomerulonephritis could be a sequela later in life.

Lymphoid hyperplasia was so pronounced in the lymph nodes that normal histologic structures were obliterated. Lymph nodes consisted of lymphoblastic cells that had numerous mitotic figures, although the capsule and perinodal structures were not infiltrated. Lymphocytic hyperplasia occurred frequently in the periportal areas of the liver and less commonly in the lung. There was marked reticular cell proliferation of the spleens; the cells appeared to resemble cells seen in reticulum cell sarcoma, but this did not appear to represent a neoplasm. The presence of lymphoid and reticular hyperplasia in the lungs along with focal areas of necrosis simulates the lesions seen in Marek's disease of chickens (Biggs, 1967), which is thought to be caused by a herpesvirus (Churchill and Biggs, 1967).

Canine herpesvirus infection could have been incidental to malignant lymphoma or the virus could be responsible, or in part be responsible, for the etiology of malignant lymphoma. Presently this cannot be answered, yet Hinz (personal communication) found that affected tissues examined contained herpes-like viruses in 25% of the dogs with ML examined by electronmicroscopy. Isolation of these viruses by in vitro technics was not accomplished. Several authors have reported the presence of herpes-like viruses in Burkitt tumor cells grown in vitro, although isolation of an agent other than Herpesvirus hominis was not achieved (Epstein et al., 1964; O'Conor and Rabson, 1965; Stewart et al., 1965b). Henle and Henle (1966) have studied extensively 10 lines of Burkitt tumor cells and found that 7 of 10 had herpes-like viral particles as detected by electron-microscopy and fluorescent microscopy. Also, it was found that the viruses in Burkitt tumor cells did not react immunologically with anti-serum produced against 10 different viruses of the herpesvirus group. Canine herpesvirus, likewise, does not react with other viruses of the herpesvirus group; however, CHV-K isolates from a dog with malignant lymphoma and the CHV (Carmichael et al., 1965a; Stewart et al., 1965a) isolated from young puppies appears identical as demonstrated by cross-serum neutralization tests. In addition, CPE in dog kidney cultures and thymus tissue cultures were similar.

Recently, Mitchell et al. (1967) isolated a herpes-like virus from P3J Burkitt lymphoma cells superinfected with Moloney sarcoma virus that caused CPE in dog thymic cells. Stewart and Durr (1967) found that a cell-free extract prepared from SL_1 Burkitt tumor cells and inoculated into newborn hamsters intracerebrally resulted in encephalitis with marked glial cell proliferation. Therefore, it appears that the

herpes-like agent found in Burkitt tumor cells can be pathogenic for the newborn hamster. Canine herpesvirus is not pathogenic for laboratory animals as reported by Carmichael et al. (1964) and Spertzel et al. (1965). The author (unpublished data) has been unable to produce lesions in hamsters inoculated by various routes with CHV-K.

It does appear clear, however, that canine herpesvirus is a good candidate virus to pursue in regard to its oncogenic potentialities. This is based on the facts that (1) the herpesvirus group is notoriously known for its latent state and (2) since Stewart et al. (1965a) have data suggesting that CHV, too, has a state of latency, this might suggest also that CHV could play a role in triggering or causing CML. In addition, the recent finding by Churchill and Biggs (1967) that Marek's disease of chickens is likely caused by a herpesvirus suggests the possibility that a similar agent could be involved in malignant lymphoma of domestic animals and man.

SUMMARY

Canine malignant lymphoma (CML) was transmissible to the Beagle neonate in 2 serial passages, and a canine herpesvirus, designated as canine herpesvirus-Kakuk (CHV-K), that was pathogenic for the germfree Beagle neonate, was isolated from a dog with CML. The clinical, hematologic, macroscopic, and microscopic findings of experimentally induced CML and a septicemia in Beagle neonates produced by CHV-K were described and discussed.

I. Experimental Transmission of Canine Malignant Lymphoma to the Beagle Neonate
Experiment I

Two serial passages of CML were accomplished in 2 litters of Beagle neonates with suspensions of viable CML whole cells. In these experiments 3 of 11 dogs inoculated with a single dose of a cell suspension developed malignant lymphoma which was clearly recognized at 53, 54, and 78 days postinoculation. Hematologic results indicated that leukemia was present in Dog 30030 and that subleukemia was present in Dogs 30032 and 30089 between 41 and 52 days. Likewise, 2 dogs had an anemia, whereas all 3 dogs had lowered platelet counts. Two dogs with overwhelming CML were preirradiated with x rays, whereas 1 dog was not irradiated. Thus, a significant feature of this work was the successful transmission of CML without pretreatment of total body irradiation. Malignant lymphoma did not occur in the third serial passage, although animals had enlargement of the superficial lymph nodes. Biopsies of

these enlarged lymph nodes revealed lymphoid hyperplasia but not malignant lymphoma. The clinical and pathologic changes were similar to those reported for naturally occurring malignant lymphoma of dogs and cats.

II. A Canine Herpesvirus Isolated from a Dog with Canine Malignant Lymphoma Pathologic for the Beagle Neonate
Experiments II, III, IV, V

The canine herpesvirus (CHV-K) was isolated from 18 germfree Beagle neonates inoculated with either a cell suspension prepared from a dog with CML or extracts prepared from puppies with the septicemic disease. Two contact control puppies died from similar septicemic conditions, whereas the 3 non-contact control puppies remained healthy. From these results it was concluded that the CHV-K could be transmitted by direct contact and artificial inoculation.

The production of a fatal septicemic disease in Beagle neonates inoculated with material prepared from a dog with CML and the isolation of the virus from the kidney of this dog indicated that the virus came from the donor dog.

This virus was responsible for a fatal septicemic disease in colostrum-deprived, germfree Beagle neonates 6 to 16 days postinoculation. The fundamental histopathologic lesion was necrosis in the liver, lung, kidney, heart, skeletal muscle, pancreas, and adrenal gland. A few intranuclear basophilic and/or acidophilic inclusions were seen in cells adjacent to the areas of necrosis. Splenomegaly and generalized lymphadenopathy were commonly seen which microscopically consisted of marked lymphocytic and reticular cell hyperplasia. The marked hyperplasia produced by this virus resembled neoplasia, although obvious neoplasms have not been induced.

Results of cross-serum neutralization tests indicated that the virus was closely related, or possibly identical to, the canine herpesviruses isolated from young puppies (Carmichael's strain: F205V, and Stewart's strain: SL18HLV). However, this was the first report in which a CHV was isolated from a dog with malignant lymphoma, and also the first report concerning the isolation of CHV from an adult dog which was pathogenic for puppies.

Results indicated that age at time of exposure is very important in reproducing the disease in puppies. Newborn, germfree Beagles inoculated prior to 8 days of age were highly susceptible, and nearly all died. One of 18 recipients inoculated at 1 day of age is presently 295 days old, having survived the infection. If the puppies were not inoculated until after 8 days, there were clinical manifestations of the disease, but few deaths occurred (Experiment V).

Factors of importance in establishing a diagnosis included: (1) age of the puppies; (2) clinical signs; (3) marked thrombocytopenia; and (4) pathologic findings. Pathologic changes in affected puppies were characteristic. Widespread hemorrhages were apparently related to the marked thrombocytopenia consistently observed in herpes-infected puppies. Necrotic and hemorrhagic lesions in the liver, lung, and kidney of dead puppies suggested this viral infection. Focal renal hemorrhages have not been reported in dogs infected with infectious canine hepatitis or distemper viruses. Microscopic examination of stained tissue sections may reveal occasional intranuclear inclusions in cells adjacent to areas of necrosis. These were often difficult to find, since they occurred only in recently infected cells. The virus caused characteristic CPE in 12 to 16 hours when grown in dog kidney cell or thymic cell tissue cultures.

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Appendix 1. Pre- and postinoculation sequential hemogram values for Dog 30030 with induced malignant lymphoma representing the 1st serial passage, Experiment I

Day[a]	WBC/cmm. blood	Differential					Erythrocytic series				
		Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
				Segs.	Bands						
0	24,917	3.0	13.5	63.5	15.5	4.5	8	5.03	45.0	15.0	280,000
21	15,915	5.5	48.5	27.5	17.5	1.0	1	3.94	34.5	10.9	463,000
41[b]	15,268	9.0	26.0	23.5	41.5	0	0	3.86	27.0	8.8	439,000
52	24,410	2.5	62.5	10.0	25.0	0	0	3.89	28.2	8.9	260,000
54	13,316	7.0	79.5	1.0	12.0	0.5	2	3.58	26.8	8.4	80,000

[a]Day 0, preinoculation blood sample

[b]At 41 days and thereafter anaplastic lymphocytes were in peripheral blood smear.

Appendix 2. Pre- and postinoculation sequential hemogram values for Dog 30032 with induced malignant lymphoma representing the 1st serial passage, Experiment I

Day[a]	WBC/cmm. blood	Differential					Erythrocytic series				
		Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
				Segs.	Bands						
0	22,894	5.0	6.5	76.0	12.0	0.5	5	5.20	46.0	15.8	390,000
21	12,261	8.0	41.5	37.0	12.5	1.0	2	3.59	34.0	10.8	290,000
41	12,727	6.0	34.5	45.5	12.0	2.0	0	3.99	30.5	10.0	320,000
52[b]	16,484	7.0	20.0	39.0	31.0	3.0	0	3.96	31.0	10.1	276,000
61	14,356	3.0	47.0	30.0	19.0	1.0	0	4.19	33.0	11.3	545,000
70	10,022	4.5	24.5	45.0	25.0	1.0	1	4.26	36.0	11.6	75,000
78	6,031	4.0	40.0	37.0	19.0	1.0	1	5.30	38.5	13.1	100,000

[a]Day 0, preinoculation blood sample

[b]At 52 days and thereafter anaplastic lymphocytes were in peripheral blood smear.

Appendix 3. Pre- and postinoculation sequential hemogram values for Dog 30089 with induced malignant lymphoma representing the 2nd serial passage, Experiment I

		Differential					Erythrocytic series				
Day[a]	WBC/cmm. blood	Mono-cytes (%)	Lympho-cytes (%)	Neutro-phils (%)		Eosino-phils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
				Segs.	Bands						
0	26,261	6.0	23.5	48.5	14.0	8.0	4.0	4.57	45.0	15.1	285,000
20	15,988	3.5	34.5	40.0	16.0	6.0	7.5	2.86	30.0	8.9	395,000
39[b]	14,400	5.0	12.5	57.0	25.5	0.0	1.0	3.93	29.0	9.4	460,000
49	9,750	3.0	20.0	63.5	13.0	0.5	5.0	2.54	20.5	6.6	157,000
53	6,100	0.0	61.0	20.0	18.5	0.5	1.5	2.31	16.5	4.5	126,000

[a]Day 0, preinoculation blood sample

[b]At 52 days and thereafter anaplastic lymphocytes were in peripheral blood smear.

Appendix 4. Pre- and postinoculation sequential hemogram values for Control Dog 30088, Experiment I

Day[a]	WBC/cmm. blood	Differential					Erythrocytic series				
		Mono-cytes (%)	Lympho-cytes (%)	Neutro-phils (%)		Eosino-phils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
				Segs.	Bands						
0	14,865	2.0	35.5	41.5	5.0	16.0	4.5	4.28	44.0	14.6	220,000
21	15,096	4.0	53.0	33.0	4.0	6.0	0.0	2.35	22.5	6.8	334,000
41	14,400	4.5	34.0	52.5	7.0	2.0	0.0	4.21	28.5	8.8	568,000
52	12,250	4.0	42.5	51.0	2.5	0.0	0.0	4.09	30.5	9.4	269,000
71	12,750	2.0	30.5	57.5	9.0	1.0	0.0	4.20	32.3	10.3	390,000
87	15,200	3.0	32.5	57.0	6.0	2.0	0.0	4.34	35.0	11.4	425,000

[a]Day 0, preinoculation blood sample

Appendix 5. Pre- and postinoculation sequential hemogram values from canine herpesvirus infected dogs, Experiments II, III, and IV

Dog No.	Day[b]	Differential[a]					Erythrocytic series				
		WBC/cmm. blood	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC 10^6 x cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
					Segs.	Bands						
30236	0	11,000	5.0	22.0	43.0	19.0	10.0	8	4.83	53.0	16.4	369,000
	11	7,000	3.0	21.0	48.0	26.0	2.0	4	2.84	27.0	9.4	144,000
30241	0	12,900	10.0	12.0	52.0	22.0	4.0	6	4.97	57.0	17.3	400,000
	11	8,900	2.0	22.0	50.0	21.0	5.0	11	3.55	33.0	11.2	178,000
30243	0	4,500	3.0	11.0	59.0	13.0	14.0	9	4.42	48.0	15.3	450,000
	11	3,300	0.0	81.0	16.0	3.0	0.0	0	2.81	27.0	9.4	24,000
	16	350	0.0	3.0	2.0	0.0	0.0	0	.98	7.5	2.5	400
30245	0	7,900	2.0	7.0	67.0	12.0	12.0	5	5.18	55.0	17.3	510,000
	11	3,100	1.0	52.0	40.0	6.0	1.0	8	3.35	35.0	11.6	32,000
30270	0	9,600	3.0	20.0	63.0	14.0	0.0	10	5.56	53.0	17.0	211,000
	4	10,500	2.0	39.0	47.0	11.0	1.0	29	5.07	47.0	14.9	221,000
	8	11,700	5.0	15.0	51.0	28.0	1.0	7	4.27	42.0	12.0	62,000
30271	0	9,600	5.0	20.0	50.0	22.0	3.0	4	5.19	47.5	17.0	468,000
	4	11,300	5.0	40.0	40.0	12.0	3.0	3	4.54	43.0	13.4	244,000
	8	11,800	2.0	19.0	63.0	15.0	1.0	1	3.67	33.0	9.8	100,000

Appendix 5--continued

Dog No.	Day[b]	Differential[a]					Erythrocytic series				
		WBC/cmm. blood	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
					Segs.	Bands						
30272	0	6,000	5.0	34.0	37.0	22.0	1.0	1	5.84	53.5	16.8	269,000
	4	11,500	1.0	30.0	47.0	21.0	1.0	8	4.98	45.0	14.8	177,000
	6	14,000	5.0	11.0	57.0	27.0	0.0	0	4.29	43.0	13.4	57,000
30273[c]	0	19,600	3.0	26.0	51.0	17.0	3.0	4	5.63	53.0	18.0	369,000
	8	32,800	1.0	18.0	61.0	18.0	2.0	2	4.55	41.0	12.6	272,000
	15	11,600	0.0	46.0	45.0	5.0	4.0	4	3.46	32.0	11.6	362,000
30276	0	12,900	3.0	22.0	40.0	33.0	2.0	10	5.26	50.0	16.9	361,000
	4	17,000	2.0	23.0	48.0	26.0	1.0	11	4.80	45.0	14.2	275,000
	7	19,400	3.0	28.0	38.0	31.0	0.0	4	3.52	34.0	12.4	83,100
30277	0	26,600	2.0	29.0	39.0	28.0	2.0	3	5.41	50.0	15.2	375,000
	7	19,800	4.0	38.0	39.0	14.0	5.0	14	5.68	50.0	15.4	372,000
30262[d]	0	9,200	8.0	13.0	33.0	46.0	0.0	11	5.16	61.0	17.7	325,000
	8	9,300	3.0	33.0	39.0	23.0	2.0	4	2.90	34.0	10.2	38,000
30263	0	12,600	3.0	22.0	60.0	14.0	1.0	3	4.73	45.5	16.4	200,000
	8	2,700	6.0	41.0	35.0	18.0	0.0	2	2.82	29.0	10.1	24,000

[a]Dog 30236: 1% basophils, 30273: 1% basophils

[b]Day 0, preinoculation blood sample

[c]Dog 30273: non-contact control that remained healthy

[d]Dog 30262: contact control that died with herpes septicemia

Appendix 6. Sequential hemogram values based on results from Appendix 5, Experiments II, III, and IV

Dog No.	Day[a]	Corr. WBC/cmm. blood	Platelets /cmm.	Erythrocytic series: RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	MCV cu.μ	MCHC (%)	Absolute lymphocytic counts: Monocytes (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
30236[b]	0	10,185	369,000	4.83	53.0	16.4	110	31	509	2,241	6,315	1,019
	11	6,731	144,000	2.84	27.0	9.4	95	35	201	1,414	4,981	135
30241	0	12,170	400,000	4.97	57.0	17.3	115	30	1,217	1,460	9,006	487
	11	8,018	178,000	3.55	33.0	11.2	93	34	160	1,764	5,693	401
30243	0	4,128	450,000	4.42	48.0	15.3	109	32	124	454	2,972	578
	11	3,300	24,000	2.81	27.0	9.4	96	35	0	2,673	627	0
	16	350		.98	7.5	2.5	76	03				
30245	0	7,524	510,000	5.18	55.0	17.3	106	31	150	527	5,944	903
	11	2,870	32,000	3.35	35.0	11.6	104	33	29	1,494	1,320	29
30270	0	8,727	211,000	5.56	53.0	17.0	95	32	262	1,745	6,720	0
	4	8,140	221,000	5.07	47.0	14.9	93	32	163	3,175	4,721	81
	8	10,935	62,000	4.27	42.0	12.8	98	30	547	1,640	8,639	109
30271	0	9,230	468,000	5.19	47.5	17.0	92	36	462	1,846	6,646	276
	4	10,971	244,000	4.54	43.0	13.4	95	31	549	4,388	5,705	329
	8	11,000	100,000	3.67	33.0	9.8	90	30	234	2,220	9,111	117
30272[c]	0	6,732	267,000	5.84	53.5	16.8	92	31	337	2,289	3,972	67
	4	10,648	177,000	4.98	45.0	14.8	90	33	106	3,194	7,242	106
	6	14,000	57,000	4.29	43.0	13.4	100	31	700	1,540	11,760	0
30273[d]	0	18,845	369,000	5.63	53.0	18.0	94	34	565	4,900	12,815	565
	8	32,157	272,000	4.55	41.0	12.6	90	31	322	5,788	25,404	643
	15	11,153	362,000	3.46	32.0	11.6	92	36	0	5,130	5,577	446

Appendix 6--continued

Dog No.	Day[a]	Corr. WBC/cmm. blood	Platelets /cmm.	Erythrocytic series: RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	MCV cu.μ	MCHC (%)	Absolute lymphocytic counts: Monocytes (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
30276	0	11,726	361,000	5.26	50.0	16.9	95	34	352	2,580	8,560	234
	4	15,856	275,000	4.80	45.0	14.2	94	32	317	3,647	11,733	159
	7	18,631	83,000	3.52	34.0	12.4	97	36	559	5,217	12,855	0
30277[d]	0	25,826	375,000	5.41	50.0	15.2	92	30	517	7,489	17,303	517
	7	17,369	372,000	5.68	50.0	15.4	88	31	695	6,600	9,206	868
30262[e]	0	8,288	325,000	5.16	61.0	17.7	118	29	663	1,077	6,548	0
	8	8,942	38,000	2.90	34.0	10.2	117	30	268	2,951	5,544	179
30263	0	12,233	200,000	4.73	45.5	16.4	96	36	367	2,691	9,052	122
	8	2,647	24,000	2.82	29.0	10.1	103	35	159	1,085	1,403	0

[a]Day 0, preinoculation blood sample

[b]30236: 101 basophils

[c]30272: 67 basophils

[d]30273 and 30277: non-contact control dogs that remained healthy

[e]30262: contact control dog that died from herpes septicemia

Appendix 7. Pre- and postinoculation sequential hemogram values from canine herpesvirus infected dogs, Experiment V

Dog No.	Day[a]	Differential						Erythrocytic series				
		WBC/cmm. blood	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
					Segs.	Bands						
30246	0	18,800	5.0	14.0	62.0	15.0	4.0	4	5.43	51.0	17.4	300,000
	1	15,300	3.0	25.0	50.0	20.0	2.0	3	3.95	34.0	11.5	486,000
	4	9,400	5.0	54.0	29.0	8.0	4.0	1	4.00	35.0	11.9	229,000
	8	18,900	6.0	30.0	26.0	35.0	3.0	5	3.17	27.0	8.7	251,000
	10	30,600	3.0	21.0	39.0	35.0	2.0	7	3.49	30.0	9.3	175,000
	14	7,700	2.0	49.0	43.0	6.0	0.0	4	3.65	28.0	9.4	289,000
	22	8,000	0.0	51.0	45.0	3.0	1.0	9	3.90	31.0	10.0	300,000
	44	13,200	2.0	38.0	57.0	2.0	1.0	1	4.48	32.0	9.5	313,000
30247	0	19,300	4.0	17.0	60.0	18.0	1.0	10	5.48	56.0	19.2	336,000
	1	10,900	5.0	41.0	37.0	14.0	3.0	6	4.81	44.0	14.6	489,000
	4	11,500	2.0	37.0	40.0	19.0	2.0	2	3.80	32.0	10.6	211,000
	8	13,100	5.0	50.0	28.0	17.0	0.0	12	3.47	30.0	10.1	205,000
	10	18,200	3.0	52.0	37.0	7.0	1.0	10	3.72	32.0	10.2	261,000
	14	10,800	0.0	66.0	30.0	2.0	2.0	4	4.24	35.0	11.6	290,000
	22	8,100	6.0	48.0	39.0	7.0	0.0	10	3.89	31.5	10.2	126,000

Appendix 7--continued

Dog No.	Day[a]	Differential					Erythrocytic series				
		WBC/cmm. blood	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
					Segs.	Bands						
30247 cont.	29	7,800	3.0	53.0	42.0	2.0	0.0	7	4.13	32.0	10.6	300,000
	44	10,100	2.0	63.0	34.0	0.0	1.0	2	4.42	34.0	10.6	23,000
30248	0	25,500	4.0	6.0	77.0	12.0	1.0	4	5.95	58.0	19.6	428,000
	1	12,900	10.0	10.0	62.0	14.0	4.0	13	3.87	36.0	12.2	378,000
	4	15,300	2.0	15.0	64.0	18.0	1.0	1	2.96	26.0	8.9	244,000
	6	6,000	4.0	71.0	15.0	10.0	0.0	0	2.81	27.0	9.0	32,000
30249	0	14,300	5.0	29.0	58.0	4.0	4.0	6	5.31	57.0	19.5	518,000
	1	8,800	3.0	38.0	48.0	9.0	2.0	4	4.51	40.5	14.0	275,000
	4	9,000	4.0	56.0	32.0	5.0	3.0	1	3.95	35.0	11.7	278,000
	8	10,700	1.0	61.0	34.0	2.0	2.0	3	3.60	30.6	10.3	115,000
	10	11,600	2.0	51.0	43.0	4.0	0.0	4	3.48	29.0	9.4	250,000
	14	10,600	1.0	63.0	30.0	4.0	2.0	19	3.49	28.0	9.6	57,000
	22	8,300	4.0	52.0	42.0	1.0	1.0	13	3.96	33.0	10.8	207,000
	29	8,100	3.0	44.0	51.0	2.0	0.0	10	4.13	33.0	9.6	220,000
	44	12,600	2.0	35.0	60.0	3.0	0.0	1	4.55	34.0	11.7	229,000

Appendix 7--continued

Dog No.	Day[a]	Differential					Erythrocytic series				
		WBC/cmm. blood	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)		Eosinophils (%)	NRBC	RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	Platelets /cmm.
					Segs.	Bands						
30250[b]	0	26,100	5.0	21.0	64.0	10.0	0.0	8	5.97	59.0	16.6	375,000
	1	15,168	9.0	53.0	28.0	7.0	3.0	5	5.63	47.0	16.2	549,000
	4	11,300	5.0	39.0	46.0	8.0	2.0	2	5.24	42.0	14.5	425,000
	8	13,600	6.0	43.0	46.0	4.0	1.0	3	4.35	35.0	12.0	420,000
	10	9,200	4.0	53.0	38.0	5.0	0.0	4	4.26	32.0	11.2	400,000
	14	10,500	5.0	43.0	44.0	5.0	3.0	12	3.53	27.0	9.3	300,000
	22	8,100	2.0	59.0	34.0	4.0	1.0	6	4.19	33.0	10.8	342,000
	29	10,300	1.0	49.0	46.0	3.0	1.0	4	4.61	34.0	11.8	414,000
	44	11,600	0.0	46.0	45.0	5.0	4.0	4	3.46	32.0	11.6	362,000

[a]Day 0, preinoculation blood sample

[b]Dog 30250: non-contact control that remained healthy

Appendix 8. Sequential hemogram values based on results from Appendix 7, Experiment V

Dog No.	Day[a]	Corr. WBC/cmm. blood	Platelets /cmm.	Erythrocytic series					Absolute lymphocytic counts			
				RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	MCV cu.μ	MCHC (%)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
30246	0	18,076	300,000	5.43	51.0	17.4	94	34	904	2,531	13,918	723
	1	14,855	486,000	3.95	34.0	11.5	86	34	445	3,714	10,399	297
	4	9,306	229,000	4.00	35.0	11.9	87	34	465	5,025	3,443	372
	8	18,000	251,000	3.17	27.0	8.7	85	32	1,080	5,400	10,980	540
	10	28,599	175,000	3.49	30.0	9.3	86	31	858	6,006	21,163	572
	14	7,404	289,000	3.65	28.0	9.4	77	34	148	3,628	3,628	0
	22	7,339	300,000	3.90	31.0	10.0	79	32	0	3,743	3,523	73
	29	7,864	271,000	4.43	34.0	11.2	77	33	315	4,168	3,224	157
	44	13,068	313,000	4.48	32.0	9.5	71	30	261	4,968	7,710	130
30247	0	17,547	336,000	5.48	56.0	19.2	102	34	702	2,983	13,687	175
	1	10,283	489,000	4.81	44.0	14.6	91	33	514	4,216	5,861	308
	4	11,275	211,000	3.80	32.0	10.6	84	33	226	4,172	6,652	226
	8	11,697	205,000	3.47	30.0	10.1	86	34	585	5,848	5,264	0
	10	16,546	261,000	3.72	32.0	10.2	86	32	496	8,604	7,280	165
	14	10,384	290,000	4.24	35.0	11.6	83	33	0	6,853	3,323	208
	22	7,364	126,000	3.89	31.5	10.2	80	32	442	3,535	3,387	0

Appendix 8--continued

Dog No.	Day[a]	Corr. WBC/cmm. blood	Platelets /cmm.	Erythrocytic series: RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	MCV cu.μ	MCHC (%)	Absolute lymphocytic counts: Monocytes (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
30247 cont.	29	7,290	300,000	4.13	32.0	10.6	77	33	219	3.864	3,207	0
	44	9,902	23,000	4.42	34.0	10.6	77	31	198	6,238	3,367	99
30248	0	24,518	428,000	5.95	58.0	19.6	97	34	981	1,471	21,821	245
	1	11,218	378,000	3.87	36.0	12.2	93	34	1,122	1,122	8,525	449
	4	15,147	244,000	2.96	26.0	8.9	88	34	303	2,272	12,421	151
	6	6,000	32,000	2.81	27.0	9.0	96	33	240	4,260	1,500	0
30249	0	13,491	518,000	5.31	57.0	19.5	107	34	675	3,912	8,364	540
	1	8,461	275,000	4.51	40.5	14.0	90	35	254	2,315	4,823	169
	4	8,910	278,000	2.95	35.0	11.7	89	33	356	4,990	3,297	267
	8	10,389	115,000	3.60	30.0	10.3	83	34	104	6,337	3,740	208
	10	11,153	250,000	3.48	29.0	9.4	83	32	223	5,688	5,242	0
	14	8,907	57,000	3.49	28.0	9.6	80	34	89	5,611	3,028	178
	22	7,218	207,000	3.96	33.0	10.8	83	33	289	3,753	3,104	72
	29	7,364	220,000	4.13	33.0	9.6	80	29	221	3,240	3,903	0
	44	12,474	229,000	4.55	34.0	11.7	75	34	249	4,366	7,859	0
30250	0	24,166	375,000	5.97	59.0	16.6	99	28	1,208	5,075	17,883	0

Appendix 8--continued

Dog No.	Day[a]	Corr. WBC/cmm. blood	Platelets /cmm.	Erythrocytic series: RBC x 10^6 cmm.	PCV (%)	Hb. Gm. /100 ml.	MCV cu.μ	MCHC (%)	Absolute lymphocytic counts: Monocytes (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
30250 cont.	1	14,446	549,000	5.63	47.0	16.2	83	34	1,300	7,656	5,056	433
	4	11,079	425,000	5.24	42.0	14.2	80	34	554	4,321	5,983	222
	8	13,204	420,000	4.35	35.0	12.0	80	34	792	5,678	6,602	132
	10	8,846	400,000	4.26	32.0	11.2	75	35	354	4,688	3,804	0
	14	9,375	300,000	3.53	27.0	9.3	76	34	469	4,031	4,594	281
	22	7,642	342,000	4.19	33.0	10.8	79	33	153	4,509	2,904	76
	29	9,903	414,000	4.61	34.0	11.8	74	35	99	4,852	4,852	99
	44	11,153	362,000	3.46	32.0	11.6	77	34	0	5,130	5,577	446

[a]Day 0, preinoculation blood sample

[b]Dog 30250: non-contact control that remained healthy

VITA

Thomas John Kakuk was born in Manitowoc, Wisconsin, on July 31, 1963. The Kakuk family moved to Stephenson, Michigan, June 1945, where the author attended the Stephenson public schools. While in high school, the author was president of the freshman and sophomore classes, received 4 letters each in football, basketball, and track, was captain of the basketball and football teams his junor and senior years, and was editor of the school paper his senior year.

He entered Michigan State University in 1955, earning a B.S. degree in wildlife management in June 1959. The author then entered veterinary school at Michigan State University, in 1959, receiving the D.V.M. degree in June 1963. In the summer of 1963, he entered the graduate school of the University of Wisconsin, Madison, where he was awarded an NIH postdoctoral pathology traineeship grant. Under this grant, he carried out research concerning fowl leukosis, receiving the M.S. degree in veterinary science and a minor in human pathology in 1965. In June 1965 he accepted a position on the canine leukemia research project, and also entered the graduate school of Michigan State University, majoring in veterinary pathology. In July 1966 he was awarded a special NIH postdoctoral fellowship concerning canine malignant lymphoma.

The author married Martha K. Smith of East Lansing, Michigan, in 1963. They have 1 child, Robert, 2-1/2, and are expecting another child in May 1968.

The author has written several scientific publications.