Canine Systemic Lupus Erythematosus

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O F THE numerous investigations of autoimmunization, the construction of animal models has been particularly informative. Several classes of these models have been elaborated: those in which lesions are induced by the injection of organ specific antigens (thyroiditis,1 encephalitis,2 aspermato-
genesis3); those in which lesions are induced by antigen-antibody complexes (serum sickness,4 glomerulonephritis5) and those in which lesions are produced by tolerated foreign immunocytes (runt disease6,7). In addition, the adminis-
tration of Freund's adjuvant alone8 or organ-specific heterologous antisera9 will result in still other types of immunologic disorders in laboratory animals. More recently, spontaneous autoimmune disorders have been described in mice10,11 and dogs.12 The autoimmune hemolytic anemia of NZB mice has been of particular interest because of its genetic background and the associated thymic lesion. While spontaneity may not always be desirable under the controlled conditions of the laboratory, this is an important feature of human autoim-
munization with which clinicians must deal. Thus, the characterization of spontaneously occurring autoimmune disease in lower mammals may provide insights unobtainable from man-made models.

The purpose of this report is to present the clinical and pathologic features of a multisystemic disease that we observed in 7 dogs. This condition is very similar to systemic lupus erythematosus in man. Its cardinal features are autoimmune hemolytic anemia, thrombocytopenia, and membranous glomeru-
lonephritis. Positive lupus preparations and other serologic abnormalities found in these animals indicate the immunologic nature of the disorder.

METHODS

Laboratory tests consisting of white blood cell counts (WBC), packed cell volume (PCV), hemoglobin levels (Hb), white blood cell differential counts, icterus index, direct antiglobulin test, blood urea nitrogen (BUN), reticulocyte count, urinalysis, Quick pro-
thrombin time, and serum transaminase values (SGO-T, SGP-T) were determined by standard methods. Platelet numbers were estimated from the peripheral blood smears.

Paper strip electrophoresis, total serum protein determinations and Hyland rapid slide
agglutination tests for rheumatoid factor and antithyroid antibody were performed on all available serum samples of affected dogs. These latter two tests were used as screening tests. A positive test for rheumatoid factor required a serum dilution of 1:20 in glycine-saline buffer, whereas a positive test for antithyroid antibody was recorded when undiluted serum was used. Titration of serum which reacted positively to these screening tests was not done. In addition, cover slip smears were prepared in the manner described by Miale\(^1\) and examined for the presence of the typical L.E. cell. Although erythrophagocytosis, nucleophagocytosis, rosette formation and large amounts of extracellular protein were noted, a smear was considered positive only when the criteria described by Hargraves\(^2\) were fulfilled.

**Normal Values of Selected Laboratory Tests in Dogs**

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
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<tbody>
<tr>
<td>White Blood Cells</td>
<td>6000-18000/mm(^3)</td>
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<tr>
<td>Band</td>
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<tr>
<td>Neutrophil</td>
<td>60-77%</td>
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<td>12-30%</td>
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<tr>
<td>Basophil</td>
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<tr>
<td>Packed cell volume</td>
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<tr>
<td>Hemoglobin</td>
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<tr>
<td>Reticulocytes</td>
<td>0.1-0.5%</td>
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<tr>
<td>Quick prothrombin time</td>
<td>7.0-10.5 seconds</td>
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<tr>
<td>Blood urea nitrogen</td>
<td>less than 20 mg. %</td>
</tr>
<tr>
<td>SGO-T</td>
<td>less than 23 SF units</td>
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<tr>
<td>SGP-T</td>
<td>less than 27 SF units</td>
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**Case Number 1**

This 6-year-old female standard poodle (R 14511) was admitted to the Angel Memorial Animal Hospital in December, 1961, because of epistaxis. Findings during physical examination were negative except for the nosebleed.

Initial laboratory findings: WBC 39,000/mm\(^3\); PCV 30 per cent; prothrombin time 7.3 seconds. Neutrophilia with a left shift, monocytosis, polychromatophilia, anisocytosis, marked thrombocytopenia, hypochromia and 2 nucleated RBC/100 WBC were seen in the peripheral blood smear. The direct Coombs test was 3+; reticulocyte count 2.1 per cent and BUN 13 mg./100 ml. The urine was normal. The next morning the dog was severely depressed and petechiae and ecchymoses were present on the skin and mucous membranes. Both elbow joints were swollen and tender. Blood and urine samples were submitted to the laboratory. Prednisone, 10 mg. every 24 hours, intramuscularly, was started and within 1 week the symptoms had abated. An LE preparation done at that time contained extracellular "LE bodies," but typical LE cells were not seen.

She remained well until March, 1962, when listlessness, nonspecific lameness, anorexia, polydipsia and dark-colored urine developed. Physical examination revealed pallor, slight icterus, severe emaciation, prostration (the dog walked unsteadily into the room, fell down and could not rise), subconjunctival hemorrhages, epistaxis, and probable splenomegaly. The temperature was 103.6 F. Intramuscular injections of 10 mg. prednisone every 12 hours were begun and the dog was placed in oxygen.

Laboratory findings were: WBC 24,000/mm\(^3\); Hb 5.3 Gms.; BUN 10 mg./100 ml.; LE preparation, negative; direct Coombs test 1+. Neutrophilia with a left shift, monocytosis, polychromatophilia, anisocytosis, hypochromia and 19 nucleated RBC/100 WBC were present. Despite vigorous therapy with corticosteroids, blood transfusions and antibiotics, the dog gradually deteriorated over the next 7 weeks with the development of extensive purpura, multiple abscesses of the skin and subcutaneous tissues, pneumonia and azotemia, the blood urea nitrogen levels surpassing 120 mg. per cent terminally. Thrombocytopenia and anemia persisted throughout the course. The direct Coombs test was inter-
mittently positive and 2 of 15 LE cell preparations were positive. The antinuclear antibody test was positive. A red cell eluate prepared from her cells was shown to sensitize the erythrocytes of 4 randomly selected normal dogs to an indirect antiglobulin test.

Post mortem examination: Advanced mineralization of the glomeruli, tubules, Bowman’s capsules and arcuate arteries were found in the kidneys. Hyalinization and sclerosis of glomeruli were also prominent. In the heart, multiple small foci of necrosis were scattered throughout the wall of the left ventricle. Reactive hyperplasia, characterized by large numbers of plasma cells, was the prominent lesion in the spleen. Segmental hyaline change was present in the walls of central arteries. Megakaryocytes were present in the bone marrow and lymph nodes were hyperplastic. Severe purulent bronchopneumonia was present.

Summary: This dog had an illness of 6 months’ duration characterized by severe thrombocytopenia and hemolytic anemia with a positive antiglobulin test with ultimate development of renal failure. Two LE preparations were positive and an eluate prepared from her red blood cells sensitized normal canine red blood cells to an indirect Coombs test.

Case Number 2

This 4-year-old white male standard poodle (R 1049) was admitted to the Angell Memorial Animal Hospital in January, 1961, because of epistaxis. According to the owner, the animal began to bleed extensively after a dog fight. Physical examination revealed a large bruise behind the left ear, marked pallor of the mucous membranes, tachycardia, polypnea, weakness, and a temperature of 101 F.

Initial laboratory findings were: WBC 39,000/mm³; PCV 11.5 per cent; icterus index 2 units; direct Coombs test 2+. Neutrophilia with a shift to the left, monocytosis and marked thrombocytopenia were present. There was autoagglutination of the red blood cells. The urine had a specific gravity of 1.012 and traces of albumin and bile were present. There were no significant findings in the centrifuged urinary sediment.

The dog was treated with transfusions of whole blood and prednisone, 10 mg./day, and he gradually improved.

At a follow-up examination on February 23, 1961, 2 weeks after returning home, he appeared in good health. The leukocyte count was 10,000/mm³; PCV 37 per cent; and the direct Coombs test was negative. Anisocytosis, polychromatophilia, hypochromia and three unclassified cells/100 WBC were seen in the peripheral blood smear. Prednisone therapy was continued in a dose of 2.5 mg./day until March 1, 1961. On March 3, 1961, the dog was returned with a temperature of 104 F., anorexia, and a moist cough. A tentative diagnosis of acute bronchopneumonia was made and treatment with 500 mg. chloromycetin intramuscularly every hours was begun. Prompt recovery ensued from the bronchopneumonia in 5 days.

Despite continuous prednisone therapy, thrombocytopenia persisted (although there was no purpura) and in May, 1961, a splenectomy was performed. The dog responded well and was discharged on the third hospital day.

Following the operation, the platelet numbers were normal. However, 2 weeks after splenectomy, when the direct Coombs test was again found to be positive, prednisone therapy was re instituted. Six months after the splenectomy, anorexia, listlessness and vomiting reappeared. Physical examination revealed pallor, generalized lymphadenopathy, depression, and a markedly dry, hyperkeratotic nose. Laboratory examination revealed: WBC 16,000/mm³; Hct 27 per cent; direct Coombs test 2+. Neutrophilia with a left shift, polychromatophilia, anisocytosis, increased platelets and 65 nucleated RBC/100 WBC were present in the peripheral blood smear. Erythroid hyperplasia was seen in the bone marrow preparation. The urine was unremarkable except for increased urobilinogen. An LE preparation was positive. Treatment with ACTH and prednisone resulted in considerable improvement. On the ninth hospital day he was begun on BW 57-322 (Imuran), 50 mg./day. The direct Coombs test became negative and within 2 weeks the hematocrit was 45 per cent. Two weeks later the dog was returned to the hospital because of epistaxis. Physical examination again revealed pallor, depression and generalized weakness.

Laboratory findings at that time were: WBC 21,000/mm³; PCV 32 per cent; pro-
thrombin time 8.1 seconds; icterus index 10 units; BUN 24 mgs./100 ml.; SGO-T 450 S.F. units and SGP-T 5200 S.F. units. Neutrophilia with a left shift, monocyto-
penia, polychromatophilia, hypochromia and frequent target cells were again noted. The urine had a specific gravity of 1.009; albumin 1+; bile; urobilinogen 1+. The centrifuged urinary sediment contained: RBC 1–3; WBC 0; casts 4–8; epithelial cells 1–2 per high dry field. Euthanasia was performed with a lethal dose of pentothal sodium at the owner’s request.

Post mortem examination: Histologic examination of the spleen following its removal in 1962 revealed congestion, atrophy of lymphoid follicles, extramedullary hematopoiesis with large numbers of megakaryocytes, and segmental hyaline changes in some central arteries. Periarterial fibosis was not found. In the kidneys, glomerulosclerosis was not prominent, but many glomeruli contained two or three widely dilated capillary loops which stood open, although empty, and were relatively thin-walled. Multiple small sharply delineated foci of necrosis were scattered throughout the liver. Hyaline degeneration of arterioles and extramedullary hematopoiesis were noted in the lymph nodes. The megakaryocytes in the femoral marrow had a fibrillar cytoplasm and their nuclei were pyknotic. Fibrous thickening of the visceral pleura was present.

Summary: A 4-year-old male poodle simultaneously developed thrombocytopenia and hemolytic anemia with a positive antiglobulin test. Prednisone was only partially effective and a splenectomy was performed. The operation was followed by an increase in platelets to normal, but there was soon an exacerbation of the hemolytic anemia, during which the LE test was positive. Further treatment with prednisone and an antimetabolite resulted in improvement, but signs of extensive hepatitis appeared together with a relapse of the thrombocytopenia. Renal and hepatic lesions were prominent at necropsy.

Case Number 3

This 5-year-old male cocker spaniel (P 12936) was admitted to the Angell Memorial Animal Hospital on November 21, 1961, because of weakness and anorexia. The animal had been treated in the out-patient clinic with salicylates for an obscure lameness 11 months previously. He had otherwise been in good health until the day before entry when he became very listless and refused to eat. On the day of admission, the dog vomited and passed dark urine. Physical examination revealed severe icterus, polypnea, weakness and splenomegaly.

Initial laboratory findings: WBC 25,000/mm³; Hb 6.3 Gm.; BUN 67 mg./100 ml.; direct Coombs test 2+; icteric index 250; reticulocyte count 4.4 per cent. Neutrophilia with a shift to the left, monocyto- penia, thrombocytopenia, anisocytosis, polychromatophilia and 23 nucleated RBC/100 WBC were present in the peripheral blood smear. An LE preparation was positive. Urinalysis: Cloudy amber; specific gravity 1.037; albumin 4+; bile 4+; urobilinogen 4+. The centrifuged urinary sediment contained WBC 1–2; RBC 0; casts 0–1; bilirubin crystals 2± and large amounts of yellow amorphous material per high dry field.

On the second hospital day prednisone was begun in a dosage of 5 mg. every 12 hours. On the third day marked hepatomegaly was found and the dog was unable to stand. The hemoglobin level continued to fall, reaching 3.8 Gm. on the sixth hospital day at which time prednisone was increased to 10 mg. every 12 hours. The following day he began to eat, could rise, and wanted to play. The urine was normal in color for the first time on the seventh hospital day. On the ninth hospital day, he was put on a decreasing dose of oral prednisone. Laboratory findings at that time were as follows: WBC 33,000/mm³; Hb 7.8 Gm.; direct Coombs test, negative; reticulocyte count 12.9 per cent; BUN 16 mg./100 ml.; SGO-T 40 S.F. units; SGP-T 56 S.F. units. Neutrophilia, shift to the left, monocyto- penia, polychromatophilia, hypochromia, target cells, and 5 nucleated RBC/100 WBC were present in the peripheral blood smear. Urinalysis: specific gravity 1.025; albumin 4+; bile, urobilinogen 1+. The centrifuged urinary sediment contained RBC 0; WBC 5–7; casts, rarely seen; epithelial cells 2–5 per high dry field. Tests for anti-thyroid antibody and antinuclear antibody were positive.
The dog improved and was discharged on December 8, 1961, with instructions for the owner to continue treatment with prednisone for one month. On December 28, 1961, he again was returned with generalized hair loss, obesity and gray-pink hyperkeratotic skin lesions on the face and under the chin. A biopsy of this eruption showed acanthosis, particularly involving the hair follicles, focal hyperkeratosis, focal telangiectasis in the papillary layer which also contained aggregations of plasma cells and lymphocytes. There were small collections of mast cells around the dermal capillaries and a few capillaries with hyalinized walls were seen. The owner related that similar lesions had been seen prior to the hemolytic crisis, but subsequently disappeared without treatment. The obesity and generalized hair loss was thought to be due to prolonged corticosteroid therapy. As of July 1, 1963, the hair had grown back and the skin lesions on the face had again disappeared; however, he remained quite obese and has persistent albuminuria.

Summary: This male cocker spaniel developed thrombocytopenia, hemolytic anemia with a positive antiglobulin test, azotemia and proteinuria. About 1 year prior to the onset of these disturbances he had “lameness” which responded to salicylates. The hematologic abnormalities responded to prednisone, but proteinuria persisted. A skin rash involving the face showed histopathologic lesions compatible with human discoid lupus. The LE preparation was positive.

Case Number 4

This 4-year-old female toy fox terrier (N 4620) entered the Angell Memorial Animal Hospital on March 30, 1960, because of purpura. She was first seen in the out-patient clinic in January, 1960, for abdominal distention, polydipsia, a dull dry coat, and alopecia. No treatment was given and these signs subsided within a few weeks. The present illness began on the day prior to entry when the owner noticed blood in the animal’s bed. The physical examination revealed petechiae and ecchymoses of the skin and mucous membranes. The gums were bleeding actively. The temperature was 103 F. Melena and slight peripheral lymphadenopathy were noted.

Initial laboratory work was: WBC 17,000/mm³; Hb 6.8 Gm. per cent; prothrombin time 7.8 seconds (normal 6-9 seconds); direct Coombs test 3+. Severe thrombocytopenia, moderate anisocytosis, polychromatophilia and 4 nucleated RBC/100 WBC were found. The leukocyte differential formula was normal. Urinalysis: clear yellow; specific gravity 1.023; albumin 4++; bile and indican, trace. The centrifuged urinary sediment showed RBC 1–3; WBC 10–20; casts 0–1; epithelial cells 1–3 per high dry field.

On the second hospital day, the dog appeared very weak and had a bloody vaginal discharge. She was given 160 ml. of whole blood, 10 mg. prednisone intramuscularly, and 2.5 mg. prednisone orally every 6 hours. By the fifth hospital day she showed marked improvement, and the peripheral blood smear now contained normal numbers of platelets. However, 4+ albuminuria was still present.

She was discharged on the seventh hospital day on maintenance dose of prednisone for two weeks. The dog remained asymptomatic for 6 months, but in October, 1960, extensive skin and mucous membrane bleeding and thrombocytopenia again appeared. The PCV was 34 per cent and the direct Coombs test was negative. Treatment with prednisone was reinstituted and during the next week the dog gradually improved. Despite continuous prednisone therapy, thrombocytopenia persisted and in January, 1961, a splenectomy was performed. Postoperatively, the dog maintained normal platelet numbers without medication and was free of purpura. However, on June 16, 1961, she returned with hemorrhagic enteritis and was subsequently treated for this disorder four times within the next 2 months. On August 23, 1961, she entered the hospital with a history of anorexia for 4 days, polydipsia, polyuria, vomiting and melena. Laboratory work revealed a hemoglobin level of 10.5 Gm., BUN 154 mg./100 ml. and normal platelets. The urine now had a specific gravity of 1.011, albumin 1+, and 0–2 hyaline casts were seen per high dry field. The dog was treated for uremia and discharged on the eighth hospital day.
On February 27, 1962, the dog entered the hospital with severe uremia. The BUN was 253 mg./100 ml. and an LE preparation was positive. The test for anti-thyroid antibody was positive. The animal was sacrificed with an overdose of pentothal sodium.

Post mortem findings: Necropsy revealed small contracted kidneys of unequal size. Both kidneys contained healed infarcts. Outside the wedge-shaped areas of infarction the glomerular capillaries and small arterioles were thickened and hyalinized, and a generalized mononuclear intertubular exudate was evident. The spleen, which was examined following the splenectomy in January, 1961, was severely congested, contained hyperplastic lymphoid follicles and mild extramedullary hematopoiesis. Segmental thickening and hyalinization of the walls of central arteries was found, but periarterial fibrosis was absent. Diffuse fatty infiltration of the liver was present. The lymphoid follicles in the body lymph nodes were hyperplastic and no evidence of extramedullary hematopoiesis was seen.

Summary: This toy fox terrier developed severe thronibocytopenia and hemolytic anemia with a positive antiglobulin test which were treated at first with corticosteroids and later by splenectomy. Removal of the spleen resulted in an 8-month remission. However, 23 months after the initial symptoms of her disease, she developed progressive uremia. The L.E. cell test was positive and anti-thyroid antibodies were found in her serum. Glomerulosclerosis with "wire loop" lesions was present at autopsy.

Case Number 5

On January 25, 1961, this 4-year-old spayed female German shepherd (R 705) was presented to Angell Memorial Animal Hospital with a history of weakness of the hind legs, anorexia, malaise, polydipsia and sporadic vomiting. These signs had been present for 6 days. Physical examination revealed a temperature of 102 F., extreme pallor, tachycardia and polypnea. Following the physical examination, the dog collapsed and was hospitalized. Specific treatment consisted of whole blood and prednisone, 2.5 mg. every 8 hours.

Initial laboratory findings were: WBC 54,000/mm³; PCV 20 per cent; direct Coombs test 2+. Neutrophilia with a left shift, monocytosis, marked polychromatophilia, anisocytosis, macrocytosis, hypochromia, and 13 nucleated RBC/100 WBC were seen in the peripheral blood smear.

Urinalysis: cloudy yellow; specific gravity 1.028; albumin 1+; bile, trace; urobilinogen, trace. The centrifuged urinary sediment contained no RBC; 0-2 WBC and rare granular casts per high dry field.

By the fourth hospital day, the dog had regained her appetite and was bright, alert, and active in her cage. She was discharged on February 3, 1961, the ninth hospital day, and at this time had a PCV of 38 per cent, WBC 20,00/mm³; direct Coombs test 1+. Home medication consisted of a decreasing dose of prednisone for a 2-week period, multiple vitamins, and an oral hematinic (Perhiemin Junior-Winthrop). On February 15, 1961, after 12 days at home, the dog was returned to the hospital with a history of progressive anorexia, vomiting, weakness and polypnea. Physical examination revealed pallor, hepatomegaly, tachycardia, polypnea and a temperature of 104 F. The laboratory findings were: WBC 30,000/mm³; PCV 20 per cent; direct Coombs test 2+. Neutrophilia, monocytosis, polychromatophilia, anisocytosis, macrocytosis and 22 nucleated RBC/100 WBC were present. The urine was dark amber in color and had a specific gravity of 1.037; bile 2+; urobilinogen 3+. The centrifuged urinary sediment contained RBC 20-30; WBC 1-2; and no casts per high dry field. Treatment consisting of 5 mg. prednisone every 8 hours was started. By the fifth hospital day the dog had again regained her appetite and appeared bright and alert. She was discharged on February 25, 1961, the tenth hospital day, with instructions for the owner to give decreasing doses of prednisone for 3 weeks. The direct Coombs test was still positive at the time of discharge and 144 nucleated RBC/100 WBC were present in the peripheral blood. After ten days at home, the dog again developed vomiting, weakness, malaise and pallor and was admitted to the hospital for the third time. In addition, the dog was jaundiced at this time and splenomegaly was detected. The laboratory find-
ings consisted of WBC 54,000/mm³; PCV 16 per cent; direct Coombs test 3+. Neutrophilia with a shift to the left, monocytosis, polychromatophilia, anisocytosis, hypochromia, macrocytosis, target cells and 85 nucleated RBC/100 WBC were again noted. The dog was treated with 10 mg. prednisone intramuscularly every 12 hours for 7 days, at which time marked clinical improvement was evident. Three hundred ml. of compatible whole blood was given intravenously on March 13, 1961, and repeated on March 15, at which time a splenectomy was performed. An uneventful recovery from surgery followed and the dog was discharged on March 23, 1961. A decreasing dose of prednisone was prescribed for 1 week. At the time of discharge, the laboratory findings were: WBC 52,000/ mm³; PCV 28 per cent; direct Coombs test 2-. The peripheral blood smear still revealed neutrophilia, monocytosis and 82 nucleated RBC/100 WBC. Periodic examinations, commencing on March 31, disclosed a consistently negative direct Coombs test, and lack of any clinical signs of exacerbation. In the differential smears, however, polychromatophilia, anisocytosis, poikilocytosis, Howell Jolly bodies, hypochromia and an occasional nucleated RBC were seen. Examination in August, 1962, revealed a reticulocyte count of 1.4 per cent and a BUN of 21 mg./100 ml. An LE cell preparation was positive. The LE preparation was again positive in January, 1963, and mild albuminuria was detected.

Histologic examination of the spleen following its surgical removal revealed severe extramedullary hematopoiesis and hemosiderosis. Periarterial fibrosis was absent, but many of the central arteries contained segmental deposits of hyaline material within their walls.

Summary: This female German shepherd was found to have severe hemolytic anemia with a positive antiglobulin test which eventually required splenectomy. The operation resulted in a prompt amelioration of the hemolytic anemia and the direct Coombs test became negative. Follow-up examinations for 20 months after splenectomy found the dog in apparent good health, but with positive LE tests and albuminuria.

Case Number 6

This 5-year-old ovariohysterectomized female terrier (P 12411) entered the Angell Memorial Animal Hospital on April 15, 1961, because of weakness, lethargy and anorexia. The owner related that 3 months before the dog had been treated at another hospital for pneumonia which was followed by rapid recovery.

The physical examination at admission to the hospital revealed severe pallor, tachycardia, extreme weakness and a temperature of 103 F. Initial laboratory findings were: WBC 24,000/mm³; PCV 13 per cent; Hemoglobin 4.1 Gm. per cent; BUN 25 mgs./100 ml.; icterus index 15 units; direct Coombs test 2+; auto-agglutination of the RBC was observed. Neutrophilia with a shift to the left, monocytosis, 20 nucleated RBC/100 WBC, anisocytosis, and polychromasia were seen in the peripheral blood smear. The platelets were decreased in number, large in size and often in odd forms.

Urinalysis: clear yellow; specific gravity 1.017; albumin 3±; bile 1+; no urobilinogen. The centrifuged urinary sediment had 0-2 RBC; 1-2 WBC; 0-2 casts and 1-2 small epithelial cells per high dry field. The dog was treated with 120 ml. whole blood and prednisone, 10 mg. every 12 hours. She improved considerably and was discharged on the eleventh hospital day with a maintenance dose of prednisone. Two days following discharge, she returned with epistaxis. Topical adrenalin and cold packs stopped the hemorrhage and the owner was instructed to continue the prednisone therapy for 1 month. Although prednisone was continued, the dog continued to have thrombocytopenia and purpura, and in June, 1961, a splenectomy was performed. Following the operation the blood findings were, for the first time since the onset of her illness, normal. However, urinalysis continued to show severe proteinuria. Sixteen months after splenectomy the dog was readmitted to the hospital because of anorexia and lethargy. Physical examination revealed pallor, hepatomegaly and a temperature of 103 F. The laboratory findings were: WBC 55,000/mm³; Hb. 5.5 Gm.; direct Coombs test, negative; reticulocyte count 1.6 per cent; BUN 24 mgs./100 ml. Neutrophilia with a shift to the left, polychromatophilia, anisocytosis, poikilocytosis, hypochromia, thrombocytopenia, and 30 nucleated RBC/100 WBC
Leukemia were seen in the peripheral blood smear. Urinalysis: cloudy brown; specific gravity 1.040; albumin 4+; bile, trace and urobilinogen 2+. The centrifuged urinary sediment contained 13–15 RBC; 25–30 WBC; 1–2 casts per high dry field. An LE preparation was negative, but tests for rheumatoid factor, anti-thyroid antibody and antinuclear antibody were positive. Prednisone, 10 mg. every 8 hours, was started on entry.

After initial signs of improvement, the dog became icteric, began vomiting blood-tinged froth and had a subnormal temperature. The icterus became more pronounced and the vomiting continued. The BUN rapidly rose to 200 mg./100 ml. and the SGO-T and SGP-T were found to be markedly elevated (2960 and 1560 S.F. units respectively.) She developed Cheyne-Stokes respiration and died on the eighth hospital day.

Post mortem examination: Histologic examination of the spleen following its removal revealed congestion, hemosiderosis, extramedullary hematopoiesis and hyalinization of some of the central arteries. There was no periarterial fibrosis.

The kidneys were found to have hyalinized and sclerotic glomeruli, thickened hyalinized Bowman’s capsules and periglomerular infiltrations of plasma cells. A small hemorrhagic infarct was present in one kidney. The liver contained large confluent areas of necrosis. An occluding thrombus was present in a muscular branch of the left coronary artery and multiple small infarcts were present throughout the myocardium of both ventricles. Large numbers of plasma cells and a paucity of lymphocytes were present in the lymph nodes. The femoral marrow was adequately cellular; however, few megakaryocytes were present.

Summary: A 5-year-old female terrier developed thrombocytopenia and hemolytic anemia with a positive antiglobulin test. Proteinuria was found at the onset of her disease. Prednisone had only a partial effect on the hematologic abnormalities, but splenectomy was followed by a 16-month remission. The terminal episode was characterized by sudden appearance of hepatic and renal failure with thrombocytopenia. An L.E. test was negative, but antinuclear and antithyroid antibodies and rheumatoid factor were found in her serum.

Case Number 7

This 2-year-old male wire-haired fox terrier (T 491) was admitted to the Angell Memorial Animal Hospital in January, 1962, because of anorexia, malaise, diarrhea, dark red urine and a lameness of the hind limb. These signs had been apparent for 3 days. Pallor, depression, splenomegaly, and a temperature of 103 F. were noted on the physical examination.

Following a 4-day course of penicillin the laboratory findings were: WBC 46,000/mm³; PCV 26 per cent; reticulocyte count 11.2 per cent; icterus index 5 units; BUN 7 mg./100 ml.; direct Coombs test 2++; SGO-T 26 S.F. units and SGP-T 19 S.F. units. Neutrophilia with a left shift, thrombocytopenia, polychromatophilia, anisocytosis and 3 nucleated RBC/100 WBC were present in the differential smear. Urinalysis: cloudy amber; specific gravity 1.045; albumin, trace; bile 4+; urobilinogen 2+. The centrifuged urinary sediment contained: RBC 1–2; WBC 1–3 and granular casts 0–2 per high dry field.

Prednisone was started at this time and a prompt recovery followed. The dog was discharged on the eleventh hospital day with a WBC of 18,000/mm³; PCV 34 per cent; reticulocyte count 1.2 per cent; and a weakly positive direct Coombs test. Prednisone was prescribed for 2 weeks at home. Physical examination 6 weeks after discharge revealed the dog to be in apparent good health. The hematologic and urinary findings were unremarkable, and the direct Coombs test was negative at this time. However, the LE preparation, anti-thyroid antibody test, and latex fixation test for rheumatoid factor were positive. The serologic test for syphilis was negative. Up to this writing, the dog has remained in apparent good health.

Discussion

The disease affecting these animals occurred in a variety of breeds: 2 poodles, 1 cocker spaniel, 1 fox terrier, 1 German shepherd, 1 wire-haired...
terrier, and 1 mongrel. The dogs ranged in age from 4 to 6 years (roughly equivalent to 30–40 years in man). Two of the 4 females had been ovariohysterectomized and only 1 of these 4 had borne a litter. None of the dogs had received any medication prior to the onset of illness and, as far as is known, none were exposed to toxic chemical agents. In 6 of the dogs the onset of the disease occurred during the winter months (November, December, January and March) and in the seventh dog, purpura first appeared in April. Each dog had been vaccinated against canine distemper and the interval between the vaccination and the appearance of this disorder was at least 3½ years.

These 7 dogs had a similar pattern of disease: hemolytic anemia, thrombocytopenia and nephritis (table 1). The disorder first became clinically evident as a result of the hematologic abnormalities, but in 3 of the animals, proteinuria was noted at the first examination. Several other abnormalities were also found including intermittent lameness of unknown cause in 2 dogs, alopecia in 2 and an eruption on the butterfly area of the face in 1 dog.

Hemolytic anemia was the most prominent feature of the disease in these dogs (fig. 1). It was characterized by hemoglobin levels as low as 3.5 Gm. per cent, reticulocytosis, normoblastemia and urobilinuria. Jaundice and spherocytosis were variable findings. There was hyperplasia of the erythroid elements in the bone marrow. In each case, the direct antiglobulin test was positive; the strength of the reaction varied between 1+ and 3+. Thus, these

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Table 1.—Prominent Findings in Dogs with Systemic Lupus Erythematosus

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LEGEND

+ = PRESENT
- = ABSENT
0 = NOT DETERMINED
Fig. 1.—Peripheral blood from a dog with systemic lupus erythematosus and autoimmune hemolytic anemia. Macrocytosis, spherocytosis and polychromatophilia are present. (Wright-Giemsa)

dogs had the typical features of autoimmune hemolytic anemia as seen in man. An eluate prepared from the red cells of dog number R 14511 sensitized normal canine erythrocytes, further substantiating the presence of an erythrocyte autoantibody. Although each dog responded initially to prednisone therapy, 4 had recurrent hemolytic episodes and eventually underwent splenectomy. Thereafter, anemia was not a major problem until azotemia appeared. In contrast to what is usually found in human beings with this condition, the direct antiglobulin test in these dogs always became negative shortly after the institution of therapy. The reappearance of a positive antiglobulin test in those dogs which were in remission was soon followed by a recurrent attack of hemolytic anemia.

The next most common feature of this canine disease was thrombocytopenia. This abnormality was found in 6 animals and was associated with an overt hemorrhagic tendency in four. The bleeding manifestations were characterized by easy bruising, ecchymoses, petechiae, and active bleeding from mucous membranes. Bone marrow aspirates of 2 of these thrombocytopenic dogs contained normal numbers of megakaryocytes. This finding, together with the prompt response to corticosteroids suggests that the thrombocytopenia in these dogs is analogous to the idiopathic (ITP) variety seen in man.
Fig. 2.—Skin biopsy from malar eruption of Case No. 3. Hyperkeratosis, parakeratosis, telangiectasis of vessels in the papillary layer of the dermis and accumulations of mononuclear inflammatory cells in the dermis are present. (Hematoxylin-eosin)

The simultaneous occurrence of idiopathic thrombocytopenic purpura and autoimmune hemolytic anemia described in man (Evan's syndrome) is associated with a high incidence of systemic lupus erythematosus.

Five of these dogs also manifested signs of renal involvement during some phase of their illness. In three, marked proteinuria was found at the outset and renal failure was the cause of death in 4 dogs of this series. Corticosteroids failed to improve the nephritis.

Of particular interest was the development of a dry scaly eruption involving the butterfly area of the face in dog number P 12936. Clinically, this lesion bore a resemblance to the eruption of lupus in man only because of its location. Histologically, however, there was a striking resemblance to human discoid lupus (figs. 2, 3). This dog had had two bouts of this rash—one of which antedated the onset of the autoimmune hemolytic anemia. It should be noted that this animal had, also, salicylate-responsive "lame-ness" 1 year to the hematologic disorder. Thus, this dog exhibited the sequential development of arthritis, butterfly rash, autoimmune hemolytic anemia, idiopathic thrombocytopenia and finally, nephritis, within a period of approximately 1 year.

The prominent gross findings at necropsy were related to anemia, uremia, or terminal bacterial infection, such as bronchopneumonia. Histologically, chronic membranous glomerulonephritis with wire loop lesions was a consistent finding (fig. 4). Extramedullary hematopoiesis including numerous megakaryocytes was a uniform finding in these dogs. In addition, hepatic
necrosis was observed in 2 dogs, and myocardial infarction resulting from thrombosis of small muscular coronary arteries was found in 2 other dogs. Periarterial fibrosis ("onion skin" lesions) in the spleen was specifically sought, but not found. However, fibrinoid necrosis, which was segmental in nature, was observed in the walls of central arteries in the spleens of 5 dogs (fig. 5). Furthermore, hematoxylin bodies were not recognized in the tissues studied from these dogs.

There has been renewed interest in autoimmune diseases in recent years, and numerous disorders of obscure cause are currently being considered as due to autoimmunization. The validity of some of these assertions must await the accumulation of more data. The widespread use of "immunologic"
Fig. 4.—Chronic membranous glomerulonephritis in a dog with systemic lupus erythematosus. “Wire loop” lesions, hyalinization of glomerular tufts, and adherions of glomerular tufts to Bowman’s capsule were found. (Periodic acid-Schiff)

tests has disclosed the presence of circulating antibodies in many diseases not hitherto thought to have an immunologic component. It is thus difficult to render a precise working definition of an autoimmune disorder. MacKay and Burnet have listed six features or “markers” of autoimmunization which seem to be acceptable guidelines in dealing with this problem. These are hyperglobulinemia, the presence of autoantibodies in the serum, the deposition of denatured gamma globulin or its derivatives in tissues, accumulations of lymphocytes and plasma cells in damaged tissues, benefit from corticosteroids, and the coexistence of multiple autoimmune disorders in the same individual. Taken by themselves, none of these “markers” are conclusive; however, when each of them occurs at one time or another during the course of an illness, they are of considerable importance. As far as is known at present, the only process which could give rise to this constellation of “markers” is an immunologic one.

Most of these “markers” of autoimmunization were found in the canine disorder described in this report. Autoantibodies, such as rheumatoid factor, LE factor (fig. 6), antithyroid antibody and positive antiglobulin tests were demonstrable in various combinations in every case (table 2). Accumulations of plasma cells were found not only in damaged tissue, but in the lymph nodes and spleens as well (fig. 7). In each case there was a prompt initial response to corticosteroids, but 4 dogs eventually underwent splenectomy be-
cause of recurrent manifestations of the hematologic components of this disease. Multiple lesions involving the red cells, platelets, kidneys, skin, and other organs, were found. No tests for the deposition of gamma globulin in damaged tissue were made, so this "marker" cannot be evaluated. Although hypergammaglobulinemia was not found in these dogs, its absence does not rule out an immunologic disturbance.*

The validity of the various serologic procedures was tested in our laboratory in the following manner: Serum samples from 25 randomly selected dogs in apparent good health were used as controls for the latex fixation and antithyroid antibody tests, total serum protein levels, and serum paper strip electrophoresis. No control serum samples reacted in the tests for rheumatoid factor or antithyroid antibody. The amount of gamma globulin present in the control serum samples ranged from 4.8 to 28.8 per cent. This variability in the amount of gamma globulin present in dog serum coincides with previous reports on the inconsistency17 of results of serum electrophoresis in healthy and diseased dogs. Our recent observation of well-defined hypergammaglobulinemia only during the prodromal stage of autoimmune hemolytic anemia in a dog may indicate that the time of sample collection from affected dogs is extremely important in the detection of abnormally high serum levels of gamma globulin. A test for antinuclear antibody was performed on the serum of 5 of these dogs. A positive test was recorded in 3 and in the asymptomatic daughter of Case Number 1. However, due to the lack of ade-

*Since submission of this report, 8 more dogs with SLE have been studied. Three of these animals had hyperglobulinemia.
Thus, using the broadest pathologic and clinical criteria it is evident that these dogs had an immunologic disorder of the autoimmune type. This was not a condition confined to one organ as is Hashimoto's disease, for example, but was a generalized process which attacked two principal systems—blood cells and blood vessels. The immunologic condition of man which exemplifies this multisystemic involvement is systemic lupus erythematosus. There are many reasons for believing that a nearly identical disorder has been found in these dogs.

There are many similarities and some differences between canine and human lupus. The similarities include the onset in young adults; the presence
Table 2.—Serologic Abnormalities in Dogs with Systemic Lupus Erythematosus

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of multiple “autoantibodies,” including the LE factor; the hematologic, renal, dermatologic and cardiac lesions; and the sequential development of one abnormality superimposed on another. The differences include the absence of any discernible photosensitivity; the lack of “onion skin” lesions in the spleen; the absence of hematoxylin bodies in tissue sections; the striking hepatic necrosis that was seen in two cases; and the lack of hyperglobulinemia. These differences may simply represent species variations. In any event, we do not believe they detract from the broad resemblances between the two forms of the disease. Hopefully, further studies on canine lupus will yield information useful in understanding human lupus.

SUMMARY

A naturally occurring immunologic disease, characterized by autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura and nephritis, is described in seven dogs. The disease primarily affected formed elements in the blood, glomeruli and blood vessels. Abnormal serum proteins, including the LE factor, rheumatoid factor and antithyroid antibody were found in affected dogs. Corticosteroids and splenectomy were effective in controlling the hematologic components of the disease, but apparently had no effect on the renal lesions. Recurrence was frequent and the prognosis was grave. Additional abnormalities, including malar eruption, intermittent lameness and alopecia were occasionally found as an integral part in the sequential involvement of several tissues during the course of the disease.

ACKNOWLEDGMENTS

We are indebted to Miss Jane Reinsborough for technical assistance, Dr. W. A. Bardawil for performing the antinuclear antibody tests, and Dr. T. C. Jones for aid in preparing the manuscript.
CANINE SYSTEMIC LUPUS ERYTHEMATOSUS

Fig. 7.—Lymph node from a dog with systemic lupus erythematosus. Large accumulations of plasma cells are within the sinusoids. (Hematoxylin-eosin)

SUMMARIO IN INTERLINGUA


REFERENCES


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Canine Systemic Lupus Erythematosus

ROBERT M. LEWIS, ROBERT SCHWARTZ and WILLIAM B. HENRY, JR.