CANINE SYSTEMIC LUPUS ERYTHEMATOSUS

Genetic Analysis of an Established Breeding Colony*

By ROBERT M. LEWIS, D.V.M., and ROBERT S. SCHWARTZ, M.D.

(*From the Departments of Surgery and Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111*)

Systemic lupus erythematosus (SLE)[1] occurs in dogs and like its counterpart in man, it is a multisystem disease usually affecting young adult females and often terminating in renal failure (1, 2). The typical patient with canine SLE sequentially or simultaneously develops autoimmune hemolytic anemia, thrombocytopenic purpura, and glomerulonephritis. In some cases symmetrical polyarthritis, dermatitis, or thyroiditis also occur. In addition to the clinical picture, the diagnosis of SLE is substantiated by laboratory findings, which include positive LE cell tests, antinuclear antibodies, complement-fixing antibodies to DNA-histone complexes, positive antiglobulin tests, and rheumatoid factor (3–5). There is no predilection for any particular breed to develop canine SLE, and no environmental stimuli that induce the disease have been identified.

Apart from its importance in veterinary medicine, the disease in dogs provides an opportunity to study the etiology and pathogenesis of SLE in a large subhuman species. In 1965 steps were taken to acquire dogs with SLE, to breed them, and to establish a colony in which genetic and environmental factors related to the disease could be analyzed. In this paper we will describe the outcome of breeding experiments designed to test the hypothesis that SLE is genetically transmitted. Our findings cannot be fully explained by any conventional genetic mechanism; instead, vertical transmission of the disease by nongenetic means seems likely.

Methods

Animals.—The colony is housed in the Animal Research Center, Southborough, Mass. In this isolated country environment the dogs are kept in 9 × 5 ft cement-floor pens, and they are fed a commercial kibbled dog food and water *ad libitum.* With the exception of a breeding colony of normal beagle dogs housed within this same facility, the SLE colony dogs have only intermittent and brief exposure to outside personnel or other animals.

All adult dogs in this facility are managed in a similar fashion. In addition to routine maintenance requirements, each animal is examined visually every day, routine health care

[1] *Abbreviations used in this paper:* ANA, antinuclear antibodies; ITP, idiopathic thrombocytopenic purpura; PBS, phosphate-buffered saline; SLE, systemic lupus erythematosus.

is provided by the veterinary staff, and every animal receives a biannual vermifuge and vaccination for canine distemper and infectious canine hepatitis.

Pregnant dogs are isolated in a nursery unit and delivery is attended by personnel trained in veterinary nursing. The puppies are raised as litters, with intensive care being provided during the first 2 wk of life. They are weaned at 4 wk and returned to the boarding area when they are 6 wk old.

When possible, female dogs are bred during their first estrus, as determined by examination of vaginal cytology (6). Two observed breedings are required to insure maximum production. In those instances where compatible matings cannot be achieved naturally, artificial insemination is utilized to maintain the breeding program.

Characteristics of the Parents.—The A breeding line originated from a mating between two purebred German shepherd dogs. The male was a normal registered show dog with no history of serious illness and no serologic evidence of canine SLE. The female had ragweed sensitivity from the age of 2 yr, but was otherwise in good health until the age of 4 yr, when she developed acute hemolytic anemia with a positive direct antiglobulin test the day after a family vacation at the beach. The disease, which was characterized by weakness, severe anemia, reticulocytosis, jaundice, hemoglobinuria, and proteinuria, was treated with high doses of corticosteroids and recovery was prompt. Positive LE cell tests were observed and antinuclear antibody was detected in her serum during the hemolytic crisis. After recovery, her annual episodes with ragweed hay fever continued and intermittently she developed eczema during the warm summer months. When 6 yr old, she delivered a litter of eleven normal puppies and these formed the nucleus of the A breeding line. 4 yr later she developed severe, unresponsive, nonbacterial colitis, and due to continuing debilitation, euthanasia was performed. Postmortem examination was not possible.

The B breeding line resulted from a mating in which both parents had SLE. The dam was a purebred German shepherd that developed acute hemolytic anemia at the age of 6 months. The disease was characterized by severe anemia, a positive direct antiglobulin test, reticulocytosis, thrombocytopenia, leukopenia, splenomegaly, and lymphadenopathy. Antinuclear antibody and LE cell tests were positive, and complement-fixing antibodies to DNA-histone complexes were detected in her serum. She responded well to corticosteroid therapy, and remained in good health for 5 yr, during which time she gave birth to five litters of puppies.

The male used in originating the B breeding line developed idiopathic thrombocytopenic purpura (ITP) at the age of 3½ yr. Before this, the only abnormality noted by the dog's owner was a severe necrotizing solar dermatitis, which occurred on the unpigmented portion of the muzzle each summer. Treatment of the ITP with corticosteroids was initially successful but three relapses occurred over a period of 10 months. Each of these episodes was characterized by severe thrombocytopenia, extensive purpura, and epistaxis. Megakaryocytes in the femoral marrow were present in normal numbers. Although steroids were effective in overcoming the clinical signs, the repeated exacerbations led to splenectomy in November 1965. Positive LE cell tests and antinuclear antibody were noted early during the disease. Since splenectomy, bleeding has not reoccurred; however, vasculitis and thrombosis, which have plagued the dog, required amputation of three toes.

The C breeding line resulted from mating two purebred French poodle dogs. The male was unaffected and the female had the following history. When 3½ yr old, she was presented with muscle pain, ill-defined joint pain, and weakness. Radiographic abnormalities were not present at this time and the clinical diagnosis was that of herniated cervical disc. Despite enforced rest at home, multiple leg lameness and joint pain persisted. In addition, she developed eczema, and focal necrotizing dermatitis 4 months later. Severe, progressive, symmetrical polyarthritis affecting the distal joints of all limbs was evident 1 yr after the initial signs of her illness. Biopsy of a joint capsule revealed lesions consistent with those of rheumatoid arthritis. In

addition, hyperglobulinemia, positive LE cell tests, and leukopenia were found. This dog has produced three litters of puppies since developing her disease, and the symptoms of symmetrical polyarthritis are moderately controlled with aspirin.

Laboratory Techniques.—Samples of whole blood, serum, and urine were collected at regular intervals. Routine laboratory methods were used in performing blood counts, urine analyses total serum protein determinations, serum electrophoresis, and bone marrow aspirate examinations. In addition, automated laboratory microtechnics were used to obtain serum levels of cholesterol, calcium, phosphorus, bilirubin, uric acid, urea nitrogen, glucose, lactic dehydrogenase, alkaline phosphatase, and transaminase in selected animals.

LE cell tests were performed according to the method of Hargraves (7). Preparations containing two or more classical LE cells were considered positive. An indirect immunofluorescent test for antinuclear antibody was performed using frozen sections of newborn rat liver as a source of nuclei and monovalent rabbit anti-dog IgG sera as the fluorescein conjugate. A fluorescein-conjugated polyvalent rabbit anti-canine immunoglobulin serum was also used to detect antinuclear antibody in 25 sera that were negative when tested with the monovalent antiserum. In no case were the results positive. Dog sera were tested both undiluted and after 1:5 dilution in phosphate-buffered saline (PBS). Sera with positive reactions were serially diluted in PBS and the highest titer giving a positive result was determined. The pattern of nuclear staining was recorded for each positive result. Tests for rheumatoid factor were performed by incubating bentonite particles coated with canine IgG, previously purified by zone electrophoresis, with 1:20 dilutions of test sera on a rotating agglutination plate. Positive tests were recorded when 50% or more of the bentonite particles were observed as large agglutinations. The titer of rheumatoid factor in positive samples was then determined by doing a similar test on serial fivefold dilutions of the serum. A microimmunoelectrophoretic method (8) was used to analyze serum samples for the presence of canine immunoglobulins (9), transferrin, and β_1C globulin.

Control animals used in these studies came from four sources: (a) normal beagle dogs housed in the same facility as the SLE colony; (b) inbred beagle dogs from a colony at Harvard Medical School; (c) inbred Labrador retriever dogs from a colony at the University of Oregon Medical School; and (d) hospitalized animals from the Angell Memorial Animal Hospital, Boston, Mass.

RESULTS

To date, 480 animals have been born in the colony from brother-to-sister matings, and third generation offspring have reached breeding age in each breeding line. Clinical signs of SLE have not yet appeared in any of the colony animals. This is not unexpected, however, since a majority of the animals are less than 3 yr old, an age at which SLE does not usually develop in the random dog population. Despite the lack of overt signs of disease, three major abnormalities have developed: excessively high neonatal mortality, multiple serologic abnormalities, and thymic lesions.

Neonatal Mortality.—Decreased fertility and increased neonatal death rates have generally been accepted as complications that may arise during the development of inbred strains of animals, but these findings have not been well documented in dogs (10). It is thought that a neonatal mortality rate of about 20% can be expected in dog breeding colonies. In our colony, the mortality rate of animals under 6 months of age was very high (Table I). Taking 6

months as the age below which death might be related to congenital causes, the neonatal mortality rates of first generation offspring (F_1) in each of the breeding lines falls within the cited figure of 20%. However, the rate in second generation (F_2) progeny was considerably higher than this in each of the breeding lines (Fig. 1), and it continued to rise in the A and B lines in the third generation (F_3).

TABLE I

Mortality Between Birth and 6 Months of Age

	F_1	F_2	F_3
A line			
No. of puppies born	11	90	68
Males born	5	36	29
Males died	0	24	29
Females born	4	51	18
Females died	0	38	16
Dead, of unknown sex	2	3	21
Total mortality	18.1%	72.2%	97.0%
B line			
No. of puppies born	43	145	38
Males born	17	71	14
Males died	2	32	6
Females born	26	64	22
Females died	4	27	18
Dead, of unknown sex	0	10	2
Total mortality	13.9%	47.9%	68.4%
C line			
No. of puppies born	7	55	23
Males born	2	30	14
Males died	0	15	6
Females born	4	25	9
Females died	0	12	3
Dead, of unknown sex	10	0	0
Total mortality	14.3%	49.1%	34.7%

Analysis of autopsy material from 184 puppies that died between birth and 6 months of age revealed a degree of consistency in both the distribution and character of the lesions present. When the most significant lesions are classified by body system (Fig. 1), the pattern of distribution is the same for all three breeding lines. Within the most commonly affected systems, evidence of bacterial infection was present in the majority of cases. The most frequently encountered lesions were purulent bronchopneumonia, purulent omphalophlebitis and hepatitis, and bacterial enterocolitis. Lesions suggestive of canine SLE were not found in these animals.

In addition to the rising neonatal mortality rate, there was a reduction in litter size with each successive generation (Table II). Although the average number of puppies in F_1 litters was within the range of expected values for the breeds composing the colony, a progressive reduction in litter size occurred in each subsequent generation of all three breeding lines. This reduction is most apparent in the F_3 progeny of the A breeding line.

Minor differences in the male to female sex ratio exist within the second and third generation offspring of each breeding line, but no significant effect of inbreeding on the sex ratio was found when each breeding line was examined
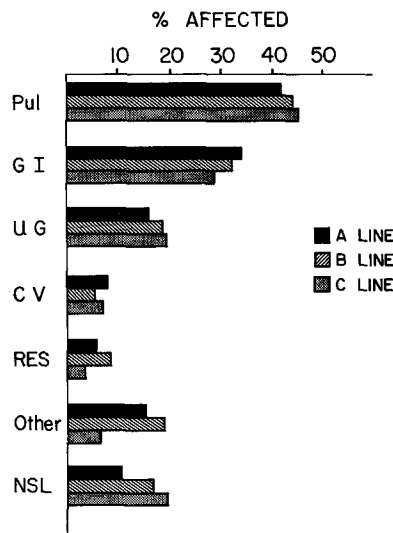

FIG. 1. Classification of lesions responsible for neonatal mortality by body system. Pul = pulmonary; GI = gastrointestinal; UG = urogenital; CV = cardiovascular; RES = reticulo-endothelial; NSL = no significant lesion; Other = all other systems.

individually (Table I). In summarizing overall production figures, in those cases where the sex could be determined 219 male and 223 female puppies have been born. No consistent variations have been observed in the neonatal mortality figures of male as compared to female puppies.

Serologic Abnormalities.—The LE cell test was selected as the primary laboratory screen to monitor the colony because of its specificity for SLE in the random dog population. Consequently, LE cell tests have been made serially over a period of years on members of the colony and a high incidence of positive tests has been found in the offspring within each breeding line (Table III). As can be seen in Fig. 2, conversion of the test from negative to positive usually occurred before 1 yr of age, and frequently when the litter was less than 6 months old. Commonly, all or most of the members of a litter developed positive

TABLE II

Puppy Production in SLE Colony

	F₁	F₂	F₃
A line			
No. of litters	1	12	12
No. of puppies	11	90	68
Range	—	2–11	3–12
Average litter size	—	7.5	5.6
B line			
No. of litters	5	23	27
No. of puppies	43	145	38
Range	4–11	3–11	1–9
Average litter size	8.6	6.3	5.4
C line			
No. of litters	1	11	5
No. of puppies	7	55	23
Range	—	4–6	4–5
Average litter size	—	5.0	4.6

TABLE III

Serologic Abnormalities and Genetic Background

Strain and generation	LE cell test	
	No. positive	% positive
A breeding line		
P	1/2	50.0
F₁	7/7	100.0
F₂	21/24	87.5
F₃	—	—
B breeding line		
P	2/2	100.0
F₁	37/38	97.3
F₂	46/57	80.7
F₃	—	—
F₁ × P	4/7	57.1
F₁ × Normal	2/6	33.3
C breeding line		
P	1/2	50.0
F₁	6/6	100.0
F₂	13/19	68.4
F₃	9/12	75.0
F₁ × P	5/7	71.4

tests at about the same time. The high percentage of positive tests first detected at 3 months of age reflects the fact that routinely, this is the age at which the animals are initially tested.

To allow genetic analysis of these data, a positive LE cell test was selected as the marker to identify the phenotype for SLE. To be included in the data, at least three members of any litter must have survived long enough to be adequately tested. An animal was considered negative only if it was older than 6 months and had had at least three consecutively negative tests.

In the A and C lines, where the original female parent had SLE and the male was normal, all of the first generation offspring were positive (Figs. 3, 4). Positive LE cell tests were found in 87.5% of the F_2 progeny in the A line and
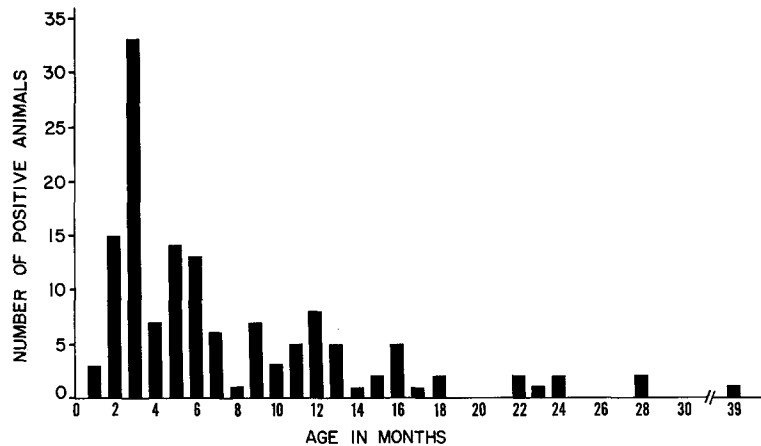

FIG. 2. Age when first positive LE cell test was detected.

68.4% of the F_2 progeny in the C line. When F_2 brother-sister matings were made in the C line, 75% of the F_3 puppies developed positive LE cell tests. Only animals with a positive test were used in these matings. Similar matings of F_2 offspring have been made in the A line, but we have yet to obtain three surviving members of any of these litters. Consequently, these data are not included in our genetic analysis. Backcross experiments (Fig. 5), in which a positive F_1 son in the C line was twice mated to his affected mother, produced a total of seven animals, five of which (71.4%) developed positive LE cell tests.

In the B line, where both original parents had SLE, 37/38 (97.3%) of the first generation offspring were found to be positive (Fig. 6 a). Sibling matings (or generationally equivalent matings) of these F_1 progeny resulted in the development of positive LE cell tests in 81% of the second generation offspring (Figs. 6 b, 6 c). To date, F_3 offspring have not been produced in sufficient numbers to be adequately tested within this breeding line.

When two different seropositive F_1 sons of the B breeding line were crossed back to their affected mother, 57% of the puppies produced had positive LE cell tests (Fig. 5). One of these same F_1 sons was mated to a normal beagle dog, and one-third of the offspring developed positive LE cell tests. In addition,
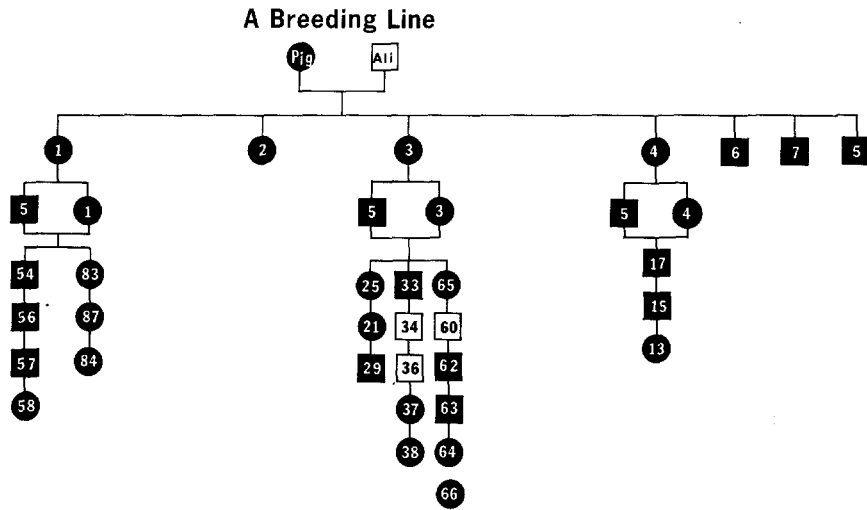
A Breeding Line

Fig. 3. Pedigree of A breeding line. The legend is the same for all breeding lines: circles = females; squares = males; black figures = positive phenotype for SLE. Numbers within figures identify individual animals.
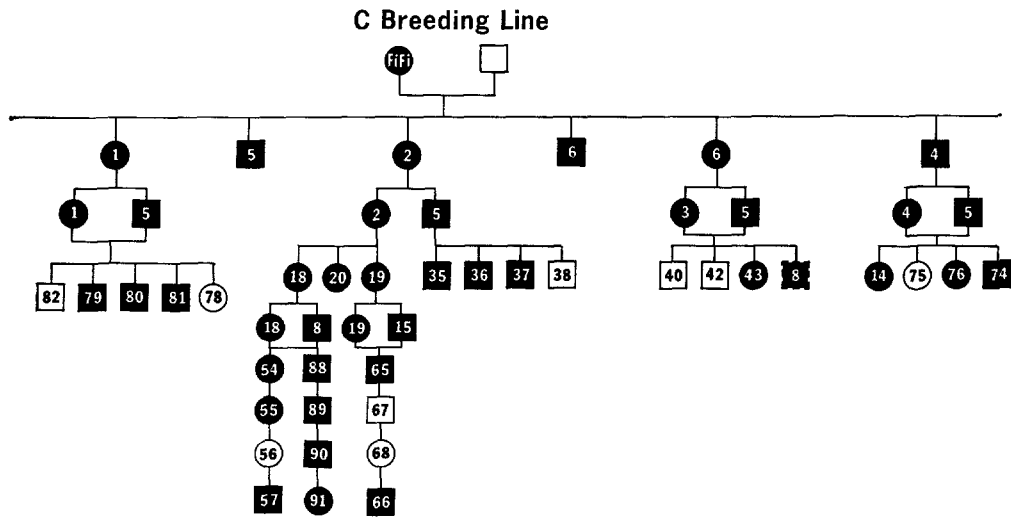
C Breeding Line

Fig. 4. Pedigree of C breeding line.

positive tests for antinuclear antibody were found in five of the six members of the litter produced by this outcross (Table IV).

A second abnormal finding, antinuclear antibody, was detected in the sera of 55/176 (31.2%) inbred dogs in the colony. 48 of these animals also had positive LE cell tests. When tested over a period of time, a rising titer of antinuclear antibody was observed in approximately half (27/55) of these animals. Using rat liver nuclei as the substrate, the staining pattern was usually diffuse over the entire nucleus. However, cells with more intense peripheral staining of

B Backcross

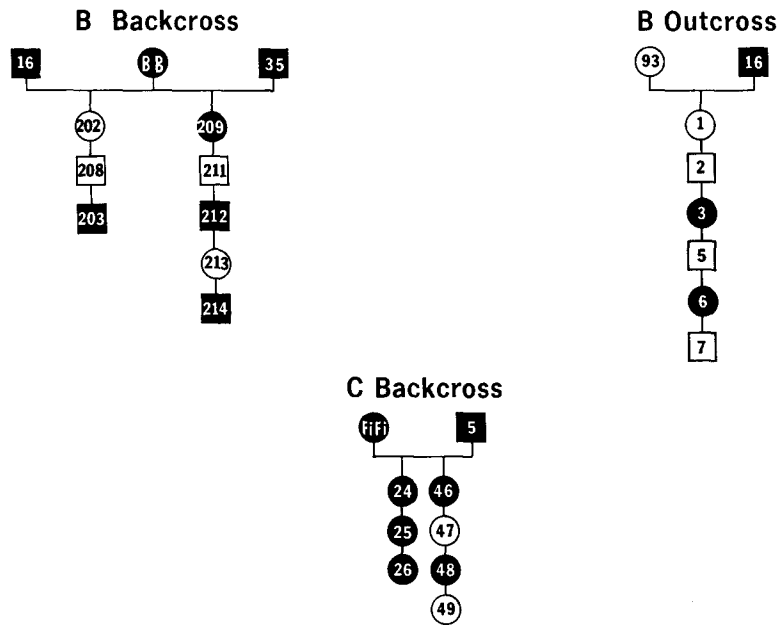**B Outcross**

C Backcross
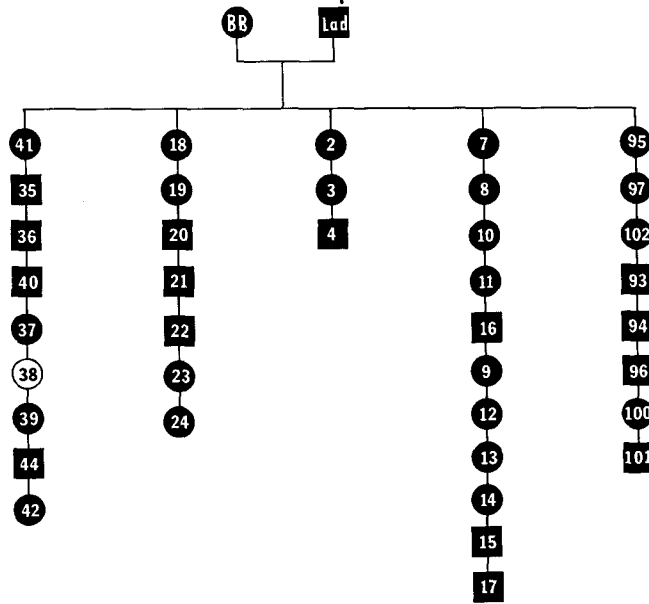
FIG. 5. Pedigree of backcross and outcross experiments.

the nucleus (rim pattern) and cells with a speckled pattern were also seen in these preparations. (Fig. 7)

Although most (48/55) of the inbred animals with antinuclear antibodies also had positive LE cell tests, a close correlation between these two laboratory tests was not found. Serial testing of 196 inbred progeny for the presence of LE cells disclosed 160 animals to be positive (81.6%). Of these only 29.0% (55/160) also had antinuclear antibody. When the distribution and frequency of these two abnormalities are compared (Tables III, IV), it becomes apparent that the development of antinuclear antibody in the dog is unrelated to the LE cell phenomenon.

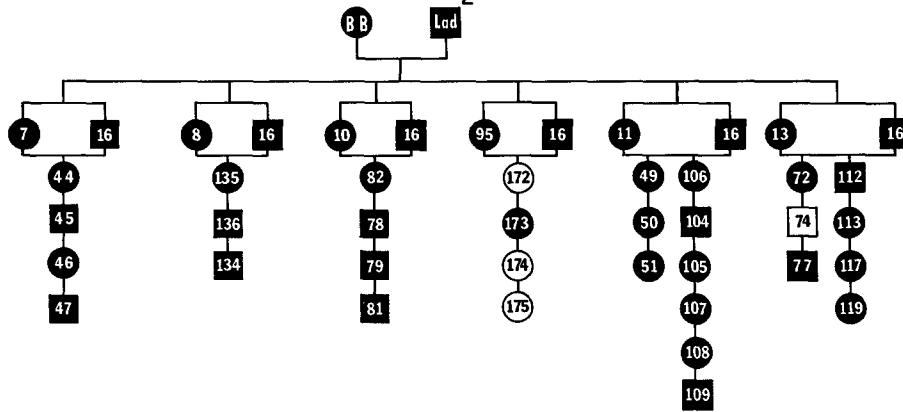A third abnormal antibody, rheumatoid factor, was detected in the sera of

B Breeding Line F₁ Offspring

BB **Lad**

41 · 18 · 2 · 7 · 95
35 · 19 · 3 · 8 · 97
36 · 20 · 4 · 10 · 102
40 · 21 · · 11 · 93
37 · 22 · · 16 · 94
38 · 23 · · 9 · 96
39 · 24 · · 12 · 100
44 · · · 13 · 101
42 · · · 14
· · · 15
· · · 17

B Breeding Line F₂ Offspring

BB **Lad**

7 · 16 │ 8 · 16 │ 10 · 16 │ 95 · 16 │ 11 · 16 │ 13 · 16
44 │ 135 │ 82 │ 172 │ 49 · 106 │ 72 · 112
45 │ 136 │ 78 │ 173 │ 50 · 104 │ 74 · 113
46 │ 134 │ 79 │ 174 │ 51 · 105 │ 77 · 117
47 │ │ 81 │ 175 │ 107 │ 119
│ │ │ │ 108
│ │ │ │ 109

B Breeding Line F₂ Offspring

BB **Lad**

14 · 35 │ 39 · 35 │ 97 · 94 │ 3 · 4
199 │ 121 │ 219 │ 63 · 27
190 │ 122 │ 222 │ 61 · 30
194 │ 123 │ 224 │ 62 · 26
196 │ 124 │ │ 64 · 28
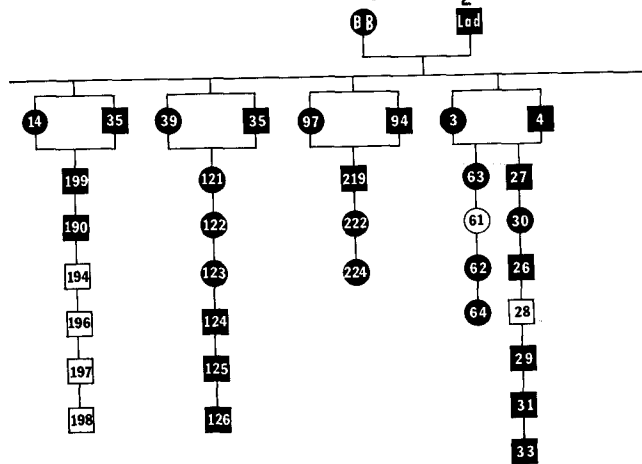197 │ 125 │ │ 29
198 │ 126 │ │ 31
│ │ │ 33

Fig. 6. Pedigree of B breeding line. (a) First generation offspring; (b) second generation offspring; (c) second generation offspring.

13 members of the colony (Table VI). None of these animals have signs of arthritis.

Despite extensive serial testing for variances in hemotologic and clinical chemistry values, no consistent abnormalities were detected in over 100 animals during a 2 yr period of investigation.

Thymic Abnormalities.—Postmortem examination revealed lesions in the thymus of 62 of 169 (36.6%) colony dogs. All these animals were over 6 months

TABLE IV

Serologic Abnormalities and Genetic Background

Strain and generation	Antinuclear antibody	
	No. positive	% positive
A breeding line		
P	1/2	50.0
F_1	4/7	57.1
F_2	4/24	16.6
F_3	—	—
B breeding line		
P	2/2	100.0
F_1	12/38	31.5
F_2	26/57	45.6
F_3	—	—
$F_1 \times$ P	2/7	29.5
$F_1 \times$ Normal	5/6	83.3
C breeding line		
P	1/2	50.0
F_1	4/6	66.6
F_2	2/19	10.5
F_3	3/12	25.0
$F_1 \times$ P	3/7	42.8

of age and they were either sacrificed as part of a population control program within the breeding colony or they had died unexpectedly.

Control tissue was collected from 32 random-bred hospitalized dogs that received complete postmortem examination. These animals were unrelated to the SLE colony dogs, they ranged in age from 8 months to 10 yr, and their deaths were from such diverse causes as automobile accidents and congestive heart failure. No evidence of SLE was found in the pathologic examination of these animals. An occasional follicle was found in the thymus from four of these control animals. It consisted of homogenous aggregates of medium-sized lymphocytes located within the medullary portion of the organ. No other changes were noted.

Gross abnormalities were not observed in the thymic tissue examined from the SLE dog colony. Microscopically, four lesions were present: (a) lymphoid follicles; (b) germinal center formation; (c) multiple granulomas; (d) reticulum cell sarcoma.

Multiple lymphoid follicles, composed of homogenous collections of medium-sized lymphocytes, were present in 37% of the animals examined (Fig. 8).
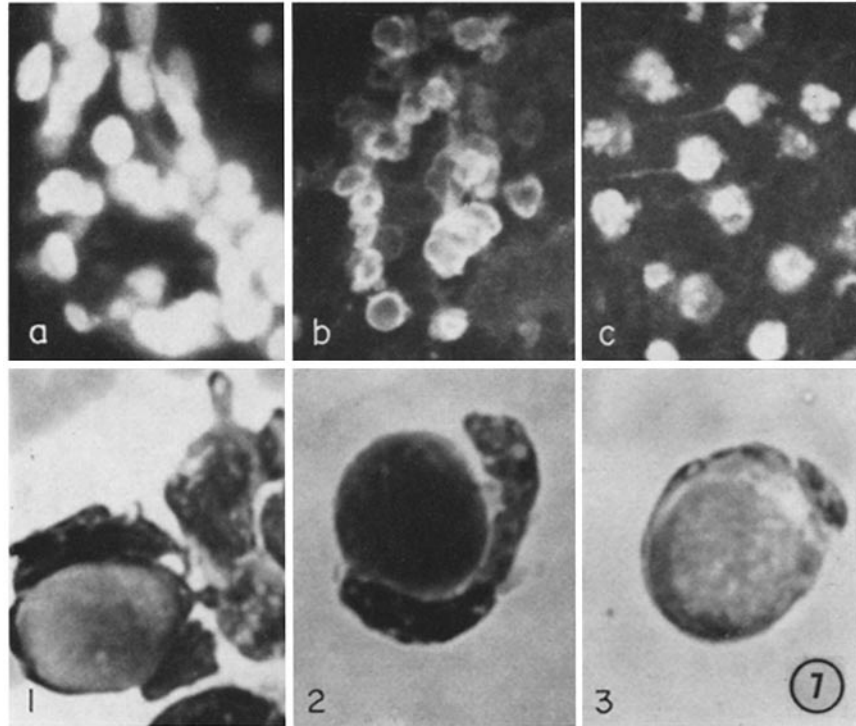

FIG. 7. Serologic abnormalities in canine SLE. (Top) Positive tests for antinuclear antibody. Rat liver nuclei illustrating diffuse (a), rim (b), and speckled (c) staining pattern. × 250. (Bottom) LE cells from third generation progeny in the C breeding line. × 1000.

These follicles occurred in the relatively hypocellular medullary portions of the organ and were clearly discernible from the normal thymic tissue by their uniform appearance and the concentric orientation of the cells. The follicles contained well-defined germinal centers, composed of reticulum cells, lymphoblasts, mitotic figures, and macrophages. Distinct collars of small lymphocytes surrounded the germinal centers. These follicles were identical in appearance to those found in the lymph nodes or spleen of the dog. In six animals, the germinal centers were grouped together in a portion of the thymus, simulating the ap-

pearance of a lymph node. In these cases, the absence of a capsule, subcapsular sinus, and medullary sinusoids preclude the possibility that the structures observed were foci of aberrant normal lymph node.
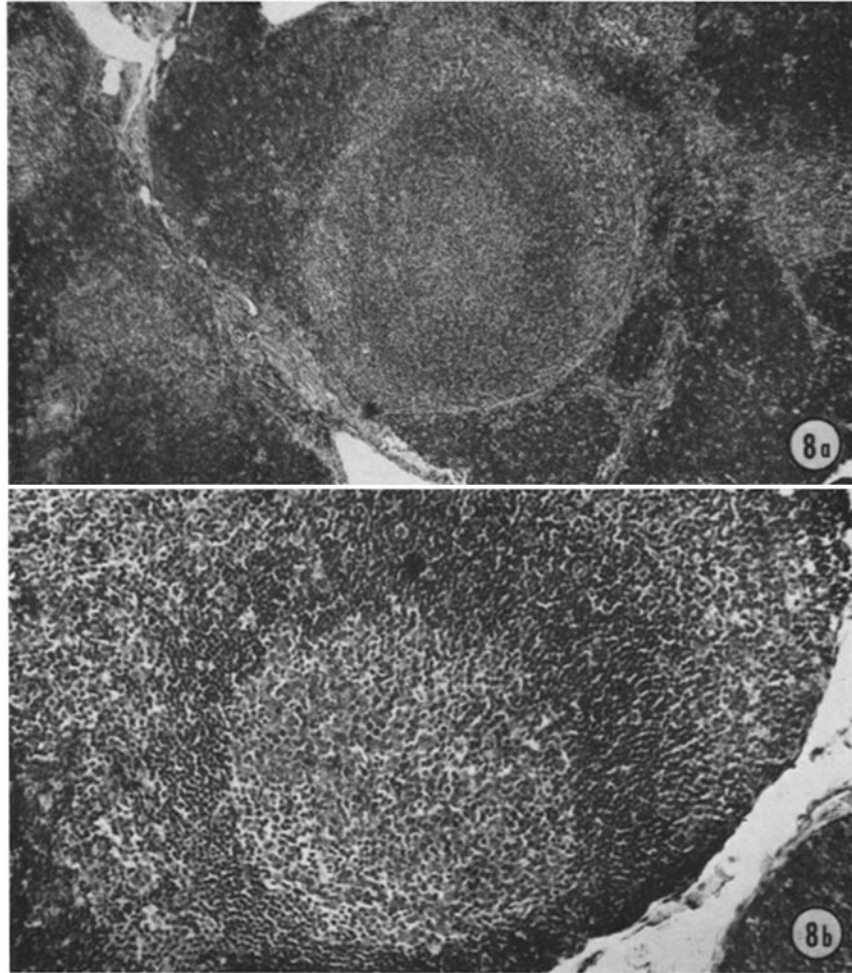

FIG. 8. (a) Lymphoid follicle with germinal center in the thymus of an F_1 dog in the B line. × 100. (b) Germinal center formation in the thymus of an F_1 dog in the C line. × 250.

Multiple, small, well-formed granulomas were present in one thymus (Fig. 9). They appeared to arise from within normal thymic lobules and had entirely replaced the normal tissue in some of these lobules. Additional granulomata were present, singly or in small clusters, throughout many parts of the affected
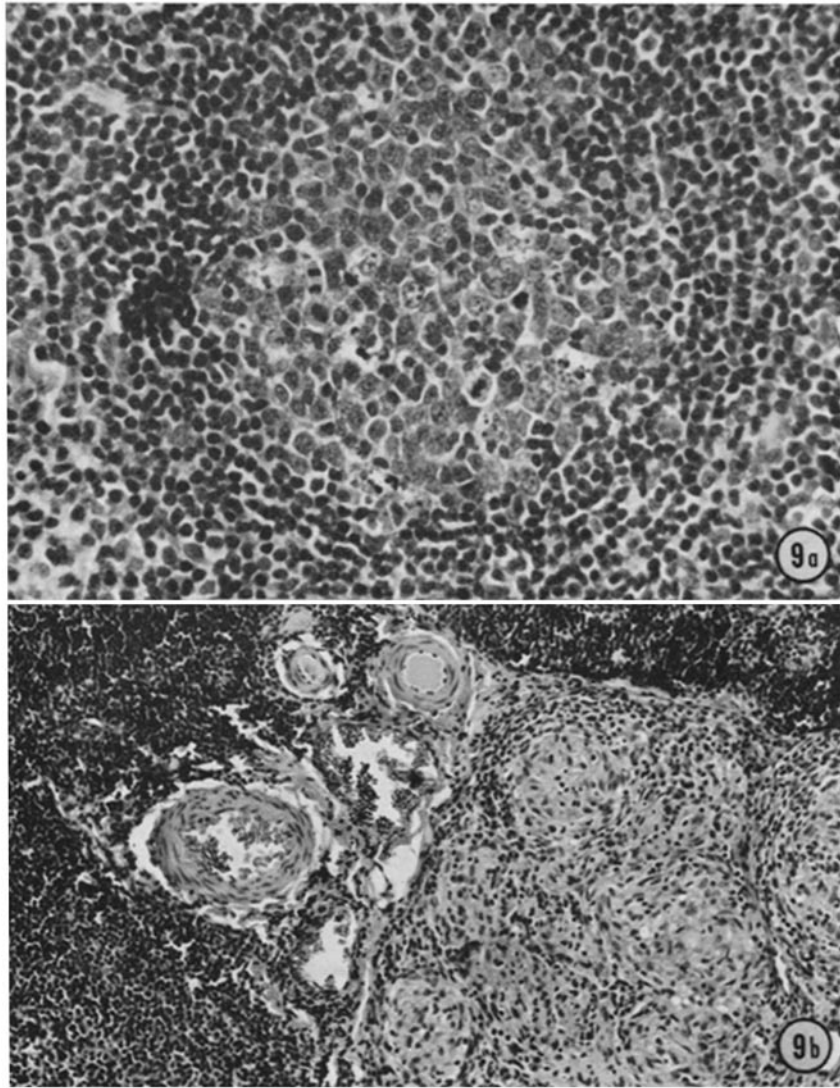
Fig. 9. (a) High power view of germinal center in dog thymus, with mitotic figures, reticulum cells, blast cells, and nuclear debris. × 400. (b) Multiple granulomas in the thymus of a B dog. × 250.

organ. No cause could be found for this lesion, despite extensive examination for bacteria, fungi, and foreign material. Similar lesions were not found elsewhere in the body at necropsy.

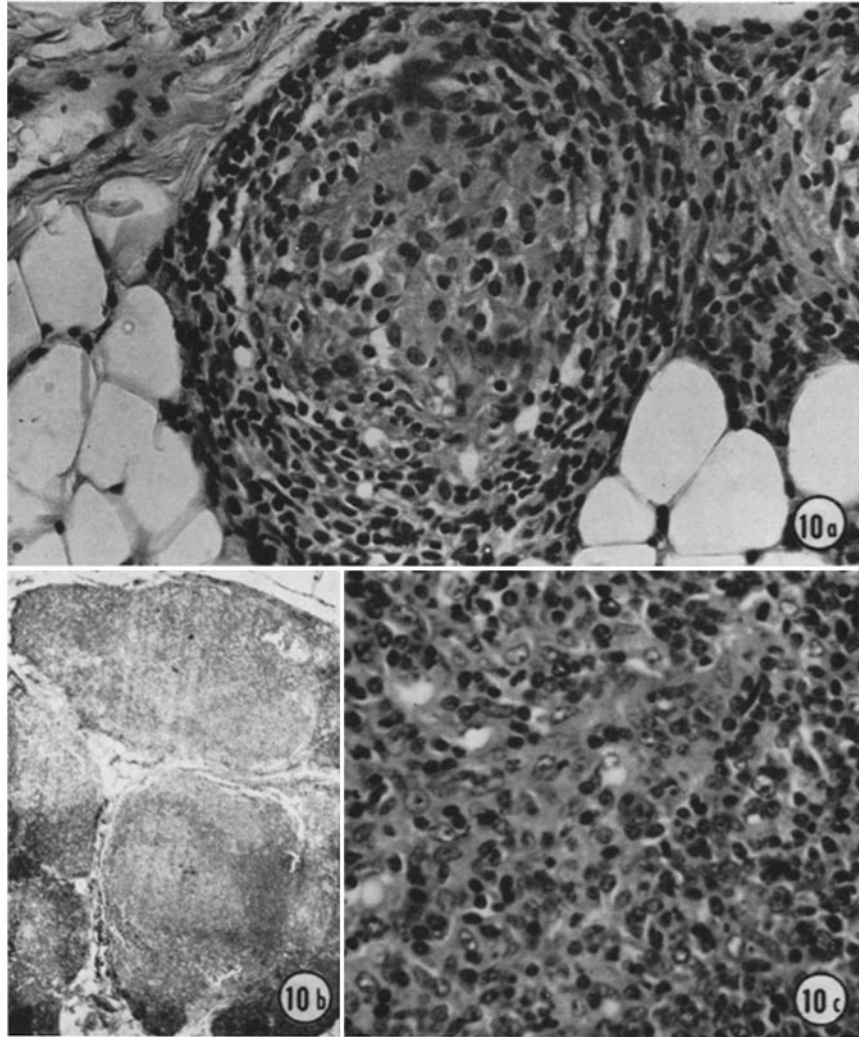Of particular interest was the observation that in one thymus examined, in

Fig. 10. (a) Discrete granulomata found in the thymus of a dog from the B breeding line. × 400. (b) Reticulum cell tumor in thymic lobule (upper), germinal center formation (lower right). × 40. (c) Pleomorphic reticulum cells replacing normal thymic tissue. × 400.

addition to lymphoid follicles and active germinal center formation, an expanding mass of pleomorphic reticulum cells replaced normal thymic tissue. This mass was confined to one lobule of the affected organ and appeared similar to the type B reticulum cell tumor described in mice (11) (Fig. 10).

DISCUSSION

The high frequency of perinatal death that occurred in conjunction with our attempt to inbreed the offspring of dogs affected with SLE cannot be adequately explained at this time. Judging by the autopsy findings, an increased susceptibility to infection occurred despite exceptional nursing care and environmental control. There was no histological evidence of SLE in any of these dogs; therefore, we assume that the cause of death in these puppies was unrelated to SLE. To what degree the disease of the parents contributed to the high mortality rate is not known, and additional means of investigating this problem, such as inbreeding under gnotobiotic conditions, are currently being explored. The perinatal mortality rate of the children of women with systemic lupus erythematosus is normal (12), although excessive number of spontaneous abortions have been noted (13).

Thymic abnormalities consisting of lymphoid follicles, many of which con-

TABLE V

Antinuclear Antibody

	Und.*	1:5	1:25	1:125	1:625
Normal cohabitant beagles (24)	0	0	0	0	0
Random hospitalized dogs (36)	0	0	0	0	0
Inbred beagle dogs (12)	0	0	0	0	0
Inbred Labrador retriever dogs (32)	1	0	0	0	0
SLE colony dogs (176)	55	22	4	1	0

* Und., undiluted serum.

tained germinal centers, were commonly found in the dogs of this colony. Formations like these also occur in guinea pigs and hamsters after direct injection of antigens into the thymus (14, 15), in New Zealand Black mice (16, 17), and in humans with SLE (18). Furthermore, hyperplasia of the lymphoepithelial elements of the thymus and the development of thymoma, well-recognized abnormalities associated with myasthemia gravis (19), have also been reported in human SLE (20, 21).

The inability to produce experimentally lesions of this type by the injection of antigen by any route other than intrathymically (14, 15), plus the exclusion from the thymus of intravenously injected vital dyes (22), indicate the existence of a blood-thymus barrier similar to the blood-brain barrier. The spontaneous development of lymphoid follicles and germinal centers in humans with SLE, in NZB mice, and in the dogs of this colony suggests that if such a barrier exists, the stimulus provoking the changes must either arise within the thymus, or arrive there by means other than diffusion through semipermeable membranes. In any case, the lesions present in the thymuses of our colony dogs have

every appearance of proliferating clones of cells arising in an apparently normal organ. The cause of these changes is unknown, but the fact that a reticulum cell tumor was found in one of the animals suggests its malignant potential.

The high frequency of thymic lesions and serologic abnormalities in the members of this colony might be explained by one or more of the following possibilities: (a) influence of environmental factor(s); (b) an effect of inbreeding per se; (c) an inordinately high rate of somatic mutation; (d) a cytoplasmic factor, defect, or abnormality of a germ cell; (e) inheritance of a genetic defect; (f) the presence of a transmissible agent.

The possibility that effects of environment or inbreeding could account for the abnormalities seems remote, since control populations with comparable inbreeding coefficients, and cohabitant normal dogs have not developed them (Tables V, VI).

Although somatic mutation of lymphocyte precursors in a genetically susceptible subpopulation has been proposed as the underlying mechanism of

TABLE VI
Rheumatoid Factor

	Positive test
Normal cohabitant beagles	0/6
Random hospitalized dogs	0/30
Inbred beagle dogs	0/12
Inbred Labrador retriever dogs	0/32
SLE colony dogs	13/124

autoimmunization in humans by Burch and Rowell (23), it seems unlikely that a mutation rate could be achieved that would produce the high incidence of abnormalities we observed in two different purebred strains of animals and one population of mongrel (mixed breed) dogs.

Results of the outcross experiment make it unlikely that some peculiar, inherited cytoplasmic factor within the germ cell could account for the abnormalities. Such a mechanism would most likely be effected through the ova, with its plentiful cytoplasm; yet, when a seropositive male dog was bred to a normal female, a high percentage of their offspring developed positive LE cell tests (33%) and antinuclear antibodies (86%) (Fig. 5).

The simplest genetic explanation for the high frequency of offspring with positive LE cell tests in our colony requires that a single, dominant, fully penetrant autosomal gene is responsible for the abnormality (Table VII). Under these conditions, when one of the original parents is affected (A line, C line), then 100% of the F_1 progeny and 75% of the F_2 progeny can be expected to be positive if the original parent is homozygous for the trait. If, however,

the affected parent is heterozygous for the trait, then only 75% of the F_1 progeny would be expected to be positive.

All seven F_1 puppies in the A line were positive. The probability of this occurring is 1.0 if the dam were homozygous for the hypothetical gene; it is unlikely that she was a heterozygote, for the probability of a heterozygote $\times$ normal mating producing 7/7 positive offspring is only 0.0078. In mating positive F_1 A dogs, all of which must be heterozygous regardless of parental zygosity, 24 puppies were produced, 21 of which were positive. The number of positive animals follows the binomial distribution with a mode of 18 positive

TABLE VII

Distribution of the Postulated Genetic Marker for Canine SLE

	No. positive	% positive	Anticipated positive
A line			
P	1/2	50	—
F_1	7/7	100	100
F_2	21/24	87.5	75
B line			
P	2/2	100	—
F_1	37/38	97.3	100
F_2	46/57	80.7	100
F_3	—	—	—
$F_1 \times P$	4/7	57.1	100
$F_1 \times P$	2/6	33.3	50
C line			
P	1/2	50	—
F_1	6/6	100	100
F_2	13/19	68.4	75
F_3	9/12	75	75
$F_1 \times P$	5/7	71	100

dogs. The probability of achieving the modal number is 0.19. The probability of obtaining 21 positive animals, or three dogs distant from the mode, is 0.14. Therefore, the hypothesis of a single dominant gene is supported by this result.

A more stringent test of the genetic hypothesis was carried out in the C line. In this combination, the sire was normal, the dam was affected, and each of the six F_1 offspring was positive. The probability of this occurring if the dam were homozygous is 1.0. If that were actually the case, all of the progeny from the backcross mating must have been positive. Since only 5/7 of those animals had positive LE cell tests, the dam could not have been a homozygote. The result of the backcross mating could have been obtained if the dam were a heterozygote ($P = 0.31$), but that seems unlikely in view of the result obtained

in the F_1 generation: the probability of a heterozygous $\times$ normal mating producing 6/6 positive offspring is only 0.016. Since the dam must be either homozygous or heterozygous for the hypothetical gene, and since neither case is upheld by the results, exclusively genetic mechanisms in the C line can be excluded.

Analysis of the B line also yields results in conflict with the hypothesis of a single, dominant gene. When both original parents are affected, as in this case, there must be no negative progeny if either one of the parents is homozygous for the trait. Since only 37/38 of the F_1 progeny in this line were positive, both affected parents would have had to be heterozygous. But the probability of obtaining 37/38 positive progeny from mating two heterozygotes is only 0.0002. The single negative F_1 dog in this breeding experiment was tested eleven times during the 2 yr of his life. In no instance was a positive LE cell test found, and repeated tests for antinuclear antibody were negative. Furthermore, autopsy of this dog revealed no abnormalities.

In another experiment with the B breeding line, four of seven puppies produced by crossing a seropositive F_1 son to his mother are positive (57%). These results are compatible with a genetic explanation, provided both the parent and the son are heterozygous for the hypothetical trait. However, as we have seen from the inbreeding experiment, it is impossible for the dam to be a homozygote, and it is highly unlikely that she is a heterozygote ($P = 0.0002$). In a third experiment with the B line, a seropositive F_1 son was mated to a normal beagle dog. Two of the six offspring had positive LE cell tests. This result is consistent with the hypothesis that the positive LE cell test is transmitted by a single dominant gene. However, it seems unusual that 5/6 of the offspring from this outcross mating have positive tests for antinuclear antibody.

In summary, six breeding experiments were carried out in these dogs. The results of two of them, the inbred A line animals and the outcross mating in the B line, are consistent with the hypothesis that these findings are due to a single dominant autosomal gene. The other four experiments, which included inbreeding of the B and C line and the backcrosses in these same two breeding lines, do not support the hypothesis. In our opinion, the results of the inbreeding experiments, although apparently due to a genetic mechanism, may in fact be the result of other factors, which may be transmitted by nongenetic mechanisms. Because the backcross experiments, which are more reliable for predicting the segregation of genes than inbreeding experiments, failed to uphold the hypothesis of a single gene, we must seek an alternative explanation of the results.

If a single gene cannot explain the results, perhaps a series of interacting genes can. Should multiple genes be located on a single chromosome and in close approximation to each other, the trait would be inherited in the same manner as a single gene defect, so this seems unlikely. If the linkage was less

complete, or if the genes were located on different chromosomes, then the frequency of the trait would be less than that of a single gene defect, due to the fact that the chance for random combination of different genes is inversely proportional to the number of genes involved. Therefore, it seems that the results obtained from our breeding experiments cannot solely be explained by any conventional genetic mechanism.

This leaves us with the sixth possibility. It is conceivable that, in addition to a genetic factor, a transmissible agent is required for the expression of the disease. Experiments have been undertaken to test this idea, and in a following paper we will present evidence that a transmissible agent, present in cell-free filtrates of tissue obtained from dogs in the SLE colony, can provoke the development of antinuclear antibodies (ANA) and positive LE cell tests when administered to normal animals.

The SLE colony is still evolving and clinical signs of SLE have not yet appeared. We therefore must stress that any relationship between positive LE cell tests, antinuclear antibodies, and the development of SLE remains unproven in these animals. However, judging by the specific correlation between the presence of LE cells and canine SLE, as well as the large body of evidence that positive LE cell tests in humans usually coincide with SLE, there is every expectation that clinical evidence of SLE will eventually occur in the colony. As a rule, LE cell tests are done in man only when there is clinical evidence of SLE; it is entirely possible that that "marker" is present long before signs of the disease are apparent. In NZB and (NZB $\times$ NZW) F_1 mice, positive anti-globulin tests and antinuclear antibodies appear months before overt disease (24); extrapolation of this to the dog suggests that clinical signs of SLE may be expected in the colony within 2 or 3 yr.

SUMMARY

Three breeding lines, originating from dogs with SLE, have been established. Two lines were initiated by mating a female with SLE with a normal male. The third line resulted from a mating of two affected dogs. Brother-to-sister matings have reached the third generation in each line. In addition, backcross and outcross matings were carried out. More than one-third of the autopsied dogs had thymic abnormalities. The commonest lesion was a lymphoid follicle; the thymus of one dog contained multiple granulomas, and in one animal a reticulum cell sarcoma of the thymus was found.

Multiple serological abnormalities, including positive LE cell tests, antinuclear antibodies (ANA), and rheumatoid factor, were found in the progeny. The development of ANA appeared unrelated to the incidence of positive LE cell tests. About 10% of the animals had rheumatoid factor in their serum. Control populations of dogs; including house pets; two other, unrelated lines of inbred dogs; and normal dogs housed in the same facility as the SLE colony

did not have these abnormalities. The incidence of positive LE cell tests in the inbred, backcross, and outcross matings was not consistent with any conventional genetic mechanism of inheritance. It is conceivable that the results can be explained by vertical transmission of an infectious agent in a genetically susceptible individual.

The authors wish to thank Drs. Park Gerald, Joseph Templeton, and Walter Tannenberg for their help in analyzing the genetic data and Miss Christina Smith for her expert technical assistance.

BIBLIOGRAPHY

1. Lewis, R. M., R. S. Schwartz, and W. B. Henry. 1965. Canine systemic lupus erythematosus. *Blood J. Hematol.* **25**:143.
2. Lewis, R. M., R. S. Schwartz, and C. E. Gilmore. 1965. Autoimmune disease in domestic animals. *Ann. N. Y. Acad. Sci.* **124**:178.
3. Lewis, R. M. 1965. An evaluation of the clinical usefulness of the LE cell phenomenon in dogs. *J. Amer. Vet. Med. Ass.* **137**:939.
4. Lewis, R. M., W. B. Henry, Jr., G. W. Thornton, and C. E. Gilmore. 1963. A syndrome of autoimmune hemolytic anemia and thrombocytopenia in the dog. *Sci. Proc. J. Amer. Vet. Med. Ass.* **1**:140.
5. Lewis, R. M., and Y. Borel. 1971. Canine rheumatoid arthritis: a case report. *Arthritis Rheum.* **14**:67.
6. Hooper, B. E., A. Hall, and H. E. Dale. 1961. Characteristics of the vaginal smear. *Small Anim. Clinician.* **1**:355.
7. Hargraves, M. M. 1954. The LE cell phenomenon. *Advan. Intern. Med.* **6**:133.
8. Scheidegger, J. J. 1955. Une microméthode de l'immunoélectrophorese. *Int. Arch. Allergy Appl. Immunol.* **7**:103.
9. Johnson, J. S., J. H. Vaughan, and S. N. Swisher. 1967. Canine immunoglobulins. II. Antibody activity in six immunoglobulin classes. *J. Immunol.* **98**:935.
10. Harrop, A. E. 1960. Reproduction in the Dog. Bailliére, Tindall, and Cassell, Ltd., London. 1st edition. 191.
11. Dunn, T. B., and M. K. Deringer. 1968. Reticulum cell neoplasms, type B, or the "Hodgkins-like lesion" of the mouse. *J. Nat. Cancer Inst.* **40**:771.
12. Donaldson, L. B. and R. R. de Alvarez. 1962. Further observations on lupus erythematosus associated with pregnancy. *Amer. J. Obstet. Gynecol.* **83**:1461.
13. Mund, A., J. Simson, and N. Rothfield. 1963. Effect of pregnancy on course of systemic lupus erythematosus. *J. Amer. Med. Ass.* **183**:917.
14. Marshall, A., and R. G. White. 1961. The immunological activity of the thymus. *Brit. J. Exp. Pathol.* **42**:379.
15. Sherman, J. D., M. M. Adner, and W. Dameshek. 1964. Direct injection of the thymus with antigenic substances. *Proc. Soc. Exp. Biol. Med.* **115**:866.
16. Burnet, M. 1966. Implications for autoimmune disease in man of studies on NZB mice and their hybrids. II. Renal and thymic disease. *Roy. Inst. Pub. Health Hyg. J.* **29**:95.
17. De Vries, M. J., and W. Hijmous. 1967. Pathological changes of thymic epithelial

cells and autoimmune disease in NZB, NZW, and (NZB × NZW) F_1 mice. *Immunology.* **12:**179.

18. Mackay, I. R., and P. DeGail. 1963. Thymic "germinal centers" and plasma cells in systemic lupus erythematosus. *Lancet.* **2:**667.

19. Murray, N. A., and J. R. McDonald. 1945. Tumors of thymus in myasthenia gravis. *Amer. J. Clin. Pathol.* **15:**87.

20. Galbraith, R. F., H. J. Summerskill, and J. Murray. 1964. Systemic lupus erythematosus, cirrhosis and ulcerative colitis after thymectomy for myasthenia gravis. *N. Engl. J. Med.* **270:**229.

21. Larsson, O. 1963. Thymoma and systemic lupus erythematosus in the same patient. *Lancet.* **2:**665.

22. Marshall, A. H. E., and R. G. White. 1961. The immunological reactivity of the thymus. *Brit. J. Exp. Pathol.* **42:**379.

23. Burch, P. R. J., and N. R. Rowell. 1965. Systemic lupus erythematosus: etiological aspects. *Amer. J. Med.* **38:**793.

24. East, J. 1970. Immunopathology and neoplasms in New Zealand Black (NZB) and SJL/J mice. *Prog. Exp. Tumor Res.* **13:**84.