

CHARACTERIZATION OF A SPONTANEOUS DISEASE OF WHITE LEGHORN CHICKENS RESEMBLING PROGRESSIVE SYSTEMIC SCLEROSIS (SCLERODERMA)*

BY M. ERIC GERSHWIN,‡ HANS ABPLANALP, JAMES J. CASTLES, RICHARD M.
IKEDA, JUDITH VAN DER WATER, JAN EKLUND, AND DARRELL HAYNES

*From the Section of Rheumatology-Clinical Immunology, Department of Medicine, and the Department of
Avian Sciences, University of California at Davis, California 95616*

Progressive systemic sclerosis (scleroderma) is a disease of unknown etiology characterized by fibrosis of skin and internal organs (1, 2). Despite observations that scleroderma is found in increased frequency in workers exposed to coal, gold, silica, and polyvinyl chloride, the vast majority of patients have had no known etiologic insults (3, 4). Indeed, with the exception of the latter industrial hazards as possible predisposing factors, there is only a rudimentary understanding of the pathogenesis of scleroderma. Much of this difficulty results from the absence of a spontaneous animal model of human scleroderma. Although scleroderma-like changes have been reported in patients undergoing bone marrow transplants and suffering from graft vs. host disease as well as in rats suffering from homologous disease, these models only mimic scleroderma (5-8). Similarly, fibrotic changes can be produced in rats injected with glycosaminoglycans, but the alterations generated appear to be only minimally related to scleroderma and have not been found to be useful models in understanding the etiology of the disease (9).

Over the past two years, our laboratory has focused attention on an inherited spontaneous disease in a closed line of White Leghorn chickens. This disease is characterized by small vessel (arterial) occlusion, skin and esophageal fibrosis, polyarthritis, and the presence of antinuclear antibodies (ANA).¹ In this report, we propose that this apparent autoimmune disease has many features analogous to human progressive systemic sclerosis (PSS).

Materials and Methods

History of Line 200 Chickens. An abnormality in the development of combs of young Leghorn males was discovered in 1942 by Dr. Paul Bernier at the Department of Poultry Husbandry, Oregon State University, Corvallis, Oreg. The combs of such affected males were noted to

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‡ Recipient of Research Career Development Award AI-00193 from the National Institutes of Health. Address reprint requests to M. Eric Gershwin, M.D.; Section, Rheumatology-Clinical Immunology; TB 192; School of Medicine; University of California; Davis, California.

¹ *Abbreviations used in this paper:* ANA, antinuclear antibody; BC, backcross; BUN, blood urea nitrogen; CPK, creatine phosphokinase; ELISA, enzyme-linked immunosorbent assay; ENA, extractable nuclear antigens; FITC, fluorescein isothiocyanate; GVHD, graft-vs.-host disease; HE, hematoxylin and eosin; PBS, phosphate-buffered saline; PCV, packed cell volumes; PSS, progressive systemic sclerosis; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminases; SRBC, sheep erythrocytes.

atrophy at an early age, with adult birds exhibiting variable degrees of missing combs. This abnormality was termed "self-dubbing" ("dubbing" being defined as the cutting or removing the comb artificially) and was seen originally in only a few males of this outbred flock. Because of its potential practical usefulness, the line was propagated by Dr. Bernier. Upon his retirement, Dr. Bernier offered this interesting bird model to one of us (Hans Abplanalp). At this point, only three males and five females were alive to propagate this line of self-dubbing chickens. Thanks to special efforts of Dr. Bernier's successor, Dr. J. Renden, we were able to establish an initial flock of 15 males and 25 hens at the Department of Avian Science at the University of California, Davis, Calif. This material is now referred to as UCD line 200. All experimental work is derived from offspring from these original chickens.

Animals. All line 200 and control line (UCD lines 003, 011, 140, 159, and 446) chickens were bred and maintained by the Department of Avian Sciences, University of California, Davis. (10). Hens were artificially inseminated and eggs collected for 2 wk. All eggs were incubated for 21–22 d in Jamesway 252 incubators (Butler Mfg. Co., Jamesway Div., Fort Atkinson, Wis.). On the day of hatch, chicks were banded and moved to floor pens or to battery brooders at the Hopkins poultry plant, University of California, Davis. Chickens were vaccinated against Marek's disease on day 1, Newcastle disease at 4–6 wk and again at 10–12 wk, and fowl pox at 10–12 wk. Birds were transferred to individual cages in windowless houses cooled by evaporation: males at 14 wk and females at 15 wk of age. The characteristics of the control chickens used in this study have previously been noted (11).

Genetics. Abnormal line 200 males were individually crossed with UCD inbred lines 003 and 011. F₁ generation chicks from these matings were mated to generate both an (F₁ × F₁)F₂ generation as well as an F₁ × line 200 backcross (BC) generation. Offspring from all crosses were monitored until ~3 mo of age for development of comb and foot lesions.

Survival. Over a course of 1 yr, 109 line 200 (abnormal) and 101 line 159 (control) chickens were monitored for survival.

Pathology. 10 UCD line 200 and control line 446 were killed at 6 wk and 6 mo (the median survival) of age. The comb, skin (from the back of the neck), esophagus, small intestine, heart, lung, and kidney were removed and preserved in formalin. Sections 5- μ m thick were prepared and stained with hematoxylin and eosin (HE) and Masson's trichrome (12). Direct immunofluorescence was performed on fresh-frozen tissue as described (10).

Hematology. At 6 mo of age, packed cell volumes (PCV), hemoglobin concentrations, and blood cell counts were determined in 20 line 200 and line 159 birds of each sex. PCV were performed in microhematocrit capillary tubes (13). Hemoglobin concentrations were assayed by the cyanmethemoglobin method (13). Blood cell counts were made by a modification of the Rees-Ecker method utilizing the following staining solution: 3.9 g sodium citrate, 0.2 ml neutral formalin, 0.5 g brilliant cresyl blue, and 100 ml distilled water (10). Fresh blood samples were diluted 1:200 in this solution in a blood-diluting pipet and the cells microscopically counted in a Neubauer hemocytometer.

Coombs's Tests. At 6 wk and 6 mo of age, heparinized blood samples were obtained from the wing vein of 20 line 200 and line 003 controls. The erythrocytes were washed three times in phosphate-buffered saline (PBS), removing the buffy coat after each wash, and the cells suspended to a final concentration of 2% in PBS. Rabbit anti-chicken γ -globulin, rabbit anti-chicken 7S Ig (Fc specific), and rabbit anti-chicken IgM (μ specific), were all heat-inactivated in a 56°C water bath for 30 min and absorbed with an equal volume of packed normal chicken erythrocytes. A direct Coombs's assay was performed in microtiter plates by addition of 25 μ l cell suspension to 25 μ l serially diluted antisera. Known positive samples (UCD line 140) and negative controls were incubated in each test (10, 11). Additionally, direct erythrocyte agglutination tests were performed in microtiter plates by adding 25 μ l 2% erythrocyte suspensions from normal chickens to serially diluted test chicken sera (10).

Cryoprecipitates. Blood was drawn into prewarmed syringes from the jugular vein of 13 line 200, 19 line 3, and 14 line 159 chickens at 6 mo of age and transferred to siliconized glass tubes kept at 38–40°C. To test for the presence of serum proteins that precipitate in the cold, the method of Weisman and Zvaifler (14) was employed. The blood was allowed to clot for 3–4 h at this temperature, and the serum was harvested by centrifugation at 500 g for 10 min at room temperature. Then, 1 ml of serum was dispensed into a 12- × 75-mm tube and placed at 4°C



FIG 1. Comb from a 4-wk-old line 200 chicken. Note the extensive swelling, distal erythema, and necrosis of the tips of the comb.



FIG 2. Polyarthrititis in 6-wk-old line 200 hen. Note the swelling of digits and peripheral necrosis.



FIG. 3. 6-wk-old line 200 hen. Note the absence of comb (self-dubbing), the loss of feathers on the neck, and polyarthritis.

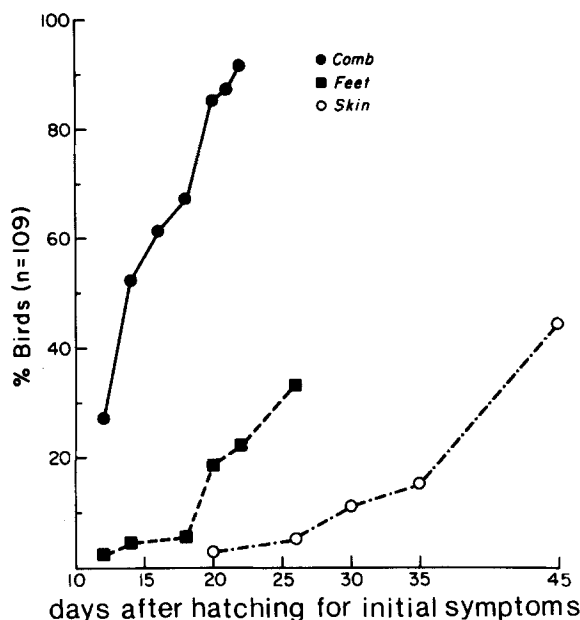


FIG. 4. The frequency of comb, skin, and joint manifestations in 109 line 200 chickens. This frequency does not significantly increase after 6 wk of age. As noted in the text, birds who develop the triad of comb, skin, and joint pathology have an accelerated mortality and are generally dead by 10 wk after hatching.

for 72 h. Samples were handled in as sterile manner as possible throughout the procedure. Known positive chicken sera (UCD line 140) were included as previously described (10).

Rheumatoid Factor. At 6 mo of age, 17 line 200, and 11 line 446 birds were bled. Rheumatoid factor was quantitated by the Rose-Waaler test (10). Sheep erythrocytes cells (SRBC) were coated with chicken 7S Ig by incubating a subagglutinating dose of the 7S Ig fraction of chicken anti-SRBC sera for 30 min at 37°C. The coated cell suspension was washed three times and resuspended in PBS to a final concentration of 5%. Test chicken sera were serially resuspended in PBS to a final concentration of 5% and serially diluted in microtiter plates. Agglutination was examined after a 2-h incubation at room temperature. Known rheumatoid factor-positive samples (UCD line 140) were included.

Anti-Thymocyte Antibodies. 15 6-mo-old line 200 and line 003 controls were assayed for the presence of anti-thymocyte antibodies. An indirect immunofluorescent test was performed on thymus cells obtained from 2-4-wk-old normal chicks (10, 11). Thymuses were removed, and the cells teased into cold RPMI-1640 media (Pacific Biologicals) with forceps, and then pressed through a stainless-steel wire screen. The cell suspension was washed three times in cold RPMI-1640 and adjusted to a concentration of 10×10^6 /ml after the final wash. 1 ml of the cell suspension was delivered to 12- \times 75 mm tubes and the cells pelleted by centrifugation. To the pellet, 0.1 ml undiluted test chicken sera was incubated at 37°C for 60 min and at 4°C for an additional 60 min. The cells were then washed three times in cold RPMI-1640 and pelleted. After removal of the supernate, 0.1 ml of diluted fluorescein isothiocyanate-(FITC) conjugated anti-chicken γ -globulin was added to the cells and incubated for 30 min at room temperature. The FITC antisera were used at a 1:15 dilution to eliminate nonspecific labeling of thymocytes. Known positive and negative samples were included in all assays.

Antinuclear Antibodies. At 6 mo of age, the titer of antinuclear antibodies in 40 line 200 and 28 line 011 controls was quantitated using HEp-2 cells as substrate (15). Known positive samples were included from UCD line 140 (10). Antibodies to DNA and extractable nuclear antigens (ENA) were performed as described (16, 17).

Biochemical Markers. The concentration of sodium, potassium, chloride, CO₂, blood urea

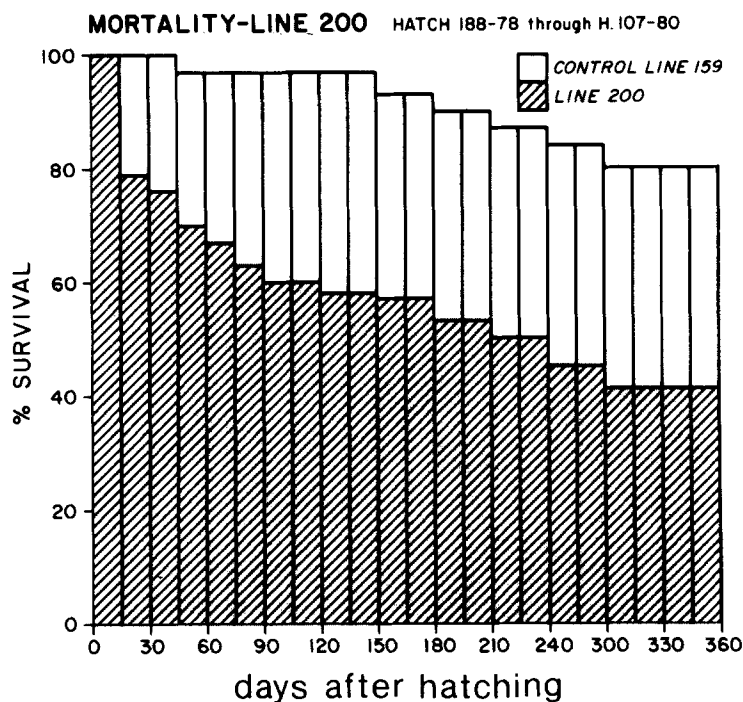


FIG. 5. The mortality characteristics of line 200 chickens. Note the early significant mortality; only 60% of line 200 birds survive 120 days after hatching compared with a <10% mortality of control line 159 at 1 yr of age.

nitrogen (BUN), creatinine, (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, uric acid, creatine phosphokinase (CPK), and aldolase were determined for 10 6-wk-old line 200 and 8 6-mo-old line 200, and 9 6-wk-old line 003 and 7 6-mo-old line 003 control chickens.

Quantitative Immunoglobulins. At 4 mo of age, sera levels of 7S Ig and IgM were quantitated by radial immunodiffusion in 11 line 200 and 14 line 446 controls. Reference standards were included in all assays (18).

Anti-Collagen Antibodies. Type II chicken collagen was purified as previously described from 2-wk-old control line 446 chicks (19). This material was coupled to SRBC and the presence of antibodies was determined by enzyme-linked immunosorbent assay (ELISA) and by hemagglutination in 46 6-mo-old line 200 chickens and 29 birds from control lines 003, 159, and 446 (19-21).

Results

Natural History of Disease Expression. Line 200 chicks, when compared with other UCD lines, are normal until ~1-2 wk of age. At that time, a series of abnormalities appear that are readily detectable by casual observation. First, swelling and erythema of the comb and wattle occurs. (Fig. 1). Subsequently, over the next several weeks, a polyarthritis involving only peripheral joints develops (Fig. 2). The severity and frequency of comb involvement peaks at 2-4 wk of age. Concurrently, the skin on the neck and back begins to swell, becomes severely indurated, loses feathers, and becomes taut (Fig. 3). These lesions, however, are not found in all birds. Indeed, the most prevalent finding is the self-dubbing comb abnormality initially observed by Bernier. This involvement of the comb is found in $\approx 90\%$ of birds at 4 wk after hatching (Fig.

4). In contrast, polyarthritis is found in only 20% of birds at 3 weeks and in 35% at 4 wk after hatching. Finally, dermal lesions are significantly more delayed than either comb or joint pathology and are found in 20% of birds at 5 wk and 45% at 7 wk after hatching (Fig. 4). There are no differences in disease expression between males and females, and thus data from both sexes are combined.

The mortality of line 200 chickens is ~20% at 1 mo of age, 40% at 4 mo of age, and 55% at 10 mo of age (Fig. 5). This compares with a <10% mortality at 1 yr in line 159 controls (Fig. 5). Birds that develop the acute episode of swelling of comb, polyarthritis, and dermal alterations account for the early accelerated mortality. The cause of death in such chickens appears to be secondary cutaneous infections of the comb, wattle, neck, and feet. Indeed, in virtually all such birds, a gangrenous necrosis of comb and digits develops after severe vascular occlusion. The vascular lesions that predispose to autoamputation of the comb are readily visible. For example, in line 200 chickens, multiple microvascular infarcts are seen. Nail beds reveal severe vasodilation, even in the absence of arthritis.

The mortality of older birds is a result of a wasting-like syndrome, characterized by listlessness, fatigue, anorexia, and loose droppings. At autopsy, such birds exhibit signs of pneumonitis but also have severe small vessel occlusive disease with involvement of esophagus, heart, and/or kidney (see below: pathology).

Genetics. In fall 1979 and spring 1980, two hatches of 150 offspring from line 200 parents were observed for 60 d. Within 3 mo, 95% of line 200 chicks developed comb abnormalities compared with 0% of UCD lines 003, 011, 159, and 446. Indeed, such comb lesions have not previously been observed at the University of California, Davis in any chicken line. In contrast, 111 F₁ offspring, generated by crossing abnormal line 200 males with hens of two UCD inbred lines noted above, revealed no detectable abnormalities. However, a backcross generation, produced by the mating of F₁ birds to parental line 200, produced 51% abnormal or 43 of 94 offspring (Table I). Finally an F₂ generation produced by crossing F₁ individuals with each genetic subline produced only 4% abnormal.

Pathology. The pathology of line 200 chickens has thus far been limited to extensive examination of 6-wk- and 6-mo-old chickens. 6-wk-old chickens have abnormalities of skin and comb, but do not appear to have significant internal manifestations, i.e., their visceral organs examined appear similar to controls (Table II). The only major exception to this observation is the presence of pneumonia in autopsied birds.

The pathology of skin and comb of 6-wk-old line 200 chickens is noteworthy for three major features: First, an intense mononuclear cell infiltrate (Fig. 6A) present diffusely throughout all layers of skin and subcutaneous tissue is apparent. Second, there is a marked proliferation of small vessels (Fig. 6B). This proliferation of small vessels is accompanied by an intense collagen deposition by a rich fibroblastic-like network and appears concurrent with the mononuclear cell infiltrate. However the proliferation of small vessels continues after the mononuclear cell infiltrate begins to wane. Finally, skin and comb develop severe occlusive disease of small arteries with vessels developing thickening of the muscle wall, narrowing of the lumen, and intimal proliferation (Fig. 6C). The overall result is replacement of subcutaneous fat and muscle with collagen (Fig. 7).

At 6 mo of age, the majority of line 200 chickens have the self-dubbed appearance but <10% have clinically visible joint or skin involvement. In contrast, they have

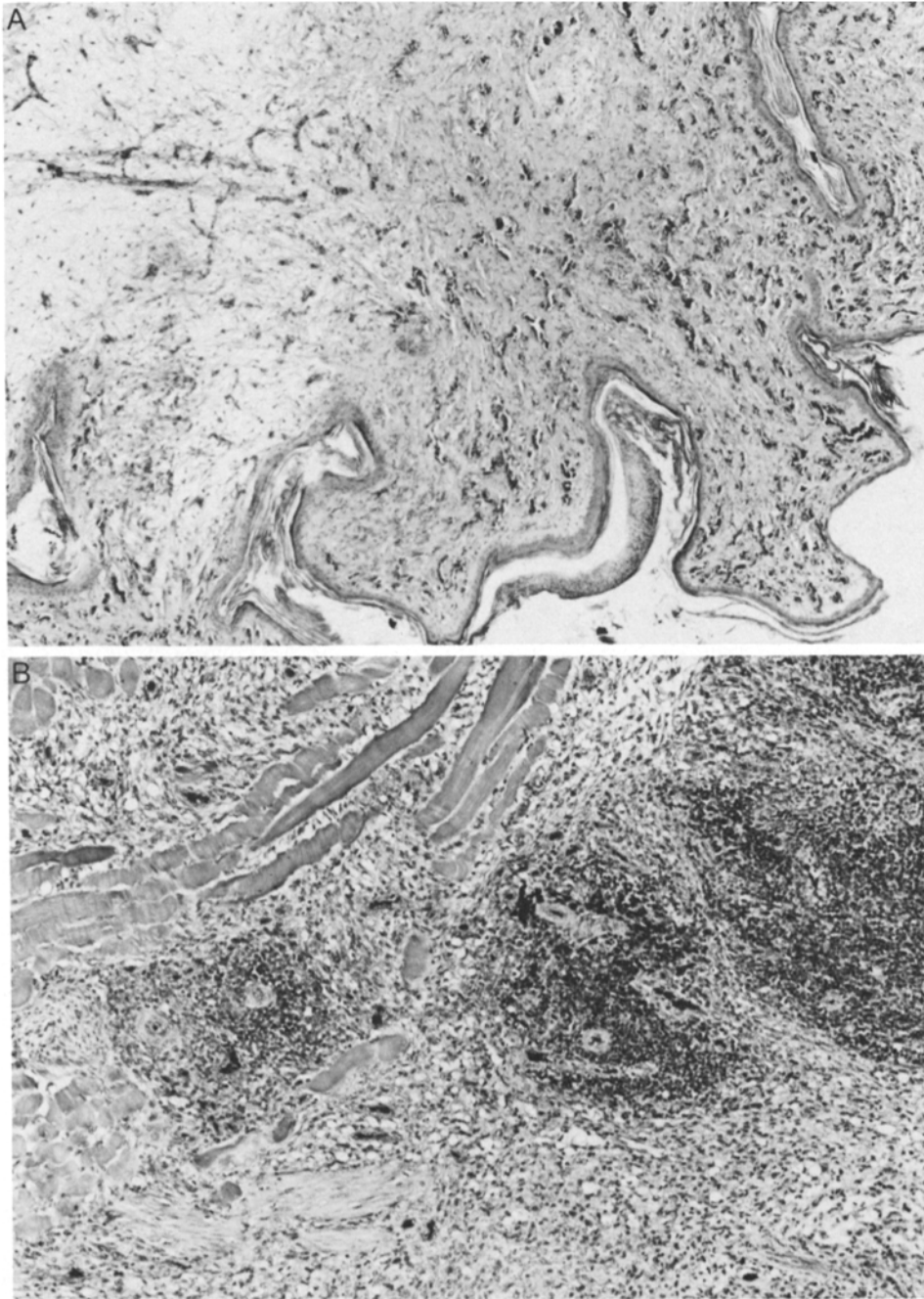


FIG. 6 A and B

FIG. 6. Line 200 chicken skin 6 wk of age. Note in (A) the intense mononuclear cell infiltrate, particularly around blood vessels, as well as the swollen appearance of vessels; HE staining; $\times 77$. In contrast, at 3 mo of age (B) there is an intense proliferation of small vessels and an increase in collagen; HE staining; $\times 77$. Finally, at 6 wk of age, (C) note the thick-walled blood vessels with lumen narrowing, intimal reduplication, smooth muscle hypertrophy, and vessels surrounded by an intense mononuclear cell infiltrate in the skin; HE staining; $\times 285$.

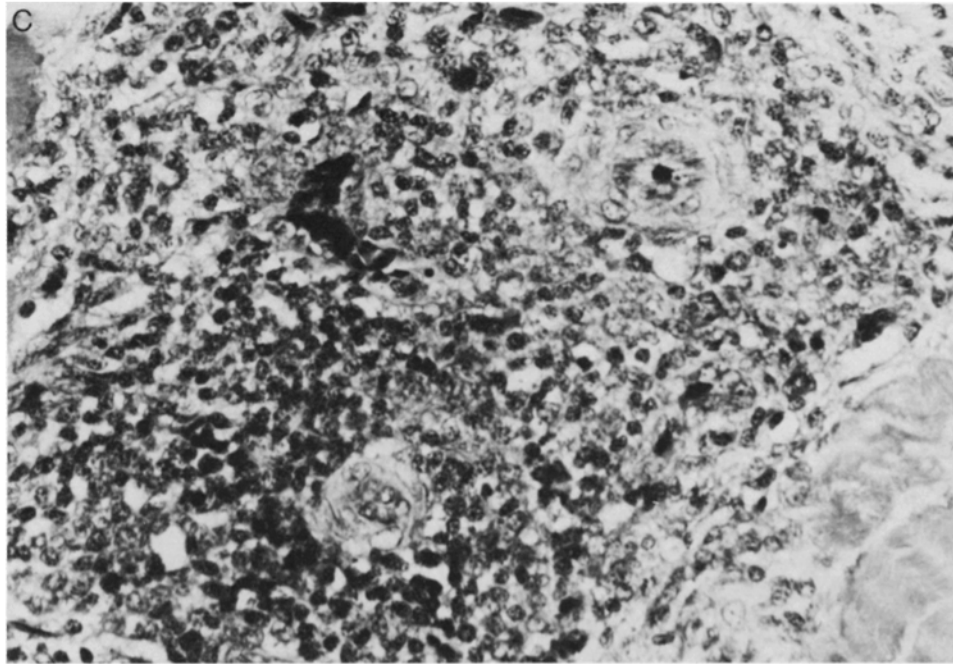


FIG. 6C

TABLE I
Genetics of Line 200

Line	Hatch	Number and percentage of abnormalities		Line description
			%	
200	Fall 1979	118/139	(85)	Selected abnormal line
200	Spring 1980	79/98	(89)	
F ₁	Spring 1979	0/111	(0)	Cross of abnormal line 200 × other inbred normals
F ₂	Spring 1980	11/247	(4)	F ₁ × F ₁
BC	Fall 1979	43/84	(51)	F ₁ × 200

extensive involvement of esophagus, heart, and kidney (Table II). The esophagus from control chickens has an intact epithelium with loose underlying connective tissue and striated muscle layer (Fig. 8A). The esophagus of chickens does not show a gradual change from skeletal muscle to smooth muscle and all levels are comparable. In contrast, 60% of 6-mo-old line 200 birds have a distinctly different esophagus than that seen in controls with extensive thickening of the underlying connective tissue by densely packed collagen. This collagen extends down into and through the muscle layer (Fig. 8B). In addition, mononuclear focal infiltrates in mucus glands are present in the subepithelial layer of the esophagus of abnormal birds (Fig. 8B); these glands, in the chicken, are analogous to human salivary glands. Finally, in addition to the

TABLE II
Incidence of Organ Involvement In Line 200 Chickens

Age	Incidence*						
	Skin	Comb	Esophagus	Heart	Lung	Small intestine	Kidney
	%						
6 wk	40	95	10	0	0	0	0
6 mo	30	90	60	40	10	0	20

* There are no comparable lesions found in birds of UCD lines 003, 011, or 159.

intense fibrosis, there is also significant small vessel proliferation in subepithelial layers of tissue. These vessels appear thick-walled, much like those in skin.

The kidneys of line 200 chickens reveal a variety of abnormalities. Because serial studies were not done, it is unclear as to whether or not these changes represent primary or secondary features of disease. However, in virtually all (greater >90%) 6-mo old line 200 chickens, significant swelling and thickening of muscular layers of renal blood vessels are present. Some vessels have surrounding round cell infiltrates. Further, ~20% of birds have a significant glomerulonephritis and, by immunofluorescence, have IgG deposits within glomeruli. IgG deposits were not found in skin, blood vessels, or the other organs described.

The thoracic cavity of line 200 chickens is most noteworthy for the appearance of pericardial effusions in 40% of 6-mo-old animals. In addition, sections of heart show moderate to severe prominence of blood vessels and cellular proliferation on the epicardial surfaces. In contrast, the lung, at 6 mo of age, appears to be relatively normal. In only 1 of 10 birds was there a significant pulmonary abnormality. In this animal, there were thickened blood vessel walls present similar to those described earlier. In addition, there was an intense cellular proliferation obliterating the aveolar space. The culture of this lung was sterile.

Hematology. At 6 mo of age the hemoglobin, hematocrit, and leukocyte counts of line 200 chickens were similar to line 159 controls (Table III). However, the differential leukocyte count revealed a moderate (9% male; 14.5% female) eosinophilia in line 200 chickens compared with <2.5% in control line 159 chickens.

Autoantibodies. Both the direct and indirect Coombs's tests performed on line 200 chickens were negative. Similarly, there were no cryoprecipitates present in any of the 5-mo old line 200 chickens; samples from abnormal line 140 birds produced cryoprecipitates. At 4 mo of age, rheumatoid factor was found in 60% of line 200 chickens examined with a mean (\log_2) titer of 5.3 ± 1.8 ; no detectable rheumatoid factor was found in 17 control line 446 birds (Table IV). There were no naturally occurring anti-thymocyte antibodies detected by the immunofluorescent methods described herein. Similarly, line 200 chickens were found to have no detectable antibodies to native DNA or ENA. Known reference standards from UCD line 140 chickens gave expected results and served as quality controls as previously described. In contrast, 26 of 40 6-mo-old line 200 chickens revealed a positive ANA test with titers of 1:20–1:160; 0 of 28 line 011 controls gave a positive ANA (Table V). Pattern reading using the HEp-2 cell line revealed the majority of positive ANA (20 of 26) to be speckled (Fig. 9). Finally, by hemagglutination, 45 or 46 line 200 chickens were

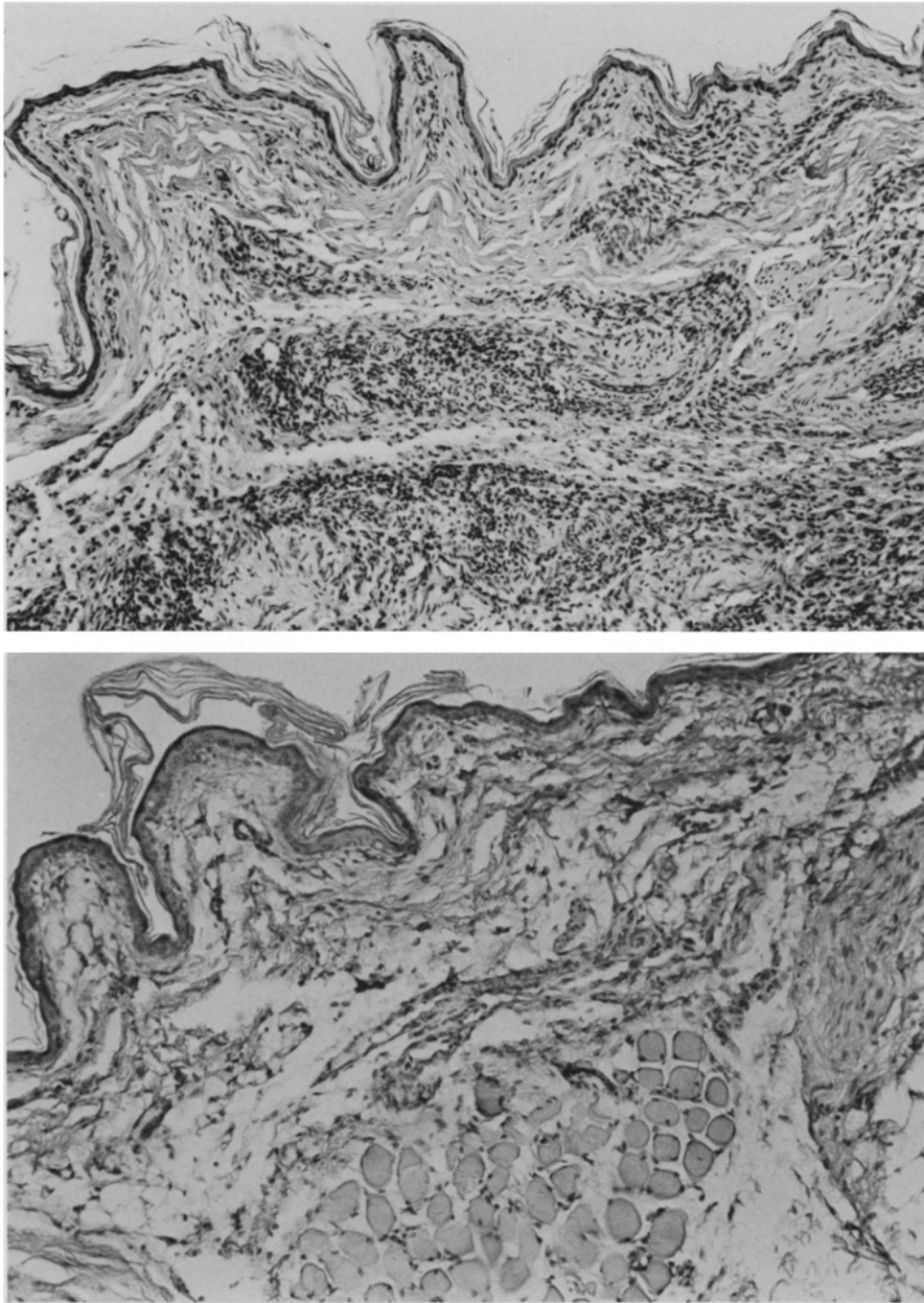


FIG. 7. Sections are of line 200 chicken skin (top). The epidermis is atrophic. Notice the dense collagen bundles that have replaced the usual delicate collagen of the superficial dermis. The remaining dermis is infiltrated with chronic inflammatory cells and adipose tissue has been replaced by fibroblasts and lymphocytes. Compare with sections of normal skin (bottom). Notice the delicate superficial dermis; the subcutaneous tissue contains fat and striated muscle; HE staining; $\times 77$.

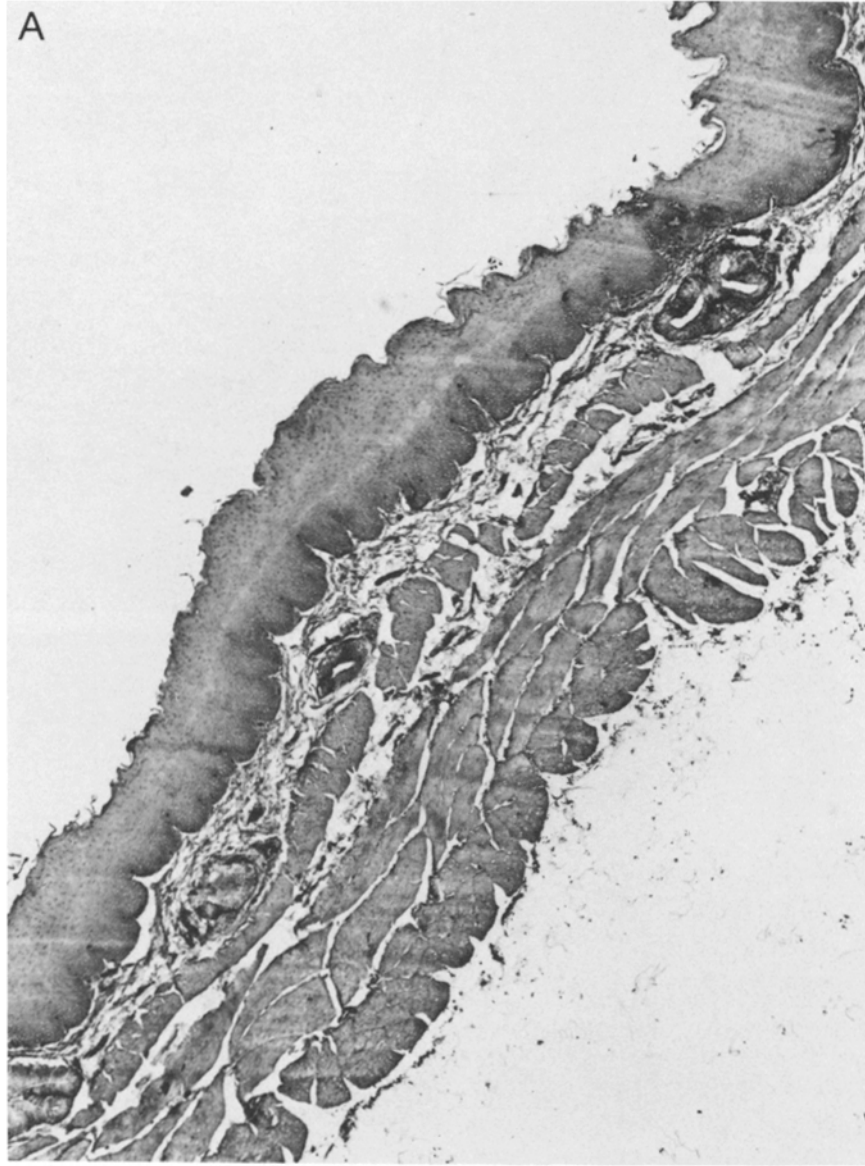


FIG. 8A

FIG. 8. Line 446, 6-mo-old esophagus (A). Note the delicate nature of the underlying collagen; this collagen does not extend into the muscle layer. In contrast, in a line 200 6-mo-old bird (B), note the lymphocytic infiltrates around esophageal mucus glands. In addition, there is an extremely thickened and dense collagen which extends down through the muscle layer; HE staining; $\times 100$.

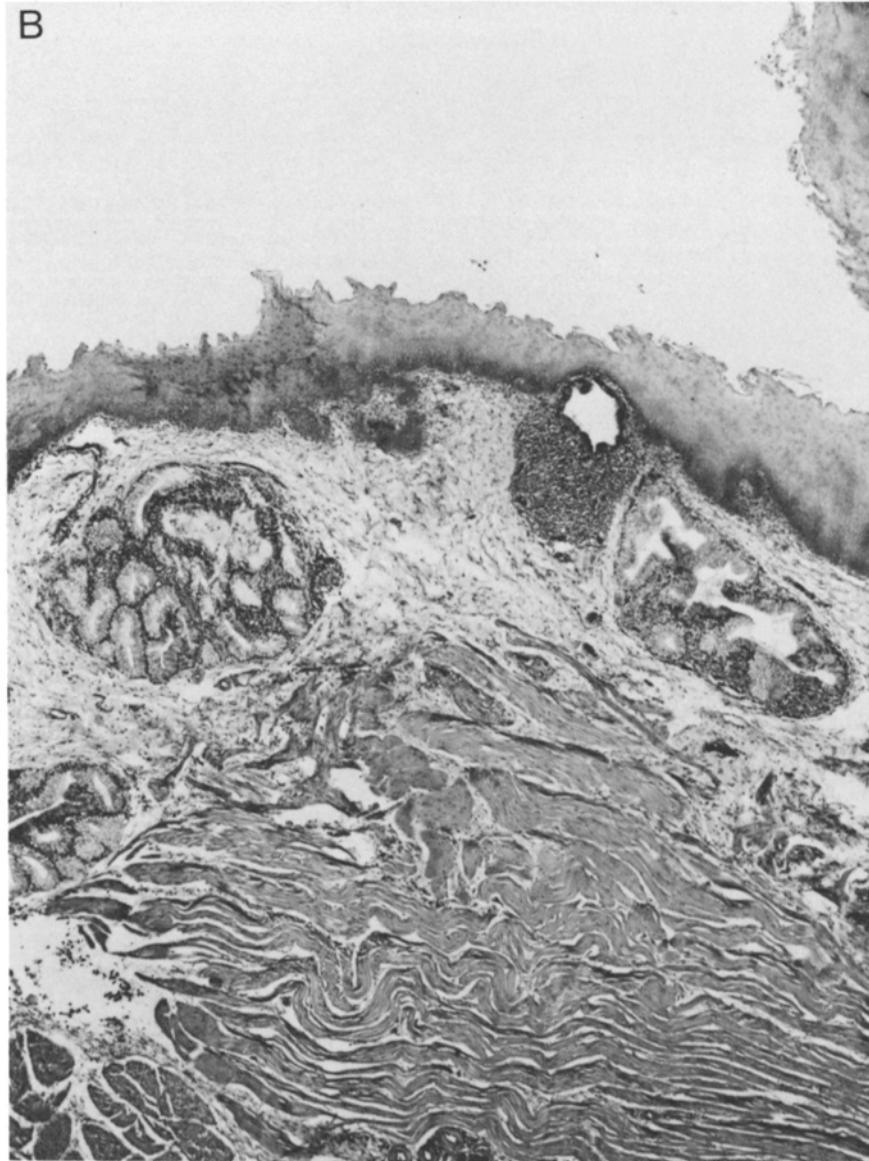


FIG. 8 B

noted, at 6 mo of age, to have antibodies to type 2 collagen with a mean titer of 3.1 ± 1.4 (Table VI). In contrast, there were no detectable anti-collagen antibodies present in 29 control chickens; ELISA gave similar results.

Quantitative Serum Immunoglobulins. At 4 mo of age, the concentration of IgM in line 200 chickens was 119 mg/100 mg; this value is similar to line 446 controls: 125 mg/100 mg. In contrast, there was approximately a twofold increment in 7S IgG 827 mg/100 mg in 11 line 200 chicks, compared with 409 mg/100% in 13 line 446 controls (Table VII).

TABLE III
Complete Blood Counts of Line 200 Chickens*

	Hemoglobin	Hematocrit	Leukocytes	Lymphocytes	Monocytes	Heterocytes	Eosinophils	Basophils
Line 200								
Male	13.6 ± 1.9‡	37 ± 2.8	24,250 ± 5074	63.7 ± 5	8.5 ± 3.6	16.5 ± 3.8	9.5 ± 5.1	2 ± 0.8
Female	8.8 ± 1.5	2.5 ± 2.0	31,375 ± 4534	62.7 ± 2	5.25 ± 0.5	16.2 ± 4	14.5 ± 1.3	1.5 ± 0.5
Line 159								
Male	13.5 ± 2	40.0 ± 20	16,615 ± 5100	64 ± 2.5	6.4 ± 1.2	25.8 ± 1.1	1.4 ± .5	2.4 ± 35
Female	9.7 ± 1.7	30.8 ± 3.0	29,397 ± 4712	76.1 ± 3	5.7 ± 1	13.3 ± 3.1	2.5 ± .5	2.4 ± .5

* 12-18 birds/group at 6 mo of age.

‡ Mean ± SEM.

TABLE IV
Rheumatoid Factor in Line 200 Chickens

Group*	Titer (log ₂)
Line 200	5.3 ± 1.8
Line 446	Not detectable

* 11-17 birds/group at 4 mo of age.

TABLE V
ANA in Line 200 Chickens*

Group	Positive/total	Pattern			
		Peripheral	Homogeneous	Speckled	Miscellaneous
Line 200	26/40	4	1	20	1
Line 011‡	0/28	—§	—	—	—

* 6 mo of age; positive ≥ 1:20.

‡ Positive ANA in other inbred lines is rare, see reference 10.

§ Not detectable.

Biochemical Markers. There were no significant differences between line 200 and control chickens at 6 wk or 6 mo with regard to sodium, potassium, chloride, CO₂, BUN, creatinine, SGOT, SGPT, alkaline phosphatase, uric acid, CPK, or aldolase.

Discussion

There are several well-catalogued mutations of chickens that have provided useful models for connective tissue and immunologic research. These models include congenital ichthyosis, muscular dystrophy, thyroiditis, and spontaneous acquired dysgammaglobulinemia (22-25). In addition, in 1945, an unusual autosomal recessive disease termed "dactylolysis" was reported by Shoffner in a short communication (26). This report described a mutation in White Leghorn chickens characterized by accelerated mortality, involution of comb, and peripheral arthritis. Unfortunately, although the author suggested that the syndrome was similar to human scleroderma, no further attention was given to these birds and the relationship between the disease of Shoffner's flock and the birds studied herein is unknown. Finally, although there are a large number of inherited diseases of collagen of dog, cat, mink, mice, and cattle, these syndromes have no similarity to those found in line 200 chickens nor any relationship to scleroderma in humans (26, 27).

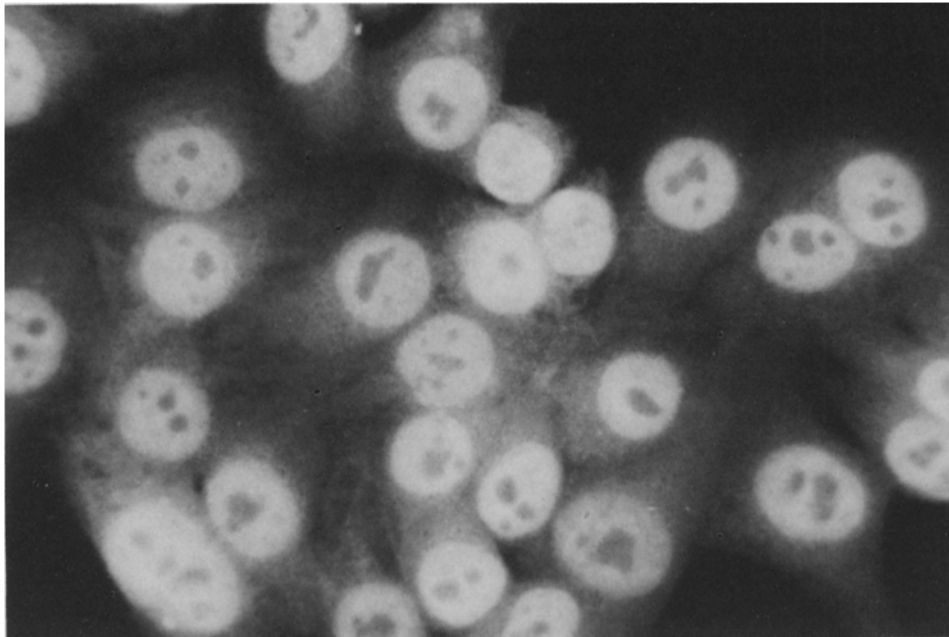


FIG. 9. Indirect immunofluorescence of line 200 sera using HEP-2 cells as substrate. Note the negative nucleolar staining with diffuse fine speckles throughout the nucleus. Because of the dense packing of these fine speckles, this staining pattern appears homogeneous about the negative nucleoli; $\times 310$.

TABLE VI
*Anti-Collagen Antibodies in Line 200 Chickens**

Group	Positive/total	Titer (\log_2)
Line 200	45/46	3.1 ± 0.04
Line 446	0/20	Not detectable

* By hemagglutination of type II chicken collagen coupled to SRBC.

TABLE VII
Quantitative Serum Immunoglobulins of Line 200 Chickens

Group*	mg/100 mg \pm SEM	
	IgM	7S Ig‡
Line 200	119 ± 24	$824 \pm 39§$
Line 446	125 ± 17	409 ± 83

* 11 birds/group at 4 mo of age.

‡ For definition of chicken 7S Ig, see references 18 and 25.

§ $P < 0.01$ by the Student's t test.

The involution of comb, toes, and the presence of skin lesions in chickens of line 200 appears to be inherited as an autosomal recessive trait, with incomplete penetrance. This conclusion is based on the absence of abnormalities in F_1 progenies resulting from reciprocal matings of normal birds of two inbred lines with line 200. Backcrosses of F_1 males with line 200 hens gave close to 50% disease frequency among progeny as would be expected for a single recessive gene, whereas F_2 progeny showed

a much lower incidence with only 4% of offspring being abnormal. This latter observation suggests the presence of modifying genes causing either enhanced expression of the disease in line 200 or reduced disease in control birds. Such a situation is in agreement with the genetic development of line 200 through selection for increased penetrance of self-dubbing over many generations. However, the precise genetic interactions that appear to regulate expression of the herein described abnormalities in line 200 require further study.

In humans, familial clustering of scleroderma is generally very rare. Of the few published reports describing scleroderma in more than one family member, siblings were usually involved, although there have been reports involving father and son and a mother and four daughters (28, 29). Because there are few well-documented familial cases of scleroderma, it has not been possible to characterize scleroderma as a heritable disorder. Moreover, a variety of immunogenetic and epidemiologic studies have failed to conclusively support an association between major histocompatibility antigens and scleroderma (30, 31). Nonetheless, it is of interest to note that close relatives of patients with scleroderma have been found to have low titers of ANA and an abnormally high frequency of chromosome breakage (31). These two factors certainly lend support to the notion of a familial predisposition to PSS.

The syndrome of line 200 chickens has a number of similarities as well as differences with human scleroderma. First, similar to the human syndrome, affected chickens develop fibrosis of skin and esophagus. Moreover, intense mononuclear cell infiltrates accompanied by collagen deposition and severe vascular occlusion appear early. In addition, as described in human scleroderma, there is marked proliferation of small vessels at the site of involvement. Nonetheless, there are a number of significant histologic differences. Unlike the histocytic infiltrate and intimal damage of small vessels of human scleroderma, affected chickens develop severe muscular hypertrophy of small vessels. This hypertrophy is a major basis for the occlusive vascular disease. Secondly, although an intense deposition of collagen occurs, the onset is significantly more acute than in humans. Further, a significant percentage of older chickens develops glomerulonephritis, a feature uncommon in human scleroderma. This glomerulonephritis, however, may be a secondary feature of infection and is probably clinically insignificant because chickens do not develop azotemia. The urine of chickens drains into the cloaca, making urinalysis difficult. The immunologic features of this syndrome in line 200 chickens include the presence of ANA, rheumatoid factors, and antibodies to type 2 collagen, but absence of positive Coombs's test, cryoprecipitates, or antibodies to thymocytes, DNA, or ENA. These immunologic features are again similar to large series of patients with scleroderma (1, 15). ANA, in particular, give a speckled pattern in ~50% of chickens.

This syndrome, however, in sharp distinction to human scleroderma, appears to be significantly more fulminant in chickens. The dramatic mortality of young chickens is a result of secondary infection of peripheral gangrenous lesions in regions vascularized by occluded vessels. Nonetheless, as emphasized earlier, these chickens have been maintained in a closed flock for nearly forty years. Moreover, as they were selected on the basis of the comb lesion, it is not surprising that this manifestation is so evident. Birds that survive the acute episode and develop the self-dubbed appearance later develop a progressive disease involving internal organs; fortunately, sufficient enough fertility is maintained to permit propagation of the line.

The use of line 200 chickens as a model of human scleroderma must be contrasted with other proposed animal models. The first proposed model of PSS was homologous disease of rats (5, 6). Experimental homologous disease is induced by injection of lymphoid cells from donors of an inbred strain into tolerant rats. Among the prominent features of homologous disease are lesions of skin, joints, and heart with impressive similarities to scleroderma. Moreover, of particular importance to scleroderma, there is immunologic reactivity directed against host skin. Indeed, affected rats reject grafts of their own skin at the same time that donor grafts are accepted. This autograft rejection can be seen to be highly specific for host skin. Chronic skin lesions of homologous disease show extreme epidermal atrophy with markedly increased thickening and collagenization of the dermis. An inflammatory reaction, if present, is usually mild in such lesions. Similarly chronic graft-vs.-host disease (GVHD) after bone marrow transplantation has been found to have scleroderma-like features in humans (7, 8, 32). Spielvogel et al. (7) have noted that patients with chronic GVHD have cutaneous abnormalities including degeneration and loss of cellularity of surface epithelium, hyperkeratosis, hyperpigmentation, and an infiltrate of the mid and deep dermis by monocytes, histiocytes, and fibroblasts (7). Moreover immune complexes are found in vessel walls and at the dermal-epidermal junction. The latter features have suggested that chronic GVHD may be more similar to either vasculitis or systemic lupus erythematosus. Nonetheless, other clinical features of both scleroderma as well as Sjögren's syndrome have also been reported in chronic GVHD. In contrast to line 200 chickens, however these models do not develop significant titers of ANA, antibodies to collagen, or rheumatoid factors. Moreover, and most important, they do not permit establishment of a distinct animal colony with a spontaneous disease.

We do not, of course, propose that this syndrome of line 200 chickens is the identical disease process known as human progressive systemic sclerosis. At best it is a new disease of chickens, in a highly inbred flock of birds, characterized by severe vaso-occlusive disease, fibrosis, and the presence of a variety of autoantibodies and hypergammaglobulinemia. A significant volume of work remains to be done to place these observations in perspective and to define the associations of specific pathologic and immune abnormalities with the clinical expression of disease and the genetic basis of the syndrome.

Summary

University of California, Davis (UCD) line 200 White Leghorn Chickens spontaneously develop a syndrome that has many analogous features to human progressive systemic sclerosis. This syndrome is characterized by progressive involution of comb, dermal fibrosis, and distal polyarthritis. These three features occur within 6 wk after hatching, and are accompanied by a 40% mortality as a result of vaso-occlusive disease, with development of secondary infection of peripheral gangrenous lesions. Birds that survive >2 mo after hatching progressively develop fibrosis of the esophagus and mononuclear infiltration of heart and kidney, with prominent occlusion of small and medium sized blood vessels. In addition, line 200 chickens develop rheumatoid factors, antinuclear antibodies, and antibodies to collagen, but do not have antibodies to thymocytes, DNA, or extractable nuclear antigens. Moreover, antinuclear antibodies when studied using HEp-2 cells as substrate demonstrate predomi-

nantly a speckled pattern. This syndrome of line 200 chickens is not detectable in F₁ crosses to several UCD inbred lines. F₁ × parental line BC₁ backcrosses have an ~50% incidence of disease, suggesting that this syndrome is inherited as autosomal recessive. However, only 4% of F₂ generation birds show abnormal symptoms, suggesting the presence of modifying genes. There is no appearance of IgG deposition, as determined by immunofluorescence, in either skin, blood vessels, esophagus, or heart. However, ~20% of chickens have a glomerulonephritis; this feature appears to be a terminal event and does not appear clinically significant. Although this syndrome of line 200 chickens has several features that are in sharp distinction to human scleroderma, the presence of common immunologic and pathologic denominators suggest that this spontaneous disease may be an appropriate model to develop a better understanding of autoimmune connective tissue diseases.

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