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Plasma free Hydroxyproline, Growth, and Sexual Maturity in the Scoliotic Chicken (40983)[1]

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Abstract. An animal model for experimental study of idiopathic scoliosis is suggested. Chickens were selected and bred for scoliosis. The scoliotic chickens were found to grow more slowly and mature sexually later than a normal control line of chickens. All chickens from the scoliotic strain had some degree of scoliosis, but the curves were more severe in the homogametic sex (male). Plasma free hydroxyproline concentrations of the scoliotic chicken were found to be twice normal values and most likely reflect an abnormality in collagen metabolism.

Scoliosis is an important spinal disorder in man (1, 2). Yet, despite extensive research, the cause of the most commonly encountered spinal curves is still unresolved and there are also few animal models for studies of the disorder.

Zorab (3) speculated that the cause of idiopathic scoliosis might result from the disorders of one or more of the following: the bony vertebral column and its adjacent ribs, the surrounding muscles and their blood or nerve supplies, the connective tissue, or unequal growth rates. By studying idiopathic scoliosis in chickens, Riggins *et al.* (4) reported that the disease, at least in chickens, is not caused by an abnormality of growth and development of the spine or an imbalance of the adjacent musculature. However, they found that several types of collagen in the scoliotic chicken are more soluble than the collagen of the normal White Leghorn controls. The significance of this finding is not clear, but it could indicate defects in the normal maturation of collagen from scoliotic chickens.

The excretion of hydroxyproline and its release into circulation is largely a function of collagen metabolism. For example, the rate of excretion of hydroxyproline is related to collagen degradation and high levels of urinary hydroxyproline are found in some patients with disorders of collagen metabolism (5). Benson (6) and Zorab (7) have found elevated urinary hydroxyproline in patients with scoliosis. Also, Smith and Francis (8) have reported increased solubility of collagen from skin of patients with scoliosis which suggests defects in collagen maturation that may influence the overall rate of collagen degradation. Subsequently, we examined scoliotic chickens for abnormalities of hydroxyproline metabolism as an additional indicator that this strain of chickens may be a useful animal model in the study of scoliosis. Data are also presented which indicate various features of sexual maturation in scoliotic chickens.

Materials and methods. Forty-eight chickens from the scoliotic strain were age-matched to 22 normal White Leghorn chickens from the Hyline Chicken Farm in Modesto, California. All birds were vaccinated against Marek disease, Newcastle disease, and fowl pox. The rationale for the selection of the controls and other details related to the husbandry have been previously reported (4, 9, 10). Individual body weights were recorded weekly. From the sixth week individual blood samples were taken weekly from wing veins using heparinated syringes, and the samples were kept

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TABLE I. BODY WEIGHTS OF CONTROL AND SCOLIOTIC CHICKENS[a,b]

Group	Sex	Body weight				
		0 weeks	6 weeks	10 weeks	14 weeks	20 weeks
Control	male	37 ± 2 (11)	525 ± 40 (11)	965 ± 82 (11)	1346 ± 116 (11)	1795 ± 120 (11)
Scoliosis	male	26 ± 1 (20)*	356 ± 43 (20)*	820 ± 74 (19)*	1230 ± 83 (19)*	1620 ± 125 (18)*
Control	female	34 ± 2 (11)	409 ± 40 (11)	744 ± 52 (11)	1022 ± 75 (11)	1409 ± 111 (11)
Scoliosis	female	27 ± 2 (24)*	291 ± 42 (24)*	662 ± 65 (22)*	985 ± 77 (21)	1316 ± 91 (20)**

[a] Expressed as mean ± SD (number of observations).
[b] Within sex, * $P < 0.005$; ** $P < 0.05$.

on ice. The plasma was separated and stored at −20° until assayed.

To measure the average age at sexual maturity in males, weekly semen samples were obtained from each cockerel from the ninth week until half of the cockerels were producing sperm. The average age of sexual maturity was assigned when half of the pullets laid their first egg. At sexual maturity, the severity of scoliosis was measured using the Cobb–Lippman method following anteroposterior spinal roentgenography (4).

Trichloroacetic acid (TCA)-soluble hydroxyproline in plasma was determined according to method of Woessner (11). Aliquots of plasma (1.0 ml) were deproteinated with 10% TCA. Following centrifugation (1000*g*, 30 min), the deproteinated samples were neutralized and hydroxyproline determined. Statistical significance was determined by the *s*tudent *t* test.

Results. Table I shows the rate of growth of the male and female chickens at various stages. Normal White Leghorn chickens hatch at significantly higher weights ($P < 0.0001$) than chickens derived from a White Leghorn line with scoliosis. The control males chickens grew faster than males from the scoliosis line throughout the 20-week period of study. In general, the female chickens gained less weight per week than did males. The control female chickens also grew faster than the scoliotic female chickens in the first 12 weeks ($P < 0.05$). The survival rate of the two groups was also different. Nine of the 48 chickens with scoliosis died during the experimental period, whereas none of the control chickens died.

Further, the normal White Leghorn chickens reach sexual maturity at an earlier age than the scoliotic strain. Table II compares comb development, a sign of sexual maturity in chickens. Comb development of the control males was evident at 3 weeks of age, but it required 5 weeks for the scoliotic chickens to show the same degree of development. Some of the control cockerels had sperm in their semen as early as 10 weeks of age, and the average time of sexual maturity was 12 weeks of age. The cockerels of the scoliotic strain did not have sperm in semen until 13 weeks of age, and the average age of sexual maturity was 17 weeks. The first egg was laid by the control pullet at 20 weeks of age and the average age of sexual maturity for the pullets was 22 weeks. The corresponding ages for the pullets of the scoliosis strain were 22 weeks for the first egg and 25 weeks for the average age of sexual maturity.

There was no scoliosis in the control

TABLE II. SEXUAL MATURITY OF SCOLIOTIC AND CONTROL CHICKENS

Strain	Age (weeks)				
	Evidence of comb development	First appearance of sperm	First egg laid	Average sexual maturity	
				Male	Female
Control	3	10	20	12	22
Scoliotic	5	13	22	17	25

TABLE III. INCIDENCE AND DEGREE OF SPINAL CURVATURE OF THE CHICKENS FROM THE SCOLIOSIS STRAIN

Sex	Total	Degree of curvature						
		0–5°	6–10°	11–20°	21–30°	31–40°	41–50°	50° +
Male	19	0	3	4	4	3	1	4
Female	21	2	1	8	5	3	2	0
Total	40	2	4	12	9	6	3	4

birds of either sex, whereas all the chickens from the scoliotic strain developed some spinal curvature. As previously reported (4), the males had a more severe curve than females. As shown in Table III, none of the scoliotic pullets had a curvature over 50°.

Table IV shows the age-related hydroxyproline levels in the plasma of the control and scoliotic strain of chickens. There is a clear elevation in the plasma of hydroxyproline in the scoliotic birds compared to controls at every age investigated ($P < 0.001$). Although attempts were made to correlate serum hydroxyproline values with the degree of curvature, no significant correlation was found with respect to hydroxyproline values estimated prior to or at the time of estimating the degree of curvature (see Discussion).

Discussion. We feel that the scoliotic chickens may serve as a good model in studies dealing with spinal abnormalities and selected aspects of idiopathic scoliosis. In a previous report we described morphological features of the lesion in chickens that appeared to be consistent with those observed in other mammals and man (4). The observations that are reported herein also appear to be consistent with selected features of the disease. For example, for some forms of congenital scoliosis, particularly infantile idiopathic scoliosis, there is delay in growth and development. Birds in the scoliotic line also appear to mature more slowly than normal White Leghorn chickens. Furthermore, it may be of significance that the most severe curves occurred in cockerels. This suggests that in chickens, as in humans, the homogametic sex develops greater spinal curvatures.

The elevated levels of plasma hydroxyproline in the scoliotic chicken cannot be explained by delayed maturation for at no time did the level of hydroxyproline in plasma fall to the level found in normal birds. The data also suggest a generalized alteration in collagen metabolism in the scoliotic chicken. The levels of hydroxyproline in trichloroacetic acid supernatant fractions of plasma were significantly higher in the scoliotic chickens than in the controls at each time period examined. Previously, we observed that collagen was more soluble in bone, tendon, and cartilage in the scoliotic line compared to the collagen in normal White Leghorns. From the

TABLE IV. HYDROXYPROLINE IN PROTEIN-FREE PLASMA FRACTIONS FROM CONTROL AND THE SCOLIOTIC CHICKENS[a]

	Hydroxyproline (μg/ml)			
	6 weeks	10 weeks	14 weeks	20 weeks
Female				
Control	2.6 ± 0.4 (11)	1.9 ± 0.3 (11)	1.9 ± 0.5 (5)	1.0 ± 0.2 (6)
Scoliotic	4.1 ± 0.4 (22)	3.9 ± 0.4 (18)	4.0 ± 0.6 (12)	2.8 ± 0.5 (7)
Male				
Control	2.9 ± 0.3 (11)	2.3 ± 0.2 (11)	2.5 ± 0.4 (6)	2.6 ± 0.7 (6)
Scoliotic	4.5 ± 0.4 (17)	4.1 ± 0.3 (6)	3.8 ± 0.8 (10)	3.8 ± 0.6 (7)

[a] Mean ± SD (number of observations). The differences between controls and scoliotic chicken was $P < 0.0001$.

standpoint of viewing the scoliotic chicken as an animal model, these observations take on significance particularly in view of the reports indicating higher than normal rates of excretion of hydroxyproline in humans with scoliosis (2, 6, 7, 12–14) and increased skin collagen solubility in patients with scoliosis (8). Although we could not measure urinary hydroxyproline in the chicken, because of the difficulties of separating urine from feces in birds, it has been observed that in mammals changes in hydroxyproline in plasma appears to parallel changes in urine (13). Subsequently, the observation of high plasma levels of hydroxyproline in scoliotic chickens appears consistent with the observations in man.

In the scoliotic strain, no idiopathic spinal curves are usually found before 4 weeks of age. After this period, the chickens began demonstrating scoliotic curves and at sexual maturity, the curves are clearly evident. We hope that by using the scoliotic chicken as a model factors that appear important to the onset of scoliosis may be identified. Further, more refined studies dealing with the maturation and typing of vertebral collagens from the scoliotic line of chickens may lead to a better understanding of reported increases in collagen content of the nucleus pulposus of scoliotic vertebra (15).

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