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Somatomedin and Thyroid Hormones in the Developing Chick Embryo (41018)

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Abstract. Embryonic chick cartilage is used to detect somatomedin activity, however, the presence of somatomedin in the developing embryo has not been reported. We have shown that thyroid hormones stimulate somatomedin activity in the rat. This study compares changes in thyroid hormones and somatomedin in the developing chick embryo. Somatomedin activity, thyroxine (T_4), and triiodothyronine (T_3) were determined in sera obtained from embryonic chicks on Days 10 through 19 of incubation and from young adult chickens. Somatomedin was determined by bioassay which measures the sulfate incorporation into pelvic cartilage of 12-day-old chick embryos. T_4 and T_3 were determined by radioimmunoassay. Somatomedin activity was not detected in 10- to 12-day-old embryos, was measurable at 13 days, and increased to a peak value at 15 days (potency of 0.93 compared to normal rat serum value of 1.0). No somatomedin was detected in the serum immediately prior to hatching or in young chicken serum. T_4 was not detected until Day 15 (0.42 μg T_4/100 ml) and increased to 1.39 μg T_4/100 ml on Day 19. T_3 was present in the serum throughout the study, with an increase occurring on Day 14 (51 ng T_3/100 ml). The appearance of somatomedin coincides with collagen formation and ossification in the chick embryo skeleton. The changes in thyroid hormone concentrations apparently do not influence somatomedin in the chick embryo as has been demonstrated in the rat.

Somatomedins are insulin-like peptides responsible for the stimulation of cartilage macromolecular synthesis (1). Somatomedin activity assayed by sulfate incorporation into embryonic chick cartilage has been detected in the serum of a variety of animal species, including man; however, its presence in embryonic chick serum has not been studied. There is conflicting data regarding the presence or absence of somatomedin activity in avian serum (2, 3).

The somatomedins were initially described as growth hormone-dependent factors (4); however, their production and/or secretion are influenced by insulin and the thyroid hormones (5, 6). We have previously shown that treatment of hypophysectomized rats with thyroxine restores growth and somatomedin activity in the absence of growth hormone (7). Thyroxine (T_4) and triiodothyronine (T_3) are present in the serum of the embryonic chick (8) and have been implicated in embryonic growth (9, 10).

The aim of this study is to determine if somatomedin activity is present in the serum of the developing chick embryo and is correlated with thyroid hormone concentration.

Materials and Methods. Eggs from white Leghorn chickens (SPAFAS) were incubated at 38° in a forced-air incubator with automatic turner (Jamesway). Embryonic stage was expressed as days of incubation and verified by the method of Hamburger and Hamilton (11).

Blood from embryonic chicks was drawn from extraembryonic blood vessels through the air sac wall by means of pipets made from glass capillary tubes. These were connected by latex tubing to a Pasteur pipet. The latter pipet was attached to a bulb-type pipet filler which provided gentle suction while blood was withdrawn. Equal aliquots of blood from 15 to 20 chick embryos of the same age were pooled. Chicken serum was obtained from blood withdrawn from a wing vein of 12- to 18-week-old white Leghorn hens. Rat serum served as the reference for the somatomedin assays and was obtained from abdominal aortic blood of normal male Sprague–Dawley rats (Madison, Wisc.)

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weighing 150–200 g. All blood was centrifuged and the serum stored at −20° until assayed.

Serum thyroxine and triiodothyronine determinations were done by radioimmunoassay using commercially available reagent kits (T_4-RIA, Roche; T_3-RIA, Mallinkrodt). Assay sensitivity levels were 0.15 μg/100 ml for T_4 and 0.25 ng/100 ml for T_3.

Serum somatomedin activity. Somatomedin activity of embryo and chicken serum was assayed by the chick cartilage sulfation method of Hall (12), modified to use supplemented Waymouth's medium.

The pelvic rudiments of 12-day-old chick embryos were dissected free, cleaned of adherent tissue, and placed in iced saline until ready for random placement into incubation vials. The incubation medium used was Waymouth's MB 752/1 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 0.35 m*M* L-serine, 0.5 g/100 ml bovine serum albumin, and 100 units/ml penicillin and streptomycin. Serum was added at concentrations of 5, 10, and 20% to provide a log dose–response curve. The three concentrations of serum were assayed with three to four pelvic rudiments per dilution.

Pelvic cartilage tissues were incubated in individual tubes containing 2 ml of serum dilutions and tubes were placed in a shaking water bath at 38° for 1 hr. Eight microcuries of carrier-free $Na_2{}^{35}SO_4$ (Schwarz/Mann) was added to each tube and the incubation was continued for 5 hr. The cartilage fragments were placed in saturated Na_2SO_4 overnight, rinsed in distilled water and 95% ethanol, air dried, and weighed to the nearest hundredth milligram on a Cahn electrobalance. The tissues were digested at 60° in 0.5-ml digestion solution which consisted of 3 m*M* cysteine HCl, 4 m*M* EDTA, 0.1 *M* NaH_2PO_4 (pH 6.5), and 0.5 mg papain. Samples of digest (100 μl) were placed on Whatman No. 1 filter paper strips (2 × 2 in), dried, and placed in scintillation vials (13). They were counted in a Packard Tri-Carb liquid scintillation counter, Model 3380 (Packard Instrument), using a scintillation solution of 4 g/liter PPO and 50 mg/liter POPOP dissolved in toluene. Results were expressed as counts per minute per milligram dry weight.

Parallel-line bioassay analysis was done on each assay using a computer program adapted for use with the Hewlett–Packard calculator, Model 65 (14). An assay was considered valid if the log dose–response for the preparation had a significant slope ($P < 0.05$) and if the response satisfied the criteria for parallelism with the reference serum ($P > 0.05$). Invalid assays were interpreted as nondetectable activity. The relative somatomedin potency of experimental sera was estimated by the antilogarithm of the horizontal distance between the log dose–response lines of the reference serum and experimental serum. Somatomedin potency of experimental serum was expressed in terms of pooled normal rat serum which served as reference standard and which by definition had a potency of 1.00.

The index of precision for 25 somatomedin assays was 0.22 ± 0.01 (mean ± SEM). Data were statistically analyzed by the Newman–Keuls test preceded by one-way analysis of variance. Differences were considered significant at the $P < 0.05$ level.

Results. *Serum somatomedin activity.* Somatomedin activity was not detected on Days 10–12 of incubation but reached a potency of 0.29 on Day 13 (Table I). Somatomedin increased daily to a maximum potency of 0.93 on Day 15, and decreased (0.74 and 0.79) on Days 16 and 17. Serum from 18- and 19-day-old embryos and young chickens had no detectable somatomedin activity.

Serum T_4 concentration. T_4 was not detected in serum from 10- to 13-day-old embryos (Table I). Serum T_4 increased from 0.42 μg T_4/100 ml on Day 15 to 1.03 μg T_4/100 ml on Day 17 of incubation. Young chicken serum had 3.15 μg T_4/100 ml.

Serum T_3 concentration. T_3 was present in the serum from Days 10–18 of incubation. Serum T_3 increased from 27 ng T_3/100 ml on Day 10 to 51 ng T_3/100 ml on Day 14 with no further change noted. The maximum concentration was 62 ng T_3/100 ml on Days 17–18. Young chicken serum had a T_3 concentration of 116 ng/100 ml.

TABLE I. SOMATOMEDIN, THYROXINE, AND TRIIODOTHYRONINE IN SERUM OF DEVELOPING CHICK EMBRYOS

Day of incubation	Somatomedin		T_4		T_3	
	n^a	Potency[b]	n^a	μg/100 ml[b]	n^a	ng/100 ml[b]
10	3	Not detectable	4	Not detectable	4	26.6 ± 5.4
11	3	Not detectable	5	Not detectable	3	34.7 ± 2.2
12	8	Not detectable	5	Not detectable	5	26.7 ± 3.9
13	5	0.29 ± 0.06[c]	14	Not detectable	12	40.5 ± 3.9
14	6	0.56 ± 0.06[c]	22	0.16 ± 0.06	12	51.3 ± 3.5[d]
15	6	0.93 ± 0.11[c]	14	0.42 ± 0.14[c]	6	50.1 ± 6.3
16	6	0.74 ± 0.11[c]	13	0.74 ± 0.11[c]	6	51.2 ± 5.8
17	5	0.79 ± 0.09	9	1.03 ± 0.10[c]	1	61.5
18	11	Not detectable	11	1.27 ± 0.15	5	61.1 ± 5.0
19	6	Not detectable	2	1.39 ± 0.31		
Chicken	8	Not detectable	7	3.15 ± 0.22[e]	7	115.5 ± 5.7[e]

[a] Number of pooled samples; a pool contained equal aliquots of serum from 15 to 20 embryos.
[b] Mean ± SEM.
[c] Significantly different from values of previous days ($P < 0.05$).
[d] Significantly different from Day 10 value ($P < 0.05$).
[e] Significantly different from chick embryo values ($P < 0.001$).

Discussion. Chick embryo cartilage is used routinely for bioassay of somatomedin; however, the presence of somatomedin in embryonic serum has not been reported. Somatomedin activity first appeared in serum of chick embryos on Day 13 of incubation, increased to a maximum on Day 15, and was not detectable prior to hatching. In contrast, cartilage derived from 11- to 12-day-old embryos has the greatest *in vitro* sulfate uptake which declines thereafter (15). Chondrogenesis also decreases after Day 12 when ossification of the skeleton begins (9). Therefore, the chondrogenic process which presumably requires somatomedin in the mammal, appears to take place independently of somatomedin in the developing chick embryo.

Collagen formation in the chick embryo, however, increases rapidly at about Days 12–13, and reaches a plateau on Days 17–19 (16). This increase in collagen formation coincides with the time of somatomedin appearance and may indicate that somatomedin may be involved in collagen synthesis as has been shown in the mammal (17).

Skeletal development of the avian embryo appears to be under pituitary control probably through a growth hormone or tropic hormones. Hypophysectomy of the chick embryo results in retarded growth of long bones and epiphyseal width as well as decreased mineralization and matrix elaboration (18). Transplantation of pituitaries partially restores these growth defects (19). An avian growth hormone was recognized only recently and a radioimmunoassay developed for its measurement in serum (20). Further, Harvey *et al.* (21) found that somatomedin activity in growing chicks (hatching to 5 weeks) was positively correlated with serum growth hormone and prolactin. No measurement of growth hormone in the chick embryo has been reported.

Our failure to demonstrate somatomedin activity in serum of young chickens by the chick cartilage bioassay is in agreement with Van den Brande *et al.* (3). They found that adult pigeon serum was inactive or inhibitory when assayed for somatomedin by rat or porcine cartilage bioassay. In contrast, Shapiro and Pimstone (2) demonstrated somatomedin activity (by porcine cartilage bioassay) in chicken and duck serum; however, the activity appeared to be about half the potency found in human or rat serum.

Thyroid hormones also influence skeletal development as evidenced by accelerated appearance of ossification centers after thyroxine treatment and retardation of growth of the tibia and epiphyses after thiourea administration (9, 22). The measurement of T_4 and T_3 in chick embryo serum has been simplified by RIA. Several studies, including the present one, show that T_3 is present in the serum of 10-day-old chick embryos and rises prior to hatching (8, 23). Thyroxine is present in the chick embryo on Day 6.5 (24) and the serum concentration gradually increases prior to hatching (25). The present study reports a similar increase in serum T_4; however, the hormone was not detected until Day 14, probably due to a less sensitive assay. Nevertheless, the serum profiles for T_3 and T_4 are very different from the somatomedin activity over the same time period. It is doubtful that in the chick embryo, somatomedin is influenced significantly by the thyroid hormones, as we have demonstrated in the rat.

Further work will be necessary to determine if this avian somatomedin is influenced by growth hormone, prolactin, or insulin.

Summary. Somatomedin activity, thyroxine, and triiodothyronine were determined in sera obtained from embryonic chicks on Days 10 through 19 of incubation and from young adult chickens. Somatomedin was determined by bioassay which measures the sulfate incorporation into pelvic cartilage of 12-day-old chick embryos. T_4 and T_3 were determined by radioimmunoassay.

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