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Serum Somatomedin Activity in Two Animal Models as Measured Using Chick Epiphyseal Plate Cartilage Bioassay (40796)[1]

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In their classical study, Salmon and Daughaday (1) found that although serum from both normal and hypophysectomized rats treated with growth hormone could stimulate incorporation of sulfate and/or thymidine into cartilage *in vitro*, growth hormone itself was ineffective. They postulated that growth hormone stimulated the production of an intermediary serum factor they call sulfation factor. It was later found that sulfation factor activity is reduced in dwarf mice (2), patients suffering from hypopituitarism (3), and pituitary dwarfs (4). Conversely, sulfation factor activity is elevated in acromegalic patients (3). Conditions which have been shown to affect sulfation factor activity are starvation (2) and age (2, 5). Sulfation factor activity has been reported to be correlated with growth rate (6) and dental maturity (7). Inhibitors of sulfation factor have also been reported (8–12).

Bioassays for somatomedin have been based on its ability to stimulate sulfate and/or thymidine incorporation into cartilage. Many of the bioassays developed have been imprecise and time consuming. The paper presented here describes the development of a bioassay for somatomedin using epiphyseal plate cartilage from young chicks. Utilizing this assay, somatomedin activity was studied in two animal models that had markedly different growth rates and/or plasma growth hormone levels. The animal models were: (a) fast-growing Yorkshire and slow-growing Ossabaw pigs and (b) lean and obese Zucker rats.

Materials and methods. Cartilage source for bioassay. Epiphyseal plate cartilage was obtained from 3- to 4-week-old White Plymouth Rock chicks. Male chicks were raised in wire bottom group cages with 24-hr light. They were fed *ad libitum* a ration described by Leach and Nesheim (13) and were offered water *ad libitum*. One group of chicks was fed from hatching on a calcium-deficient diet where the percentage calcium was decreased from 1.20 to 0.25%.

Bioassay for somatomedin activity. Chicks were fasted for 2 days and then killed by cervical dislocation. Incisions through the hyaline cartilage caps were made exposing the epiphyseal growth plates at the proximal ends of the tibiotarsus, fibula, and tarsometatarsus. The upper portions of the epiphyseal plates were removed and the zone of calcifying cartilage carefully dissected away and discarded. The remaining cartilage was cut into approximately 3 × 3-mm fragments and stored in cold 0.9% NaCl until its use in the bioassay. Single-cartilage fragments were incubated in individual 12 × 75-mm tubes. The incubation medium contained 500 $\mu\ell$ of an enriched Kreb's phosphosaline buffer (14). Varying amounts of serum and Kreb's phosphosaline buffer were added to make a total of 950 $\mu\ell$ of medium. The tubes were incubated in a metabolic shaker at 39°C. After 4 hr incubation, 50 $\mu\ell$ of Kreb's phosphosaline buffer containing 2 μCi of [^{35}S]sulfate (specific activity = 894 mCi/mmol), obtained from New England Nuclear, Boston, Massachusetts, was added to each tube and incubaton continued for another 3 hr.

The cartilage fragments were then transferred to 12 × 75-mm tubes containing 1 ml distilled water and boiled for 5 min. They were then rinsed in two distilled water

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rinses for 0.5 hr each, dried, weighed on a Cahn electrobalance and placed in counting vials to which 0.5 ml formic acid was added. The vials were placed in a waterbath at 95°C for 30 min after which 10 ml of Triton X scintillation fluid were added before counting. Values were expressed as counts per minute/milligram cartilage weight above buffer control values. All serum samples were from individual animals. Each sample was run in quadruplicate at four dilutions—2.5, 5.0, 7.5, and 10.0%.

After plotting log dose–response curves, regression analysis and index of precision (λ) were computed for each serum sample. Homogeneity of regression on the linear portions of the dose–response curves was tested with an F test. Analysis of covariance was used to determine differences between mean dose–response curves.

Radioimmunoassay for insulin. Insulin levels were determined using the double antibody technique of Hales and Randle (15). The antisera against porcine insulin prepared in guinea pigs was obtained from Miles Laboratory (No. 64-104-16) and was used at an initial working dilution of 1:40,000. The anti-guinea pig gamma globulin was produced in sheep at The Pennsylvania State University farms. Porcine insulin (Lilly Research Laboratories, No. 615-D63-10) was radioiodinated with ^{125}I at room temperature by a modification (16) of the method of Greenwood *et al.* (17). Porcine insulin (Lilly Research Laboratories, No. 615-D63-10) was used as standards.

Radioimmunoassay for growth hormone. Growth hormone levels were determined using modifications of the technique and radioiodination procedure described for the insulin radioimmunoassay. The materials used in setting up this assay were supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health. The rat growth hormone (NIAMD-Rat GH-I-2) used for radioiodination was dissolved in 0.01 M NH_4HCO_3 buffer (18) at pH 8.3. Rat growth hormone standards (NIAMD-Rat GH-RP-1) were dissolved in 0.01 M NH_4HCO_3 at pH 8.3 to a dilution of 1 μg/ml and frozen. Just before the standard was added to the standard curve tubes, it was further diluted to 200 ng/ml with phosphate-buffered saline containing 1% egg white. Anti-rat growth hormone (NIAMD-Anti-Rat GH-GHS-2) was used at a 1:10,000 initial working dilution. Anti-monkey gamma globulin was obtained from Antibodies Inc., Davis, California, and used at a 1:20 dilution. Hormone levels within animal models were compared using a Student's t test.

Source of serum for somatomedin bioassay. Five-week-old Sprague–Dawley rats, eight sham operated, eight hypophysectomized, were obtained from Zivic–Miller Laboratories, Inc. (Alison Park, Pa.). Serum was collected from the rats by decapitation 2 weeks after their arrival. Weight gain data was collected during this period in order to test for completeness of hypophysectomy.

Serum was obtained from seven feral Ossabaw and seven domestic Yorkshire strains of pigs. The pigs were fed standard corn–soybean growing and finishing diets *ad libitum* until their slaughter at 10 months of age.

Five female obese Zucker rats (19) and six lean littermates were fed Purina rat chow *ad libitum*. All rats were sacrificed between 16 and 19 weeks of age except for one lean rat, which was 28 weeks of age. The rats were sacrificed by decapitation and their serum was obtained.

Results. Sham operated Sprague–Dawley rats gained 67.3 ± 3.6 g and the hypophysectomized rats lost 2.8 ± 2.0 g during the 2 weeks between their arrival and sacrifice. Serum was tested for somatomedin activity (Fig. 1). The mean dose–response curve for the hypophysectomized rats was 72% of the mean dose–response curve for the sham operated rats ($P < 0.01$). There was no difference between the slopes of the two mean dose–response curves indicating that the assay was measuring the same serum component in both groups of animals.

A study was conducted to compare epiphyseal plate cartilage from normal and calcium deficient chicks. It was thought that use of calcium deficient chicks would increase the precision of the assay since the

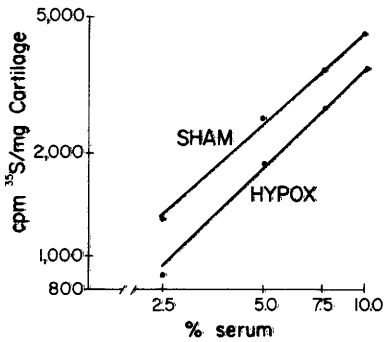

FIG. 1. The effect of serum from hypophysectomized versus sham-operated rats on sulfate incorporation. Each mean dose–response curve represents an average from eight animals. SEM around the curves for hyped and sham-operated rats are ±312 and ±346 cpm, respectively.

epiphyseal plate of such chicks is wider and easier to slice. Serum from the Yorkshire and Ossabaw pigs was used in this study. The data obtained using the cartilage from the calcium-deficient chicks showed much greater variation than did the data obtained using the cartilage from chicks on a normal diet (Table 1). The standard errors of the mean for the slopes were larger and the indices of precision greater. Also, the cartilage from the calcium-deficient chicks had a reduced ability for sulfate incorporation. Therefore, no other assays were performed using cartilage from chicks on a calcium-deficient diet.

Initial work on characterization of the hormone profiles of the Yorkshire and Ossabaw strains of pigs indicated that the serum levels of immunoreactive growth hormone in fasted Ossabaw pigs were significantly lower than in fasted Yorkshire pigs at 1, 3, and 6 months of age. Fasted immunoreactive insulin levels at these ages were not significantly different (20). When serum from 10-month-old Yorkshire and Ossabaw pigs was tested for somatomedin activity, it was found that there was no difference between the mean dose–response curves (Fig. 2). There was no difference between the slopes of the two mean dose response curves.

Serum immunoreactive growth hormone and insulin were measured in two studies using 14- to 16-week-old lean and obese Zucker rats pair fed until sacrifice (Table 2). The immunoreactive growth hormone levels were substantially lower in the obese rats in both studies (significantly lower in Study I). The immunoreactive insulin levels were significantly higher in the obese rats in both studies.

Serum from 16- to 19-week-old rats (except for one lean rat which was 28 weeks old) pair fed until sacrifice was tested for somatomedin activity (Fig. 3). The mean dose–response curve for the obese Zucker rats was 59.5% of the mean dose–response curve for the lean Zucker rats ($P < 0.01$). There was no difference between the average slopes of the two mean dose–response curves.

Discussion. The average index of precision for the somatomedin bioassay described here ($\lambda = 0.18$; $n = 41$) fell within

TABLE I. THE EFFECT OF A CALCIUM DEFICIENT DIET ON THE SULFATE INCORPORATION OF CHICK CARTILAGE

Diet	Slope[a]		Index of precision[b] (λ)
	Yorkshire	Ossabaw	
Calcium deficient	0.434 ± .061	0.350 ± .073	0.31
Normal	0.558 ± .032	0.497 ± .031	0.24

[a] Each value represents the average of seven animals ± SEM.

[b] Each value represents the average of 14 values.

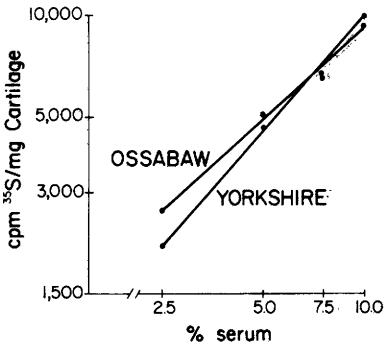

FIG. 2. The effect of serum from Yorkshire versus Ossabaw pigs on sulfate incorporation. Each mean dose–response curve represents an average from seven animals. SEM around the curves for Yorkshire and Ossabaw pigs are ±321 and 539 cpm, respectively.

TABLE II. IMMUNOREACTIVE GROWTH HORMONE AND INSULIN LEVELS IN GENETICALLY LEAN AND OBESE RATS

Strain	Growth hormone (ng/ml)		Insulin (μU/ml)	
	Study I	Study II	Study I	Study II
Lean (6)	284 ± 68	140 ± 71	60 ± 7	28 ± 9
Obese (6)	31 ± 12*	81 ± 36	179 ± 20*	140 ± 33*

* Differs significantly from lean group, $P < 0.01$.

the range of currently used bioassays for somatomedin activity. In one of the later studies, lean versus obese Zucker rats, the average index of precision was 0.13 (n = 11). The log dose–response curves were linear from 5% serum making the assay as sensitive as currently used bioassays except for the hypophysectomized rat costal cartilage bioassay. The three main advantages of the chick epiphyseal plate bioassay are: (a) the time and labor are greatly reduced; (b) bioassay cartilage is very active, uniform from study to study, and readily available; and (c) the cost is also reduced.

The bioassay measures overall stimulatory activity on mucopolysaccharide synthesis rather than individual somatomedin

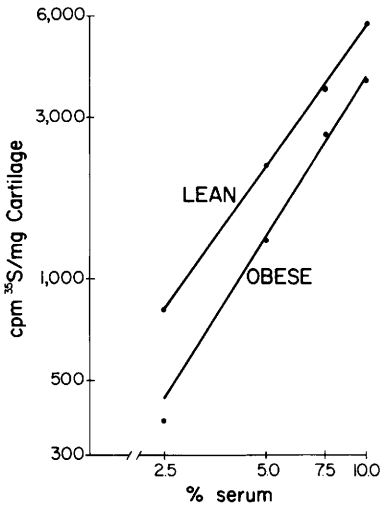

FIG. 3. The effect of serum from lean versus obese Zucker rats on sulfate incorporation. The mean dose–response curves for the lean and obese Zucker rats represent averages from six and five serum samples, respectively. SEM around the curves are ±259 and ±234 cpm, respectively.

levels and, therefore, represents the summation of somatomedin(s) activity in serum. It measures this activity consistently within species as indicated by there being no differences among the average slopes of mean dose–response curves. That the somatomedin activity as measured in this bioassay is growth hormone dependent was demonstrated by the hypophysectomized versus sham-operated rat study. A comparison of current bioassay procedures with this one is shown in Table 3. Similar comparisons were made by Van den Brande and DuCaju (23).

Somatomedin is the proposed mediator for the actions of growth hormone on cartilage metabolism (24). In several different clinical situations, somatomedin has been shown to be correlated with growth rate (3). Somatomedin levels were reported to be lower in dwarf mice than their heterozygous controls (2). The results of the studies reported here show that the obese Zucker rat has lower serum growth hormone levels and somatomedin activity than their lean littermates.

The obese Zucker rats have been shown to be hyperphagic (19) and hyperlipemic (22). They have rapid body weight gain (19), elevated fat deposition, and reduced lean mass deposition (26). When pair fed to lean littermates, the Zucker rats divert an abnormally large proportion of the limited caloric intake to fat storage at the expense of muscle and bone growth (27). Insulin levels in the Zucker rats are elevated with the rise in insulin levels following the onset of obesity (27).

The implication of growth hormone and somatomedin in this syndrome warrants more investigation. Whether the decreases in growth hormone levels and somatomedin

TABLE III. COMPARISONS OF CURRENT SOMATOMEDIN BIOASSAY TECHNIQUES

Authors	Animal	Organ	Incubation time (hr)	Isotope	Precision (λ)
New assay	chick	epiphyseal plate cartilage	3	$^{35}S(SO_4)$	0.19
Salmon and Daughaday (1)	hypophysect. rat	costal cartilage	24	$^{35}S(SO_4)$	0.26
Daughaday and Reeder (14)	hypophysect. rat	costal cartilage	24	^{3}H(Thym)	—
Yde (21)	normal rat	costal cartilage	24	$^{35}S(SO_4)$	0.20
Hall (22)	chick embryo	pelvic rudiments	6	$^{35}S(SO_4)$	0.20
Van den Brande and DuCaju (23)	normal pig	costal cartilate	48	$^{35}S(SO_4)$ ^{3}H(Thym)	0.15 0.19

activity precede or follow the onset of obesity remains unclear. Fatty acids have been shown to inhibit somatomedin stimulation of cartilage metabolism (9, 10). Whether the lower somatomedin activity in the Zucker rat serum is a result of lower somatomedin levels, inhibition of somatomedin activity by the higher fatty acid content or both has not been determined. However, the concomitant decreases in growth hormone levels, somatomedin activity, and lean mass deposition with increases in insulin levels and fat deposition are noteworthy. A more complete investigation of the roles of growth hormone and somatomedin in this syndrome could contribute to the understanding of somatomedin action.

There was no difference in somatomedin activity between Ossabaw and Yorkshire pigs at 10 months of age. Additional experiments are being planned to measure somatomedin activity in the Ossabaw and Yorkshire pigs at an earlier age.

In summary, a chick epiphyseal plate cartilage bioassay was shown to be effective in measuring somatomedin activity. Our data indicate that the Zucker rat animal model could serve as an important tool in the study of somatomedin action.

Summary. A somatomedin bioassay measuring sulfate incorporation into young chick epiphyseal plate cartilage was developed and used to determine somatomedin activity in two animal models. The incubation schedule consisted of 4 hr preincubation and 3 hr incubation. The average index of precision, λ, of the bioassay was 0.18 with increased precision evident in later studies. The sensitivity fell within the range of currently used somatomedin bioassays. Using radioimmunoassay techniques, serum insulin and serum growth hormone levels were measured in lean and obese Zucker rats. Serum from obese Zucker rats was found to have reduced growth hormone levels, reduced somatomedin activity, and elevated insulin levels when compared to their lean littermates. There was no difference in somatomedin activity between slow-growing Ossabaw and fast-growing Yorkshire pigs.

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1. Salmon, W. D., and Daughaday, W. H., J. Lab. Clin. Med. **49**, 825 (1957).
2. Herbai, G., Acta Endocrinol. **66**, 333 (1971).
3. Daughaday, W. H., Salmon, W. D., and Alexander, F., J. Clin. Endocrinol. Metab. **19**, 743 (1959).
4. Hall, K., Acta Endocrinol. **66**, 491 (1971).
5. Tato, L., DuCaju, M. V. L., Prevot, C., Rappaport, R., Clin. Endocrinol. Metab. **40**, 534 (1975).

6. Hall, K., and Olin, P., Acta Endocrinol. **69**, 417 (1972).
7. Hall, K., and Filipsson, R., Acta Endocrinol. **78**, 239 (1975).
8. Dehnel, J. M., McConaghey, P. D., and Francis, M. J. O., J. Endocrinol. **62**, 355 (1974).
9. Delcher, H. K., Eisenbarth, G. S., and Lebovitz, H. E., J. Biol. Chem. **248**, 1901 (1973).
10. Eisenbarth, G. S., Beuttel, S. C., and Lebovitz, H. E., Biochim. Biophys. Acta **331**, 397 (1973).
11. Eisenbarth, G. S., and Lebovitz, H. E., Endocrinology **95**, 1600 (1974).
12. Garland, J. T., Lottes, M. E., Kozak, S., and Daughaday, W. H., Endocrinology **90**, 1086 (1972).
13. Leach, R. M., and Nesheim, M. C., J. Nutr. **86**, 236 (1965).
14. Daughaday, W. H., and Reeder, C., J. Lab. Clin. Med. **68**, 357 (1966).
15. Hales, C. N., and Randle, P. J., Biochem. J. **88**, 137 (1963).
16. Niswender, G. D., Reichert, L. E., Midgley, A. R., and Nalbandov, A. V., Endocrinology **84**, 1166 (1969).
17. Greenwood, F. C., Hunter, W. M., and Glover, J. S., Biochem. J. **89**, 114 (1963).
18. Sinha, Y. N., Shelby, F. W., Lewis, U. J., and Vanderlaan, W. P., Endocrinology **91**, 784 (1972).
19. Zucker, L. M., and Zucker, T. F., J. Hered. **52**, 275 (1961).
20. Wangsness, P. J., Martin, R. J., and Gahagan, J. H., Amer. J. Physiol. **233**, E104 (1977).
21. Yde, H., Acta Endocrinol. **57**, 557 (1968).
22. Hall, K., Acta Endocrinol. **63**, 338 (1970).
23. Van den Brande, J. L., and DuCaju, V. V. L., Acta Endocrinol. **75**, 223 (1974).
24. Daughaday, W. H., Hall, K., Rabin, M. S., Salmon, W. D., Van den Brande, J. L., and Van Wyk, J. J., Nature (London) **235**, 107 (1972).
25. Zucker, L. M., Ann. N.Y. Acad. Sci. **131**, 447 (1965).
26. Deb, S., Martin, R. J., and Hershberger, T. V., J. Nutr. **106**, 191 (1976).
27. Zucker, L. M., J. Nutr. **91**, 247 (1967).
28. Zucker, L. M., and Antoniades, H. N., Endocrinology **90**, 1320 (1972).

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