Biological Profile of Various Growth Hormone Preparations. (26612)

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Biological activity of primate, beef and whale pituitary growth hormone as evidenced by body wt gain and the tibia assay was investigated by Li, *et al.*(1). These preparations were observed to have almost identical growth-promoting potencies in the tibia test, but were not comparable on the basis of body weight gain; thus the interest to investigate the activities of these preparations in stimulating incorporation of radio-sulfate in costal cartilage of hypophysectomized rats.

Methods. Female rats, Holtzman strain, were hypophysectomized* at 21 ± 2 days of age, housed at an environmental temperature of 78 ± 2°F and fed Purina laboratory chow *ad lib.* Two weeks after hypophysectomy, animals weighing between 65-80 g were used for experimentation, 8 animals/dosage group.

1. *Dose-response study.* Animals were given a single intraperitoneal (I.P.) injection of the test preparations. Hormone preparations were dissolved in isotonic saline so that each ml contained the desired dosage. Eight hours later, 20 μc S^{35} as sodium sulfate dissolved in 1 ml isotonic saline (without added carrier) was administered I.P. Sixteen hours later (24 hrs after hormone injection) the animals were sacrificed and the VII costal cartilage removed. This procedure has been shown to be a reliable index for growth hormone activity(2,3). 2. *Duration of response study.* Animals were given a single I.P. injection of 12 μg test preparation. At various time intervals over a 48-hr period, animals were sacrificed and VII costal cartilage removed. *Four hours prior to sacrifice,* 10 μc S^{35} was administered I.P.

Cartilage was cleaned of adhering tissue, weighed, and treated by the procedure previously described(2). Radio-sulfate was counted in a gas-flow end window counter and results reported as CPM/mg tissue, wet weight.

Results. 1. *Dose-response study.* Growth hormone preparations isolated from human, monkey, and beef pituitary glands appear to have equal activity in promoting radio-sulfate incorporation in costal cartilage of hypophysectomized rats. Material obtained from whale, pig, and sheep pituitaries demonstrated equal activity but approximately 50% of that observed for human, monkey, or beef preparations (Table I). Calculated potencies presented in Table IV are based on 3-12 μg doses. With the exception of human and pig preparations, the response appeared to plateau at doses above 12 μg.

When these 6 preparations were compared for their ability to increase width of epiphyseal plate, human, monkey, and beef materials were observed to have almost identical activity (Table III). Pig and whale preparations demonstrated 100% and 50% more activity, respectively, whereas the sheep preparation was least active. 2. *Duration of response study.* Activity of these preparations, based upon duration of action of a single 12 μg dose, was studied. Radio-sulfate incorporation was increased relative to un-

TABLE I. Effect of Various Growth Hormone Preparations on Radio-Sulfate Incorporation of Costal Cartilage.

Preparation	Control	cpm/mg tissue ± S.E. Human	Sheep	Pig	Whale	Monkey	Beef
Control	29.0 ± 1.9						
3 μg GH		43.1 ± 4.0	37.0 ± 3.1	33.5 ± 2.9	35.5 ± 1.3	41.3 ± 3.2	40.0 ± 3.3
6 μg "		50.2 ± 3.6	37.4 ± 2.8	38.3 ± 3.0	37.1 ± 2.0	45.7 ± 6.1	47.0 ± 2.3
12 μg "		53.0 ± 5.7	49.1 ± 4.4	48.7 ± 4.1	50.7 ± 4.2	56.7 ± 5.1	59.4 ± 8.2
24 μg "		71.5 ± 4.9	45.8 ± 4.9	66.2 ± 9.6	49.8 ± 4.5	55.7 ± 9.0	59.8 ± 9.9

* Hormone Laboratories, Chicago, Ill.

TABLE II. Duration of Activity of Single Dose of Various Growth Hormone Preparations on Radio-Sulfate Incorporation of Costal Cartilage.

Hr post-hormone	cpm/mg tissue ± S.E.						
	Control	Human	Sheep	Pig	Whale	Monkey	Beef
24	10.8 ± .4	15.1 ± 2.6	17.1 ± 3.6	13.4 ± 1.1	14.7 ± 2.2	11.0 ± 2.2	13.9 ± 3.1
28	9.7 ± 1.0	16.7 ± 2.0	12.7 ± 1.0	18.6 ± 1.4	16.1 ± 2.9	16.7 ± 1.1	13.6 ± 2.4
32	9.0 ± .2	17.1 ± 1.8	14.4 ± 1.5	12.0 ± 1.2	18.1 ± 1.9	13.2 ± 1.5	16.4 ± 1.1
36	6.2 ± .5	10.0 ± 1.1	8.8 ± 1.2	12.2 ± 1.8	12.4 ± 1.1	8.3 ± .7	8.2 ± 1.2
40	5.5 ± .6	10.8 ± 1.9	8.9 ± .6	8.4 ± .7	9.8 ± 1.9	8.2 ± .7	12.0 ± 1.4
48	7.0 ± 2.2	8.7 ± .9	8.4 ± 1.0	8.9 ± 1.2	11.0 ± 1.1	9.2 ± 1.1	8.3 ± .9

TABLE III. Assay of Various Purified Growth Hormones by the Tibia Test.*

Growth hormone	Response† Total dose in 4 days			Slope	Index of precision
	20 μg	60 μg	120 μg		
Human	213 ± 2 (8)	235 ± 2 (8)	256 ± 2 (6)	52.9	.129
Monkey	210 ± 4 (4)	242 ± 5 (6)	261 ± 3 (5)	65.7	.147
Beef	206 ± 3 (5)	242 ± 1 (4)	248 ± 6 (4)	56.3	.095
Pig	226 ± 4 (7)	243 ± 4 (5)‡	279 ± 4 (6)§	60.8	.168
Whale	220 ± 4 (5)	250 ± 2 (5)	268 ± 4 (5)	62.6	.130
Sheep	209 ± 2 (4)	231 ± 3 (5)	249 ± 4 (6)	52.6	.157

* Unpublished results (private communication from C. H. Li).
† In terms of mean tibia width ± stand. error. No. of rats in parentheses.
‡ 40 μg total dose.
§ 100 " " " .

treated animals during the first 24 hours with all preparations and remained elevated during the 48-hour experimental period (Table II). Increased uptake of isotope continued through the 32nd hour and was observed in all groups. Peak response varied from 24 hours (sheep) to 32 hours (whale). These observations of prolonged effect of growth hormone compared with the relatively short-lived effects of ACTH are of significant interest and should provide useful methods for study of metabolism and mechanism of action of growth hormone.

Discussion. Using the tibia assay and body weight gain, Li *et al.*(1) found that whale and beef growth hormone had greater effect on body weight gain than did human or monkey hormones, but the 4 preparations appeared to have almost identical growth-promoting activity in stimulating epiphyseal tissue.

TABLE IV. Comparative Potencies of Growth Hormone Derived from Several Mammalian Species.*

Species	S^{35} assay	Tibia test
Human	1.00	1.00
Monkey	.920	1.12
Beef	1.054	.90
Pig	.438	2.13
Whale	.477	1.54
Sheep	.481	.83

* Calculations based on data presented in Tables I and III.

We have investigated these hormone preparations for their ability to stimulate incorporation of radio-sulfate into costal cartilage. It was observed that human, beef, and monkey preparations were nearly equally active, and the calculated potencies derived from the 24-hr radio-sulfate assay and 4-day tibia assay were not significantly different.

Similar correlations were not obtained with whale and pig preparations. Recent data from Dr. Li's laboratory (Table III) show whale and pig growth-hormone preparations to possess greater activity in the tibia assay than human, beef, and monkey materials. In the 24-hour radio-sulfate uptake assay, whale and pig material were observed to have approximately 50% the activity of human, monkey, or beef growth hormone. If modification of the growth hormone molecule to

a more active form, by rat tissues, is required, rate of utilization would be relatively slower and a 24-hour test might well be too short a period for quantitative comparison.

It is interesting to note that, following a single injection, whale and pig growth hormones had the same duration of activity and magnitude of response as did human, monkey, and beef preparations in the time intervals studied. This could be a further indication that the difference between the 2 assays is a function of time dependent upon modification of the growth hormone molecule and/or slower rate of utilization of whale and pig material.

The fact that the 2 assays for growth-promoting activity give quantitatively different results suggests that the short-term assay is of value for qualitative measurement and that quantitative comparisons require a longer test period.

Summary. Growth hormone isolated from 6 different species was compared for its ability to increase width of epiphyseal plate and to stimulate radio-sulfate incorporation by costal cartilage. Human, beef, monkey, and sheep preparations manifested almost identical effects in the 2 assays. Whale and pig hormones appeared to elicit greater effect in the tibia test. Whale growth hormone was observed to have greater duration of activity to stimulate radio-sulfate incorporation than other preparations studied. Short-term radio-sulfate assay appears to be of value for qualitative detection of growth-promoting activity.

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Determination of "Heparinoid" Substances in Urine with a Dye-Binding Technic.* (26613)

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Although crystalline heparin of known chemical composition and with reproducible physiological properties is available for clinical and laboratory study, the basis of its action in blood clotting and other metabolic activities has not been completely elucidated. Complicated chemical methods have revealed very small amounts of heparin in the circulating blood, too minute, however, for quantitative determination.

Early investigators were not in agreement as to the fate of heparin, whether endogenous or administered. Howell and McDonald(1) were first to report the presence of heparin in urine of dogs following injection. Copley and Schnedorf(2) using a relatively simple dye-precipitation technic, found that from 9.9-35.6% of injected heparin was excreted in urine of dogs within 110 minutes. There was no activity before administration. Jaques(3), using a metachromatic technic, and Astrup(4), with an anticoagulant procedure, reported daily excretion in urine of man of amounts up to 0.7 mg heparin without prior injection. When injected, excretions of 2-15%(3) and 1.6-12%(4) were obtained.

The excreted material is not unchanged heparin, as seen in the disparity between metachromatic activity and the lesser clot-

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