Effects of Avian and Rat Pituitary Extracts on Tibial Growth and Blood Composition.* (26829)

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Data are available which indicate that differences exist in molecular structure as well as in physiological effects of growth hormone preparations obtained from various species (1). Thus, employing the rat tibia test, hypophyseal extracts from human, whale, monkey and beef sources were reported to be nearly equal in potency, yet differ in their ability to promote gains in body weight(2). Solomon and Greep(3) compared the growth promoting potential of pituitary extracts from 9 different vertebrate forms and concluded from rat epiphyseal cartilage data that the hypophysectomized recipients were more sensitive to amphibian and mammalian preparations than they were to extracts prepared from fish. Also, these authors reported that the hypophysectomized rat was insensitive to avian pituitary extracts; however, the results presented were for one dose level which was assayed in only 4 hypophysectomized animals.

Additional information on the growth hormone content of chicken pituitaries seemed desirable, especially in light of the multiple differences with respect to protein anabolic and carbohydrate metabolism known to exist between aves and mammalia, differences which are readily influenced by administration of growth hormone(4). Whether the avian growth hormone molecule differs structurally from that of other species, or whether avian metabolism is sufficiently different from that of mammals to render the bird resistant to mammalian growth hormone, is not clear. However, adult chickens have been reported to be very resistant to the protein anabolic as well as to the carbohydrate influence of growth hormone prepared from mammalian sources(5,6).

The purpose of this study, therefore, was to investigate comparative potency of avian, rat and purified beef growth hormone as assayed in the immature hypophysectomized female rat. The parameters chosen for assaying these preparations were the tibia cartilage width, blood urea, oxyhemoglobin, hematocrit and whole blood specific gravity (WBSG).

Methods. Chicken heads from 9 to 12-week-old White Rock birds of both sexes (equally divided) were obtained immediately after death (Broadway Poultry Co., Boston, Mass.), the anterior pituitary separated rapidly from the posterior lobe, weighed on a Roller-Smith torsion balance, and pulverized manually in a Potter homogenizer containing a known amount of saline. These crude extracts were then made alkaline, butanol added as a preservative, and stored at 0-4°C. Each extract was made fresh 48 hours prior to first injection. Rat pituitaries were obtained from young adult Sprague-Dawley descendents of both sexes (equally divided) and were extracted in the same manner. Purified beef growth hormone (NIH-GBH-2) was donated by the Endocrinology Study Section.

Pituitary preparations were injected subcutaneously once each day (in equally divided doses over a 4-day period) into female rats hypophysectomized at 26 days of age (Charles River Breeding Laboratories) and allowed to recover an additional 17 days prior to use. Control rats received 0.25 ml of 0.9% saline solution per injection. Eight hours after last injection all recipients were fasted 16 hours, after which a terminal blood sample was drawn from the vena cava, and the right tibia excised and placed in 10% formol to be stained with silver nitrate according to the method of Greenspan *et al.*(7). Microhematocrits, oxyhemoglobin(8) and specific gravity(9) determinations were carried out on fresh whole blood; serum urea was measured by the method of Rosenthal(10). Completeness of hypophysectomy was judged at

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TABLE I. Assay of Avian, Rat and Purified Beef Growth Hormones.

Donors		Recipients						
No. of ant. pit.	Ant. pit. wt, mg	No. of rats	Body wt gain, g	Tibia width, μ	Blood urea, mg %	Oxyhemoglobin, g %	Hematocrit, %	Blood specific grav.
Total dose/4 days				Avian pituitary extract				
NaCl control*	—	15	0	153 ± 2†	62.8 ± 1.1	16.5 ±.27	36.2 ± .9	1.048 ±.0006
½ ant. pit.	3.3	9	4	162 ± 2	55.4 ± 1.3	15.7 ±.49	32.9 ±1.0	1.044 ±.0008
1 *idem*	6.6	10	4	191 ± 6	55.6 ± .9	15.6 ±.33	32.5 ± .9	1.045 ±.0009
2 "	13.2	11	1	236 ± 5	52.9 ± 1.3	14.3 ±.38	31.3 ± .8	1.044 ±.0005
4 "	26.4	8	1	285 ±15	49.2 ± .6	15.1 ±.35	31.5 ±1.1	1.045 ±.0011
				Rat pituitary extract				
¼ ant. pit.	1.6	8	3	242 ± 4	53.3 ± 1.0	—	—	—
½ *idem*	3.2	8	4	293 ± 6	54.1 ± 1.2	16.1 ±.40	34.2 ± .5	1.046 ±.0005
1 "	6.5	8	6	312 ± 9	49.4 ± 1.3	15.5 ±.25	33.7 ± .7	1.045 ±.0005
				Purified beef growth hormone				
NaCl control	—	11	.3	156 ± 4	66.0 ± 2.5	17.3 ±.20	36.2 ± .5	1.049 ±.0004
100 μg	—	8	5	207 ± 7	56.1 ± 1.6	16.9 ±.26	34.0 ± .6	1.047 ±.0005
200 "	—	6	7	225 ± 9	58.1 ± 1.7	16.4 ±.33	31.9 ± .9	1.047 ±.0006
400 "	—	7	8	303 ±15	45.5 ± 2.1	16.0 ±.31	31.9 ± .5	1.046 ±.0004
1600 "	—	8	16	407 ±13	56.3 ± 2.7	15.9 ±.48	32.0 ±1.1	1.046 ±.0009

* Control rats received 0.25 ml NaCl daily.

† All values are means ± stand. error.

autopsy by adrenal weights and examination of the sella turcica.

Results. Table I compares the results of injecting graded doses of avian and rat anterior pituitary tissue with those of a highly purified beef preparation. Equal growth-promoting properties were evident when tibias of rats receiving 13.2 mg avian hypophyseal tissue were compared with those of rats receiving 1.6 mg rat pituitary extract. By bioassay standards of significance(7,11) a significant increase in cartilagenous width required 6.6 mg avian hypophyseal extract ($p < .01$) whereas the minimum rat pituitary dose employed (1.6 mg) evoked a highly significant increase in tibial size ($p < .001$).

While the lowest dose of avian pituitary extract did not effectively increase tibial cartilage width, it decreased significantly urea ($p < .01$), hematocrit ($p < .05$), and WBSG levels ($p < .01$). Oxyhemoglobin was depressed significantly only when the equivalent of 2 or more avian pituitaries were injected ($p < .05$).

Discussion. The results of this study confirm and extend an earlier report on avian hypophyseal growth substance(s) as evidenced by the epiphyseal cartilage assay test (3), and present observations as to certain hematological alterations induced by these adenohypophyseal extracts. Comparing tibial cartilage widths, the chicken anterior pituitary apparently contains one-eighth of the growth-stimulating potential found in equal amounts of the rat adenohypophysis. It is possible that the hypophysectomized rat is considerably more resistant (8 times?) to avian hypophyseal extracts; however, it would be necessary to conduct assays in immature hypophysectomized chicks to distinguish a decrease in hypophyseal hormone content from an alteration in recipient resistance.

The hematological response to avian pituitary extracts in absence of tibial growth is of interest, for as such, these indices may aid in evaluation of such avian preparations. The depression in oxyhemoglobin, hematocrit and WBSG levels induced by the purified beef preparation are in accord with observations using similar preparations in normal and diabetic dogs(12). However, considerable variability occurred in all blood parameters at the highest dose level of purified growth hormone employed. From the blood data in Table I it would appear that the minimal effective dose (as judged by tibial enlargement) would nonetheless evoke a marked depression in urea levels.† The qualitative si-

† With saline control tibial widths ranging 145-160 μ a significant response is generally considered in excess of 190 μ(7, 11).

milarity in tibial and hematological responses to both crude hypophyseal extracts and purified preparations would implicate the growth hormone molecule in effecting these alterations. However, since assay of other hypophyseal hormones contained in these crude extracts was not carried out their possible influence on the parameters chosen for study cannot be assessed here.

The failure to observe a linear response in body weight gain may be due in part to the short fasting time employed (and therefore variable intestinal contents), or may indicate a sensitivity of the hypophysectomized rat to the crude hetero-specie material injected.

Summary. Chicken and rat hypophyseal extracts were compared with a purified beef growth hormone preparation in the hypophysectomized, immature female rat using the tibial cartilage assay and hematological alterations. The avian adenohypophysis contains 1/8th of the growth-promoting material found in an equivalent amount of rat hypophyseal extract. Significant depression in hematocrit and WBSG levels occurs at doses of avian extracts which are unable to promote epiphyseal cartilage growth.

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Extraction of a Glucose Uptake Stimulator from Tumors.* (26830)

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The presence of a glucose uptake stimulator in a retroperitoneal fibrosarcoma has recently been reported from this laboratory(1). This tumor had been removed from a patient exhibiting repeated episodes of spontaneous hypoglycemia associated with increased plasma insulin-like activity. Following removal of the tumor, the patient became euglycemic and had plasma insulin-like activity indistinguishable from normal, which indicated that the tumor was the source of a glucose uptake stimulator(2). These observations suggested the possibility that a glucose uptake stimulator might be found in other neoplastic tissues even in the absence of hypoglycemic symptoms in the tumor-bearing patients. This paper reports the results of assays for a glucose uptake stimulator in various malignant tumors.

Methods. Tumors obtained from the operating room within minutes of removal from the patients were carefully dissected away from surrounding tissues, cut into pieces 3-4 cm thick, and stored at -20°C until use. In most cases it was possible to obtain tissues showing no histological evidence of neoplastic changes from the areas surrounding the tumor. Fractions were prepared from these tissues in the same manner as that used for

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