Tissue Distribution of Radioiodinated Human Growth Hormone in the Mouse[1] (37073)

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(Introduced by B. Theodore Cole)

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Human growth hormone (HGH) has been utilized in the long-term treatment of children with hypopituitary dwarfism (1). The duration of HGH therapy in these children has ranged from 6 mo to 5 yr. During long-term treatment, antibodies to HGH have been detected which occasionally contribute to growth inhibition (2, 3). Since HGH is employed clinically for extended time periods, it would be of interest to determine whether this hormone concentrates in a specific target organ or whether it distributes in a ubiquitous fashion. Toward this end, an animal model was employed in the present investigation to study the distribution of radioiodinated HGH. Such a study would provide clues to possible areas of concentration in the human.

Materials and Methods. Hormone procurement. A highly purified preparation of HGH was supplied through the courtesy of the Abbott Pharmaceutical Co., North Chicago, IL.[2] In addition, human chorionic gonadotropin (HCG) was used as a tropic hormone control.[3]

Radioiodination procedures.[4] Both HGH and HCG were labeled with ^{125}I by the method of Hunter and Greenwood (4). The labeled hormones were both isolated and freed from unbound iodine by gel filtration on Sephadex G-100. Radioactivity counts were measured in an ionization chamber. Pooled fractions of ^{125}I-HGH displayed a specific activity of 86.64 $\mu Ci/\mu g$ and a protein content of 1.94 $\mu g/ml$, while the ^{125}I-HCG had a specific activity of 78.42 $\mu Ci/\mu g$ and a protein content of 3.35 $\mu g/ml$. The radiohormones were suspended in 0.05 *M* phosphate buffer (pH 7.5) stabilized with 1.0% bovine serum albumin. The free iodine (^{125}I) content of the stored radio-preparations was determined periodically by thin-layer radiochromatography.

Antibody studies. Goat anti-HGH was obtained commercially[5] for use in hormone inhibition studies *in vivo*. Nonimmune goat serum was used as a control. The anti-HGH and/or nonimmune goat serum (0.1 ml) was administered iv 5.0 min prior to the injection of ^{125}I-HGH. Specificity of the antibody for ^{125}I-HGH could be demonstrated by autoradiographic immunodiffusion in agar.[5]

Statistical procedures. The method of averages (6) was used throughout this study. Discrete quantitative data was recorded as the mean, standard error, and standard error of the mean (SEM). Points on the bar graphs were considered significant if nonoverlapping occurred at 1 SEM ($\pm$) value.

Animal studies. Normal adult male and female mice of the A7(C57$\times$A) strain,[6] weighing 25–35 g, were employed for the radioisotope tissue distribution studies. Im-

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[2] Human growth hormone was prepared for the Abbott Co. by Dr. Matzusari (the Raben method) working in the laboratory of Dr. Shizume in Japan.

[3] Purified HCG was kindly supplied to us by Dr. Merritt R. Callantine, Endocrinology Section, Parke-Davis, Ann Arbor, MI.

[4] Radiolabeling and isolation procedures were performed by B. J. Green and Fred Jefferies, under the supervision of Dr. Jonathan Miller, Abbott Radiopharmaceuticals, North Chicago, IL.

[5] Miles Laboratories, Kankakee, IL.

[6] The mice were kindly supplied to us by Dr. W. U. Gardner, Department of Anatomy, Yale Univ.

mature male mice (10–20 g), of the same strain were also utilized for study. The mice received 30–40 μCi (0.5–1.0 μg protein) of either ^{125}I-HGH or ^{125}I-HCG intravenously at various time intervals from 1.0 to 18 hr prior to death to determine the time of optimum localization. At the time of sacrifice, 12 tissues were removed, weighed, and assayed for radioactivity in an automatic gamma well-counter (Nuclear Chicago). Counts were accumulated for a period of time to insure less than 5% statistical counting error, and corrections were made for radioactive decay and machine counting efficiency. The tissues were counted against a 100 μl blood sample from the same animal. Organ and tissue samples were taken to approximate the weight of the blood sample and were calculated as counts per minute per milligram of tissue. Results were expressed as tissue:blood (T/B) ratios.

Biologic activity. The ^{125}I-HGH was tested for biologic activity by the 10-day body weight test of Evans and Simpson (7). Sprague Dawley hypophysectomized (hypox) immature female rats were commercially obtained. Six of the rats were administered 0.1 ml of ^{125}I-HGH on alternate days for 10 days. A second group of seven hypox rats were similarly administered physiological saline. On the first, fifth, and tenth days, the animals from both groups were weighed. In addition, the total organ weights of the seminal vesicle and testis were recorded for the experimental and control hypox animals on day 10 following sacrifice.

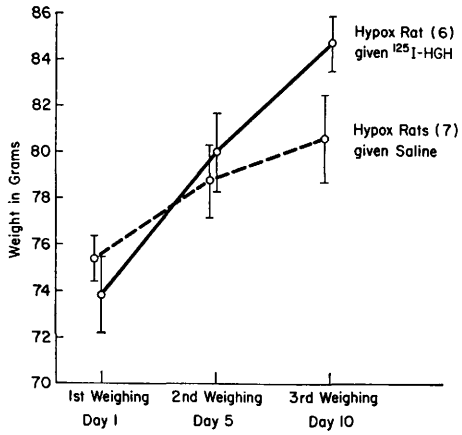

FIG. 1. The 10-day body weight test was employed to test the biologic activity of ^{125}I-labeled human growth hormone (HGH). The number in parentheses indicates the number of animals studied in each group. The vertical bars, enclosed at each end, indicate one SEM (±) value.

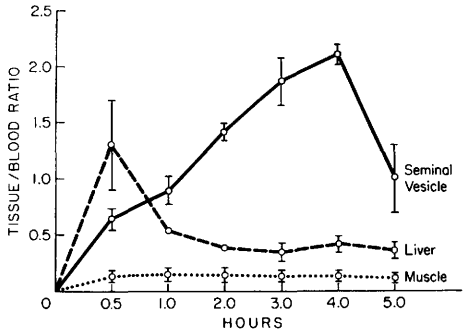

FIG. 2. ^{125}I-labeled human growth hormone (HGH) was injected at time 0, and the mice were sacrificed at time intervals indicated. The points represent the mean (T/B ratio) value of 2 mouse determinations at each time interval. The vertical bar is the range of these values.

Results. Radioiodination procedures. Labeling of HGH with ^{125}I with subsequent passage through Sephadex G-100 resulted in a monophasic curve exclusive of the free iodine peak. Homogeneity of the ^{125}I was further confirmed by paper electrophoresis. Free radioiodine, as determined by thin-layer chromatography, contributed less than 1% of the total activity of the radiohormone preparation.

Biologic activity. The ^{125}I-HGH was tested for biologic activity using the 10-day body weight test. The hypox rats administered ^{125}I-HGH had significantly higher total body weights (SEM) and an increased growth rate as indicated by the slope of the line in Fig. 1. At the end of 10 days, the average weight of the seminal vesicle in the ^{125}I-HGH treated rats was 1.6 times as great as that of control animals. The total weight of the testis in the experimental group, although increased, was not significantly different (1 SEM unit) from that of the control groups.

Animal studies. Time study. The concentration of ^{125}I-HGH from 1 to 18 hr (only 1 to 5 hr shown) by various tissues of adult male mice is displayed in Fig. 2. The ^{125}I-HGH was injected at time zero and mice were sacrificed at time intervals indicated. The

graph in Fig. 2 indicates that the concentration of radiohormone in the SV steadily increased from time zero to 3 hr postinjection. Optimum radio-uptake by the seminal vesicle (SV) occurred at the 3–4 hr interval with an abrupt decrease thereafter. The radioconcentration value of ^{125}I-HGH in the SV attained a value of 2.1 times that of blood. Uptake of ^{125}I-HGH also occurred in the liver during the first 30 min, and briefly achieved a concentration slightly greater than unity (1.3 T/B ratio). Muscle tissue did not take up the radiohormone at any time interval studied. Concentration of the radiohormone in the SV was not observed at 18 hr postinjection (T/B = 0.259). The tissue:blood ratios in the liver and muscle were 0.732 and 0.079, respectively.

Sexual specificity. ^{125}I-HGH was injected into normal adult male and female mice and studied at 3.0 hr postinjection. The bar graph in Fig. 3 demonstrates the organ specificity of HGH in the SV of male mice and the apparent lack of a target organ in females. It is obvious from the bar graph that the SV was the only organ to concentrate the radioisotope and achieve a value greater than unity (1.85 T/B ratio). It may be noted that other accessory sex organs, such as the prostate, did not take up measurable quantities of radioiodinated HGH.

Organ distribution. The ^{125}I-HGH and ^{125}I-HCG injected separately into intact male mice and uptake studied at 3.0 hr postinjection (Fig. 4). Again, only the SV displayed radioisotopic uptake greater than blood in the case of HGH. ^{125}I-HCG was rapidly excreted by the kidney of male mice and failed to demonstrate specific tissue localization. The rapid excretion of ^{125}I-HCG in male mice has been previously reported in this laboratory (8). All other tissues and organs studied with both radiohormones failed to attain T/B ratios greater than unity. With the exception of the lungs (T/B = 0.61), all the remaining organs and tissues displayed T/B ratios less than 0.5. The thyroid, with T/B ratios 15 to 18 times that of the SV, is considered a distinct entity since iodine is accumulated by this organ.

Immature mouse study. ^{125}I-HGH tissue distribution studies were undertaken in immature versus adult intact male mice. It was hoped these studies would elucidate whether androgen or the lack thereof influenced the uptake of radiohormone (HGH) in the SV of the mouse. As shown in Fig. 5, radio-uptake levels in the SV of immature mice increased substantially but were not significantly different from adult, male mice. Therefore, the lack of high circulatory levels of androgen slightly enhanced the uptake of ^{125}I-

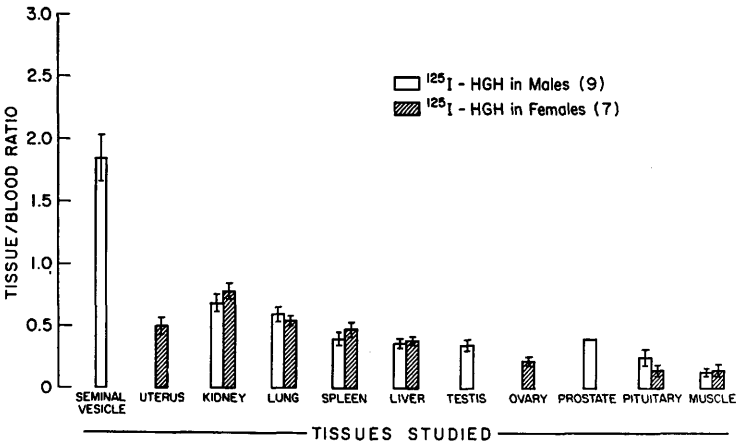

FIG. 3. Male and female intact mice were injected iv with ^{125}I-labeled human growth hormone (HGH) and studied 3.0 hr later. The number in parentheses indicates the number of animals studied in that group. Each bar represents the mean of the sample; one (±) SEM is signified by the vertical line through the top of each bar.

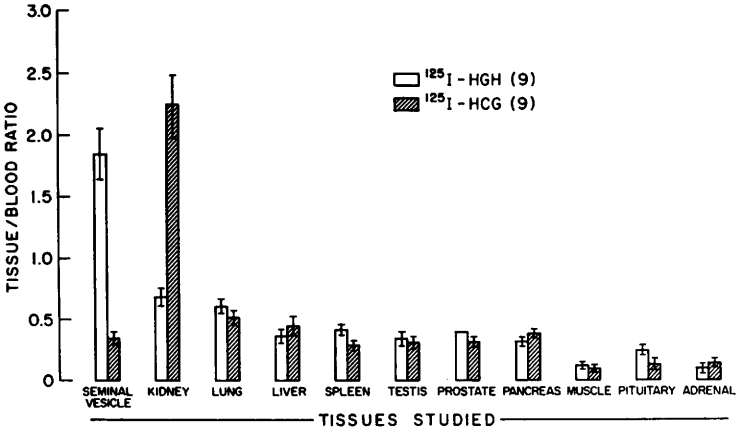

FIG. 4. Intact male mice were injected with either ^{125}I-HGH (human growth hormone) or with ^{125}I-HCG (human chorionic gonadotropin). See Fig. 3 legend for an explanation of the symbols. The mice were studied at 3.0 hr postinjection.

HGH in the SV. Radio-uptake in no other organs and/or tissues studied changed significantly in confirmation of the preceding studies.

In vivo *antibody inhibition.* In order to determine the organ specificity of ^{125}I-HGH in the male mouse, inhibition studies using goat anti-HGH were performed. The bar graph in Fig. 6 displays a marked reduction of SV radio-uptake resulting in a T/B ratio of 0.85 when specific antibody was administered 5.0 min prior to the radiohormone injection. These data indicated that ^{125}I-HGH levels in the SV were less than unity and that the SV was not actively concentrating the radiohormone. The use of normal goat serum did not significantly alter the uptake of ^{125}I-HGH beyond the normal uptake levels shown in Fig. 6. It should be noted that antibody inhibition decreased the excretion rate of ^{125}I-HGH in the kidneys and the lungs and substantially increased radio-uptake in the liver. The antigen–antibody aggregates (HGH:anti-HGH) were presumably phagocytized and/or mechanically entrapped by the liver.

Discussion. Chemically pure hormones

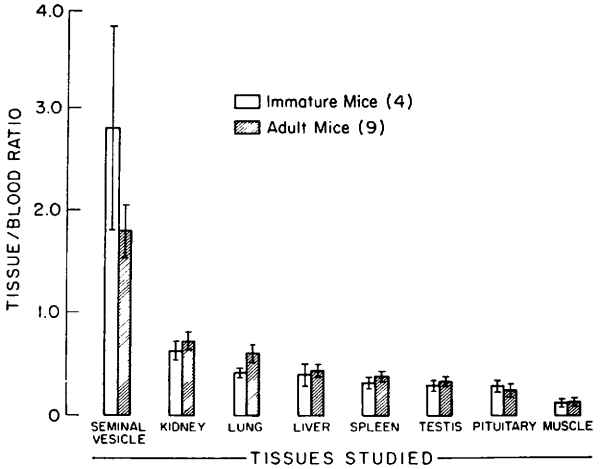

FIG. 5. Intact adult and immature male mice were injected with ^{125}I-labeled human growth hormone. See Fig. 3 legend for an explanation of the symbols. The mice were studied at 3.0 hr postinjection.

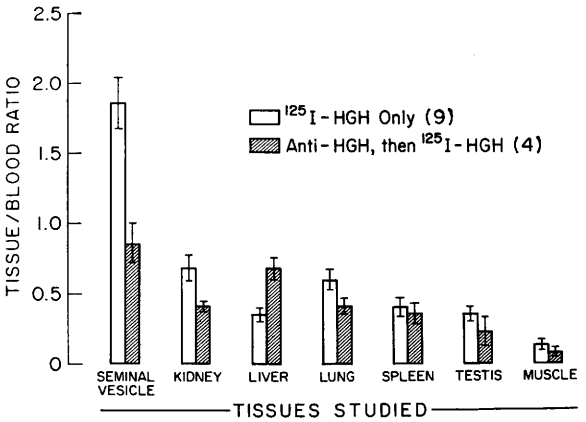

FIG. 6. Goat anti-HGH (human growth hormone) antiserum was administered into adult intact male mice 5.0 min prior to the injection of ^{125}I-HGH. The mice were studied at 3.0 hr postinjection. See Fig. 3 legend for an explanation of the symbols.

exert multiple effects and HGH produces many and varied metabolic alterations within the organism. HGH is known to act as a biologic synergist acting in consort with either ACTH, FSH, or LH (9). HGH is thought to facilitate the response of tropic hormones by aiding in the general maintenance of the secretory epithelium and connective tissue stroma of the accessory sex glands. This stimulation may be of a direct or indirect nature. The effect may be indirect in which ACTH (for example) would stimulate the adrenal cortex to produce sufficient androgen to restore the male accessory organs. In the present case of the mouse SV, HGH appears to act together with androgen directly on the gland to produce a similar effect. The absence of high circulating androgen in the immature mice seems to permit an enhanced uptake of HGH in the mouse SV. In this situation, HGH alone may be stimulating the mouse SV. That is, the presence of androgen in the adult mouse tends to decrease the uptake of HGH by the adult mouse SV (see Fig. 5). That ^{125}I-HGH does act upon the SV tissues in a direct fashion was confirmed by antibody inhibition studies. These later studies also provided evidence that free ^{125}I was not being concentrated by the mouse SV. A comparable synergism of preputial gland and seminal vesicle growth has been previously reported in the rat due to the action of testosterone and pituitary growth hormone (10). Both the vascularity and the linear rates of blood flow in the rat SV increase following injection of testosterone propionate in both intact and castrated animals (11).

The high hepatic radio-uptake observed at 30 min postinjection in the present mouse studies are consistent with previous findings in the human. Hepatic clearance is known to account for 91% of the total metabolic clearance of growth hormone in normal human subjects (12). The uptake of ^{125}I-HGH rapidly subsided in the mouse liver while the incorporation of this radiohormone in the SV consistently increased to the 3 to 4 hr interval. At 18 hr postinjection, however, the SV had lost most of its radiohormone (T/B = 0.259) while radio-uptake had once again increased in the liver (T/B = 0.732). Apparently, the HGH reentered the circulation and was again utilized by the liver. In the rodent liver, growth hormone enhances the incorporation of amino acid into the total protein of the liver and into the proteins of the nuclei, mitochondria, microsomes, and soluble fractions of hepatic cells (13). Growth hormone administration in the rodent also causes a quantitative increase in the ribonucleic acid content in the nucleolar fraction from hepatic cell nuclei (14).

It may be argued that the effects of HGH on the secondary sex organs of the mouse may be due to the prolactin activity of the human growth hormone preparation. Recent

evidence using tritiated leucine labeled antiserum to ovine prolactin (cross-reacts with human) in tissue culture has demonstrated the existence of 2 separate hormones in the human (15). Thus, a prolactin effect may not have influenced the present findings concerning HGH uptake in the mouse SV. The biologic and biochemical studies in the present report would tend to support the presence of a single, growth-stimulating component. However, one cannot rule out the possibility that the selective retention of ^{125}I-HGH in the mouse seminal vesicle was due to the presence of prolactin receptor sites. The answer to this question would require further experimentation employing (a) a prolactin-free HGH preparation (bovine growth hormone), and/or (b) a labeled prolactin preparation in conjunction with the ^{125}I-HGH.

The present study has demonstrated a concentration of HGH in the seminal vesicle of the mouse. In addition to the antibodies formed against HGH in the long-term treatment of humans, there is now a suggestion of secondary sex gland stimulation. Such a stimulation, maintained for extensive periods by long-term usage, might lead to gland hyperplasia, cellular irritation, and/or conceivably, anaplasia. The uptake and concentration of HGH in the human sex accessory organs has yet to be demonstrated. The present investigation provides a rationale for future studies of native growth hormone in the human. Finally, it is suggested that the mouse seminal vesicle may provide a biological model for an *in vivo* assay of HGH.

Summary. The distribution of human growth hormone (HGH) labeled with ^{125}I was studied in mature and immature intact mice. The ^{125}I-HGH appeared to be biochemically homogeneous and possessed both biologic and immunologic activity. Uptake of ^{125}I-HGH in mice occurred initially in the liver, but the highest radioconcentration values occurred in the seminal vesicle (SV) of the male mice at 3.0 hr postinjection. Other tissues and organs in the male mice did not take up the radiolabeled HGH. Radio-uptake in a sexually equivalent organ of the female could not be demonstrated. ^{125}I-HGH levels were higher in the SV of immature than in mature male mice; however, this difference was not significant. The use of anti-HGH antiserum employed as a blocking agent specifically inhibited the uptake of ^{125}I-HGH in the SV of the mouse.

Growth hormone was discussed as a biologic synergist in an attempt to explain the localization of ^{125}I-HGH in the SV. Uptake of the radiohormone in the liver may have resulted from either (a) a blood clearing mechanism, or (b) stimulation of hepatic protein anabolism. It was suggested that continual stimulation of the sex accessory glands could result in hyperplasia and, conceivably, anaplasia.

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