Mean juxtaglomerular indices of kidneys from various experimental and control groups

No. of animals	No. of kidneys evaluated	Sex	Treatment	Mean JGI ± S.E.M.
5	9	♂	Castrated	1.60 ± 0.09
5	10	♂	Untreated	1.61 ± 0.07[a]
6	12	♀	2 mg Progesterone daily	1.72 ± 0.08
5	10	♀	0.2 mg Testosterone daily	1.34 ± 0.03
4	8	♀	Untreated	1.64 ± 0.09[b, c]
8	15	♀	0.05 ml sesame oil daily	1.74 ± 0.10[a, c]

[a] Compared to testosterone treated females $p < 0.01$. [b] Compared to testosterone treated females $p < 0.025$. [c] Compared to progesterone treated females $p \geq 0.05$.

all of the other groups. The zona glomerulosa was unremarkable in all groups. Submaxillary salivary glands of untreated males and testosterone treated female animals had convoluted tubular epithelial cells filled with intensely eosinophilic granules characteristic of male morphology. All of the other groups had primarily female morphologies with inconspicuous tubular epithelia and a sparsity of intracytoplasmic granules.

Discussion. Evaluation of the JGI in female mice treated with testosterone has revealed a statistically significant reduction as compared to various control groups. Several possible mechanisms exist by which the JGI could be reduced including a direct response to the androgen. One explanation would be competition of testosterone with aldosterone resulting in decreased salt retention and exhaustion (degranulation) of the juxtaglomerular cells. This explanation, however, seems unlikely since our mice showed no adrenal cortical hyperplasia, a condition which is known to parallel increased aldosterone activity[12, 13]. Testosterone itself causes minor salt retention[14] which could account for the decrease in JGI. The failure of adrenal participation is difficult to assess since it has been shown that JGI changes can be mediated by alterations in cation intake in adrenalectomized animals[15]. In addition, aldosterone is not directly responsible for renin secretion[16]. Another explanation of the decrease in JGI is that the increased blood flow to the kidney as a result of testosterone administration[17] increases glomerular filtration rate and pressure, thereby lowering the JGI[4, 7]. A final possibility could be increased erythropoietin production stimulated by testosterone[18] resulting in decreased JGI.

The inability of progesterone to alter the JGI is difficult to explain[19]. Progesterone administration, unlike testosterone will enhance aldosterone secretion[20] and, therefore should have resulted in a marked decrease in JGI.

In conclusion, the results of this study have suggested that the JGI in Swiss mice is unaffected by sex or orchidectomy and that the JGI is lowered significantly by the chronic administration of high doses of testosterone to female mice[20].

Résumé. La testostérone, administrée de façon chronique à des souris femelles provoque une diminution statistiquement significative de l'index juxtaglomérulaire. Les femelles traitées par la progestérone ou les souris mâles et femelles des groupes contrôlés ne présentent aucune variation. Ces constatations sont discutées en relation avec l'effet de la testostérone sur le flux sanguin rénal, la pression de perfusion glomérulaire et la secrétion d'aldostérone, de rénine et d'érythropoiétine.

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Immunospecificity and Localization of Radiolabeled Human Growth Hormone in the Mouse

Human growth hormone (HGH) has recently been utilized in the clinic for the long term treatment of children with hypopituitary dwarfism[1]. Antibodies to HGH, which are known to contribute to growth inhibition, have been detected in these children[2]. The increased usuage in the clinic and the laboratory synthesis of this protein trophic hormone has prompted investigation into the distribution of radiolabeled HGH in intact experimental animals. In recent studies, the seminal vesicle of the mouse was reported as a target organ for ^{125}I-HGH[3]. The present investigation was undertaken to further clarify the immunospecificity and localization of HGH in the mouse. In addition, studies were undertaken to determine whether HGH radio-uptake occurs in the rat.

Materials and methods HGH, prepared by the RABEN method[4], was supplied and radiolabeled through the courtesy of the Abbott Radiopharmaceutical Laboratories,

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North Chicago, Illinois. After filtration through Sephadex G-100, the radiolabeled hormone contained a specific activity of 86.64 μCi/μg and a protein concentration of 1.94 μg/ml. Radiolabeled human chorionic gonadotrophin (^{125}I-HCG) was used as a trophic hormone control and has been previously characterized[5,6]. The ^{125}I-HGH was tested for biologic activity by the 10-day body weight test of EVANS et al.[7]. Goat anti-HGH was obtained from Miles Laboratories, Kankakee, Illinois for use in antibody-hormone inhibition studies in vivo.

Adult male mice of the A7(C57 × A) strain, originally obtained from Dr. W. U. GARDNER, Dept. of Anatomy, Yale University, were employed for the radioisotopic tissue distribution studies. Each of the mice, weighing 25–35 g, received i.v. 30 to 40 μCi (0.5–1.0 μg protein) of ^{125}I-HGH or ^{125}I-HCG. At 3 h postinjection, the animals were autopsied and 12 tissues were removed, weighed, and assayed for radioactivity in a gamma wellcounter. The results were expressed as the percent dose uptake of isotope per gram of tissue (%/g).

Radioisotopic techniques[8] were applied to fixed tissue sections of liver and seminal vesicle from the ^{125}I-HGH and ^{125}I-HCG treated animals. Intact and hypophysectomized male Sprague-Dawley rats were also employed to test the species specificity of radiolabeled HGH.

Results and discussion. The radioisotopic tissue distribution studies were first performed on adult male mice using both radiohormone preparations; then on mice previously administered goat anti-HGH antiserum. The uptake levels of ^{125}I-HGH were significantly higher in the seminal vesicle than in any other tissue studied with the exception of the thyroid (Table). The %/g of ^{125}I-HGH was 7.1 times higher in the seminal vesicles than in comparable tissue of control mice injected with ^{125}I-HCG. The mean ($\bar{x}$) uptake levels of radiolabeled HGH was somewhat greater in the kidney than in comparable HCG levels; however, this difference was not significant. The kidney uptake level is presumably related to excretion of the radiohormones. Concommittantly, radioisotope levels in the other organs and tissues (i.e., liver and testis) were insignificant.

HGH radio-uptake levels in the untreated mice (see above) were then compared to mice previously treated with anti-HGH antiserum 5.0 min prior to radio-hormone injection. The data in the Table demonstrate a 4-fold reduction of seminal vesicle (SV) radio-uptake resulting in a %/g level of 0.556 when specific antibody was employed. The use of nonspecific goat serum had no effect when similarly administered. The %/g levels in the liver were slightly elevated, although not significantly, from control radio-uptake levels. Other organs and tissue levels were either decreased or not altered. These results implied that HGH radio-uptake was inhibited in the SV due to the formation of antigen-antibody (HGH: anti-HGH) aggregates. These immune complexes may have been phagocytized and/or mechanically entrapped by the liver as indicated by the slight rise in radioactivity for this organ.

Both radiohormone preparations were also injected into 6 adult intact and 6 hypophysectomized male Sprague-Dawley rats. None of the organs and tissues studied in any of these rats were responsive to the radiohormones. All the organ and tissue radio-uptake levels were equivalent to that of the mouse liver and testis (Table). ^{125}I-HGH uptake in the rodent seminal vesicle is apparently a characteristic only of the mouse.

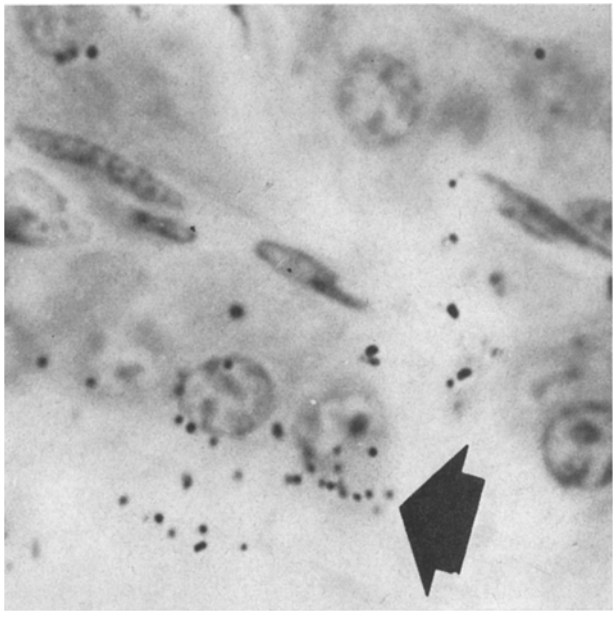
Autoradiographic analysis revealed the presence of isotopic foci in the tunica mucosa of the seminal vesicle. Radioactivity in the mucosal layer was associated with the columnar epithelial cells, often in the perinuclear region of the cells (Figure). However, some isotopic activity was also present in the connective tissue stroma underlying the epithelium. Corresponding tissue sections from control animals and from the liver of experimental animals were unremarkable.

Present studies have demonstrated that the adult male mouse concentrates ^{125}I-HGH in the seminal vesicle at 3 h postinjection. Secondly, the use of goat anti-HGH serum significantly depressed the uptake of ^{125}I-HGH in the mouse seminal vesicle. Thirdly, isotopic localization

The percent dose uptake per gram of tissue (%/g) is demonstrated for various tissues of radiohormone injected male mice

Radiohormone employed	Tissues studied			
	Seminal vesicle	Kidney	Liver	Testis
^{125}I-HGH $\bar{x}$	2.240	1.048	0.405	0.420
(7) SE	0.704	0.387	0.118	0.138
^{125}I-HGH[a] $\bar{x}$	0.556	0.575	0.551	0.113
(5) SE	0.071	0.258	0.116	0.020
^{125}I-HCG $\bar{x}$	0.309	0.591	0.249	0.175
(4) SE	0.075	0.104	0.035	0.025

[a] Indicates antibody inhibition study (anti-HGH). HGH, human growth hormone; HCG, human chorionic gonadotrophin. The figures represent the mean ($\bar{x}$) and standard error (SE) of the mean for the animal studies indicated in the parentheses.



Tissue autoradiogram displaying isotopic granule distribution overlying columnar epithelial cells in the mouse seminal vesicle. Isotopic foci were also observed to a lesser extent, in the connective tissue stroma. Note the granule localization in the perinuclear region of the cells (arrow), oil × 400.

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was observed in the secretory epithelium and, to a lesser extent, in the connective tissue stroma of the gland. Lastly, ^{125}I-HGH did not localize in any of the organs of the intact and/or hypophysectomized male rat.

HGH is known to act as a synergist with other hormones and to play a supportative role in many biologic phenomena[9]. The present investigation (autoradiograph) suggests that growth hormone may be an active participant in the functioning and maintenance of the mouse SV secretory epithelium. Previous studies have implicated androgen (testosterone) as a prime suspect for the synergistic action of HGH in the mouse[3]. Currently, it is uncertain whether the observed HGH stimulation of the SV could be attributed to the prolactin or the growth stimulating activity of the hormone preparation.

This study has demonstrated radio-uptake inhibition in the SV when specific antiserum was administered prior to the radiohormone injection. This uptake inhibition may have resulted either in steric blockage at the HGH cell receptor site or inability of the immune complex (HGH: anti-HGH) to penetrate the target cell membrane. Whatever the cause, the immune aggregates do not accumulate in the SV and are probably eliminated by the reticuloendothelial tissues, i.e., the liver. It becomes obvious why antibodies to HGH detected during longterm treatment for hypopituitary dwarfism contributes to growth inhibition in children.

Finally, it is of endocrinologic and systematic interest that cell receptor sites for HGH are present in the mouse but have not been detected in the rat. If indeed the stimulation could be attributed to a prolactin effect of the radiohormone, then a different mechanism or system of cell receptors for HGH must be present in the rat as compared to the mouse. This is not unexpected as many differences in reproductive physiology appear to exist between these 2 rodents[10].

Zusammenfassung. Bei männlichen Mäusen wurde die Bindung von ^{125}I-HGH untersucht. Im Vergleich zur Bindung an andere Gewebe derselben Tiere konnte eine zellspezifische Bindung im Bereich der Samenblasen festgestellt werden. Die Befunde werden in Hinblick auf eine Steroid-ergänzende Tätigkeit des HGH diskutiert.

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Epiphyseal-Hypothalamic Interaction. An in vitro Study with Some Sheep Pineal Fractions

Evaluation of the hypophysiotropic activity was carried out using our usual test (MOSZKOWSKA et al.[1]). In this test, the secretion of pituitary gonadotropins from pituitaries incubated in vitro in the presence of a cortical extract (controls) is compared with the secretion of gonadotropins from pituitaries incubated in the presence of a hypothalamic extract. Using this test we were able to evaluate the action of various pineal fractions on the hypothalamic-hypophysiotropic activity by comparing the gonadotropin releasing factor content of hypothalami incubated alone and in the presence of the pineal fractions.

In previous studies on the hypothalamic-hypophysiotropic activity in the rat, we have verified that the pineal Sphadex G-25 fraction F3 is capable of inhibiting this activity (MOSZKOWSKA et al.[1–3]). New information on the effect of the Sphadex G-25 fraction F3 was obtained from in vitro experiments using the mouse hypothalmus.

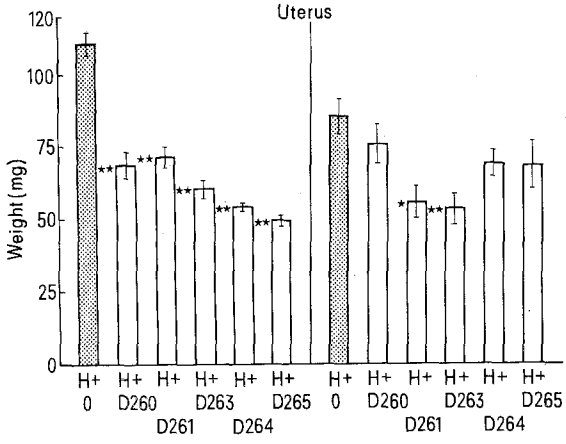

Fig. 1. Weights of the uterus of mice after injection of the incubation liquid (left) and extract (right) of mice hypothalamus incubated with and without a sheep pineal fraction UM-2R. D 260, D 261, D 263, D 264, D 265 are the codes of 5 different Sephadex G 25 columns from which UM-2R fractions are prepared. H, hypothalamus.

In continuation of these results, we carried out in vitro experiments with pineal fractions obtained by Sephadex G-25 filtration of an aqueous pineal extract followed by ultrafiltration of the low molecular weight Sephadex G-25 fractions, on the Amicon membranes UM-2 and UM-05 (EBELS and BENSON[4]).

Methods of extraction and separation of UM-2R and UM-05R. The frozen sheep pineal glands were homogenized in distilled water, filtered on a Sphadex G-25 column, equilibrated and eluated with distilled water. The extraction and separation was carried out in darkness and at 2 °C. The low molecular fraction thus obtained underwent a double ultrafiltration through 2 Amicon diaflomembranes. The first filtration on the membrane UM-2 gave a residue UM-2R and a filtrate. This filtrate, after a further filtration on UM-05, gave a second residue UM-05R and again a filtrate. The two residues were studied in vitro, and will be referred to in the following sections as fractions UM-2R and UM-05R.

From information obtained from the Amicon catalogue, the substance of the residues obtained from UM-2R have molecular weights greater than 1000, while those obtained from UM-05 have molecular weights between 500 and 1000. For details see EBELS and BENSON[4].

Bioassay. For the study of each pineal fraction 6 male mice hypothalami were incubated for $^1/_2$ h in a Krebs

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