TABLE I. The Data Represent the Average of 6 Experiments with Each Serum, During Which Rat Epididymal Adipose Tissue Was Incubated for One Hour at 37°C in a Medium Composed of 4 ml Krebs-Bicarbonate Buffer, 1 ml of the Serum and 1.0 μg/ml Tritium-Labeled Bovine Growth Hormone.

Serum added	Mean (c/m/g)	S.D.	Average wt of fat (g)
None	4,252	1,307	.273
Guinea pig	2,854	864	.254
Rabbit	3,570	848	.267
Calf	4,242	948	.236
Bovine albumin 1%	4,261	1,091	.271
Human	5,599	1,073	.254
Rat	6,630	1,252	.243
Rabbit anti-bovine growth hormone	1,735	284	.256

serum. Somewhat analagous findings have recently been reported to occur in the response of adipose tissue to insulin(5). These data suggest that species differences in serum may be one of the significant differences which determine responsiveness to growth hormone.

Three different experiments were performed to determine whether the radioactivity uptake could be interpreted as hormone uptake. First, the finding that antiserum to the hormone diminished the uptake of radioactivity was noted (Table I). Second, the uptake of radioactivity was determined when C^{14}-(acetylated) human albumin was added to the buffer, and in 6 separate experiments it was found that the c/m/g adipose tissue was one-fourth of the c/m/g found when comparable amounts of acetylated hormone were used. Third, when the adipose tissue was homogenized in saline, rather than chloroform:methanol, for 6 experiments, and when trichloracetic acid was added to make a final concentration of 5%, it was found that 50% of the c/m were precipitated. This suggested that one hour after incorporation into adipose tissue much of the radioactivity was bound to protein, and probably still to growth hormone.

When adipose tissue was obtained from rats weighing 50, 100, 200, and 400 g, no significant differences in the uptake of growth hormone were found.

Summary. The rat epididymal adipose tissue uptake of tritium-labeled bovine growth hormone was found to depend upon the species of serum which was added to the incubation medium.

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Distribution of Tritium Labeled Human Growth Hormone in Rats and Guinea Pigs.* (30729)

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Because guinea pigs are relatively unresponsive to growth hormone(1), we have compared the tissue distribution of human growth hormone in rats and guinea pigs. The technique used was to determine the concentration of radioactivity in tissue homogenates one hour after intravenous injection of tritium labeled human growth hormone. No significant difference was found between rats and guinea pigs. Autoradiographic studies demonstrated that the radioactivity had been concentrated in proximal renal tubular cells of both rats and guinea pigs.

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Methods. Biologically active labeled human (3.2 acetyl/mole) and bovine (12 acetyl/mole) were prepared by our previously reported method(2,3).

Three rats and three guinea pigs were injected intravenously (tail vein and femoral vein) with 50 $\times$ 10^6 d/m (90 μg) tritium labeled human growth hormone. One hour after injection the animals were sacrificed and homogenates of tissues using a glass hand homogenizer were prepared in distilled water, 6 N HCl (sternum and tibia only), and 1:1 chloroform:methanol (epididymal adipose tissue only). Radioactivity in aliquots of the homogenates (0.1 ml) was counted using a Nuclear Chicago Liquid Scintillation Counter and 5 ml of dioxane mixture per sample. Dioxane mixture contained 7 g PPO, 0.3 g POPOP, 100 g naphthalene, in 1000 ml dioxane. The c/m/0.1 ml of each homogenate was determined in duplicate aliquots cycled twice through the counter. Quenching was determined by addition of a known standard (the tritiated acetate) to each counting vial. The validity of this method for correcting for quenching was confirmed by counting 0.1 ml and 0.2 ml aliquots of blood and kidney homogenate.

Three rats and guinea pigs were injected with 256 $\times$ 10^6 d/m (0.45 mg) human growth hormone, 255 $\times$ 10^6 d/m (0.22 mg) bovine growth hormone, and 240 $\times$ 10^6 d/m tritiated acetate. Tissue sections (5 μ) of liver, pancreas, kidney, tibia, and aorta were prepared from formalin fixed specimens of each animal. The slides were coated with an invisible film of gelatin (except over the tissue), covered with film from Kodak Autoradiographic Stripping Plates and kept for 5 weeks in the freezer. The film was developed and fixed, and then the sections were studied with a phase contrast microscope and were then stained with modified Giemsa.

Using a double antibody precipitation technique several tissue homogenates were studied to determine if the radioactivity was still attached to human growth hormone. Guinea pig anti-human growth hormone serum was prepared by injecting an emulsion of 1 ml human growth hormone solution (2 mg/ml) in 1 ml incomplete Freund's adjuvant at 1, 2, and 3 weeks. At the fifth week 3 daily subcutaneous injections of 2 mg human growth hormone were given, and the guinea pigs were bled during the sixth week. Duplicate mixtures were prepared which contained 0.5 ml rabbit anti-guinea pig gamma globulin serum (1-10) and 0.5 ml guinea pig anti-human growth hormone serum (1-2000). After 4 hours at room temperature 0.2 ml of the tissue homogenate was added. Tissue homogenates had previously been centrifuged to remove any radioactivity that could be sedimented at 4500 rpm. After 16 hours at 0°C the tubes were centrifuged at 4500 rpm for 15 minutes, and the residue was suspended in 5 ml dioxane mixture and counted in the liquid scintillation counter.

Male guinea pigs and male Sprague-Dawley rats were obtained from Adams Caviary, Los Angeles, Calif. Bovine growth hormone (NIH-GH-B8) and human growth hormone were obtained from the Endocrinology Study Section of National Institutes of Health. Acetic anhydride-T (3560 mc/mM) was obtained from Nuclear Chicago Corp. Rabbit anti-guinea pig gamma globulin was obtained from Hyland Laboratories, Los Angeles, Calif. Dioxane, naphthalene, PPO, and POPOP were obtained from Eastman Kodak Co. and Nuclear Chicago Corp.

Results. After injecting the tritium labeled human growth hormone into rats and guinea pigs the tissue radioactivity and weights were compared and are summarized in Table I. To approximate the total body distribution of radioactivity, muscle, fat, bone and blood were assumed to represent 40%, 20%, 20% and 10% of the body weight of both rats and guinea pigs. Thus, the values for diaphragm and gastrocnemius (which were similar) were extrapolated to represent 40% of the body weight, for sternum and tibia (which were similar) to 20% of the body weight, and epididymal adipose tissue to 20% of the body weight. From 40-80% of the injected radioactivity could then be accounted for. The small intestine and its contents were homogenized and counted together.

The tissue distribution of radioactivity was similar in the rats and guinea pigs. Muscle, intestine, liver, kidney, urine, fat, bone and

TABLE I. Tissue Distribution of Tritium Labeled Human Growth Hormone: Tissue weights are each the average of 6 animals, and c/m are each the average of 3 animals. Diaphragm and gastrocnemius were averaged for muscle c/m; sternum and tibia were averaged for bone c/m; epididymal adipose tissue was the fat.

	% of body wt		c/m/mg tissue		% of recovered c/m	
Tissue	Rat	Guinea pig	Rat	Guinea pig	Rat	Guinea pig
Muscle	40.	40.	8.5	7.7	41.	37.
Fat	20.	20.	2.2	3.0	5.	9.
Bone	20.	20.	2.0	3.9	4.	7.
Liver	4.1	4.0	19.6	18.4	13.	13.
Kidney	.83	1.1	98.	23.	9.	4.
Intestine	6.5	10.	10.	8.3	16.	17.
Heart	.38	.35	7.	4.0	.4	.2
Lung	.62	.74	9.4	4.0	1.7	.8
Spleen	.86	.18	14.	18.	.5	.2
Pancreas	.25	.17	15.	8.4	.3	.16
Testes	1.3	.13	5.	3.0	.19	.08
Brain	1.1	1.6	4.8	4.9	.22	.32
Aorta	.02	.03	16.	6.2	.07	.05
Adrenal	—	.04	—	12.	—	.09
Bile	—	.02	—	7.4	—	.06
Urine	1.0	1.0	134.	69.	10.	7.
Blood	10.	10.	4.	3.7	8.	6.

blood accounted for the majority of the injected radioactivity. Only small amounts of radioactivity were found in brain, testes, spleen, heart, lungs, pancreas, aorta, adrenal, and bile. Of the organs tested, bone, fat, brain, testis, heart, and lung were the least different from the concentration of radioactivity in the blood. Urine, which had collected in the bladder during the interval between injection of growth hormone and sacrificing the animal, was consistently much more radioactive than blood and slightly more radioactive than kidney. This was true for both rats and guinea pigs.

When aliquots of homogenate from liver, kidney, intestine, and muscle were investigated for the presence of labeled human growth hormone antigen, the data in Table II were obtained. For each tissue duplicate controls, which lacked only the guinea pig anti-human growth hormone serum, were run.

TABLE II. Average of Duplicate Double Antibody Precipitation Experiments for Each Tissue. Controls contained no guinea pig anti-human growth hormone serum.

	% of control c/m in antibody precipitate	
Tissue	Rat	Guinea pig
Liver	90	100
Kidney	158	150
Muscle	132	114
Intestine	—	116

Mixtures which contained guinea pig anti-human growth hormone serum consistently precipitated more radioactivity than the controls when kidney and muscle homogenates were used, and not when liver and intestine homogenates were used. This was true for both rats and guinea pigs. When aliquots of homogenates from lung, pancreas, aorta, spleen, and heart were suspended in 5% trichloracetic acid, it was found that 80-90% of the radioactivity was precipitated.

Autoradiographs of formalin fixed tissues (liver, kidney, tibia, pancreas, aorta) were prepared from each of 6 animals. In both rats and guinea pigs the proximal renal tubular cells were found to contain definite labeling. This labeling was present for both the human and bovine growth hormone injected animals, but was not present in animals which received the same dose of radioactivity as free acetate. The labeling was localized along the brush border in these proximal tubular cells as can be seen in Fig. 1. Minimal labeling was present in the liver sections, but the specific pattern could not be determined definitely, and no labeling was found in the tibia, pancreas and aorta sections.

Discussion. Other attempts to determine the *in vivo* distribution of growth hormone were made by Salmon *et al*(4) using iodinated hu-

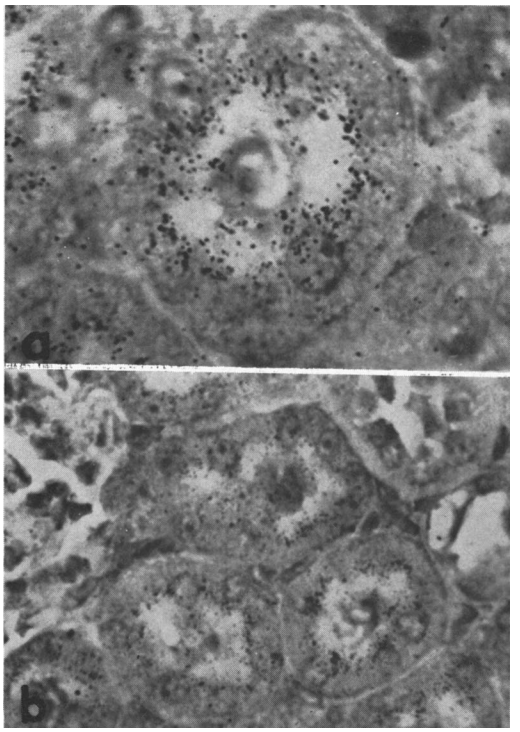

FIG. 1. Photomicrographs of rat kidney cortex stained with modified Giemsa. Rats had received injections of tritium labeled human growth hormone one hour before they were sacrificed.

man growth hormone intravenously injected into rabbits and by Sonenberg *et al*(5) using iodinated bovine growth hormone injected into rats. The physiologic potency of the iodinated human growth hormone was not demonstrated, but it disappeared from plasma with a half-time similar to the usual half-time for unlabeled growth hormone. Radioactivity in that study was found principally in the rabbit liver, kidney, spleen, and lung, with only small amounts in muscle and fat. The iodinated bovine growth hormone was demonstrated to stimulate the weight gain of hypophysectomized rat as much as unlabeled hormone. Hypophysectomy and alloxan diabetes did not alter the distribution of the hormone. When their data are calculated according to the tissue weights found in the present study, the iodinated bovine growth hormone and the tritium labeled human growth hormone were distributed in a similar manner in rats.

Autoradiography has been used to study the body distribution of iodinated insulin (6). The radioactivity was present in connective tissues, as well as heavily concentrated in proximal renal tubular cells and in the hepatic Kupffer cells which were near the portal spaces. This distribution of the iodinated insulin to kidney and liver is similar to our findings with tritiated human and bovine growth hormone. The growth hormone was not greatly concentrated in the elastic or connective tissues, although the major portion of it was distributed to skeletal muscle. Pituitary hormones, labeled with fluorescent dyes, and injected into rats, have been localized in proximal renal tubular cells by Vilar *et al*(7).

These data support the concept that the growth hormone in plasma is significantly concentrated in the liver and kidney in both rats and guinea pigs, and that skeletal muscle, fat, intestine and bone are the other major tissues to which growth hormone is distributed. The growth hormone in the proximal renal tubular cells most likely represents reabsorption of growth hormone.

Intravenously injected bovine growth hormone had been demonstrated to disappear rapidly and at a similar rate from rabbit, rat, and guinea pig plasma(2). The present studies suggest that injected growth hormone appears in comparable amounts in the same tissues of rats and guinea pigs. Therefore, these data suggest that the unresponsiveness of guinea pigs to injected growth hormone does not result from abnormal tissue distribution, or from excessive loss in urine or bile.

Summary. 1. Biologically active labeled human growth hormone, prepared by limited acetylation with tritiated acetic anhydride, was injected intravenously into rats and guinea pigs. 2. In both rats and guinea pigs the radioactivity was distributed primarily to skeletal muscle, liver, intestine, kidney, bone, fat, and urine. 3. Autoradiographic studies indicated that intravenously injected human and bovine growth hormone were concentrated in proximal renal tubular cells of both rats and guinea pigs.

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Renal Response to Unilateral Vagotomy During Water Diuresis.* (30730)

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Bilateral cervical vagotomy promotes prolonged inhibition of mild water diuresis(1) and increased antidiuretic activity of blood (2,3). These reports support the concept of vagal afferent control of antidiuretic hormone secretion, which has evolved from the observation that left atrial stretch induces diuresis in dogs with intact vagus nerves, but has no effect when vagal transmission is blocked(4). Since vagal afferents responsible for the induction of certain respiratory and cardiovascular phenomena are confined to or predominate in a particular vagus nerve (*e.g.*, 5,6), the present experiments were performed to determine the influence on renal function of unilateral vagal section during mild water diuresis.

Methods. Mongrel dogs (12.7-21.8 kg) of both sexes were anesthetized with pentobarbital sodium (30 mg/kg, i.v., with supplementation as required). After a priming intravenous injection of creatinine and sodium para-aminohippurate (PAH), warm 5% glucose in water, containing appropriate amounts of these substances, was infused continuously by pump at the rate of 2.5 ml/min throughout the experiments. The ureters were exposed through a mid-ventral incision, and were cannulated with polyethylene tubing. Short segments of the cervical vagus nerves (vagosympathetic trunks) were isolated approximately 5-7 cm below the lower border of the larynx, and double loose ligatures were placed around each nerve. Parafilm was placed between the cut surfaces of the skin to prevent adherence and the wound was closed with a towel clip. A femoral artery was cannulated for determining mean blood pressure with a mercury manometer, and an indwelling needle was inserted into an external jugular vein for obtaining blood samples at 30-minute intervals during subsequent clearance studies. An animal was considered suitable for an experiment if urine flow was reasonably stable during three 15-minute control periods and the urine was hypotonic; during the control phase, the mean $\pm$ S.E. for urine osmolality was 165 $\pm$ 15 mOs/kg H_2O. At the end of the last control period, the neck wound was opened and one vagus nerve was gently lifted and cut with scissors; manipulation of the nerve preparatory to sectioning was not accompanied by bodily movements or changes in blood pressure. It was found previously(1) that sectioning the vagi with or without ligation and in the presence or absence of a locally applied anesthetic did not alter the results. Right vagotomy was performed in 5 dogs and the left nerve was sectioned in a second group of 5 dogs. Upon completion of urine collections (9-16 fifteen-minute periods) after unilateral vagotomy, the contralateral vagus was sec-

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