with cholesterol and lard develop a high incidence of mammary cancer and lung adenocarcinoma. In the absence of other lipids, cholesterol alone causes the development of a high incidence of lung adenocarcinoma. The incidence of mammary cancer in the mice of this group is very low, apparently due to the poor development of the ovaries and a low estrogen titer in the females receiving cholesterol. The appearance of giant basophilc cells in the pituitary would be indicative of a low estrogen titer in these mice. The fact that cholesterol and lard cause the development of both mammary and lung cancer, while cholesterol alone causes almost exclusively the development of lung adenocarcinomas supports our point of view that there is a relationship between the nature of the malignancies in mice and the composition of the lipids in their diets.

Some slides were examined by Dr. Stephen S. Sternberg, Sloan-Kettering Institute, to whom I am greatly indebted.

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Received July 12, 1965. P.S.E.B.M., 1966, v121.

Bovine Growth Hormone Uptake by Epididymal Adipose Tissue.*† (30728)

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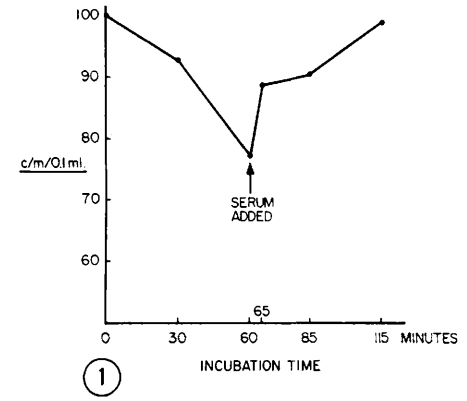
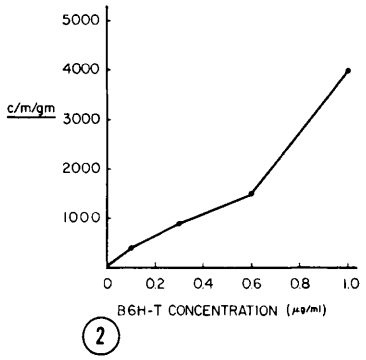
Recognition of species specificity of growth hormone has stimulated interest in the modification of bovine growth hormone in order to make it useful in man. The reasons for this species specificity are unknown, although differences in the amino acid composition of growth hormone from different species are thought to contribute to it. Since guinea pigs are also relatively resistant to growth hormone(1) we have studied them. In a previous report it was noted that bovine growth hormone disappeared from the plasma of guinea pigs after intravenous injection as rapidly as it did in rats(2). Further, it has now been found in this laboratory that the tissue distribution of human growth hormone intravenously injected into rats and guinea pigs is similar. This report presents data suggesting that guinea pig plasma retards, and rat plasma stimulates, *in vitro* uptake of bovine growth hormone into rat epididymal adipose tissue.

Methods. All experiments were performed using 5 ml Krebs-bicarbonate buffer, saturated with 95% oxygen and 5% carbon dioxide in 10 ml stoppered flasks. The order in which materials were added to the incubation flasks was found to be important. The rat was killed after the buffer was warmed to 37°C, and saturated with the oxygen and carbon dioxide mixture. Epididymal adipose tissue (200-300 mg, from male Sprague-Dawley, 100-150 g rats), albumin or serum, and finally the tritium-labeled bovine growth hormone were promptly added. The flasks were shaken (100 strokes/minute) in a metabolic shaker at 37°C during each experiment. Adipose tissue was removed from the flask, rinsed for 15 minutes in ice-cold saline, homogenized in 1 ml chloroform:methanol (1:1), and then 0.1 ml aliquots were

* Supported by USPHS Grant AM-04235.
† Mr. Reuben Gambetta provided technical assistance.





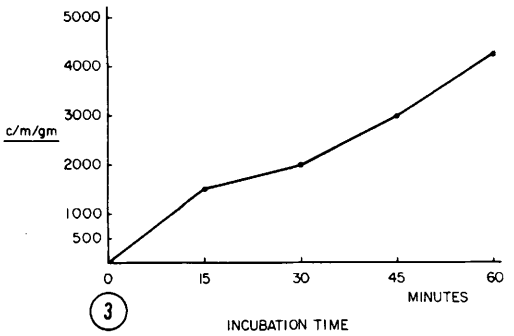


FIG. 1. Each point represents the average of 12 separate experiments. Tritium-labeled bovine growth hormone (1.0 μg/ml) was incubated at 37°C in 5 ml Krebs-bicarbonate buffer and 1 ml human serum was added at 1 hour. The c/m/0.1 ml buffer was determined at intervals.

FIG. 2. Each point represents the average of 6 separate experiments in which rat epididymal adipose tissue was incubated at 37°C for 1 hour in Krebs-bicarbonate buffer which contained 1% bovine albumin and varying concentrations of tritium-labeled bovine growth hormone. The c/m/g adipose tissue are indicated.

FIG. 3. Each point represents the average of 6 separate experiments in which rat epididymal adipose tissue was incubated for varying time intervals at 37°C in Krebs-bicarbonate buffer which contained 1% bovine albumin and 1 μg/ml tritium-labeled bovine growth hormone. The c/m/g adipose tissue are indicated.

counted in 5 ml dioxane mixture (10% naphthalene, 0.7% PPO, and 0.03% POPOP in dioxane) in a Nuclear Chicago Liquid Scintillation Counter. Quenching was determined by addition of a known standard to each flask and the c/m/g of adipose tissue were calculated using this correction. Bovine growth hormone (NIH-GH-B6)‡ was labeled with tritium as previously described and was determined to be biologically comparable to unlabeled growth hormone(2).

Results and discussion. When only labeled growth hormone, without adipose tissue or serum was incubated in the buffer, the concentration of radioactivity in the buffer decreased gradually. Forty duplicate experiments indicated that the relative concentration of radioactivity at time 0, 1, 2, 3, and 4 hours was 100%, 65%, 59%, 56%, and 56% when the buffer contained only 0.3 μg/ml labeled growth hormone. No decrease in the concentration of radioactivity occurred when albumin or serum was added to the buffer before the labeled hormone. Fig. 1 illustrates data which suggest that the labeled bovine growth hormone binds to the sides of the flasks, and when serum was added at 60 minutes the radioactivity increased gradually to the original concentration. This problem of proteins binding to glass has been described previously with several different proteins(3,4).

The uptake of tritium-labeled bovine growth hormone by adipose tissue depends upon incubation time and concentration of the hormone as indicated in Fig. 2 and 3. The growth hormone uptake was significantly reduced by addition of rabbit anti-bovine growth hormone serum when compared to rabbit serum, and it was dependent upon the species from which the serum was obtained (Table I). In particular, guinea pig serum reduced the uptake of bovine growth hormone by rat epididymal adipose tissue when compared to the uptake in the presence of rat

‡ Bovine growth hormone (NIH-GH-B6) was supplied by the Endocrine Study Section, Nat. Inst. Health.

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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Received July 22, 1965. P.S.E.B.M., 1966, v121.

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.

* This research was supported by USPHS Grant AM-04235.