Hepatic Lipid Metabolism in Hypophysectomized and Growth Hormone-Treated Hypophysectomized Rats (34686)

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(Introduced by L. D. Abbott, Jr.)

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The effects of hypophysectomy (Hx) and growth hormone (GH) administration on lipid metabolism have been the subject of considerable investigation in recent years, but few attempts have been made to define progressive alterations occurring after Hx or after the onset of GH administration. Jensen *et al.* (1, 2) have made perhaps the most comprehensive study along these lines in defining the changes in lipid composition in testes and liver following Hx. They have found that the most drastic changes occur during the first 3 weeks after the operation. ^{32}P incorporation into tissue phospholipid has also been reported to decrease following Hx, but the period elapsed after the operation was not reported (3). The effects of GH administration in promoting lipolysis in adipose tissue, thereby producing alterations in blood free fatty acids, are reasonably well defined, but the effects on hepatic metabolism are less clear. ^{32}P incorporation into liver phospholipids has been reported to increase (4) or to exhibit no change (5) after GH administration. However, Greenbaum and his coworkers (5) have demonstrated an increase in liver lipids due to GH treatment and ascribe this to an increase in di- and triglyceride synthesis.

This report deals with the results of experiments in which we studied progressive changes in hepatic lipid metabolism following Hx and administration of bovine GH (BGH) to Hx rats. The changes were assessed by determining incorporation of ^{3}H-glycerol into lipids of liver slices from these rats. These experiments revealed a decreased incorporation of label into all lipids except an acidic lipid fraction following Hx, reaching a minimum by 3 weeks postoperation. GH administration caused a gradual, but incomplete, reversal of this effect of Hx over a 10-day period.

Materials and Methods. All chemicals were obtained commercially and used without further purification. 2-^{3}H-glycerol (sp act, 200 mCi/mmole) was purchased from New England Nuclear Corporation, Boston, Mass.

A highly purified preparation of bovine growth hormone was prepared by the method of Dellacha and Sonenberg (6) except that G-100 Sephadex was used in place of G-75 for the final gel filtration step and was the generous gift of Dr. M. Sonenberg. Normal and hypophysectomized male Sprague-Dawley rats weighing 85 g were purchased from Zivic-Miller Laboratories, Allison Park, Pa. Hx rats were shipped to our laboratories 1 to 2 days after the operation. They were used 1 to 8 weeks post-Hx and maintained on Purina laboratory chow and water *ad libitum* until the time of sacrifice.

The animals were killed by decapitation and the liver was perfused *in situ* via the portal vein with a solution containing 0.1 *M* sucrose, 0.1 *M* KCl and 0.025 *M* phosphate buffer, pH 7.4. Liver slices (1 g $\pm$ 10 mg) were prepared with a Stadie-Riggs apparatus and transferred to a 25-ml Erlenmeyer flask containing 10 ml of medium. After the flask contents reached thermal equilibrium, 5 μCi of ^{3}H-glycerol were added and the reaction mixture was incubated in an air atmosphere for 2 hr at 37° while shaking at 100 cycles/min. After the incubation period, the slices were collected by centrifugation, washed once with fresh incubation medium, and the lipids extracted by the method of Folch *et al.*

(7). The crude lipid extracts were washed once with 0.4% $CaCl_2$, then twice more with pure upper phase containing 2 mg of glycerol/ml. The washed lipids were dissolved in 10 ml of chloroform containing 0.02% BHT, and aliquots were taken for determination of radioactivity and for chromatographic analyses.

Lipids from 100 mg of wet tissue were applied to a 2-g silicic acid column (100–200 mesh) and eluted with 25 ml of ether, 15 ml of ethanol–ether (2:8, v/v), 20 ml of ethanol–ether (7:3, v/v), 30 ml of methanol–ethanol (6:4, v/v) and 10 ml of methanol. Fourteen fractions were collected in scintillation vials; two 10-ml fractions followed by eight 5-ml and four 10-ml fractions. The solvents were evaporated, 5 ml of Bray's solution (8) were added to each vial and the radioactivity was determined in a Nuclear-Chicago Mark I scintillation spectrometer. This separation scheme yielded four discrete fractions of the following composition: neutral lipids (vials 1–3); acidic lipids (vials 4–6), consisting of phosphatidic acid, cardiolipin, serine phosphoglycerides, and some inositol phosphoglycerides; cephalin (vials 7–10), consisting of ethanolamine and inositol phosphoglycerides; and lecithin (vials 11–14), consisting of choline phosphoglycerides and sphingomyelin. The lipid content of each of these fractions was determined by thin-layer chromatography as previously described (9).

Results. The incorporation of glycerol into total lipids by liver slices from Hx rats decreased rapidly following Hx (Fig. 1). One week postoperation the total amount of glycerol incorporated was 74% of the normal values. This decreased to 46% that of normal levels at 2 weeks post-Hx. From the third to eighth week, the amount of label incorporated remained diminished, ranging from 32 to 43% of that found in normal rats of the same age.

The incorporation of glycerol into neutral lipid, acidic lipid, cephalin, and lecithin fractions is shown in Fig. 2. A decrease in incorporation of glycerol into all lipid classes except the acidic lipids was noted after Hx. Some differences in pattern of labeling may exist in the early periods following the operation, but the data are not conclusive.

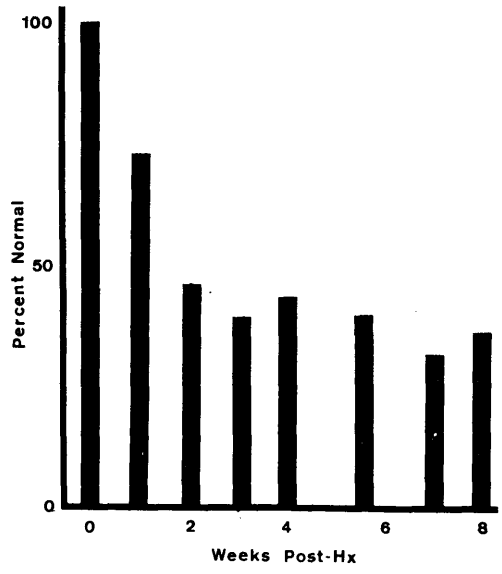

FIG. 1. *In vitro* incorporation of ^{3}H-glycerol into total lipids by liver from normal and Hx rats. One g of tissue slices was incubated with 10 ml of solution containing 0.1 *M* sucrose, 0.1 *M* KCl and 0.025 *M* Na phosphate buffer, pH 7.4. 5 μCi of ^{3}H-glycerol were added and the reaction mixture was incubated for 2 hr at 37° in air. The slices were processed as described under Materials and Methods. The 100% control values are means of at least two separate determinations on liver slices from 12 normal rats which were equivalent in age to 0-, 2-, 3-, 4-, 5.5-, and 7-weeks post-Hx rats. The experimental values are means of 2 separate determinations done in duplicate from 14 Hx rats which were killed in pairs at the times indicated.

We next examined the effects of BGH administration on hepatic lipid metabolism in 3- to 4-weeks post-Hx rats. The incorporation of isotope into total lipids of liver slices derived from normal, Hx, and BGH-treated Hx rats is shown in Fig. 3, Panel A. It is apparent that no significant changes in the synthesis of total lipid occurred in normal or Hx rats over the experimental period. However, administration of BGH for 2 days caused a small increase in incorporation. After 5 days of BGH treatment this increase was 60% over values for Hx animals. This increase continued up to 10 days of treatment, the incorporation then being 75%

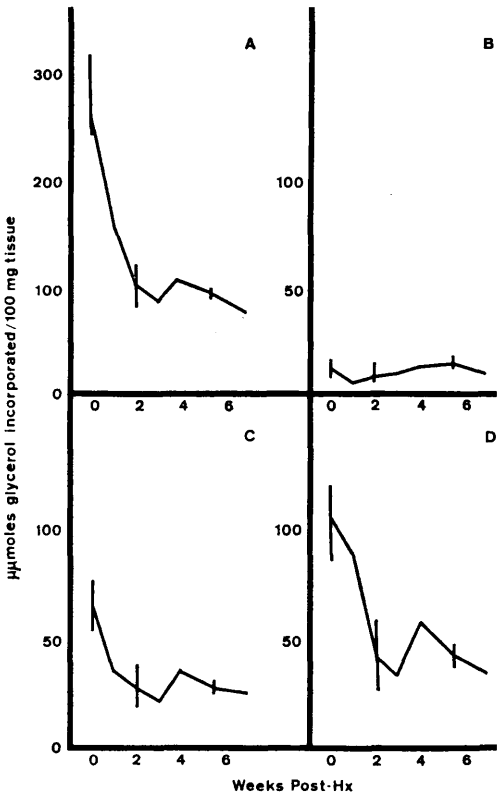

FIG. 2. *In vitro* incorporation of ^{3}H-glycerol into lipid classes by liver slices as a function of time after Hx. Experimental conditions and number of animals employed were the same as in legend to Fig. 1. Panels A through D represent neutral lipids, acidic lipids, cephalin, and lecithin, respectively. Bars indicate the range of values.

greater than the slices from Hx animals. The labeling of neutral lipids, cephalin, and lecithin from liver slices of these normal, Hx, and hormone-treated Hx rats is shown in Panels B, C, and D, respectively. No increase occurred in normal and Hx rats, but BGH treatment stimulated the synthesis of all lipids except the acidic lipids. Incorporation of label into the lecithin and cephalin fractions increased more rapidly than into neutral lipids. Five days of hormone treatment increased the synthesis of neutral lipids only 55% while cephalin labeling was up 70% and lecithin up 78% compared to values obtained with Hx rats.

Discussion. We have used the incorporation of radiolabeled glycerol to study new synthesis of lipids. To quote a recent article concerned with hepatic lipid metabolism, "The presence of radioactive glycerol in the particular lipids studied must indicate synthesis *de novo* of that molecule. This contrasts with results for some other labeled precursors which may be incorporated without resynthesis of all three bonds to the glycerol moiety" (10). Therefore the incorporation of label into a lipid measures total synthesis, or turnover, rather than only indicating turnover of a particular portion of the molecule.

A consistent decrease in the incorporation of labeled glycerol into the neutral lipids, cephalin, and lecithin of liver was observed during the first 3 weeks post-Hx (Figs. 1 and 2). These data are in accord with the results of Jensen and Privett (2), who found that maximum changes in hepatic lipid content occurred at the same interval following Hx. The length of time after Hx which is necessary to produce a maximal decrease in these parameters is probably a dual response to diet and pituitary ablation, as it is known that Hx rats consume less food than normal rats of the same age (11). The Hx rats used in this study gained only about 4 g/week during the experimental period while the normal rats gained 25–30 g/week. Although pair feeding might have allowed for an improved comparison, it would not resolve the problem, as this type of regime in no way provides for altered energy requirements or altered physical activity. The decline in glycerol uptake and lipid synthesis after Hx may reflect the rate of decrease in cellular proliferation, although we do not as yet have sufficient data on this parameter. It is also possible that it reflects the disappearance of GH from the tissue.

The precise alterations in metabolism which lead to the decreased incorporation of glycerol into lipid by liver slices from Hx rats have not yet been established. However, certain observations are relevant to this problem. Levels of α-glycerol phosphate were measured during the course of some of these experiments (12) and found to be similar in slices from normal and Hx rats, the mean values being 1.36 and 1.35 μmoles/g of wet weight, respectively. Therefore, dilution of

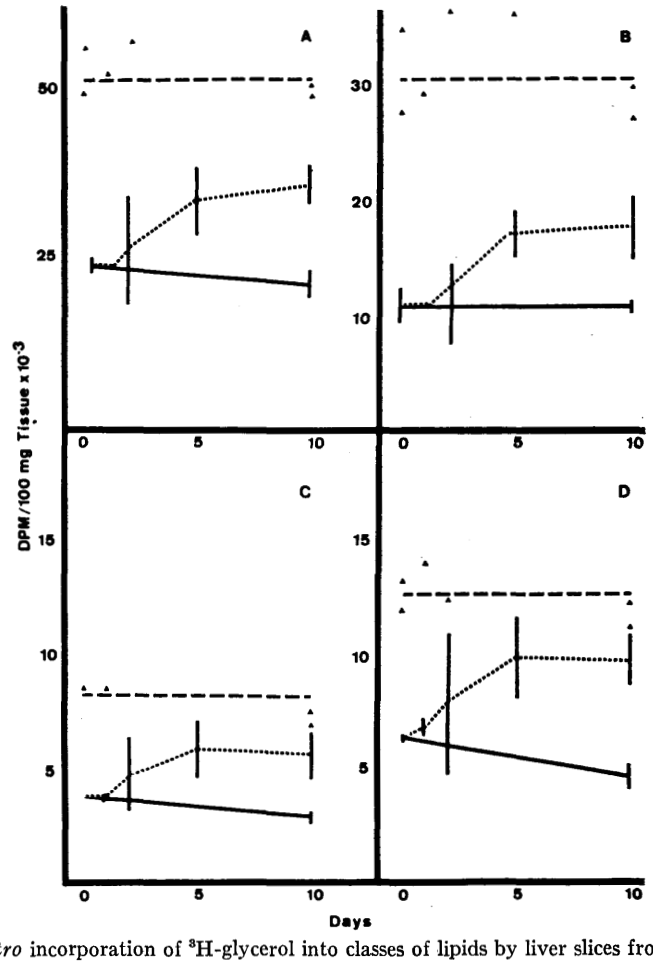

FIG. 3. *In vitro* incorporation of ^{3}H-glycerol into classes of lipids by liver slices from normal (---), Hx (—), and BGH-treated rats (....). Experimental conditions were the same as in legend to Fig. 1. Ten Hx rats received 1 mg of BGH in glycine buffer daily and pairs were killed at each time indicated. Four Hx rats which served as the BGH-controls were injected with glycine buffer alone and pairs were killed at the times indicated. Eight normal rats of the same age as the Hx rats were killed at the indicated times. The values are the means of two separate determinations done in duplicate. Panels A through D represent total lipids, neutral lipids, cephalin, and lecithin, respectively. Bars indicate the range of values.

the isotope by a larger α-glycerophosphate pool in Hx rats is unlikely. However, tissue from these rats may have an altered permeability to glycerol or lower rate of substrate phosphorylation. The amount of label found in the acidic lipids was comparable in the normal and Hx animals, but does not indicate the turnover rates of phosphatidic acid as it represents a static measurement. Jensen and Privett (2) found somewhat increased levels of phosphatidic acid in the livers of Hx rats. These findings together would suggest that its turnover may be slower in these animals. The turnover of diglycerides may also be slower after Hx, as the percentage of label accumulated in this fraction in our experiments was greater in Hx than in normal rats. This may be due to lower activity of enzymes which catalyze the conversion of diglycerides to triglycerides and phospholipids or to decreased concentrations of the other metabolites involved in these conversions. We are presently investigating these possibilities.

BGH treatment of Hx rats stimulated

incorporation of glycerol into all lipids except the acidic lipids of liver slices. However, this increase failed to reach normal values after 10 days of treatment with large amounts of hormone, a result in accord with observations on ^{32}P incorporation (3). The large increase in labeling of the cephalin and lecithin fractions is also in agreement with early reports of Greenbaum and his co-workers (4), but we do not have sufficient data to comment on later observations (5) in which they observed a decrease in ^{32}P incorporation into phospholipids 6 hr following hormone treatment of normal rats. This possible disparity of results may be attributable to differences in hormone treatment and/or the use of normal vs. Hx rats. We first observed a slight increase in glycerol incorporation 2 days after BGH injections, while the decrease noted by Greenbaum's group was after much shorter periods. It is interesting to speculate that BGH might have a biphasic effect on phospholipid synthesis, reminiscent of its action on plasma FFA levels in Hx rats (13, 14).

Summary. Changes which occur in hepatic lipid metabolism of hypophysectomized and bovine growth hormone-treated hypophysectomized rats were studied. Synthesis of lipids, as measured by the ability of tissue slices to incorporate ^{3}H-glycerol into lipids, decreased rapidly following hypophysectomy. Three weeks after hypophysectomy, glycerol incorporation was approximately 40% of that in normal rats and remained depressed over the experimental period. Synthesis of acidic lipids was not appreciably affected by hypophysectomy but labeling of all other classes of lipids, particularly triglycerides, was diminished. Daily administration of bovine growth hormone over a 10-day period to animals 4-weeks posthypophysectomy resulted in a 75% increase in total lipid synthesis. Incorporation of label into the lecithin and cephalin fractions exhibited the greatest increase of those lipid fractions studied during the first 5 days of bovine growth hormone treatment.

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