loaded rats, induced by ureteral obstruction, and accompanied by an increase of H^3-inulin ratios without change in the Na^{22} ratios is very strongly suggestive that C^{14}-urea is trapped in the lumen of the distal tubule, together with H^3-inulin, while Na^{22} is being reabsorbed. This together with the rapid fall in the $^{TF}/P$ ratios of C^{14}-urea (and H^3-inulin) following release of the obstruction is strongly suggestive that urea is retained in the distal tubules because of their restricted permeability. These data suggest then that the restricted permeability of urea is adequate to explain increased distal tubular fluid urea concentrations at least under the conditions of ureteral obstruction.

1. Lassiter, W. E., Gottschalk, C. W., Mylle, M., Am. J. Physiol., 1961, v200, 1139.
2. Gottschalk, C. W., Morel, F., Mylle, M., ibid., 1965, v209, 173.
3. Capek, K., Fuchs, G., Rumrich, G., Ullrich, K. J., Pfluger's Arch., 1966, v290, 237.
4. Carrasquer, G., Diana, A. Louise, Proc. Soc. Exp. Biol. & Med., 1967, v125, 823.
5. Steinhausen, M., Pfluger's Arch., 1963, v277, 23.
6. Lassiter, W. E. Mylle, M., Gottschalk, C. W., Am. J. Physiol., 1964, v206, 669.
7. Carrasquer, G., Baldwin, A. Louise, ibid., 1965, v209, 1001.

Received April 6, 1967. P.S.E.B.M., 1967, v125.

Role of Growth Hormone and Dietary Adaptation on Incorporation Of Leucine into a Mitochondrial Protein.* (32217)

PATRICK F. DELANEY AND PAUL F. FENTON
Department of Biology, College of the Holy Cross, Worcester, Mass., and the Division of Biological and Medical Sciences, Brown University, Providence, R. I.

Nitrogen excretion studies on intact animals(1) led us to postulate that one function of pituitary growth hormone might be to speed the adaptation of the organism to the rapid utilization of fats to provide energy for protein synthesis under conditions under which little carbohydrate is available. Increasingly the metabolic effects of hormones are being sought at the subcellular level. Korner(2) has shown that incorporation of labeled amino acids into proteins of nuclei, mitochondria, microscomes and soluble fraction of liver was diminished by hypophysectomy and restored toward normal by growth hormone administration. In more recent work he has concentrated his attention on the growth hormone effect on protein synthesis in the ribosomal system. However, in the light of our observations on the effect of growth hormone on the nitrogen-sparing action of fat, we turned our attention to the well known protein synthesizing system of mitochondria. Since these organelles oxidize both carbohydrates and lipids, they might possibly be a site of adaptive changes which we postulated to occur after high fat feeding and after growth hormone administration.

Methods. Male mice of the C57/Fn strain, 3 to 5 months old, were fed either diet #343 containing 60% fat or diet #307 containing 60% carbohydrate(1) for at least 2 weeks prior to the experiment. Growth hormone,† 10 mg/kg body weight, was injected intraperitoneally 5-5-1/2 hours before the animals were decapitated and exsanguinated. Several livers were pooled and the mitochondria isolated by the method of Truman and Korner (3). The incubation medium was essentially medium B of Truman and Korner using either succinate or octanoate and β-hydroxybutyrate as substrate. When using the latter substrate, the mitochondria were washed 4 times with 0.25 M sucrose and twice with 0.15 M KCl. Each ml of the incubation medium contained the mitochondria from 1 g of liver, 0.5 μc of

* Supported by grants from USPHS, AM-09186 and HD-01227.

† Growth hormone preparation NIH-GH-B8 Bovine was the gift of Endocrinology Study Section, NIH.

TABLE I. Effect of Growth Hormone on Incorporation of Leucine-C^{14} into Protein in Isolated Mitochondria (Succinate Substrate).

Previous ration*	No. of trials	Growth hormone	Leucine-C^{14} incorporation, counts/min/mg protein/hr
High fat	(5)	—	151 ± 23
	(4)	+	320 ± 65
High carbohydrate	(4)	—	240 ± 42
	(5)	+	528 ± 63

* High fat ration consisted of 30% casein (Labco Vitamin-free), 55% hydrogenated fat (Crisco), 5% corn oil (Mazola), 5% starch, 5% salt mixture (Hubbell, Mendel, Wakeman).

High carbohydrate ration consisted of 30% casein, 60% starch, 5% salt mixture (Hubbell, Mendel and Wakeman), and 5% corn oil (Mazola).

Vitamins, with the exception of B_{12}, were intimately mixed with the casein prior to preparation of the rations.

leucine-C^{14}, and 1 mg of a synthetic amino acid mixture. The flasks were incubated in a metabolic shaker at 37° for one hour under an atmosphere of air. The reaction was stopped by adding $HClO_4$ containing unlabeled leucine, and the protein was isolated as directed by Truman and Korner. The dry protein thus obtained was dissolved in 0.5 N NaOH. The nitrogen content of this solution was measured by the method of Johnson(4). The C^{14} content was determined by liquid scintillation counting of 0.2 ml of the alkaline protein solution in 10 ml of Bray's solution (5).

Results and discussion. When succinate was used as substrate we found that mitochondria from fat-adapted and carbohydrate-adapted animals incorporated labeled leucine into mitochondrial protein to about the same extent. Treating mice, regardless of their dietary history, with growth hormone produced an increase in the degree of *"in vitro"* incorporation of amino acid (Table I). These results confirm the observations of Korner(2).

On the basis of our studies with intact animals(1) we suspected that, when octanoate was used as substrate, mitochondria from animals adapted to high fat rations would incorporate labeled amino acid into protein to an extent not affected by previous growth hormone treatment. Mitochondria from carbohydrate-adapted mice would be expected to incorporate amino acid to a lesser degree, and previous growth hormone treatment would be expected to increase incorporation to the level observed in the fat-adapted animals. The results are shown in Table II and are compatible with our suggestion that growth hormone may act in part by bringing about adaptive changes which facilitate the utilization of lipids to provide energy for protein synthesis and that these adaptive changes can occur without growth hormone administration if the animal is given time to adapt itself to a high fat ration.

Mice fed a high fat ration *ad libitum* consume more calories than those fed a high carbohydrate diet. It is difficult to say whether a small difference in caloric intake prior to the experiment would drastically affect the reaction of mitochondria to growth hormone.

In our studies with intact animals(1) carbohydrate-feeding gave a maximal nitrogen-sparing action which could not be increased by growth hormone administration. The pronounced effect of growth hormone when succinate was used as substrate (Table I) is not in harmony with the earlier findings and suggests an additional mode of action of growth hormone than the one we have already proposed.

Summary. Isolated mitochondria from animals previously treated with growth hormone incorporated more amino acid into protein when succinate was substrate. In the presence of β-hydroxybutyrate and octanoate the mitochondria from fat-adapted animals incorporated amino acid at a greater rate than mitochondria from carbohydrate-fed ones. In this latter study, growth hormone enhanced incorporation rate only in mitochondria from

TABLE II. Effect of Growth Hormone on Incorporation of Leucine-C^{14} into Protein in Isolated Mitochondria (Octanoate Substrate).

Previous ration*	No. of trials	Growth hormone	Leucine-C^{14} incorporation, counts/min/mg protein/hr
High fat	(4)	—	294 ± 37
	(5)	+	280 ± 25
High carbohydrate	(3)	—	171 ± 23
	(5)	+	287 ± 26

* Composition of rations as indicated in Table I.

animals fed a high carbohydrate ration.

1. Katorski, B., Delaney, P. F., Fenton, P. F., Am. J. Physiol., 1965, v209, 910.
2. Korner, A., Biochem. J., 1960, v74, 462.
3. Truman, D. E. S., Korner, A., Biochem. J., 1962, v83, 588.
4. Johnson, M. J., J. Biol. Chem., 1941, v137, 575.
5. Bray, G. A., Anal. Biochem., 1960, v1, 279.

Received November 18, 1966. P.S.E.B.M., 1967, v125.

Healing of Urinary Bladder Wounds. Uptake of S^{35}-Sulfate in Acid Mucopolysaccharides.* (32218)

FINN RASMUSSEN (Introduced by G. Asboe-Hansen)
University of Copenhagen, Department of Dermatology, with Connective Tissue Research Laboratories, Rigshospital, Copenhagen, Denmark

Wound healing processes involve biochemical and morphological changes usually in the connective-tissue elements of the tissue. The importance of collagenous fibers for tensile strength is well-documented. The fibers and cells are embedded in the amorphous ground substance, containing acid mucopolysaccharides, electrolytes, hormones, and many other substances. The acid mucopolysaccharides are polysaccharides containing hexosamine and uronic acid. They are present as macromolecules, linked to proteins. Even if the exact mode of action is unknown, they undoubtedly participate in biologic processes such as inflammation, tissue repair and ageing.

The aim of this study was to examine the synthesis of sulfomucopolysaccharides in healing linear wounds in the urinary bladder of rabbits, by measuring the uptake of $S^{35}O_4$. The results of biochemical and morphological studies on mucopolysaccharides and collagen in the same wounds have previously been reported(1,2).

Material and methods. Fifty-five male albino rabbits weighing about 2.5 kg were kept on an adequate laboratory diet for at least one week before operation. Five animals were not operated, but were killed at the time of operation and served as controls. The animals were anesthetized by intravenous injection of 60 mg Nembutal® supplemented by ether inhalation. The abdomen was opened through a low midline incision. The urinary bladder was emptied by puncture and aspiration. An incision through all layers was made in the midline of the anterior wall from the top to the neck. The bladder was closed in 2 layers with continuous sutures of 4-0 nontraumatic silk. In the first layer the suture was passed through the entire bladder wall near the wound edge. The second suture was seroserous. The abdominal wall was closed in 2 layers with silk sutures.

The operated animals were divided into 11 groups, sacrificed 2 to 28 days after the operation (Table I). They were killed by intravenous injection of 300 mg nembutal. Forty-eight hours before sacrifice the rabbits were given an intraperitoneal injection of 1.5 mCi $S^{35}O_4$ in aqueous solution. The original carrier free S^{35}-labelled sodium sulfate obtained from the Radiochemical Centre, Amersham, England, was diluted by adding 0.04% sodium sulfate to a final activity of 1 mCi per ml. The wounds were removed, and, after drying and defatting, the tissue was homogenized in 0.5 N NaOH. The uptake of $S^{35}O_4$ was determined by the method of Moltke(3) as modified by Marckmann(4). After wet ashing of 2.5 ml homogenate with fuming nitric acid in a sand bath, the $S^{35}O_4$ was precipitated as barium sulfate. The samples were counted at infinite layer thickness with a Geiger-Müller tube to a statistical error below 3%.

In 2 animals the ureters were ligated through an abdominal incision before the injection of radiosulfate. The animals were

* Supported by grants from Fonden til lægevidenskabens Fremme, Købmand i Odense Johann og Hanne Weimann f. Seedorffs legat and Reinholdt W. Jorck og Hustrus Fond.