6. Frawley, T. F., Thorn, G. W., Proc. 2nd Clin. ACTH Conf., Blakiston Co., Philadelphia, 1951, v1, 115.

7. Andreyev, L., Pugsley, L. I., J. Physiol., 1933, v80, 96.

8. Chauncey, H. H., Levine, D. M., Kass, G., Shwachman, H., Henriques, B. L., Kulczyeki, L. L., Arch. Oral Biol., 1962, v7, 707.

9. Eddy, W. A., Heft, H. L., Rosenstock, S., Ralston, R., J. Dent. Res., 1933, v13, 511.

10. Bates, J. F., Arch. Oral Biol., 1960, v3, 1.

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An Effect of Growth Hormone on Hexose Phosphate Metabolism.* (30610)

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In the course of studies on the effect of pituitary growth hormone (GH) on the intermediary metabolism of carbohydrate(1,2,3), its influence on the concentration of hexose-6-phosphate in muscle was examined. An increase in hexose-6-phosphate has been shown to occur in muscle after administration of epinephrine(4,5,6) and cortisol(7), and also in starvation and in diabetes(4,8,9). Recently, an accumulation of hexose-6-phosphate compounds has been found in the heart muscle of hypophysectomized diabetic rats treated with bovine growth hormone (BGH) and cortisol, while BGH alone did not induce any significant changes in these metabolites(7).

Levels of glucose-6- and fructose-6-phosphate were determined in the skeletal muscle of 4 groups of rats. These groups included normal animals, normal animals receiving GH, hypophysectomized rats, and hypophysectomized rats receiving GH. The rats were given an intraperitoneal glucose load, in the fasting state, to provide an adequate amount of carbohydrate. This procedure appears indispensable for detection of an increase in hexose-6-phosphate. Since preliminary experiments in the laboratory(10) have shown that the highest concentration of hexose-6-phosphates occurred one hour after the glucose injection, the 1-hour level was used.

Materials and methods. Normal female rats weighing 200 g, and hypophysectomized female rats weighing 150 g, were fasted for 18 hours and injected with 0.5 g of glucose per 100 g of body weight, in the form of 10% glucose in water. One hour later the rats were killed by a blow on the head, and 1 g of leg muscle tissue was rapidly removed and frozen in a Wollenberger clamp cooled in liquid nitrogen(11). The muscle was pulverized in a chilled mortar and extracted with perchloric acid, after which the supernatant solution was neutralized to pH 7.4(4). Glucose-6-phosphate was determined by adding glucose-6-phosphate dehydrogenase to the assay mixture(12) and by measuring the change in optical density at 340 mμ. Fructose-6-phosphate was assayed by estimating the increase in absorbance after addition of phosphoglucose-isomerase(12). Other groups of normal and hypophysectomized rats were injected intramuscularly with GH† (1 mg/100 g) for 4 days (the last injection 6 hours before death) after which glucose-6-phosphate and fructose-6-phosphate were assayed in the same manner.

Results and discussion. The results of the tests show a significant elevation of the glucose-6-phosphate level, one hour after glucose injection, in the skeletal muscle of normal rats after GH treatment, when compared with the glucose-6-phosphate content of the muscle of non-treated controls. The differences in the concentration of glucose-6-phosphate were consistent and striking, and the

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† Bovine GH was obtained from the Endocrinology Study Section of N.I.H., Bethesda, Md.

TABLE I. Glucose- and Fructose-6-Phosphate in Rat Muscle, With and Without Growth Hormone (GH).

Animal (rats)	No.	Treatment	Glucose-6-phosphate		Fructose-6-phosphate	
			Concentration in μmoles/1 g wet muscle			
1. Normal	12	None	1.11	.22*	.16	.04*
2. "	13	Glucose load	1.13	.31	.20	.06
3. "	10	Glucose load plus GH	2.05	.26	.27	.06
4. Hypophysectomized	10	None	.96	.19	.12	.02
5. "	8	Glucose load	1.19	.15	.30	.05
6. "	8	Glucose load plus GH	1.67	.42	.30	.04

* Standard deviation of the mean.

P value was less than 0.05. Hypophysectomized rats demonstrated the same changes but to a lesser degree. Elevations of fructose-6-phosphate were also noted, but were less impressive and not statistically significant (Table I).

These findings are in agreement with the general concept of the effect of GH on glucose metabolism. It is known that GH, after a short initial period of increase in glucose utilization, induces an inhibition of glucose uptake by muscle as well as an impaired glucose tolerance and a resistance to the hypoglycemic action of insulin. These facts can readily be explained by an increase in intracellular glucose-6-phosphate which depresses the phosphorylation of glucose by hexokinase within the cell(13). The resulting accumulation of intracellular glucose(4, 15) probably impairs the uptake of blood glucose by the tissue, although all the necessary factors for the transport of sugar through the cell membrane (insulin, enzymes and coenzymes) are readily available.

The process which leads to the accumulation of hexose-6-phosphates under the influence of BGH, as well as after epinephrine or cortisol treatment, has not yet been firmly established. An increase in the glucose- and fructose-6-phosphate concentration in muscle may result from the inhibition of phosphofructokinase (PFK) activity(4,14,16). It has been shown that such a block at the PFK level can be induced by perfusing the isolated rat heart with fatty acids, ketone bodies or pyruvate. The respiration of these substances may lead to accumulation of Krebs cycle intermediates, such as citrate which is an inhibitor of PFK activity. An increase in the citrate concentration has been observed in diabetes(17) and after epinephrine(18) and GH(19,20) administration. Each of the latter 3 conditions is characterized by the mobilization of free fatty acids.

Summary. Glucose- and fructose-6-phosphate were assayed in the skeletal muscle of normal and hypophysectomized female rats, with and without growth hormone treatment, one hour after a glucose injection. The results show a statistically significant increase in the glucose-6-phosphate content of the muscle of rats receiving GH when compared to glucose-6-phosphate levels in controls, as well as less striking increases in fructose-6-phosphate levels.

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1. Weil, R., Altszuler, N., Kessler, J., Am. J. Physiol., 1961, v201, 251.

2. Ho, P. P., Weil, R., J. Mt. Sinai Hosp., 1963, v30, 528.

3. Weil, R., Ho, P. P., Altszuler, N., Am. J. Physiol., 1965, v208, 887.

4. Newsholme, E. A., Randle, P. J., Biochem., 1961, v80, 655.

5. Kipnis, D. M., Helmreich, E., Cori, C. F., J. Biol. Chem., 1959, v234, 165.

6. Ellis, S., McGill, J., Anderson, H. L., Jr., Fed. Proc., 1957, v16, 294.

7. Regen, D. M., Davis, W. W., Morgan, H. E., Park, C. R., J. Biol. Chem., 1964, v239, 43.

8. Regen, D. M., Davis, W. W., Morgan, H. E., Fed. Proc., 1961, v20, 83.

9. Morgan, H. E., Cadenas, E., Regen, D. M.,

Park, C. R., J. Biol. Chem., 1961, v236, 262.

10. Weil, R., unpublished data.

11. Wollenberger, A., Ristau, O., Schoffa, G., Pflügers Arch. ges. Physiol., 1960, v270, 399.

12. Bergmeyer, H. U., Editor, Methods of Enzymatic Analysis, 1963, p134.

13. Randle, P. J., Ann. Rev. Physiol., 1963, v25, 291.

14. Newsholme, E. A., Randle, P. J., Manchester, K. L., Nature, 1962, v193, 270.

15. Garland, P. B., Randle, P. J., ibid., 1963, v199, 381.

16. Garland, P. B., Randle, P. J., Newsholme, E. A., Nature, 1963, v200, 169.

17. Parmeggiani, A., Bowman, R. H., Biochem. & Biophys. Res. Comm., 1963, v12, 268.

18. Ellis, S., Pharmacol. Rev., 1956, v8, 485.

19. Ikkos, D., Luft, R., Gemzell, C. A., Almquist, S., Acta Endocrinol., 1962, v39, 547.

20. Henneman, D. H., Henneman, P. H., J. Clin. Invest., 1960, v39, 1239.

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Collagenolytic Activity During Active Bone Resorption in Tissue Culture.* (30611)

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Previous work has demonstrated that the resorption of bone in tissue culture(1,2) is accompanied by degradation of bone collagen (3,4). Based on the nature of the degradation products and the specificity of the enzymes present in the tissue culture medium, it was proposed that an enzyme (or enzymes) was liberated during bone resorption which possessed collagenolytic activity(4). A collagenolytic factor has been recently shown to be present in the bones of animals previously given parathyroid extract(5). In the present study, purified, reconstituted H^3-proline- and H^3-hydroxyproline-labeled collagen fibrils were added to an *in vitro* culture system. These experiments provided direct evidence that a collagenolytic factor is released during active bone resorption.

Experimental. Preparation of H^3-hydroxyproline and H^3-proline-labeled guinea pig skin collagen. Guinea pigs weighing approximately 150 g to 175 g were injected intraperitoneally with 3 millicuries of chromatographically pure H^3-proline after they had shown a consistent weight gain for 3-4 days. A second injection of 3 millicuries of H^3-proline was administered intraperitoneally 3 hours later and the animals were sacrificed 9 hours after the first injection. The skins were removed in the cold room (2°C) and kept cold during mincing. They were extracted with cold, 1 M NaCl solution, 0.05 M Tris, pH 7.4 (15 ml/g wet weight of tissue) 48 hours. The supernatant was separated from the skin residue by filtration through several layers of cheese cloth, filtered through medium- and then fine-sintered glass filters and completely clarified by centrifugation at 30,000 rpm for 3 hours in a Spinco Model L Ultracentrifuge. The clear, cold viscous supernatant was then acidified to pH 3.0 with acetic acid which resulted in a flocculent precipitate. This was centrifuged at low speed, resuspended in 0.3 M acetic acid and dialyzed at 2°C against 0.3 M acetic acid. The solution was clarified by ultracentrifugation as described and the clear supernatant made to a final concentration of 5% NaCl by the slow addition of concentrated NaCl solution. The resultant flocculent precipitate was harvested by low speed centrifugation, resuspended in 0.3 M acetic

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