Effect of Hypophysectomy and Growth Hormone on Glucose Uptake by Rat Epididymal Fat Tissue.* (26729)

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The effect of hypophysectomy and of growth hormone on carbohydrate utilization has been the subject of numerous studies, both *in vivo* and *in vitro*. A critical analysis of such investigations appeared recently (1,2). The *in vitro* studies, performed mainly on the cut rat diaphragm, gave conflicting results in that some workers reported that diaphragms from hypophysectomized rats took up more glucose than normal diaphragms(3-5), while others found a diminished(5) or normal(6) uptake. More recently, it has been reported that the uncut "intact" diaphragms(7) or perfused hearts (8) of hypophysectomized rats took up less glucose than the respective tissues of normal rats.

The present studies have extended these investigations to rat epididymal fat tissue. This tissue is very active metabolically and is sensitive to insulin(9). The glucose uptake by epididymal fat pads excised from normal, hypophysectomized and growth hormone-treated hypophysectomized rats was determined. In addition, the effect of serum from these animals on glucose uptake was determined in an attempt to correlate it with the changes in glucose uptake by the excised tissues.

Materials and methods. Epididymal fat tissue was obtained from normal and hypophysectomized male Sprague-Dawley rats (purchased from Hormone Assay Laboratories, Inc.), weighing 150-225 g and fed *ad lib*. The hypophysectomized animals were used 7-21 days following operation. Animals receiving growth hormone were injected with 30 μg of bovine growth hormone, i.p., (Somar A, Lot R50109; gift of Endocrinology Study Section, Nat. Inst. of Health) 24 hr prior to sacrifice. The animals were decapitated and their blood was collected and allowed to clot. Serum was removed, pooled, and frozen for later use. Each epididymal fat pad was removed quickly, cut into 2 or 3 sections weighing 50-100 mg, and put into preweighted vials containing 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) with a glucose concentration of 100 mg %. Vials were immediately reweighed to determine tissue weight. They were then incubated for 2 hr at 37°C with constant shaking. A control vial, containing the incubation medium, but no fat tissue, was also incubated. At the end of incubation, the vials were placed in an ice bath to terminate further glucose uptake. The glucose concentration of each sample was determined in aliquots of Somogyi zinc-barium filtrate(10), by the method of Nelson(11). Glucose uptake is expressed as μg glucose disappearing/g tissue/hr incubation time. The effect of serum on glucose uptake was measured by incubating epididymal fat tissue from normal rats with the test serum and comparing glucose uptake from the serum with glucose taken up from the glucose-buffer solution. Each serum sample was assayed against a control glucose-buffer sample using fat tissue from the same animal.

Results. The effect of hypophysectomy and growth hormone treatment on glucose uptake by rat epididymal adipose tissue is shown in Fig. 1. Glucose uptake by adipose tissue from the hypophysectomized animals was 733 $\pm$ 39 μg/g/hr. This is a significant ($P = <0.01$) decrease from the glucose uptake by normal tissue which is 1165 $\pm$ 88 μg/g/hr. A single injection of growth hormone (30 μg) in hypophysectomized animals, 24 hr prior to sacrifice, increased significantly ($P = <0.01$) the glucose uptake by the excised fat pads to 970 $\pm$ 60 μg/g/hr.

The effect of serum from hypophysectomized and growth hormone-treated hypophysectomized rats on glucose uptake by normal

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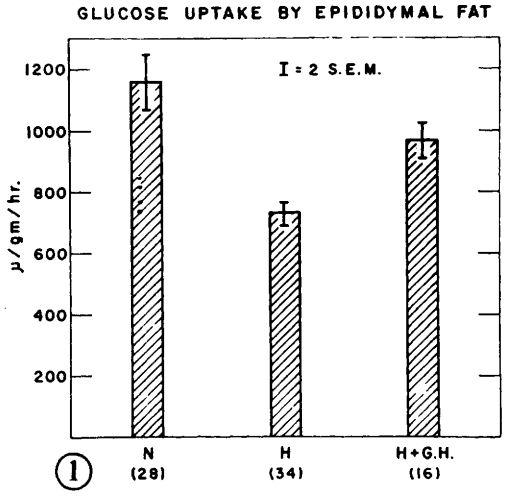

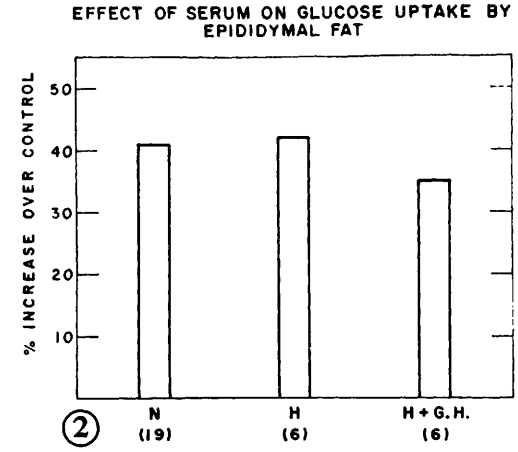

FIG. 1. Glucose uptake by epididymal fat tissue of normal (N), hypophysectomized (H), and growth hormone-treated hypophysectomized (H + G.H.) rats. Uptake is expressed as μg glucose disappearing/g adipose tissue/hr incubation. Numbers in parentheses represent No. of experiments. S.E.M. = Stand. error of the mean.

FIG. 2. Effect of sera from normal (NS), hypophysectomized (HS), and growth hormone-treated hypophysectomized (HS + G.H.) rats on glucose uptake by normal epididymal fat tissues. Activity is expressed as % increase in glucose uptake above that observed in glucose-buffer medium. Numbers in parentheses represent No. of experiments.

fat tissue is shown in Fig. 2. Values are expressed as % increase in glucose uptake over that obtained with normal fat pads incubated in glucose-buffer media. Glucose uptake in the latter was 1025 ± 94 μg/g/hr. It is evident that the sera from normal animals increased glucose uptake by 41% above control level. The sera from hypophysectomized rats, fed *ad lib.*, increased glucose uptake to the same extent as those from normal rats. Likewise, the single injection of growth hormone did not influence the effect of sera on glucose uptake.

Discussion. The observed diminished glucose uptake by the fat tissue of the hypophysectomized rat agrees with the recent findings that the excised "intact" diaphragm(7) and the perfused heart(8) of the hypophysectomized rat take up less glucose than the respective tissues of normal rats. It is also in harmony with the *in vivo* findings of Steele *et al.*(12) that in the hypophysectomized dog in the steady postabsorptive state, rate of endogenous glucose removal from the plasma is lower than in the normal dog.

The decreased uptake of glucose by the tissues of the hypophysectomized animal cannot be ascribed at present to a deficiency of any one specific hormone. The characteristic atrophy of the thyroid glands found in these animals, results in an over-all decrease in metabolism, which may contribute to the observed decrease in glucose uptake by the excised adipose tissue. It is also probable that the decreased uptake of glucose reflects a compensatory decrease in insulin secretion. Supporting this possibility is the demonstration of atrophy(13) and decrease in size of the Golgi bodies(14) of the pancreatic beta cells of hypophysectomized rats.

The present finding that administration of growth hormone to the hypophysectomized rat increases the low glucose uptake of the excised epididymal fat tissue is in agreement with the *in vivo* observation by Altszuler *et* al.(15,16) that in the hypophysectomized dog a growth hormone regimen increases rate of uptake of plasma glucose by the tissues, concomitant with an increase in hepatic glucose production. The increased uptake of glucose by the tissue of these growth hormone-treated animals may be due to increased insulin secretion. There is substantial evidence that growth hormone stimulates secretion of insulin (for references see 17). However, growth hormone administration was shown also to lower blood glucose concentration in acutely depancreatized dogs (18) and hypophysectomized-eviscerated

rats(19). Also, addition of growth hormone *in vitro* increases glucose uptake by the diaphragm from normal(20) and hypophysectomized(19) rats and by epididymal fat tissue from normal rats(21).

The present studies reveal that sera from hypophysectomized rats increase glucose uptake by normal epididymal tissue to the same extent as sera from normal rats. Since the ability of serum to increase glucose uptake by fat tissue has been utilized as an index of "insulin-like" activity(22) it would appear that sera of hypophysectomized animals have the same activity as sera from normal animals. This finding is at variance with that of Randle(23), who reported that in a majority of his hypophysectomized rats no insulin activity could be detected in the plasma. The absence of insulin activity is not readily explainable, since his rats were not fasted and insulin was presumably being secreted. In view of the lack of specificity of the aforementioned method of insulin assay, it is feasible that extirpation of the pituitary gland may remove or reduce the effectiveness of contra-insulin agents, thereby giving the appearance of a normal insulin-like activity, even though the actual amount of insulin in the serum may be reduced.

The lack of change in the effectiveness of serum to increase glucose uptake observed at 24 hr following injection of growth hormone agrees with findings of Randle(23). This does not necessarily dispute the possibility that growth hormone increased secretion of insulin, and that this was responsible for the increased uptake by excised fat tissue.. Since 90% of the injected insulin is cleared from the serum within 20 minutes(24,25) and is no longer detectable within 60 minutes(25), it is conceivable that any insulin released as a result of the growth hormone injection may have been cleared from the serum in the 24-hr period following the injection.

Summary. The epididymal fat tissue from hypophysectomized rats took up a significantly smaller amount of glucose than did the normal tissue. A single injection of bovine growth hormone into hypophysectomized rats increased glucose uptake by epidymal fat tissue excised 24 hr later. Sera from hypophysectomized and growth hormone-treated hypophysectomized rats increased glucose uptake by normal fat tissue to the same extent as sera from normal animals.

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1. de Bodo, R. C., Altszuler, N., *Vitamins and Hormones,* 1957, v15, 205.

2. ———, *Physiol. Rev.,* 1958, v38, 389.

3. Krahl, M. E., *Ann. N. Y. Acad. Sci.,* 1951, v54, 649.

4. Krahl, M. E., Park, C. R., *J. Biol. Chem.,* 1948, v174, 939.

5. Perlmutter, M., Greep, R. O., *ibid.,* 1948, v174, 915.

6. Li, C. H., Kalman, C., Evans, H. M., *Arch. Biochem.,* 1949, v22, 357.

7. Kipnis, D. M., *Ann. N. Y. Acad. Sci.,* 1959, v82, 354.

8. Morgan, H. E., Henderson, M. J., Regen, D. M., Park, C. R., *ibid.,* 1959, v82, 387.

9. Wertheimer, E., Shapiro, B., *Physiol. Rev.,* 1948, v28, 451.

10. Somogyi, M. J., *J. Biol. Chem.,* 1945, v160, 69.

11. Nelson, N., *ibid.,* 1944, v153, 375.

12. Steele, R., Wall, J. S., de Bodo, R. C., Altszuler, N., *Am. J. Physiol.,* 1956, v187, 25.

13. Kinash, B., Haist, R. E., *ibid.,* 1954, v178, 441.

14. Batts, A., *Ann. N. Y. Acad. Sci.,* 1959, v82, 302.

15. Altszuler, N., Steele, R., Wall, J. S., de Bodo, R. C., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 744.

16. Altszuler, N., Steele, R., Wall, J. S., Dunn, A., de Bodo, R. C., *Am. J. Physiol.,* 1959, v196, 121.

17. Campbell, J., *Hypophyseal Growth Hormone, Nature and Actions.* (Smith, Gaebler and Long, Eds.) McGraw-Hill Book Co., Inc., N. Y., 1955, p270.

18. Kurtz, M., de Bodo, R. C., Kiang, S. P., Ancowitz, A., *Am. J. Physiol.,* 1950, v163, 727.

19. Park, C. R., Brown, D. H., Cornblath, M., Daughaday, W. H., Krahl, M. E., *J. Biol. Chem.,* 1952, v197, 151.

20. Ottaway, J. H., *Nature,* 1951, v167, 1064.

21. Winegrad, A. I., Shaw, W. N., Lukens, F. D. W., Stadie, W. C., Renold, A. E., *J. Biol. Chem.,* 1959, v234, 1922.

22. Martin, D. B., Renold, A. E., Dagenais, Y. M., *Lancet,* 1958, v2, 76.

23. Randle, P. J., *Hypophyseal Growth Hormone, Nature and Actions,* (Smith, Gaebler and Long, Eds.) McGraw-Hill Book Co., Inc., N. Y., 1955, p413.

24. Elgee, N. J., Williams, R. H., Lee, N. D., *J. Clin. Invest.*, 1954, v33, 1252.
25. Grodsky, G. M., Forsham, P. H., *ibid.*, 1960, v39, 1070.

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Relation of Sialic Acid to Rh_0 (D) Antigen. (26730)

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Dodd *et al.*(1) reported specific inhibition of Rh_0 (D) antibody by sialic acids. We proposed to examine this inhibition using various neuraminic acid derivatives with Rh_0 (D) saline antibodies and to investigate the effect of neuraminidase, an enzyme which will remove sialic acid from erythrocytes, on agglutination of the cells by specific antiserum.

Materials and methods. N-acetylneuraminic acid (NANA) was prepared from human blood plasma according to Martensson *et al.*(2). Crystallization from glacial acetic acid yielded crystals with a melting point of 184-185°C. No glycolic acid could be detected by the method of Klenk and Uhlenbruck(3). The material moved as a single spot when analyzed by paper chromatography in the following solvents: butanol: acetic acid:water (4:1:5). Rf 0.32; butanol: propanol:0.1 N HCl (1:2:1). Rf 0.45. *Crude bovine sialic acid* (I) was prepared from mucin of bovine submaxillary glands according to Blix *et al.*(4). The dried mucin was suspended in water, sialic acid was liberated by acid hydrolysis and isolated by ion exchange chromatography(5). This material assayed 74% NANA by the resorcinol reaction(6). When examined by paper chromatography, 3 components reacting as sialic acid were observed. A small amount of ninhydrin-positive material was also present. The fastest-moving sialic acid component was identified as N,O-diacetylneuraminic acid by the fact that on further acid treatment it was converted to NANA and also gave a hydroxamic acid test for esters. According to glycolic acid analysis, the material contained 14% N-glycolylneuraminic acid (NGNA). *Crystalline bovine sialic acid* was prepared from crude bovine sialic acid (I) by crystallizing it twice from methanol-ether and once from glacial acetic acid. This material had a melting point of 186-187°C. According to glycolic acid analysis the material contained 16% NGNA. Two distinct spots were observed when the crystals were analyzed by paper chromatography in butanol: propanol:0.1 N HCl. The two spots were identified as NANA, Rf 0.45, and NGNA, Rf 0.34. *Crude bovine sialic acid*(II) was prepared by dissolving bovine submaxillary mucin in water and heating at 100°C for 30 minutes. The liberated sialic acid was isolated by methanol extraction(4). This material contained 61% NANA and 9% NGNA. It showed 5 components reacting as sialic acid when analyzed by paper chromatography. Three components were identified as NANA, NGNA, and N,O-diacetylneuraminic acid. The other 2 components are reported by Blix(7) to be triacetylneuraminic acid and an isomerization product. *Porcine sialic acid* was isolated from porcine submaxillary mucin according to Blix(6). The material contained 63% NANA and 45% NGNA. It showed NANA and NGNA spots by paper chromatography. *Human brain gangliosides* were isolated from acetone powders of fresh human grey matter by extraction with methanol-chloroform (2:1), solvent distribution according to Folch *et al.*(8), dialysis and finally silicic acid chromatography. The final product contained 23% NANA, 31% hexose (as galactose), 5.5% hexosa-

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