Insulin Stimulated Transport of 3-*O*- Methyl Glucose Across the Rat Jejunum* (33690)

REUBEN S. DUBOIS AND CLAUDE C. ROY
(Introduced by H. Claman)
Department of Pediatrics, University of Colorado Medical Center, Denver, Colorado

The intestinal transport of hexose is increased in both alloxan (1–7) and clinical diabetes (8). Although insulin was reported to stimulate the transport of glucose in intestinal loops (3, 6), insulin is generally believed not to affect the transport of glucose in the intestinal epithelial cells, and therefore, the intestine was classified as "insulin insensitive" (1, 2, 4, 5, 7–10).

Using a new *in vitro* technique for the extracorporeal perfusion of isolated rat jejunal segments with an intact circulation (11), the lack of insulin effect was confirmed in both normal and alloxanized animals (12). However, in rats made diabetic with anti-insulin serum (AIS), the absorption of 3-*O*-methyl-D-glucose (3-O-MG) responded to insulin.

Methods. Induction of the diabetic state. Male Sprague-Dawley rats weighing 250–350 g were made diabetic with either alloxan 50 mg/kg i.v. after a 48-hr fast or with AIS i.v. The guinea pig AIS (Burroughs Wellcome & Co.) was given in amounts titrated (13) to neutralize 2 units of insulin. Four of the 14 control animals were given 2 ml of normal guinea pig serum (Colorado Serum Co.) i.v. Fasting blood sugars in the unanesthetized state varied between 74–90 mg/100 ml in the controls, 250–900 mg/ml in the alloxanized group and 105–180 mg/100 ml in the AIS-treated animals.

Perfusion of intestinal segments. All animals were fasted 24 hr prior to surgery. The alloxanized animals were used at least 72 hr after the induction of diabetes, while the AIS-treated rats were used in less than 18 hr. Under sodium pentobarbital anesthesia (4.3 mg/100 g of body wt. i.p.) a 20-cm segment of rat upper jejunum was completely removed from the animal and perfused extracorporeally through the superior mesenteric artery. The blood perfusate consisted of 20% washed bovine red cells suspended in a physiological electrolyte solution with 2.5% dextran (mol. wt. 78,000), 2.5% bovine serum albumin, 100 mg/100 ml of D-glucose and 100 mg/100 ml 3-O-MG. It was oxygenated by means of a rotating mesh screen using humidified 95% oxygen and 5% carbon dioxide, and circulated at a rate of 3 ml/min by a servo-controlled constant flow roller type pump with an asymmetrical head (see Fig. 1). The portal vein was cannulated and the portal venous effluent was collected in 1-min aliquots. The lumen of the intestinal segment was perfused at 1 ml/min with 100 mg/100 ml of 3-O-MG, made isotonic with saline, and to which 2 μC% of 3-*O*-methyl-D-glucose—^{14}C (New England Nuclear Corp. 5.3 mCi/mmole) were added.

Perfusion characteristics and calculation of absorption. Once placed in the tissue chamber at 37°, the intestinal preparation was perfused extracorporeally for about 5 min before the constant infusion of luminal substrate was started. The concentration of substrate in the venous effluent rose promptly and was coupled with a pronounced decrease in the luminal effluent concentration of 3-O-MG. This accumulative tissular and vascular phase lasted an average of 8–10 min and was followed by a steady rate of substrate influx into the venous effluent and of substrate disappearance from the lumen. At that stage, an intra-arterial infusion of saline at 0.5 ml/min was begun and continued for a period of 12 min. It was followed by a second consecutive 12-min period of perfusion during which insulin (10 mU/ml beef–pork commercial mixture) was infused intra-arterially at 0.5 ml/min.

Plasma ^{14}C was assayed in a Packard No.

* Presented in part at the Am. Gastroenterol. Assoc. Meeting, Philadelphia, May 1968.

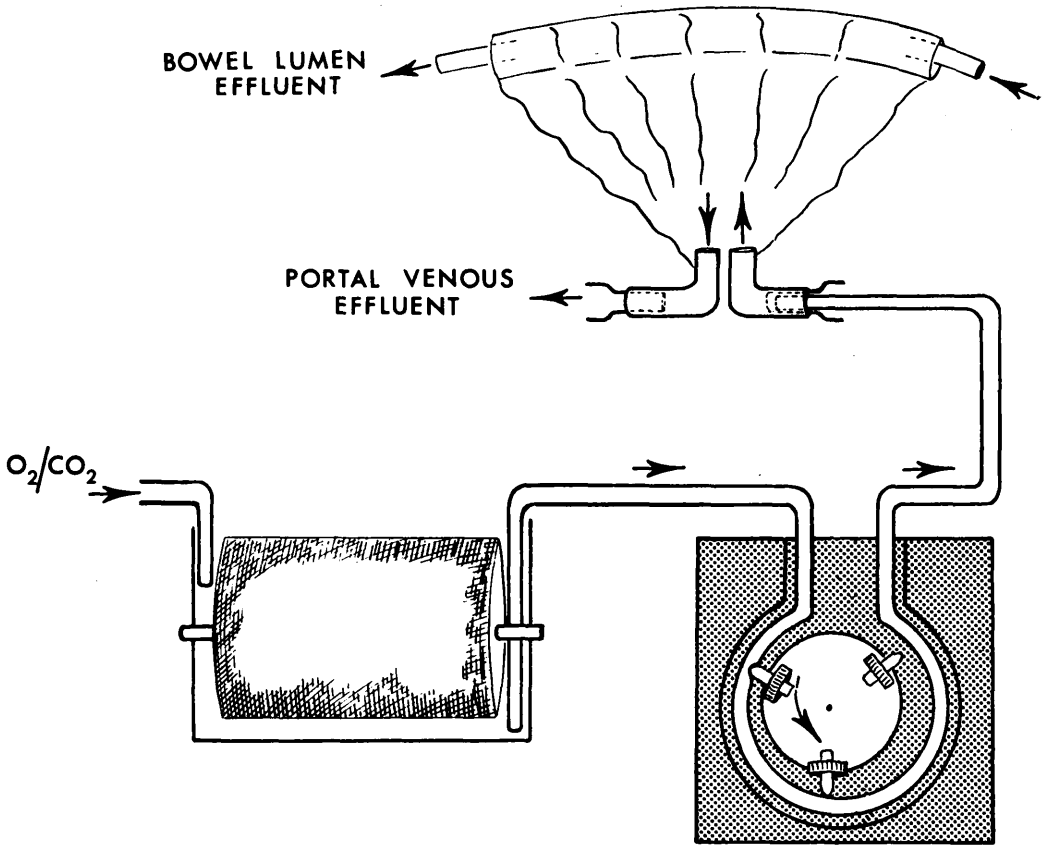

FIG. 1. Schematic diagram of perfusion apparatus and isolated segment of small bowel.

300 Tricarb liquid scintillation spectrometer. The percentage minute absorption was calculated by dividing the product of the substrate concentration in the portal venous effluent times the minute volume by the mean luminal substrate concentration. The latter was considered equal to the average of the substrate concentrations in the proximal and distal intestinal catheters.

Results. The percentage minute absorption of 3-O-MG before and during the insulin infusion did not vary significantly between the four animals treated with normal guinea pig serum and the 10 uninjected rats; therefore, these animals were pooled into one control group.

The effect of experimental diabetes on the percentage minute absorption of 3-O-MG in the 3 groups is compared in Table I. It is readily apparent from the values shown that there are wide variations from animal to animal within each group. Nevertheless, the absorption seen in the alloxan-diabetes group is significantly increased over both the control and AIS group.

As shown in Table II, the effect of intra-arterial infusion of insulin (5 mU/min) on the mean percentage minute absorption of 3-O-MG in both control and alloxanized animals is negligible. On the other hand, insulin appears to stimulate the transport of substrate in the AIS group. It is interesting to

TABLE I. The Effect of Diabetes on the Percentage Minute Absorption of 3-O-CH_3-glucose.

	No.	$\bar{X} \pm$ SEM	p^a	
Controls	14	6.5 ± 0.8		
Alloxan	5	9.6 ± 0.9	<.05	NS
AIS	10	4.6 ± 0.5	<.05	

[a] Mann-Whitney U non-parametric test.

TABLE II. The Effect of Insulin on the Percentage Minute Absorption of 3-O-CH_3-glucose.

	No.	Saline ($\bar{X} \pm$ SEM)	Insulin ($\bar{X} \pm$ SEM)	p[a]
Controls	14	6.5 ± 0.8	6.3 ± 0.8	NS
Alloxan	5	9.6 ± 0.9	9.6 ± 1.3	NS
AIS	10	4.6 ± 0.5	6.6 ± 0.8	<0.001

[a] t test on paired observations.

note that the apparent, but statistically not significant, decrease in 3-O-MG transport in these animals is brought back to the levels observed in the control group by insulin.

Discussion. These perfusion experiments with 3-O-MG, an actively transported monosaccharide with an absorption rate close to D-glucose (14), confirm a recent report (7) and are in accord with the increased uptake of the glucose analog, 6 deoxy-D-glucose (4) observed in intestinal preparations from alloxan diabetic rats. Since 3-O-MG and 6 deoxy-D-glucose are not metabolized (15, 16), a possible interpretation is that the alloxan-induced diabetic state leads to an anatomical modification of the intestinal mucosa (17) or to a functional alteration characterized by an increased rate of transport (4). It was suggested that the increased absorption of hexose in alloxan diabetes is coupled with an increased absorption of sodium (6). It is unlikely that hypoinsulinism per se leads to the increased transport of 3-O-MG across the alloxanized rat intestine since insulin failed to modify absorption in our alloxanized group.

The insulin deficiency syndrome produced by neutralizing endogenously secreted insulin with AIS (18) did not alter the transport of glucose in everted gut sacs from hamsters studied within 18 hr of the induction of the diabetic state (19). Similarly, the present report fails to show a statistical difference between the mean percentage minute absorption of 3-O-MG in AIS-treated and control animals. However, intra-arterial insulin did stimulate transport in the former group, while it had no effect in the latter. The recent demonstration of an insulin effect on the utilization of glucose by kidney slices from alloxanized animals prompted the suggestion that the general concept of tissue sensitivity to insulin be reexamined (20). The present study dissociates transport from utilization by the use of 3-O-MG and suggests that, in a specific experimental condition of insulin deprivation, insulin may have an effect on intestinal transport.

Summary. The transport of 3-*O*-methyl-D-glucose was studied in 20-cm segments of upper jejunum removed with an intact circulation from the animal and perfused extracorporeally. Intestinal preparations from alloxanized animals had a significantly higher percentage minute absorption of the substrate. Insulin failed to modify the transport rate in both the control and the alloxan-treated animals. However, insulin stimulation of transport was observed in experiments carried out in a group of rats where diabetes was induced by anti-insulin serum.

This work was supported by the University of Colorado Medical Center Research Grant No. 90701 and by USPH Grants HD-65 and AM-08925.

1. Pauls, F. and Drury, D. R., Am. J. Physiol. **137**, 242 (1942).

2. Laszt, L. and Vogel, H., Nature **157**, 551 (1946).

3. Lequin, H. C. and Steyne-Parve, E. P., Biochim. Biophys. Acta **58**, 439 (1962).

4. Crane, R. K., Biochem. Biophys. Res. Commun. **4**, 436 (1961).

5. Banargee, S. and Varma, S. D., Proc. Soc. Exptl. Biol. Med. **118**, 494 (1965).

6. Aulsebrook, K. A., Experientia **21**, 346 (1965).

7. Flores, P. and Schedl, H. P., Am. J. Physiol. **214**, 725 (1968).

8. Vinnik, I. E., Kern, F., and Sussman, K. E., J. Lab. Clin. Med. **66**, 131 (1965).

9. Levin, R. and Goldstein, M. S., Recent Progr. Hormone Res. **11**, 343 (1955).

10. Mehnert, H., Foster, H., and Haslbeck, G., Diabetologia **3**, 23 (1967).

11. Dubois, R. S., Vaughan, G. D., and Roy, C. C., *in* "Perfusion and Preservation of Organs" (J. C. Norman, ed.) Appleton, New York (1968).

12. Dubois, R. S. and Roy, C. C., Western Soc. Pediatric Res., Los Angeles, Abstr. **21** (1967)

13. Wright, P. H. and Malaisse, W. J., Diabetologia **2**, 178 (1966).

14. Wilson, T. H. and Landau, B. R., Am. J. Physiol. **198**, 99 (1960).

15. Crane, R. K. and Krane, S. M., Biochim. Biophys. Acta **20**, 568 (1956).

16. Csaky, T. Z. and Glenn, J. E., J. Appl. Physiol. **12**, 145 (1958).

17. Jervis, E. L. and Levin, R. J., Nature **210**, 391 (1966).

18. Wright, P. H., Am. J. Med. **31**, 892 (1961).

19. Binder, H. J., Spiro, H. M., and Spencer, R. P., Biochim. Biophys Acta **135**, 350 (1967).

20. Mahler, R. J. and Szabo, O., Proc. Soc. Exptl. Biol. Med. **125**, 879 (1967).

Received Sept. 25, 1968. P.S.E.B.M., 1969, Vol. 130.

Effect of Hydrocortisone on Age-Dependent Changes in Lipid Metabolism of Primary Human Amnion Cells *in Vitro** (33691)

GRACE C. YUAN[1] AND R. SHIHMAN CHANG[1]

Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts 02115

A previous communication described age-dependent changes in lipid metabolism of primary human amnion (PHA) cells *in vitro*. These changes were: (a) increase in incorporation of palmitate-1-^{14}C into cell lipids relative to oxidation of palmitate-1-^{14}C into $^{14}CO_2$, or conveniently referred to as an increase in lipid/CO_2 ratio, and (b) increase in incorporation of cholesterol-26-^{14}C into cell lipids (1). A subsequent publication described the prolongation of postmitotic life span of PHA cell by hydrocortisone (2). We now ask the question whether hydrocortisone would reverse the age-dependent change in lipid metabolism of PHA culture.

Materials and Method. Cell culture. The preparation and maintenance of replicate PHA cell cultures, the determination of cell density and the nutrient medium used in the feeding of the cultures and the procedure for hydrocortisone experiemnts were essentially the same as those previously described (2) except that no mycostatin was added to the medium.

Isotopes. Palmitate-1-^{14}C, oleate-1-^{14}C, and cholesterol-26-^{14}C with specific activity, respectively, of 6.5, 8.7, and 26.8 mCi/mmole were purchased from New England Nuclear Corporation. The fatty acids were added to nutrient medium as sodium salts to final concentration of 0.5 μCi/ml. Cholesterol-26-^{14}C was first dissolved in alcohol and then dispersed in nutrient medium also to 0.5 μCi/ml. The final concentration of alcohol in medium was less than 0.1% which was not toxic to PHA culture.

Incorporation experiment. This is in general similar to that described previously (1,2). Briefly, 11 days after explantation the amnion cells from one placenta were harvested and distributed to culture tubes each receiving the same number of cells. Three days later, half of the cultures were fed with media containing hydrocortisone sodium succinate at 0.1 μg/ml and the other half were fed with the same medium but without hydrocortisone. This was arbitrarily designated day zero. Cultures were fed with hydrocortisone and control media twice weekly. Starting on day 31, 2 cultures from each set were sacrificed for nuclei count (3), 2 were fed with medium containing palmitate-1-^{14}C, 2 with oleate-1-^{14}C, and 2 with cholesterol-26-^{14}C. Seventy-two hr later, CO_2 was trapped, lipids were extracted, and radioactivity was monitored by procedures described previously (1).

Results. Table I shows the effect of hydrocortisone on the uptake of oleate-1-^{14}C relative to oxidation of oleate-1-^{14}C into $^{14}CO_2$. In control cultures, age-dependent increase in the lipid/CO_2 ratio was consistently observed in all experiments. In hydrocortisone-treated cultures, however, this age-

* Supported in part by Research Grant (HD 00703) from National Institutes on Health.

[1] Present address: G. C. Yuan, Department of Nutrition and Food Sciences, MIT, Cambridge, Massachusetts; and R. S. Chang, School of Medicine, University of California, Davis, California.