Effect of Insulin on Glucose Uptake by the Uterus of the Rat, *in vitro*, During Contraction and Relaxation.* (27338)

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It is a well known clinical observation that in the diabetic patient muscular exercise increases glucose utilization and decreases insulin requirements. Ingle *et al.*(1,2) have shown that muscular exercise increases considerably the rate of entry of glucose into tissues in absence of insulin. Sacks and Smith(3) among others have also shown that muscular exercise has the same specificity with regard to the molecular structure of the sugars which may enter into the cells as that shown by insulin. However, the mechanism by which muscular exercise increases glucose uptake is unknown. Goldstein(4) has suggested the existence of a humoral factor produced during muscular contraction which would be responsible for the entry of glucose into cells.

The experiments reported here were carried out to test the validity of the assumption that glucose uptake takes place during the period of relaxation, possibly as a secondary effect thereof.

Materials and methods. Uteri were obtained from rats of our colony, weighing 120-160 g. Estrus was induced by previous injection of 500 μg of diethylstilbestrol dipropionate subcutaneously; for some experiments uteri were obtained from rats 1-3 days before the end of pregnancy. All animals were sacrificed by a blow on the head and decapitation. Each organ was divided into halves, one being used as control. For most experiments the hemiuteri were closed by tying both ends; in some instances this was not done, in which cases both the inside and outside of the muscle were bathed by the buffer. The hemiuteri were incubated in Warburg vessels at 37° in 2 ml of the appropriate buffers for 60 minutes, 60-70 cycles per minute.

For measurements during spontaneous uterine contraction Gey and Gey(5) bicarbonate buffer was used, whereas for uteri in permanent relaxation a magnesium-free low calcium buffer as described by Jalon(6) was employed. The buffers contained 3 mg glucose/ml. Insulin, when used, was added at a 0.1 U/ml dose. Tensilon, whenever used to increase the intensity and frequency of uterine contractions of estrous rats, was added at a concentration of 60 μg/ml of buffer. Tetanic contraction was produced by addition of 0.1 ml of a 1/500,000 acetylcholine solution per ml of buffer. In cases where muscular activity (periodic contraction, tetanic contraction) or relaxation were registered a standard graphic procedure was used(7). Medium glucose concentration was measured by the Somogyi-Nelson method(8,9). Glucose uptake was expressed as mg glucose disappearance per 100 mg wet weight of uterus per hr. Statistical analysis of the results was performed according to Student's paired observations method(10).

Results. The table shows a summary of the chief results obtained: glucose uptake is lower for uteri during tetanic contraction than during relaxation, the difference between means being statistically significant. The estrous uterus is sensitive to insulin 100,000 μU/ml during spontaneous contraction, whereas no difference was found for glucose uptake in presence or absence of insulin, when uteri from rats at the end of pregnancy were used. It should be pointed out that when the pregnant uterus is removed from the abdominal cavity an intense spontaneous contraction ensues on cutting, which cannot be prevented by addition of relaxing agents such as curare injected *in vivo* at near lethal doses.

Uterine muscle is sensitive to insulin both during tetanic contraction and permanent relaxation. When the intensity and frequency of the spontaneous contractions are enhanced by addition of Tensilon (3-hydroxyphenyl-

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TABLE I. Means of Glucose Uptakes, by Rat Uterine Muscle.

	mg/100 mg of wet uterus/hr of incubation, ± S.E. of mean	
Relaxation*†	.244 ±.027	
Tetanic contraction*‡	.166 ±.023	
	Insulin‖	Control
Relaxation*†	.326 ±.055	.258 ±.037
Tetanic contraction*‡	.268 ±.024	.188 ±.029
Periodic spontaneous contraction*§	.272 ±.031	.230 ±.010
Permanent contraction¶	.231 ±.014	.221 ±.013
	Tensilon**	Control
Periodic spontaneous contraction*§	.444 ±.033	.217 ±.030

* Rat uterus in estrus, from animals pre-treated with estrogens.

† Relaxation produced by incubation in hypocalcic medium.

‡ Tetanic contraction produced by action of acetylcholine chloride.

§ Periodic spontaneous contraction obtained by incubation in Gey and Gey's medium.

‖ Insulin = 100,000 μU/ml.

¶ Rat uterus, from animals at end of gestation.

** 3-hydroxyphenyl-dimethylethylammonium bromide.

dimethyl-ethylammonium bromide), glucose uptake increases considerably.

The tetanic contraction obtained by addition of acetylcholine does not represent a perfect tetanus: when muscular activity was registered graphically, the plateau obtained after addition of acetylcholine was not really a straight flat line, but consisted of oscillations indicating the existence of short and feeble phases of contraction and relaxation.

There was no difference in glucose uptake whether the hemiuteri were closed at both ends, or open so that the content and buffer could interchange.

Discussion. These data support the hypothesis that glucose enters the muscle cells during the period of relaxation: less glucose is in fact taken up by the rat uterus during the acetylcholine-induced tetanic contraction of the non-pregnant uterus than during relaxation. These findings agree with those of other authors which indicate that muscular contraction increases glucose-uptake.

It is suggested that the difference in sensitivity to insulin observed between the uteri in permanent contraction obtained from rats at the end of pregnancy and those in acetylcholine-induced tetanic contraction might be taken as indirect evidence of the conclusion stated above. This different insulin sensitivity might well be due to hormonal factors occurring at the end of pregnancy, but we feel that it might also be related to the fact that the tetanus induced by addition of acetylcholine was less perfect than that obtained with uteri from pregnant rats near delivery. It would therefore seem that the uterine muscle in almost absolute contraction (end of pregnancy) is no longer sensitive to insulin, while sensitivity to this hormone is not lost in the muscle in imperfect tetanus, which still shows short and feeble periods of relaxation.

Summary. a) Glucose uptake and insulin sensitivity have been determined *in vitro* for uteri from normal and pregnant rats (at the end of pregnancy) during periods of relaxation, spontaneous contraction, Tensilon-enhanced spontaneous contractions and acetylcholine-induced tetanic contraction. b) Though glucose was taken up by the muscle in tetanic contraction, there was greater glucose uptake of the uteri during the period of relaxation. c) Uteri during the imperfect acetylcholine-induced tetanic contraction are sensitive to insulin, whereas those in almost absolute contraction, obtained from rats at the end of pregnancy, are not. d) Glucose uptake is also increased when the intensity and frequency of the contractions of the non-pregnant uterus are stimulated with Tensilon.

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