

on the growth and behavior of this tumor are under investigation. These will be correlated with plasma ketosteroid and corticoid levels. If it could be demonstrated that chemotherapeutic agents affected growth and steroid biosynthetic capacity in a similar fashion in this tumor, the study of plasma steroid response to therapeutic agents may prove to be a useful screening technic.

Summary. A transplantable adrenal mouse carcinoma produced elevated plasma ketosteroid in the host. Non-tumor-bearing mice subjected to gonadectomy-adrenalectomy have negligible levels of plasma ketosteroid. Tumor-bearing mice have unchanged plasma levels following this procedure indicating steroid production by the tumor. ACTH and $\Delta 1-9\alpha$ fluorohydrocortisone have no influence on the plasma ketosteroid level of tumor-

bearing animals. Distant metastases were found in adrenalectomized-gonadectomized males and adrenalectomized females, but not in animals not subjected to these procedures.

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Inhibition of Glucose Uptake by Acidosis *in vitro*.^{*} (23833)

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In diabetic acidosis there is a relative insensitivity to insulin(1). Chari(2) has concluded that a high concentration of acetate is probably one of the factors causing a derangement of carbohydrate metabolism. The work of Guest *et al.*(3) shows however, that the excess hydrogen ion itself is an extremely important factor. They induced a simple ammonium chloride acidosis in dogs and demonstrated a reduced insulin sensitivity and a less favorable glucose tolerance. Guest attributes these effects to an inhibition of hexokinase at the lowered pH. Comparable experiments were conducted *in vitro* to determine whether extra-hepatic tissues were involved.

Method. Hemidiaphragms from rats weighing 150 ± 10 g were incubated at 38°C in Krebs-Ringer solutions buffered with bi-

carbonate- CO_2 at pH 7.4 and 6.8. Each solution contained 250 mg/L glucose and some insulin, and was equilibrated with 5% CO_2 in O_2 . The glucose uptake of each hemidiaphragm was determined by measuring the disappearance of glucose from 2 ml of the nutrient solution in 1 hour. The glucose determinations were by the anthrone method(4).

Results. When the insulin concentration exceeded 0.08 i.u./ml the glucose uptake was the same at pH 7.4 as at 6.8, but with decreasing insulin concentrations the muscles at the lower pH took up significantly less glucose. With 0.008 i.u./ml insulin the difference was 25%, and it was 80% when no insulin was added to the nutrient medium. The absolute glucose uptake decreases with decreasing insulin concentrations so that the percentage differences become large. Table I lists both the percentage differences and the absolute differences in g/kg/hr at each insulin concentration. The latter are shown graphically in Fig. 1. From these results it may be concluded

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TABLE I.

Units of insulin conc., i.u./ml	No. of pairs	% difference of glucose uptake	Absolute difference of glucose uptake, g/kg/hr
0	6	80.6 ± 10.9	.434 ± .081
.008	8	25.8 ± 5.0	.379 ± .073
.016	6	20.6 ± 2.6	.307 ± .038
.032	6	25.8 ± 3.9	.306 ± .043
.048	8	7.5 ± 4.5	.102 ± .063
.080	6	1.8 ± 7.1	.032 ± .129
.160	18	4.1 ± 2.9	.062 ± .122

% difference =

$$\frac{\text{uptake at pH 7.4} - \text{uptake at pH 6.8}}{\text{uptake at pH 7.4}} \times 100.$$

that acidosis will inhibit glucose uptake in the rat diaphragm and that the effect of the acidosis can be overcome by massive increases in the insulin concentration.

At an insulin concentration of 0.008 i.u./ml there is a suitably consistent inhibition of glucose uptake at a lower pH so that the effect of various degrees of acidosis can be studied. The difference of glucose uptake between paired muscles incubated at pH 7.4 and 7.1 was almost as great as that between pH 7.4 and 6.8. There was no enhancement

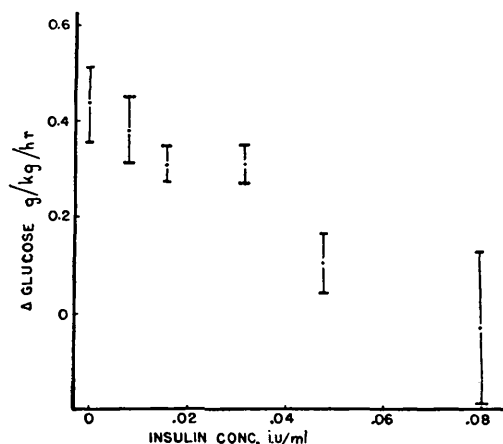


FIG. 1. Inhibition of glucose uptake at pH 6.8 is expressed as the difference between uptake at pH 7.4 and 6.8. This is plotted against insulin conc.

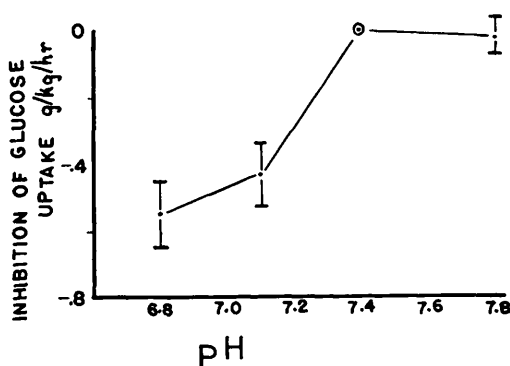


FIG. 2. Effect of pH changes on glucose uptake. Insulin conc. is constant at 0.008 i.u./ml.

of glucose uptake by raising the pH to 7.8 (Fig. 2). At this same insulin concentration, the difference of glucose uptake was the same whether the pH was lowered to 6.8 by the addition of hydrochloric acid or of acetic acid. If it is presumed that the acetate ion penetrates the cell more readily than does the chloride ion, then the similarity of effect on glucose uptake of these two acids suggests that the acidosis affects a surface process and not an intracellular process.

Summary. Glucose uptakes of rat diaphragms *in vitro* were compared at pH 7.4 and 6.8. The acid medium inhibited glucose uptake but the degree of inhibition could be decreased by adding insulin to both media. At insulin concentrations in excess of 0.08 i.u./ml, glucose uptake was the same at each pH. With 0.008 i.u./ml insulin a pH of 7.1 was almost as inhibitory as pH 6.8. There was no enhancement of glucose uptake at pH 7.8.

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