Studies on Binding of Glucose by Insulin.* (26714)

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(Introduced by C. G. Loosli)
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These experiments were concerned with the possible binding of insulin and glucose. Levine and Goldstein(1), using rats, showed that insulin increased the distribution volume of sugars, the first 3 carbons of which were of the same configuration as glucose. This selectivity of insulin for sugars configurationally related to glucose suggested the possibility of a binding site complimentary to the first 3 carbons of glucose. It was felt that this hypothesis could be tested by dialyzing mixtures of glucose and insulin against saline at pH 7.4.

Insulin did not retard the free distribution of glucose across a semipermeable membrane. Therefore, it appears that insulin does not bind glucose sufficiently to affect its free distribution in solutions containing a physiological salt concentration and pH.

Methods and materials. Crystalline beef insulin§ was weighed out and dissolved in 0.01 N NaOH, and to this was added an equal volume of 0.03 M Tris buffer, pH 8.9, (Tris-hydroxymethyl aminomethane). The pH of this solution was adjusted to 7.4-7.5 with 0.01 N HCl.

Egg albumin solutions (Armour's) used as the protein control were prepared in the same manner as the insulin solution. *Glucose* solutions were made up in distilled water. All solutions were prepared fresh for each experiment.

Dialysis for 48 hours gave the most consistent results. It was found that Visking 20/32 dialysis tubing passed insulin and that 12/32 tubing did not, as was previously observed by Craig and King(2).

Glucose was determined by the Glucostat method(3). All solutions were deproteinized with NaOH and $ZnSO_4$.

Experimental procedure. 1. 6 ml insulin (1 mg/ml) were placed in a dialysis bag with 1 ml of glucose (1.62 mg/ml) and dialyzed against 50 ml of normal saline at 2°C.

2. Number 1 was repeated using 1 ml of 2.4 mg/ml glucose instead of 1.62 mg/ml.

3. 6 ml of egg albumin (1 mg/ml) were placed in a dialysis bag with 1 ml of 1.62 mg/ml glucose and dialyzed against 50 ml of normal saline.

4. Number 3 was repeated using 1 ml of 2.4 mg/ml glucose instead of 1.62 mg/ml.

5. (Control) 3 ml of 0.01 N NaOH and 3 ml of Tris buffer were placed in a dialysis bag with 1 ml of 1.62 mg/ml glucose and dialyzed against 50 ml of normal saline.

All dialyses were done in 125 ml stoppered Erlenmeyer flasks in a 2°C water bath.

Results. Repeat experiments indicated that there was no appreciable difference between glucose concentrations inside and outside the dialysis bag in any of the glucose-insulin or glucose-egg albumin solutions tested. The initial glucose was accounted for in all experiments indicating no appreciable loss of glucose due to experimental manipulation or bacterial contamination.

There was no retardation of glucose distribution by insulin across a semipermeable membrane. Concentration of glucose within the bag was equal to concentration outside when dialyzed in presence of insulin. This was also noted when glucose was dialyzed in presence of egg albumin.

It appears that insulin does not retard glucose and probably does not combine with it.

Discussion. Analysis of Table I: If one mole of insulin were to bind one mole of glucose, there would have been 8 and 12.3 micromoles of glucose distributed on both sides of the semipermeable membrane in Flasks 1 and 2 respectively. Assuming this to be the case, there would have been 25.2

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TABLE I.

Flask No.	Location	Vol, ml	Glucostat determin'n, μg	Predicted conc. on basis of free distribution	% of total glucose recovered
1	Inside bag	7	28	27.5	98
	Outside "	50	27		
2	Inside "	7	42	42	98
	Outside "	50	41		
3	Inside "	7	31	27.5	100
	Outside "	50	28		
4	Inside "	7	46.8	42	105
	Outside "	50	44		
5	Inside "	7	28	27.5	101
	Outside "	50	29		

μg of glucose/ml in Flask 1 and 38.4 μg of glucose/ml in Flask 2. This would have been the case if the glucose bound to insulin were *not* liberated when the solution was deproteinized. In this event only 89% of the glucose would have been recovered from Flask 1 and 92% from Flask 2 instead of the observed 98%.

If one mole of glucose were bound to insulin and liberated upon deproteinization, then there would have been an increase of 1 micromole of glucose within the bag in both Flasks 1 and 2. In this case there would have been 50.9 μg/ml instead of the observed 28 μg/ml in Flask 1 and 64.1 instead of 42 μg/ml in Flask 2.

All of the initial glucose was recovered, therefore, deproteinization liberated any glucose which may have been bound. Since there was no disparity between concentration of glucose inside and outside the bag, it is assumed that no glucose was bound. It would be inconsistent with the initial postulate to propose that less than one mole of glucose/mole of insulin were bound. In the light of these experiments it is assumed that, at physiologic ionic strength and pH, insulin does not bind with glucose.

Summary. Insulin does not retard distribution of glucose through a semipermeable membrane under the conditions of this experiment. Conditions in different media may show different results. It was decided not to investigate the initial premise further since the conditions of these experiments were similar to the pH and salt concentration encountered *in vivo*.

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Development of a Strain of Psittacosis Virus Partially Resistant to 5-Fluorouracil in a Tissue Culture System.* (26715)

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The development of resistance to chemotherapeutic agents by psittacosis virus has been described(1-6). The present paper describes the development of resistance to 5-fluorouracil (FU) by strain TTF psittacosis virus in a tissue culture system.

Materials and methods. "Wild-type" vi-

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