as 1 in 32. Although prior antibody did not prevent infection, no information is available about the presence or type of clinical illness accompanying the rise in titer. Since even homologous antibody was so ineffective in preventing reinfection, nothing can be said about the role of heterologous antibody. The evidence is strong, however, that repeated infections are important in broadening of the serologic response.

The recent description of additional rhinovirus serotypes which have distinct similarity to previously characterized types(16) has given added importance to the study of any antigenic interrelationships that might exist. Grouping these agents, if possible, would be a great aid in handling and identifying them. Evaluation of these relationships in terms of protection from clinical disease will require the use of volunteers or the epidemiologic study of a significant number of natural infections.

Summary. The antigenic relationships of the ECHO-28 strains and B632 were studied in guinea pigs. The cross reaction demonstrated between B632 and JH was reciprocal, while the one between B632 and 2060 was not. Inoculation of guinea pigs with both B632 and 2060 virus in different order brought out the antigenic relationship of these types. In human sera, the age-specific prevalence of ECHO-28 and B632 antibody was compared. A titer rise to one or both types was found in 41 of 355 paired sera. This increase in antibody occurred in some cases in spite of homologous prior antibody. In most cases, the rises in antibody level occurred against only one virus, but there was a strong suggestion that with prior antibody, an increase in antibody to both viruses was more likely.

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Glycogen Synthesis in Rat Liver Slices: Role of Glucose Concentration, Mesothelium and Insulin.* (30845)

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Glucose uptake has been studied extensively under *in vitro* conditions(1,2), but little attention has been given to the actual extent to which glucose penetrates the tissue under

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different conditions. Preparations, such as the diaphragm, are covered by a mesothelial surface and the possible regulatory effect of this membrane on exchange has not been considered. Previous studies in this laboratory utilizing histochemical techniques, provided evidence that a limiting factor in such experiments is the incomplete penetration of solutes into tissue slices under different conditions (3). Grounds exist to suspect that in tissues covered by mesothelium uptake of solutes can be regulated by mesothelial permeability(4). The original evidence was based on the penetration of tetrazolium salts, which are non-physiological histochemical solutes. To establish a more direct relationship between these mechanisms and physiological exchange, comparable studies must be conducted with physiological solutes such as glucose. The present investigation is based on the same type of histochemical approach using a modification of the procedure of Deane *et al*(5). Slices of rat liver were incubated in a glucose medium and subsequently fixed. Cross sections were stained for glycogen and the depth of glycogen deposition was used as a measure of glucose penetration. The objectives of this investigation were: a) to establish the limits of glucose penetration relative to concentration in the medium, b) to determine the influence of mesothelium on glucose penetration and c) to study the effect of insulin on mesothelial permeability.

Methods. Male Sprague-Dawley rats weighing 200 to 250 g were fasted for 24 hours and sacrificed by decapitation. The liver was quickly removed and placed in iced saline. Freehand slices were incubated for 3 hours at 37°C in a metabolic shaker in flasks gassed continuously with water saturated oxygen. The media consisted of a Krebs-Ringer phosphate(6) to which selected concentrations of glucose were added. Where indicated, glucagon-free crystalline zinc insulin was introduced to give a final concentration of 2 units per ml. Following incubation, slices were fixed for 24 hours in iced acetic-alcohol-formalin (5:85:10). The sections were cut to provide slices in a plane perpendicular to the flat surface and stained for glycogen either by the indirect ammoniacal silver method or by the Gomori methenamine-silver nitrate technique, with appropriate diastase controls(7,8). Glycogen was found to be stained only in a clearly defined band of cells at the slice surface. The width of this glycogen zone was calculated for each slice by examining sections microscopically and averaging 10 measurements made with an ocular micrometer.

A Krebs-Ringer phosphate medium was used rather than the high potassium, low sodium solutions which favor maximal *de novo* glycogenesis by rat liver slices(9). The latter solutions do not represent a physiological milieu for isolated cells and are known to alter plasma membrane permeability(10, 11). It should be pointed out that cells of liver slices incubated in physiologically balanced salt solutions establish and maintain (as in the *in vivo* state) internal high potassium and low sodium levels(12-14). In addition, the stimulating action of insulin on glycogen synthesis in rat hemidiaphragm was found to be abolished in the presence of a high potassium, low sodium medium(15). Inasmuch as hemidiaphragm is covered on both surfaces by mesothelium, any effect that insulin might have on these membranes would be obscured by the use of a high potassium, low sodium solution.

Results and discussion. Our first objective was to determine the extent to which glucose penetrates liver slices and whether this could be altered by variations in glucose concentrations. Slices taken perpendicular to the face of the lobe provided surfaces for exchange which were free of mesothelium.

1. *Glucose concentration.* The results (Table I) demonstrate that glycogen is deposited only in the first few cell layers at the surface of the slice. The actual width of this glycogen band depended on the concentration of glucose in the medium; significant increases occur at both 600 and 900 mg% glucose. It is noteworthy that even under optimal circumstances the band of glycogen was limited, occupying a width of only 0.138 mm with glucose concentrations in the medium as high as 900 mg%. When one takes into account the fact that the depth of oxygen penetration for liver slices is 0.23 mm

TABLE I. Influence of Glucose Concentration on Width of the Glycogen Band at Cut Surface of Liver Slices.

Glucose concentration (mg %)	Width of glycogen band (mm)	
	Experimental*	Predicted†
300	.081 ± .008	—
600	.122 ± .005	.114
900	.138 ± .004	.140

* These values represent averages of 6 experiments ± SEM.

† From $d = \sqrt{8\ DC/A}$; if A is constant, $d_2 = d_1 \sqrt{C_2/C_1}$, $d_1 = 0.081$ mm and $C_1 = 300$ mg %.

Liver slices were incubated for 3 hr at 37°C under oxygen. The incubation medium was composed of the following solutes mixed in the indicated proportions: NaCl (0.9%), 8.5; KCl (1.15%) 0.4; KH_2PO_4 (2.11%), 0.1; $CaCl_2$ (1.22%), 0.3; $MgSO_4 \cdot 7H_2O$ (3.82%), 0.1; $NaHCO_3$ (1.3%), 0.3; PO_4 buffer (0.1 M), 0.3; D-glucose (0.3 M) diluted in sucrose (0.3 M), 2.0.

(16), it is clear that the limited depth of glycogen deposition cannot be ascribed to the restricted penetration of oxygen. The band of glycogen deposition most likely reflects glucose penetration which in turn is influenced by its concentration in the medium. The validity of this hypothesis could be checked by calculating the width of glycogen deposition at 600 and 900 mg% from measurements of the glycogen band at 300 mg% and then comparing these figures with the experimental measurements. From Warburg's formula for penetration of solutes into tissue slices(16),

$$d = \sqrt{8\ DC/A} \quad \text{(formula 1)}$$

where d = depth of solute penetration, D = diffusion coefficient of solute through the tissue, C = concentration of solute in the medium, A = rate of consumption of solute per unit volume of tissue. The following relationship can be established when rate of consumption of solute, A, is constant(3,4):

$$d_2 = d_1 \sqrt{C_2/C_1} \quad \text{(formula 2)}.$$

Since the depth of penetration, d_1, is known, at 300 mg%, C_1, it is possible to calculate depth of penetration at 600 and 900 mg% by substituting each of these values for C_2. The mathematically derived values for d_2 at 600 and 900 mg% glucose are in close agreement with those obtained experimentally (Table I). The fact that such an agreement does exist would seem to indicate that the rate of glycogen deposition in each cell remains constant in the face of increases in glucose concentration in the medium from 300 to 900 mg%. The increase in overall glycogen synthesis in tissue slices associated with increases in glucose concentration is thus the result of increased glucose penetration; the number of liver cells involved in the synthesis of glycogen increases, while the rate of glycogen synthesis in each cell remains unchanged.

2. Permeability of mesothelium. The limiting action of mesothelium on glucose uptake was studied by preparing slices parallel to the face of the lobe so that the slice exhibited an extensive mesothelial as well as a cut surface. For these studies, slices were incubated in 800 mg% glucose. The glycogen band along the cut surface was found to be twice as wide as that at the mesothelial surface (Table II). The factors considered in the preceding experiments dealing with glucose concentration make it unlikely that the rate of glycogen deposition per unit volume of tissues differs at the mesothelial and cut surfaces. A more plausible explanation would be that as a result of the restriction of glucose passage imposed by the mesothelium, the concentration of glucose bathing the underlying parenchymal cells is appreciably less than that at the cut surface. Inspection of formula 2 indicates that it is possible to calculate the concentration of glucose (C_2) in the tissue just under the mesothelial barrier; depth of penetration at both surfaces can be

TABLE II. Effect of Insulin on Glucose Penetration at Mesothelial and Cut Surfaces. Incubations conducted in 800 mg % D-glucose.

	Cut surface		Mesothelial surface	
	Control	Insulin	Control	Insulin
Width of glycogen band (mm)*	.134 ±.003	.134 ±.003	.077 ±.006	.120 ±.004
Concentration of glucose (mg %)	800	800	262† (1.00)	598† (2.28)

* These values represent averages of 12 experiments ± SEM.

† From formula 2, $C_2 = C_1 (d_2/d_1)^2$.

Experimental conditions as for Table I. Where indicated, insulin was added to give a final concentration of 2 units per ml.

measured, d_1 and d_2, and concentration at the cut edge is equal to that in the medium (C_1 = 800 mg%). Such calculations indicate that to account for the limited penetration that occurs at this surface, the concentration of glucose in the submesothelial space should be about one third of that in the medium.

3. *Insulin.* Liver slices from the same group of animals were simultaneously incubated in a medium containing 800 mg% glucose and insulin. Whereas the glucose penetration at the cut surface remained unaffected, insulin seemed to increase substantially glucose penetration at the mesothelial surface; the glycogen band now was almost identical in width to that at the cut edge (Table II). Since there was no discernible effect on the parenchymal cells at the cut surface, the enhancing action across the serosal layer must be attributed to an action on the mesothelial covering. Apparently insulin increased the permeability of the mesothelium to glucose, allowing a much higher concentration of this sugar to be established in the submesothelial space. Calculation of the glucose concentration achieved in the submesothelial space indicates that it is twice that present in control slices.

The above experiments demonstrate that penetration of glucose into tissue slice preparations is frequently restricted and is affected in a predictable manner by variation in concentration. Unwarranted conclusions regarding cellular metabolism could be drawn in terms of overall tissue action in experiments where concentrations are varied since such manipulations alter only the number of participating cells. The problem is further compounded in preparations covered by mesothelium since this membrane significantly restricts glucose penetration and in addition is highly responsive to insulin. There is a clear-cut indication that glucose uptake in such preparations could be regulated by mesothelial permeability as has been demonstrated for tetrazolium salts(4).

Summary. The penetration of glucose into liver slices under *in vitro* conditions was determined histochemically by staining for glycogen. The width of the glycogen band was found to depend on glucose concentration in the medium. The extent to which glucose penetrates mesothelial surfaces was about half that observed at cut surfaces. Although insulin increased glucose penetration 2-fold along the mesothelial surface, there was no comparable effect at the cut edge.

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