amount of formazan deposited in the light and dark adapted retinae with glutamate as substrate was less and the total amount of formazan stain in the retinae appeared to be greater. An increase in time of incubation from 10 to 15 minutes with the substrates tried, succinate and glutamate, increased the amount of formazan so much that any difference between the light and dark adapted retinae could not be detected visually. Additional nitroBT in the media has the same effect. These results suggest that any difference observed is due to a constant difference in amount of formazan deposited and that some co-factor or electron transport carrier is altered. An alternative hypothesis is that the light intensities used damage some enzyme system in the extra mitochondria space which inhibits certain electron transport reactions within the mitochondria where nitroBT reduction is thought to take place (3). The present data do not permit one to distinguish between these alternatives. However, these data do suggest that light of the intensity used does not alter appreciably the permeability either of the cells or the mitochondria because some substrates used did not show the effect. Since all of the substances which seem to inhibit the reduction of nitroBT in the dark adapted retinae are substrates for enzymes which do or can use NAD as a coenzyme and since NADH preincubation also inhibits nitroBT reduction in both dark and light adapted retinae, one is tempted to speculate that NADH production by any enzyme system present will produce the effect. The data in Table I do not fully support such a conclusion since substrates for some NAD requiring enzymes show no effect.

The effect reported here appears to be specific for the retina because it could not be demonstrated in excised iris, or cerebral cortex tissue of the rabbit.

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Effect of Hypoinsulinism on Intracellular Transport of Glucose in the Small Intestine. (29886)

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One of the mechanisms of action of insulin suggested is that it facilitates the entry of glucose inside the cells(1-4). Insulin has been found to increase the transfer of pentose (5), amino acids(6) and potassium(7) from the extracellular fluid to the intracellular fluid. Thus insulin is necessary for intracellular transport of glucose, pentose, amino acids and potassium. The intestinal absorption of glucose involves transference of the sugar from the mucosal to the serosal side of the intestine. Absorption of glucose from the intestinal lumen, however, increased in insulin deficiency produced either by injection of alloxan in rats(8,9) or by development of scurvy in guinea pigs(10). It has been reported that scorbutic guinea pigs suffer from hypoinsulinism(11).

Materials and methods. Albino rats and guinea pigs weighing between 250 g and 300 g were used. Rats were made diabetic by intraperitoneal injection of alloxan, 200 mg/kg body weight. Scurvy was produced by feeding guinea pigs a scorbutogenic diet(12) for 4 weeks.

After a fasting period of 24 hours, normal guinea pigs, scorbutic guinea pigs, normal rats and alloxan diabetic rats were killed by stunning followed by decapitation. The small intestine was removed, flushed with ice-cold Ringer fluid, everted, cut into pieces of 5 cm in length starting from the duodenal end and

TABLE I. Intracellular Transport of Glucose in Small Intestine of Animals Under Different Treatments.

Condition of animals	Total tissue water, ml/100g tissue	Extra cellular water, ml/100g tissue	Glucose, mg/ml incubation medium	Glucose, mg/ml total tissue water	Glucose, mg/ml intracellular water
Normal rat	82 ± 1.2 (80 – 85)	30 ± 1.3 (27 – 33)	2.32 ± .32 (2.2 – 2.4)	2.57 ± .08 (2.48 – 2.67)	2.89 ± .11 (2.74 – 3.27)
Alloxan-diabetic rat	76 ± 1.4 (73 – 80)	32 ± 1.6 (29 – 35)	2.46 ± .08 (2.24 – 2.78)	4.75 ± .38 (4.04 – 5.5)	6.62 ± .42 (5.2 – 8.03)
t					19.6
Normal guinea pig	85 ± 1.6 (82 – 88)	27 ± 1.8 (23 – 30)	4.09 ± .12 (3.9 – 4.36)	3.33 ± .84 (4.14 – 4.6)	4.57 ± .19 (4.4 – 5.0)
Scorbutic guinea pig	82 ± 4.5 (79 – 84)	29 ± 2.1 (26 – 34)	3.87 ± .05 (3.78 – 3.98)	5.42 ± .50 (4.5 – 6.4)	6.3 ± .41 (5.0 – 7.5)
t					3.8

Values are Mean ± S.E. 4 animals in each group.
Figures in parentheses indicate range of values.

used for the different studies. The number of animals under each treatment was four.

Determination of thiosulfate space. One piece of the intestine was placed in a siliconized conical flask to which 6 ml Krebs-Ringer-bicarbonate saline containing 1 g sodium thiosulfate and 300 mg glucose per 100 ml were previously added. The flask was then oxygenated, stoppered and incubated in a water bath maintained at 37°C. After incubation for half an hour the tissue was removed, intestine opened by cutting along the long axis, washed gently in ice-cold Krebs-Ringer-bicarbonate saline, blotted, weighed, homogenized with 1 ml each of 10% sodium tungstate and ⅔ N sulfuric acid, volume made up to 10 ml with glass distilled water and filtered. Thiosulfate in the filtrate and in the medium was estimated by iodometric titration with 0.005 N potassium iodate using starch solution as the indicator and the thiosulfate space was calculated(13). *Determination of total tissue water.* The everted piece of intestine was weighed, dried at 70°C to constant weight and the percentage of total tissue water was calculated(13). *Determination of glucose uptake.* Each piece of the intestine was taken in a siliconized conical flask containing 6 ml of about 0.3% glucose solution prepared in Krebs-Ringer-bicarbonate saline, oxygenated, stoppered, incubated at 37°C for one hour, tissue removed, washed in cold Krebs-Ringer-bicarbonate saline, blotted, weighed, homogenized with 10 ml of 0.45% zinc sulfate, homogenate neutralized with 2 ml of suitably diluted 2 N sodium hydroxide, the tube containing the neutralized homogenate placed in boiling water for 3 minutes, cooled, filtered, and glucose in the filtrate estimated(14). Glucose uptake was expressed as glucose concentration per ml of tissue water and glucose concentration per ml of intracellular water(5).

Results. The results are given in Table I. Intracellular water of the intestine of the diabetic rat or the scorbutic guinea pig contained more glucose after incubation in a medium containing glucose than did the intestine of normal animals similarly incubated.

Discussion. The transport of glucose inside the cells of the intestine of diabetic and scorbutic animals was found to be increased as compared with normal animals. The absence of insulin facilitated the transfer of glucose from the medium to the inside of the intestinal cells while in muscle the phenomenon was reversed(1,2). The ineffectiveness of insulin in facilitating permeability of glucose or other sugars having a similar configuration inside the cells of other tissues has also been reported. Glycogen synthesis by the cells of the cerebral cortex of guinea pigs is not affected by insulin(7,15,16). Obviously the glucose molecule is able to enter either the cell or the sub-cellular compartments like mitochondria where enzymes for glycogenesis might be located, even without the aid of insulin. Similar ineffectiveness of insulin on glucose utilization reactions has been observed in retina(17), rabbit erythrocytes(18), Ehrlich ascites tumor cells(19) and leucocytes(19). It seems from these observations that the mechanism of insulin action differs from tissues to tissues. The

process of transport of glucose in the intestine is, therefore, different from the transport mechanisms operating in muscles and is independent of the direct action of insulin.

Summary. Effect of insulin insufficiency on the transport of glucose in the intestinal cells was studied by incubation of small intestines of rats and guinea pigs in a medium containing glucose. Insulin deficiency was produced by injection of alloxan to rats and by feeding a scorbutogenic diet to guinea pigs. Concentrations of glucose in the intracellular water of the intestine incubated in glucose-saline were found higher in the intestines obtained from diabetic rats and from scorbutic guinea pigs as compared to normal. Insulin does not seem to have any direct role in the intracellular transport of glucose in the small intestine.

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Erythrocyte Hemolysis by Isolated Rat Liver Lysosomes.* (29887)

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Present knowledge of lysosomes has been summarized recently(1). Interaction between lysosomes and other membranous parts of cells is one of the most important yet least understood reactions. Purified lysosomes recently have become available and their composition and reactions of their lipoprotein membrane have been studied(2,3). This paper reports studies of *in vitro* hemolysis of erythrocytes by isolated rat liver lysosomes.

Experimental. Male Sprague-Dawley rats weighing about 200 g were used for collecting blood for erythrocytes and livers for isolating subcellular fractions—mitochondria, microsomes and lysosomes. Blood was collected by decapitating the rats and was immediately mixed with equal volume of ice-cold saline phosphate buffer pH 7.4 (0.9% saline in 0.1 M phosphate buffer). Livers also were removed immediately and were chilled in ice, washed and homogenized in a Waring blendor for 20 seconds in 0.25 M

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