Summary. Rat liver homogenates were incubated aerobically with and without added ribonuclease. Simultaneous determinations of respiratory rate and capacity to convert mevalonic acid to non-saponifiable material were made. Loss of the synthetic capacity of the homogenates, considered as a reflection of the failure to maintain a functional level of ATP, was followed by a decline in rate of respiration. Synthetic capacity and rate of respiration were maintained by the presence of added polyanions.

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Glucose Transport by the Intestinal Mucosa of the Dogfish.* (27219)

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Few data are available in the literature on the transport of non-electrolytes across the intestine of marine fishes. In the present study we have used a simple preparation of intestinal mucosal membrane of the dogfish to study the transport of glucose, and to show the effect of temperature on transport.

Methods. Spiny dogfish (*Squalus acanthias*) used in this study were caught in the gulf of Maine during the months of July and August. They weighed 8-12 lb. and were kept in an enclosure in the ocean for one to 4 days. On the day of experiment, they were removed and immobilized by spinal transsection. The abdomen was opened immediately, the small intestine was removed and placed in an ice-chilled dogfish Ringer's solution without glucose. A duodenojejunal segment was cut open through the mesenteric line, and mounted on a cork ring, with which the tissue was submerged in ice-chilled Ringer's solution. Spiral valves of the mucosa were excised. The remaining mucosa was stripped away from serosal and muscular layers. This mucosal membrane was then cut into 3 or 4 sections. Each section was mounted over a cylindrical glass tube, 1.7 cm in diameter and 4.0 cm in length. The mucosal side was on the outside, and the submucosal side on the inside of the tube. Glucose in varying amounts was added to the dogfish Ringer's solution at the start of each experiment. Then, one ml of the fresh glucose-Ringer's solution was pipetted into the tube against the submucosal surface. Next, the tube was placed in a plastic beaker containing 5 ml of the same solution. The cylindrical tube, beaker, membrane and solutions were shaken in a Dubnoff shaker at 36°C, 26° and 16° for an hour. A gas mixture, CO_2-O_2 (5-95%) was bubbled into the outside solution throughout the experiment. Dogfish Ringer's solution was composed of: NaCl 280 mM, KCl 12 mM, $CaCl_2$ 10 mM, $NaHCO_3$ 4.5 mM, NaH_2PO_4 0.5 mM and urea 360 mM. The final solution was 960 milliosmoles/liter. After one hour of incubation, the inside or submucosal fluid was collected for measurement of volume and glucose concentration. The outside or mucosal fluid was also collected for measurement of glucose concentration. Glucose analysis was that of Nelson (1). The mucosal membrane was blotted gently with filter paper and weighed.

Results. The complete intestinal segment with intact mucosal, muscular and serosal layers, did not transport glucose. Experi-

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TABLE I. Site and Rate of Intestinal Transport of Glucose.*

Part of intestine†	Rate of glucose transport mmol/kg tissue wet wt/hr			Avg
I	243	245	225	
	187	246	203	222
	233	243	216	
	188	210	234	
II	203	233		218
III	239	228		233
IV	210	200		205

* Initial concentration of glucose was 12 mM.
† The order (I-IV) of intestinal segment was from duodenal-jejunal to ileum.

ments, therefore, were performed only on the isolated mucosal membrane. Microscopic examination of the membrane showed intact mucosal cells, a few muscle fibres and no serosa. Table I summarizes the results obtained on transport of glucose by 4 different levels of intestine, from duodenum to ileum. The duodenojejunal segment gave the highest rate of transport and the ileal segment the lowest rate of transport; however, differences detected were minimal, and probably insignificant. Data presented here were obtained from experiments on the duodenojejunal segment only. This segment was chosen because it was easier to dissect than were the others.

Temperature. Fig. 1 presents results on rate of glucose transport *vs* concentration at 36°C and 26°C. Each point is the average of 2 to 4 experiments. At glucose concentration of 30-40 mM, a maximal rate of transport was reached, both at 36° and 26°. However, at 36° the rate of glucose transport was 4.0-4.5 times greater than that at 26°. When the intestine was incubated at 16° there was no net glucose transport.

Effect of inhibitors. Phlorizin, sodium azide, iodoacetic acid, 2,4-dinitrophenol and uranyl acetate in varying concentrations were added to the outside and inside fluids. Temperature of the bath was 36° in all experiments of this type. Table II shows that all of these compounds except 2,4-dinitrophenol inhibited glucose transport in varying degrees depending upon the concentration of the inhibitor.

Fig. 2 presents Lineweaver-Burk's plots of data(2). The ordinate is the reciprocal of rate of transport; the abscissa, the reciprocal of glucose concentration of the initial fluids. A linear relationship between 1/V and 1/S was suggested by the close fit of calculated regression lines to the data(3). At 36°, Km and V_{Max} were 20 mM and 636 mmol/kg/hr respectively; at 26° they were 16 mM and 78 mmol/kg/hour respectively. In the presence of phlorizin the calculated Km and V_{Max}

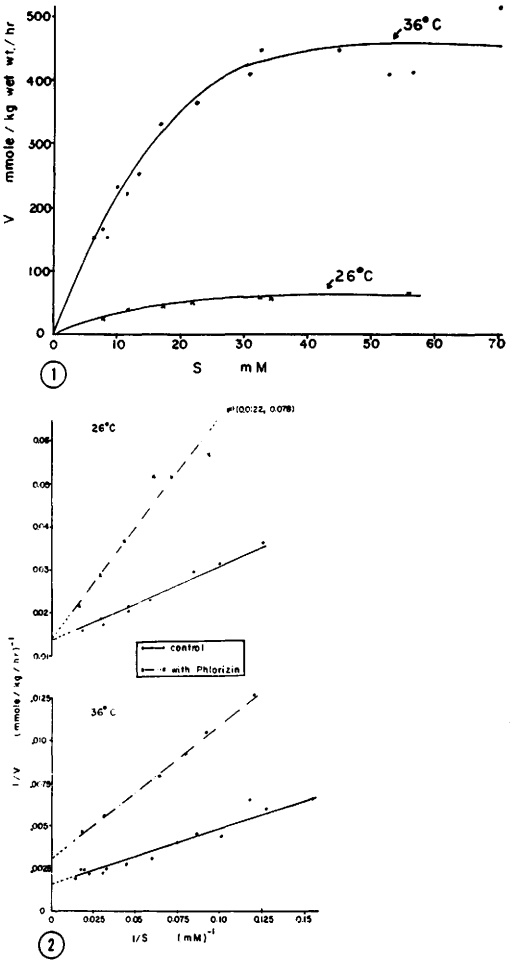

FIG. 1. Intestinal transport of D-glucose at 36° and 26°. A mucosal membrane of duodenojejunal segment was mounted on a cylindrical tube. The submucosal side was filled with 1 ml of dogfish Ringer solution containing glucose and was incubated in 5 ml of the same solution.

FIG. 2. Lineweaver-Burk's plots of intestinal transport of glucose with and without phlorizin. Ordinate is reciprocal of rate of transport; abscissa, reciprocal of glucose concentration. Solid line is a control without phlorizin; dotted line is glucose transport in presence of 0.05 mM phlorizin.

TABLE II. Inhibition of Glucose Transport by Some Inhibitors.

Inhibitor	Conc.	% of inhibition*
NaN_3	3×10^{-3}M	40.7 (3)
	4×10^{-3}M	82.8 (3)
	5×10^{-3}M	100 (1)
	8×10^{-3}M	100 (2)
2,4-DNP	2.5×10^{-5}M	0 (2)
Iodoacetic acid	3×10^{-4}M	22.5 (3)
	10^{-3}M	49 (3)
	1.5×10^{-3}M	100 (3)
Phlorizin	2.5×10^{-5}M	32.5 (2)
	5×10^{-5}M	45.2 (2)
	5×10^{-4}M	100 (2)
Uranyl acetate	5×10^{-6}M	37.0 (2)
	10^{-5}M	46.3 (3)

* No. in parentheses indicates No. of experiments.

were 27 mM and 339 mmol/kg/hour at 36°, and 34 mM and 72 mmol/kg/hour at 26° respectively. At 26° the regression lines of glucose transport with and without phlorizin intercept the ordinate apparently at the same point, *i.e.*, they have the same maximum. At 36°, the 2 lines intercept the ordinate at 2 different points, *i.e.*, the uninhibited and inhibited rates of transport reach different maximal levels.

Discussion. Data shown here indicate that glucose is able to move from the mucosal fluid to the submucosal fluid against concentration gradients. The rate of transport is greater at 36° than at 26°. Certain metabolic inhibitors including phlorizin inhibit this transport. Such transport of glucose can be an active process in view of net movement up a concentration gradient, and of the maximal rates reached as concentration of glucose increased. However, in preparation of the mucosal membrane the spiral valve was cut leaving a small opening between mucosal and submucosal solutions. If movement of glucose had occurred down the concentration gradient, it would indicate the presence of a leak in the membrane. But glucose moved up the concentration gradient. This leaves the possibility of glucose movement by bulk flow *via* a siphon or pressure effect. However, there was neither change of pressure nor change of volume on either side. Therefore, we have inferred that the hole was small and that leakage was insignificant compared to movement by active transport. Two additional facts excluded the possibility of "leakage" movement of glucose. These facts are: first the effect of temperature, and second, the effect of the inhibitors.

Experiments with inhibitors show that phlorizin, at 5×10^{-5}M reduced glucose transport to 55% of the control value. This concentration is far less than that needed for inhibition of oxidative phosphorylation(4,5). Therefore, phlorizin appears to be bound or adsorbed to the glucose carrier system as has been postulated by others(5,6). Our data show the phlorizin is a competitive inhibitor for glucose transport at 26°. This means that at this temperature glucose transport is conducted mainly by a carrier system at the surface of the intestinal mucosa. Phlorizin binds with the carrier system selectively, producing a competitive inhibitory effect. However, at 36° the inhibitory effect of phlorizin is non-competitive, a more complicated transport mechanism for glucose at this temperature is suggested. Although Csaky and Fernald(7) have shown a linear relationship between the temperature of incubation and rate of disappearance of glucose in an isolated intestinal loop of frog, an influence of temperature on glucose transport in the presence of phlorizin has not been cited by other investigators. It will be interesting to see whether a similar phenomenon may occur with glucose transport by other cellular membranes.

Summary. A simple technic using the mucosal membrane of dogfish gut to study the transport of glucose was presented. It was found that the mucosal membrane can transport glucose from the mucosal to the submucosal fluid against concentration gradients. This transport was greater at 36° than at 26°, and was inhibited by phlorizin, sodium azide, iodoacetic acid and uranyl acetate. Michaelis-Menten analysis of data has shown that inhibition of glucose transport by phlorizin was competitive at 26° and non-competitive at 36°. It is postulated that a carrier system for glucose transport operates at the low temperature, while a more complicated mechanism handles glucose transport at 36°.

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Oxidative Assimilation and C^{14} Distribution in *Azotobacter agilis*. (27220)

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Azotobacter agilis(1) has a high rate (Q_{O_2}) of oxygen consumption in the presence of a readily oxidizable substrate but exhibits low endogenous activity and relatively low efficiency of oxidative assimilation. *A. agilis* oxidatively assimilates approximately 15-20% of the glucose utilized, the remainder being oxidized to carbon dioxide and water. Values around 18% were reported for acetate and succinate and 22% for pyruvate or lactate (1). This study is concerned with both the influence of the growth substrate on assimilation and distribution of assimilated material within the cells.

Materials and methods. Cells were grown for 20 hours in Burk's medium(2) gelled with 2% agar and containing 1% glucose, acetate or succinate as the carbon and energy source. All manometric tests were carried out at 30° C using standard technics. The cell suspensions were prepared in 0.05 molar phosphate buffer of pH 7.2 containing magnesium chloride in a final concentration of 0.005 molar and were adjusted to a Klett-Summerson colorimeter reading (green filter, #42) of 300. The methods for cell fractionations and measurements of radioactivity were those described in a study on *Bacillus cereus*(3), all samples being converted to CO_2 and measured in a Dynacon (Nuclear Chicago Corp.). This procedure yields more accurate and reproducible values than barium carbonate plating and counting methods.

Results. Of the various compounds tested manometrically, acetate, pyruvate, succinate, fumarate, malate, α-ketoglutarate, ethanol and glycerol were oxidatively assimilated by *A. agilis*, generally in the range of 20-25%, by acetate-, succinate-, or glucose-grown cells. Xylose, arabinose, glutamate, glycine, glyoxylate, glycolate, and formate were not oxidized by *A. agilis*. Lag periods of the order of 30-50 minutes were observed for oxidation of most substrates (excluding growth substrate) except no lag was noted for oxidation of acetate, pyruvate and ethanol by all cells and, in the case of succinate-grown cells, fumarate and malate also were oxidized without lag. Glucose, however, was an exception. It was oxidized readily by glucose-grown cells but not by cells grown on acetate or succinate. Long lag periods, 100-200 minutes, were commonly observed and the rate of glucose oxidation in suspensions containing 4 μ-moles of glucose never approached by more than one-sixth the rate observed with glucose-grown cells. On the other hand, the rate of oxidation of succinate by acetate-grown cells approached that of succinate-grown cells after approximately a 50-minute lag period. Increasing the initial concentration of glucose 10-fold or more decreased to some extent the duration of the lag period, particularly with succinate-grown cells. Typical response of acetate- or succinate-grown cells to oxidation of glucose and of acetate-grown organisms to succinate oxidation is illustrated in Fig. 1. The behavior with glucose was confirmed with the use of uniformly labeled C^{14}-glucose. Glucose oxidation went to completion within about 40 minutes with glucose-grown cells while oxygen consumption in the presence of glucose by succinate-grown cells, corrected for endogenous respiration, amounted to 5.2% of the theoretical value for complete oxidation at the end of 1 hour, and 14.1, 41.0, and