Development of brush-border membrane hexose transport system in chick jejunum

AHMED TAHIR SHEHATA, JOSEPH LERNER, AND DAVID S. MILLER
Biochemistry Department, University of Maine, Orono 04469; and
Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672

SHEHATA, AHMED TAHIR, JOSEPH LERNER, AND DAVID S. MILLER. *Development of brush-border membrane hexose transport system in chick jejunum.* Am. J. Physiol. 240 (Gastrointest. Liver Physiol. 3): G102–G108, 1981.—Hexose transport was characterized in jejunal slices and brush-border membrane (BBM) vesicles from chicks of several ages (range: 2 days before hatch to 21 days after hatch). With slices, initial rates of 0.1 mM 3-*O*-methyl-D-glucose transport were low in −2- and 0-day chicks, increasing to maximal levels 2–7 days after hatching; rates declined to adult levels over the next 2 wk. Phlorizin did not inhibit uptakes in −2- or 0-day slices. In contrast, about 80% of the uptakes in tissue from 2-day or older chicks was phlorizin inhibitable. The apparent K_t for phlorizin-sensitive transport was the same in slices from 2- (maximal transport rates) and 21-day (minimal posthatch rates) chicks; the apparent V_{max} was 2.5 times greater in 2-day slices. With BBM vesicles, initial rates of Na-dependent D-glucose transport and maximal overshoot levels were greater in 2-day vesicles than in 21-day vesicles. As in slice experiments, K_t values were the same in the two preparations, but V_{max} values were 1.4 times higher in the 2-day preparation.

Gallus domesticus; brush-border membrane vesicles; intestinal slices; D-glucose; 3-*O*-methyl-D-glucose; phlorizin-sensitive transport

THE SMALL INTESTINE absorbs certain nutrients, i.e., sugars and amino acids, primarily by processes that are selective, saturable, concentrative, and dependent on cellular metabolism and medium Na. Such transport is mediated by carriers that are embedded in the brush-border membrane (BBM) of the intestinal epithelial cell and is powered by potential energy stored in the transmembrane Na gradient. This electrochemical potential gradient for Na is maintained by the enzyme, Na-K-ATPase, which is located on the basal-lateral surfaces of the cell (for example, see Ref. 23). Previous studies with tissue slices and sacs have demonstrated that the fundamental characteristics of the glucose transport system change during the first few weeks after birth (hatch) and that early developmental patterns for avian and mammalian small intestine are similar (1, 4, 5, 16, 19, 24). Accordingly, in birds and mammals, significant transport activity has been observed a few days before birth, with rapid increases in activity occurring at birth. In mammalian tissue, transport activity has been shown to decrease after the 1st wk, but corresponding data for avian intestine are difficult to interpret because of methodological problems (see DISCUSSION).

We have investigated the changes that take place in the chick intestinal glucose transport system with the developmental state. Using a combined tissue slice-BBM vesicle approach, we studied 3-*O*-methyl-D-glucose (3-*O*-MG, nonmetabolizable glucose analogue) transport in slices and D-glucose transport in vesicles. BBM vesicle techniques permit the study of transport in the absence of cellular metabolism (11, 26). Thus, functional changes occurring at the BBM with development can be evaluated and compared with those occurring in whole tissue. The results of the present study indicate that changes in slice hexose transport are closely paralleled by changes in BBM transport and that the major kinetic difference between tissue from younger (2-day) and older (21-day) chicks is found in the V_{max} term.

METHODS

Animals. Hubbard-Cross chicken eggs were incubated in the University hatchery, and, upon hatching, animals were removed to an environmentally controlled brooder. Chicks were fed a high-protein mash diet ad libitum. For transport experiments, jejunal tissue was obtained from chicks at the following ages: 2 days before hatch (−2 days), day of hatch (0 days), 2 days after hatch (+2 days), +7 days, +14 days, +21 days, and +15 wk (adult).

Whole tissue studies. Accumulation of 3-*O*-MG was determined in jejunal slices, using the procedure of Lerner and Steinke (18). Briefly, slices were incubated at 37°C in oxygenated (95% O_2-5% CO_2) Krebs-Henseleit buffer (with the following composition in mM: NaCl 118, KCl 4.75, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and at pH 7.6), containing ^{3}H-labeled (New England Nuclear) and unlabeled substrate and unlabeled inhibitors, where indicated. During incubation, tissue was shaken at a rate of 100 cycles/min. Increasing the shaking rate had no effect on 3-*O*-MG uptake. Thus, a possible differential effect of unstirred layers on transport was not a factor in our slice experiments (27). After a predetermined period, the tissue was separated from medium by filtration, briefly washed (ice-cold 300 mM mannitol), blotted, and extracted in 2.5% trichloroacetic acid. The extracted tissue was weighed, and an aliquot of the clarified extract was counted using standard liquid scintillation procedures.

Membrane vesicle studies. BBM vesicles were prepared from mucosal scrapings of jejunal tissue by the $CaCl_2$ precipitation procedure of Evers et al. (6). The final pellet was suspended in vesicle buffer [containing in

mM: mannitol 240, $CaCl_2$ 2, Tris (tris(hydroxymethyl)-aminomethane)-HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) 50, and at pH 7.6]. Transport experiments were conducted using modifications of the Millipore filtration procedure of Hopfer et al. (13). A modification of the automated procedure of Kessler et al. (14) was used for short-time (1–5 s) incubations. In both cases, reactions were started by mixing 10 μl of vesicle suspension with 25 μl of medium (140 mM NaCl or KCl with 50 mM Tris-HEPES, 2 mM $CaCl_2$, and ^{3}H-labeled and unlabeled substrate at pH 7.6) and terminated by adding 1 ml of ice-cold stop solution (100 mM NaCl, 240 mM mannitol, 2 mM $CaCl_2$, 1 mM phlorizin, and 20 mM Tris-HEPES at pH 7.6). Final concentrations in the incubation media were 100 mM NaCl or KCl, 2 mM $CaCl_2$, 69 mM mannitol, and 50 mM Tris-HEPES at pH 7.6. Vesicles were collected on 0.45-μm Millipore filters and washed with 3 ml of ice-cold stop solution. Filters were air-dried and then counted.

BBM preparations were routinely assayed for marker enzyme activities. Na-K-ATPase and oligomycin-sensitive Mg-ATPase were determined by the procedures of Miller et al. (21) and alkaline phosphatase by the procedure of Heidrich et al. (8). Membrane protein was determined using the Bio-Rad protein assay (Bio-Rad, Richmond, CA). In some experiments, vesicle diameters were measured using a modification of the procedure of Groseclose and Hopfer (7). Membranes were fixed in vesicle buffer with 1% glutaraldehyde, collected on Formvar-coated grids, and negatively stained with 1% phosphotungstic acid (pH 6.5). Vesicles were viewed in a RCA electron microscope at 50 kV, and representative micrographs were selected for measurements of vesicle diameter. Assuming a spherical shape, mean vesicle surface areas were calculated using the formula: $S = \pi d^2$.

Calculations. Uptakes are expressed as nmol per g wet wt (whole tissue) or pmol per mg membrane protein. Vesicle uptakes were corrected for the small amount of glucose binding to filters. Data are given as means ± SE. Sample means were judged to be significantly different when $P < 0.05$, using an unpaired t test.

RESULTS

Whole tissue studies. One-minute uptakes of 0.1 mM 3-*O*-MG by slices from chicks of varying ages are shown in Fig. 1. Total uptake increased rapidly shortly after hatch, plateaued between 2 and 7 days, and fell to adult levels after 21 days. Using 1 mM phlorizin, a specific inhibitor of BBM hexose transport (15, 28), we separated total uptake into inhibitable and uninhibitable components. Significant uptake in the phlorizin-sensitive component was not detected in slices from −2- and 0-day chicks (Fig. 1). By 2 days after hatch, uptake in this component had reached a maximal level that was maintained for at least 5 additional days. Phlorizin-sensitive transport declined over the next 2 wk, and adult levels of transport activity were found in tissue from 21-day chicks. Thus, the posthatch developmental pattern for transport in this component parallels that found for total uptake. In contrast, transport in the phlorizin-insensitive component declined slowly over the period studied. This component represents diffusive uptake, nonspecific binding, and, possibly, uptake mediated by brush-border and basal-lateral Na-independent (phlorizin-insensitive) carriers.

Detailed experiments were conducted with tissue from 2- and 21-day chicks, because they represented posthatch ages of high and low phlorizin-inhibitable transport (Fig. 1). Time courses of total and phlorizin-sensitive uptakes of 3-*O*-MG by slices from 2- and 21-day chicks were linear for at least 3 min of incubation (Fig. 2 and legend). At all incubation times, total and phlorizin-inhibitable uptakes by 2-day slices were significantly higher than corresponding uptakes for 21-day slices. At both ages, 3-*O*-MG transport was concentrative, inasmuch as tissue-

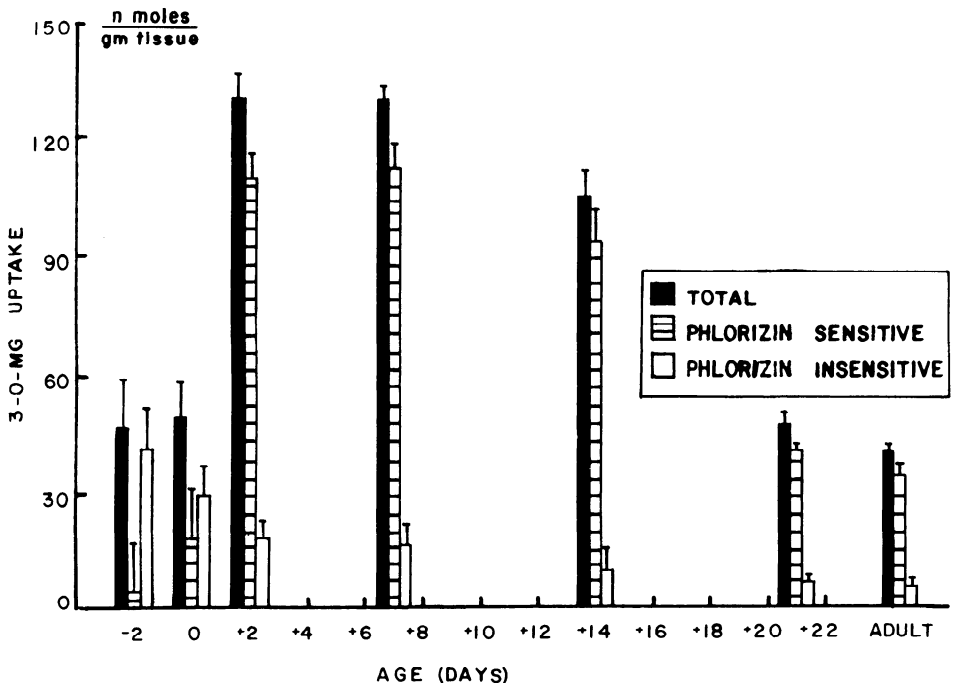

FIG. 1. One-minute uptakes of 0.1 mM 3-*O*-methyl-D-glucose (3-*O*-MG) by jejunal slices from chicks of different ages. Data are given as means ± SE from 10–30 birds.

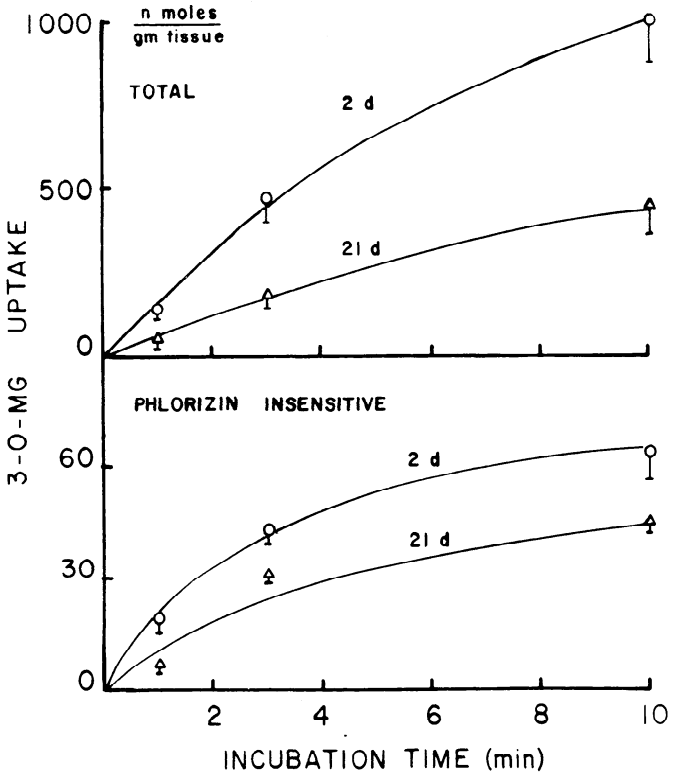

FIG. 2. Time course of 0.1 mM 3-*O*-methyl-D-glucose (3-*O*-MG) uptake by jejunal slices from 2- and 21-day chicks. Data for total and phlorizin-sensitive uptakes (total minus insensitive uptake, not shown) are linear functions of incubation time through 3 min. Data are given as means ± SE from at least 10 birds.

to-medium ratios exceeding unity were found after 3-min incubations (Fig. 2) (assuming unit tissue density, an uptake of 100 nmol/g tissue is equivalent to a tissue-to-medium ratio of 1). To determine the kinetic basis for the observed changes in transport with development, we measured initial (3-min) uptakes of 3-*O*-MG as a function of substrate concentration in slices from 2- and 21-day chicks. In both preparations, the phlorizin-inhibitable component exhibited saturation kinetics (Fig. 3), and double-reciprocal plots of these data were linear with no evidence of heterogeneity at either age (Fig. 4). The apparent K_t for the phlorizin-inhibitable component was the same in tissue from both age groups, but V_{max} was 2.5-fold higher in 2-day slices than in 21-day slices (Table 1). These whole tissue data suggest that a decrease in apparent carrier capacity per g tissue was responsible for the alteration in 3-*O*-MG transport rates found with development.

With regard to kinetic analysis of the small, uninhibitable component, plots of initial uptake vs. substrate concentration were linear over the concentration range studied (Fig. 3). These data suggest that if Na-independent and/or phlorizin-insensitive processes were involved, they possessed very low-substrate affinity. Apparent permeability coefficients (K_d, calculated from the slopes of the lines in Fig. 3) appeared to be about 70% higher in 2-day tissue than in 21-day tissue; this finding is in agreement with the data in Fig. 1.

Brush-border membrane vesicle studies. To determine if the observed changes in hexose transport with development were a result of altered BBM function, we studied D-glucose transport in jejunal BBM vesicles from 2- and 21-day chicks. BBM fractions were characterized using specific marker enzyme analyses. Alkaline phosphatase activities (BBM marker) were higher in mucosal homogenates from 2-day chicks (0.67 ± 0.004 μmol·mg protein^{-1}·h^{-1}) than in homogenates from 21-day chicks (0.64 ± 0.005 μmol·mg protein^{-1}·h^{-1}), a finding in agreement with the chick and rat data of Moog (22). BBM enrichments (ratio of specific activity in BBM fraction to that in mucosal homogenate) of this marker averaged 13 ± 2 for 2-day chicks and 19 ± 5 for 21-day chicks. These enrichments are within the range of values (10–30) reported for mammalian and avian BBM preparations (20, 26). In addition, BBM fractions contained little activity associated with basal-lateral (Na-K-ATPase) or mitochondrial (oligomycin-sensitive Mg-ATPase) contamination.

In the presence of an initial, inwardly directed, Na gradient, uptakes of 0.05 mM D-glucose by vesicles from both age groups were rapid, rising to levels several times those found at equilibrium (60 min; Fig. 5). The rapid rise in uptake above equilibrium levels (overshoot) was abolished by addition of 1 mM phlorizin to the medium (in presence of a NaCl gradient) or by replacement of the initial NaCl gradient with KCl gradient. This Na-dependent and phlorizin-inhibitable overshoot in brush-border vesicles is characteristic of the Na-glucose co-

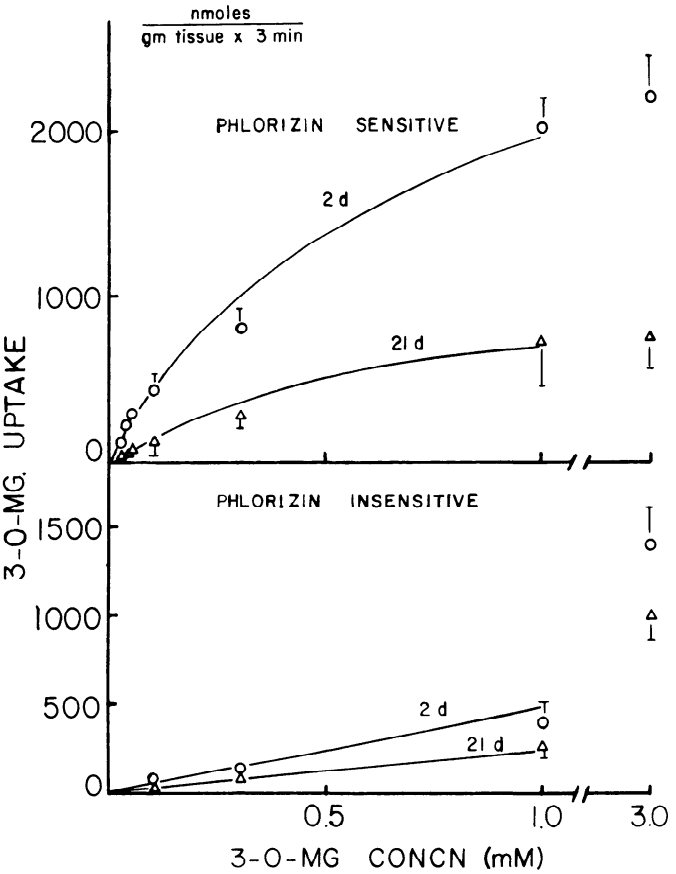

FIG. 3. Concentration dependence of 3-*O*-methyl-D-glucose (3-*O*-MG) uptake (3 min) by jejunal slices from 2- and 21-day chicks. Data are given as means ± SE from 10 birds.

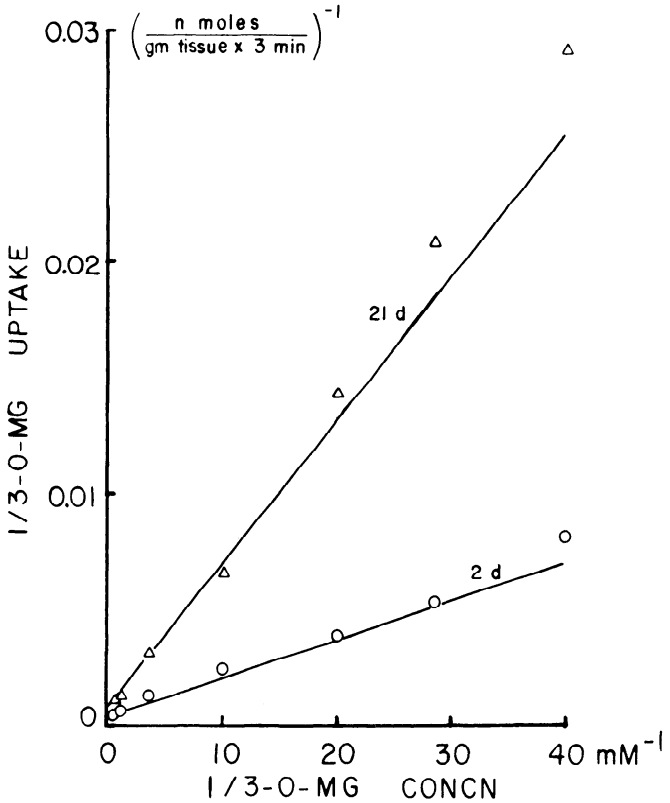

FIG. 4. Double-reciprocal plot of phlorizin-sensitive uptake data from Fig. 3.

TABLE 1. *K_t and V_{max} values for hexose transport in jejunal slices and BBM vesicles from 2- and 21-day chicks*

Age, days	K_t	V_{max}
	Slices (3-O-MG)	
2	0.7 ± 0.1 (10)	3000 ± 300 (10)
21	0.8 ± 0.2 (10)	1160 ± 50 (10)†
	BBM vesicles (glucose)	
2	0.2 ± 0.02 (4)	887 ± 40 (4)
21	0.2 ± 0.06 (4)	637 ± 90 (4)*

Data are given as means ± SE. Numbers in parentheses are the number of birds (slices) or preparations (vesicles). Values were determined from data in Figs. 4 and 8. V_{max} units are nmol·g tissue^{-1}·3 min^{-1} for slices and pmol·mg protein^{-1}·2 s^{-1} for vesicles. BBM, brush-border membrane; 3-*O*-MG, 3-*O*-methyl-D-glucose. *Significantly lower than 2-day value, $P < 0.05$. †Significantly lower than 2-day value, $P < 0.01$.

transport mechanism that is present in the intestinal and renal proximal tubule BBM (26). The observed overshoot suggests that uptake occurred into an osmotically active intravesicular space. Additional experiments (not shown) with both 2- and 21-day vesicles indicated that uptake increased linearly with the reciprocal of medium osmolality ($1/\pi$). Extrapolation of these lines to $1/\pi = 0$ (infinite osmolality and, hence, zero intravesicular volume) indicated no detectable glucose binding to vesicles.

Comparison of the time courses of 0.05 mM D-glucose uptake by 2- and 21-day BBM vesicles indicated that, except for the 60-min points, uptakes by 2-day vesicles were about 1.5–2 times greater than those found for 21-day vesicles (Fig. 5). Sixty-minute (equilibrium) values were similar in the two preparations, indicating that both possessed the same intravesicular volume per mg membrane protein, about 0.8 μl/mg protein. This value agrees well with those reported for BBM vesicles from chicken and rat intestine (7, 20). We also determined mean vesicle surface areas from representative electron micrographs of negatively stained preparations. As shown in Fig. 6, preparations from both age groups exhibited similar surface area distributions; mean values for 2- and 21-day vesicles were nearly identical.

Because vesicle uptakes are generally measured under Na^+ gradient conditions, kinetic studies must be carried out under conditions where forces driving transport are relatively constant. Two procedures have been used, i.e., equilibrium exchange (nongradient conditions; see Ref. 11) and initial rates (approximately constant gradient conditions; see Ref. 28). In the present study, we used the latter method to compare kinetic constants for D-glucose uptake by vesicles from 2- and 21-day chicks. As

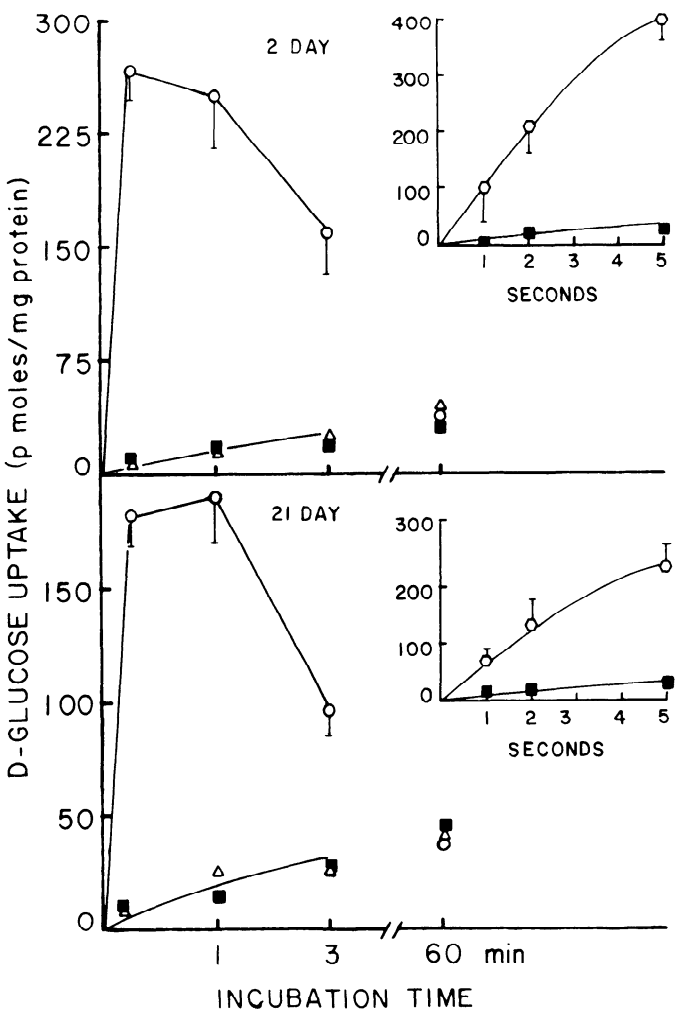

FIG. 5. Time course of 0.05 mM D-glucose uptake by jejunal brush-border membrane vesicles from 2- and 21-day chicks. Data are shown for uptakes in presence of an initial 100 mM NaCl gradient (*circles* and *hexagons*), an initial 100 mM KCl gradient (*triangles*), and an initial 100 mM NaCl gradient plus 1 mM phlorizin (*squares*). Data for short-term uptakes are shown in insets. Data are given as means ± SE from at least 4 preparations.

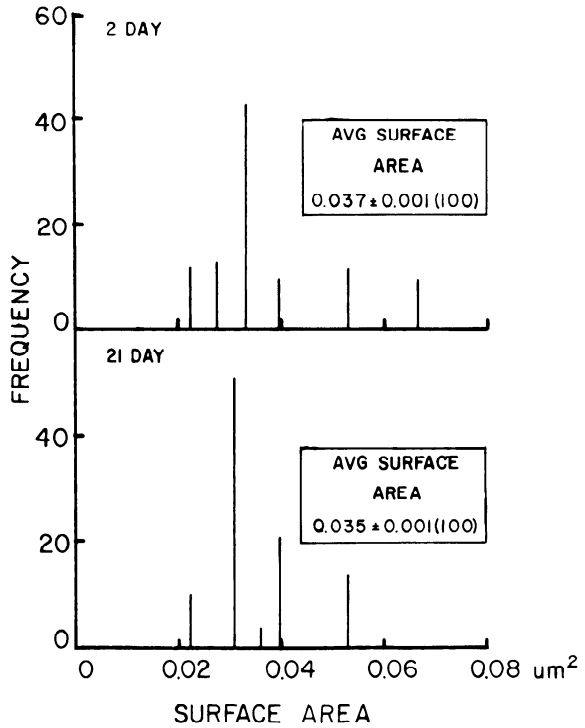

FIG. 6. Distribution of calculated surface area for randomly selected brush-border membrane vesicles from 2- and 21-day chicks.

shown in Fig. 5, uptakes of 0.05 mM D-glucose were linear through 2 s in both preparations. Other experiments showed that uptakes were linear through 2 s for all substrate concentrations through 1 mM (not shown). Longer incubation times resulted in clear-cut decreases in rates of uptake, indicative of reduced driving forces and, possibly, substrate backflux. We interpret these time-course data to mean that, even with the highest glucose concentration studied, forces driving transport were nearly constant during the first 2 s of incubation; this incubation time was thus used for experiments concerned with the concentration dependence of uptake. As demonstrated for 3-*O*-MG in jejunal slices, total D-glucose uptake by vesicles exhibited saturation kinetics; the phlorizin-uninhibitable component increased linearly with substrate concentration (Fig. 7). At each concentration studied, total and phlorizin-sensitive uptakes were significantly greater in 2-day vesicles than in 21-day vesicles; uptakes by the phlorizin-insensitive component were similar in both preparations. At each age, double-reciprocal plots of vesicle data for phlorizin-sensitive uptakes indicated that transport occurred through a single mediated component (Fig. 8). This component possessed a K_t of 0.2 mM for both preparations, but the V_{max} for 2-day vesicles was 1.4 times higher than the V_{max} for 21-day vesicles (Table 1). This change in V_{max} is somewhat lower than that found for whole tissue (Table 1). This discrepancy probably reflects the composite nature of apparent V_{max} values for vesicular transport (see DISCUSSION).

DISCUSSION

During the first 2.5 wk of embryogenesis, the developing chick obtains nutrients from the yolk sac and its extensive vascular system (25). Several studies indicate that hexose transport is detectable in the avian small intestine during the last half week of the incubation period and that transport activity increases dramatically shortly after hatching (3, 9, 19). These studies provide data on total nutrient transport (mediated plus nonme-

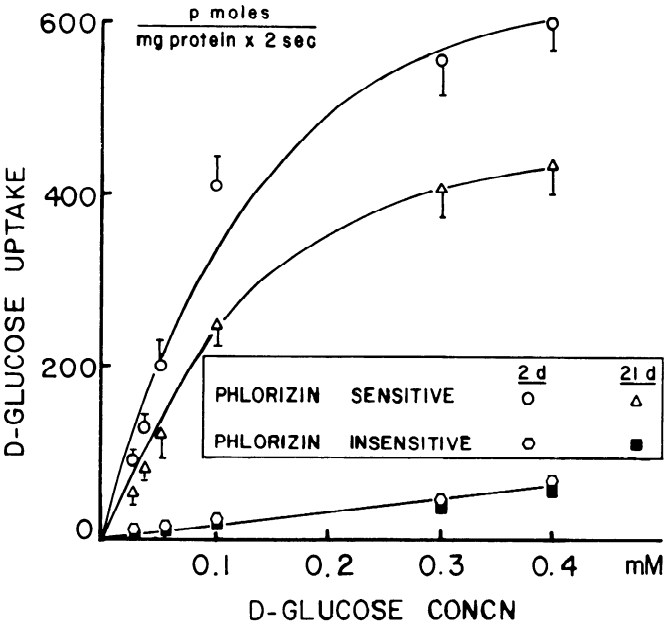

FIG. 7. Concentration dependence of D-glucose uptake (2 s) by jejunal brush-border membrane vesicles from 2- and 21-day chicks. Data are given as means ± SE from 4 preparations.

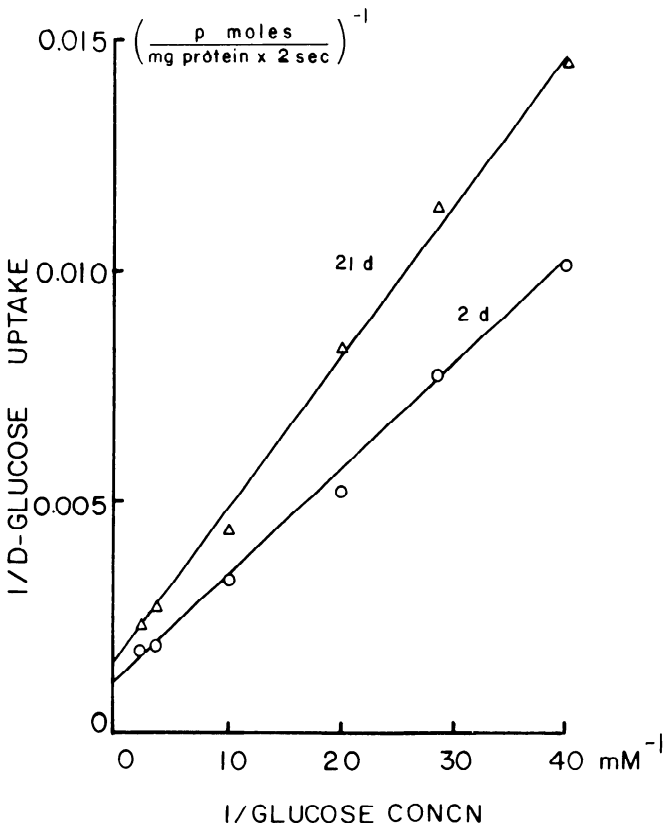

FIG. 8. Double-reciprocal plot of phlorizin-sensitive uptake data from Fig. 7.

diated), because no corrections were made for nonspecific processes or binding. The present data for total 3-*O*-MG uptake in jejunal slices from −2- to 7-day chicks are in agreement with the cited studies. Our findings for 7- to 21-day chicks, i.e., decrease in total transport over the time period, agree with the jejunal sac data of Raheja et al. (24) but not with the slice data of Bogner and Haines (3) or Holdsworth and Wilson (9); these latter authors found no decrease in transport after the 1st wk of life. They did, however, use tissue from the entire small intestine for their transport studies. In this regard, Lerner et al. (17) have demonstrated that both rates of nutrient transport and developmental timetables can vary substantially with the region of the gut studied.

In the present study, we used a blocking concentration of phlorizin, a specific inhibitor of the BBM Na-hexose cotransporter, to determine the portion of total uptake that was mediated by that transport system. In slices from −2- and 0-day chicks we found no evidence for cotransporter-mediated uptake. In contrast, uptakes in tissue from chicks older than 2 days were about 80% inhibitable by phlorizin, indicating that most of the transport was mediated by the cotransporter. Clearly, in the first 2 days after hatching, BBM hexose carriers were either synthesized or activated. Our slice data also showed that rates of mediated transport were constant and maximal from 2 to 7 days after hatch; they declined to adult levels over the next 2 wk. With BBM vesicles, rates of specific glucose transport were significantly lower in tissue from 21-day chicks than in tissue from 2-day chicks. The close agreement between vesicle and slice data strongly suggests that changes in the BBM play an important role in determining overall intestinal transport function during the early postnatal period. The use of the combined slice-vesicle approach minimizes the possibility that observed changes in vesicle transport might be due to the differential effects of the membrane isolation procedure on brush borders from different age chicks (10, 12). To our knowledge, the present study is the first to demonstrate such developmental changes in intestinal transport, using this combined approach. Preliminary combined slice-membrane vesicle data for other nutrients have shown several different developmental patterns for transport activity over the first 3 wk of life. For example, rates of myo-inositol transport increased steadily over this period, while rates of β-alanine and glutamic acid transport decreased; rates of choline transport remained the same (Shehata et al., unpublished observations). These findings suggest that Na-dependent intestinal BBM transport systems for different nutrients follow different developmental timetables. Taken together, these findings cannot be explained by any single simple change in the transport characteristics of the tissue.

The kinetic analysis used here for slices and vesicles provides apparent K_t and V_{max} values for the hexose transport system under initial rate conditions. In analogy with enzyme kinetics, K_t values should be independent of tissue preparation, and the absence of a change in K_t with development clearly indicates that certain fundamental characteristics of the carrier, e.g., substrate affinity, do not change. Because we are not measuring transport in a purified system, changes in V_{max} are more difficult to interpret. In the absence of complicating factors, one could assume that the observed decrease in V_{max} was indicative of a decrease in carrier number and/or maximal turnover rate per carrier. However, unlike K_t, V_{max} values are clearly dependent on tissue geometry, more specifically on the relation between BBM surface area and tissue weight (slices) or protein content (vesicles). With intact tissue, accurate measurements of intestinal surface areas are difficult to obtain, because of the extensive amplification of the surface by villi and microvilli (2). With vesicles, our data show that both mean intravesicular space per mg membrane protein and mean vesicular surface area were the same in BBM from 2- and 21-day chicks. However, potential alterations of, for example, the fraction of nonvesicular protein or the density of carrier proteins per unit surface area clearly confound the situation.

Finally, because kinetic parameters for vesicles were obtained under gradient conditions, the possibility of unequal dissipation of initial electrochemical gradients in the two vesicle preparations should be commented upon. On the one hand, initial experiments have indicated that 15-s uptakes of ^{22}Na by 21-day vesicles are about 70% higher than uptakes by 2-day vesicles, suggesting increased Na permeability in the 21-day preparation (Shehata et al., unpublished observations). On the other hand, time-course data clearly show that glucose uptakes by 2- and 21-day vesicles were linear for at least 2 s at all concentrations studied and that extrapolated lines for these data passed through the origin (see RESULTS). These findings suggest that driving forces for glucose transport did not change detectably during the 2-s incubation period. Although we cannot rule out the possibility that changes in rates of dissipation of the initial gradients differentially affected V_{max} values, our data indicate that such changes are, at most, small.

We thank Linda Butler and Sally Dion for excellent technical assistance.

This study was supported by National Institutes of Health Grants ES-01678, AM-19445, and RR-05764, National Science Foundation Grant 7836821, and Maine Agricultural Experiment Station Grant 5-6-48400.

Received 12 May 1980; accepted in final form 14 August 1980.

REFERENCES

1. BAMFORD, D. R., AND P. A. INGHAM. Sugar absorption by foetal and neonatal rat intestine in vitro. *J. Physiol. London* 248: 335–348, 1975.
2. BAYER, R. C., C. B. CHAWAN, F. H. BIRD, AND S. W. MUSGRAVE. Characteristics of the absorptive surface of the small intestine of the chicken from 1 day to 14 weeks of age. *Poult. Sci.* 54: 154–169, 1975.
3. BOGNER, P. H., AND I. A. HAINES. Functional development of active sugar transport in the chick intestine. *Am. J. Physiol.* 207: 37–41, 1964.
4. BUTT, J. H., AND T. H. WILSON. Development of sugar and amino acid transport by intestine and yolk sac of the guinea pig. *Am. J. Physiol.* 215: 1468–1477, 1968.
5. DEREN, J. J., E. W. STRAUSS, AND T. H. WILSON. The development

of structure and transport systems of the fetal rabbit intestine. *Dev. Biol.* 12: 467–486, 1965.
6. EVERS, C., W. HASSE, H. MURER, AND R. KINNE. Properties of brush border vesicles isolated from rat kidney cortex by calcium precipitation. *Membr. Biochem.* 1: 203–219, 1978.
7. GROSECLOSE, R., AND U. HOPFER. Small intestinal sugar and amino acid transport in semistarvation. *Membr. Biochem.* 2: 135–148, 1978.
8. HEIDRICH, H. G., R. KINNE, E. KINNE-SAFFRAN, AND K. HANNIG. The polarity of the proximal tubule cell in rat kidney. *J. Cell. Biol.* 54: 232–245, 1972.
9. HOLDSWORTH, C. D., AND T. H. WILSON. Development of active sugar and amino acid transport in the yolk sac and intestine of the chicken. *Am. J. Physiol.* 212: 233–238, 1967.
10. HOPFER, U. Isolated membrane vesicles as tools for analysis of epithelial transport. *Am. J. Physiol.* 233 (*Endocrinol. Metab. Gastrointest. Physiol.* 2): E445–E449, 1977.
11. HOPFER, U. Kinetics of Na^+-dependent D-glucose transport. *J. Supramol. Struct.* 7: 1–13, 1977.
12. HOPFER, U. Transport in isolated plasma membranes. *Am. J. Physiol.* 234 (*Renal Fluid Electrolyte Physiol.* 3): F89–F96, 1978.
13. HOPFER, U., K. NELSON, J. PERROTTO, AND K. J. ISSELBACHER. Glucose transport in isolated brush-border membrane from rat small intestine. *J. Biol. Chem.* 248: 25–32, 1973.
14. KESSLER, M., V. TANNENBAUM, AND C. TANNENBAUM. A simple apparatus for performing short-time (1–2 seconds) uptake measurements in small volumes; its application to D-glucose transport studies in brush border vesicles from rabbit jejunum and ileum. *Biochim. Biophys. Acta* 509: 348–359, 1978.
15. KINNE, R. Properties of the glucose transport system in the renal brush border membrane. In: *Current Topics in Membrane and Transport*, edited by F. Bonner and A. Kleinzeller. New York: Academic, 1976, p. 209–267.
16. LERNER, J. *A Review of Amino Acid Transport Processes in Animal Cells and Tissues.* Orono, ME: Univ. of Maine at Orono Press, 1978.
17. LERNER, J., P. H. BURRILL, P. A. SATTELMEYER, AND C. JANICKI. Developmental patterns of intestinal transport mechanisms in the chick. *Comp. Biochem. Physiol. A:* 54: 109–111, 1976.
18. LERNER, J., AND D. S. STEINKE. Intestinal absorption of glutamic acid in the chicken. *Comp. Biochem. Physiol. A:* 57: 11–16, 1977.
19. LEVIN, R. J. Digestion and absorption of carbohydrate...from embryo to adult. In: *Digestion in the Fowl* (*Proceedings of the Eleventh Poultry Science Symposium*), edited by K. N. Boorman and B. M. Freeman. Edinburgh: British Poultry Sciences, 1976, p. 96–110.
20. MAX, E. E., D. B. P. GOODMAN, AND H. RASMUSSEN. Purification and characterization of chick intestine brush border membrane. *Biochim. Biophys. Acta* 511: 224–239, 1978.
21. MILLER, D. S., D. B. PEAKALL, AND W. B. KINTER. The enzymatic basis for DDE-induced eggshell thinning in a sensitive bird. *Nature London* 259: 122–124, 1976.
22. MOOG, F. Developmental adaptations of alkaline phosphatases in the small intestine. *Federation Proc.* 21: 51–56, 1962.
23. MURER, H., AND R. KINNE. Sidedness and coupling of transport processes in small intestinal and renal epithelia. In: *Biochemistry of Membrane Transport*, edited by G. Seminza and E. Carafoli. Berlin: Springer, 1977, p. 292–304.
24. RAHEJA, K. L., J. TEPPERMAN, AND H. M. TEPPERMAN. The effect of age on intestinal glucose transport in the chick (*Gallus domesticus*). *Comp. Biochem. Physiol. A:* 58: 245–248, 1977.
25. ROMANOFF, A. L. Structural and functional development. In: *The Avian Embryo.* New York: Macmillan, 1960.
26. SACKTOR, B. Transport in membrane vesicles isolated from the mammalian kidney and intestine. In: *Current Topics in Bioenergetics*, edited by D. Rao Sanadi. New York: Academic, 1977, p. 39.
27. THOMPSON, A. B. R. Unstirred water layer and age-dependent changes in rabbit jejunal D-glucose transport. *Am. J. Physiol.* 236 (*Endocrinol. Metab. Gastrointest. Physiol.* 5): E685–E691, 1979.
28. TOGGENBURGER, G., M. KESSLER, A. ROTHSTEIN, G. SEMENZA, AND C. TANNENBAUM. Similarity in effects of Na gradients and membrane potentials on D-glucose transport by, and phloridzin binding to, vesicles derived from brush borders of rabbit intestinal mucosal cells. *J. Membr. Biol.* 40: 269–290, 1978.