Uptake Capacity In Vitro for Glucose and Methionine and In Situ for Oleic Acid in the Proximal Small Intestine of Posthatch Chicks

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ABSTRACT

The uptake capacity of the duodenum and jejunum was determined in vitro for glucose and methionine using 14C-inulin as an unabsorbed reference substance in posthatch chicks. Fatty acid uptake in situ was also determined in the duodenum.

Methionine uptake capacity increased in both duodenum and jejunum between hatch and 7 d and was constant between 7 and 14 d. Calculation of apparent Michaelis constant (Kt) for both substrates indicated little change with age and thus the amount of carrier in the intestine may be increasing. Uptake capacity of the duodenum for glucose increased between hatch and 7 d of age but no changes were found between 7 and 14 d. In the jejunum, uptake capacity was constant from hatch to 14 d of age. In situ uptake of oleic acid in the duodenum did not change between hatch and 14 d of age. These results suggest that intestinal uptake capacity changes little after 7 d of age, and thus feed intake may be the major factor controlling nutrient uptake in chicks.

(Key words: chick, intestine, glucose, methionine)

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INTRODUCTION

In the newly hatched chick, rapid development of the gastrointestinal tract allows the ingestion of sufficient exogenous feed to replace the yolk sac as the major nutrient source within 48 to 72 h after hatch (Pinchasov and Noy, 1993). Although the chick's intestinal system is anatomically complete in the embryonic stage (Overton and Shoup, 1964; Lim and Low, 1977; Chambers and Grey, 1979) the absorptive surface changes considerably posthatch and the rate of enterocyte proliferation is enhanced (Cook and Bird, 1973; Moran, 1985). Rapid changes in the morphology of the proximal small intestine occur in the immediate posthatch period from 4 to 10 d of age; villi increase in size and number with greater absorptive surface per unit of intestine. The number of enterocytes per villus also increase with age but these changes are more pronounced in the jejunum then in the duodenum (Uni et al., 1995).

Uptake of nutrients is dependent on their digestion prior to absorption from the gastrointestinal tract (GIT). Digestion in the GIT is due mainly to pancreatic enzyme activity; secretion of trypsin, amylase, and lipase was found not to increase per gram of feed intake between 4 and 14 d posthatch (Uni et al., 1995). In previous studies in which absorption has been determined in newly hatched chicks, some reports have shown that digestion of protein and starch may be less than maximal in very young birds (Noy and Sklan, 1995; Uni et al., 1995). Krogdahl and Sell (1989) have suggested that pancreatic lipase activity was limiting lipid absorption in some diets in young chicks.

The factors limiting nutrient uptake from the intestine have not been clearly clarified. Thus, the absorptive capacity of the intestine at different ages is relevant to this question and can be examined by in vitro or in situ techniques. Some in vitro determinations of intestinal uptake of glucose and nonessential amino acids have been carried out in chicks during embryonic development and up to 6 d old (Bogner and Haines, 1964; Holdsworth and Wilson, 1967; Shehata et al., 1981, 1984) or between 1 to 3 wk posthatch (Gonzalez and Vinardell, 1992). These studies utilized different intestinal sites and different probe molecules and substrate concentrations. The site of major uptake of amino acids, glucose, and fatty acids in the chick is the duodenum and the proximal jejunum (Hurwitz et al., 1979; Sklan et al., 1979; Sklan and Hurwitz, 1980). This study compared uptake capacity in duodenum and jejunum of posthatch chicks until 14 d of age for methionine, an essential and generally limiting amino acid, and for glucose. In situ uptake of oleic acid was also determined in the duodenum.

MATERIALS AND METHODS

The labeled materials 14C-inulin, 3H-glucose, 3H-methionine, 3H-oleic acid were from the Radiochemical Center.2 Care and treatment of the chicks complied

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with the guidelines of the Animal Care Committee of the Hebrew University.

Arbor Acres chicks were obtained from a commercial hatchery and were examined for *in vitro* or *in situ* uptake at 6 to 12 h posthatch, or were placed in electrically heated battery pens (2 × 1.5 m) on an 18 h light:6 h dark lighting schedule. Room temperature was maintained at 25 ± 2°C and temperature under the battery heaters was 30 and 27°C for Weeks 1 and 2, respectively. Birds consumed *ad libitum* water and a feed that met or exceeded NRC requirements (1994). At 6 to 12 h after hatch or at 7 and 14 d old, intestinal segments were removed from six chicks from the apex of the duodenum or a point 15 cm distal to the bile duct. Birds were fed until examined. Intestinal segments were washed with cold 0.9% NaCl before study. *In vitro* uptake of glucose or methionine was determined by incubation of 1-cm slices in Ringers solution gassed with O2 at 37°C using 3H-labeled glucose or 3H-labeled methionine as described by Karasov et al. (1986). In preliminary studies, a substrate concentration range from 1 to 10 mM was indicated and 15 μCi/mL of probe molecule was used. 14C-Inulin (6 μCi/mL) was used as an unabsorbed reference substance to correct for adsorption. In some tests, at 14 d, solutions were gassed with N2 instead of O2 to determine passive uptake.

In additional chicks, *in situ* uptake of 3H-oleic acid was determined from ligated 10-cm duodenal loops centered at the apex of the duodenum as previously described (Sklan and Budowski, 1977; Sklan et al., 1979). A buffered solution of 8 mM taurocholic acid and 6 mM oleic acid containing 2 μCi/mL 3H-oleic acid with 14C-inulin (1 μCi/mL) as an unabsorbed reference was injected into the loop (0.2 mL).

Radioactivity was measured by liquid scintillation with quench correction determined using internal standards of both 3H and 14C (Sklan et al., 1979).

The unidirectional uptake was calculated by the change in ratio of the respective probe molecule to the unabsorbed reference marker and uptake was expressed in micrograms per minute per gram of wet intestinal weight. In the ligated segments, mucosal uptake was defined as the amount of substrate disappearing from the lumen per centimeter segment. Serosal transport was taken to be the difference between the amount of label disappearing from the incubation medium and the amount of label remaining in the intestinal tissue. Results are expressed as percentage dose per minute per centimeter.

Results are presented after analysis of variance using the General Linear Models procedures of SAS® (SAS Institute, 1986).

**RESULTS**

*In vitro* uptake by passive diffusion of methionine and glucose was determined at 14 d in the duodenum and jejunum in the absence of O2. Assimilation under these conditions was maximal at 10 mM and was less than 33 and 38% of uptake in the presence of O2 for methionine and glucose, respectively.

Methionine uptake per gram of tissue at three ages over a concentration range from 1 to 10 mM is shown in Figure 1. At hatch, uptake was similar at low concentrations in both duodenum and jejunum. With increasing age, no change in uptake at 1 and 1.5 mM was found. However, at concentrations of 3 mM and greater, uptake was higher at 7 and 14 d than at hatch (P < 0.05). There were no significant differences between the uptake at the different substrate concentrations in both duodenum and jejunum at all ages tested, although the concentration plateau was less well defined at 7 and 14 d of age. Determination of apparent Michaelis constant (Kt) and maximum velocity (Vmax) with Lineweaver-Burk plots yielded apparent Kt values ranging from 1.5 to 4.3 mM (CV = 3.8 to 8.6%) and Vmax between 110 and 149 μmol/g per min (CV = 4.7 to 9.4%) at the different ages.

Determinations of glucose uptake in chicks from hatch to 14 d old are shown in Figure 2. At hatch, duodenal uptake was lower at glucose concentrations of 3 mM and greater than jejunal uptake. Duodenal uptake at hatch at concentrations of 5 and 10 mM was lower than that observed at 7 and 14 d. Jejunal uptake changed little with age, although the uptake exhibited a less well defined plateau at 7 and 14 d. Determination of apparent Kt and Vmax with Lineweaver-Burk plots yielded apparent Kt values ranging from 0.30 to 0.61 mM and Vmax values between 20 and 90 μmol/g per min.

*In situ* uptake of oleic acid was determined in ligated segments of the duodenum at three ages, as shown in Table 1. Little change in mucosal uptake or serosal transport per centimeter of intestine was found between hatch and 14 d samples.

TABLE 1. Uptake of oleic acid *in situ* in duodenum of chicks of different ages

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>Mucosal uptake (% dose/min/cm)</th>
<th>Serosal transport (% dose/min/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00434 ± 0.00073</td>
<td>0.00243 ± 0.00053</td>
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<tr>
<td>7</td>
<td>0.00473 ± 0.00026</td>
<td>0.00247 ± 0.00008</td>
</tr>
<tr>
<td>14</td>
<td>0.00366 ± 0.00033</td>
<td>0.00228 ± 0.00013</td>
</tr>
</tbody>
</table>

1Results are means of samples from six birds ± SD. Differences between values in columns were not different (P > 0.05).

**DISCUSSION**

This study indicates that the absorptive capacity of the proximal small intestine for glucose, methionine, and fat changed little after 7 d in the posthatch chick. In a previous study on the development of the nutrient transport systems of the whole intestine in the posthatch chick, transport of glucose became more active at hatch, peaked at about 2 d posthatch, and thereafter decreased.

**TABLE 1. Uptake of oleic acid *in situ* in duodenum of chicks of different ages**

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1Results are means of samples from six birds ± SD. Differences between values in columns were not different (P > 0.05).
In this study, birds were examined until 6 d of age (Bogner and Haines, 1964). Holdsworth and Wilson (1967) determined methyl glucoside and glycine uptake in embryos and until 6 d posthatch in whole intestines. They noted that $K_t$ values for methyl glucoside remained constant, whereas $V_{\text{max}}$ increased between hatch and 3 d of age. Transport capacity of glycine behaved differently, remaining constant after hatching. The $K_t$ values in that study were similar to the values found for glucose and methionine in the present study. Shehata et al. (1981) also reported maximal glucose uptake in the chick jejunum at 2 to 7 d of age, and in further studies Shehata et al. (1984) also noted differences in development of nutrient transport systems in the jejunum with age, with alanine and glutamine transport declining, whereas choline transport remained unchanged. These studies were paralleled by those of Gonzalez and Vinardell (1992) and Planas et al. (1982), who determined changes in vitro uptake of 3-oxy-methyl glucose and leucine at single substrate concentration at 1 to 3 wk of age and found little change. Passive glucose transport did not change with increasing age, although active transport per gram of tissue peaked at 2 mo and then declined in mice (Bird et al., 1992).

In this study, we attempted to integrate these findings and measured the capacity of the duodenum and jejunum to absorb glucose and methionine in chicks from hatch to 14 d of age over a physiological range of concentrations. Both of these compounds were absorbed by mechanisms that indicated saturation kinetics, although carrier facilitated and passive transport occur simultaneously. The latter comprised less than one third of uptake at the highest concentrations tested. Unidirectional uptake capacity of glucose from the duodenum was low at hatch and was higher at 7 d. However, in the jejunum little change was observed after hatch. In contrast, uptake of methionine in both duodenum and jejunum increased between Day 0 and 7.

The apparent $K_t$ and $V_{\text{max}}$ found here for L-methionine and glucose were similar to those reported previously in chicks (Lerner and Taylor, 1967; Shehata et al., 1981) and rats (Larsen et al., 1964). Determination of the apparent $K_t$ in this study indicated that this parameter changed little with increasing age for both

**FIGURE 1.** Uptake of methionine *in vitro* in slices of duodenum (●) and jejunum (○) per gram of tissue per minute for chicks 0, 7, and 14 d old at different concentrations. Results are means of samples from six birds per point and bars show SD when these did not fall within the circles. Uptake of methionine on Day 0 at 3 to 10 mM was lower then on Days 7 and 14 ($P < 0.05$).
Concentration, mM

FIGURE 2. Uptake of glucose in vitro in slices of duodenum (•) and jejunum (○) per gram of tissue per minute for chicks 0, 7, and 14 d old at different concentrations. Results are means of samples from six birds per point and bars show SD when these did not fall within the circles. Uptake from duodenum at 3 to 10 mM was less than from the jejunum and from uptake at 7 and 14 d (P < 0.05).

Fatty acid uptake was also examined in this study. However, in order to determine the uptake of fatty acids from aqueous medium the substrate is presented as mixed bile acid-fatty acid micelles and thus effective concentrations at the brush border do not change linearly with increasing fatty acid concentrations. In situ techniques give better estimates of fatty acid uptake than in vitro techniques, which are highly dependent on defined physical contact with the brush border (Sklan et al., 1979). A further difficulty exists in ligating the jejunum at Day 0, as this was impaired by the yolk sac. Therefore, in this study, duodenal fatty acid uptake was determined at a single micelle concentration, and this did not change, or possibly decreased slightly with age of the chick.

These findings thus allow us to speculate on some of the processes connected with nutrient uptake in the developing chick. Secretion to the duodenum of trypsin, amylase, and lipase per gram of feed intake does not increase between 4 and 14 d after hatch and digestibility in broiler strains was consistently high and this indicated that sufficient digestive enzymes were present...
except possibly at 4 d for N and for starch in light strain birds (Noy and Sklan, 1995; Uni et al., 1995). Thus, feed entering the GIT is consistently digested to a high degree, with no accumulation of digested but unabsorbed nutrients (Riesenfeld et al., 1980; Sklan et al., 1980): hence neither digestion nor uptake reach the stage at which they are rate limiting. Uptake capacity does not increase with age and some changes in the rate of passage of feed through the intestine occur posthatch (Noy and Sklan, 1995), which may influence both digestion and absorption. Thus, intake of feed appears to be a major factor controlling the amount of substrate for digestion and absorption in young chicks.

REFERENCES


