Energy Utilization in Newly Hatched Chicks

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ABSTRACT The changes in body weight and composition were examined in broilers that either had immediate access to feed and water or had not been fed for 48 h posthatch. Chicks without access to feed decreased in BW by 7.8% in the 48 h posthatch, which was equivalent to 5.3 kcal/45 g chick/d. However, during this period the small intestines increased in weight and protein content by 80% or more. The decrease in yolk fat and protein could account for most of the changes in body composition in the feed-deprived chick. In contrast, fed chicks grew by 5 g and used 4.5 kcal/d for maintenance; during this period small intestines increased in weight by 110%.

Intestinal absorption of exogenous nutrients was determined from hatch through 4 d posthatch by administration of a bolus of labeled glucose, methionine, or oleic acid, together with a nonabsorbed reference substance. Absorption of fatty acids was more than 80% at hatch and was higher than that of glucose and methionine, which was low especially when the bolus was administered as a solution. Absorption of all components tested increased with age and was more than 80% on Day 4. Duodenal *in situ* uptake determinations in hatching chicks indicated that uptake of oleic acid was high from yolk and saline solutions compared with glucose and methionine, which exhibited low uptake from yolk but higher uptake from saline solutions. These studies indicate that, although the small intestine has the capacity to absorb carbohydrates and amino acids at hatch, uptake may be dependant on the development of suitable conditions, including sufficient pancreatic and brush border enzymes for digestion and adequate sodium for function of the glucose-sodium cotransporters.

(*Key words*: chick, yolk, absorption, growth)

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INTRODUCTION

In the developing avian embryo, the sole energy supply is from the yolk (Romanoff, 1960). Twenty percent of the BW of newly hatched chicks is yolk, which provides immediate posthatch energy (Romanoff, 1960). Chicks normally forage and ingest feed following hatch, and growth commences approximately 24 h after initiation of feed intake. Under practical conditions, many birds do not have access to feed until 36 to 48 h after hatching, and, during this time, body weight decreases (Noy and Sklan, 1998). Chicks that underwent deutectomy at hatch and had access to feed began growing on Day 4 with a growth rate parallel to that of intact birds (Murakami *et al.*, 1992; Uni *et al.*, 1998). Several authors have suggested that yolk is used for maintenance, whereas exogenous energy is utilized for growth (Anthony *et al.*, 1989), although the deutectomy studies mentioned previously (Murakami *et al.*, 1992) contradict this suggestion.

Birds must undergo metabolic adaptations while moving from embryonic yolk dependence to utilization of exogenous feed. Pancreatic and brush border enzymes must be available in sufficient quantities for digestion, and, in addition, the uptake processes need to be able to transfer the required quantities of nutrients. The presence of pancreatic enzymes in the intestine has been observed during late embryonic development (Marchaim and Kulka, 1967); however, quantitative determination in the intestine immediately posthatch has not been carried out to date. From Day 4, secretion of pancreatic enzymes per gram of feed intake changed little with age (Uni *et al.*, 1996), and digestion on Day 4 of starch, protein, and fat was 85, 78, and 87%, respectively (Noy and Sklan, 1995). *In vitro* and *in situ* studies have suggested that the intestine has excess absorptive capacity at hatch for glucose, methionine (Noy and Sklan, 1996), and oleic acid (Noy and Sklan, 1998); however, *in vivo* absorption has not been determined close to hatch.

This study examines the energy supply from yolk and exogenous feed in the immediate posthatch period.

MATERIALS AND METHODS

The following labeled compounds were obtained from Amersham International[2]: inulin-[^{14}C]-carboxylic acid, ^{3}H-glucose, ^{3}H-sucrose, ^{3}H-methionine, and ^{3}H-oleic acid. ^{141}Ce was from New England Nuclear.[3]

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All procedures were approved by the Animal Care and Welfare Committee of our Institute. Male chicks (Ross) were taken immediately following hatch, defined as the time when birds completely cleared the shell. Some chicks were held for 48 h without access to feed, whereas other chicks received starter [meeting or exceeding NRC (1994) requirements] and water for *ad libitum* consumption. Six chicks were sampled for yolk weight and body composition at hatch and on Days 1, 2, 4, and 5. Intestines were removed and washed with cold 0.15 *M* saline and with air before analysis.

In Vivo Absorption

Chicks were administered a dose of 0.5 to 0.7 μCi ^{3}H-methionine, ^{3}H-glucose, or ^{3}H-oleic acid together with 0.3 μCi ^{141}Ce adsorbed per 0.1 g feed (Sklan *et al.*, 1975) or in 0.1 mL 0.02 *M* potassium phosphate buffer (pH 7.8) intubated with a 100-μL pipette. In some experiments, ^{3}H-sucrose was used. Digesta were obtained at different times after the dose was administered by flushing the gizzard, duodenum (from the pylorus to the distal point of entry of the bile ducts), jejunum (Meckel's Diverticulum marked the end point of the jejunum), ileum (the ileocaecal junction marked the end of the ileum), and the ceca with 0.15 *M* NaCl. Means from five birds were used per data point.

In Situ Uptake

In situ uptake of ^{3}H-oleic acid, ^{3}H-glucose, and ^{3}H-methionine were determined from ligated 5- to 7-cm duodenal loops centered at the apex of the duodenum by injecting the labeled compounds (0.2 μCi in 0.1 mL 0.1 *M* NaCl for glucose and methionine and 0.1 *M* NaCl and 4 m*M* taurocholic acid for oleic acid) as previously described using ^{14}C-inulin (1 μCi/mL) as nonabsorbed reference substance (Noy and Sklan, 1996). Five birds were used per data point.

Analyses

Radioactivity was measured by liquid scintillation with quench correction determined using internal standards of ^{3}H, ^{14}C, and ^{141}Ce (Sklan, 1979).

Calculations

When an unabsorbed reference substance is fed, the ratio between any dietary component and the unabsorbed reference substance can be used to calculate changes between intestinal segments. A decrease in the ratio is defined as disappearance (digestion, absorption, catabolism) and an increase as secretion or production. If the unabsorbed reference substance is administered as a bolus together with the respective probe molecule, assuming that the rate of passage of both molecules along the gastrointestinal tract is similar, then decreases in the ratio of probe to the unabsorbed reference along the gastrointestinal tract can be used to calculate the relative absorption (Hurwitz, 1972). In this study, labeled probe molecules were administered together with ^{141}Ce, the unabsorbed reference substance (Sklan *et al.*, 1975), and calculation of the relative absorption of glucose, sucrose, methionine, and oleic acid was carried out from the ratio in the administered dose and in the ileum.

The unidirectional uptake *in situ* was calculated by the change in ratio of the respective probe molecule to the nonabsorbed reference marker during incubation. 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 (Noy and Sklan, 1998). Results are expressed as a percentage dose $\times$ $min^{-1} \times cm^{-1}$.

Statistical Analysis

Least squares means of results are presented after analysis of variance using the General Linear Models procedures of SAS. Differences between means were tested using *t* tests, and significance was declared at $P < 0.05$ unless otherwise stated (SAS Institute, 1986).

RESULTS

Chicks without access to feed had decreased BW during the 48 h posthatch compared with fed chicks, whose BW increased. In this period, yolk weight (yolk sac plus yolk) declined exponentially; this decrease was more rapid in fed birds than in feed-deprived birds (Figure 1). Fat and protein contents of the yolk also decreased exponentially, and fed chicks utilized fat and protein more rapidly than feed-deprived chicks ($P < 0.05$; Figure 1). The weight and length of the small intestines increased posthatch, and, when expressed as a fraction of BW, intestinal weight and length increased relative to BW until 5 to 7 d posthatch (Figure 2). Interestingly, in birds that did not ingest exogenous feed during the initial 48 h, intestinal weight and length increased during this period, although more slowly than in fed birds. Increases in intestinal weight and length relative to BW were similar in fed and feed-deprived chicks.

Chicks consumed 6.5 g feed in the first 48 h posthatch and increased BW by 5 g (Table 1). During this period, yolk size decreased by approximately 60%, transferring almost 1 g fat and protein for utilization by the bird, and the small intestines increased in weight by more than twofold. Birds without access to feed had a decrease in BW by 3.5 g during the 48 h posthatch, and slightly less yolk, yolk fat, and protein were used than in feed-deprived birds. However, during this period, despite the lack of feed intake, the small intestines increased in weight by 80%.

Examination of the changes occurring between 2 and 4 d posthatch showed similar growth increments in fed

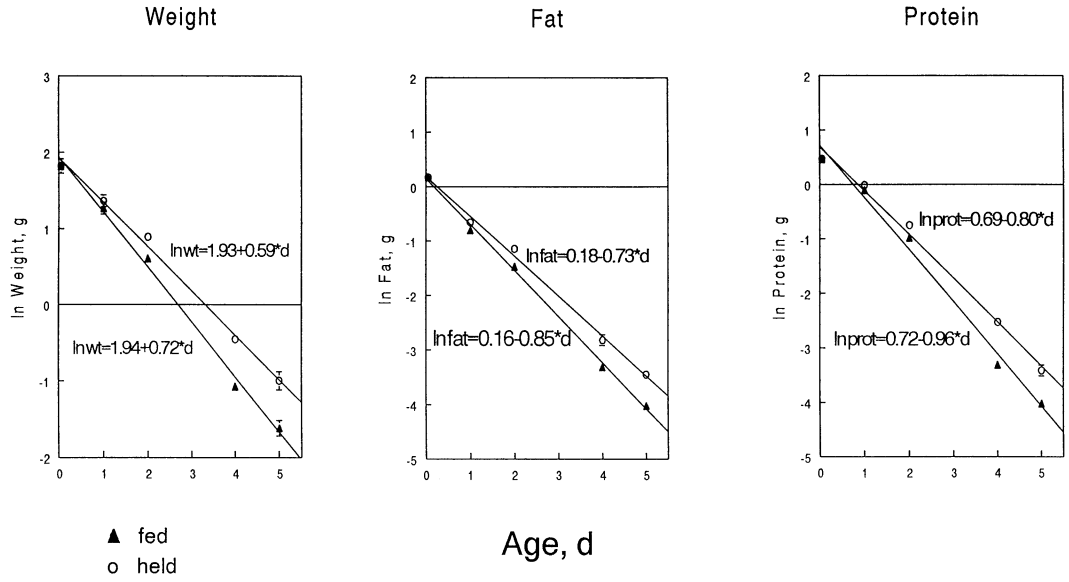

FIGURE 1. Changes in yolk weight and fat and protein contents with time after hatching in fed (triangles) and feed-deprived (circles) birds. Results are ln of means, and SD are shown where they do not fall within the data points from six birds/data point. The slope was different for weight, fat, and protein ($P < 0.05$) between fed and feed-deprived birds.

and feed-deprived chicks, although less fat was retained and intestinal development was less in the feed-deprived chicks.

Glucose absorption at hatch was low from buffer solutions and increased from 43% close to hatch to 52% on Day 1, 76% on Day 2, and nearly 90% on Day 4 posthatch (Figure 3). In some experiments, sucrose absorption was examined, and results were similar to those for glucose (data not shown). A similar pattern of low absorption at hatch was found for methionine, which increased from 53% at hatch to nearly 80% on Day 4. In contrast, oleic acid absorption was greater than 85% at hatch and increased slightly with age (Figure 3).

Uptake from solid feeds exhibited a similar pattern, although glucose (and sucrose) absorption at hatch was slightly higher and was 75 to 77% until 48 h after hatch. Methionine uptake was 58% at hatch, which increased to nearly 80% at Day 4. Oleic acid was absorbed at over 80% at hatch and increased to approximately 90% on Day 4 (Figure 3).

In situ duodenal uptake was determined from buffer or from yolk on the day of hatching (Figure 4). Mucosal uptake of glucose was fourfold higher from buffer than from yolk, and methionine mucosal transport was 3.5-fold more from buffer than from yolk. In contrast, oleic acid uptake was one-third lower from buffer than from yolk. Similar trends were observed for mucosal uptake and serosal transport.

DISCUSSION

This study has indicated that, in posthatch chicks, the yolk is utilized, in part, for growth of the intestines. In the immediate posthatch period, fat is more completely absorbed than glucose or methionine; however, once suitable conditions for uptake of these nutrients are present, absorption increases.

Examining changes in chicks with no access to feed in the 48 h posthatch indicated that BW decreased while yolk was utilized. The amount of fat and protein that disappeared during the 48 h posthatch corresponded to 5.3 kcal/bird per d. If these nutrients were all utilized for energy, then this would represent the maintenance requirement of the chicks. During this period, the intestinal weight increased by 0.88 g, of which 0.12 g was protein that originated, at least in part, from the yolk. It is possible that other body organs were mobilized for the purposes of supplying protein and possibly energy. However, the decreased content of the yolk accounted for most of the changes observed in overall body composition. Literature reports that the basal metabolic rate for a 45-g broiler was 6.5 kcal/bird per d (Kuenzel and Kuenzel, 1976) and resting metabolic rate was 10.6 kcal/d calculated using oxygen consumption data (Meltzer, 1983). In the fed birds in this study, the energy intake over the observed growth, using values for growth from Hurwitz *et al.* (1978), yields an estimate of approximately 4.5 kcal/d for maintenance. In contrast, in chicks with no access to feed, 5.3 kcal/bird per d disappeared, and this value represents feed-deprived metabolism and tissue growth. Thus, it appears that the maintenance requirements of chicks during the 48 h after hatch in a temperature-controlled environment of 32 C are in the range of 4.5 kcal/45 g bird per d, which is lower than the values reported previously.

In the hatching bird, the yolk contained 1.6 g protein, almost all of which disappeared by Day 4 after hatch. This protein may be the source for the amino acids required for the preferential gastrointestinal growth observed in all newly hatched chicks, including chicks that

had no access to feed. Examination of the protein intake in fed birds during the 4 posthatch d indicated that approximately 7 g protein were ingested, and 30% of this protein was retained, of which 0.48 g was in the small intestines. This protein retention is low compared with that found in older broilers (50%; Buyse *et al.*, 1996) and may be due to low digestion and uptake from the exogenous feed in the immediate posthatch period. Retention was thus examined in posthatch chicks.

The use of marker procedures to measure digestion is usually carried out in a steady state with respect to input-output of the marker. The percentage absorption can be calculated from the change in ratio of the observed component to marker between defined points along the gastrointestinal tract. Absolute disappearance can also be determined if the marker intake is known. However, a steady state of input-output is only reached after feeding the nonabsorbed marker for several days. Thus, it is not possible to reach this state in the newly hatched bird, although digestion and absorption have been determined at Day 4 posthatch (Noy and Sklan, 1995). In this study we used a nonsteady state procedure, administering both labeled probe molecule and nonabsorbed marker molecules together as a single bolus with the assumption that rate of passage is similar for the probe and marker (Hurwitz, 1972). This procedure then allows calculation of the relative absorption between any two points along the gastrointestinal tract from the change in ratio between the probe and the reference substance divided by the ratio of the inflow. In preliminary studies, we found similar absorption values by sampling from 30 min after administration of the bolus through 4 h.

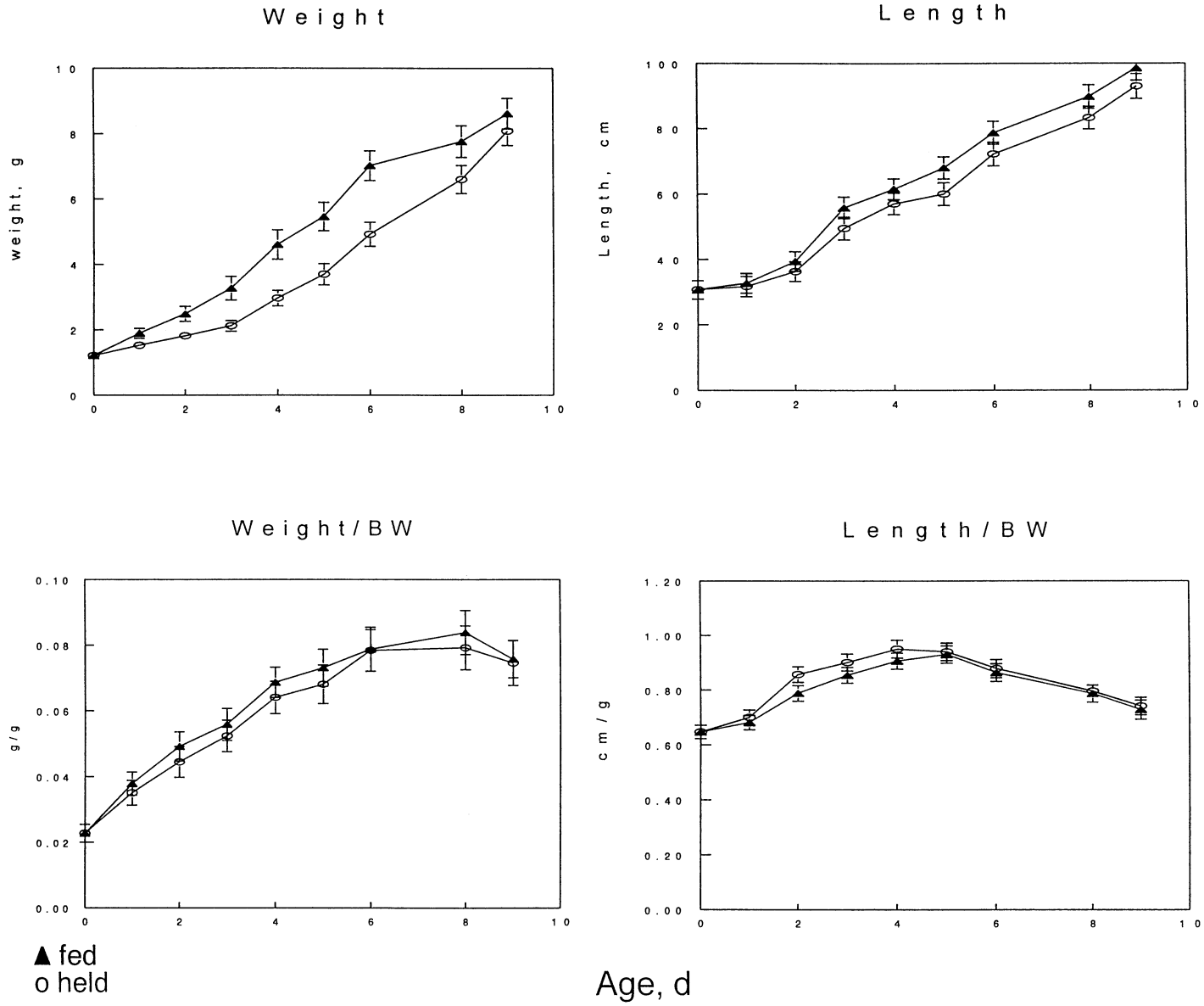

FIGURE 2. Weights and length of small intestines (pylorus to ileo-ceacal junction) and as a fraction of BW in fed (triangles) and feed-deprived (circles) birds. Results are means, and SD are shown where they do not fall within the data points from seven birds/data point. The differences between feed-deprived and fed birds were significant for intestinal weight between 1 and 7 d and for length between 5 and 7 d ($P < 0.01$).

TABLE 1. Feed intake, BW, and yolk changes (grams) between hatch and 4 d old[1]

		Change 0 to 2 d		Change 2 to 4 d	
	0 d	Fed	Feed-deprived	Fed	Feed = deprived
Feed intake					
Wet weight		6.5	0	23.8	23.1
Fat		0.25	0	0.90	0.87
Protein		1.38	0	5.07	4.92
BW					
Wet weight	45.2 ± 1.0	5.0 ± 0.6	–3.5 ± 0.7*	16.9 ± 1.2	16.0 ± 1.4
Protein	7.14 ± 0.44	0.36 ± 0.05	–0.47 ± 0.03*	1.57 ± 0.06	1.47 ± 0.10
Fat	4.20 ± 0.28	–0.45 ± 0.06	–0.92 ± 0.02*	1.10 ± 0.03	0.3 ± 0.02*
Yolk					
Wet weight	6.88 ± 1.60	–4.25 ± 0.27	–3.78 ± 0.20	–2.1 ± 0.2	–2.0 ± 0.18
Fat	1.19 ± 0.28	–0.95 ± 0.06	–0.84 ± 0.05	–0.26 ± 0.01	–0.20 ± 0.01
Protein	1.59 ± 0.24	–1.08 ± 0.04	–0.95 ± 0.08	–0.48 ± 0.02	–0.51 ± 0.02
Small intestine					
Wet weight	1.11 ± 0.10	1.37 ± 0.08	0.88 ± 0.10*	2.12 ± 0.03	1.91 ± 0.04*
Protein	0.13 ± 0.00	0.21 ± 0.01	0.12 ± 0.00*	0.27 ± 0.01	0.20 ± 0.01*

[1]Means ± SD from six birds per data point.
*Differ from fed chicks ($P < 0.05$).

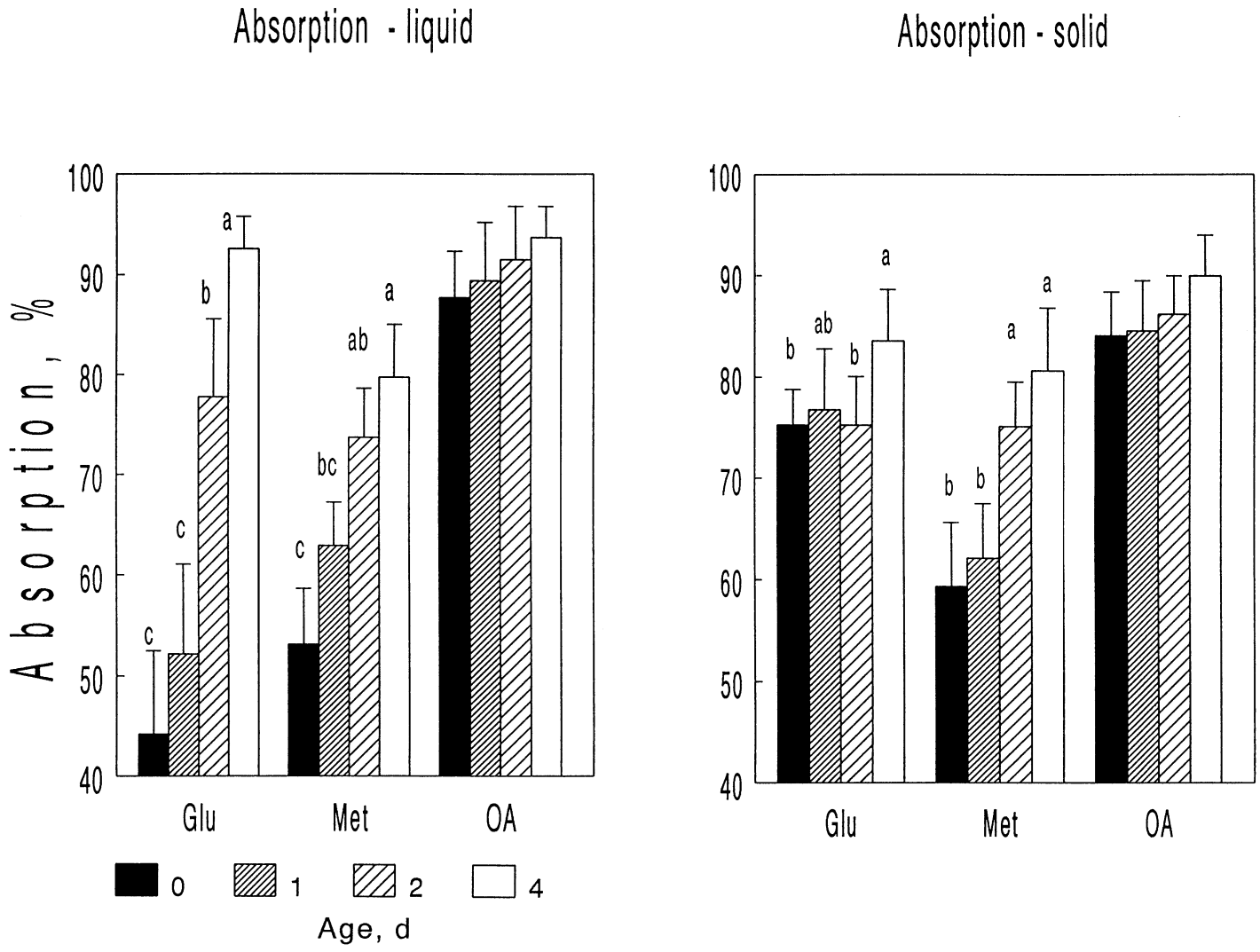

FIGURE 3. Percentage absorption of glucose (Glu), methionine (Met), and oleic acid (OA) at hatch (0) and at 1, 2, and 4 d posthatch where the labeled probe and nonabsorbed reference substance were administered in potassium phosphate buffer solutions (left panel) or adsorbed onto feed. Results are means, and bars are SD from five birds at each age. Bars not labeled with the same letter differ ($P < 0.05$).

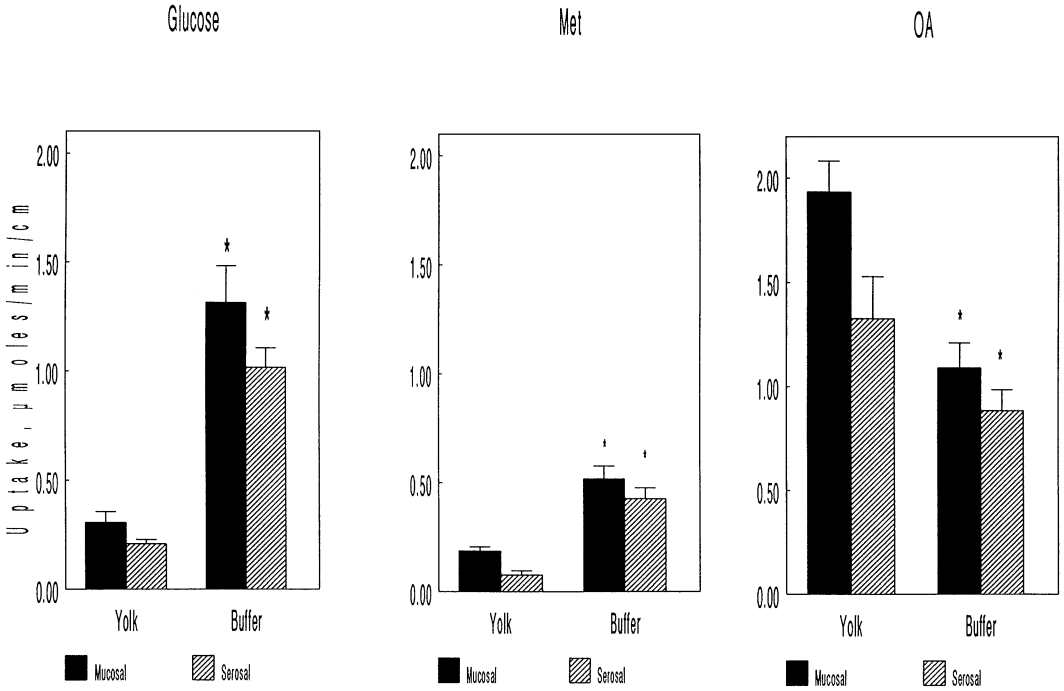

FIGURE 4. *In situ* duodenal mucosal uptake (filled bars) and serosal transport of glucose (left panel), methionine (Met; center panel), and oleic acid (OA; right panel) from yolk or from buffered solutions in hatched chicks. Results are means, and bars are SD from five birds. Asterisks indicate where uptake from yolk differed from uptake from buffer solutions ($P < 0.05$).

However, the most uniform distribution throughout the intestine was found after 60 min, and this time was used in further experiments.

We carried out one series of experiments between 0 to 4 d posthatch with solutions of probe molecules and ^{141}Ce in 20 m*M* potassium phosphate buffer (pH 7.6). Absorption of glucose and methionine was in the region of 50% within 4 h of hatch, and this absorption increased to approximately 80% by 48 h after hatch. This result was in contrast to oleic acid, which was more than 85% absorbed within hours of hatching. In a second series of experiments, labeled molecules were adsorbed onto feed particles before administration to the chick. Under these conditions, uptake of the water-soluble probes was somewhat higher than that from buffer but was still lower than absorption of oleic acid close to hatch. These results would appear to be different from previous *in vitro* (Noy and Sklan, 1996) and *in situ* studies (Noy *et al.*, 1996; Noy and Sklan, 1998) in young chicks, where glucose and methionine uptake appeared to be similar or greater than that of oleic acid and did not appear to be low at hatch.

We also performed *in situ* experiments in which duodenal uptake was determined from yolk or from buffered solutions introduced into ligated loops in chicks 1 to 4 h posthatch. Oleic acid showed close to twofold higher uptake from yolk than from buffer solutions. Glucose and methionine showed very low uptake from yolk, but uptake was approximately threefold higher from buffered solutions. These observations may be explained as follows: first, oleic acid in the hydrophobic environment of the yolk-filled small intestine is conveyed to the brush border and taken up more efficiently than from aqueous solutions. On the other hand, glucose and methionine are water-soluble, nonlipophillic molecules, and the presence of yolk will inhibit their reaching the brush border membrane for uptake. Second, our previous *in vitro* and *in situ* uptake determinations (Noy *et al.*, 1996; Noy and Sklan, 1996; Noy and Sklan, 1998) were carried out with solutions containing 0.15 *M* Na^+, whereas here, in the *in vivo* and *in situ* yolk uptake studies, Na concentrations were below 0.05 *M*. Uptake of glucose and methionine, at least in part, requires sodium cotransport (Stevens *et al.*, 1984), and sufficient sodium was apparently present in the aqueous-buffered *in situ* and *in vitro* uptake studies. However, it may be that duodenal sodium concentrations *in vivo* in hatching birds are not sufficient for cotransport prior to intake of exogenous sodium sources.

Thus, on the basis of the experiments performed here, uptake of exogenous protein and carbohydrates are low immediately posthatch. Previous studies have, however, indicated that carbohydrate digestion was 85% and protein digestion was 78% at 4 d posthatch (Noy and Sklan, 1995; Uni *et al.*, 1995). It appears from the present study that digestion of nonlipid materials increases in the immediate posthatch period, reaching 80% or higher by Day 4 as in the previous report. If N uptake near hatch is of the order of magnitude found here for methionine (50 to 65%), then this may explain the lower N retention between 0 and 2 d as compared with older birds.

Studies by Moran (1988) in the posthatch poult indicated that subcutaneous glucose was more effective for growth and energy supply than oral administration,

which the author attributed to an immature intestine. This conclusion would be parallel to the low uptake of glucose from the small intestine as found in this study. Other authors (Jin *et al.*, 1998) have also suggested that carbohydrate and protein utilization is lower close to hatch than in older birds.

It appears that in the immediate posthatch period there is preferential growth of the small intestine using precursors derived from the yolk, which also supplies energy for maintenance. Fatty acids are taken up efficiently from yolk and ingested feed; however, carbohydrates and amino acids from exogenous feed are absorbed when the conditions are appropriate. These conditions include the presence of adequate enzymatic activity and sufficient sodium to operate the sodium glucose and amino acid cotransporters.

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