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Metabolism of Isolated Chick Small Intestinal Cells. Effects of Ammonia and Various Salts†

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ABSTRACT: The metabolic responses to ammonia were studied in cells isolated from the small intestine of the chick. Control cells produced $^{14}CO_2$ and lactate and incorporated ^{14}C from [6-^{14}C]glucose into lipid and protein linearly with time. However, their incorporation of ^{14}C from glucose into RNA and DNA declined with time. Ammonia caused a generalized stimulation of glycolysis and of the tricarboxylic acid cycle. This was demonstrated by an increased rate of lactate and $^{14}CO_2$ production from [U-^{14}C]glucose and an increased rate of $^{14}CO_2$ production from [2-^{14}C]pyruvate, [1,5-^{14}C]citrate, [1,4-^{14}C]succinate, and α-[1-^{14}C]ketoglutarate. Cells from chicks beyond 10–15 days of age responded to ammonia by producing more lactate than cells from younger chicks. Ammonia concentrations as low as 1 mM stimulated glucose and citrate metabolism. Ammonia concentrations in the small intestine were 1.2–1.9 mM and reached 6–10 mM in the ceca. It is concluded that quantities of ammonia normally present in the gastrointestinal tract can stimulate carbohydrate metabolism by intestinal cells and alter their life span.

The effects of ammonia (*i.e.* NH_3 plus NH_4^+) upon the metabolism of brain and liver have been extensively studied (Saheki *et al.*, 1971; Worcel and Erecinska, 1962; McKhann and Tower, 1961). Normally peripheral tissues are protected from this highly toxic metabolite by detoxication mechanisms which maintain tissue fluid ammonia below 0.1 mM. Cells continuously exposed to unusually high concentrations of ammonia are those of the gastrointestinal mucosa. Endogenous and bacterial enzymes acting upon nitrogenous substrates release large amounts of ammonia in the bowel (Warren and Newton, 1959; Wilson *et al.*, 1968a). *In vivo* dialysates of human colon contents have been shown to range from 2.7 to 44 mM in ammonia (Wrong *et al.*, 1965). Such concentrations are sufficient to destroy other cells of the body (Dang and Visek, 1968). Normally from 15 to 30% of the urea synthesized by the liver in simple stomached mammals is recycled from the blood into the gastrointestinal tract where it is hydrolyzed to ammonia and CO_2 (Walser and Bodenlos, 1959). This process releases about 3.5 g of ammonia N per day in man. During uremia, ammonia in colon dialysates of human patients has been as high as 75 mM (Wilson *et al.*, 1968b; Wrong *et al.*, 1970).

The present studies were conducted with isolated cells from the small intestine of chicks. The purpose was to examine their response to varying concentrations of ammonia and to gain information about their metabolic characteristics which allow them to function in an environment containing ammonia concentrations which would destroy or seriously impair the function of other cells. The results are compared to those of similar studies conducted concurrently in our laboratory with isolated brain cells (Gibson, 1973). In most of the experiments ammonia was generated from urea by urease since this is a usual and probably major source of ammonia in the gastrointestinal lumen.

Experimental Procedure

Materials. Male, white, 7- to 49-day-old, Leghorn chickens obtained from Dekalb-Marshall Hatcheries, Ithaca, N. Y., provided all of the cells. In exploratory studies a Krebs–Ringer phosphate buffer was used which contained: NaCl, 120 mM; KCl, 4.8 mM; $CaCl_2 \cdot H_2O$, 2.6 mM; K_2HPO_4, 12.5 mM; KH_2PO_4, 3.1 mM; and $MgSO_4 \cdot 7H_2O$, 1.2 mM. Subsequently, a modified Krebs–Ringer phosphate buffer essentially as described by Ram *et al.* (1963) with Ca added proved more satisfactory. The latter contained: NaCl, 12.5 mM; Na_2HPO_4, 85 mM; KCl, 5 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; and $MgSO_4 \cdot 7H_2O$, 1.3 mM. Its pH was adjusted to 7.4 with HCl. All buffers and solutions contained 1 mg/ml of bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.). Buffers used for cell isolation contained 1 mg/ml of hyaluronidase. All cell preparations and experiments were carried out

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in siliconized glassware or in plastic laboratory ware. ^{14}C-Labeled substrates were obtained from New England Nuclear Corp. (Boston, Mass.).

Preparations of Epithelial Cells. Intestinal epithelial cells from three or four chickens were prepared for each experiment as described by Kimmich (1970). Intestinal segments were incubated for 30 min at 37° in hyaluronidase containing buffer and the resulting suspension was strained through nylon (110 mesh). The nylon was wrapped around the retained material and the mass was subjected to slight pressure with forceps to increase the yield of cells. After the suspensions were centrifuged at 100*g* for 1 min, the cells were resuspended in incubation media and centrifuged again. Following this washing, the cellular pellet was resuspended in 4–8 vol of buffer.

Measurement of Metabolic Activity. $^{14}CO_2$ from ^{14}C-labeled substrates was collected employing methods similar to those of Kimmich (1970). The cell suspensions (0.6 ml) were added to 3.4 ml of buffer containing substrate in 25-ml Erlenmeyer flasks equipped with center wells. Small glass vials containing filter paper (2 × 2 cm) and 0.3 ml of hyamine hydroxide were placed in the wells (Husain and Paradise, 1973). The incubation flasks were closed with serum stoppers and maintained at 37° in a water bath for the period of incubation while shaken at 90 cpm. Following incubation the reactions were stopped with 1.0 ml of 1.2 N $HClO_4$ injected through the stopper. Appropriate blanks were prepared by adding $HClO_4$ to the buffered medium before the cell suspension and incubation followed at 37° in the manner described for flasks containing viable cells. After an additional hour of shaking, the vials plus hyamine and filter paper were removed and dropped directly into 15 ml of liquid scintillation cocktail (Aquasol, New England Nuclear, Boston, Mass.). Samples for counting were held for 3 to 5 hr at room temperature before being cooled for radioactive assay in a scintillation spectrometer. The channels ratio method was used for determining quench corrections. Counting times were adjusted to give a maximum counting error of 1% or a maximum counting time of 10 min.

Cell viability, estimated from the percentage of cells which excluded nigrosin dye, usually ranged from 70 to 80% (Kaltenbach *et al.*, 1958).

Lipid, protein, RNA, and DNA were isolated according to Glazer and Weber (1971). RNA and DNA concentrations were determined by the methods of Ceriotti (1955) and Hubbard *et al.* (1970), respectively. Lactate was determined by the method of Hohorst (1965) and ammonia by the method of Chaney and Marbach (1962). Protein was determined using the biuret reaction (Gornall *et al.*, 1949) with bovine serum albumin as the standard. Cell protein was precipitated with $HClO_4$, centrifuged, and redissolved with 1 N KOH before assay.

Treatments. Ammonia was generated in the incubation medium by treating appropriate quantities of urea with crystalline Jack bean urease (Visek *et al.*, 1967) for 30 min; then the pH was adjusted with HCl to 7.4. The modified KRP buffer proved more satisfactory than standard buffer for maintaining pH when ammonia was released into the medium. To determine if the observed effects were due to ammonia and not to other moieties, the effects of equimolar concentrations of various ammonium, sodium, and potassium salts were also compared.

Ammonia in Intestinal Contents. Dry matter in segments of the intestinal contents was determined on aliquots dried in preweighed dishes at 70° for 12 hr. A second aliquot from each segment was diluted with $^1/_{12}$ N H_2SO_4 in a scintillation vial and capped. After thorough mixing a weighed aliquot of the supernatant was transferred to incubation flasks like those used for $^{14}CO_2$ collection. Ammonia released by addition of 1 ml of saturated K_2CO_3 was collected on filter paper moistened with 0.3 ml of 1 N H_2SO_4. The ammonia collected was diluted with $^1/_{12}$ N H_2SO_4 and assayed by the method of Chaney and Marbach (1962).

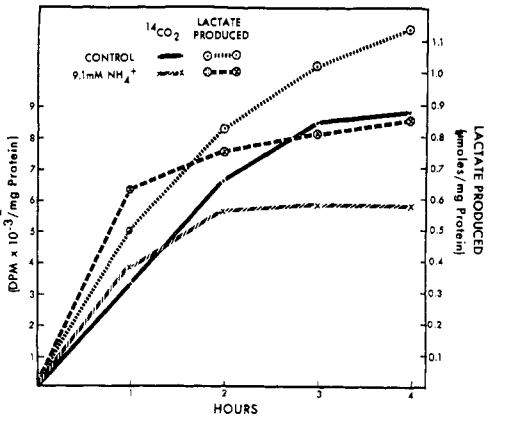

FIGURE 1: $^{14}CO_2$ and lactate production from [U-^{14}C]glucose by isolated cells of 21-day-old chicks incubated in modified KRP buffer and 5 mM glucose. Ammonia for treated cells (9.1 mM) was generated by incubating urea with urease.

Results

Initial experiments showed that the isolated cells released $^{14}CO_2$ and produced lactate at a nearly linear rate for 2–3 hr (Figure 1). When incubated with ammonia, their rate of lactate and $^{14}CO_2$ production from [U-^{14}C]glucose was faster than control during the first hour. However, by the end of 2 hr, CO_2 and lactate production had virtually ceased. The cells incorporated ^{14}C from [6-^{14}C]glucose into lipid, protein, RNA, and DNA as shown in Figure 2a,b. Incorporation of ^{14}C into lipid and protein was almost linear with time (Figure 2a). The incorporation into RNA and DNA was not linear as a function of time (Figure 2b) probably because of decreased pentose phosphate shunt pathway activity. A decline in metabolism *via* the pentose phosphate shunt is indicated by the decline in $^{14}CO_2$ from [1-^{14}C]glucose compared to that from [6-^{14}C]glucose. The ratio of [1-^{14}C]glucose/[6-^{14}C]-glucose metabolized to $^{14}CO_2$ *in vitro* was 1.31, 1.07, and 0.93 in cells incubated in control media after 20, 40, and 60 min, respectively.

In our initial studies we assumed that cells from chicks varying from 1 to 6 weeks in age would respond similarly to ammonia (Kimmich, 1970). However, cells from chicks older than 15 days produced more lactate when incubated with glucose and approximately 10 mM NH_4^+ than cells from 5- to 10-day-old chicks (Table I).

Increasing the pH stimulated glucose metabolism and inhibited citrate metabolism (Table II). However, incubation of cells from 47-day-old chicks with ammonia (10 mM) stimulated the metabolism of glucose by about 23% and instead of an inhibition in citrate metabolism, caused a 50% increase. The response was the same at pH 7.1 or 7.4.

NaCl, $NaHCO_3$, and Na_2SO_4 did not alter $^{14}CO_2$ production from [U-^{14}C]glucose appreciably compared to control (Tables III and IV). NH_4Cl, NH_4HCO_3, $(NH_4)_2SO_4$, and urea +

TABLE I: Effect of Age of Chick on the Response in Lactate Production to Incubation of Isolated Cells of the Small Intestine *in Vitro* in KRP Buffer with 8–10 mM NH_4^+ Generated from Urea by Urease.[a]

Days of Age	Ratio of Lactate NH_4^+ Treated/Control
5–6[b]	1.030 ± 0.005
8–10[b]	1.051 ± 0.016
14–19[c]	1.147 ± 0.025
29–35[d]	1.317 ± 0.075
45–50[e]	1.396 ± 0.167

[a] Data presented as mean ± standard error of the mean from independent experiments representing the ratio of the mean of three or four controls to the mean of three or four treated flasks. [b] Mean of two experiments. [c] Mean of seven experiments. [d] Mean of eight experiments. [e] Mean of three experiments.

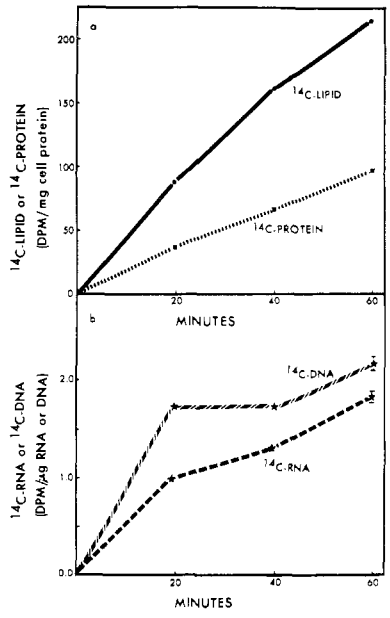

FIGURE 2: Incorporation of [6-^{14}C]glucose into lipid and protein (a) and RNA and DNA (b) of isolated cells from chick small intestine. Cells were incubated for the indicated time in normal KRP buffer containing 5 mM glucose. Each point is a mean of four observations.

urease all simulated $^{14}CO_2$ production from [U-^{14}C]glucose at all ages, but KCl stimulated $^{14}CO_2$ production only in cells from chicks 35 and 47 days of age (Tables III and IV). Lactate production in 35- and 47-day-old chicks was stimulated by KCl, NH_4Cl, NH_4HCO_3, $(NH_4)_2SO_4$, and urea + urease.

The stimulation in $^{14}CO_2$ and lactate production from [U-^{14}C]glucose occurred with 1 mM NH_4^+ produced from urea + urease. A stimulation of 20.5 and 16% occurred in $^{14}CO_2$ and lactate production, respectively, from [U-^{14}C]glucose by cells from 50-day-old chicks. The per cent increases in $^{14}CO_2$ and lactate production produced by NH_4^+ concentrations of 5–10 mM were 31.7 and 24.3%, respectively. With cells from 5-day-old chicks the stimulation in lactate production with KCl and urea + urease was much less pronounced than by cells from 35-day-old chicks (Table IV). Incorporation of [U-^{14}C]-glucose into lipids was not consistently affected by various ammonium salts (Table IV).

The production of $^{14}CO_2$ from [2-^{14}C]pyruvate was stimulated by 10 mM KCl, 9.8 mM NH_4HCO_3, and urea + urease ($[NH_4^+]$ = 10 mM). Relative to the Na_2SO_4 control, $(NH_4)_2SO_4$ also stimulated release of $^{14}CO_2$ from [2-^{14}C]pyruvate (Table V).

All sources of ammonium increased the metabolism of [1,5-^{14}C]citrate and [6-^{14}C]citrate to $^{14}CO_2$ by cells from 5-, 35-, and 46-day-old chicks (Tables VI and VII). Maximum stimulation of citrate metabolism occurred at about 5 mM NH_4^+. Incorporation of [1,5-^{14}C]citrate into lipids was markedly stimulated by ammonia (Table VII). Ammonia (9.5 mM) generated from urea by urease also stimulated $^{14}CO_2$ production from [1,4-^{14}C]succinate and α-[1-^{14}C]keto-glutarate.

The ammonia concentrations in gastrointestinal contents of chicks exceeded 1 mM in all segments of the gastrointestinal tract and reached 6–10 mM in the cecum. Except for the small intestine, 21-day-old chicks had lower gastrointestinal ammonia concentrations than 49-day-old chicks.

Discussion

The studies described provide data confirming that cells isolated from the mucosa of the chick small intestine are a

TABLE II: Effects of pH and NH_4^+ on the Metabolism of [U-^{14}C]Glucose and [1,5-^{14}C]Citrate by Isolated Cells from the Small Intestine of 47-Day-Old Chicks.[a]

	[U-^{14}C]Glucose			[1,5-^{14}C]Citrate	
	$^{14}CO_2$[b]	Lactate[c]	$[NH_4^+$–N][d]	$^{14}CO_2$[b]	$[NH_4^+$–N][d]
		pH 7.1			
Control	2626 ± 18 (1.00)	278 ± 5 (1.00)	0.1	8,969 ± 70 (1.00)	0.1
$+NH_4^+$	3230 ± 14 (1.23)	478 ± 5 (1.72)	9.9	13,467 ± 66 (1.50)	9.9
		pH 7.4			
Control	3044 ± 30 (1.10)	310 ± 7 (1.12)	0.1	7,607 ± 140 (0.85)	0.1
$+NH_4^+$	3618 ± 28 (1.37)	535 ± 7 (1.92)	9.9	11,991 ± 40 (1.34)	10.0

[a] Cells were incubated for 1 hr in modified KRP buffer (pH 7.1 or 7.4) with 5 mM [U-^{14}C]glucose or 1 mM [1,5-^{14}C]citrate. Data expressed as mean ± standard error of the mean of three observations per treatment. Ratio of treated/control (pH 7.1) presented in parentheses. [b] Dpm/mg of cell protein per hr. [c] nmol of lactate produced/mg of cell protein per hr. [d] mmol of NH_4^+–N/l.

TABLE III: Effects of Na^+, K^+, and Various Ammonium Salts on the Metabolism of [U-^{14}C]Glucose by Isolated Cells from the Small Intestine of 47-Day-Old Chicks.[a]

	$^{14}CO_2$[b]	Lactate Production[c]	$[NH_4^+$–N][d]
Control	2735 ± 5 (1.00)	431 ± 19 (1.00)	0.1
NaCl (10 mM)	2745 ± 29 (1.00)	422 ± 8 (0.98)	0.1
KCl (10 mM)	3470 ± 42 (1.27)	692 ± 33 (1.61)	0.1
NH_4Cl	3402 ± 32 (1.24)	544 ± 12 (1.26)	9.9
$NaHCO_3$ (10 mM)	2863 ± 45 (1.05)	441 ± 45 (1.02)	0.1
NH_4HCO_3	3545 ± 33 (1.30)	506 ± 25 (1.17)	10.0
Na_2SO_4 (10 mM)	2761 ± 25 (1.01)	438 ± 6 (1.02)	0.1
$(NH_4)_2SO_4$	3408 ± 100 (1.25)	542 ± 13 (1.26)	10.0
Urea + urease	3565 ± 26 (1.30)	525 ± 38 (1.22)	9.8

[a] Cells were incubated for 1 hr in modified KRP buffer (pH 7.4) with 5 mM glucose plus [U-^{14}C]glucose (6.81×10^5 dpm). Data are corrected to 1×10^6 dpm of [^{14}C]glucose and expressed as mean ± standard error of the mean of three observations/treatment. Ratio of treated/control presented in parentheses. [b] Dpm/mg of cell protein per hr. [c] nmol/mg of cell protein per hr. [d] nmol of NH_4^+–N/l.

TABLE V: Effects of Various Na^+, K^+, and NH_4^+ Salts on the Metabolism of [2-^{14}C]Pyruvate by Isolated Cells from the Small Intestine of 48-Day-Old Chicks.[a]

	Treated/Control		
	$^{14}CO_2$	^{14}C-Labeled Lipids	$[NH_4^+$–N][b]
NaCl (10 mM)	0.99 ± 0.01	1.17 ± 0.02	0.1
KCl (10 mM)	1.23 ± 0.01	0.63 ± 0.03	0.1
NH_4Cl	1.00 ± 0.01	1.11 ± 0.03	9.3
$NaHCO_3$ (10 mM)	1.00 ± 0.01	1.46 ± 0.06	0.1
NH_4HCO_3	1.14 ± 0.02	1.62 ± 0.07	9.8
Na_2SO_4 (10 mM)	0.94 ± 0.01	1.14 ± 0.02	0.1
$(NH_4)_2SO_4$	1.00 ± 0.02	1.21 ± 0.10	9.9
Urea + urease	1.13 ± 0.01	1.56 ± 0.03	10.0

[a] Cells were incubated for 1 hr in KRP buffer (pH 7.4) with 6.25 mM Na-pyruvate + [2-^{14}C]pyruvate (2.53×10^6 dpm). Data are presented as mean ± standard error of the mean of four treated flasks divided by mean of control flasks ± standard error of the mean. Cells incubated in control media metabolized 143 and 0.31 nmol of [2-^{14}C]pyruvate/mg of cell protein per hr to $^{14}CO_2$ and ^{14}C-labeled lipids, respectively. [b] mmol of NH_4^+–N/l.

suitable experimental model for metabolic studies. Similar intestinal cell preparations have been used extensively to study absorptive processes (Reiser and Christiansen, 1971a,b; Sayeed and Baue, 1973). The linear production of $^{14}CO_2$ and lactate and the linear incorporation of glucose into lipid and protein indicate that pathways of lipid and protein synthesis and overall metabolism were functioning. The decline in the ratio of [1-^{14}C]glucose to [6-^{14}C]glucose metabolized to $^{14}CO_2$ indicates that the activity of the pentose phosphate pathway declined with duration of incubation. This undoubtedly was also responsible for a decreasing rate of incorporation of [6-^{14}C]glucose into RNA and DNA with increasing time of incubation (Figure 2).

The response to ammonia was a generalized stimulation of glycolysis and the tricarboxylic acid cycle. Ammonia is released in all segments of the alimentary tract of mammals by the action of bacteria; the main precursor in man is endogenous urea (Walser and Bodenlos, 1959). Ammonia concentrations determined in the stomach, cecum, and colon of horses have varied from 5 to 10 mM and in sheep they have

TABLE IV: Effects of NH_4^+, Na^+, and K^+ on the Metabolism of [U-^{14}C]Glucose by Isolated Cells from Small Intestine of Chicks.[a]

	5-Day-Old Chicks				35-Day-Old Chicks			
	$^{14}CO_2$[b]	^{14}C-Labeled Lipids[b]	Lactate Production[c]	$[NH_4^+$–N][d]	$^{14}CO_2$[b]	^{14}C-Labeled Lipids[b]	Lactate Production[c]	$[NH_4^+$–N][d]
Control	1822 ± 6 (1.00)	323 ± 6 (1.00)	604 ± 16 (1.00)	0.1	3036 ± 17 (1.00)	258 ± 7 (1.00)	503 ± 19 (1.00)	0.1
NaCl (10 mM)	1835 ± 6 (1.01)	312 ± 4 (0.97)	604 ± 10 (1.00)	0.1	2969 ± 12 (0.98)	294 ± 5 (1.14)	480 ± 19 (0.95)	0.1
KCl (10 mM)	1903 ± 53 (1.04)	303 ± 2 (0.94)	632 ± 3 (1.05)	0.1	3609 ± 17 (1.19)	274 ± 2 (1.06)	679 ± 21 (1.35)	0.1
NH_4Cl	2452 ± 32 (1.35)	284 ± 1 (0.88)	718 ± 55 (1.19)	10.2	3761 ± 34 (1.24)	248 ± 3 (0.96)	626 ± 20 (1.24)	10.5
Urea + urease	2966 ± 25 (1.63)	334 ± 5 (1.03)	620 ± 32 (1.03)	10.0	4450 ± 26 (1.47)	280 ± 1 (1.09)	686 ± 14 (1.36)	10.6

[a] Cells were incubated in modified KRP buffer (pH 7.4) for 60 min with 5 mM glucose plus [U-^{14}C]glucose (9.29×10^6 and 8.97×10^6 dpm on 5- and 35-day-old chicks, respectively). Data are expressed as mean ± standard error of the mean of three observations/treatment. Ratio of treated/control presented in parentheses. [b] Dpm/mg of cell protein per hr per 10^6 dpm of [U-^{14}C]glucose. [c] nmol/mg of cell protein per hr. [d] mmol of NH_4^+–N/l.

TABLE VI: Effects of Various Na^+, K^+, and NH_4^+ Salts on the Metabolism of [1,5-^{14}C]Citrate by Isolated Cells from the Small Intestine of 46-Day-Old Chicks.[a]

	$^{14}CO_2$	
	Treated/Control	$[NH_4^+-N]$[b]
NaCl (10 mM)	0.940 ± 0.006	0.1
KCl (10 mM)	1.081 ± 0.008	0.1
NH_4Cl	1.230 ± 0.009	9.6
$NaHCO_3$ (10 mM)	0.765 ± 0.002	0.1
NH_4HCO_3	0.955 ± 0.009	9.6
Na_2SO_4 (10 mM)	0.993 ± 0.006	0.1
$(NH_4)SO_4$	1.256 ± 0.006	9.5
Urea + urease	1.161 ± 0.003	9.5

[a] Cells were incubated for 1 hr in modified KRP buffer (pH 7.4) with 1 mM citrate plus [1,5-^{14}C]citrate (6.914×10^5 dpm). Data are presented as mean ± standard error of the mean of three or four treated flasks divided by the mean of control flasks containing no additional salts. Cells incubated in control buffer produced 3676 dpm of $^{14}CO_2$/mg of cell protein per hr per 10^6 dpm of [1,5-^{14}C]citrate. [b] mmol of NH_4^+–N/l.

approached 10 mM in all segments of the digestive tract (Hecker, 1971). The present experiments shown that ammonia in the digesta of avian species may reach 10 mM in the ceca and large bowel but remains about 1–1.5 mM in the small intestine. The lower ammonia concentrations in the cecum and colon of 21- *vs.* 49-day-old chicks suggest that intestinal ammonia increases as the chick matures.

As in other tissues, phosphofructokinase of jejunal mucosa is one of the rate-controlling enzymes of glycolysis and may be responsible for adaptation to ammonia (Srivastava and Hubscher, 1966). Pritchard and Lee (1972) reported that citrate did not alter lactate production by the chick intestine at 7 days of age but caused decreased lactate production in older chicks. Lohmann and coworkers (1966) showed that glycolysis of chick intestinal epithelium changed from anaerobic to predominately aerobic at about 6 days after hatching. Wallace and Newsholme (1967) found that phosphofructokinase activity in the liver of chickens increased several-fold in the first 8 days of life. If a similar change in intestinal phosphofructokinase activity occurs, a relatively sudden change in the glycolytic response to ammonia and citrate could occur after 7 days. The maximal velocity of phosphofructokinase activity in rat jejunal mucosa increases about 3.3-fold with 0.3 mM NH_4^+ and saturating concentrations of fructose 6-phosphate (5 mM) (Tejwani and Ramaiah, 1971). These authors suggested that the relatively high concentrations of NH_4^+ and fructose 6-phosphate with a relatively high ADP–ATP ratio may be responsible for the high aerobic glycolytic rate and the lack of a Pasteur effect in this tissue. K^+ has also been observed to stimulate phosphofructokinase but not to the same degree as NH_4^+ (Lowry and Passonneau, 1966). In the present experiments, addition of 10 mM KCl stimulated the metabolism of [U-^{14}C]glucose and lactate production by cells from older chicks *in vitro* (Tables III and IV). KCl also stimulated the metabolism of [2-^{14}C]pyruvate (Table V) and [1,5-^{14}C]citrate (Table VI). The stimulatory effects of KCl on citrate metabolism in 46-day-old chicks were not observed in 5- or 35-day-old chicks (Table VII).

The data show clearly that ammonia concentrations normally in the small intestine of the chick are sufficient to stimulate metabolism of intestinal cells *in vitro*. According to present data, isolated cells of the chick small intestine respond differently to ammonia than isolated rat brain cells (Gibson, 1973).

An area which needs further study is the influence of ammonia upon the entry of organic acids into cells. The differences in citrate metabolism with changes in pH (Table II) may have been due to differential rates of citrate uptake. It would be expected that higher pH would increase ionization of citrate and less would be metabolized because less would enter cells by diffusion. Little is known of the processes which move Krebs cycle acids across animal cells, although a common step in their transfer by intestinal cells has been suggested (Atfield *et al.*, 1971). The present data do not exclude the possibility that a faster rate of metabolism is the result of a greater uptake of organic acids by intestinal cells when ammonia is present. If this were the case, a similar stimulatory effect on glucose uptake would have to be postulated, however.

Simpson and Angielski (1973) suggested that the concentration of bicarbonate ion regulates citrate transport across the kidney mitochondrial membrane. Apparently, bicarbonate had a similar effect in the present experiments because citrate metabolism was decreased in the presence of $NaHCO_3$ (Table VI).

TABLE VII: Effects of NH_4^+, Na^+, and K^+ on the Metabolism of [1,5-^{14}C]Citrate by Isolated Cells from the Small Intestine of the Chick.[a]

	5-Day-Old Chicks			35-Day-Old Chicks		
	$^{14}CO_2$[b]	^{14}C-Labeled Lipids[b]	NH_4^+–N[c]	$^{14}CO_2$[b]	^{14}C-Labeled Lipids[b]	NH_4^+–N[c]
Control	4097 ± 130 (1.00)	9.8 ± 0.3 (1.00)	0.1	5025 ± 18 (1.00)	7.9 ± 2.0 (1.00)	0.1
NaCl (10 mM)	3719 ± 26 (0.91)	5.6 ± 0.3 (0.57)	0.1	4789 ± 42 (0.95)	9.7 ± 2.0 (1.23)	0.1
KCl (10 mM)	3941 ± 6 (0.90)	8.9 ± 0.8 (0.91)	0.1	5004 ± 30 (1.00)	8.0 ± 1.2 (0.89)	0.1
NH_4Cl	6056 ± 25 (1.48)	17.2 ± 1.6 (1.78)	10.1	8093 ± 44 (1.61)	16.0 ± 2.0 (2.03)	10.4
Urea + urease	6871 ± 62 (1.68)	26.3 ± 1.2 (2.68)	10.2	11887 ± 25 (2.34)	47.4 ± 0.9 (6.00)	10.4

[a] Cells were incubated in modified KRP buffer (pH 7.4) for 60 min with 1 mM citrate plus [1,5-^{14}C]citrate 6.83×10^5 and 5.01×10^5 dpm for 5- and 35-day-old chicks, respectively. [b] Dpm/mg of cell protein per hr per 10^6 dpm of [1,5-^{14}C]citrate. [c] nmol of NH_4^+–N/l.

Predicting *in vivo* effects of ammonia from present data must be done with caution. However, there is evidence that ammonia changes the characteristics of the intestine *in vivo*. The process of urea hydrolysis has been suggested as one factor responsible for the differences in the gastrointestinal tract between conventional and germ-free animals (Visek, 1972). The mucosal cell surface is greater in conventional animals compared to germ-free animals (Gordon and Kardoss, 1961). Not only are more mucosal cells found in conventional animals, but they are replaced 30–40% more rapidly as shown by progress up the villi and extrusion into the lumen of the intestine (Lesher *et al.*, 1964). The present studies (Figure 1) give evidence compatible with the conclusion that ammonia shortens the normal metabolic life span of cells incubated *in vitro*. If the accelerated rate of metabolism due to ammonia occurs in the living animal, shortening of the cell life span can result and provide the need for more rapid turnover of intestinal epithelial cells seen in conventional animals compared to their germ-free counterparts. Zimber and Visek (1972) observed a depression in incorporation of [^{3}H]thymidine into DNA of colon and ileum of ammonia intoxicated mice showing that ammonia can alter the kinetics and life span of intestinal cells. Dang and Visek (1968) have shown that ammonia released by urease destroys blood cells. When ammonia production in the intestinal tract was suppressed by immunization against urease, Zimber (1970) observed a reduction of thymidine incorporation into DNA of the colon mucosa in mice. The latter evidence was interpreted as an indication of decreased cell synthesis.

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