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# Effect of Dietary Carbohydrates on Bacterial Cholyltaurine Hydrolase in Poultry Intestinal Homogenates

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**The bile salt hydrolase activity in intestinal homogenates reflects composite activities of the gastrointestinal microbial consortia. We have proposed that specific transformations of conjugated bile acids by the intestinal microflora result in the production of metabolites which depress the growth of poultry. The influence of dietary carbohydrates on the physical and kinetic properties of cholyltaurine hydrolase activity, one such bile acid-transforming enzyme in gastrointestinal homogenates of young chickens, was characterized by using a sensitive radiochemical assay. Cholyltaurine hydrolase activity in crude extracts of ileal homogenates was increased twofold by 0.25% Triton X-100 and a freeze-thaw cycle. The pH optimum for cholyltaurine hydrolase from ileal homogenates was very broad and reflected the pH range of poultry intestinal contents (i.e., 5.8 to 6.4). The carbohydrate component of the diet did not affect the apparent temperature optimum (41°C) or stability profile, nor did it affect the apparent $K_m$ for taurocholic acid hydrolysis (approximately 0.43 mM). The enzymes in intestinal homogenates were active on all taurine-conjugated bile acids tested. The carbohydrate component of the diet did, however, affect the specific activity of cholyltaurine hydrolase in ileal homogenates from chickens. The levels of cholyltaurine hydrolase activity (rye > sucrose > corn) in homogenates from birds fed the different diets were directly related to the amount of growth depression (rye > sucrose > corn) associated with feeding these dietary carbohydrates. These data suggest that intestinal levels of cholyltaurine hydrolase are correlated with the amount of carbohydrate-induced growth depression in poultry.**

The intestinal tract of chickens is colonized by bacteria soon after the eggs are hatched (22). A stable population is established within 7 to 14 days of hatch, when gram-positive bacteria represent the majority (65 to 85%) of organisms isolated from the duodenum, ileum, and cecum (20). *Escherichia coli*, enterococci, and lactobacilli predominate in the small intestine. Obligately anaerobic bacteria outnumber facultatively anaerobic bacteria 100:1 in the cecum (20). The hydrolysis of glycine- and taurine-conjugated bile acids in the gastrointestinal tract is catalyzed exclusively by bacterial enzymes, referred to collectively as bile salt hydrolases (7, 8). This activity is expressed constitutively by a large number of microorganisms (6, 8, 12) which colonize the gastrointestinal tract of humans and other animals (1, 3, 14). The deconjugation of bile salts has been reported to be catalyzed by bacteria capable of attachment to epithelial cells (2), nonattached bacteria, and cell-free supernatants from intestinal contents (18). Species and strains belonging to the genera *Clostridium*, *Lactobacillus*, *Peptostreptococcus*, *Bifidobacterium*, *Fusobacterium*, *Eubacterium*, *Enterococcus* (*Streptococcus*), and *Bacteroides* (7, 8) have been reported to catalyze this reaction. In addition, species of *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Lactobacillus*, and *Enterococcus* which colonize the chicken gut have been shown to catalyze the hydrolysis of conjugated bile acids (2, 13). The bile salt hydrolase activity has been partially purified, however, only from *Clostridium perfringens* (16) and *Bacteroides fragilis* (1, 23).

We have proposed (5) that the deconjugation of bile salts by intestinal bacteria leads to the production of growth-depressing metabolites and that antibiotic feed additives elicit their beneficial effects by reducing the population of bile salt hydrolase-producing microorganisms. The assay conditions used to measure this activity in ileal homogenates were based on those used to characterize the partially purified *C. perfringens* enzyme (16). Since the cholyltaurine hydrolase activity in ileal homogenates represents composite activities of the gastrointestinal microbial consortia, we considered it necessary to characterize several aspects of this activity to ensure that optimum assay conditions were used to measure the effects of feed additives and diet on the level of this activity in ileal homogenates. Moreover, since we have previously demonstrated that growth-promoting antibiotics reduce intestinal levels of bile salt hydrolase activity (5), we wanted to test whether an association existed between growth-depressing diets and intestinal bile salt hydrolase activity. It has been demonstrated that poultry diets formulated with rye or sucrose, in place of corn, depress weight gains, and these alternate dietary carbohydrates serve as experimental diets for assessing antibiotic-mediated growth promotion (24–27). Thus, we anticipated that a qualitative shift in the intestinal population of bacteria in response to various dietary carbohydrates might be reflected by changes in the kinetic and physical properties of cholyltaurine hydrolase.

The present reports describes the influence of dietary carbohydrates on the physical and kinetic properties of cholyltaurine hydrolase in gastrointestinal homogenates of young chickens.

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## MATERIALS AND METHODS

**Animals and diets.** Day-old male (Arbor Acre × Peterson) broiler chicks were housed overnight and fed a nonmedicated practical corn-based diet (Lab Chick Chow S-G, no. 5065; Ralston-Purina Co., St. Louis, Mo.). The following day, the chicks were sorted and blocked on a weight basis. The chicks were assigned to pens randomly and fed corn-, sucrose (25)- or rye (12)-based diets for 9 to 14 days. Feed and water were available ad libitum. The chicks were maintained in battery brooders on raised wire floors in a room maintained at 35°C and subjected to continuous lighting. Five birds from each diet were randomly selected for the preparation of gastrointestinal homogenates.

**Preparation of gastrointestinal homogenates.** The chickens were asphyxiated with $CO_2$, the crop, small intestine, and ceca were rapidly excised, and the various anatomical sections were dissected and placed into tared specimen cups previously flushed with $CO_2$. The crop was defined as the distended sac between the cranial and caudal esophagus. The ileal segment was defined as the region from the yolk sac stalk to the ileo-cecal junction. Similar anatomical segments from each group (five birds) were pooled, gross weights were recorded, and the specimens were introduced into an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) containing an atmosphere of 10% $H_2$–5% $CO_2$ in nitrogen.

Anaerobic sodium acetate buffer (5 mM, pH 5.6) containing 0.336% EDTA and 0.156% 2-mercaptoethanol was added to each specimen (1:10, wt/vol), which was then homogenized in a Waring blender. The homogenates were centrifuged at 2,000 × g for 5 min at 10°C to remove large feed particles and tissue debris. The supernatant fluid, consisting of bacteria attached to epithelial cells, free bacteria, and soluble intestinal contents, was removed and either retained at 4°C in the glove box for immediate use or mixed with Triton X-100, frozen in an acetone-dry ice bath, and stored anaerobically at −15°C for future use. This low-speed supernatant was referred to as the crude extract and retained nearly 100% of the original cholyltaurine hydrolase activity for up to 3 months when stored at −15°C.

The initial dilution normalized the cholyltaurine hydrolase activity to a gram wet-tissue weight basis. This variable was chosen in preference to a dry-weight or protein basis to minimize the effects of the dietary contents (19).

**Fractionation and permeabilization of crude extracts.** A sample (10 ml) of the crude extract was placed in a screw-capped tube and centrifuged at 10,000 × g for 20 min at 10°C. The resulting supernatant was decanted, and the pellet was suspended in a small amount of anaerobic acetate buffer (described above). Both the supernatant and pellet fractions were adjusted to a final volume of 10 ml with anaerobic acetate buffer and retained in an ice bath. Whole cells in the crude extract were permeabilized with Triton X-100 or lysozyme treatment followed by an acetone-dry ice freeze-thaw cycle. Triton X-100 was introduced into a tube, and sufficient crude extract was added to yield either 0.05 or 0.25% (vol/vol) final concentration. A freeze-thaw cycle consisted of immersing the extract into an acetone-dry ice bath for at least 5 min followed by incubation in a 35°C water bath until the extracts were completely thawed (15). If the extract was not used immediately after freezing in the acetone-dry ice bath, it was stored at −15°C and thawed as needed. Lysozyme (1-6876; Sigma Chemical Co., St. Louis, Mo.) was added to crude extracts as a powder (final concentration, 3 mg/ml). The treated extract was incubated for 30 min at room temperature before the freeze-thaw cycle. Whenever possible, manipulations of extracts were performed in the anaerobic chamber. When that was not possible, strict anaerobic techniques were used.

**Cholyltaurine hydrolase assay.** Bile salt hydrolase activity was measured radiochemically by quantitating the amount of [carboxy-$^{14}$C]cholic acid ([$^{14}$C]CA) hydrolyzed from tauro-[carbonyl-$^{14}$C]cholic acid ([$^{14}$C]TCA) (18). Reaction mixtures were prepared in 1-dram (1 fluidram = 3.696 ml) screw-cap vials containing 0.5 ml ~~of~~ anaerobic acetate buffer (described above) and supplemented with TCA and sufficient [$^{14}$C]TCA to yield a final concentration of 2.0 mM TCA with a specific radioactivity of 0.025 μCi/μmol. Test extract (0.5 ml) was added, and the mixture was incubated for 30 min at 41°C (unless otherwise indicated). To ensure linear rates, the test extracts were diluted such that <5% of the substrate was hydrolyzed during the incubation period (21).

All reactions were terminated by lowering the pH to 2.0 with 6 N HCl. Ethyl acetate (1.0 ml) was added to partition the [$^{14}$C]CA into the organic phase and 0.5-ml samples were removed and added to 10 ml of Aquasol-II (Du Pont Co., Wilmington, Del.) in glass scintillation vials (5). [$^{14}$C]CA extraction efficiency was nearly 100% (5). Radioactivity was measured in a model 1215 Rack-beta II liquid scintillation counter (LKB Instruments, Inc., Rockville, Md.) Counts per minute were corrected by using an external standard channels ratio and a $^{14}$C quench curve.

**Determination of the pH optimum.** To determine the effect of pH on cholyltaurine hydrolase activity, the radiochemical assay reaction mixture contained 0.5 ml of 0.2 M sodium acetate or potassium phosphate buffer containing 0.336% EDTA–0.156% 2-mercaptoethanol and 0.5 ml of test extract. The pH of final reaction mixtures minus radioactive substrate was measured.

**Determination of temperature optimum.** The effect of temperature ($T$) on the rate of TCA hydrolysis by intestinal homogenates was measured by incubating complete reaction mixtures for 30 min at various temperatures. The effect of temperature on the stability of cholyltaurine hydrolysis was determined by incubating multiple reaction mixtures minus substrate at different temperatures. At timed intervals, reactions mixtures incubated at specific temperatures were removed and placed on an ice bath. When all temperature incubations had been completed, the level of cholyltaurine hydrolase activity remaining was determined by adding substrate and incubating the reaction vessels at 41°C for 30 min. The rate constant $k(\text{min}^{-1})$ was determined from the slope of a plot of $(\text{rate}_T/\text{rate}_{41°C}) \times 100$ versus time at $T$.

**Determination of the $K_m$ for cholyltaurine hydrolysis.** The effect of substrate concentration on the rate of hydrolysis was determined by preparing reaction mixtures with a constant concentration of radioactive substrate and adjusting the specific radioactivity with unlabeled cholyltaurine. All reactions were incubated at 41°C. The duration of incubation was reduced at lower substrate concentrations such that <5% of the substrate was converted to product during the incubation. This adjustment in incubation time ensured the measurement of linear rates (21).

**Substrate specificity studies.** The specificity for hydrolysis of taurine-conjugated bile acids was measured with nonradiolabeled bile salt substrates and by separating the substrate from the product by high-pressure liquid chromatography (HPLC). TCA, taurochenodeoxycholic, taurodeoxycholic, taurolithocholic, and taurosulfolithocholic acids were obtained from Sigma Chemical Co. Stock solutions (5 mg/ml) were prepared in methanol. A sufficient

amount of each stock solution of bile salt was added to a 4-dram vial to yield a final concentration of 0.5 mg/ml, and the methanol was evaporated. The final volume of the reaction mixtures was 3.0 ml (1.5 ml of acetate buffer and 1.5 ml of Triton X-100 [0.25%, vol/vol] freeze-thawed extract). A 250-μl sample was withdrawn from each reaction mixture at time zero and placed in an Eppendorf centrifuge tube which contained sufficient 6 N HCl to give a final pH of 2.0. The reactions were incubated at 41°C in the glove box, and samples were withdrawn at 1, 2, and 3 h. All samples were acidified and centrifuged for 10 min to remove denatured protein. The resulting supernatant was passed through a Sep-Pak $C_{18}$ cartridge (Waters Associates, Inc., Milford, Mass.) prewetted according to the manufacturer's instructions. The bile salts were eluted from the cartridge with 3.0 ml of methanol. Methanol was evaporated and the residue was suspended in 1.0 ml of HPLC mobile phase (methanol–acetonitrile–0.03 M monobasic potassium phosphate, pH 3.4 [60:10:30, vol/vol/vol]) (17). The samples were analyzed on a model 334 HPLC (Beckman Instruments, Inc., Fullerton, Calif.) equipped with a Du Pont μBondapak $C_{18}$ reverse-phase column, 30 cm by 3.9-mm inside diameter, 10-μm particle size. Data were acquired and analyzed by using a Maxima Chromatography Workstation, model M2PC (Dynamic Solutions Corp., Ventura, Calif.).

**Chemicals.** All chemicals used were of reagent grade. HPLC-grade solvents were used in the substrate specificity experiments. [$^{14}$C]TCA (55.7 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill.

## RESULTS

The distribution of cholyltaurine hydrolysis activity in ileal homogenates of chickens fed a semisynthetic sucrose-based diet is shown in Table 1. Treatment of the crude extract with 0.25% Triton X-100 followed by a freeze-thaw cycle increased the activity twofold. Fractionation of untreated and Triton-treated extracts revealed that the increased activity due to Triton X-100 was mainly attributable to increases in the pellet fraction. This observation suggests that a significant amount of enzyme activity in previously prepared crude extracts was undetected, possibly because it was intracellular and sequestered from the substrate.

The effects of alternate permeabilization techniques on cholyltaurine hydrolase activity in ileal homogenates from sucrose-fed birds are shown in Table 2. The use of 0.25% Triton X-100 with a freeze-thaw cycle resulted in extracts with the highest specific activity. Lysozyme treatment either alone or in combination with a freeze-thaw cycle had an insignificant effect on the crude extract activity. Thus, all further experiments were conducted with crude extracts treated with 0.25% Triton X-100 and a freeze-thaw cycle.

TABLE 1. Distribution of cholyltaurine hydrolase activity in ileal homogenates[a]

| Location | Sp act (mean ± SD) | |
|---|---|---|
| | Control | Triton X-100 |
| Crude extract | 1,244 ± 24 | 2,471 ± 64 |
| Supernatant[c] | 835 ± 17 | 949 ± 64 |
| Pellet[c] | 438 ± 13 | 1,271 ± 170 |

[a] Ileal homogenates were prepared from chicks fed the semisynthetic soybean protein sucrose-based diet.
[b] Nanomoles of CA released per 30 min per gram of ileum; $n = 3$.
[c] Crude extract was centrifuged at 10,000 × $g$ for 10 min.

TABLE 2. Cholyltaurine hydrolase activity following various membrane disrupting treatments[a]

| Treatment | Freeze-thaw | Sp act (mean ± SD)[b] |
|---|---|---|
| Control | − | 500 ± 38 |
| | + | 882 ± 36 |
| 0.05% Triton X-100 | + | 926 ± 34 |
| 0.25% Triton X-100 | + | 1,141 ± 69 |
| Lysozyme | − | 603 ± 22 |
| | + | 911 ± 52 |

[a] The cholyltaurine hydrolase was obtained from ileal homogenates of chicks fed the semisynthetic soybean protein sucrose-based diet.
[b] Nanomoles of CA released per 30 min per gram of ileum; $n = 3$.

The effect of temperature on the rate of cholyltaurine hydrolase activity in ileal homogenates is shown in Fig. 1. Measurable cholyltaurine hydrolase activity was observed over the temperature range of 22 to 85°C, regardless of the type of diet the chicks were fed. Two temperature effects were apparent: a broad plateau occurred between 37 and 47°C, and a sharp apparent optimum occurred at 55°C. The design of this experiment prevented the differentiation between the effects of temperature on the rate of catalysis and enzyme stability. The thermal stability of cholyltaurine hydrolase activity in ileal homogenates was measured by incubating the extracts at different temperatures for varying lengths of time and then assessing the remaining activity at 41°C. The thermal inactivation rate constant ($k$) was calculated from the slope of a plot of percent original activity versus time at each temperature evaluated. The effects of temperature on $k$ are shown in Fig. 2. Subsequent assays for cholyltaurine hydrolase activity were conducted at 41°C, the normal body temperature of chickens.

The effect of pH on cholyltaurine hydrolase activity is shown in Fig. 3. No distinct optimum in pH was noted for cholyltaurine hydrolase activity in the ileal homogenates from birds fed diets containing any of the carbohydrate

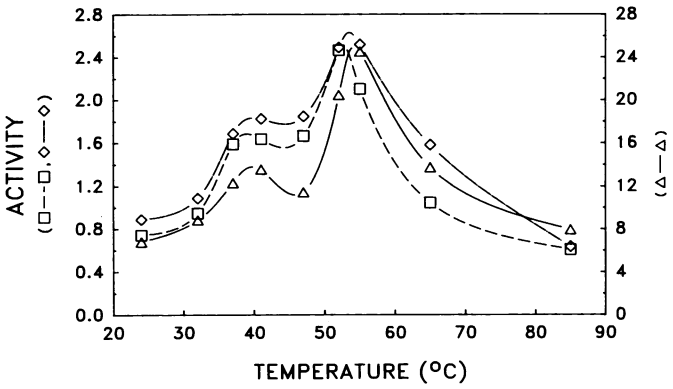


FIG. 1. Effect of temperature on rate of hydrolysis of TCA by ileal homogenates from chickens fed different sources of dietary carbohydrate. Symbols: □, corn; ◇, sucrose; △, rye. Complete reaction mixtures were prepared and sealed in the anaerobic glove box and transferred to heated chambers. Reaction mixtures were incubated for 30 min at the appropriate temperature and terminated, and the concentration of [$^{14}$C]CA released was determined. Activity is defined as micromoles of CA released per 30 min per gram of ileum.

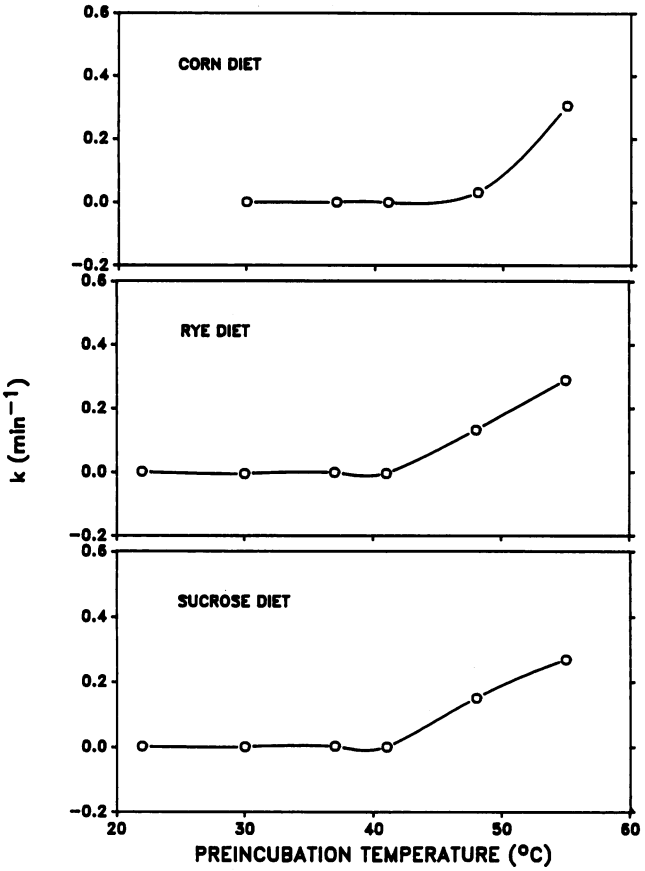


FIG. 2. Effect of temperature on stability of cholyltaurine hydrolase in ileal homogenates from chickens fed different sources of dietary carbohydrates.

sources. The effects of pH on cholyltaurine hydrolase activity in crop and cecal homogenates were similar to those in ileal homogenates and did not exhibit distinct pH optima (data not shown).

The kinetic parameters for bile salt hydrolase activity in ileal homogenates of birds fed diets containing various carbohydrates were determined. A Lineweaver-Burk plot of

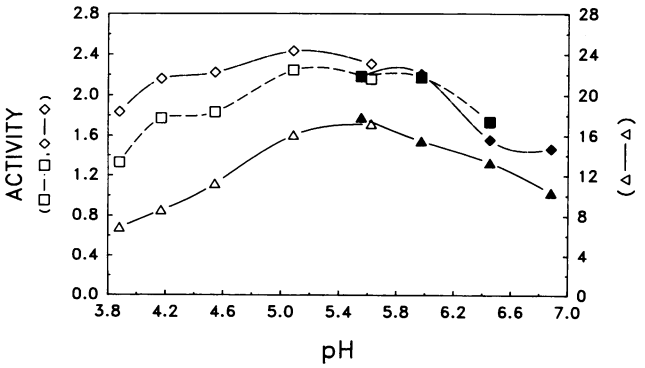


FIG. 3. Effect of pH on hydrolysis of TCA by ileal homogenates from chickens fed different sources of dietary carbohydrates. Symbols: □, ■, corn; ◇, ◆, sucrose; △, ▲, rye; open, sodium acetate buffer; closed, potassium phosphate buffer. Activity is defined as micromoles of CA released per 30 min per gram of ileum.

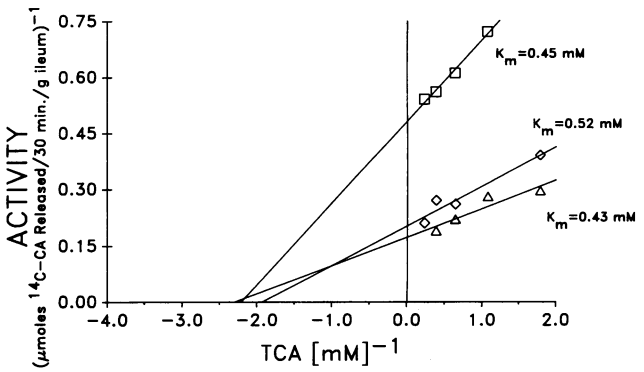


FIG. 4. Lineweaver-Burk plot for taurocholate hydrolysis by ileal homogenates from chickens fed different sources of dietary carbohydrate. Symbols: □, corn; ◇, sucrose; △, rye.

representative homogenates, using cholyltaurine as substrate, is shown in Fig. 4. The $V_{max}$ of cholyltaurine hydrolysis was affected by dietary carbohydrate in ileal homogenates, but the $K_m$ of the enzymes for cholyltaurine was not.

The substrate specificity for taurine-conjugated bile acids was determined in ileal homogenates from chickens fed diets containing rye, corn, or sucrose as the predominant carbohydrate source. Table 3 shows the percent hydrolysis of taurine-conjugated bile acids after 3 h of incubation at 41°C. All taurine-conjugated bile acids were effective substrates for the bile salt hydrolase activities present in the ilea. Dietary carbohydrates did not affect substrate specificity.

The effect of dietary carbohydrate on the distribution of cholyltaurine hydrolase activity within the chicken gastrointestinal tract and on the weight gain of 9-day-old chicks is shown in Table 4. In all cases, the activity increased distally from the crop to the cecum, as would be expected for an activity associated with anaerobic bacteria. In each segment, homogenates from rye-fed chicks exhibited greatly enhanced activity compared with homogenates from either corn- or sucrose-fed chicks.

## DISCUSSION

Entire intestinal segments were homogenized to include activities associated with both adherent and nonadherent bacteria as well as any free extracellular enzyme which might be present. The cholyltaurine hydrolase activity in intestinal homogenates was increased by treatments affecting cellular integrity. These results suggest that intestinal cholyltaurine hydrolase activity in intestinal homogenates is represented by extracellular and intracellular enzyme pools. This distribution reflects the possible contribution of activities from extracellular (e.g., *C. perfringens* [12]) and intra-

TABLE 3. Substrate specificity for bile salt hydrolase activity in ileal homogenates

| Diet | % Hydrolysis of taurine-conjugated bile acid[a] | | | | |
|---|---|---|---|---|---|
| | TCA | TCDCA | Bile salt TDCA | TLCA | TsLCA |
| Corn | 93.5 | 93.0 | 70.1 | 85.3 | 66.9 |
| Sucrose | 88.4 | 95.8 | 76.4 | 92.0 | 59.5 |
| Rye | 71.2 | 86.6 | 74.0 | 82.7 | 35.7 |

[a] TCDCA, Taurochenodeoxycholic; TDCA, taurodeoxycholic; TLCA, taurolithocholic; TsLCA, taurosulfolithocholic acid.

TABLE 4. Influence of dietary carbohydrate source on development of cholyltaurine hydrolase activity in selected poultry gastrointestinal homogenates

| Diet | 9-Day wt gain (g) | Bile salt hydrolase activity (mean ± SD)[a] | | |
|---|---|---|---|---|
| | | Crop | Ileum | Cecum |
| Corn | 152.3 | 36 ± 3 | 1,270 ± 89 | 40,905 ± 1,800 |
| Sucrose | 114.0 | 47 ± 5 | 1,824 ± 103 | 23,916 ± 1,970 |
| Rye | 108.6 | 174 ± 15 | 23,990 ± 1,518 | 128,717 ± 2,130 |

[a] Nanomoles of CA released per 30 min per gram of homogenate; $n = 3$.

cellular (e.g., *B. fragilis* [9, 12, 23]) enzymes. The presence of extracellular cholyltaurine hydrolase in chicken ileal homogenates is similar to the extracellular activity in rat cecal contents reported by Norman and Widstrom (18).

Preincubation of ileal extracts from birds fed rye-, corn-, and sucrose-based diets at 55°C for 10 min resulted in losses of 94, 95, and 93% of the original activity, respectively. Thus, the peak of activity at 55°C (Fig. 1) represents a competition between kinetic and stability effects of temperature on cholyltaurine hydrolase activity. The temperature stability of ileal homogenate cholyltaurine hydrolase activity agrees favorably with that reported for bile salt hydrolases from other bacteria where it has been measured. For example, *Enterococcus* (*Streptococcus*) *faecium* bile salt hydrolase lost 40% of its activity after 5 min at 50°C and 75% after 5 min at 75°C (1). The cholyltaurine hydrolase activity of a *Peptostreptococcus intermedius* strain was inactivated by heating at 60°C for 10 min (10). The true "optimum" temperature for cholyltaurine hydrolase activity, the maximum temperature at which the enzyme exhibits a constant activity over a time period at least as long as the assay time (21), was 41°C (Fig. 2), which corresponds to the body temperature for broiler chickens (22). The temperature profiles for cholyltaurine hydrolase in ileal homogenates of birds fed the different diets were similar, suggesting that the carbohydrate content of the diet did not cause a shift in the microbial consortia elaborating this activity.

The apparent pH optimum for partially purified bile salt hydrolase enzymes from *B. fragilis* were reported by Stellwag and Hylemon (23) to be 4.2 and by Aries and Hill (1) to be 5 to 6. The *C. perfringens* enzyme exhibited a broad optimum of between 5.0 and 5.8 (16). Whole cells of *Lactobacillus acidophilus* exhibited an optimum of 6.0 (6), and acetone powders of *P. intermedius* exhibited an optimum of 5.6 (10). These optima for enzymes from pure cultures contrast the optimum pH of 6.5 observed with extracellular enzymes from rat cecal contents (12). The very broad pH optima for cholyltaurine hydrolase activity observed in ileal homogenates from chickens (Fig. 3) probably reflect the contributions from numerous bacterial bile salt hydrolases. The observed range of activity favorably corresponds with the pH range of 5.76 to 6.42 measured in contents from poultry small intestines (4).

The substrate specificity for bile salt hydrolase activity in ileal homogenates (Table 3) and the $K_m$s for cholyltaurine hydrolysis (Fig. 4) were not affected by the source of carbohydrate utilized in the poultry diet. The $K_m$ for hydrolysis of cholyltaurine in these homogenates closely agrees with the value of 0.45 mM reported by Stellwag and Hylemon (23) for partially purified bile salt hydrolase enzyme from *B. fragilis*. The $K_m$ for hydrolysis of cholyltaurine by a partially purified enzyme from *C. perfringens* was 37 mM (16). In both of these studies cholyltaurine hydrolysis exhibited the highest $K_m$ of the various substrates tested. The kinetic properties of cholyltaurine hydrolysis by either whole cells or extracts from other bacteria (e.g., streptococci or lactobacilli) have not been reported.

The physical and kinetic characteristics of the enzymes responsible for hydrolyzing cholyltaurine in intestinal homogenates did not change in response to differences in dietary carbohydrates fed to the chickens. The apparent pH optima, temperature profile, $K_m$, and substrate specificities reflected the combined contributions of numerous bacterial cholyltaurine hydrolase activities. The most striking effect of diet on this hydrolase activity was the uniformly high level of activity measured in homogenates from chickens fed the rye-based diet (Table 4). Focusing on the ileum, which is the site of conjugated bile acid-mediated active transport of fats and lipids (29), the specific activity of cholyltaurine hydrolase in homogenates from rye-fed chickens was nearly 20 times the level in homogenates from corn-fed chickens and >10 times the level measured in sucrose-fed chickens. In addition, homogenates from chickens fed a sucrose-based diet exhibited 44% more cholyltaurine hydrolase activity than those from corn-fed chickens.

The increase in bile salt hydrolase activity associated with rye and sucrose components of the diet probably reflects an increase in the number of bacteria present in the small intestine. For example, Stutz (24–26) has demonstrated that diet significantly affects weight gain and an increase in the size of the *C. perfringens* population in the ileum of broiler chickens. Untawale and McGinnis (27) reported that feeding rye caused a significant numerical increase in lactobacilli and enterococci both in the intestinal lumen and on the epithelial wall. Since bile salt hydrolase activities from pure cultures or partially purified enzymes exhibit variations in temperature and pH optima (1, 6, 10, 12, 16, 23), had a certain representative of the ileal flora capable of catalyzing bile salt hydrolysis been selected or enriched by the dietary treatment, a shift in these enzyme parameters should have been measured. Since these effects were not seen, we concluded that the dietary carbohydrates sucrose and rye increased the number of bacteria capable of hydrolyzing bile salts while not affecting the diversity of this group. We intend to specifically test this conclusion by using selective media we have modified to differentiate colonies based on their capacity to deconjugate deoxycholyltaurine (M. P. Dashkevicz and S. D. Feighner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q72, p. 295; unpublished data).

The levels of cholyltaurine hydrolase activity in homogenates from chickens fed the different diets directly parallel the amount of growth depression associated with feeding these dietary carbohydrates (Table 4) (11, 24, 25, 28). Thus, these data further suggest that changes in a specific bacterial biotransformation (i.e., elevation of bile salt hydrolase specific activity) contribute to carbohydrate-induced growth depression in poultry. Conversely, a decrease in the level of bile salt hydrolase activity in ileal homogenates has been associated with antibiotic-mediated growth promotion (5). Taken together, these observations suggest that bacterial cholyltaurine hydrolase may represent a specific target for achieving growth promotion with nonantibacterial agents.

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