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Growth of Rats Fed Bile Salts, Urea and Chlortetracycline.*. (30440)

WILLARD J. VISEK, H. C. DANG, S. R. KIRBY AND G. A. SPERLING
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

Bile acids are secreted into the gastrointestinal lumen in conjugated form and may undergo hydrolysis by microorganisms but not by endogenously produced enzymes(1,2). Feces of germfree and antibiotic treated animals have been found to contain no detectable deoxycholic acid(3,4), often the most prominent bile acid in feces of animals and man(5,6). *In vitro* incubation with deoxycholic acid causes marked damage to the intestinal villi(7) and extensive histological changes in the small intestinal mucosa of mice have been reported following 2 days of feeding on a diet containing 2% sodium deoxycholate(8). Microorganisms in the intestine liberate ammonia by urea hydrolysis and strains known to hydrolyze bile acids also produce urease(9,10). Ammonia production in the gastrointestinal tract by microorganisms may lead to toxic manifestations and is reduced with oral administration of antibacterial substances(11). The effects of varying the type or quantity of bile acids in the diet of growing animals with antibiotics in the diet have been studied(12,13). However, the authors have not found published data for growing animals following simultaneous feeding of bile salts, urea and antibacterial substances.

Methods. Weanling male rats of the Holtzman strain having initial weights of 48-60 g were fed and housed in individual cages with wire floors. Their purified basal diet calculated to meet the dietary requirements of growing albino rats(14) contained the following ingredients in per cent: vitamin free casein 14; sucrose 75; stripped lard† 5; glucose monohydrate 1.8075; dl methionine 0.18; minerals 3‡; vitamins in glucose 1§; antioxidant 0.0125.‖ Additions were made

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† Most volatile fraction from prime steam lard obtained by molecular distillation. Contains less than 5 ug of tocopherols per g of fat and fulfills fatty acid requirements of the rat. Distillation Products Industries, Rochester, N. Y.

‡ Minerals per kg of diet: $Ca(PO_4)_2$ 11.1 g; $Ca(H_2PO_4)_2$ H_2O 11.1 g; $KHCO_3$ 4.67 g; $MgCO_3$ 1.42 g; NaCl 834 g; Na_2HPO_4 549 mg; $MnSO_4$ H_2O 156 mg; $FeSO_4$ $7H_2O$ 126 mg; $ZnCO_3$ 23 ug; $CuSO_4$ 5HO 20 ug; KIO_3 253 mg; Na_2SeO_3 87.8 ug.

§ Crystalline vitamins provided per kg of diet: thiamine HCl 1.25 mg; riboflavin 2.5 mg; pyridoxine HCl 1.2 mg; niacinamide 15 mg; calcium pantothenate 8 mg; choline dihydrogen citrate 1582 mg; vit. B_{12} 5 ug; vit. A 2000 I.U.; vit E 60 I.U.

‖ Santoquin, Monsanto Chemical Co., St. Louis, Mo.

TABLE I. Gain in Weight and Efficiency of Feed Utilization for Rats Fed a Basal Diet Supplemented with Bile Salts or 100 mg/kg of Chlortetracycline (6 Animals per Treatment Group).

Treatment groups	28 Day wt gain (g)*	Gain/feed ratio†
1. Basal	103.5	.359
2. " + CTC 100 mg/kg	92.7	.356
3. " + .1% deoxycholate	96.8	.355
4. " + .5% "	53.1	.263
5. " + 1.0% "	No survivors after 5 days	
6. " + .1% dehydrocholate	95.3	.370
7. " + .5% "	77.0	.328
8. " + .5% dehydrocholate + chlortetracycline (100 mg/kg)	78.1	.316
9. " + .1% cholate	104.0	.373
10. " + 2.0% "	No survivors after 21 days	
11. " + .1% taurocholate	112.0	.364
12. " + 2.0% "	12.0	.072
13. " + 2.0% taurocholate + CTC (100 mg/kg)	14.8	.087
14. Basal	97.7	.371
15. " + .5% ox bile	71.2	.330
16. " + 1.0% " "	49.7	.251

* Avg of 6 animals per group. Error mean square for growth 230.6.

† Error mean square for gain/feed 0.0014.

to the total basal diet unless they exceeded 1% whereupon they were made at the expense of sucrose. With the exception of ox bile all additives were of reagent or USP grade. All animals were provided with distilled water and their assigned diets *ad libitum* for the experimental period of 28 days. Weekly weights and feed consumption were recorded. A factorial experiment described below followed preliminary experiments in which ox bile and sodium salts of bile acids were tested for differential toxicity (Table I). Groups 1 through 13 of the preliminary experiments were started simultaneously, whereas 14 through 16 were started on a subsequent date.

The factorial experiment was a 4 × 2 × 2 arrangement with 16 treatment groups of 5 animals. Four concentrations of sodium deoxycholate as a percentage of the diet were used: 0, 0.1, 0.3, and 0.5. Each concentration of deoxycholate was tested with or without 1% urea and with or without 100 mg/kg of chlortetracycline¶ singly or in combination. Data obtained were treated by analysis of variance.

Results. Sodium deoxycholate proved to be more toxic than ox bile or the other bile salts as shown by growth depression and mortality (Table I). Sodium cholate (0.1%) caused no depression in growth and the results suggested that growth might be increased in the presence of 0.1% sodium taurocholate. All animals receiving 1.0% of deoxycholate died in 5 days or less but all animals fed 2.0% taurocholate survived the entire 28 days although their average gain was only 12 g. Addition of chlortetracycline failed to counteract the depression in growth produced by 2.0% taurocholate. All of the animals receiving 2.0% sodium cholate died between the 7th and 21st days. Animals that died regardless of treatment showed severe diarrhea. Diarrhea was also noted in the 28 day survivors of the groups to which 0.5% or more of ox bile or any bile salt were fed. At death or at 28 days animals fed 0.5% deoxycholate, 0.5% dehydrocholate, 0.5% cholate, 0.5% ox bile, or 1.0% taurocholate showed hyperemia of the distal small intestine. Group 8, fed 0.5% dehydrocholate plus chlortetracycline, had enlargement and increased weight of the ceca plus contents.

The average gains in body weight of the 78 animals surviving 28 days in the factorial experiment were reduced when deoxycholate was added to the diet and the depression approached linearity when plotted as a function of deoxycholate concentration. Each 0.1% of sodium deoxycholate added reduced gain in body weight by an increment of about 10% below the average of 108 g for all groups receiving no deoxycholate. The differences between concentrations of deoxycholate were highly significant ($P<0.001$). The lowest concentration of deoxycholate (0.1%) also depressed feed intake but at higher concentrations growth depression was greater than could be attributed to reduced feed intake alone. Data on the conversion of feed to gain in body weight paralleled the differences in growth.

In the preliminary experiments (Table I) and in the factorial experiment (Table II) chlortetracycline led to a depression of growth in animals fed the basal diet without

¶ Gift of American Cyanamid Co., Princeton, N. J.

TABLE II. Gain in Body Weight (GMS) and Efficiency of Feed Utilization for Male Weanling Rats Fed Sodium Deoxycholate, 1% Urea and 100 mg/kg Chlortetracycline for 4 Weeks.*

Deoxycholate, %	Without chlortetracycline		With chlortetracycline		Avg by deoxycholate concentration
	Without urea	With urea	Without urea	With urea	
0	114.0	118.2	98.4	101.8	108.1
G/F†	.385	.400	.375	.342	.375
.1	98.2	90.4	90.0	90.4	92.3
G/F	.352	.347	.323	.336	.340
.3	82.8	71.2	75.6	59.2	72.2
G/F	.311	.289	.294	.275	.292
.5	50.0‡	48.2	43.8	81.2‡	55.8
G/F	.226	.245	.219	.322	.253
Mean gain	86.2	82.0	77.0	83.2	
Mean G/F	.318	.320	.303	.319	

Means of 8 groups with and without chlortetracycline and with and without urea regardless of deoxycholate concentration

	Without chlortetracycline	With chlortetracycline
Gain (8 groups)	84.1	80.1
G/F (8 ")	.319	.311
	Without urea	With urea
Gain (8 ")	81.6	82.6
G/F	.311	.319

* Each value is an avg for 5 animals unless otherwise indicated. Error mean square for growth 173.9.

† Ratio of gain to feed consumed. Error mean square 0.0013.

‡ 4 animals.

other additives. Growth depression has been recorded several times in this laboratory with rats fed chlortetracycline in this basal diet. All groups receiving chlortetracycline regardless of the concentration of deoxycholate showed a greater depression in growth when urea was absent. The group fed urea alone showed the greatest growth but when urea was added in the presence of deoxycholate growth depression was also greater than that of the corresponding concentration of deoxycholate alone. Statistical analysis demonstrated a highly significant 2-way interaction between deoxycholate and urea ($P<0.005$) and between deoxycholate and chlortetracycline ($P<0.01$). Likewise there was a significant 3-way interaction among groups fed deoxycholate, chlortetracycline and urea ($P<0.05$). These interactions became apparent (Table II) when the mean of 81.6 for 8 groups receiving no urea is compared to 82.6, the mean for all 8 groups receiving urea. Urea appeared to increase growth for all 8 groups in contrast to the apparent growth depression when only urea was added in the presence of deoxycholate. Similarly the mean of 84.1 for 8 groups fed no chlortetracycline was slightly higher than 80.1 for the groups fed chlortetracycline. The individual effects of chlortetracycline and urea when added in the presence of deoxycholate were masked in these averages which include data where chlortetracycline and urea were added in combination. Their interactions were revealed by statistical analysis to be highly significant.

Discussion. The basal diet was compounded to meet the published requirements of the albino rat(14). It was employed despite its inability to produce maximum growth.** Highly purified ingredients were used to facilitate possible future comparisons involving changes in concentrations of specific nutrients. The NRC tabulation does not include components which are often included in purified diets of rats and it is recognized that the results of this study might have been altered with their inclusion since the effects of antibacterial agents on nutrition of substances like folic acid, biotin, and vit. K are well known for the rat(14).

** R. G. Warner, unpublished data.

The apparent toxicity as indicated by growth depression of urea when added in the presence of deoxycholate gives evidence that bile acids and urea both produced endogenously by warm blooded species may provide the gastrointestinal microflora with substrates which permit production of substances having ability to depress nutrient utilization and growth of the host. It has been speculated that bacterial products of bile acid hydrolysis lead to malabsorption and the entity known as the blind loop syndrome(7). Work of others has suggested that antibiotics counteract the growth depression by non-conjugated bile acids because microbial production of products having growth depressing properties is inhibited (12,13). However, lithocholic acid has been found to decrease growth equally in germfree or conventional chicks(15). The evidence indicates that urea is converted *in vivo* into ammonia and CO_2 by microbial urease(s) (11), but the products of bile acid hydrolysis are more varied and complex(1,2). Thus the products and mechanisms responsible for growth depression in the present studies are uncertain. Evidence has been presented that ureolytic activity in the gastrointestinal lumen is reduced concurrently with increased growth following urease immunity(16,17). Urea hydrolysis and bile acid hydrolysis are carried out by extracellular microbial enzymes(18). It is therefore possible that bile acid hydrolysis may also be controlled by immune mechanisms induced by appropriate antigens.

Summary. Addition of ox bile and bile salts to a purified diet produced differential effects on growth in rats. Deoxycholate produced growth depression ($P<0.001$) at all concentrations. Growth was reduced further by 1% urea or 100 mg/kg of chlortetracycline. Addition of urea with chlortetracycline tended to abolish growth depression produced by either alone.

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