

AMINE FORMATION AND METABOLIC ACTIVITY OF MICROORGANISMS IN THE ILEUM OF YOUNG SWINE FED CHLORTETRACYCLINE

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The favorable effect of low levels of chlortetracycline (Aureomycin) in promoting an increased rate of gain in young swine is widely accepted as a contribution to our agricultural economy (Jukes, 1955). In young swine farrowed by hysterectomy and reared in isolation, with relative freedom from the more common infections to which baby pigs are exposed, significant increases in rate of growth occurred when their diets were supplemented with low levels (10 to 40 ppm) of chlortetracycline (Hill and Larson, 1955). In view of this result an attempt has been made to learn if there are detectable effects of chlortetracycline on organisms of the intestinal tract, in addition to suppression of those producing disease.

Stokstad (1954) has stated that antibiotic effect in promoting increased growth apparently is confined to action on bacteria within the intestinal tract.

Several investigators have reported experiences suggesting that chlortetracycline suppresses the formation of toxic substances such as amines in the intestinal tracts of young animals. Sieburth *et al.* (1954) found support for this contention when they observed that a marked dilation of the blood vessels of the small intestine, and a reduction of the pungency of odors of the intestinal contents, occurred in chicks whose feeds were supplemented with 10 ppm of chlortetracycline.

Michel (1956) and Michel and Francois (1956) reported that the microflora of the intestinal contents of swine were very active in the decarboxylation of amino acids. Aspartic acid and glutamic acid were most readily decarboxylated; arginine, histidine, alanine, and tryptophan also were attacked; lysine, ornithine, and methionine were rarely affected. There was great variation from animal to animal. Decarboxylation of tryptophan was not consistently inhibited by chlortetracycline, but decarboxylation of aspartic acid, glutamic acid, and arginine was markedly reduced in the presence of chlortetracycline.

Melnykovich and Johansson (1955) reported a reduction of amine formation in the gut of rats fed chlortetracycline. In studying the effect of tetracyclines on enzymatic activities of intestinal bacteria of rats, Melnykovich (1956) found that, although the decarboxylase activities of these organisms were not inhibited in the presence of suitable substrate and chlortetracycline, the adaptive formation of certain amino acid decarboxylases was prevented if tetracyclines were added to the medium before growth was initiated.

Our recent experiments have been directed to chromatographic, electrophoretic, and manometric studies of ileum contents because bacteriological cultures of intestinal contents from young swine receiving chlortetracycline as a feed supplement showed a greater suppression of clostridia and enterococci in the ileum than in other portions of the intestinal tract (Larson and Hill, 1955).

EXPERIMENTAL PROCEDURES AND RESULTS

To test possible inhibition of formation of toxic substances in the gut by chlortetracycline, ileum contents were examined from 29 young swine fed basal diets (Hill and Larson, 1955), and from 29 whose diets were supplemented with approximately 40 ppm of chlortetracycline. Samples from litter mates were pooled yielding 10 samples for each group. These animals ranged in age from 1 day to 14 weeks. Although cultures from meconium of baby pigs did not yield any organisms, a pooled sample of ileum contents from 2-day-old pigs yielded 130×10^6 *Escherichia coli*, 0.04×10^6 enterococci, 57×10^6 lactobacilli, 0.0003×10^6 clostridia, and 0.2×10^6 yeasts per gram of wet weight (18.75 per cent solids). Microorganisms also were abundant in ileum contents of older pigs. If the amino acid decarboxylase activity of these microorganisms were inhibited by chlortetracycline, a reduction in amine formation should be detectable.

For the separation of amines, fresh samples of

ileum contents were hydrolyzed with *N* NaOH at 25 C overnight. Samples equivalent to 1 g of starting material were subjected to the liquid-liquid extraction procedure of Block *et al.* (1958) to separate amines from amino acids and amino sugars. The resulting ether extract containing a small amount of phosphoric acid was neutralized, saturated with a mixture of $K_3PO_4:Na_2SO_4$, 1:6, and extracted with butanol. Amines in the butanol extract were converted to hydrochlorides, concentrated and chromatographed on paper (Bremner and Kenten, 1951) with ascending development in butanol-acetic acid-water, 100:24:100, and stained with ninhydrin or Ehrlich's reagent. A minimum of 2 μ g of each amine was detectable by this chromatographic procedure.

In attempts to make quantitative estimations by liquid-liquid extraction procedures, the recovery of known amounts of amines from aqueous solutions or mixtures with ileum contents was 30 to 60 per cent. Estimation of amounts extracted was made by visual comparison of the intensity of stained spots from extracts of experimental samples with spots obtained from standards by direct chromatography because colors produced were not satisfactorily measurable by densitometry. Five microgram amounts of each known amine produced an intensity which was assigned a value of 2 on an arbitrary scale of 0 to 10. Amines for which standards were available and their respective R_F values (R_F = distance traveled by substance/distance traveled by solvent front) obtained with our conditions were: agmatine (0.03-0.04), putrescine (0.08-0.11), cadaverine (0.14-0.16), histamine (0.17-0.19), ethanolamine (0.31-0.33), tyramine (0.58-0.61), tryptamine (0.66-0.69), and indole (0.93). Some spots were obtained whose R_F values did not correspond to any of the standards available. However, they occurred consistently enough to be recorded. The experimental results for amines separated by paper chromatography are presented in figure 1.

The ileum contents from the nonsupplemented groups exhibited a greater amount and variety of amines than those from the groups fed chlortetracycline. Readily demonstrable amounts of amines occurred in ileum contents of swine as young as 4 days as well as in older animals. Well defined spots with R_F values corresponding to agmatine, putrescine, cadaverine, histamine, ethanolamine, tyramine, tryptamine, and a number of unidentified spots were elicited from ileum

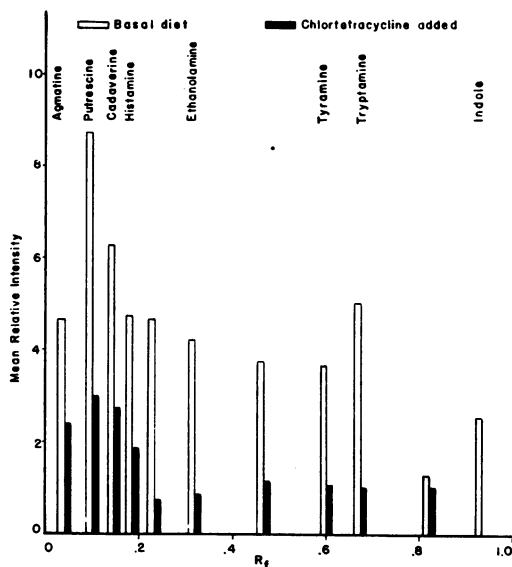


Figure 1. Effect of dietary chlortetracycline on amine formation *in vivo* in ileum contents of young swine. The unlabeled bars represent unidentified compounds with R_F values of 0.23, 0.48, and 0.83. Mean values of 10 matched groups.

contents of animals on basal diets. The spots from ileum contents of chlortetracycline supplemented animals were not well defined. An extract of ileum contents from 4-day-old piglets fed a basal milk diet yielded well defined spots on paper chromatograms with R_F values corresponding to agmatine, putrescine, cadaverine, histamine, ethanolamine, tyramine, tryptamine, and unidentified spots at R_F values of 0.23 and 0.48. An extract from two litter mates on a diet differing only in supplementation with chlortetracycline at 10 ppm (receiving a total of 16.5 mg during 4 days) yielded pale spots corresponding to agmatine, cadaverine, histamine, and an unidentified spot at an R_F of 0.23.

From 22-day-old litter mates, with 4 animals on each regime, extracts from the basal group yielded spots corresponding to putrescine, ethanolamine, tyramine, tryptamine, and an unidentified spot with an R_F value of 0.23. No spots were demonstrable from the extract of ileum contents of the chlortetracycline-fed litter mates. A similar trend occurred in other samplings.

Paper electrophoresis was employed to separate amines directly from unextracted ileum contents. Electrophoresis was carried out by conventional methods using a horizontal closed

strip of Whatman no. 1 paper wetted with barbiturate buffer at pH 8.6 and ionic strength of 0.05, equilibrated for 1 hr. Samples of ileum contents and known amines, yielding characteristic zones, were applied at appropriate areas and a direct current of 200v at 5.5 milliamperes was imposed for 90 min at approximately 1.75 milliwatts per cm². Discrete and well defined spots with characteristic zones were demonstrable with ninhydrin staining.

Ileum contents investigated by this procedure were obtained by aspiration from two miniature swine in which fistulas described by Mann and Bollman (1931) had been prepared surgically by J. H. Grindlay of the Mayo Foundation. A short loop of the terminal portion of the ileum was isolated to make the fistulous tract; the free end of the transposed loop was drawn through a stab wound and sutured to the skin. Leakage

was prevented since waves of peristalsis passed from the surface of the skin to the lumen of the ileum. Samples were obtained by passage of a suitable catheter through the opening without sacrificing the animal. It was necessary, however, to anesthetize the animals for this procedure. Spots corresponding to agmatine, cadaverine, putrescine, and tyramine were elicited from ileum contents of animals receiving basal diets. After chlortetracycline was included in the diet for 1 week, only pale diffused spots were discernible, indicating that amine formation was reduced in the presence of this agent.

In previous reports, the suppression of oxygen uptake by ileum contents of animals fed chlortetracycline was noted (Larson and Hill, 1955). Since the oxygen content of the digestive tract is low, studies were made to determine the effect of chlortetracycline on metabolic activities in the absence of oxygen. Ileum contents from young swine were analyzed by Warburg manometry (Umbreit, Burris, and Stauffer, 1957). Weighed amounts were placed in Warburg flasks containing bicarbonate buffer to attain pH 7.0, and activity was measured at 30 C in an atmosphere of 95 per cent nitrogen and 5 per cent CO₂. When 10 μg of chlortetracycline per ml of preparation were added, an inhibition of release of carbon dioxide was observed. Variations were encountered, but an average reduction of 42.1 per cent was observed in 10 samples during 90 min of reaction, and of 46.4 per cent during 120 min. The results of 10 separate runs, using 12 animals, are shown in figure 2.

DISCUSSION

In view of the established decarboxylase activity of *E. coli*, clostridia, and enterococci (Gale, 1946; Najjar, 1955), the occurrence of detectable amounts of amines in ileum contents was anticipated. The finding of less amines in extracts from ileum contents of chlortetracycline-fed animals was a favorable concurrence with our observation that populations of enterococci and clostridia of those animals were reduced by chlortetracycline. Extracts of seven control samples yielded tyramine which is produced from decarboxylation of tyrosine mainly by enterococci, while only one extract of the chlortetracycline-fed animals yielded more than a trace of tyramine. Because chlortetracycline has not been found to reduce populations of *E. coli*, the finding of amines

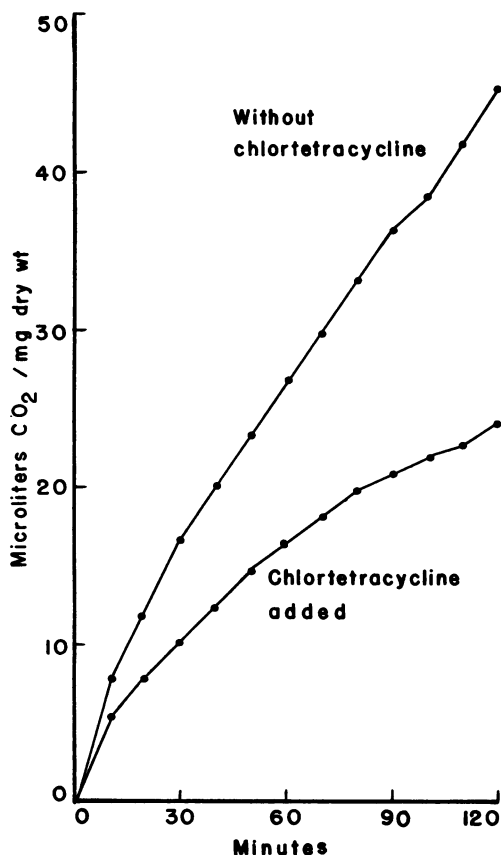


Figure 2. Effect *in vitro* of chlortetracycline on the metabolic activity of ileum contents of young swine. Mean value of 10 samples.

which were the products of decarboxylase activity of *E. coli*, namely agmatine, putrescine, histamine, and cadaverine, in samples from both groups was as expected. However, they were less abundant in samples from the chlortetracycline-fed group. Products of tryptophan breakdown were infrequently detected in ileum samples from chlortetracycline-fed swine.

The reduction of metabolic activities of bacterial populations of ileum contents containing chlortetracycline is a striking facet of the effect of chlortetracycline. Although the carbohydrate breakdown products may not be as deleterious as some of the amines of protein dissimilation, lessened metabolic activity may contribute to the sparing of nutrients for the host animal.

These experimental findings offer support for the view that the rate of gain and feed efficiency of young swine were improved because some metabolic activities of microflora of intestinal contents were reduced when chlortetracycline was administered. Amino acid decarboxylase activity was lessened, and metabolic activities were retarded when diets were supplemented with chlortetracycline. A reduction in amines and an increased availability of nutrients resulting from the sparing of amino acids and the inhibition of dissimilation of carbohydrates may be some of the factors that contribute to the favorable effect of chlortetracycline for young swine.

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SUMMARY

To examine the possibility that metabolic activities of intestinal microorganisms of young swine may be reduced when chlortetracycline is added to the diet, ileum contents were collected from two groups of young swine, one of which was fed a basal diet, and the other fed the basal diet supplemented with 40 ppm of chlortetracycline. Paper chromatography and paper electrophoresis were used to separate amines extracted from ileum contents. A greater amount and variety of amines were obtained from those

receiving the basal diet without chlortetracycline supplementation. Amines produced by decarboxylases from *Escherichia coli* were found to be common to both groups, but with lesser amounts obtained from the chlortetracycline-fed group.

The metabolic activities of the microflora of the ileum were markedly reduced when small amounts of chlortetracycline were imposed.

Findings of reduced metabolic activities of the microflora of ileum contents of young swine receiving low levels of chlortetracycline suggest that chlortetracycline may in this way contribute to the thriftiness and well-being of these young animals.

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