tyrocidine than with gramicidin but the latter, although slower in action, ultimately causes a greater degree of hemolysis than the former.

Comparative experiments have been done to determine the influence of horse serum on the rate and amount of hemolysis caused by gramicidin and tyrocidine. The hemolytic activity of both substances is decreased in the presence of only 1% of horse serum. Five percent of serum causes further inhibition of the hemolytic activity of gramicidin and completely prevents hemolysis by tyrocidine. Increasing the amount of serum beyond 5% did not result in a further decrease of the hemolytic activity of gramicidin.

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Influence of Vitamins and Coliform Bacteria on Sulfaguanidine Tolerance by Young Chickens.*

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Sulfaguanidine is well established as an effective bacteriostatic agent for treatment of enteric disorders, but prolonged feeding of this drug in large amounts is known to cause a variety of pathological changes, including loss of weight or failure to maintain normal growth in experimental animals such as rabbits,[1] rats,[2] and chickens.[3] Black *et al.*[4,5] reported that a basic cause of growth inhibition by sulfaguanidine is its interference with intestinal bacteria which normally synthesize growth-promoting substances used by the animal body. At least some of the harmful effects of the drug can be counteracted with liver extract,[4,6] p-aminobenzoic acid,[4] vitamin K,[5] or biotin concentrate[6] but not with nicotinic acid.[7] Presumably these agents act by supplying vitamins ordinarily procured from intestinal bacteria, or by preventing the interference of sulfaguanidine with essential metabolic processes. To our knowledge no substances which increase the harmful effects of sulfaguanidine have been reported.

The wide implications and apparent acceptance of this concept of interference with synthesis by the intestinal flora led us to undertake a further investigation of the relation of vitamins and intestinal bacteria to the action of sulfaguanidine. The results obtained with young growing chickens are presented here to indicate the complexity of the relationship; they are regarded as neither an evaluation of the phenomena involved nor a criticism of previous work.

Experimental methods. Single-comb, white Leghorn chicks of uniform size and vigor were individually fed 3 times daily on an adequate ration designated as Nebraska 8-S.[8] Controlled feeding was begun 24 hr after hatching and individual records of food intake and body weight were recorded throughout the experimental period of 3 to 4 weeks.

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The following supplements were added to the ration in daily portions as required for each group of birds:

(1) Sulfaguanidine—1% of body weight of the bird per day

(2) Vitamin mixture
  (a) p-aminobenzoic acid—12 mg per bird per day
  (b) thiamin—0.6 mg per 100 g of ration
  (c) riboflavin—0.8 mg per 100 g of ration

(3) *Escherichia coli* (24-hr cultures on nutrient agar or Long's medium[9])
Water suspension of cells corresponding in turbidity to tube 8 of the McFarland[10] nephthelometer series—0.2 ml per g of ration.

Droppings were collected on clean Kraft paper placed beneath the wire mesh floor of each brooder compartment. The period of sampling was limited to 2 hr in order to provide fresh samples for laboratory use. Aliquots of moist feces were weighed and decimal dilutions to 1:10 million were then made in sterile water. Bacteriological examinations included direct microscopic counts, plate counts on enriched tryptone-glucose-cysteine agar[11] both with and without 0.045% potassium tellurite, gas production in brilliant green-bile-lactose broth[12] and coliform counts on desoxycholate agar[13] plates. Incubation at 37°C for 2-4 days was employed. Because of the extent of these data only summaries of average coliform counts are included in the tabulated results.

Results. Preliminary experiments showed that daily feeding of sulfaguanidine at levels of 3-5% of the body weight of the baby chicks caused a rapidly fatal toxicity. Lower levels of 1-2% retarded growth and caused some mortality but permitted the survival of a majority of the birds. Repeated bacteriological examinations of feces from sulfaguanidine-treated birds and controls bore out Corwin's[1] observation that the drug does not lower the total bacterial content of the feces even though there is marked inhibition of the coliform group, for a concomitant increase in Gram-positive cocci occurs. These findings indicated that 1% sulfaguanidine would give marked growth inhibition for studying the influence of selected vitamins, and that the coliform bacteria were the most likely group among the intestinal flora to play a decisive role.

Table I summarizes the data obtained from birds which received the standard 8-S ration alone and with the vitamin mixture or the *E. coli* suspension, both in the presence and absence of 1% sulfaguanidine. Repetition of each phase of this investigation 2 to 4 times yielded the same trends in every in-

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TABLE I.

Relation of Sulfaguanidine, *E. coli*, and Vitamins to Survival and Fecal Coliform Counts of Young Chicks.

Supplement to 8-S ration	No. birds	No. deaths	Avg coliform bacteria/g feces × 1000
None	16	1	3,372
Vitamin mixture*	15	0	4,056
E. coli suspension	16	1	3,529
Sulfaguanidine, 1%	28	7	24
" " + vitamin mixture*	21	12	1,847
" " + *E. coli* suspension	21	15	242

*Thiamin, 0.6 mg/100 g ration + riboflavin, 0.8 mg/100 g ration + p-aminobenzoic acid, 12 mg per bird per day.

stance; therefore, only total or average results are reported.

The most striking aspect of these experiments was the increased mortality rate shown by birds receiving either the vitamin mixture or the *E. coli* suspension in conjunction with sulfaguanidine. Control groups receiving 8-S ration alone and with the vitamins or bacterial suspension showed death rates of 0-6%. Those receiving only sulfaguanidine in the 8-S ration suffered a mortality rate of 25%. Addition of both the vitamins and sulfaguanidine to the ration increased the rate to 57%. A suspension of *E. coli*, recently isolated from the feces of a normal chick, combined with sulfaguanidine produced even greater harm, causing a death rate of 71%. In short the presence of a mixture of p-aminobenzoic acid, thiamin and riboflavin doubled the number of deaths resulting from administration of 1% sulfaguanidine and a suspension of *E. coli* nearly tripled the deaths from this cause.

By contrast with the above differences in mortality rate, all birds receiving sulfaguanidine alone and with supplements of vitamins or *E. coli* gained about the same amount, *i.e.*, 15 to 20 g per 100 g of feed consumed. This growth was only ⅓ of the increase maintained by chicks not receiving the drug, regardless of whether or not bacterial and vitamin supplements were included in the diet.

Counts of coliform bacteria made at weekly intervals on desoxycholate agar showed that feces of birds receiving no sulfaguanidine contained in excess of 3 million coliform bacteria per gram of moist sample. The feeding of *E. coli* did not significantly increase the number present in the droppings but the vitamin mixture did tend to give higher counts. Addition of sulfaguanidine to the 8-S ration caused progressive dimunition of these bacteria until practically none were present after 3 weeks. In the presence of the vitamin mixture or the *E. coli* suspension the drug decreased the coliform bacteria, but appreciable numbers persisted throughout the period of observation.

Conclusions. The death rate among baby chicks fed an adequate ration plus sulfaguanidine at the level of 1 g per 100 g body weight per day is less than half that occurring when a mixture of p-aminobenzoic acid, thiamin and riboflavin or a suspension of *E. coli* are administered with the drug. These facts, together with the tendency of both the vitamins and bacterial suspension to sustain the coliform bacteria in the feces in the presence of an inhibitory concentration of the drug, suggest that the tolerance of sulfaguanidine by young birds may be related to the activities of the coliform bacteria in the intestinal tract.

Our findings do not, in themselves, constitute a contra-indication to the clinical use of sulfaguanidine, for the level of drug employed here is much in excess of the effective therapeutic dosage. They serve only to emphasize the apparently fundamental and complex relationship of vitamins and intestinal bacteria to the action of sulfaguanidine *in vivo*.

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