USE OF SULFASUXIDINE, STREPTOTHRICIN, AND STREPTOMYCIN IN NUTRITIONAL STUDIES WITH THE CHICK*

BY P. R. MOORE, A. EVENSON, T. D. LUCKEY, E. MCCOY, C. A. ELVEHJEM, AND E. B. HART

(From the Departments of Biochemistry and Agricultural Bacteriology, College of Agriculture, University of Wisconsin, Madison)

(Received for publication, June 27, 1946)

Sulfonamides are known to alter the intestinal flora of animals, with a consequent change in the synthesis of certain vitamins by the intestinal bacteria (1-5). This altered synthesis may in turn lead to an increased dietary vitamin requirement for some animals or invoke new requirements in others. For example, the inclusion of 1 per cent of sulfasuxidine in the diet of chicks increases their folic acid requirement about 3-fold (6).

The question then naturally arises whether other bactericidal or bacteriostatic agents could produce still different changes in the intestinal flora and lead to increased requirements for other vitamins or to new types of deficiencies. Further, a reverse phenomenon could possibly be encountered; namely, the inhibition of certain bacterial groups which might decrease the growth of the animal either through the consumption and consequent immobilization of vitamins or through the production of toxic compounds. Finally, a drug or combination of drugs that would completely inactivate all bacteria in the intestinal tract would be highly desirable, since investigators would be provided with an essentially sterile animal and vitamin requirements could be studied uncomplicated by "intestinal vitamins" or toxic substances. This investigation was carried out with the latter view in mind.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Wisconsin Alumni Research Foundation, Swift and Company, Commercial Solvents Corporation, and by funds granted by the American Dairy Association.

We are indebted to Merck and Company, Inc., Rahway, New Jersey, for the crystalline vitamins and streptothricin; to Wilson and Company, Inc., Chicago, for gelatin; to The Wilson Laboratories, Chicago, for liver fraction L and whole liver substance; to Allied Mills, Inc., Peoria, Illinois, for soy bean oil; to Dr. B. L. Hutchings of the Lederle Laboratories, Inc., Pearl River, New York, for synthetic folic acid; to Sharp and Dohme, Inc., Philadelphia, Pennsylvania, for sulfasuxidine; to the Abbott Laboratories, North Chicago, Illinois, for haliver oil; to E. I. du Pont de Nemours and Company, Inc., New Brunswick, New Jersey, for crystalline vitamin D3; and to Dr. J. M. McGuire of Eli Lilly and Company, Indianapolis, Indiana, for streptomycin.
Day-old white Leghorn cockerels obtained from a commercial hatchery were maintained in electrically heated cages with raised screen bottoms and fed a basal ration consisting of dextrin 61 gm., alcohol-extracted casein 18 gm., gelatin 10 gm., Salts 5 (7) 6 gm., soy bean oil 5 gm., l(-)-cystine 300 mg., i-inositol 100 mg., thiamine hydrochloride 0.3 mg., riboflavin 0.6 mg., calcium pantothenate 2.0 mg., choline chloride 150 mg., nicotinic acid 5 mg., biotin 0.02 mg., pyridoxine hydrochloride 0.4 mg., 2-methyl-1,4-naphthoquinone 0.05 mg., and a-tocopherol 0.3 mg. In addition each chick received weekly by dropper 1200 U. S. P. units of vitamin A and 120 A. O. A. C. units of vitamin D₃. After 3 days on the basal diet chicks within a 10 gm. weight range were divided into uniform groups of six and given the supplements shown in Table I. Streptomycin, streptothricin, and sulfasuxidine supplements were ground in a mortar with 10 gm. of the casein prior to inclusion in the diet. All experiments were terminated when the chicks were 4 weeks old and representative groups were chosen for bacteriological analysis of their cecal contents (8).

Results

The addition of 5000 units of streptothricin per 100 gm. of basal diet had no significant effect on growth or survival of the chicks, while levels of 10,000 units or more proved toxic. The addition of 500 γ per cent of folic acid proved ineffective in counteracting this toxicity. Liver eluates, whole liver substance, Wilson’s liver fraction L singly or in combination with 500 γ per cent of folic acid were likewise found ineffective. Histamine hydrochloride at a level of 0.02 per cent was found non-toxic to the chicks.

Rather unexpected results were obtained with streptomycin in that increased growth was observed when this compound was fed together with adequate amounts of folic acid (compare Groups 11 and 12 with Group 4). Similarly, chicks receiving sulfasuxidine and 500 γ per cent of folic acid exhibited increased growth over those fed folic acid alone. A combination of sulfasuxidine and streptomycin in addition to the 500 γ per cent of folic acid produced a decreased growth when compared to either compound alone but an increased growth over the 500 γ per cent of folic acid control group. This observed growth stimulation by sulfasuxidine and streptomycin is interesting, since it suggests the inhibition of intestinal bacteria that are either producing toxic materials or are rendering certain dietary vitamins unavailable to the animal, an effect different than that usually encountered with sulfonamides. In this latter connection it is worth noting that Parsons (9) has induced a thiamine deficiency in humans by...
**Table I**

**Growth, Toxicity, and Bacterial Counts* in Chicks**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Supplement added per 100 gm. basal ration</th>
<th>No. of chick in each group</th>
<th>Average weight at 4 weeks</th>
<th>Total count</th>
<th>E. coli</th>
<th>Escherichia</th>
<th>Enterococci</th>
<th>Lactobacili</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>1</td>
<td>155</td>
<td>100</td>
<td>10</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Norit eluate† = 5% or 2% liver fraction L</td>
<td>0</td>
<td>220</td>
<td>100</td>
<td>10</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5% whole liver substance</td>
<td>0</td>
<td>245</td>
<td>100</td>
<td>10</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>500 γ % folic acid</td>
<td>0</td>
<td>220</td>
<td>25,000</td>
<td>100</td>
<td>1000</td>
<td>25,000</td>
<td>25,000</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5000 units streptothricin</td>
<td>0</td>
<td>150</td>
<td>25,000</td>
<td>100</td>
<td>1000</td>
<td>25,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10,000 units streptothricin</td>
<td>5</td>
<td>50</td>
<td>2500</td>
<td>100</td>
<td>1000</td>
<td>25,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>25,000 units streptothricin</td>
<td>6</td>
<td></td>
<td></td>
<td>1</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Streptothricin, 1000 units 1st wk., 5000 units 2nd wk., 10,000 units 3rd wk., 25,000 units 4th wk.</td>
<td>2</td>
<td>120</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5000 units streptothricin + 5% whole liver substance</td>
<td>0</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1% sulfasuxidine§ + 500 γ % folic acid</td>
<td>0</td>
<td>280</td>
<td>50,000</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>50,000</td>
<td>0.001</td>
</tr>
<tr>
<td>11</td>
<td>10,000 units streptomycin</td>
<td></td>
<td>+ 500 γ % folic acid</td>
<td>0</td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>50,000 units streptomycin + 500 γ % folic acid</td>
<td>0</td>
<td>300</td>
<td>1,000</td>
<td>0.01</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>1,000</td>
<td>0.001</td>
</tr>
<tr>
<td>13</td>
<td>10,000 units streptomycin + 500 γ % folic acid + 1% sulfasuxidine</td>
<td>0</td>
<td>240</td>
<td>1,000</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>1,000</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Bacterial counts are given in terms of the number of bacteria per cc. of cecal contents × 10^6.
† Weights for Groups 1 and 2 are based on twenty-four chicks.
‡ An alcohol-water-ammonia eluate of a charcoal adsorbate of liver fraction L. Expressed as equivalents per cent of the gm. of starting material per 100 gm. of ration.
§ Succinylsulfathiazole.
|| A concentrate containing 300 units per mg.
feeding large amounts of live yeast which supposedly is utilizing dietary thiamine for its own metabolism, thus leaving inadequate amounts available to the host. However, the possibility that these agents are acting systemically cannot be overlooked. Parenthetically, streptomycin did not appear to increase the biotin requirement of the chick above 20 γ per cent in contrast to the report of Emerson and Smith (10) who have induced biotin-like deficiency symptoms in rats by the oral administration of streptomycin. This different response indicates that the chick relies almost entirely on an external source of biotin and any synthesis occurring in the intestine is negligible. Alternatively, a partial synthesis of biotin might occur in the chick intestine but the organism responsible for this synthesis is not affected by streptomycin as in the case of the rat.

All of the sulfonamides failed to sterilize the intestinal tracts of the chicks and no significant decreases in the total count of the intestinal bacteria were observed (Table I). The number of animals used for the bacteriological studies was limited but the results substantiate the findings of Smith and Robinson (11) that orally administered streptomycin produces a marked reduction in the coliform bacteria of the feces. It is interesting to note that in the chicks receiving a low folic acid diet (Group 1) the coliform bacteria were the predominant microorganisms, while in the presence of folic acid (Group 4) the Lactobacilli predominate. Experiments are now in progress to verify this observation further.

SUMMARY

Streptothricin at levels of 5000 units per 100 gm. of purified diet is readily tolerated by the chick but when given at levels of 10,000 units or more is toxic. No toxicity was observed when streptomycin was fed at levels of 50,000 units per 100 gm. of diet. Sulfasuxidine and streptomycin singly or in combination lead to increased growth responses in chicks receiving our basal diet supplemented with adequate amounts of folic acid. Streptothricin, streptomycin, sulfasuxidine, or a combination of streptomycin and sulfasuxidine failed to sterilize the intestinal tract of chicks, but produced a marked reduction in the coliform bacteria of the cecal contents.

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