Effect of Terramycin on Fecal Microflora of Rats. II. Therapeutic Level of Terramycin.* (20430)

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(Introduced by Edgar Zwilling)

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A previous report(1) of the effect of dietary terramycin (100 mg per kg), in 3 rations, indicated statistically significant increases in *Proteus spp.*, regardless of count of *Escherichia coli*. This observation is in disagreement with Sieburth *et al.*(2,3) who reported the simultaneous decrease in *E. coli* and increase in *Proteus*.

In the expectation of obtaining exaggerated effects of terramycin, yielding counts more susceptible of statistical analysis and to verify the previous findings, the experimental procedure previously described(1) was repeated, except that 666 mg terramycin per kg ration was administered, total counts of lactobacilli were made using Rogosa's medium(4), and the experimental period was 5 weeks. Essentially, the procedure consisted of making weekly bacterial counts, using appropriate media and conditions, of feces of groups of animals receiving 3 diets with and without terramycin. The amount of terramycin fed the animals approximated the dosage administered in human therapy. The diets differed in concentrations of protein and carbohydrate. The counts and changes in counts obtained were subjected to a standard analysis of variance(5). Table I includes the mean values of the logarithms of the real numbers, and the degree of variability of the following experimental groupings: (a) among rations, (b) between composited treated and untreated animals, and (c) between replicates. Table II shows the two values making up each subgroup (mean logs of all enumerations) and the subgroup means. The least significant difference (L.D.S.) at the 5% level of probability between subgroup means is also included in Table II.

Results and discussions. The groups of microorganisms studied will be discussed in order of their appearance in Tables I and II.

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Total anaerobic count. The addition of terramycin to all rations resulted in a marked decrease in the viable anaerobes ($P<0.001$). This reduction was independent of ration (absence of significant interaction between ration and terramycin). Ration, *per se*, however, exerted an effect on the anaerobic count, but at low probability ($P<0.10$), highest counts being obtained with the high carbohydrate ration.

Total aerobic count. The addition of terramycin to all 3 rations resulted in a marked reduction of aerobic count ($P<0.05$). There was no interaction apparent between diet and terramycin, and no effect of diet *per se*.

Proteus spp. The number of *Proteus spp.* was markedly increased by the addition of terramycin to any of the 3 rations ($P<0.001$). The increase was not influenced by population of *E. coli*, an observation in disagreement with Sieburth and his associates(2,3). Ration *per se* exerted no effect on the *Proteus* count nor was there an apparent interaction between ration and terramycin.

Fecal streptococci. Terramycin *per se* resulted in a slight increase in fecal streptococci count, although the increase was not significant at the 5% level ($P<0.10$). No interaction between ration and terramycin was noted. A significant ration effect ($P<0.05$) however, was observed between the high protein and the basal ration, lower counts obtained with the former. This was the only significant ration effect observed in this study.

Total lactobacilli. The total lactobacilli, enumerated in Rogosa's medium were markedly reduced by addition of terramycin to any of the 3 rations. No interaction obtained between ration and terramycin. Many colonies developing on this medium were found to be streptococci and yeasts, and the counts here reported are from numbers of colonies, without identification, and are considered to be provisional.

TABLE I. Mean Values of the Logs of All Enumerations of Total Viable Anaerobes, Total Viable Aerobes, *L. acidophilus*, *E. coli*, *Proteus spp.*, Fecal Streptococci and Total Viable Lactobacilli, in the Feces of Albino Rats on 3 Diets with and without Terramycin. Statistical data include ration differences, drug differences, duplication of experiment and significant probabilities.

	Organisms						
Exp. groupings	Total anaerobes count, $\times 10^6$	Total aerobes count $\times 10^6$	*L. acidophilus* count, $\times 10^3$	*Proteus spp.* count, $\times 10^3$	*E. coli* count, $\times 10^4$	Fecal streptococci count, $\times 10^3$	Total lactobacilli count, $\times 10^6$
Rations							
Basal	2.968	2.503	2.595	2.960	2.821	3.624	1.817
Protein	2.847*	2.230	2.378	3.162	2.702*	1.878‡	1.687
Carbohydrate	3.167	2.606	1.945	2.955	3.472	2.739	1.956
Terramycin							
(−)	3.372†	2.685‡	3.334†	1.996†	2.272§	2.389*	2.644†
(+)	2.616	2.207	1.277	4.055	3.724	3.106	1.005
Replication							
I	3.026	2.174‡	2.170	2.782	3.226	2.838	1.812
II	2.962	2.718	2.441	3.269	2.769	2.657	1.837

* P <.10. † P <.001. ‡ P <.05. § P <.01.

L. acidophilus. Terramycin *per se* caused a significant reduction on *L. acidophilus* (P< 0.05). No interaction between diet and terramycin was observed, although higher counts were obtained with the basal and the high protein rations than with the high carbohydrate ration. This substantiates the observation that dextrin fails to maintain a high aciduric intestinal flora(1,6,7).

E. coli. Increased dosage of terramycin did not result in a significant interaction between ration and terramycin, as was observed (1) with the low nutritional level. A ration effect, however, was noted at the 10% level of probability, between the high carbohydrate and the high protein diets, higher counts obtained with the former. Terramycin *per se* caused an *increase* (P<0.01) in *E. coli,* an observation in disagreement with the findings of most workers. Attention is particularly directed to the fact that the tergitol-7 agar(8) used in this and prior work(1) has been found to be from 30 to 40% more productive in recovery of *E. coli* than other differential media(9). During the experimental work reported here, hundreds of cultures isolated from this medium were found to give IMViC (10) reactions of "typical" *E. coli.* Although no other studies were made of the organisms recovered, it is believed that the productivity and selectivity of this medium are remarkable, and the counts reliable.

Conclusions. A comparison of this and the previous report is presented in Table III, from which the following conclusions may be drawn: (a) a significant diet and terramycin interaction was observed only in low terramycin dosage, only in counts of *E. coli;* (b) a significant diet effect was observed in *Proteus* counts, only at low dosage, and in counts of fecal streptococci only at high terramycin dosage; (c) significant decreases in total anaerobes, total aerobes, *L. acidophilus,* were found at both dosages; (d) increase of *Proteus* at both dosages; (e) decrease of fecal streptococci at low dosage but slight increase at high dosage. Comparisons illustrate the need for severe analysis of experimental conditions and procedures, and in general indicate validity of the findings.

Summary. 1. In the feces of albino rats, statistically significant reductions in counts

TABLE II. Mean Logs of 5 Weekly Values Including L.S.D. among Subgroups of All Animals on Different Rations with and without Terramycin.

Organisms	Exp.	Basal —	Basal +	High protein, low carbohydrate, terramycin —	High protein, low carbohydrate, terramycin +	High carbohydrate, low protein —	High carbohydrate, low protein +	L.S.D. for subgroups*
Total anaerobes $\times 10^6$	I	3.179	2.743	3.502	2.334	3.596	2.800	.398
	II	3.398	2.550	3.254	2.289	3.300	2.970	
	Mean	3.289	2.647	3.378	2.316	3.448	2.886	
Total aerobes $\times 10^6$	I	2.209	2.228	2.259	1.727	2.577	2.045	.640
	II	3.242	2.333	2.898	2.033	2.925	2.878	
	Mean	2.726	2.280	2.579	1.880	2.750	2.461	
L. acidophilus $\times 10^5$	I	3.204	1.021†	3.203	1.320	3.219	1.051	1.193
	II	3.939	2.214	3.292	1.695†	3.149	.357	
	Mean	3.581	1.618	3.248	1.508	3.184	.704	
E. coli $\times 10^4$	I	2.239	4.064	2.120	4.094	2.796†	4.045	.977
	II	1.729†	3.252†	1.772	2.820	2.977	4.069†	
	Mean	1.984	3.658	1.946	3.457	2.886	4.058	
Proteus spp. $\times 10^3$	I	1.672	4.106	2.189	3.337	1.232	4.160	.989
	II	1.852	4.211	2.645	4.477	2.386	4.041	
	Mean	1.762	4.159	2.417	3.907	1.809	4.100	
Fecal streptococci $\times 10^3$	I	2.526	4.492	2.534	1.496	2.756	3.220	1.332
	II	3.236	4.242	1.454	2.123	1.825	3.157	
	Mean	2.881	4.367	1.994	1.762	2.291	3.188	
Total lactobacilli $\times 10^6$	I	2.581	.823	2.547	.702	2.725	1.494†	.794
	II	2.737†	1.185	2.395	1.104	2.879	.724†	
	Mean	2.659	1.004	2.471	.903	2.802	1.109	

* L.S.D. for subgroups computed at 5% level of probability.
† One missing value calculated.

TABLE III. Comparative Statistical Analyses of 2 Sets of Different but Comparable Experimental Data on Effects of Dietary Terramycin on Fecal Microflora.

	T*	Organisms: Anaerobes	Aerobes	*L. acidophilus*	*E. coli*	*Proteus spp.*	Fecal streptococci
Terramycin	A	Decrease† $(P < .01)$	Decrease† $(P < .01)$	Decrease† $(P < .01)$	Decrease‡ $(P < .01)$ Increase§ $(P < .05)$	Increase‖ $(P < .05)$	Decrease† $(P < .001)$
	B	Decrease† $(P < .001)$	Decrease† $(P < .05)$	Decrease† $(P < .05)$	Increase‖ $(P < .01)$	Increase‖ $(P < .001)$	Increase¶ $(P < .1)$
Diet effect	A	None	None	None	None	Present $(P < .05)$	None
	B	Present¶ $(P < .1)$	"	Present¶	Present¶	None	Present $(P < .05)$**
Diet terramycin interaction	A	None	"	None	" ‡§	"	None
	B	"	"	"	None	"	"

* A = 100 mg terramycin/kg ration. B = 666 mg terramycin/kg ration.
† Decrease in all diets. ‡ Decrease in high carbohydrate ration. § Increase in basal ration.
‖ Increase in all diets. ¶ Not significant. ** Increase in high protein ration.

of the total anaerobes, total aerobes and total lactobacilli were obtained by the addition of terramycin to the 3 diets employed. On the other hand, *E. coli, Proteus spp.* and, to a slight extent fecal streptococci, were increased in numbers. The increase in *Proteus* was unaccompanied by a decrease in *E. coli.* 2. The only ration effect was observed with the counts of fecal streptococci: the animals receiving the high protein rations showed a lower count than those receiving either the high carbohydrate or the basal ration. 3. The effect of terramycin upon counts of all organisms studied was found to be independent of ration in which the terramycin was incorporated (lack of statistically significant interaction between diet and terramycin). 4. The effects here reported were compared to the effects observed in a previous study.

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Failure of Certain Vitamins to Affect the Survival of the Eviscerate Rat. (20431)

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It was considered possible that vitamin deficiencies might ensue rapidly in the eviscerate animal and thereby limit survival. Eviscerate rats were injected with different preparations of vitamins without a favorable effect upon survival.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets. The technic of aseptic evisceration has been described(1). When the animals reached 250 $\pm$ 2 g, they were anesthetized (intraperitoneal injection of 18 mg of cyclopal sodium) and subjected to the second stage of evisceration. Sterile solutions containing 0.9% NaCl, glucose, insulin, crystalline penicillin G, and streptomycin sulfate with and without vitamins were administered by a 6-place continuous injection machine which delivered fluid into the saphenous vein of the right hind leg at the rate of 20 ml per 24 hours. The initial glucose load was 44 mg per 100 g of rat per hour and was decreased to 36 mg after 48 hours. The insulin dose was 4 units per rat per 24 hours. The dose of penicillin was 10,000 units per rat per 24 hours, and the streptomycin dose was 20 mg per rat per 24 hours. Temperature was 26.5 $\pm$ 0.5°C. Survival time was determined by a device which amplified the D.C. potential generated by the heart to actuate a recording mechanism.

The following vitamins were used. A mixture of B vitamins contained the following amounts per ml: Vit. B_{12}, 1 μg; folic acid, 1 mg; thiamine hydrochloride, 2 mg; riboflavin, 2 mg; pyridoxine hydrochloride, 1 mg; pantothenate sodium, 10 mg; and nicotinamide, 25 mg. Rutin and ascorbic acid were mixed together in a 1 to 1 weight ratio. The above substances were added to the solutions given by continuous intravenous injection. The oil soluble vit. A, D, and E were given together by continuous subcutaneous injection at the rate of 1 ml per rat per 24 hours. Cod liver oil was the source of vit. A and D; mixed tocopherols were added.

Experiments and results. Eviscerate rats given vitamins were compared simultaneously with eviscerate rats not given vitamins but