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Oral Administration of Bacitracin.

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(Introduced by John F. Norton.)
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Bacitracin is the antibiotic substance or substances produced by the growth of the "Tracy I" strain of *Bacillus subtilis*. This material is now being produced on a pilot scale and has been made available for study. Several reports have been published regarding the efficacy of this antibiotic, when administered topically or systemically, but information concerning its oral use is very meagre.

The purpose of this paper is to present a report of the findings following oral administration of bacitracin to dogs. The results concern the effect of bacitracin on the intestinal flora, and bacitracin levels in the feces, blood and urine.

Scudi, Clift and Krueger[1] reported, that following the oral administration in dogs of 3,000 u and 6,000 u of bacitracin per kg body weight, no detectable bacitracin was found in the blood stream, nor was any found in the urine during the 24 hours after administration of the antibiotic. Following oral administration of 1500 u/kg body weight to 2 dogs, less than 5% of the oral dose was recovered in the stool. From these findings they concluded that the antibiotic was largely destroyed in the intestinal tract.

Meleney[2] reported the use of oral bacitracin in conjunction with systemic administration, in the treatment of a case of colitis. Clinically, good results were obtained.

Six dogs have been studied. All dogs were put on a uniform diet several days preceding and during the experiment. Bacitracin in solution was administered by stomach tube twice daily, at 8:30 A.M. and 4:30 P.M. The amounts given varied from 2000 u/kg body weight per day to 20,000 u/kg body weight per day. Heparinized blood samples were taken at one and 2 hours after administration of the morning dose. Fecal specimens were obtained by means of a fecal hook each morning before the administration of bacitracin and in 4 of the 6 dogs, 24 hour urine samples were collected. The dogs exhibited no gross change in activity. Body weight was maintained and appetite as measured by food consumption was not affected.

To study the effect of bacitracin on the intestinal flora, a procedure was used similar to that of Poth,[3] for the study of the effect of sulfonamides and streptomycin on the intestinal flora of dogs.

A portion of each specimen of feces was diluted 1-10 with 0.5% potassium sulfite. We have shown that in optimum proportions bacitracin is inactivated by potassium sulfite. After standing for 30 minutes at room temperature, tenfold serial dilutions in saline were carried through 10^{10}, and samples from these dilutions were seeded into test media.

To determine the relative percentages of gram + and gram — organisms, fluid thioglycollate medium was inoculated and incubated 48 hours at 37°C. Smears were made and examined to determine the highest dilution at which gram + and gram — organisms were found.

The presence of fecal streptococci was determined by inoculating tubes of sodium azide medium and incubating at 37°C for 48 hours. Direct smears were made from all tubes showing growth and examined for the presence of streptococci.

Anaerobic agar was inoculated and heated to 80°C for 15 minutes and incubated 37°C for 48 hours to determine the presence of spore forming anaerobes.

[1] Scudi, J., Clift, M., and Krueger, R., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 9.

[2] Meleney, F., *Quarterly Progress Report to Office of Surgeon General*, April 1, 1948.

[3] Poth, E., Knotts, F., Lee, J., and Inui, F., *Arch. Surg.*, 1942, **44**, 187.

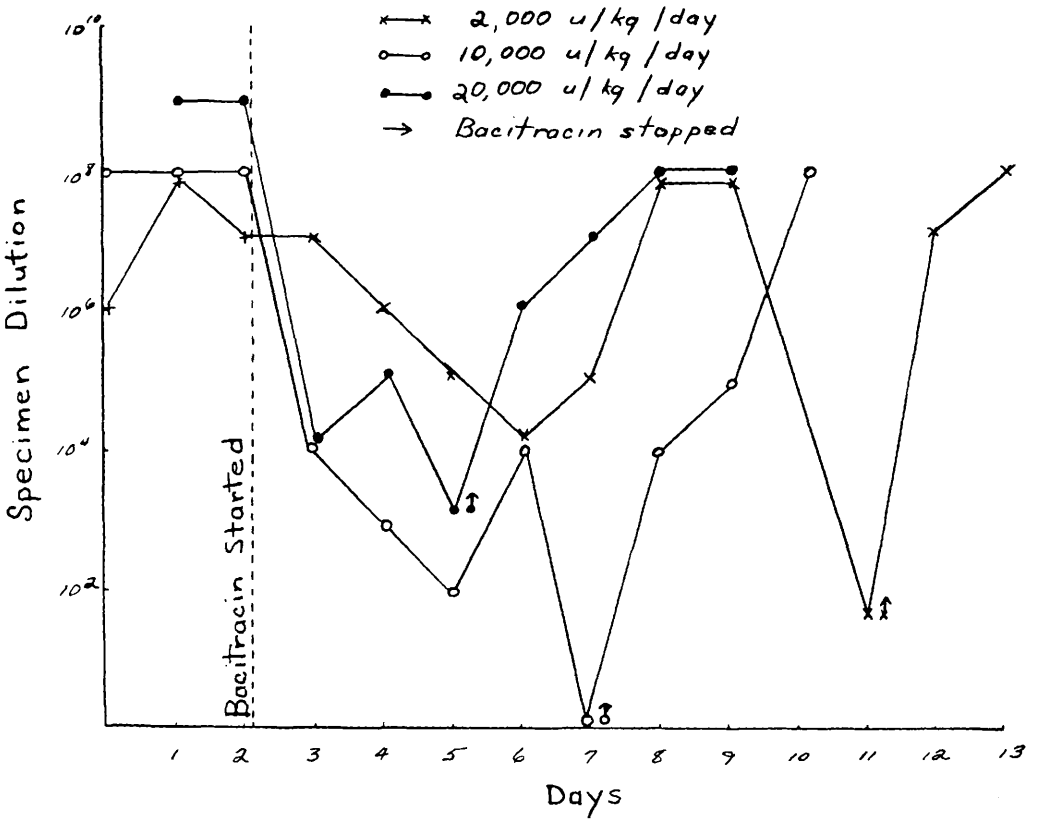

FIG. 1.
Effect of Bacitracin on Fecal Streptococci.

To determine the number of coliform organisms, poured plates of eosin-methylene blue agar were prepared.

Fecal, blood and urine specimens were assayed for bacitracin by the method described by Bond and Nook.[4] A hemolytic streptococcus was the test organism and the standard bacitracin was that supplied by the Food and Drug Administration.

The effect of bacitracin on the intestinal flora of dogs may be seen in the following figures and tables:

Fig. 1 shows the effect of bacitracin on fecal streptococci. At the dosage level of 2,000 u/kg per day, there was a marked variation in total count with the lowest count on the last day of treatment. At a dosage of 10,000 u/kg per day the count dropped abruptly and was zero on the fifth day of treatment. When treatment was stopped there was a rapid rise so that normal levels were reached in 3 days. The 20,000 u treatment curve is similar to the 10,000 u except that treatment was stopped on the third day, which was too soon to allow the count to drop to zero.

Fig. 2 shows the effect of bacitracin on the spore forming anaerobes. The results are comparable to those found for the fecal streptococci. A 2,000 u dose decreased the count but the level did not remain constant during the course of treatment. Both the 10,000 u dose and the 20,000 u dose produced an immediate and rapid drop to zero which remained until cessation of treatment. At that time there was a rapid and sharp rise to normal levels within 2 to 3 days.

Fig. 3 expresses graphically the relative percentage of gram + and gram — organisms with a dose of 10,000 u/kg per day. The pretreatment samples showed a greater incidence

[4] Bond, G., and Nook, M., *Science*, 1948, **107**, 228.

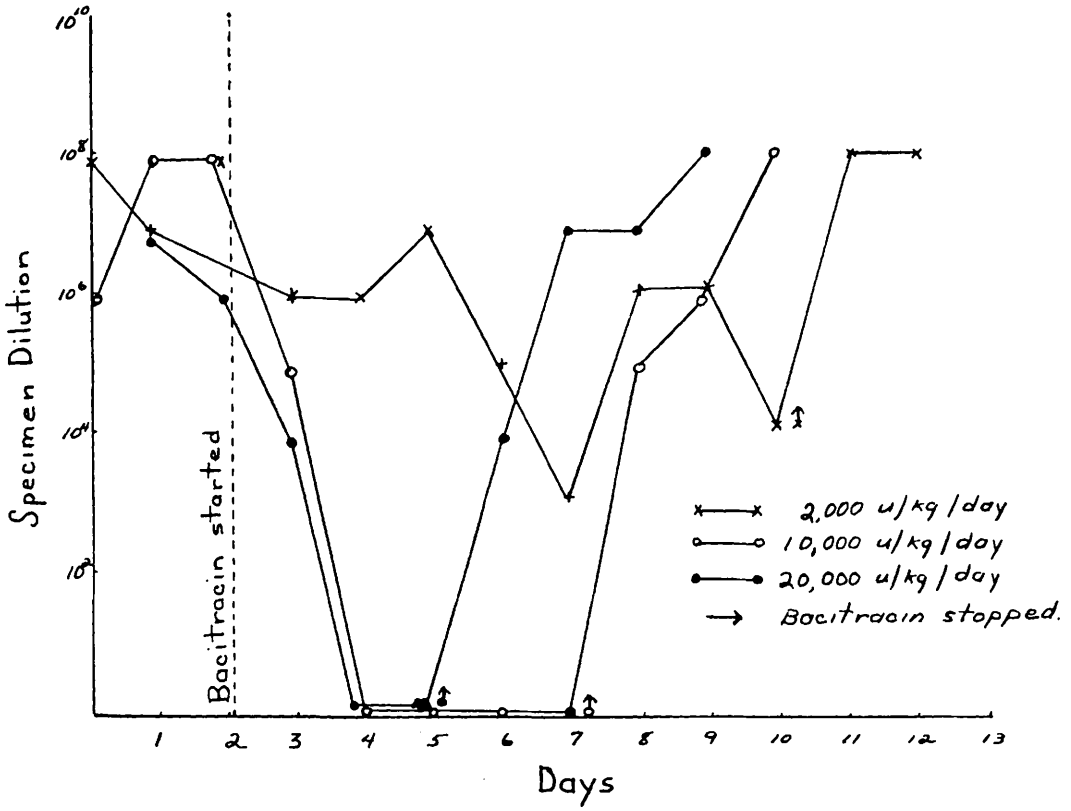

FIG. 2.
Effect of Bacitracin on Spore Forming Anaerobes.

of gram + organisms; but within 24 hours after treatment was started, there was a shift so that gram — organisms predominated during the course of treatment. Forty-eight hours after cessation of treatment the ratio was back to the normal relationship. The figures in the graph are highest dilutions at which the organisms were found.

No significant change was noted in the coliform organisms.

Table I shows the amount of bacitracin per g of feces. At the 2,000 u dose, the feces of 1 dog contained measurable amounts of bacitracin in 3 of 4 days and for 2 days after cessation of treatment. The other dog on the 2,000 u dosage did not show measurable levels until the last day of treatment and the first day after treatment had stopped. With 10,000 u both dogs showed definite levels of bacitracin persisting 1 to 3 days after cessation of treatment. With 20,000 u both dogs showed high levels of bacitracin for the 3 days of treatment and these persisted for 2 days after treatment. It was not possible to determine the total bacitracin in the feces.

Table II shows the blood levels expressed in units of bacitracin per cc of plasma obtained following the oral administration of bacitracin. 2,000 u/kg per day was not sufficient dosage to produce detectable blood levels. 10,000 u/kg per day produced levels of similar magnitude in 2 dogs 1 hour after administration. The 2 hour levels in all cases were lower than the 1 hour level and in one 2 hour sample no bacitracin could be demonstrated. No levels were found 24 hours after cessation of treatment. With 20,000 u/kg per day the plasma levels were higher in all cases than with 10,000 u. Again the 2 hour levels were lower than the 1 hour and no bacitracin could be demonstrated 24 hours after cessation of treatment.

In Table III urine levels of bacitracin are given for 4 of the 6 dogs studied. The urine was collected at 24 hour intervals and assayed.

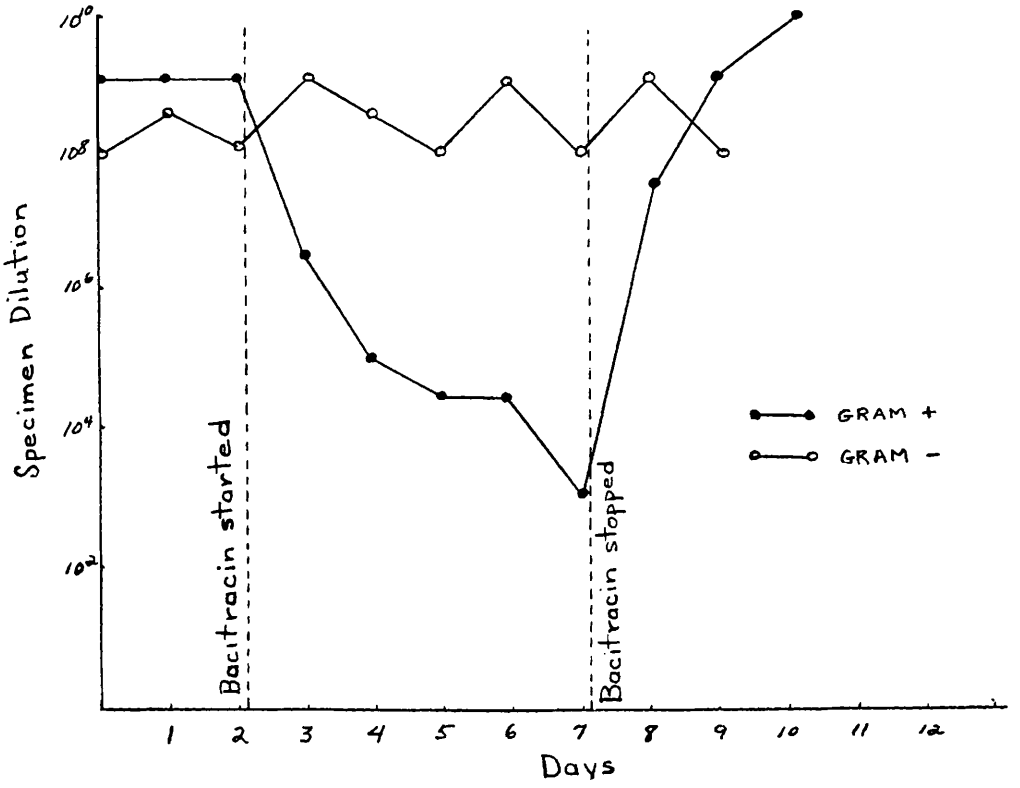

FIG. 3.
Effect of Bacitracin (10,000 u/kg/day) on Gram+ and Gram— organisms.

TABLE I.
Fecal Level of Bacitracin Following Oral Administration.
Expressed as units of Bacitracin per gram of feces.

Dose, u/kg/day	Pre-treatment	Treatment period								Post-treatment		
		Day 1	2	3	4	5	6	7	8	Day 1	2	3
2,000	0	.09	.56	0	.6					1.19	.6	0
2,000	0	0	0	0	0	0	0	0	.1	.4	0	
10,000	0	1.5	1.9	2.5	3.0					3.4	2.7	.6
10,000	0	1.0	12.0	4.2	8.7					.4	0	
20,000	0	75.0	75.0	15.0						7.0	8.0	0
20,000	0	90.0	85.0	100.0						18.0	9.0	0

2,000 u daily gave very low and irregular results. With 10,000 u a urinary level was found for each day of treatment and for 3 days following treatment. 0.22 u/cc of urine was the highest level obtained at this dosage. The 2 dogs on the 20,000 u dose showed high urinary levels for the 3 days of treatment and in one case detectable levels persisted for 2 days post treatment. The highest level obtained was 1.65 u/cc of urine.

Summary. From the small series of animals receiving oral bacitracin, the following significant facts were obtained: 1. Fecal streptococci and spore forming anaerobes were greatly reduced in the intestine at a dosage between 2,000 and 10,000 u/kg per day. 2. Coliform organisms were little affected by bacitracin. 3. Bacitracin persisted in the feces as long as 3 days following cessation of treatment. 4. Bacitracin was absorbed from

TABLE II.
Blood Levels of Bacitracin Following Oral Administration. Expressed as units per cc of plasma.

Dose, u/kg/day	Pre-treatment	Treatment period: Day 1 1 hr	Day 1 2 hr	Day 2 1 hr	Day 2 2 hr	Day 3 1 hr	Day 3 2 hr	Day 4 1 hr	Day 4 2 hr	Post-treatment Day 1	Post-treatment Day 2
2.000 u.	0	0	0	0	0	0	0	0	0	0	0
2,000 u.	0	0		0		0		0		0	0
10,000 u.	0	0		.013		.048		.045		0	0
10,000 u.	0	.085	.08	.064	.045	.046	0	.058	.047	0	0
20,000 u.	0	.1	.095	.088	.07	.22	.1			0	0
20,000 u.	0	.11	.098	.12	.08	.55	.17			0	0

TABLE III.
Urine Levels of Bacitracin Following Oral Administration.

Dose, u/kg/day	Pre-treatment	Treatment period: Day 1 u/cc	Day 1 Total u	Day 2 u/cc	Day 2 Total u	Day 3 u/cc	Day 3 Total u	Day 4 u/cc	Day 4 Total u	Post-treatment: Day 1 u/cc	Day 1 Total u	Day 2 u/cc	Day 2 Total u	Day 3 u/cc	Day 3 Total u
2,000	0	0	0	.045	10.5	0	0	0	0	.046	11.9	0	0	0	0
10,000	0	.06	8.21	.22	49.5	.096		.03	6.75	.1	9.0	.04	12.7	.025	4.75
20,000	0	.55	2550	.8	2080	.18	15.0			.075	15.0	.054	17.8	0	0
20,000	0	.9	3690	.5	1550	1.65	792			.17	73	0	0	0	0

the intestinal tract as evidenced by significant blood levels. 10,000 u kg per day was sufficient to produce blood levels as high as 0.085 u/cc of plasma 1 hour after administration. 5. Additional evidence of absorption was obtained by the finding of high levels of bacitracin in the urine. The highest obtained with 10,000 u was 0.22 u/cc and with 20,000 u 1.65 u/cc of urine.

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Attenuation of Vaccinial Virus by Interfacial Adsorption.*

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Previous mention has been made of the possibility of attenuating the virus of vaccinia by interfacial adsorption.[1] The data obtained at that time are those given in the present report. They illustrate the attenuation of this virus so that it will no longer produce the lesions which are characteristic of untreated vaccinia when injected endermically into rabbits but will nevertheless produce an immunity to the action of the untreated virus in these animals.

The method of attenuation employed was the same as that which was used in inducing the clotting of non-clotting blood plasmas[2] and in the attenuation of insulin[3,4] and of toxins such as ricin,[1] tetanal toxin,[1] staphylococcal toxin,[5,6] and snake venom.[1] It was shown there that, while emulsified chloroform is on the whole to be preferred over other adsorbing agents, inert emulsified gases are in some instances equally efficient in bringing about an attenuation.[6]

The virus† used in the present experiments was produced in chick embryos and suspended in saline. The saline suspension was treated by shaking with an equal volume of chloroform for 24 hours and all chloroform subsequently removed by evaporation under reduced pressure at a temperature below 40°C. Newly acquired animals which had not been exposed to vaccinia were used and each animal was isolated from all others during the period of treatment.

After completing these experiments, the writer found that Kelser[7-10] had prepared a rinderpest vaccine by allowing a saline suspension of the finely ground tissue from animals killed in the acute stages of rinderpest to stand in the ice box for a few hours in the presence of .75% chloroform. Kelser later prepared a rabic vaccine by the similar treatment of a 33⅓% fixed-virus brain-emulsion in physiological saline in the presence of 1.0% chloroform.

The writer subsequently found that Kelser's method could be applied to vaccinia to attenuate it sufficiently so that it will produce no lesions when administered endermically. Long standing of toxins with chloroform had,

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† The writer is indebted to Dr. John Buddingh for the virus preparations used in these experiments.

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