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A Study of the Effect of Gramicidin Administered by the Oral Route.*

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Gramicidin, an extract isolated by Dubos[1] from a spore-bearing soil bacillus, has been found to be bactericidal against gram-positive organisms not only in the test tube but also *in vivo*. Although studies have now been reported on the effect of this substance on the bacterial flora of wounds,[2] no detailed study has been made to determine whether or not gramicidin is capable of altering the intestinal flora through its action in killing the gram-positive components. The purpose of this work, therefore, was to ascertain whether or not this bactericidal agent possessed the ability of changing the intestinal flora of animals from one in which both gram-positive and negative members were present to one in which only the latter could be detected.

Mice were used as the experimental animals. They were fed a diet consisting of Purina Dog Chow which was found to be capable of producing a bacterial flora in the intestine which was rich in *L. acidophilus*. Since this bacterium is gram-positive it should be susceptible to gramicidin. *L. acidophilus* is easily detectable in plates made of a suitable medium by its characteristic colony morphology. Determinations were made at specific intervals of the percentage of the entire viable fecal flora which was made up of *L. acidophilus* before the beginning of the experiment and during the period when gramicidin was fed by mouth. The medium used was a tomato-yeast peptone agar described previously.[3] One stool from each experimental animal was obtained just at the moment of evacuation and emulsified in 5 cc of saline. One-tenth cc of this emulsion was then plated into the tomato agar. The plates were incubated at 37°C for 48 hours and then examined, the percentage of acidophilus colonies being

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1 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 311.

2 Herrell, W. E., and Heilman, D., *J. Clin. Invest.*, 1941, **20**, 583.

3 Weinstein, L., Weiss, J. E., Rettger, L. F., and Levy, M. N., *Arch. Int. Med.*, 1933, **52**, 384.

calculated by determining their number in 100 colonies. Before gramicidin was administered 3 determinations of the acidophilus counts were made at intervals of 3 days to establish the level of the organisms in the feces of each of the experimental animals. Fifty mice were used in the experiment, and they were divided into 5 groups of 10 each. The bactericidal agent, tyrothricin,† which contains the 2 substances, gramicidin and tyrocidine, was administered in distilled water by means of a ureteral catheter attached to a syringe, the catheter being passed into the stomach. One group of animals was given 0.1 mg of tyrothricin daily for 21 days; a second group was given 0.5 mg daily for the same period; and a third set of animals was fed 1 mg of the bacterial extract daily for an equal length of time. Two groups of mice were kept as controls, one group of 10 receiving 0.1 cc of a 1:10 dilution of 95% alcohol in water to correspond to the alcohol which was administered to the test mice as the vehicle for the tyrothricin, and the other group receiving nothing in addition to the basic diet. In the latter part of the experiment the tyrothricin was fed in the same amounts but was given twice a day, morning and late afternoon.

Results. Studies of the fecal flora prior to the administration of tyrothricin showed that the basal diet was capable of producing a fecal flora which was rich in *L. acidophilus*, some of the animals having counts of this organism as high as 90% of the total viable intestinal organisms. Feeding of the bactericidal agent daily for a period of 3 weeks produced no marked reduction in the number of the aciduric bacteria. The counts on the stools of the experimental animals showed a variation through a range similar to that exhibited by the control mice. Because of the failure to influence the fecal bacteria by feeding tyrothricin in the doses stated once a day, the substance was fed twice a day in corresponding quantities. This procedure, however, did not alter the results and no reduction of the numbers of *L. acidophilus* was detectable.

Since *L. acidophilus* was not killed when tyrothricin was administered by mouth, studies were made of the action of the bacterial extract on this organism in the test tube. Varying amounts of tyrothricin were added to 5 cc amounts of tomato-yeast extract broth and the tubes inoculated with 1 cc of a 48-hour culture of a strain of *L. acidophilus* isolated from one of the experimental animals. The tyrothricin-containing cultures were then incubated at 37° C for 48 hours and 0.2 cc of each mixture plated on tomato-yeast ex-

† The tyrothricin used in this study was obtained from Lederle Laboratories, Inc.

tract agar and examined for typical colonies after 48 hours' incubation. Two different experiments were carried out; it was found that in one instance 0.005 mg and, in the other case, 0.0125 mg of tyrothricin were the largest amounts which would allow *L. acidophilus* to grow; 0.0125 mg and 0.025 mg were found to kill the aciduric organism. These quantities are 1/40 to 1/80 of the largest amount of tyrothricin fed once a day and 1/80 to 1/160 of the largest amount of the bacterial extract fed twice a day.

One week after the conclusion of the experiment all the animals were killed by stunning; 5 of each group were autopsied and sections of the liver, kidney, stomach, duodenum, jejunum, and colon studied microscopically. All of the tissues were found to be normal and no difference could be detected between the organs of the control animals and those which had been treated with tyrothricin.

Comment. Although *L. acidophilus* was found susceptible to the action of tyrothricin *in vitro* even when small amounts of the bacterial extract were used, this substance had no effect when used *in vivo*. This was true even after the administration of 160 times the quantity capable of killing the organism in the test tube. The reason for this discrepancy is not apparent at the moment, but two possibilities are available in explanation. One is that since the bacterial extract has been shown by Dubos[4] to be a polypeptide, it may be destroyed by the pancreatic or intestinal juices of the animal. Another possible explanation for the failure of tyrothricin to kill *L. acidophilus* in the living animal may be that of the bactericidal agent is inhibited by fecal material; evidence for this hypothesis has been demonstrated in this laboratory.[5]

The failure to produce lesions in the gastro-intestinal tracts of the treated animals may be due to the fact that the tyrothricin was fed in amounts too small to produce such an effect.

Conclusions. Tyrothricin in small amounts is bactericidal against *L. acidophilus in vitro*. Tyrothricin has no effect on the same organism when it is administered by mouth to animals harboring this bacillus even when 160 times the *in vitro* killing dose is given. In the amounts used in these experiments tyrothricin was not found to produce lesions in the gastro-intestinal tract.

[4] Hotchkiss, R. D., and Dubos, R. J., *Proc. Am. Soc. Biol. Chem.*, 1941, **35**, 63.

[5] Rammelkamp, C. H., and Weinstein, L., unpublished observations.