THE PRODUCTION OF BACTERICIDAL SUBSTANCES BY AEROBIC SPORULATING BACILLI

By RENÉ J. DUBOS, Ph.D., and ROLLIN D. HOTCHKISS, Ph.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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From cultures of an aerobic sporulating bacillus isolated from soil (strain B.G.), there has been crystallized a substance—gramicidin—which exerts a selective bacteriostatic and bactericidal effect against Gram-positive microorganisms (2–5, 9–12). The morphological, cultural, and biochemical characteristics of the organism which produces gramicidin, coincide in general with those of the species described as *Bacillus brevis* in Bergey's Manual of descriptive bacteriology;[1] it must be pointed out, however, that different strains of *B. brevis* vary greatly in the amount of gramicidin which they produce when grown in peptone media, and that strain B.G. from which the substance was first isolated, appears to be one of the most efficient in this respect.

It has been known for a long time that certain microbial cultures exhibit marked antagonistic activities toward other, unrelated species; in particular the antagonistic properties of aerobic, sporulating bacilli have been recognized by a number of workers. Pringsheim (15) described the inhibitory effect exerted by a sporulating bacillus (*Bacillus vulgatus*) on the growth of diphtheria bacilli and other microorganisms on agar media. In 1924 Much (14) observed that a certain strain of *Bacillus mycoides* produced in broth a substance which caused the lysis of several Gram-positive cocci and Gram-negative bacilli. Similar observations were reported by Rosenthal (16–18) who worked with a number of strains of aerobic sporulating bacilli isolated by Duclaux (6) from Cantal cheese, and by Weiland working with a strain of *Bacillus mesentericus* (21).

Recently Stokes and Woodward (19) have described a method for the isolation from soil of microorganisms endowed with bactericidal properties, and reported the preparation from cultures of several of these species of an alcohol-soluble, water-insoluble fraction which carries the bactericidal activity. Finally, from cultures of an aerobic sporulating bacillus Hoogerheide (7, 8, 13) has crystallized a substance which appears identical with gramicidin both in chemical composition and biological activity.

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[1] The authors wish to record their indebtedness to Dr. N. Smith, Dr. R. Gordon, and Dr. F. O. Clark of the United States Department of Agriculture, who have generously carried out a number of tests involved in the identification of the culture. More recently Dr. Smith has informed us that the cultures T.C. and LBa mentioned in the present paper are also strains of *B. brevis* whereas the *Tyrothrix* cultures are strains of *B. subtilis*.

The observations of Much (14) and Rosenthal (16, 18) appear of special interest since these workers reported that their strains exhibited lytic activity not only against Gram-positive organisms, but also against Gram-negative species. An effort was therefore made to isolate from natural sources strains of aerobic sporulating bacilli possessing the biological activities of the cultures studied by Much and Rosenthal.

Isolation of Aerobic Sporulating Bacilli Exhibiting Bactericidal Activity

The material (soil, sewage, manure, cheese, etc.) to be investigated for the presence of aerobic, spore-forming antagonists was heated at 70°C. for 30 minutes to destroy the non-sporulating forms. The heated material was then inoculated into suspensions of living cells of *Escherichia coli* or *Staphylococcus aureus*; these bacterial suspensions, containing approximately 5×10^8 cells per cc., were prepared by resuspending the bacterial cells centrifuged from 8 hour old broth cultures into phosphate buffer (M/15) at pH 7.3. Frequent microscopic and cultural tests were made in an attempt to determine the presence of an antagonistic flora capable of destroying the staphylococci or colon bacilli. Cultures exhibiting antagonistic activity were immediately inoculated into new suspensions of living cells of the same test organisms. In all cases it was found that the addition of small amounts of peptone or gelatin (0.01 per cent) to the bacterial suspension greatly accelerated the disappearance of the staphylococci or colon bacilli. Under optimum conditions, complete disappearance of the staphylococci could often be observed in 18 to 24 hours at 37°C.; it usually took 48 to 72 hours to cause the destruction of the Gram-negative bacilli. At this stage isolation of the active strain of antagonist was readily obtained by heating the mixed culture at 75°C., and plating it on peptone agar.

Many different strains of aerobic sporulating bacilli endowed with properties antagonistic to other microorganisms were isolated by the use of this technique; 7 from soil, 3 from manure, 2 from sewage, and 2 from cheese; all were found active against Gram-positive microorganisms and also, but to a smaller degree, against Gram-negative bacilli. It was also possible to obtain from the Culture Collection of the Lister Institute, London, through the courtesy of its curator, Dr. St. John Brooks, 6 of the *Tyrothrix* cultures isolated by Duclaux in 1887, and tested by Rosenthal. As described by the latter worker, the *Tyrothrix* cultures—especially *Tyrothrix scaber*—were found to exhibit bactericidal properties, although they appear much less active than the cultures isolated in the present work. Finally, it was found that *Bacillus brevis* (strain B.G.) from which gramicidin was first isolated, can also cause the destruction of Gram-negative bacilli resuspended in very dilute (0.003 per cent) peptone solutions.

A complete descriptive study of these different strains of aerobic sporulating bacilli has not been carried out; it can be stated, however, that they appear to belong to different bacterial species since they differ in many

morphological, cultural, and physiological characteristics such as staining reactions, morphology, colony appearance, manner of growth in broth, inhibitory effect of glucose, thermophilic properties, liquefaction of starch, production of bactericidal substances, etc.

Separation of a Soluble Bactericidal Fraction by Extraction of the Cultures with Ethyl Alcohol

It has been shown elsewhere (5) that the bactericidal principles produced by *Bacillus brevis* can be obtained in solution by extracting the cells or peptone cultures of this organism with ethyl alcohol or acetone at acid reaction. The following experiments describe the procedures used to prepare alcoholic solutions possessing bactericidal activity, from cultures of several of the organisms mentioned in the preceding chapter.

The cultures were grown in two different media, (*a*) 1 per cent tryptone, 0.5 per cent NaCl, tap water—pH 7.0, and (*b*) 1 per cent gelatin, 0.05 per cent $MgSO_4$, 0.2 per cent KH_2PO_4, 0.4 per cent Na_2HPO_4, 0.5 per cent NaCl, tap water—pH 7.0.[2] The media were distributed in shallow layers (2 cm. thick) and autoclaved at 15 pounds pressure for 30 minutes. They were inoculated with peptone cultures of the selected organism previously heated at 75°C.; 0.5 cc. inoculum was used per liter of medium. Incubation was allowed to proceed for 6 days at 37°C.

At the end of the incubation period, the cultures were adjusted to pH 4.7 with concentrated HCl; this required 3.5 to 4.5 cc. of acid per liter of culture. The acidified cultures were allowed to stand for 24 hours at room temperature; they were then centrifuged and the supernatant fluid discarded. The precipitates were taken up in 95 per cent alcohol, using 50 cc. of this solvent per liter of original culture. On the following day the alcoholic solutions were clarified by filtration through filter paper; they were then diluted with 10 volumes of 1 per cent solution NaCl in tap water. A precipitate formed which contained the active principle; it was separated by filtration and desiccated over P_2O_5 *in vacuo*. The yield of precipitate varied markedly from one culture to another; the largest yields were recovered from cultures of *Bacillus brevis* (strain B.G.), of culture T.C.[3] (isolated from a Turkish cheese), and of culture LBa[3] (obtained from sewage). Up to 500 mg. of dry material was recovered from 1 liter of culture of these organisms.

The dried material was dissolved in 95 per cent alcohol to give solutions containing 20 mg. per cc. The alcoholic solutions, diluted in distilled water give opalescent colloidal solutions which precipitate on addition of electro-

[2] When purified gelatin was used, growth was much stimulated by the addition to the medium of small amounts of yeast extract or meat infusion which probably supplied some accessory growth factors.

[3] Cultures T.C. and LBa were isolated at the laboratory of the Hospital for Incipient Tuberculosis, Ray Brook, New York, in cooperation with Dr. D. Yagin and Mr. L. Baisden.

lytes. The aqueous solutions exhibit marked bactericidal effect when added to suspensions in buffer solutions of a great variety of microorganisms. They are also effective in protecting mice against infection with pneumococci and streptococci.

Before describing in greater detail the procedure of the bactericidal tests and the results obtained, it appears of interest to report at this time the following observation. As stated above, cultures of the different strains of aerobic sporulating bacilli used in the present study all yield an alcohol-soluble, water-insoluble fraction endowed with bactericidal activity; the yield of this material varied enormously from one culture to the other (from 20 mg. to 500 mg. per liter of medium) but surprisingly enough, the bactericidal activity per unit weight of the different preparations thus obtained appeared of the same order. In all cases, for instance, it took approximately 0.01 mg. of the dry materials to kill 10^{10} staphylococci in 5 hours at 37°C.; approximately 0.01 mg. of material, administered intraperitoneally, was sufficient to protect mice against 10,000 fatal doses of Type I pneumococcus. The similarity in solubility properties, and in biological activity, of the material obtained from the different cultures, suggests that the strains of sporulating bacilli used in the present study all produce in different amounts similar types of substances endowed with bactericidal activity. In fact, it can be stated at the present time that a substance apparently identical with gramicidin in crystalline structure, analytical composition, and biological properties, has been isolated from culture T.C. which, in many growth characteristics, differs markedly from *Bacillus brevis* (strain B.G.) from which gramicidin was first isolated. Culture T.C. also yields another bactericidal substance similar to, if not identical with tyrocidine, also crystallized from cultures of *Bacillus brevis* (strain B.G.).

Gramicidin and tyrocidine are two crystalline substances which have been separated by differential solubilities in acetone-ether mixtures from the alcohol-soluble, water-insoluble fraction obtained from cultures of *Bacillus brevis* (strain B.G.) (9). Although both substances are essentially polypeptides consisting in part of *d*-amino acids, they exhibit differences in chemical composition which have been considered elsewhere (9); they also differ markedly in biological properties, and some of these biological differences will be described in the following experiments.

Bactericidal Activity in Vitro of Gramicidin and Tyrocidine

Five hours old cultures of *Escherichia coli* and *Staphylococcus aureus* in meat infusion peptone broth were centrifuged and the cells resuspended in two different media; (*a*)

M/15 mixed phosphate buffer, pH 7.3; (*b*) supernatant of the broth culture from which the *E. coli* cells had been collected; this supernatant fluid was filtered through a Berkefeld candle and adjusted to pH 7.3 before use. The bacterial suspensions (in buffer or metabolized broth) gave approximately 3×10^9 colonies per cc. when plated in meat infusion peptone agar. Graded amounts of gramicidin and tyrocidine, diluted in distilled water, were added to 3 cc. volumes of the bacterial suspensions. The mixtures were incubated at 37°C. and streaked on meat infusion peptone agar after 3 hours and

TABLE I

The Effect of Gramicidin and Tyrocidine on Bacterial Suspensions in Vitro

Amount of substance added to 3 cc. bacterial suspension		Bacterial suspension in buffer				Bacterial suspension in metabolized broth			
		Growth on agar plates*		Lysis†		Growth on agar plates		Lysis	
		E. coli	Staphylococci	*E. coli*	Staphylococci	*E. coli*	Staphylococci	*E. coli*	Staphylococci
	mg.								
Gramicidin	0.500	++++	−	0	0	++++	−	0	0
	0.100	++++	−	0	0	++++	−	0	0
	0.010	++++	−	0	0	++++	−	0	0
	0.005	++++	−	0	0	++++	+	0	0
	0.002	++++	+	0	0	++++	+++	0	0
Tyrocidine	0.500	−	−	L	L	++++	−	0	L
	0.100	−	−	L	L	++++	−	0	L
	0.050	−	−	L	L	++++	−	0	L
	0.025	+++	−	L	L	++++	++++	0	L
	0.010	++++	++++	0	0	++++	++++	0	0
Controls.....	0	++++	++++	0	0	++++	++++	0	0

* ++++ = abundant growth on meat infusion peptone agar.
− = no " " " " " "

† L = destruction of cellular structure as revealed by microscopic analysis; large amounts of tyrocidine cause a precipitation of cellular material which masks the lytic phenomenon.
0 = no lysis.

12 hours incubation. The lytic effect of gramicidin and tyrocidine on the bacterial cells was also determined by microscopic examination. Although the results of growth on agar plates were about the same when the mixtures were cultured after 3 hours or 12 hours incubation, lysis was not evident at the first period of observation. The results presented in Table I report growth on agar plates and lysis of the bacterial cells after the mixtures of bacterial suspensions and gramicidin or tyrocidine had been incubated for 12 hours.

The results presented in Table I confirm the great activity of gramicidin against staphylococcus, a Gram-positive organism, and its ineffectiveness against *E. coli*, a Gram-negative species. Tyrocidine, on the contrary,

exhibits bactericidal activity against both test organisms, resuspended in buffer solutions. This correlation between the reaction of the cell to the Gram stain and its differential susceptibility to gramicidin has been extended to a number of other bacterial species; pneumococci, streptococci, staphylococci, diphtheria and diphtheroid bacilli, aerobic and anaerobic sporulating Gram-positive bacilli, have all been found to be susceptible to both gramicidin and tyrocidine. On the contrary, the following Gram-negative groups, *Escherichia, Klebsiella, Shigella, Salmonella, Hemophilus, Neisseria,* are resistant to gramicidin but susceptible to tyrocidine.

Another generalization appears justified at the present time. Treatment with tyrocidine often results in the lysis of the bacterial cells (streptococci, diphtheria and diphtheroid bacilli are an exception to this rule). On the contrary, when the cells of susceptible bacterial species are treated with gramicidin, they retain their characteristic morphology and staining reactions long after they have lost the capacity to grow when inoculated into plain broth or on plain agar.

It is also apparent from the results presented in Table I that both gramicidin and tyrocidine are more effective when tested in buffer solutions than in the presence of the constituents of meat infusion peptone broth. In particular, the activity of tyrocidine against *E. coli* (and other Gram-negative bacilli) is remarkably inhibited when the bacterial cells are resuspended in peptone solutions or meat infusion peptone broth.

The Activity of Gramicidin and Tyrocidine against Bacterial Infections

As reported elsewhere (9, 10), the intraperitoneal injection of gramicidin exerts a protective action against infection of mice with pneumococci and streptococci; gramicidin is ineffective *in vitro* against Gram-negative bacilli and also fails to protect mice against infection with *Klebsiella pneumoniae.* Tyrocidine, on the contrary, can be shown to exert a bactericidal effect *in vitro* against Gram-negative as well as Gram-positive microorganisms; however, all attempts to obtain a protective effect with this substance against Gram-negative infections have so far failed.

Varying amounts of tyrocidine have been administered to mice by the intraperitoneal, subcutaneous, intravenous, or oral routes, and failed to protect these animals against infection with *Klebsiella pneumoniae* or *Salmonella aertrycke.* In fact, the feeding of large amounts of tyrocidine to mice even failed to modify the normal Gram-negative intestinal flora. It can be stated in passing that large amounts of young active cultures of aerobic sporulating bacilli (*Bacillus brevis* strain B.G., culture T.C., and *Tyrothrix scaber*) have been fed to mice and guinea pigs in an attempt to

modify the intestinal flora as suggested by Rosenthal (17); it was indeed possible to recover these bacterial species from the feces for a number of days or even weeks after these cultures had been fed to guinea pigs, showing that the sporulating bacilli had become established in the intestinal tract. There was also definite indication that the Gram-positive components of the normal intestinal flora had been displaced by the aerobic sporulating bacilli, but in no case could we observe any significant reduction of the number of coliform bacilli.

TABLE II

The Protective Effect of Gramicidin and Tyrocidine against Infection of Mice with Type I Pneumococcus*

Treatment (intraperitoneal)		Infecting dose of pneumococci†											
		10^{-4}						10^{-7}			10^{-8}		
	mg.												
Gramicidin	0.025	S	S	S	S	S	S	—	—	—	—	—	—
	0.01	S	S	S	S	S	S	—	—	—	—	—	—
	0.005	S	S	S	S	S	S	—	—	—	—	—	—
	0.002	S	S	S	S	S	S	—	—	—	—	—	—
Tyrocidine	0.250	D4	D5	S	S	S	S	—	—	—	—	—	—
	0.100	D5	S	S	S	S	S	—	—	—	—	—	—
	0.050	D4	D4	D5	S	S	S	—	—	—	—	—	—
	0.025	D2	D2	D4	D4	D5	D8	—	—	—	—	—	—
Controls.....	0							D2	D4	D5	D4	D4	D5

* In this particular experiment all mice treated with gramicidin were alive and well when discarded 9 days after inoculation. Usually a few scattered deaths are observed whatever the dose of gramicidin used for treatment.

† S = survival of the animal.
D = death " " " Numeral indicates number of days elapsing between inoculation and death.

All these observations would indicate that, like other classical antiseptics, tyrocidine is essentially ineffective *in vivo*. Surprisingly enough, however, crystalline preparations of this substance can exert a definite protective action against pneumococcus infections in mice. This is illustrated in the following experiment.

Mice were infected intraperitoneally with 10,000 fatal doses of Pneumococcus Type I; within 15 minutes after infection they were treated intraperitoneally with varying amounts of gramicidin or tyrocidine diluted in distilled water.

The results presented in Table II show that one single injection of 0.050 to 0.100 mg. of tyrocidine administered intraperitoneally is sufficient to

protect mice against 10,000 fatal doses of pneumococcus; tyrocidine is however much less active than gramicidin, since the same protective effect could be obtained with 0.002 mg. of the latter substance.

Gramicidin and tyrocidine differ in many other biological properties; for instance 0.3 to 0.5 mg. of gramicidin injected intraperitoneally is sufficient to kill a 25 gm. mouse in 48 hours; 2 mg. of tyrocidine is required for the same toxic effect; the latter substance therefore is less toxic than the former but it will be recalled that it is also much less effective against the Gram-positive bacterial cell both *in vitro* and *in vivo*.

TABLE III

Hemolytic Activity of Gramicidin and Tyrocidine in Vitro

Bactericidal agent		1 cc. of 10 per cent washed red cells—Hemolysis after incubation for the following lengths of time:			
		15 min.	3 hrs.	8 hrs.	24 hrs.
	mg.				
Gramicidin	0.400	−	−	−	−
	0.200	−	−	−	−
	0.100	−	−	−	−
	0.050	−	−	−	−
	0.020	−	−	−	−
Tyrocidine	0.400	++++	++++	++++	++++
	0.200	++++	++++	++++	++++
	0.100	+++	+++	+++	+++
	0.050	+	++	++	++
	0.020	−	+	+	+
Control.....	0	−	−	−	−

++++ = complete hemolysis.
− = no hemolysis.

Studies of the effect of gramicidin and tyrocidine on the physiological functions of the susceptible bacterial cells have also revealed profound differences in the mechanisms of action of the two substances; these studies will be reported later. At this time, mention will be made only of the effect of the two bactericidal substances on the mammalian erythrocyte.

Hemolytic Action of Gramicidin and Tyrocidine in Vitro.—Rabbit erythrocytes were washed free of serum and resuspended in a volume of 5 per cent aqueous solution of glucose sufficient to give a concentration of cells corresponding to 1/10 that of the blood. Graded dilutions of gramicidin and tyrocidine in 10 per cent glucose were added to the cell suspension and the mixtures incubated at 37°C. Hemolysis readings were made after 15 minutes, 3 hours, 8 hours, and 24 hours incubation.

As shown in Table III tyrocidine causes an immediate hemolytic effect which does not increase appreciably with prolonged incubation. On the contrary, no hemolytic effect could be observed with gramicidin, even after 24 hours incubation.

DISCUSSION

The antagonism exerted by certain types of microorganisms against other microbial species is a fact of common observation (12, 20) but the mechanism of the antagonistic action may vary so profoundly from one case to another that it hardly permits of any general systematic formulation. "Antibiosis" (12) may be due, for instance, to competition for oxygen or other essential nutrients, to liberation into the culture medium of acidic or basic products which interfere with growth, to the production of other metabolites which may kill the cells, etc., etc. The antagonistic action of certain aerobic sporulating organisms discussed in the present paper, offers on the contrary a fairly well defined entity. From a great variety of sources (soil, sewage, manure, cheese, etc.) strains can be isolated of aerobic sporulating bacilli, differing in morphological, cultural, and physiological characteristics, which all produce in peptone media an alcohol-soluble, water-insoluble fraction endowed with bactericidal activity. Among the first saprophytic, aerobic sporulating bacilli to be described, were those isolated by Duclaux (6) from Cantal cheese; on account of their origin, Duclaux gave to these organisms the generic name of *Tyrothrix* (now to be placed in the genus *Bacillus*). In 1925 Rosenthal (16, 18) showed that the strains of *Tyrothrix* isolated by Duclaux slowly release into the culture medium a substance endowed with lytic and bacteriostatic activity. The antagonistic action recognized by Rosenthal was probably due to the alcohol-soluble, water-insoluble fraction described in the present and other reports. The name tyrothricin has been proposed for this alcohol-soluble, water-insoluble fraction (10).

Tyrothricin has now been obtained by growing different species of aerobic sporulating bacilli on several media; (*a*) tryptone solution, a medium rich in tyrosine and tryptophane, (*b*) gelatin solution, a medium deficient in these aromatic amino acids, (*c*) synthetic media, consisting of mixtures of amino acids, with or without tryptophane and tyrosine. The yields of tyrothricin have varied considerably on the different media with the different organisms. It seems worth reporting that very large yields have been obtained by growing *Bacillus brevis* (strain B.G.) in a gelatin medium. Since gelatin is deficient in aromatic amino acids, and since tyrothricin is rich in tyrosine and tryptophane, it is evident that the organism is capable of rapidly syn-

thesizing large amounts of these aromatic amino acids. It will be recalled also that many of the amino acids which constitute tyrothricin are of the unnatural *d*-type; since the *d*-amino acids are not present in gelatin, it appears that these substances are also synthesized by the bacillus in the course of its growth.

Crude tyrothricin is bactericidal *in vitro* not only against Gram-positive microorganisms, but also against Gram-negative species. Failure to recognize this fact in earlier publications was due to the following reasons: (*a*) the activity of the crude product is very much greater against Gram-positive than against Gram-negative species; (*b*) the activity against Gram-negative bacilli is markedly inhibited in the presence of broth constituents, and all the earlier bactericidal tests were carried out directly in broth cultures.

Tyrothricin, prepared from *Bacillus brevis* (strain B.G.) has yielded two crystalline products, the chemical nature of which has been outlined elsewhere (9, 10). One of these substances has been called gramicidin on account of its selective bacteriostatic and bactericidal effect against Gram-positive microorganisms. The other substance is an organic base which has been called tyrocidine to recall the generic name of *Tyrothrix* and because the substance is rich in the amino acid tyrosine.

In spite of their common origin and of the fact that both substances are polypeptides, gramicidin and tyrocidine differ not only in certain chemical properties, but also in biological activity. Gramicidin is effective only against Gram-positive microorganisms; tyrocidine, when tested in buffer solution in the absence of broth, affects both Gram-positive and Gram-negative species. Tyrocidine causes immediate hemolysis of washed red cells, whereas gramicidin has no hemolytic effect. Tyrocidine also causes lysis of many bacterial species; there is definite evidence, however, that the lytic effect in this case is not a direct one, but is only a secondary autolytic process which follows upon death of the cell (2).

Although the effect of gramicidin is to some extent inhibited by the presence of peptones and serum, this inhibitory effect is especially marked in the case of tyrocidine; in fact, it is very difficult to recognize any effect of tyrocidine on Gram-negative bacilli when these organisms are suspended in peptone solutions.

It will be shown elsewhere that tyrocidine immediately destroys the metabolic activity not only of bacterial but also of animal cells. This effect can be recognized by the immediate loss of oxygen uptake, of acid production, of reducing ability. On the contrary these essential metabolic functions are respected by gramicidin even in the case of the most susceptible bacterial cells.

All available evidence, therefore, indicates that tyrocidine behaves like a general protoplasmic poison, whereas the effect of gramicidin is of a much more subtle nature. In fact it will be shown elsewhere that the effect of gramicidin is to some extent reversible. For instance staphylococci "killed" with gramicidin and which are unable to grow on meat infusion peptone media can be made to grow in the presence of certain tissue components (1).

Since gramicidin is not a gross protoplasmic poison, and since it is less inhibited by peptones than are most antiseptics, it becomes easier to understand why under certain conditions it retains much of its activity in the presence of animal tissues. In fact, gramicidin, when applied locally at the site of the infected area, does exhibit a definite activity against infection with pneumococci and streptococci (9, 11, 12). It appears, however, that gramicidin is almost completely inactive against systemic infection when injected intravenously (4). Whether this ineffectiveness is due to physical properties which prevent diffusibility of the substance throughout the tissues or whether it is due to the inhibitory effect of tissue components upon its activity, cannot be decided at the present time.

Tyrocidine, although inactive against infection with Gram-negative bacilli, appears to exhibit definite activity against pneumococcus infection in mice. The results reported in Table II have been obtained with preparations recrystallized several times and can hardly be explained by a contamination of tyrocidine with gramicidin. Tyrocidine is much less active than gramicidin against pneumococcus infections in mice; on the other hand, when tested *in vitro* against these same microorganisms resuspended in buffer solutions, tyrocidine is almost as active as gramicidin. This discrepancy appears of special interest since it offers a concrete example of two substances having a common origin, definite similarity in chemical structure, but differing widely in "chemotherapeutic" action. It is hoped that a comparison of the chemical structure of the two substances, and a knowledge of the mechanism of their physiological action against bacterial and tissue cells, may throw light on some of the factors which govern the effectiveness of antiseptic agents in the animal body.

SUMMARY

Several species of aerobic sporulating bacilli recently isolated from soil, sewage, manure, and cheese, as well as authentic strains obtained from type culture collections, have been found to exhibit antagonistic activity against unrelated microorganisms.

Cultures of these aerobic sporulating bacilli yield an alcohol-soluble, water-insoluble fraction,—tyrothricin,—which is bactericidal for most Gram-positive and Gram-negative microbial species.

Two different crystalline products have been separated from tyrothricin. One, which may be called tyrocidine, is bactericidal *in vitro* for both Gram-positive and Gram-negative species; the other substance, gramicidin, is effective only against Gram-positive microorganisms. In general, tyrocidine behaves like a protoplasmic poison and like other antiseptics, loses much of its activity in the presence of animal tissues. Gramicidin on the contrary exerts a much more subtle physiological effect on the susceptible bacterial cells and, when applied locally at the site of the infection, retains *in vivo* a striking activity against Gram-positive microorganisms.

Addendum.—Heilman and Herrell (*Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 182) have recently described a marked hemolytic effect of gramicidin in tissue culture, whereas, in the experiments reported in the present paper, no hemolysis was observed when washed sheep red cells were resuspended in isotonic glucose solution. We have now established that gramicidin does indeed cause a slow hemolysis of erythrocytes resuspended in buffer or saline solutions, but the addition of small amounts of glucose to the system is sufficient to prevent any hemolytic action.

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