

Gramicidin S and its use in the Treatment of Infected Wounds

ANTISEPTICS of biological origin are now well known since the pioneer investigations of Fleming on penicillin and Dubos on tyrothricin. In our laboratory early in 1942 an attempt was made to isolate the strains of *Bacillus brevis* from Russian soils in order to prepare tyrothricin similar to that of Dubos. In the course of this work we isolated from soil a new strain of aerobic sporulating bacillus possessing some unique characteristics. It is well known that alcoholic extract of the acid precipitate of the culture of *B. brevis* contains an amorphous body, designated by Dubos and Hotchkiss (1941) as tyrothricin, which can be afterwards fractionated by special procedures into two individual crystalline substances, gramicidin and tyrocidine hydrochloride. In distinction from this, alcoholic extract of the acid precipitate from our strain consists almost entirely of the antibacterial substance, which is not amorphous but is directly crystallizable from the alcoholic solution. This crystalline substance can be further purified and obtained in the form of colourless needles with the melting point 267–268°. Hence it is different from gramicidin (m.p. 228–230°) and tyrocidine hydrochloride (m.p. about 240°). The bacteria producing this substance were designated as the strain of Gause-Brazhnikova, and the substance itself as gramicidin S (Soviet gramicidin).

Chemical properties and antagonistic effect of gramicidin S. Gramicidin S differs from tyrothricin, gramicidin of Dubos and tyrocidine hydrochloride by its easy solubility in chloroform. We had an opportunity of comparing directly gramicidin S with the original preparation of tyrothricin, obtained from Lederle, Inc., New York. Further, we were able to compare the properties of our strain of bacillus with that used by Lederle for the commercial production of tyrothricin. It was occasionally discovered that a batch of tyrothricin obtained from Lederle is heavily contaminated with spores of some bacillus. This bacillus was found to be identical with that described by Dubos, and with its aid we prepared a batch of tyrothricin identical with that offered by Lederle. It was observed by us that the spores of the Dubos strain of bacteria are much more resistant to the action of ethyl alcohol than the spores of the strain of Gause-Brazhnikova.

Although gramicidin S is similar to tyrothricin in many respects, a direct comparison shows that it is about four times more efficient in killing staphylococci than tyrothricin. Numerous tests made with various strains of pyogenic cocci on the nutritive media containing 10 per cent of human blood serum have shown that 25 gamma of gramicidin S per 1 c.c. of the medium is sufficient to kill staphylococci, whereas 100 gamma of tyrothricin is required for the same effect. These experiments were made with the commercial preparation of gramicidin S, which is now available in bulk. (Staphylococci are killed by pure crystalline gramicidin S at a concentration of 3 gamma, and *B. coli* at 50 gamma per 1 c.c. of the nutritive medium.) In the case of streptococci and pneumococci, 6 and 12 gamma of gramicidin S respectively are sufficient for the killing action, whereas about 3 gamma of tyrothricin has the same effect. It follows from these data that gramicidin S is more regular in its action upon various genera of pyogenic cocci, whereas tyrothricin has a weak action upon staphylococci and a strong effect upon strepto-

cocci and pneumococci. Because septic wounds containing staphylococci cause the most trouble, the advantage of gramicidin S for surgical practice is obvious.

The action of gramicidin S upon gas bacilli was studied by a number of methods. It was found that 10 gamma of this substance per 1 c.c. of the medium is sufficient to kill *Cl. Welchii* and *Cl. histolyticus*.

The toxicity of gramicidin S equals that of tyrothricine: the median toxic dose for intraperitoneal injections in rats is 15–20 mgm. per kgm. weight.

The use of gramicidin S in the prophylactic treatment of experimental infections was also studied. Experimental lacerated wounds of muscles in guinea pigs were infected with *Cl. Welchii*, treated either by gramicidin S solution (experiment) or by physiological saline (controls), and repaired. The mortality in experimental animals was 5 per cent, whereas in controls it attained 53 per cent. Similar experiments were made on rats, where the experimental wounds were infected by garden soil. The mortality of controls was 100 per cent, whereas in the rats treated by gramicidin S it was only 40 per cent.

Clinical results. Clinical application of gramicidin S was studied in 573 cases. The original 4 per cent alcoholic solution of gramicidin was diluted by water to make the concentration of gramicidin 400–800 gamma per c.c. of the liquid. These solutions were applied daily either locally, or introduced into cavities. The results of clinical observations can be summarized as follows.

The first group of cases includes septic gunshot wounds of the hip; the suppuration following heavy burns of the abdomen, breast, hip and hands; abscesses of the abdominal wall; heavy anaerobic phlegmones, etc. The application of gramicidin S has led to: (1) rapid disappearance of bacteria in the wound and successful epithelization; these processes were controlled by cytological and bacteriological observations; (2) successful preparation of the wound for subsequent surgical treatment (secondary joint or the transplantation of the skin); (3) in some cases the septic state disappeared through the elimination of the local suppuration process.

The second group of cases includes septic gunshot wounds of the larynx, chronic otitis, etc. The application of gramicidin S dramatically arrests the suppuration and rapidly improves the general state of the patient.

The third group of cases consists of empyemas. Removing the pus by a syringe and introducing 50–100 c.c. of a water solution of gramicidin S into the pleural cavity two or three times is usually sufficient to eliminate the infectious process entirely.

The fourth group of cases includes osteomyelitis. The heavy suppurations of gunshot fractures are rapidly arrested by the local application of gramicidin S. In the cases of chronic osteomyelitis the application of gramicidin S immediately after the performance of sequestrectomy arrests the further spread of suppuration.

Details of experimental and clinical work with gramicidin S have been published in a monograph entitled "Soviet Gramicidin and Wound Healing", which is issued in Moscow in Russian. Copies of this monograph are available, and can be sent on application.

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