SUMMARY: Virulent tubercle bacilli were incubated in Kirchner semi-solid agar medium with the membrane fraction prepared from guinea-pig peritoneal exudate cells. A marked inhibition of mycobacterial growth was observed in the presence of added melittin which is known as an activator of membrane phospholipase A. The bactericidal activity in a buffer environment was also demonstrated by the fraction alone and that stimulated by melittin.

In the accompanying paper (1), the membrane fraction (MF) prepared from mouse peritoneal exudate cells was reported to be mycobactericidal in a buffer environment of reduced pH and stimulated by melittin. As a supplement to this observation, an attempt was made to examine the bacteriostatic activity of a similar fraction (MF) of guinea-pig origin.

Peritoneal exudate cells consisting of polymorphonuclear leukocytes and mononuclear cells in almost the equal percentages were harvested from five guinea pigs and MF was prepared therefrom in the same way as described before (1). The bacteriostatic test was conducted in Kirchner semi-solid agar medium with or without graded concentrations of melittin (Sigma).

Kirchner semi-solid agar medium (agar 0.1%, bovine serum 3%, and kanamycin 100 μg per ml) was dispensed into small test tubes (1 x 10 cm) in 3-ml amounts. The inoculum was 100 μl of 0.1 mg of H37Rv (a kanamycin-resistant substrain of H37Rv tubercle bacilli) per ml suspension. MF was used in an amount of 100 μl (300 μg as protein and 65 μg as phospholipids). The absolute amount of melittin to be added was 10, 5, 2.5 and 1.25 μg dissolved in 10 μl of water.
Fig. 1. Melittin-stimulated bacteriostatic effect of the membrane fraction of H37Rv tubercle bacilli in Kirchner semi-solid agar medium.

MF, a melittin solution and a bacterial suspension were sequentially layered on the surface of semi-solid agar. Reading the bacterial growth at 37 C was made every week, and Fig. 1 is the observation in 4 weeks.

In the absence of MF, vigorous bacterial growth occurred in all tubes with or without melittin. The growth was observed not only as a subsurface band consisting of confluent colonies but also as pellicle growth creeping up along the inner surface of the tube. In the tubes added with MF, however, discrete colonies of smaller number appeared in a deeper layer of semi-solid agar responding to the increasing concentrations of melittin.
With a similar MF fraction from guinea pigs, the bactericidal test on H37Rv tubercle bacilli was conducted by the same procedure as described before (1). The incubation system was consisting of 100 μl of 0.05 M acetate buffer (pH 5.6), 10 μl of 100 mM CaCl₂, 100 μl of an H37Rv tubercle bacilli suspension (1 mg per ml), and 100 μl of MF (488 μg as protein and 185 μg as phospholipids) with or without 10 μl of a 250 μg per ml melittin solution. After termination of incubation at 37 C for given hours, the mixture was added with 0.7 ml of the acetate buffer and the mixture incubated for additional 30 min. Enumeration of viable bacilli was made in the same way as in the preceding paper (1).

As shown in Fig. 2, MF was highly active in killing tubercle bacilli. Viable counts decreased down to one-tenth of the starting counts in 8 hr and to one-hundredth in 23 hr. This effect was greatly enhanced by addition of melittin, which alone was almost inactive in killing tubercle bacilli in the employed concentration.

Fig. 2. Bactericidal activity of the membrane fraction on H37Rv tubercle bacilli.
The above two observations are not only the confirmation of the previous results (bactericidal effect) with mouse MF but also such an important extension that MF is bacteriostatic even in a neutral and serum-containing environment in which tubercle bacilli can multiply vigorously.

REFERENCE