esterase; also it did not produce a hypercholesterolemic effect when added to a high cholesterol diet(3,4). Both findings would suggest that a complex of cholesterol and bile salt is formed in the lumen which then enters the intestinal wall. It appears that entrance of free cholesterol into the intestinal wall does not depend on emulsification or solubility of cholesterol in fat droplets.

Summary. Entrance of cholesterol-4-C^{14} into the free cholesterol pool of mucosa and its subsequent transfer to the lymph requires the presence of a specific type of bile salt. This process does not require the presence of dietary fatty acid. It is suggested that free cholesterol in the lumen of the intestine complexes with bile salt which then enters the free cholesterol pool of mucosa.

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Observations on Bacteriostatic and Bactericidal Action of Erythromycin.* (23980)

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Haight and Finland(1,2,3) have demonstrated that a growing culture of a sensitive strain of streptococcus is rapidly sterilized by erythromycin, whereas a fully grown culture of the same organism is insensitive to the bactericidal action of the drug. They conclude that "erythromycin may be either bacteriostatic or bactericidal depending on sensitivity of the organism and on concentration of the antibiotic. Erythromycin exerts its effect only against multiplying bacteria."

In the present work the effect of erythromycin on sensitive strains of *Staphylococcus aureus* was determined under various conditions. The results showed a striking effect of population density on bactericidal action of the drug.

Materials and methods. A strain of *S. aureus* very sensitive to erythromycin was kindly furnished by Dr. Lawrence Kunz of Mass. General Hospital. The organisms were grown on nutrient agar slants and stored in a refrigerator. Log-phase cells were obtained by inoculating Trypticase Soy broth with small numbers of cells and incubating at 37°C until a concentration of approximately 10^8 organisms/ml was obtained (Klett reading = 30). Growth was followed either turbidimetrically with Klett Electrophotometer or by viable plate counts. For the latter, 0.1 ml samples of proper dilutions of culture were spread onto blood agar plates, read after 24 hours' incubation at 37°C. Purified crystalline erythromycin (980 μg/mg) was the generous gift of Lilly Research Laboratories. Stock solutions containing 2000 μg/ml of the drug were prepared in distilled water. These solutions were tested on pour plates and found to inhibit growth of organism at concentration of less than one μg/ml. When measured by viable plate counts, the doubling times of cultures containing initially 10^5 and 10^8 organisms/ml were identical (26 min.). Furthermore, this same doubling time was obtained when growth of cultures containing 10^8 organisms/ml was followed turbidimetrically.

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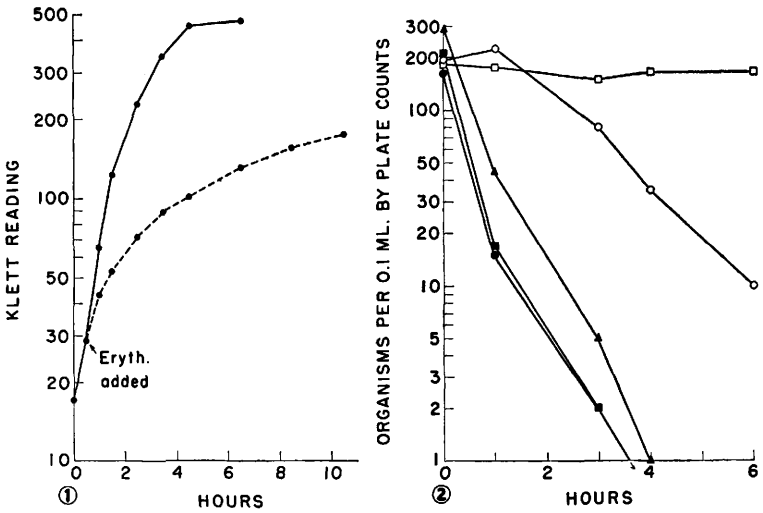

FIG. 1. Effect of erythromycin on growth of *S. aureus*. 50 ml cultures in 125 ml Erlenmeyer flask incubated with rotary shaking. 2 ml samples aseptically removed at intervals for turbidity determination. Erythromycin to make 4 μg/ml added as indicated. For other conditions see text.

FIG. 2. Effect of dense culture filtrate on action of erythromycin. Log-phase cells, diluted in warm broth to 10^8 organisms/ml were inoculated into media as follows: Broth ●——●; 1 part broth, 1 part saline ▲——▲; 1 part broth, 1 part drug-free culture filtrate ■——■; 1 part broth, 1 part filtrate with drug ○——○; organisms suspended in saline □——□. Erythromycin, 4 μg/ml. Each medium supplemented with 4 μg/ml of erythromycin at time of inoculation.

Results. Effects of erythromycin on growth of S. aureus: Fig. 1 shows results of experiment in which erythromycin (4 μg/ml) was added to one of a pair of flasks of growing bacteria, when the cell population in each had reached about 10^8 viable units/ml. The erythromycin permitted a striking further increase in turbidity, although at a rate that was significantly less than that of control culture. Similar results have been obtained with concentrations of the drug up to 200 μg/ml.

That cells responsible for this increase in turbidity were still sensitive to erythromycin was demonstrated by plating onto agar containing the drug. Growth was suppressed by concentration of 0.8 μg/ml.

In similar experiments it has been observed that increasing turbidity of the dense culture was paralleled by an increase in hemocytometer count, but no change in character of cells on Gram staining was detected. In contrast to above results, when the culture was diluted to 10^3 organisms/ml in broth, the drug was rapidly bactericidal (Table I).

Modification of action of erythromycin. To explain the difference in effects of erythromycin on heavy and light suspensions of bacteria, the culture fluid from a heavy suspension was examined. For this experiment cultures identical to those represented in Fig. 1 with and without erythromycin were prepared. After 24 hours' incubation the cultures were centrifuged and supernatants were sterilized by Seitz filtration. The pH of filtrates was 7.0. When filtrate from erythromycin-grown cells was added to an equal volume of broth containing erythromycin, the bactericidal action of the drug on low cell densities was depressed though not eliminated (Fig. 2). In contrast, the filtrate from cells grown without erythromycin had no effect. Dilution of broth with 0.85% saline resulted in a medium in which the bactericidal rate was

TABLE I. Effect of Erythromycin on Light and Heavy Cell Suspensions.

Hr	Light cell suspension Organisms/ml	Heavy cell suspension Organisms/ml	Klett reading
0	1810	181 × 10^6	30
3	10	218 "	91
10	0	130 "	162

At zero time a sample of heavy suspension was used to inoculate a second flask of medium at cell concentration indicated. For other conditions see text.

unchanged. In saline erythromycin exerted no killing effect.

Discussion. In our experiments visibly turbid cultures of *S. aureus* continued to grow in the presence of erythromycin at a concentration that was rapidly bactericidal to low-density log-phase cultures. Turbidity of culture increased from Klett reading 29 to 228. At the end of 24 hour incubation, microscopic examination of cells showed no change in size or shape, while hemocytometer counts showed increase in cell numbers. It is thus apparent that the increase in Klett reading was due to a net increase in number of cells in the suspension. This increase in cells, however, was not accompanied by a rise in number of viable units. Such results could be explained if growth and killing were proceeding simultaneously at rates that gave an approximately constant viable count. On the other hand, this discrepancy may be related to the data in Fig. 1 which on plain graph paper form a straight line. Thus, the increase in cell material was not attended by any net increase in synthetic ability. However, attempts to find this effect of the drug reflected in altered content of ribo- or desoxyribonucleic acid or protein in cells have been unsuccessful.

Pittenger *et al.*(4) reported that growth of *S. aureus* is significantly depressed by addition of erythromycin over most of the visible growth curve range. They found relatively low concentrations of the antibiotic to have a prompt inhibitory effect even when added to cells which reached the late log phase of growth. However, inhibition was progressively less complete as size of the inoculum increases. The present work is in agreement with that of Haight and Finland in showing erythromycin to be bactericidal to low density cultures. However, this paper also demonstrates that dense cultures of rapidly growing organisms are not subject to this bactericidal action. It is not clear whether the observations of Pittenger *et al.* demonstrate the same phenomenon, because they followed growth only turbidimetrically, and thus did not distinguish bacteriostatic from bactericidal action.

It would appear that only rapidly dividing organisms are killed by the drug. It might therefore be asked whether the protective action of the filtrate is due to some growth-inhibiting action on cells at low density. It can be seen in Fig. 2, by comparison with the medium diluted with saline, that mere dilution of medium by filtrate would not confer protection. Protection could occur if the filtrate contained a cytotoxic substance. Because the filtrate contains considerable erythromycin activity, this possibility cannot be tested with the same strain of organism. However, the filtrate supported rapid growth of a drug-resistant mutant derived from the same strain, using the filtrate as medium. Thus, a generally cytotoxic effect would appear to be ruled out.

This finding suggests that the protective action observed is most likely the result of decrease of growth rate by release of a specific factor, which appears to be released in the presence of erythromycin. It remains to elucidate its nature and the mechanism of its action.

Summary. 1. Erythromycin was bactericidal to low cell concentrations of growing *S. aureus* but not to heavy suspensions of the same growing cells. 2. Turbidity of the heavy suspension continued to increase after addition of erythromycin, although at a reduced rate. This increase was not paralleled by any increase in viable units. 3. Viable cells in a drug-treated suspension were still as sensitive to erythromycin as before addition of the drug. 4. Addition of a filtrate of a drug-treated, dense suspension to a dilute suspension of growing cells delayed onset and reduced rate of sterilization of these cells by erythromycin.

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