CHAPTER - IV

DISCUSSION AND SUMMARY

DISCUSSION

Mycobacillin was originally discovered as an antifungal antibiotic by Bose and Mazumdar in 1955 (124). Our work demonstrates that this antibiotic also possesses a strong antiprotozoal activity. In fact, the minimum inhibitory concentration of the drug for the growth of C. albicans was about 150 μg/ml which is considerably more than that needed for the supression of the growth of the cultural form of L. donovani. At a concentration of 30 μg/ml of the antibiotic, the L. donovani promastigotes completely failed to grow in the liquid medium (Fig. 3). In presence of lower concentrations of the antibiotic, the cell growth continued at a slower rate. This might be due to the partial loss of viability of the cells or due to a longer generation time. In case of C. albicans rapid loss of viability was observed in presence of the antibiotic (208). Even though our evidences are not very strong, it appears that a drastic loss in viability also occurs when the antibiotic comes in contact with the protozoa.

Respiration in presence of exogenous glucose, after the exhaustion of the endogenous respiration, was found to be affected quite drastically in presence of the antibiotic (15 μg/ml), but a lag period of about 60 mins was required before the respiration was completely suppressed (Fig. 5).

The situation with the fungal systems is, however, quite different. In this case, even massive doses of the antibiotic did not have any effect either on endogenous respiration or on respiration in presence of glucose (209). However, inhibition of the respiratory chain or the glycolytic pathway does not appear to be the primary effect of the antibiotic. This was conclusively proved by our experiments with cell-free extract of the organism. We demonstrated considerable respiration in cell-free extracts only in presence of an artificial electron acceptor, phenazine methosulphate (Fig.10). This respiration remained completely unaffected for more than 60 mins in presence of 30 μg/ml of the antibiotic. The lack of any direct effect of the antibiotic on respiration is not surprising since only a very few antibiotics have been shown to have a direct effect on the enzymes of metabolic pathways. The possibility that the antibiotic may act as an uncoupler of oxidative phosphorylation can not however be eliminated. We repeatedly noted that on contact with the antibiotic for about 60 mins, all motility of the cells was lost indicating a possible effect on the ATP-pool of the cells. We have already mentioned that our efforts to prepare viable mitochondrial preparations capable of showing high P:O ratio have failed so far. In absence of such data no conclusion can be drawn regarding the effect of mycobacillin on oxidative phosphorylation.

The first evidence that mycobacillin might have a direct effect on cellular permeability was provided by Banerjee and Bose during their course of study with Candida albicans as the test organism (210). Ultraviolet absorbing materials were released into the medium and a good correlation was observed between this release and the loss in viability. In our case also, a rapid leakage of intracellular materials into the medium was observed even in presence of low doses of the antibiotic (Figs. 13,14). These authors observed strong agglutination of the yeast cells on prolonged contact with the antibiotic (208). No distinctive morphological change of L. donovani on treatment with the antibiotic was however noted by us. The agglutination reaction of the yeast cells is possibly a secondary phenomenon since a drastic loss in viability was observed long before the agglutination started.

The saturation kinetics of the release of intracellular materials with increasing mycobacillin concentration (Fig.13) suggests the presence of a fixed number of sites for mycobacillin interaction. The release was also found to be basically linear with time. The direct effect of the antibiotic on cell membrane was further confirmed by our experiments on transport of glucose or glycine in mycobacillin treated cells. The net uptake of these metabolites as well as the binding constants were found to have changed on treatment

of the cells with the antibiotic.

A large number of polypeptide antibiotics have been shown to have their primary effect on the cell membrane. Some of these, like valinomycin, are supposed to act in a specialized manner for the specific transport of ions across the membrane barrier. On the other hand, some antibiotics like gramicidin S, tyrocidin are supposed to have non-specific detergent type of action. Some of our results, like the saturation kinetics of the release of intracellular materials with increasing concentration of the antibiotic suggest a specific interaction. On the other hand, lysis of the prepared membrane vesicles with the antibiotic strongly indicates a detergent type of action. It is therefore not unlikely, that even though the antibiotic in low doses act at specific sites on the cell-membrane, it may, under changed circumstances and in larger concentrations produce a detergent-like action. It was observed by Banerjee and Bose from studies on mycobacillin with DU-NOUY'S tensiometer, that the antibiotic is a surface active agent, capable of inducing the release of intracellular materials from the cells of Candida albicans. (226).

Ghosh and coworkers (157-159) had previously shown that nystatin, a typical polyene antibiotic, also acted on the membrane of L. donovani promastigotes. Since sterols could

counteract the action of nystatin, they concluded that the sterols of the membrane were responsible for the binding and subsequent anti-protozoal activity of the antibiotic. In our hand, however, no experiment with sterols was successful because of the massive lysis of the cells in presence of these compounds. That mycobacillin can specifically bind with sterols was further proved by the work of Halder et al (213) who showed a definite change in the spectrum of the antibiotic in presence of some of these sterols. Moreover, these workers had further shown that the antifungal activity of mycobacillin could be completely reversed when those sterols were present in the medium. Even though we failed to reverse the anti-protozoal activity of mycobacillin with sterols, employment of desensitized plasma was found to be capable of completely reversing the effect of the antibiotic. Preincubation with desensitized plasma completely prevented the release of intracellular materials (Table 6), inhibition of respiration (Fig. 21) and the fall in uptake of nutrients (Figs. 22 and 23). It is therefore likely that some steroid-like material which is bound to the plasma, irreversibly binds the antibiotic to the plasma and thus makes it unavailable for the antibiotic action.

Employing radioactive mycobacillin one can clearly answer some of the questions raised in the above sections. The membrane vesicles, recently prepared in our laboratory may also prove to be a powerful tool for the study of these interactions, since the vesicles may act as model membranes in this case.

SUMMARY

I. Mycobacillin, a cyclic polypeptide antibiotic, at a concentration of 30 μg/ml, effectively inhibited the growth of the promastigotes of L. donovani in liquid growth medium. Several other antibacterial antibiotics like penicillin, streptomycin and tetracycline were found to be completely ineffective at identical concentration (weight/volume). At concentrations below 30 μg/ml, this polypeptide antibiotic only partially inhibited the growth of the protozoan in liquid medium.

II. The oxygen uptake by intact cells of the protozoan in presence of exogenous glucose was found to be reduced by about 35% within first 30 minutes of contact of the cells with the antibiotic present at a concentration of 15 μg/ml. A prolonged period of about 70 minutes was needed before the respiration was completely stopped. The degree of inhibition of respiration increased with increasing concentration of the antibiotic. Penicillin, streptomycin and tetracycline were found to be completely ineffective in inhibiting the respiration of L. donovani even at high concentration (100 μg/ml). Both methyl and butyl esters of mycobacillin were found to be effective in inhibiting the respiration of the organism, but their activity was considerably less than the parent mycobacillin.

III. Experiments on the oxygen-uptake in presence of glucose by cell-free extract (of L. donovani) fortified with co-factors and phenazine methosulphate in presence of mycobacillin at two different concentrations (15 μg/ml and 30 μg/ml) showed that the antibiotic had no direct effect on glycolysis or respiration of the organism..

IV. Appreciable leakage of intracellular materials took place when the cells of L. donovani were incubated with mycobacillin. Penicillin, streptomycin and neomycin at identical concentration (50 μg/ml) did not induce any such leakage of intracellular materials. Such release of intracellular materials, probably due to loss of permeability barrier of the cells, was found to take place even with lower concentrations of the antibiotic.

V. The net uptake as well as the rate of entry of ^{14}C-labelled glucose and glycine into the protozoan cells preincubated with mycobacillin, were found to decrease significantly. The extent of such decrease in the rate of entry of these metabolites was directly dependent on the concentration of the antibiotic in the preincubation medium and the period of such preincubation.

VI. Isolated cell-membranes of L.donovani promastigotes were slowly solubilized by mycobacillin when a suspension of the membrane vesicles was incubated with the antibiotic (60 μg/ml).

VII. Experiment on incorporation of ^{14}C-labelled leucine into proteins in presence of mycobacillin in the cell-free extract of L.donovani showed that the antibiotic had no direct effect on the biosynthesis of proteins in the organism.

VIII. Mycobacillin preincubated with desensitized rabbit-plasma, failed to induce any release of intracellular materials from the cells of L.donovani. Similarly, the inhibition of respiration and entry of glucose and glycine into the protozoan cells by mycobacillin, was found to be antagonized by desensitized rabbit plasma.