



LIFE CYCLE OF THE MYCOBACTERIUM TUBERCULOSIS

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The existence of life cycles in certain bacteria has been known for many years. Various authors have termed it pleomorphism, while others called it a mutation. Life cycles in bacteria should be expected in accordance with the general biological law: that in the lower simple forms of life there is a capacity for irregular and sudden metamorphosis to adjust the organisms to changing environment with special forms for special circumstances. Marked irregularities in the cycle should be expected in bacteria, because the influences of environment operate on exposed single cells. We might be tempted to state that: the morphology of an organism is its physical expression to the environment in which it lives.

Some years ago we made the casual observation that when T. B. grew on a solid transparent medium a peculiar cloudiness was seen beneath the surface growth. For a time this turbidity was attributed to chemical changes in the medium brought about through the growth of the organism. When finally a culture was observed in which the cloudiness spread through the medium beyond the area covered by the bacterial growth, another explanation had to be found. Careful attempts to remove portions of this sub-surface media, smearing and staining it by the usual procedures proved shortly the existence of a sub-surface growth of acid-fast rods, but then we were constantly confronted with the possibility that the bacilli were accidentally pushed into the medium in attempting to obtain smears of the sub-surface material.

The technic of demonstrating the organisms was crude and nothing more was said or done about the matter, pending the development of a more suitable procedure. Finally, in 1930-32, in cooperation with my colleague, Dr. W. H. Butler, the problem was attacked from a new angle.

Next in order, histological methods were tried. The cultures were killed with formalin fixation, the entire slant removed from the tube with a special instrument, dehydrated with alcohol, infiltrated with celloidin, and cut into sections of five micra. In this manner, we were able to demonstrate beyond a doubt that the *Mycobacterium tuberculosis* grows not only on the surface of the medium but grows rather luxuriantly beneath the surface of the solid medium. Morphological changes were observed in the organisms, depending upon the composition of the medium and its hydrogen-ion concentration.

The discovery of these sub-surface growths was the first step which led to new findings that have not heretofore been reported, namely, the occurrence of penicillar forms and the formation of *Pettenkofferia* which have been previously described by Kuhn, ('24) as appearing in cultures of cholera vibrio of Metchnikoff, and Alice Evans, ('32) in a streptococcus culture obtained from a case of epidemic encephalitis.

The presence of a filterable phase in the *M. tuberculosis* has been suspected for a long time and the occurrence of the *Pettenkofferia* seems to substantiate this supposition. It should be mentioned here that *Pettenkofferia* always appear in chains of metamorphosing organisms. They appear first as minute dots, gradually increasing in size until they justly merit the name of balloon bodies. Lastly they disintegrate. The particles composing the *Pettenkofferia* are ultramicroscopic, in fact the dissolution of the balloon bodies is reminiscent of smoke issuing from an overturned hot air balloon, the wisps of smoke disappearing in the wind. One can readily see that such particles could have escaped the scrutiny of the most careful worker, had bouillon been used throughout the experiments. Our own suspicions of the presence of a filterable phase gained confirmation and the third phase of the work was undertaken in an attempt to confirm our suspicions.

Due to the fact that the Oklahoma Legislature set aside funds for research in 1935, we were able to secure the services of a technically trained assistant, Miss Gertrude Wilber. Without her splendid cooperation and that of Dr. H. W. Butler, who gave unstintingly and unpaid for his time, the following work would not have been accomplished.

This part of the work dealt with the filterable phase of the *Mycobacterium tuberculosis*; standard cultures were obtained from several sources. These were sub-cultured in measured amounts of glycerine bouillon and when adequate growth was present, filtered through Berkefeld filters, coarse, normal, and fine. Centrifuged specimens of the filtrates showed no organisms. Slants were seeded from all filtrates routinely on various media. To the remaining filtrate an equal quantity of fresh glycerine

bouillon was added and the flask returned to the incubator for further observation. For a time the cultures were routinely refiltered after six days of incubation, because by that time there was visible evidence of bacterial activity such as clouding of the media and the appearance of surface veiling.

Later in the season, due to uncontrollable factors of temperature incident to the great heat, the filtering was done as soon as evidence of growth was present. This varied from hours to days.

The second series of filtrates were again subjected to the same procedures as previously used, with the exception that no fresh bouillon was added to the filtrates. All these first and second filtrates are still under observation.

Slants made from the filtrates were killed at twenty-four and seventy-two hours, seven days, and sixteen days. Smears were made prior to killing. Celloidin sections from this material completed at present, give sufficient evidence to confirm the findings in the smears.

The first discernable organisms appearing in the filtrate were 'G' forms, a name applied by Hadley to small coccoid forms in filtrates of the Shiga bacillus. In due course of time, the adult forms appeared. In checking some old cultures which were left over from previous experimental work (in smears and celloidin sections), we were able to demonstrate the presence of a reversion to the 'G' forms as well. We feel quite confident that our series demonstrates the presence of an ultramicroscopic, filterable phase because centrifuged specimens of our filtrates have not shown any organisms and yet we were able to grow the 'G' forms and subsequently the adult organisms answering the classical description of the *Mycobacterium tuberculosis*.

The question might be raised as to why animal experiments have not been used to check our findings of broth and slant cultures. The answer in our opinion is that animal inoculations would complicate matters to such an extent that it would be unsafe to draw definite conclusions from them. Animals have been inoculated repeatedly with material from cold abscesses and pleural exudates but all with negative results. Also, had we inoculated animals with the filtrates, we would have been unable to determine with certainty the absence of a previous or subsequent bacillary infection.

