VI. NOTES ON LIFE CYCLE PHENOMENA AND FILTRABILITY OF THE TUBERCLE BACILLUS

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Finality in science is an illusion. Irrespective of the import of the newer observations in bacteriology, no one today holds rigidly to the monomorphous theory of bacteria established in the youth of this science. My own part in this work has been confined to studies with the tubercle bacillus, supplementary or complementary to that of others whose work is omitted in this brief review for lack of space. Changes that I have observed are fundamentally not greatly unlike those reported with other species. The variations, therefore, seem to belong to all forms of bacteria and are not the result of a few isolated observations.

Notwithstanding the almost overwhelming array of new evidence, we must not be too hasty in modifying or overthrowing a stable order for an untried theory. For example, the majority of tuberculous infections can still be accounted for by the monomorphous theory of bacterial growth and reproduction. Likewise our knowledge of the Klebs-Loeffler bacillus apparently accounts for most of the disease diphtheria. The same may be said of other bacteria. Occasionally, however, there are peculiarities of growth and morphology of bacteria that even a tyro should not fail to observe and study. Such changes are the difference in virulence, colony formation, speed of growth, branching, lack of acid-fastness, formation of granules with appearances of sprouting forms, not to mention the so-called filtrable forms. Only a few of my observations concerning unusual phenomena will be cited, preceeding which I shall mention some controls.

Concurrent with the crucial experiments reported formerly,¹ I worked in a veritable wilderness of cultures, which were obtained

¹ Amer. Rev. Tuberc., 17, 53; 17, 77; 18, 630.
in some relation to tuberculosis, either associated in cultures or in lesions. More than two hundred such strains were studied, some with three or more sub-groups. In respect to most of these strains I failed to show any relationship to the tubercle bacillus or to the disease, although it was equally true that I failed to prove that they were not related, except in their inability to become tuberculogenic. Many variable features were observed which established most of the strains as unstable and pleomorphic. For example, strains were observed to vary in morphology of both cell and colony. Wide chemical and biological differences were also noted from generation to generation, which prevented classification according to any recognized system. Although many seemed to be in intimate contact with the tuberculous process and were pathogenic, neither culture nor animal inoculation, including serial passage, could establish them as tuberculogenic unless they originated directly from a tubercle bacillus or a granule of a tubercle bacillus.

I mention this collateral work now because of its control value. These apparently barren experiments were interspersed among the successful experiments, affording valuable controls against spontaneous tuberculosis and against the finding of acid-fast bacilli in normal lymphatic structures. I may say parenthetically that neither in this large series nor in a large series recently studied, have we been able to find acid-fast bacilli in lymph nodes of guinea pigs, unless we were also able to relate them to contact with some form of the tubercle parasite. We have thoroughly studied the problem of spontaneous tuberculosis in this laboratory and know under what conditions it will appear. Our observations have been, that it will result in any laboratory if the guinea pigs are not properly dieted and are not kept away from contact with other tuberculous animals. A guinea pig in this respect is not different from a child. In view of this possibility, it cannot be denied, therefore, that any proof dependent upon animal passage alone is subject to serious criticism. Under the best of controlled conditions the disease is liable to develop, although it is true that it may be reduced to an insignificant minimum.
LIFE CYCLE PHENOMENA OF TUBERCLE BACILLUS

My experiences pertaining to variability of the tubercle bacillus, can best be illustrated in the short space allotted by a brief review of two aspects of the problem. First, observations of a life cycle different from the usual fission reproduction, and second, a summary of some new work on filtration.

For the first, I shall cite a few observations on a strain taken from a localized caseous pneumonia of a patient coming to autopsy (A175) at the Chicago Municipal Tuberculosis Sanitarium. The number of this strain was 159, and various subgroups included many strains of variable forms. Smears from the lung tissue revealed literally thousands per field of acid-fast bacilli, bearing at the ends or, most commonly, in the middle large purplish oblong granules measuring about 0.7 by 1.2μ. It was so nearly a pure culture that the problem of emulsification in salt solution and single cell picking became an easy matter. In addition, dark-field studies were made in salt solution and boullion suspensions for periods up to three days. In the dark fields, the granules within the bacilli appearing with a reddish refraction could be seen to divide first horizontally, then parallel to the bacillus, producing tetrads. Simultaneously they lost their red refraction and took on gradually a blue tint. No sprouting was observed as with a previously studied culture (no. 52) (American Review of Tuberculosis, January, 1928, plate I and plate II, figs. 1, 2 and 3), but stained smears revealed that many such changes as sprouting and the formation of comma-shaped, horse-shoe-shaped and pessary forms had taken place in the course of reproduction.

The single cell pickings, however, were more convincing. Refractile granules were picked and inoculated subcutaneously into guinea pigs. One granular bacillus with one large granule and three small granules produced a slight and atypical tuberculous infection. Six and ten granules respectively produced a more typical tuberculosis, while an arbitrary dosage of about fifty in a larger series produced typical tuberculosis; smaller doses were all negative. This is, therefore, the first absolute proof that “Much’s granules” will produce tuberculosis, although the granules observed by Much, no doubt, consist of a variety of forms differing in different circumstances.
Finally, a large number of single cells were picked both from the original and the first subcultures in an attempt to get pure cultures. After many attempts without results, one mold-like growth was obtained from the first subculture on Petroff's medium that produced a dry mucoid colony of a semi-acid-fast mycelial growth that killed guinea pigs with an atypical tuberculosis. Typical acid-fast bacilli were found in guinea pigs, but on artificial medium the culture gradually lost luxuriance and died out. This would ordinarily have been considered a mold contamination, but the mycelial threads contained acid-fast granules that seemed to warrant reporting.

The attempt to obtain a pure culture was repeated many times: finally we succeeded in isolating a colony from a single granule (and several from two or more granules). This is a typical human-type tubercle bacillus that has remained pure and unchanged for six years.

Since then, twenty-eight Maximow slide cultures with Long's medium have failed to demonstrate anything more than a sluggishly growing microorganism growing slowly and forming granules but no "micro-gonidia" or other changes observed while in the original host.

These experiments go further to explain the difference of opinion that exists concerning such variability than any I can suggest. In the human host, we had a most unusual type of acid-fast bacillus with large granules. These granules seemed to change into tetrads under the dark field. They caused an atypical tuberculosis in guinea pigs in small doses. In a picked single granule in culture tube, a Staphylococcus tetragenus culture grew. Other cultures seemed to confirm this observation. Fixed and stained smears showed a number of acid-fast forms, apparently coming from granules in a number of ways. A complete series of transitions could be followed in both dark field and stained specimen. Our special senses must be trusted to a certain extent in such elementary observations. Certainly photographic lenses may be depended upon. Yet, when this culture became stabilized on an artificial medium, it grew from generation to generation with little change.
Another microorganism from autopsy no. 182 was even more interesting in that many semi-acid-fast forms could be caused to revert to acid-fastness and correspondingly develop tuberculogenic qualities. More recently a similar return to acid-fastness has been observed by a strain in culture.  

Although all the work reported is strongly presumptive, I have tried to present only as facts phenomena that could in some way be verified by photographic reproduction; reproduction by an unbiased artist; single cell culture studies; or animal inoculation experiments where the bacteriological findings were compatible with the pathology. I have tried to allow for appearances and illusions whenever there was doubt. The whole study reveals that certain strains of tubercle bacilli (if not all strains at certain times) have variations and modes of growth and reproduction in addition to the usual fission reproduction.

The filtration experiments have been repeated because of the numerous negative and indefinite reports recently in the literature and, particularly, because of the objections raised concerning contamination. My first experiments were conducted under conditions entirely acceptable to the older school of bacteriology regarding filtration. The filters were tested with fresh B. coli cultures for leaks (besides other precautions). If the filtrate did not produce growth in glucose boullion, surely there should be no question about any other positive results. There would be a more justifiable excuse to question negative results under such circumstances. In fact, filtration experiments are subject to such variable conditions (electrical charge on the filters, viscosity of medium, pressure, etc.) that it is a wonder any positive results are ever obtained when all aspects are rigidly controlled. As a technician, I had considerable public health bacteriological experience and my work was acceptable to several competent Laboratory Directors. In the same environment I had personally prepared, previously, more than a hundred litres of Cole's antipneumococcus serum without apparent contamination. Nevertheless, I felt that the work should be repeated on a larger scale.

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1 Revista Medico-Chirurgica do Brasil, 37, No. 9, Setembro de 1929.
with three types of Berkefeld filters nearly always in *duplicate*. In other words, I wished to conduct a "mass experiment." In the new experiments I was assisted by Miss Asya Stadnichenko, for whose aid I wish to express my appreciation. The material consisted of twelve specimens, including pus from cold abscesses; pus from non-tuberculous abscesses; macerated cultures of tubercle bacilli, macerated livers of fetuses from tuberculous mothers, and macerated animal organs.

The results seem to show that where no tubercle bacilli are present (as shown by controls) the animals all survive; where there is an avirulent tubercle bacillus, the animals also survive; where the culture is virulent there is an "asthenic" death in about two months, without tubercule, without acid-fast bacilli, and without the power to infect subsequent animals. With certain cold abscesses and other forms of septic material, there is likewise a "filtrate disease," but it is transmissible in series to other guinea pigs; in other cases there is a direct return to acid-fast bacilli and usually atypical tubercle formation, or there is an apparent return on one or two passages in animals. The work on three fetuses was negative for filter-passing forms, although one was positive in a control animal.

The theory of a "filtrate toxin" or a "bacillo-casein" cannot explain the phenomenon of transmissibility of the virus or the slow return to acid-fastness and tuberculogenic qualities. Neither does it account for the discrepancy between the results with virulent and non-virulent strains. These can only be a filtrable part of the tubercle bacillus or a spontaneous tuberculosis in animals. The phenomenon seems to be occurring too regularly and too consistently in its time and manner of appearance to be spontaneous tuberculosis, especially since the controls fail to show a like change.

Finally, the "transmissible asthenia" seems to be entirely too common to explain on any such basis. As a theoretical explanation it may be thought of as a toxic death from very small gonidia or fragments of virulent tubercle bacilli that may go through an existence of their own, occasionally returning to acid-fastness but otherwise going on indefinitely until they run their course.
itary factors may not permit the establishment of a stable form in their freshly liberated state. They may have to return to stability with some protective mechanisms or become extinct. Rapid passage may keep them in existence until stability is reached. The size of the forms may militate against any form of cell defense (tubercle or foci of inflammation), yet a toxic effect may be produced and maintained till the animal succumbs.

The question of filtrability of the tubercle bacillus is, therefore, not only open but seems to demand further experimental work. Especially should this be performed where there are any unusual phenomena in either the host or the parasite.