

idly, attaining a maximum growth in ten days. During the first five days they were first yellow-white, then yellow, and by the tenth day they were a deep orange color. Each of these subcultures had the general morphological characteristics of the acid-fast group. They were composed of numerous rounded colonies, and occasionally one or more of these was piled on one another. The edges of the growth were usually smooth and at times became indented as the growth progressed. The staining characteristics were the same as the original colonies. Dilution cultures were made to obtain single colonies. After dilution growth appeared in two days. This was in the form of white, rounded colonies which did not start to assume the yellow color before the fourth or fifth day. By the tenth day they had attained a maximum growth and a deep orange color. The rounded colonies, by the third or fourth day, showed from two to five lobulations which gave the appearance of dividing starfish eggs. None of these showed the crusting projections found in many of the colonies of the acid-fast group. All appeared on the surface of the medium and did not cause lysis of it. By the end of the sixth week each colony had developed a fine, veil-like growth, orange in color about its base. These were very similar to those seen about the bases of many single colonies of acid-fast organisms. At the tenth day, sixth week and third month the organisms stained the same as those of the original colonies, except that there were fewer acid-fast rods to be found.

The organism grew easily and rapidly on blood agar; it made almost a complete growth in five days, and showed no further growth after ten days. On this medium it had lost its chromogenic power and appeared as dirty white, slightly heaped-up colonies. Often these had a moth-eaten appearance and irregular borders. They had about the same appearance on plain agar, although some piled up in clean white, rounded groups. On these media they were entirely non-acid-fast and were of a coccoid, granular type, slightly larger than ordinary cocci, although a few rods the size of acid-fast organisms still were present.

The organism gave a luxuriant growth on Long's medium. It formed a pellicle on which rounded, granular, light-orange colonies developed. Many of these granular colonies sank to the bottom of the flask, but others developed in their place on the pellicle. These again were almost wholly non-acid-fast rods, although a few acid-fast granules were present. The growth was less luxuriant on plain and dextrose broth, and in these two types of medium it was found at the bottom of the tube as a dirty white precipitate. Growths of this sort were made up of the non-acid-fast, coccoid granular organism.

Five guinea-pigs were inoculated with suspensions of the second generation of the chromogenic organism. Each pig was given 1/50 of 1 mgm subcutaneously in the right groin. Four weeks after this inoculation, all were in excellent condition and were reinoculated with 1 mg of the same culture. This was again given subcutaneously in the right groin.

Of these five animals, two at autopsy had no demonstrable tuberculosis. The other three had caseous nodes at the sites of inoculation and varying numbers of tuberculous lesions in spleens, livers and lungs. Acid-fast rods were demonstrated in smears from the caseous material, as well as in smears of the affected organs. The amount of tuberculosis each animal had was much less than would be expected following a much smaller dose of our parent strain of H-37 human tubercle bacilli.

We believe that this organism is a non-acid-fast mutant of our strain of H-37 human tubercle bacilli. Although chromogenic, it maintains cultural characteristics similar to the acid-fast group when grown on special media. Its growth on simple media is composed almost entirely of non-acid-fast granules. Granular forms may be the primary type of organisms of the acid-fast group, with the chromogenic organism as an intermediate phase, and the acid-fast bacillary forms the typical, well-known varieties.

We also have evidence that sterile Berkefeld filtrates of cultures of the chromogenic organism will cause normal cultures of human and bovine tubercle bacilli and timothy grass bacilli to lose their acid-fastness and grow as forms similar to the coccoid granular type of the chromogenic mutant.

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