incubation may be of value for the future development of artificial incubation and commercial incubators.

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SPECIAL ARTICLES

MENDELISM AMONG BACTERIA?

DURING the summer of 1926 at the American Museum's station for the study of insects we isolated a strain of yellow pigmented bacterium that was pathogenic to the wood fly *Lucillia serricatta*. This organism was described in the American Museum Novitates No. 251[1] as *Bacillus lutzii* Brown. At the time the true generic affiliation was doubtful but it was suspected from the appearance in old cultures of spheric refractile bodies and from the fact that very young cultures—three to six hours old—reacted Gram positive that it belonged in the genus Bacillus. Since then a prolonged study has shown the refractile bodies not to be true spores and, as the brief period of Gram positiveness disappeared after the seventh or eighth transfer, we now believe the organism to be a member of the Bacteriacaea probably in Flavobacterium. The generic nomenclature in bacteriology and the scope of these genera are so ill-defined in many cases that it is difficult to assign some strains to any group. We at least do not feel qualified to originate any new generic names until the present ones are more fully understood.

Early in the work it became apparently difficult to keep the strains free of a yeast-like organism. Another difficulty appeared in the loss of color in one of the substrains. Both, while annoying at the time, have been found to be merely phases in the life history that, if we are correct in our inferences, may prove to be of more than passing interest.

As startling and as improbable as it may seem, we are of the opinion that we have been witnessing an exhibition of a case of Mendelism among asexual organisms. This statement may at outset seem contradictory to fundamentals, since Mendelism entails inheritance from two parents. However, a moment's reflection will reconcile it to some extent. It is a well-known and fundamental fact that great numbers of asexual organisms go through a process of conjugation during which portions of the nuclear material are exchanged. This is true in both the plant and animal kingdoms and now we propose that it is true in the intermediate bacterial kingdom.

Briefly, the life cycle of *F. lutzii* as exhibited under laboratory conditions and observed by us is as follows:

When the substrat becomes of such a nature as to interfere with the normal mode of existence as a rod-shaped organism, the rods undergo a series of morphological changes that terminate in a resistive stage. If the change in substrat is gradual, as might be caused by the natural accumulation of wastes in an old culture, many filamentous forms are developed and seem capable of existing long after the rods have died off. Upon transplanting these filaments the resultant culture is always one of pure rod forms. However, if the introduction to toxic material is sudden and if that toxic substance is of a cumulative type such as lead, a rapid change different in character occurs. A group of rods, eight in number we believe from lengthy observation, gather together and fuse into a mass. This mass is far more intensely stained by 1:10 aqueous basic fuchsin than the individual rods themselves are. This stage has been called by Löhnis[2] a symplasm.

While within this protective envelope the bacterial mass completely fuses and then gradually subdivides into two ill-defined parts and next into four spheric stain-resisting bodies. Each of these spheroids then passes through fission at right angles and tetrads are produced. These tetrads disintegrate into sixteen resistant "cocci." In time or upon being transplanted to a non-toxic nutrient medium each "coccus" again divides once, the increased volume due to this division causes the envelope to rupture and the "cocci" are released. If the substrat is suitable, they grow at once into normal rods and reassume the ability to take up aqueous basic fuchsin. These rods are of identical form, size, physiological and serological character with the initial rods.

If a single asexual "species" embraces strains varying in a character, such as color in our case, and if that character be Mendelian in its makeup, it is wholly conceivable that a stage of conjugation or, as the analogous bacterial stage may be called, symplasmism would bring this to light if carefully followed up. Apparently the difficulty heretofore obscuring its recognition has been the lack of a strikingly recognizable variable character. Our color character was ideal for this work as it was very definite and easily recognized.

Originally we set out to determine the cause of the loss of color in the substrain noted above. We at first believed it to be some obscure environmental cause and set out to eliminate as many as possible. We had found that by making daily transfers all strains kept their original color and that the only form present was rods. However, in the white strains

[1] F. M. Brown. American Museum Novitates No. 251. 1927.

[2] F. Löhnis and N. R. Smith. *Jour. Agric. Research*, 5: 676–702, 1916; *Jour. Agric. Research*, 23: 401–432, 1923.

whenever the culture was allowed to age a fortnight or more symplasms were formed and the outcome of transplanting was problematical so far as color was concerned; often both white and yellow colonies appeared on a single slant. Our theories relating metabolism and the color change were disproved by the fact that in these cases both colors developed under identical conditions in the same slant, so we were back where we started, looking for an answer.

Single-cell cultures of both yellow and white strains were now made and carried over a period of five months, being transferred every two weeks by means of single-cell isolations. This was carried on with some forty substrains. At the end of that time (eleven transfers having been made) substrains that showed no change of color from the first single-cell isolation were taken and considered to be pure strains of that color. Serological work proved them all to be identical. Inasmuch as our yellow strains had shown no variation in color since the split-off of the single white substrain over a year before, we turned our attention particularly to the white strains that were apparently pure and, covering the ground again, we proved to our own satisfaction that metabolism had nothing to do with the color change. At the same time we carried along a transfer from the original and parent culture that had been kept sealed with paraffin and on ice for fifteen months. No changes were observed with this strain.

We were about to give it up for a bad job when we mixed broth cultures of the two phases and plated it just to see what would happen. We got a pretty, variegated slant. The entire material was set aside and attention turned to other things. Two weeks or so passed and the time for the routine monthly transplants came around. Along with others, the tube of mixed strains was carried on. A few days later, on checking over the new transfers we were surprised to find that the mixed transfer was almost devoid of yellow areas. Interest was again stirred and, since there were a great many symplasms in the mixed strains tube, we entered upon a series of experiments to determine whether or not they had anything to do with it. Finally we evolved the following.

Since we had found that lead in a solid medium caused a rapid formation of the symplasm, we inoculated a lead-acetate nutrient agar slant with a freshly prepared mixed broth culture with a ratio of 1:1 million of cells of each strain. When many of the symplasms were observed to have ruptured and the resistant "cocci" were abundant in the smears we inoculated plain nutrient broth with material from the lead medium and after twenty-four hours plated thinly on nutrient agar. Numerous repetitions of this experiment always resulted in the white phase appearing in far greater numbers than the yellow. We now felt that we were on the right track and that the symplasm was the key to the situation. The technique was improved by isolating single symplasms from a saline suspension of the material on a lead-acetate slant and planting them in nutrient broth. Nutrient agar plates from these broth cultures showed a majority of white-phase colonies in every instance but one when a pure white phase was recovered. A second passage of white phase cells through the same process always resulted in a preponderance of white phase colonies. The ratio found in counting 500 colonies from a single such plating was 362:138, or about 8:3, white:yellow.

But if we consider the symplasm to represent a sexual generation, theoretically the first filial Mendelian generation should show but one of the variations, the dominant. This probably would be true if only two individuals entered into the symplasm and each individual were an alternate variant. Since neither supposition is true in this case, the superficial indication of Mendelism can not appear. Since eight cells enter into the symplasm the results in the F_1 generation will be those normally observed in the F_2—namely, in the thirty-two individuals from a single symplasm we would expect eight homozygous whites, sixteen heterozygous whites and eight yellows, provided the white cells are dominant as our work tends to indicate. But this is based on the supposition that equal numbers of white and yellow cells go to make a symplasm, and we can not control this, so a deviation from the expected ratio of 9:3 to about 8:3 simply means that in the symplasm used white character genes were slightly less in number than yellow. This might appear no matter how many colonies were counted unless in the mixture forming the symplasm there were exactly equal proportions of white and yellow cells. In the latter case the law of averages would balance things provided a sufficient number of colonies was counted to allow that law to function properly.

Our one stumbling-block in understanding all of this more clearly is this: How did an apparently pure recessive yellow strain throw the apparently dominant white strain? Frankly, we don't know.

Investigation by others along the same lines with color production and such characters as rough and smooth colonies, virulent and avirulent strains will be of great interest to us. Subcultures of our pure line strains are on deposit with the American Type Collection at the McCormick Institute in Chicago. We will welcome correspondence and further information as to heredity among asexual organisms.

We wish to acknowledge here the stimulation accorded us by Dr. Ralph R. Mellen, now of the Western Pennsylvania Hospital, Pittsburgh, Pa., through discussion, correspondence and his own admirable papers treating the subject of microbic heredity, and also by Dr. F. E. Lutz, of the American Museum of Natural History, for his interest in general.

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COD-LIVER OIL AND THE ANTIMONY TRICHLORIDE REACTION FOR VITAMIN A

DURING the months of January, February and March, 1928, I was in the Lofoten Islands, Norway, making a rather comprehensive survey of matters of importance in connection with cod livers and the oil produced from these livers. Many samples of oil were exported by me to the United States for animal assay and other lines of investigation. In addition to work of this character I was desirous of securing definite information as to the vitamin values of absolutely fresh Norwegian cod-liver oils produced by different methods from livers treated in a variety of ways. The use of biological methods of assay being out of the question, I equipped myself with a Lovibond tintometer and proceeded to assay my oil samples for vitamin A by the antimony trichloride reaction which was being widely used in England for the quantitative estimation of this vitamin in cod-liver oil.

In the course of my tests I made an observation which appeared to cast grave doubt upon the validity of the claim that the blue color produced in cod-liver oil by the addition of antimony trichloride is due to vitamin A. Since my return from Europe a very comprehensive and critical study has been made in our laboratories of the antimony trichloride reaction, with the result that we believe we have demonstrated most conclusively that this reaction does not afford a reliable means of determining the vitamin A content of cod-liver oil. We are now preparing full details for early publication. Our first Norwegian observation which cast doubt upon the validity of the claims made for the color reaction follows.

It is pretty generally accepted that cod-liver oil deteriorates when left open to light and air and that this deterioration is indicated, among other things, by a decrease in the vitamin A potency. In order to learn just how rapidly the vitamin A of fresh Norwegian cod-liver oil is destroyed when free access of light and air is permitted, I took two small tin pans of the same size and introduced the same volume of fresh oil into each and took a third sample for immediate colorimetric examination. I then placed one pan of oil in a clean, dry, dark closet and left the other pan of oil outdoors during the day uncovered, thus affording free access to rain, snow, sunlight and such particles of cinders and other dirt as might be blown into it. At night the pan was brought into the laboratory until morning. In the event of a driving rain or a particularly heavy fall of snow during the day, the pan was rescued and brought into the laboratory, but the oil was actually exposed to the elements for a total of seventy-nine hours during a period of two weeks.

It is doubtful if any sample of cod-liver oil was ever treated in a more shameful manner, during the time that its companion sample was supposed to be conserving its vitamin A in the seclusion of the dark closet.

At the end of two weeks these two oils were examined by me in the tintometer, using the antimony trichloride procedure. When I found that the cod-liver oil which had been standing open to air and light gave *a deeper blue color* than the original oil or the oil kept in the dark, I concluded that I had made some error in the technique and discarded all solutions and reagents and did not bother to finish the test. However, after making up a new oil solution with a different sample of chloroform and using a different bottle of antimony trichloride, I again found that the cod-liver oil which had been standing outdoors for seventy-nine hours possessed a higher value than either the original oil or the oil I had carefully protected in the dark closet. I then began to wonder if my eyesight had been injured through the monotony incident to looking at nothing but codfish, cod livers and cod-liver oil for a rather long interval. The idea that the antimony trichloride reaction was inaccurate did not at that moment suggest itself to me. However, when I examined the three samples of oil again on the following day and verified my previous findings, my confidence in the accuracy of the reaction was materially lessened.

Starting from the above initial observation, we believe that we have collected a mass of experimental evidence which very definitely indicates that the antimony trichloride reaction is not an accurate measure of the vitamin A potency of cod-liver oil. If this is true we must still consider the animal assay as the only accurate method by which cod-liver oil may be assayed for vitamin A.

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