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The effect of wetting on the pathogenicity and viability of the tubercle bacillus.

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In papers published from this laboratory during the past three years attention has been directed to changes produced in cultures of pellicle forming bacteria by adding surface tension depressants to the media. The changed form of growth from pellicle to diffuse growth thus induced has been attributed[1] to differences in degree of wetting of the bacteria.

We have found that the tubercle bacillus can be made to grow beneath the surface of glycerine broth by appropriately depressing the surface tension of the medium. The tubercle bacillus, like other pellicle forming organisms, we believe, grows on the surface because of its high fat content which tends to resist wetting. In a recent paper Larson and Larson[2] have shown that practically all aerobic bacteria may be grown on the surface of broth by growing them on media from which they may store up fat in increased amounts.

The present study concerns the effect of wetting on the viability and pathogenicity of the tubercle bacillus. A virulent culture of this organism was grown in glycerine broth to which had been added sufficient soap to depress the surface tension to 44 dynes. Growth under these conditions is under the surface of the medium and is slower than when grown under the usual conditions. Inoculation of such cultures subcutaneously into the flank of guinea pigs was followed by a slight enlargement of the inguinal glands of the side inoculated. Within the following ten days this glandular enlargement had receded, after which the animals showed no signs of tubercular infection.

The mechanism of this rapid loss of pathogenicity is difficult of analysis at the present time. However, if the hypothesis is

[1] Larson, W. P., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 62-63.

[2] Larson and Larson, *Jour. Inf. Dis.*, 1922, xxxi, 407.

accepted that pellicle formation of the tubercle bacillus is an indication of minimum wetting, the same arguments may be used to explain altered pathogenicity. When the tubercle bacilli are growing diffusely throughout the medium they are, according to this hypothesis, wetted, and therefore when introduced into the animal body are penetrated by the antibodies or bactericidal substances present, and destroyed.

Calmette[3] has recently been able to deprive the tubercle bacillus of its pathogenicity by growing it in bile broth. Since bile is a surface tension depressant it is reasonable to assume that its action upon the growth of the tubercle bacillus is much the same as that of soap. Calmette does not state whether the amount of bile used in his medium caused the tubercle bacillus to grow diffusely through a liquid medium. We have found that when bile is added to glycerine broth so that the surface tension is approximately 44 dynes it grows beneath the surface as it does when soap is used. Growth in bile medium is more rapid and more profuse than in the soap broth. We are of the opinion that the attenuation of the tubercle bacillus, as observed by Calmette, is due to a wetting phenomenon.

We have also investigated the effect of sodium rescinoleate on the pathogenicity of tubercle bacilli in sputum. Equal amounts of T.B. sputum and 2 per cent. solution of sodium rescinoleate were mixed and allowed to stand for several hours. A quantity of the mixture was then injected subcutaneously into a series of guinea pigs. The controls were inoculated with half the volume of the untreated sputum. The animals inoculated with the soap-sputum mixture developed slight enlargement of the lymph glands draining the inoculated area. Fourteen days later the glands had returned to normal in all the animals, none of which developed further signs of tuberculosis.

The controls all died with the characteristic picture of experimental tuberculosis.

The question naturally arose as to whether the sodium rescinoleote had killed the tubercle bacilli or merely altered their pathogenicity. This problem was attacked by placing a small fragment of pelicle from a young culture on glycerine broth into a

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[3] Calmette, A., *Ann. de l'Inst. Pasteur. Par.*, 1921, xxxv, 561-570.

1 per cent. castor oil soap solution, and making cultures at given intervals. The organisms which were thus transferred from the soap solution to glycerine broth sank to the bottom of the flask immediately and there was no growth even after a prolonged period of incubation. If, however, the cultures were made on the surface of egg medium, or supported in the surface of glycerine broth by means of a wire gauze, growth developed with characteristic pellicle formation.

From this experiment we conclude that, under these experimental conditions, the pathogenic properties of the tubercle bacilli are lost before the property to reproduce. The fact that we obtained growth in the above experiment when the organisms were supported in the surface of the glycerine broth, and a negative culture when the inoculun fell to the bottom of the flask will be attributed by most bacteriologists as due to the influence of oxygen. Bacteriologists have for years considered pellicle growth as evidence of obligatory aerobiosis. But those who hold to this view have not offered an explanation as to the force which supports the organisms on the surface of a liquid medium.

With few exceptions most pellicles may be sedimented by slight agitation or centrifugation.

This indicates clearly that they are of higher specific gravity than the medium upon which they are growing. It follows therefore that they are supported on the surface of a fluid medium by some force. Since "demand for oxygen" is not a force it follows further that they are not on the surface because of their demand for oxygen, although the oxygen present may, undoubtedly does, influence their development. The fact that tubercle bacilli will grow diffusely through the medium by appropriately depressing its surface tension is evidence that it will grow under conditions where the oxygen tension is not at its maximum. Its tendency to grow only in or upon the surface of glycerine broth is probably due to the concentration of surface tension depressants there, which create optimal conditions of wetting.

The foregoing observations suggest that an important function of the fat in the cell membrane is to regulate the "wetting" of the cell.

Since glycerine is the carbon compound par excellence from which bacteria synthesize fats, as shown by Larson and Larson,[4]

the necessity for this substance in the nutrition of the tubercle bacillus is obvious.

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The action of potassium cyanide on the chlorophyll mechanism of Nereocystis.

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In connection with studies on the nature of the action of cyanide on cell respiration, it seemed of interest to test the effect which cyanide might have upon the mechanism of photosynthesis.

Strips 1x 10 cm. cut from the frond of the large, Pacific coast kelp Nereocystis were used, and oxygen production was taken as a measure of photosynthesis using Winkler's method for determination of oxygen.

That potassium cyanide (.00008 mol in the experiment given) can produce an apparently complete but reversible inhibition of photosynthesis is shown by the following condensed statement of results from a typical experiment.

TABLE I.

Each number in the table is the average quantity of oxygen produced (+), or consumed (—) in three duplicate determinations. Concentration of KNC in first test, .00008 mol.

Strip placed in.	First test.	Second test. All in light. No KNC.
	O_2 in c.c. thiosulfate.	O_2 in c.c. thiosulfate.
Light	+6.65	+6.89
Light KNC	+ .18	+6.82
Darkness	—1.10	+6.63
Darkness KNC	— .39	+5.80

[4] In this paper the term fat is used as a convenient expression for the ether acetone soluble substances.