

egg albumin and water follows a similar curve, our experiments further suggest that the acid effects of alkaline buffer solutions with high carbon dioxide tension on living cells depend rather on the relative impermeability of the membrane to the metallic cations preventing free entrance of the sodium bicarbonate than on a specific solubility of carbon dioxide in the cell membrane.

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The mechanism of serum fastness.

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Soon after the discovery of the agglutinins it was observed that some strains of a given microorganism were not agglutinated by a specific immune serum. Such non-agglutinable strains are said to be serum fast. The mechanism of serum fastness is not well understood. Ehrlich's explanation of this phenomenon on the basis of suppressed receptors does enable us to visualize the condition, but a suppression of receptors is probably far from what actually takes place.

In our studies¹ on pellicle formation it has been shown that pellicle forming bacteria are rich in acetone-ether soluble substances. Bacteria which ordinarily do not grow in pellicle were caused to do so by growing them in broth to which had been added glycerine or carbohydrates which they did not ferment. By growing the staphylococcus on glycerine broth for a few generations it began to grow in a pellicle, and finally formed as

¹ *Jour. Inf. Dis.*, 1922, **xxxi**, 407-415.

definite a pellicle as the tubercle bacillus, or other pellicle forming organisms.

The acetone-ether soluble substances of staphylococci grown in this way increased from 7.9 per cent. to 39.9 per cent.

It was concluded that pellicle formation was due to the increased amounts of acetone-ether soluble substances by virtue of which the bacteria resist wetting, and are thus supported on the surface of the medium by surface tension.

The present study concerns the effect of wetting on the agglutination reaction.

A laboratory strain of the staphylococcus aureus was grown in parallel cultures on ordinary broth and three per cent. glycerin broth respectively. Rabbits were immunized with killed cultures of the staphylococcus grown on ordinary broth. In the interest of brevity the strain grown in ordinary broth will be referred to as the "lean" strain and that grown in glycerine broth as the "fat" strain. An agglutinating serum was thus obtained which agglutinated the lean strain in a dilution of 1-90.

Parallel agglutination tests were then made with the fat and lean strains in serum dilution of 1-50. After four hours in the incubator the lean strain was completely precipitated, while there was no visible change in the tests with the fat strain. However, after 30 hours there appeared to be some precipitation. At this point a count was made to determine the percentage of bacteria still remaining in suspension as compared with the control,—which contained the same number of bacteria without the serum. The count was made by a laboratory worker not personally interested in the experiment. The count revealed that 30 per cent. of the organisms had been precipitated by the serum, while 70 per cent. still remained in suspension. The test tube containing the fat strain which had been in contact with the agglutinating serum for 30 hours was then centrifuged and the supernatant fluid removed, and to this supernatant fluid was added the proper amount of the lean strain. Agglutination was found to be prompt. This experiment indicates that there had been very little adsorption of the antibodies by the fat strain.

The fat strain was then cultivated on ordinary broth, making daily transplants, and the agglutinability of each generation tested. It was found that the agglutinability of the fat strain was completely restored after three generations of culture on or-

dinary broth. The first generation on ordinary broth showed a tendency to agglutinate, but the reaction was much slower than in the control test. The experimental production of serum fast staphylococci suggests that the mechanism of serum fastness may be due to a lack of wetting. This would then explain why the tubercle bacillus which is so rich in fat-like substances gives inconstant serum reactions.

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The precipitin test in the diagnosis of tuberculosis.

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The precipitin test has been found to give reliable results in the diagnosis of active tuberculosis. The antigen is prepared by disrupting tubercle bacilli, preferable an old culture, with carbon dioxide by the method described by Larson, Hartzell and Diehl.¹ The disrupted bacteria are filtered through paper in order to remove the shells. The clear filtrate is layered over the serum to be tested, and the tubes incubated for a period up to two hours. A definite cloudy ring at the interface of the two fluids indicates a positive reaction. The cloud often appears within the first five minutes. In the far advanced cases, however, the reaction develops more slowly, but is usually very definite. Upon standing several hours the ring gradually becomes dispersed.

Thus far the blood serum of 190 cases have been examined. Of these, 100 were patients in the University Hospital and Dispensary, but not in the tubercular clinics. Ninety cases, representing all stages of tuberculosis, were from a local sanatorium. From the 100 cases not suspected of having tuberculosis, eleven positive reactions were obtained. Six of these have since been

¹ *Jour. Inf. Dis.*, 1918, xxii, 271.