

content of the apparatus, introducing particular gases according to needs and in drying the valves (by a current of air) in case they become moist. The temperature of the water bath is regulated to $\pm 0.001^\circ \text{C}$. The conductivity is measured by a Leeds Northrup 470 cm. slide wire, a resistance box of Curtis coils, using an audio oscillator as a source of current. With this arrangement the accuracy depends upon the ability to maintain a constant base line rather than upon the accuracy with which the conductivity is measured. Aside from the rigid exclusion of outside air from the apparatus and general cleanliness, the regularity of the base line depends largely upon smooth working of the valves and the regularity of pumping. The conductivity change is never quite zero, even in the absence of tissue in the respiration chamber, and a small correction must be applied for absolute values. This may depend upon a reaction between the solution and the glass. A similar objection applies to the colorimetric method.

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On the phase reversal of the lipoid-aqueous systems in the bacterial cell wall.

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In previous communications, Mellon^{1, 2} has shown that the principle of ion antagonism is applicable to bacteria. Under the conditions of the experiment with strain N.D.—67 the principle operated in a Na:Ca ratio as high as 100 or 150 to 1. This strain, which is quite stable in H_2O and NaCl solutions, is quickly precipitated by CaCl_2 solutions in strengths as dilute as 10^{-6} cm. The physico-chemical mechanism suggested was reversal of the aqueous-lipoid system in the wall of the cell whereby CaCl_2

¹ Mellon, Ralph R., *J. Med. Res.*, 1922, lxiii, 345.

² Mellon, Ralph R., Hastings, W. S., and Anastasia, C., *J. Immunol.*, 1924, ix, 365.

made the lipid layer the external phase. This theory is in accord with the work of Clowes³ with the simple oil-water emulsions.

It was determined to test the validity of this conception by the surface tension method. Mudd⁴ has shown that the tubercle bacillus when placed at the interface between an aqueous and a lipid or a lipid solvent will always be drawn into the latter by virtue of the large amount of wax contained in this organism. It was thought that the occurrence of the same phenomenon would be strong evidence for a reversal of the aqueous-lipoidal relations of our N.D.—67 by CaCl_2 .

Accordingly the organisms were placed in CaCl_2 solution in concentrations ranging from 10^{-1} to 10^{-6} and this solution shaken with organic lipoidal solvents, such as cyclohexanol, amyl alcohol, capryl alcohol and pelargonic acid. In every instance the organisms were found on the organic side of the phase boundary when the latter formed. They did not pass over to the aqueous side under the time of observation, which was several days.

A series of higher alcohols was used that showed a progressively increased solubility in H_2O as follows: Di-butyl carbinol < than Di-propyl carbinol which is < than Di-ethyl carbinol. In accordance with the theory, the organisms acted on by CaCl_2 did not leave the aqueous phase for the Di-ethyl, but they did so completely for the Di-butyl carbinol, while the Di-propyl was about a 50-50 transference.

Furthermore the organisms when extracted by alcohol:ether 2:1 showed removal of the lipid, which prevented their affinity for the lipid solvent. This was shown by the fact that when acted on by CaCl_2 they no longer left the aqueous solution for the cyclohexanol. Thus the reversal of phase relations between the lipid-aqueous systems of the cell wall is supported by these experiments.

³ Clowes, G. H. A., *J. Phys. Chem.*, 1916, **xx**, 407.

⁴ Mudd, Stuart, and Mudd, Emily, B. H., *J. Exp. Med.*, 1924, **lx**, 647.