## 3060

# A study by new methods of the surfaces of normal and sensitized acid-fast bacteria.

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Technique and theory of a simple method for study of certain physical chemical properties of the surfaces of bacterial and other cells have been reported.[1] In its present development the method is chiefly useful for examination of cells or other test objects in whose surface lipins are present or suspected, and whose range of size is from that of a bacterium to that of a large white blood cell. It is hoped that other workers will extend and adapt it to their purposes in the study of permeability, cytolysis, action of protective colloids and other problems.

The present report concerns the serum sensitization of acid-fast bacteria. These are tested as follows:

A drop of oil (Tricaprylin, Kahlbaum) and a drop of bacterial suspension are placed upon a carefully cleaned slide. A clean cover slip is placed on them in such a way as to spread the oil into a film under one end of the cover slip, and the watery suspension into a film adjoining the oil. The preparation is then studied under the dark-field microscope. The oil-water boundary surface or interface appears as a bright line, and the bacteria as bright shimmering objects against a dark background. If the preparation has been properly made the oil encroaches slowly on the aqueous phase so that the interface moves across the field and overtakes the sedimented or suspended bacteria and bacterial clumps.

The behavior of the bacteria when overtaken by the interface differs according as their surface is physico-chemically[2] similar to and therefore miscible with the oil or with the water. Normal acid-fast bacteria when touched by the oil are shot violently into the oil phase. If in clumps, the bacteria are dispersed explosively

[1] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, xl, 633, 647; 1926, xliii, 127.

[2] Harkins, W. D., *Colloid Symposium Monographs*, New York, 1925, ii, 162.

by the interfacial stresses and the scattered bacteria suddenly appear in the oil phase. After sensitization with serum the bacteria are no longer easily wet by the oil; they are stable in the interface and if free slide along it; the interface advancing against a sensitized clump attached to the glass bends backward instead of flowing ahead over the bacteria. The clumps are strongly coherent and are comparatively little dispersed by the interfacial stresses. Serum-treated bacteria which show detectable changes in the direction indicated may be said to give a positive interface reaction.

The visible changes effected in the tubercle and other acid-fast bacteria by strong sensitization are thus: (1) that their predominantly oil-miscible (or chemically non-polar) surface has become a water-miscible or chemically polar surface. (2) that the cohesion of the bacteria has been greatly increased. These changes are brought about by high concentrations of normal serum or by high or low concentrations of homologous immune serum. The effect with immune serum is specific, as has been shown both by the titers and by adsorption tests.

Agglutination tests as ordinarily conducted are notoriously unreliable with the acid-fast bacteria. Certain strains, for instance, show little or no agglutination even in potent homologous sera (v. Table II). Additional information of value may be had, however, by a simple modification of the usual macroscopic agglutination procedure. A given amount of an even bacterial sus-

TABLE I.
Comparative tests with three species of cold-blooded "tubercle" bacilli. Little serological relationship shown.

| | | Fish T. B. Antiserum | Turtle T. B. Antiserum | Frog T. B. Antiserum |
|---|---|---|---|---|
| Fish T. B. Suspension | Agglutination | 80 | All neg. | 20 |
| | Resuspension | 320 | All neg. | 2½ |
| | Interface | 160 | 5 | 10 |
| Turtle T. B. Suspension | Agglutination | 40 | 1280* | 80 |
| | Resuspension | All neg. | 320 | 10 |
| | Interface | 2½∓ | 80 | 5 |
| Frog T. B. Suspension | Agglutination | 5 | 10 | 2560* |
| | Resuspension | 2½ | 10 | 2560* |
| | Interface | 2½ | 5 | 640 |

The numbers indicate the titers for the several reactions, *e. g.*, 80 means that the highest dilution of serum producing detectable effect was 1:80.
*Titer not reached.

pension is added to each of the serum dilutions and the tubes are allowed to stand overnight in the ice-box. Agglutination is read in the ordinary way, and the tubes are then centrifuged until clear. The supernatant fluid is poured off and a few drops of salt solution are added to the sediment in each tube. The tubes are arranged in a rack and shaken uniformly until the control shows an even suspension. The organisms which have been treated with the higher concentrations of serum resuspend in flocculi whether or not they showed agglutination by the ordinary procedure. The size and coherence of the flocculi increases up to the highest serum concentrations even where there was a prezone by the usual method. The interface reaction similarly has shown sensitization of the washed bacilli to be maximal after treatment with sera of maximal concentrations, and the interface reaction is positive with inagglutinable strains. The agglutination prezone and the inagglutinability of certain strains are thus due to inhibition of clumping, and not to a failure to bind agglutinins. Agglutinins are bound, but something prevents the bacteria from clumping until they are forcibly brought together in the bottom of the centrifuge tube.

TABLE II.
Three saprophytic strains not definitely differentiated serologically. (3)

| | | "Mist" Bac. Antiserum | Smega Bac. Antiserum | Pseudotuberculosis Bac. Antiserum |
|---|---|---|---|---|
| "Mist" Bac. Suspension | Agglutination | 20 tr. | 10 tr. | 20 tr. |
| | Resuspension | 40 | 40 | 80 |
| | Interface | 40 | 20 | 20 |
| Smegma Bac. Suspension | Agglutination | 40 | 20 | 40 |
| | Resuspension | 320 | 80 | 80 |
| | Interface | 40 | 40 | 80 |
| Pseudotuberculosis Bac. Suspension | Agglutination | 10 tr. | All neg. | All neg. |
| | Resuspension | 20* | 80 | 320 |
| | Interface | 80 | 80 | 160 |

Tr. indicates only a trace of agglutination in any tube of series.
*Titer not reached. Bacteria are sensitized even when ordinary agglutination reaction completely fails to show it.

The interface and the resuspension reactions have both been found to be more reliable detectors of the binding of antibodies by the acid-fast bacteria than the ordinary agglutination procedure.

The immune sera used in this study were kindly furnished us by Dr. J. Fürth[3] and Dr. J. D. Aronson. The fish bacillus in Table I is a new species isolated by Aronson.[4]

## 3061

### A study of the electrical field surrounding heart muscle.

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In a study of the electrical field surrounding excited cardiac muscle, the writer has applied the present conception of electricity as understood by the electron theory.

It has been possible to advance two sources of experimental evidence, based upon the mathematical theory of a specifically defined electrical conception, from which it seems possible to come to but one conclusion, namely that an element of heart muscle when passing through the stages of excitation, contraction and recovery, exhibits first electrical polarity in one direction for a very limited period of time, and subsequently a reversed polarity for a relatively prolonged period of time.

Before being accepted as applicable to the study in question, the mathematical theory was tested experimentally with complete agreement between experimental and theoretical results.

The above conclusion depends on:

(1) The fact that the measured electrical field surrounding both cold and warm blooded hearts, under certain conditions, can apparently be described accurately in terms of an equation derived as suggested above.

(2) The striking and complete agreement between the deflections theoretically predicted, on the basis of the hypothesis advanced, for varying positions of two electrodes on a strip of cardiac muscle, with those obtained by actual experiment.

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[3] Fürth, J., *J. Immunol.*, in press.

[4] Aronson, J. D., forthcoming publication.

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