Use of Silicones in Aerobiology

S. M. Pady and C. D. Kelly

Department of Botany and Department of Bacteriology, McGill University, Montreal

In studies of organic and inorganic materials of the air, particularly in the epidemiology of rusts and other fungus diseases (*4*) and in allergy studies with pollen grains (*1*), it has been common practice to collect the specimens on glass slides covered with some adhesive such as vaseline, glycerine-jelly, or oil. In our investigations of airborne fungi and bacteria we have encountered temperatures as low as −48° C. At such low temperatures ordinary adhesives become too viscous or congeal and are unsatisfactory. The silicones made by the Dow Corning Corporation[1] seemed to offer possibilities as a substitute for the usual adhesives because of their retention of physical properties at low as well as high temperatures, and they were accordingly investigated. They have proved to be so satisfactory that it is considered worth while to call these new techniques to the attention of workers in this and related fields.

Slides coated with silicone grease (DC-4-ANC-128-A) have been used successfully in collecting rust, smut, and *Alternaria* spores from the air. Such preparations are superior to vaseline-coated slides for the following reasons: (1) The background is white, making an effective contrast. (2) The consistency remains unchanged at temperatures ranging from −75° C to over 200° C. (3) The slides can be sterilized, if desired, in dry heat for 2 hr at 180° C. Many other silicones are available which might also be used for this and similar purposes.

In ordinary studies of airborne fungi and bacteria, exposure of agar plates from airplanes has been common practice (*3, 5*). However, Proctor and Parker (*1*, p. 49) have shown that agar plates exposed to low temperatures will freeze and give sterile readings even in nonsterile air. This problem has been overcome by coating the bottom of a Petri plate with silicone grease, sterilizing at 180° C for 2 hr, exposing the plates from an airplane and pouring in melted agar on return to the laboratory. Under these conditions, bacteria and fungi produce satisfactory colonies. Fungi grow through the overlying agar to the surface and sporulate normally; bacteria and yeasts develop between the two layers, forming typical subsurface colonies. Some bacteria are freed when agar is poured in and colonies develop in or on the agar. Isolations from any of these colonies can be made without difficulty. The principal advantage of this method is that plates may be exposed to extremely low temperatures for an indefinite period without any physical change or danger of freezing. By this technique cultures have been obtained from plates which were exposed to air temperatures as low as −48° C.

It would appear to us that these two methods might prove very useful in many phases of aeromycology and aerobacteriology, particularly where exposures are to be made at low or high temperatures. In routine exposures for stem and leaf rust studies, as well as for other cereal pathogens, silicones appear to be more satisfactory than vaseline. They should also be very satisfactory for the collection of pollen grains in allergy and other studies. For collecting microfauna and even small insects, the silicones would probably work equally well. They should prove of value also in fields other than aerobiology, particularly in snowflake and ice crystal investigation, and in studies of dust at high altitudes or even in the stratosphere.

[1] Experimental material supplied by Dr. M. J. Hunter, Research Director, whose cooperation is gratefully acknowledged.

References

1. Moulton, F. R. (Ed.). *Aerobiology.* (AAAS Symp. Vol. 17.) Wash., D. C., 1942.
2. NRC Committee on Apparatus in Aerobiology. *Phytopath.*, 1941, **31**, 201.
3. Pady, S. M., Kelly, C. D., and Polunin, Nicholas. *Nature*, Lond., 1948, **162**, 379.
4. Stakman, E. C. and Christensen, C. N. *Bot. Rev.*, 1946, **12**, 205.
5. Wolf, Fred T. *Bull. Torrey bot. Club*, 1943, **70**, 1.

Relation of Surface Phagocytosis to the Fibrinous Character of Acute Bacterial Exudates[1]

Mary Ruth Smith and W. Barry Wood

Department of Medicine and The Oscar Johnson Institute for Medical Research, Washington University School of Medicine, St. Louis

Evidence has recently been presented that encapsulated bacteria which cause acute respiratory disease are phagocyted in the body in the absence of opsonins by a mechanism termed "surface phagocytosis" (*2, 8*). Leucocytes operating in the presence of various tissue structures have been observed to ingest and destroy type I pneumococci, Friedlander's bacilli, group A hemolytic streptococci, and staphylococci (*7*). Although the encapsulated bacteria escape phagocytosis when floating freely in a fluid medium, they are readily phagocyted when trapped against tissue structures by the leucocytes. Also, when caught in a sufficiently dense concentration of leucocytes, they may be pinned between the surfaces of two or more cells and thus phagocyted (*6*). Of the microorganisms so far studied, only type III pneumococcus is resistant to surface phagocytosis (*5*). Its ability to escape the leucocytic pseudopods has been shown to be due to an outer "slime layer" of capsular polysaccharide which is present only when the organism is multiplying rapidly. When the slime layer is lost with aging of the bacterial population, type III pneumococcus becomes susceptible to surface phagocytosis.

Since leucocytes utilize tissue structures in phagocyting encapsulated bacteria in the absence of antibody, it would seem likely that they may utilize in a similar manner the fibrinous strands that characteristically occur

[1] Aided by a grant from the Commonwealth Fund.

in acute bacterial exudates. To test this possibility, the following experiments were performed.

Type I pneumococci (A-5 strain) harvested from 4-hr broth cultures, were washed in gelatin-Locke's solution and centrifugalized (*8*). To the pneumococcal centrifugate were added rat leucocytes (*2*) which had been washed in the cold (4° C) in both gelatin-Locke's solution and citrated rat plasma[2] containing platelets from

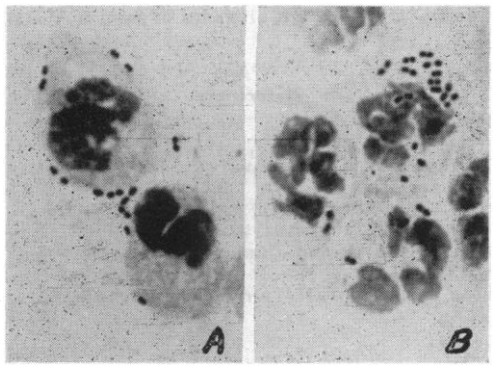

FIG. 1. *A*—Phagocytic test in unclotted plasma. *B*—Phagocytic test in clotted plasma.

the buffy coat. (The platelets were included to insure clot retraction when thrombin was added to the plasma.) The final leucocyte-pneumococcus suspension in plasma contained approximately 5–10 phagocytic cells and 25–30 pneumococci per oil immersion field. Phagocytic tests were carried out on glass cover slips coated with dry film[3] to prevent spontaneous clotting of the plasma. When clotting was desired, one part of gelatin-Locke's solution containing 20 mg % of purified beef thrombin[4] was added to five parts of the cell-plasma suspension. In all control preparations one part of gelatin-Locke's solution without thrombin was added. The cover slip preparations were covered with hollow ground slides, sealed with vaseline, and incubated for 1 hr at 37° C. Smears from the incubated preparations were stained with methylene blue. Clotted samples were fixed in Zenker-formol solution, sectioned, and stained by the Gram-Weigert technique.

As is seen in Fig. 1, phagocytosis failed to occur in unclotted plasma (*A*), whereas in clotted preparations (*B*) the phagocytosis was marked.

In order to observe the manner in which the leucocytes utilize the fibrinous strands in phagocyting unopsonized pneumococci, pneumococcus-leucocyte mixtures in clotted plasma were studied in the warm stage of the microscope. Near the margins of the clot, phagocytosis was seen to result only when leucocytes succeeded in pinning the pneumococci against the fibrin strands. From this observation it was evident that the mechanism of surface phagocytosis in fibrin clots is essentially the same as that previously described in the lung (*2*, *7*, *8*).

[2] Rat plasma has been shown to contain no opsonins to the A-5 strain of pneumococcus I.

[3] General Electric, Organosilicon Product, 9987.

[4] Obtained through the courtesy of Dr. T. E. Weichselbaum.

Fibrin formation is a common feature of acute inflammation. Fibrinous exudates occur in acute bacterial infections of the lungs, pleura, peritoneum, meninges, and other tissues of the body. A significant portion of the fibrinous material in purulent exudates has recently been identified as desoxyribose nucleoprotein (*1*). Although beta hemolytic streptococcal exudates (group A) are relatively poor in both fibrin and desoxyribose nucleoprotein (*3*), most other bacterial exudates contain appreciable amounts of reticular substance. The present study demonstrates that strands of reticulum enable leucocytes to phagocyte encapsulated bacteria in the absence of antibody. Thus it may be concluded that the fibrinous properties of early bacterial exudates contribute to antibacterial defense by promoting surface phagocytosis. In chronic infections, on the other hand, where most of the leucocytes in the exudate are nonviable, the fibrinous strands may act as a mechanical barrier to recovery by interfering with adequate drainage of the lesions.

References

1. SHERRY, S., TILLETT, W. S., and CHRISTENSEN, L. R. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 179.
2. SMITH, M. R. and WOOD, W. B. *J. exp. Med.*, 1947, **86**, 257.
3. TILLETT, W. S., SHERRY, S., and CHRISTENSEN, L. R. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 184.
4. WOOD, W. B. *Ann. int. Med.*, 1947, **27**, 347.
5. WOOD, W. B. and SMITH, M. R. In press.
6. ———. *Science*, 1947, **103**, 86.
7. ———. *Trans. Ass. Amer. Physicians*, 1947, **60**, 77.
8. WOOD, W. B., SMITH, M. R., and WATSON, B. *J. exp. Med.*, 1946, **84**, 387.

A Method for Preventing Moisture Condensation During Photography of Tissue Cultures in Hanging Drops

Mary J. Hogue

Department of Anatomy, School of Medicine, University of Pennsylvania

When tissue cultures grown in hanging drops over depression slides are taken from an incubator (37° C) and kept at room temperature, moisture condenses on the cover slip in the form of drops. These drops make it impossible to get clear photographs of the tissues. Various means have been devised to prevent condensation—the most common one is to keep the culture at incubator temperature. To accomplish this, some form of heating apparatus is placed on or near the stage of the microscope, but this may be a tedious process.

A simple procedure for preventing moisture from collecting on the cover slip has been devised. It consists of saturating the atmosphere around the culture with moisture and keeping the temperature fairly constant. Brass rings, such as those used by W. H. Lewis in his tissue culture work, are used. He found them useful for photomicrography, since they held the cultures at a uniform height above the slide.