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

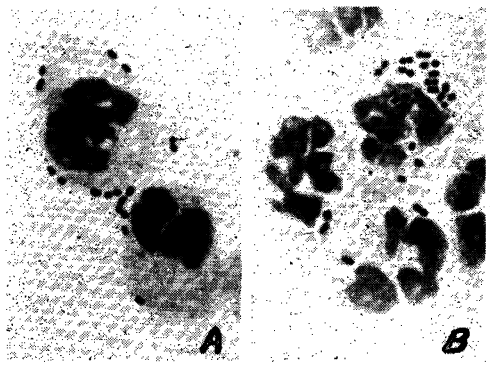

FIG. 1. *A*—Phagocytic test in unclotted plasma. *B*—Phagocytic test in clotted plasma.

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.

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

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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.

These brass rings are 1 mm thick with a 25-mm outside diam and a 19–20 mm inside diam. About one-fourth of the ring is cut away, leaving it incomplete. The ring, held by forceps, is heated over a flame and then dropped into a dish of salvoline (6 parts vaseline and 1 part paraffin). It quickly sinks below the surface. It is then picked up, the excess salvoline is drained off and the ring, now completely covered with salvoline, is placed on an ordinary glass slide, well cleaned with 70% alcohol. When cool and hard, the salvoline holds the ring in place. The space enclosed by the ring is then filled with distilled water, most of which is at once drained off, leaving the surface of the slide within the ring covered with a thin film of water.

The cover glass containing the tissue culture is removed from its depression slide and placed on the brass ring. The salvoline holds it firmly in place. The layer of water inside the ring keeps the atmosphere around the culture saturated with water at a fairly constant temperature, so that water does not condense on the cover slip. If necessary, more water can be added to the space in the ring by means of a fine pipette placed at the opening in the ring. The tissue culture never comes in contact with the water. After photographing, the cover slip with its tissue culture is returned to its glass depression slide and the brass ring is ready for use again. Sometimes it may be necessary to add more salvoline to the top of the ring. This is easily done with a glass pipette filled with salvoline. The slide inside the ring must be completely free of salvoline. If it is not, then a freshly cleaned slide must be used; otherwise the distilled water will not form a thin even film over the glass slide. Glass rings or plastic rings can be used instead of brass rings.

Enzymatic Decomposition of Lignin[1]

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Numerous claims have been made in the literature purporting to demonstrate the ability of microorganisms to utilize lignin as a sole carbon source, or purporting to demonstrate the existence of enzymes acting specifically on the lignin molecule. These reports are based on techniques which presuppose that lignin isolated by acid or alkaline treatment of plant material undergoes no important changes during its isolation (*8, 9, 10, 13, 15*) or that various color reactions can be used to detect the presence or disappearance of lignin (*2, 6*). Some work has been based on the assumed similarity in structure between lignin and tannic acid (*1*). Advances in our understanding of the chemistry of lignin indicate that these suppositions are very dubious (*5, 12, 14*). The work of Brauns (*3, 4*) in isolating so-called "native lignin" by a process which minimizes structural alterations in the lignin molecule seemed to us to warrant an experimental reexamination of the ability of microorganisms to utilize isolated lignin and of the possible existence of lignin-oxidizing enzymes. Results of these studies to date are summarized here in a preliminary manner.

Lignin was prepared from 12-yr-old red sprucewood by the method of Brauns, and used in both nutritional and enzyme studies with fungi.

After a survey of a large number of species of wood-rotting fungi, good growth of several of these organisms was obtained after two weeks' incubation on media in which lignin was the limiting carbon source. This material will be reported in a separate communication (*7*).

Studies on a lignin-oxidizing enzyme were carried out, using the absorption of oxygen measured in a Warburg manometric apparatus as an indication of enzyme activity. A reliable source of enzyme was found to be commercial mushroom spawn,[2] which is an intimate mixture of mycelium of *Agaricus campestris* and well-decomposed horse manure. Conditions used in the preparation of this material are such that it is essentially a pure culture of this organism. A dry, stable preparation of the enzyme has been obtained by the following procedure:

Mushroom spawn is mixed with three times its weight of distilled water in a Waring Blendor for 3 min. The resulting mash is subjected to a pressure of 15,000 psi in a Carver press and the press juice is dialyzed for 48 hr in Visking cellulose sausage casing against cold running tap water. The dialyzed solution is removed from the casing, cooled to 5° C, and to it is added two volumes of cold acetone. The resulting precipitated solution is cooled to 5° C, and the brown precipitate is centrifuged down and washed twice with cold 66% acetone. The precipitate is finally dried at 35° C under aspirator pressure. One lb of spawn yields 2–3 g of this dry preparation. The concentration of solids is reduced from 20 mg/ml in the original press juice to 6 mg/ml in the dialyzed solution, and to 2 mg in the acetone precipitate.

The enzyme is activated by citrate and phosphate ions and it is therefore buffered to pH 6.0 with McIlvaine's buffer (*11*) for activity measurements. Under these conditions 75 cu mm of oxygen are absorbed in the first hour when 1 ml of an aqueous solution containing 3 mg of enzyme is incubated with 1 ml of an aqueous lignin suspension containing 30 mg lignin/ml. The enzyme is quite stable in acid solution (pH 4.0–6.5), but loses activity rapidly at pH values higher than 7. The pH optimum for enzyme activity is 5.7–6.0, with a temperature optimum close to 40° C. Under optimum conditions, the activity of a solution of enzyme is proportional to its concentration.

It is believed that this enzyme is not identical with any of the known phenol oxidases, since mushroom sporo-

[1] This work was done under Contract No. N7 onr 397-4 between the University of Maryland and the Office of Naval Research. The project was initiated at the suggestion of the Prevention of Deterioration Center, National Research Council.

[2] The mushroom spawn used in these experiments was obtained from the L. F. Lambert Company, Coatesville, Pennsylvania.