# Acid-Fast Membranes of Lipid Pneumonia by Fluorescence Microscopy. (17828)

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An insoluble membrane may be formed around certain oil globules within a protein solution or lung tissue, that stains with the acid-fast technic. Such membranes have been described for unsaturated oils like cod liver, paraffin oil and rabbit fat in the lung and in serum(1,2,3). The increased sensitivity of the auramin fluorescence for revealing acid-fast bacteria suggested that it should be useful in the examination of tissues from cases of lipid pneumonia(4) due to aspiration of unsaturated oil, *e.g.* cod liver oil.

Tissue embedded and sectioned in paraffin was mounted, deparaffined and stained in 0.1% Auramin O (C.I. No. 655) in 3% phenol for 2-5 minutes and destained in salt-acid alcohol (0.5% NaCl in 70% EtOH with 1 ml HCl) until the dye remained only in the acid-fast material; usually in 5 to 10 minutes with at least 2 changes of the bleach. On examination with long wavelength ultraviolet radiation the auramin in the acid-fast material fluoresces strongly yellow, Fig. 1B-D, revealing the membranes from the lipid pneumonia rather better than does the usual Ziehl-Neelsen staining, Fig. 1A, with carbol-fuchsine. As the eye sees small bright details on a dark background better than the converse, the finer details are readily visible. Thioflavine TG (C.I. No. 815) may also be used. Sections stained in 1942 and mounted with a cover glass held in place at the edges by gold size had not faded by 1950. Polyvinyl alcohol may be used without a cover glass to make a lasting mount, but mounting in balsam, etc. is not satisfactory because the balsam itself fluoresces and spoils the contrast and the auramin gradually fades in cemented mounts(5).

The brightest fluorescence is obtained with the crossed filter method using a blue-ultraviolet transmitting filter on the radiation source or the condenser of the microscope and a complementary yellow filter in or on the eyepiece of the microscope. A mercury arc, or a high amperage tungsten filament lamp, may be used as sources although the latter is suitable only for a monocular microscope(6). The acid-fast material is seen as bright yellow on a yellowish background, since the blue-white fluorescence of the protein in the tissues is seen as yellow. For color differentiation a Corning No. 5860 filter may be used with the Hg arc which transmits no visible light. Other combinations are possible and fluorescing counterstains may also be used(5). The intensity of the fluorescence image is low, despite the high contrast, and best results will be obtained when working in a room with subdued illumination.

Ceroid, an acid-fast, sudanophillic hyaline material formed in certain instances of experimental cirrhosis found in macrophages within the liver(7) should be visible also with this technic. Serum and lipid combinations have been reported to increase antibody formation(8) and may prove to be acid-fast. Meyer(9) has discussed acid-fast materials found in animal tissues, especially those reported by Cuénot(10) in the connective tis-

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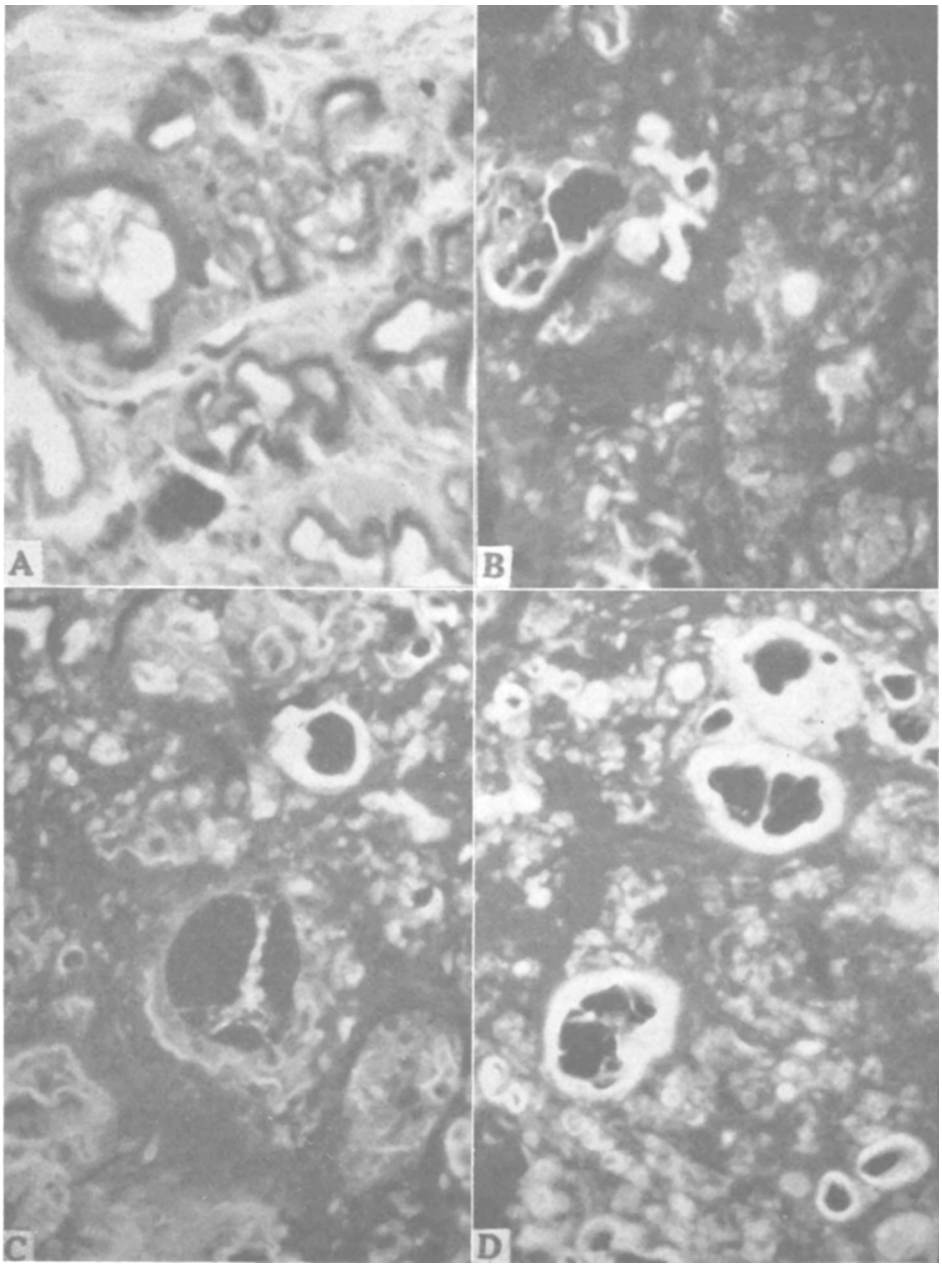


FIG. 1.
Lung sections showing acid-fast material from lipid pneumonia due to cod-liver oil, 300×.
A. Ziehl-Neelsen stained section; B-D, Fluorescence image after treatment with carbol-auramin.

sue of the earthworm with regard to the possibility of their being symbiotic bacteria. The fluorescence microscope should be useful for the examination of these materials and should aid in understanding the phenomena involved.

*Summary.* Fluorescence microscopy using carbol-auramin or carbol-thioflavine which

are specific for acid-fast materials is recommended for the study of the membranes formed in lipid pneumonia where unsaturated lipids are suspected and for other familiar lipo-protein combinations, as more sensitive than the brightfield microscopy with Ziehl-Neelsen staining.

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## Some Biochemical Aspects of Herpes Infection.* (17829)

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Inoculation of an adapted strain of herpes simplex virus on the chorio-allantoic membrane of an egg is followed by viral growth not only in this membrane but also in the liver, heart, spleen, and brain of the embryo (1). The pathology and histology of the herpetic infection of these tissues has been described by Anderson(1) while the pathology of the same infection in tissue culture has been given by Rivers, Haagen and Muckenfuss(2). This work is a preliminary study of some of the biochemical changes which characterize the host tissue during the course of this viral infection. It was from the viewpoint of learning the nature of the alterations which take place in the metabolism of the infected tissues that measurements were made: of the rate of growth of the tissues; of two of the respiratory enzyme systems; of changes in the nucleic acid composition; and of the protein content. The results of these findings and some of their significance are discussed in the following.

*Experimental.* In all experiments the Armstrong 1166 strain of herpes simplex virus was used. Previously it had undergone 54 passages in mice and 37 passages in eggs. The inoculation was made on the chorio-allantoic membrane of 12-day-old embryonated eggs. The tissues were harvested after 3 days and enzyme determinations were made on pooled samples of this moist tissue. Other pooled samples were frozen and brought to dryness *in vacuo*. These desiccated samples were used for nucleic acid and nitrogen determinations.

*Methods.* The protein nitrogen was determined on samples of tissue which were precipitated from an aqueous suspension with trichloroacetic acid and washed repeatedly with alcohol and ether. The nitrogen was measured by the Koch-McMeeken modification of Nessler's method(3). To determine the number of nuclei per unit weight of liver, a 10% homogenate of the tissue was prepared in 1.0% citric acid(4). The citric acid hardened nuclei were stained with methyl green and counted in a hemocytometer after appropriate dilution. The phosphate was determined by the modified Fiske and Subbarow procedure(5). Succinoxidase determinations were made using the method of Potter and Schneider(6). The $\alpha$-ketoglutaric acid oxidase activity was measured by the method described by Ackermann(7). The desoxyribosenucleic acid (DNA) was determined by both the Schmidt-Thannhauser(8) and by the Schneider(9) procedures. The

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