Demonstration of Glycogen in the Human Liver by the Electron Microscope. (18652)

COUNCILMAN MORGAN AND ROBERT W. MOWRY. (Introduced by R. W. G. Wyckoff)
From the Laboratory of Physical Biology and Laboratory of Pathology and Pharmacology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

The purposes of this paper are to show that glycogen is readily visible in thin tissue sections examined by the electron microscope and to suggest that its removal by diastase offers a convenient way of differentiating it from other cellular constituents since differential staining of electron microscopic preparations has so far been unsuccessful.

Procedure. Thin blocks of human liver were fixed in alcohol-formalin (10% formalin in 95% alcohol), known to be an excellent fixing agent for glycogen(1). After fixation the blocks were dehydrated in absolute alcohol, cleared in xylol and embedded in paraffin. Sections were stained for glycogen by Best's carmine stain, the Bauer reaction and the periodic acid-leucofuchsin method and examined under the light microscope(2). From livers showing abundant glycogen small blocks were excised from the paraffin embedded tissue and the paraffin removed by xylol followed by acetone. The tissue was then embedded in methacrylate and sectioned by the methods outlined by Newman, Borysko and Swerdlow(3) except that a glass edge(4) was used in place of a steel knife. Thin sections were floated onto formvar coated screens and the methacrylate removed by amyl acetate. The sections were shadowed with palladium. All observations were made with an RCA type EMU electron microscope.

To determine that glycogen was not lost during methacrylate embedding sections one and five μ thick were cut from each methacrylate block mounted on glass slides and stained for glycogen. Examination in the optical microscope showed that the reembedding had not appreciably altered the tissue and that as much glycogen was visible within the nuclei and cytoplasm as in sections taken from the original paraffin block. It should be emphasized at this point that poor preservation of cellular structure, largely resulting from post-mortem autolysis, is more evident in thin sections. In the light microscope 5 μ thick sections show what one is accustomed to consider as well preserved liver. In progressively thinner sections superimposition of structures diminishes and clumping and distortion of the cytoplasm become more and more striking.

In the electron microscope many nuclei are shown to contain varying amounts of a moderately dense substance having the form and distribution of glycogen as previously described(5) and especially as seen in stained, one μ thick sections in the optical microscope. The nuclei are generally enlarged and the chromatin is concentrated at the periphery. In the majority of nuclei the material presumed to be glycogen aggregates as small globules at the periphery (Fig. 1). Less commonly it forms an incomplete coating on the nuclear membrane and encloses irregular particles of similar density (Fig. 2). Rarely is the nucleus largely filled (Fig. 3).

In occasional nuclei small globules of apparent glycogen are enveloped by very dense, irregular material resembling chromatin (Fig. 4). Since in such nuclei no other structures can be identified as nucleoli and since they are of similar size it is possible that these may be glycogen filled nucleoli. In the cytoplasm of cells from blocks of tissue shown to contain granules of cytoplasmic glycogen in the optical microscope irregular, sharply demarcated particles of amorphous material similar in density to that seen within the nuclei are

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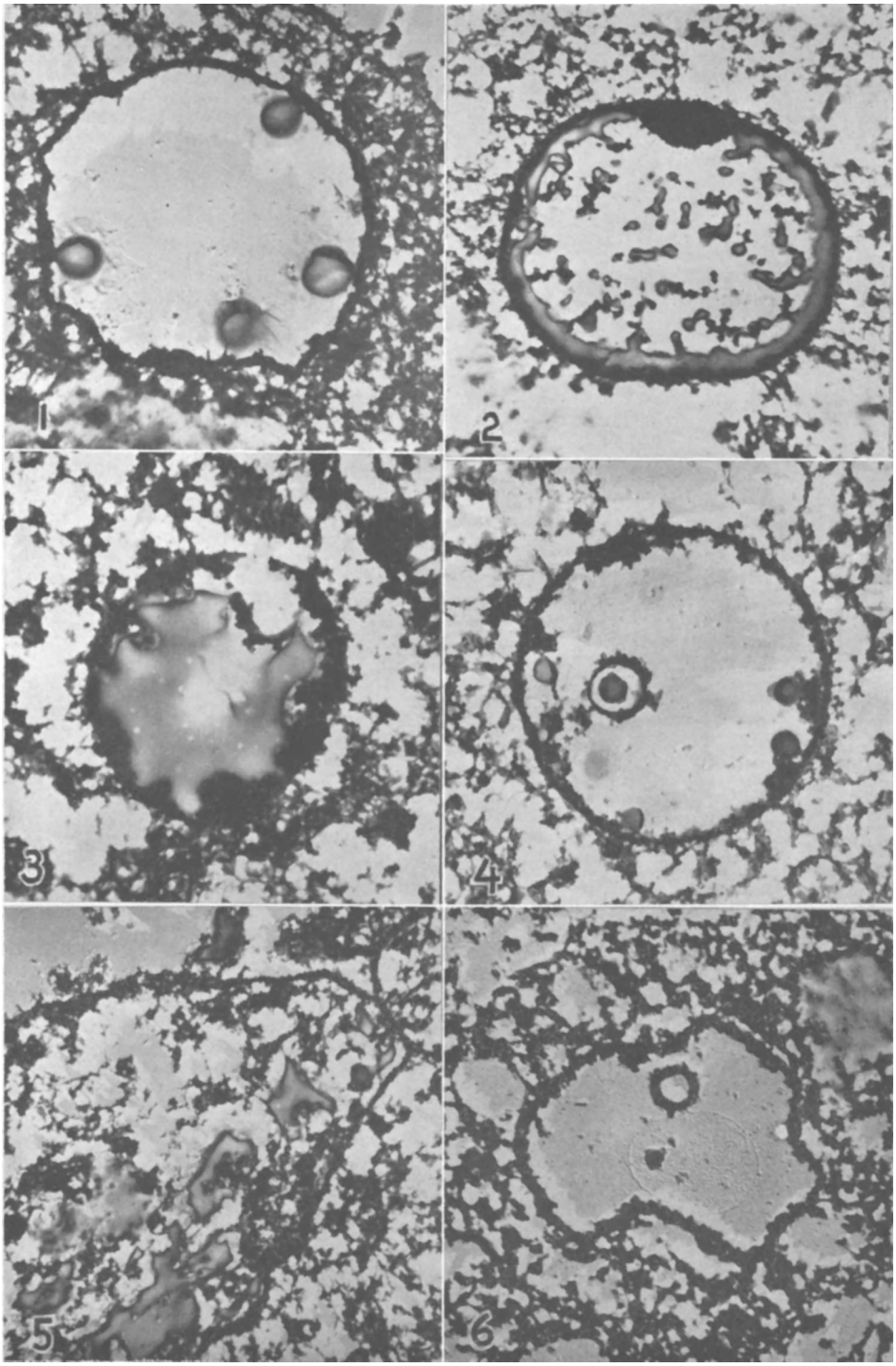

FIG. 1. Liver cell nucleus containing 4 glycogen droplets. Magnification = 3,466×.
FIG. 2. Intranuclear glycogen, much of which adheres to the nuclear membrane. Magnification = 3,866×.

FIG. 3. Nucleus largely filled by glycogen. Magnification = 6,000×.
FIG. 4. Nucleus containing 5 droplets of glycogen, one of which lies within a possible nucleolus. Magnification = 5,200×.
FIG. 5. Cytoplasm containing irregular, sharply circumscribed, moderately dense masses resembling glycogen in nuclei. Magnification = 2,933×.
FIG. 6. Typical nucleus in duplicate section after diastase digestion. Magnification = 4,800×.

observed (Fig. 5).

To determine whether diastase affects this material 5 μ, 1 μ and ultrathin sections were incubated at 37°C in 0.1% USP IX malt diastase in Lillie's pH 6.0 saline phosphate buffer for 1 hour(6,7). Examination of stained sections in the light microscope as well as ultrathin sections in the electron microscope reveal complete removal of the presumed nuclear and cytoplasmic glycogen. Often in nuclei a faint ring is left on the formvar film, suggesting the outline of a pre-existing deposit of glycogen (Fig. 6). Occurring in the cytoplasm are masses of material which are poorly demarcated, larger and uniformly less dense than the presumed glycogen particles (best seen in Fig. 5 and 6). These are not removed by diastase. Other cytologic features appear unaltered. Sections incubated in the buffer solvent without diastase show no effect upon the glycogen. Because of the ribonuclease-like activity demonstrated after prolonged incubation of sections in malt diastase, and apparently due to contaminating traces of ribonuclease, duplicate sections were incubated in a solution of crystalline ribonuclease (Armour's, bovine pancreas) with control sections of pancreas (8). No removal of material regarded as nuclear or cytoplasmic glycogen is observed in the electron microscope.

Summary. In sections of human liver examined by the electron microscope, material is visible in the nuclei and cytoplasm, which, because of its resemblance in morphology and location to glycogen seen in stained sections examined in the light microscope and because of its removal by diastase, is presumed to be glycogen.

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Hemolysin from the Yeastlike Phases of Some Pathogenic Fungi. (18653)

S. B. SALVIN. (Introduced by C. L. Larson)
From the Rocky Mountain Laboratory (Hamilton, Mont.), National Microbiological Institute, National Institutes of Health, Public Health Service.

The only hemolysin previously described in a pathogenic fungus was isolated from the ground mycelium of *Aspergillus fumigatus*(1). The present investigation is concerned with an hemolysin or hemolysins from the yeast-like phases of *Histoplasma capsulatum, Blastomyces dermatitidis, Candida albicans,* and *Cryptococcus neoformans.*

The fungi used were from the collection maintained in this laboratory. *H. capsulatum* (strain 6515) and *B. dermatitidis* (strain 6046) were grown for 5 days in flasks containing an amino-acid broth at 37°C, and kept in constant rotation during this period of growth (2). *C. albicans* (strain 3148) and *C. neoformans* (strain 3715) were grown for 7-10 days at 25°C in a similar amino-acid broth in 1-liter Blake bottles(3). The cells, after exposure to 0.5% formalin for 72 hours, were thoroughly washed in saline at 3°-5°C, and stored in 1:10,000 merthiolate until ready to

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