Glycogen Metabolism in Chlamydia-Infected HeLa-Cells

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A difference in glycogen metabolism between two strains of Chlamydia agents was observed which can serve as a taxonomic marker to distinguish C. psittaci from C. trachomatis.

Inclusions in HeLa cells infected with Chlamydia trachomatis appear to contain glycogen when cells are stained by the peridate-Schiff (PAS) technique (1, 3, 4). C. psittaci infections do not produce PAS-positive-stained inclusions in the host. The compounds involved in the histochemical reaction have not been chemically identified in chlamydial infections.

HeLa 229 cells, chlamydia agents C. trachomatis strain TW-3 (5) and C. psittaci strain meningopneumonitis (MN) were prepared as described by Jenkin (6). Infectivity titrations were performed by the method of Jenkin and Ernick (Bacteriol. Proc., p. 133, 1963). Glycogen was isolated from HeLa cells by adding equal volumes of 33% KOH to cells and digesting the mixture at 70 C. Oyster glycogen (1 mg/sample; Calbiochem., Los Angeles, Calif.) was added as a carrier. Two and one-half volumes of 95% ethanol were added to the digest. The glycogen was sedimented by centrifuging at 1,000 × g for 30 min in the PR-2 centrifuge (International Equipment Co., Needham Heights, Mass.). Supernatant fluid was discarded; the pellet was redissolved in 1.0 ml of 0.1% KCl and reprecipitated with 95% ethanol. The pellet was washed three times, dissolved in 1.2 ml of 0.1 n KCl solution, and counted with 15 ml of Bray's scintillation fluid (2) in a Packard Tri-Carb liquid scintillation counter. Counts were obtained from triplicate samples in two independent experiments. HeLa cells, cultivated for 24 hr, were trypsinized and transferred into 1-oz bottles (4.0 × 10⁴ cells per bottle). Eighteen hours later, the cells were infected with 0.2 ml of strains TW-3 or MN, each organism infecting at least 60% of the cells. A normal yolk sac homogenate was prepared and served as a control. Strains MN and TW-3 were adsorbed to HeLa cells for 2 hr at room temperature with constant shaking by using a Belco shaker. At the end of the adsorption period, the HeLa cells were washed with Hank's balanced salt solution (BSS) to remove residual yolk sac material. Each bottle received 1 ml of Eagle's minimal essential medium (MEM) with 5% fetal calf serum or glucose-U-¹⁴C (1 µCi/ml, 309 mCi/mM; Amersham/Searle Co., Des Plaines, Ill.) in MEM, incubated at 37 C, and shaken continuously; samples were removed at 12-hr intervals.

Cells infected with strain TW-3 exhibited a marked increase of glycogen accumulation during the period from 48 to 60 hr of incubation. Sixty hours after incubation, the activity of the ¹⁴C-glycogen in MN-infected cells and uninfected cells was 50% lower than in TW-3-infected cells (Fig. 1)

![Fig. 1. Labeling of glycogen during 24-hr pulses of glucose-U-¹⁴C of strain TW-3 infected (●), strain MN infected (×), and control (○) HeLa cells.](http://jb.asm.org/Downloaded from http://jb.asm.org/
Studies of glycogen accumulation were made as described in the legend to Fig. 2. Labeled glycogen declined more rapidly in normal cells and cells infected with strain MN than in cells infected with strain TW-3 during the first 48 hr after the inoculation. Cells infected with strain MN showed a second decline in labeled glycogen after 84 hr of infection (Fig. 2). In TW-3-infected cells, the rate of glycogen accumulation slowly declined after 84 hr of infection although the total radioactivity continued to rise.

The results of the investigation of glycogen accumulation in TW-3-infected HeLa cells showed that TW-3 infection alters glycogen metabolism in the host cell. The association of PAS-positive staining with glycogen accumulation in cells infected with TW-3 is not completely compatible with the results of this report since normal cells accumulate glycogen at a similar rate as TW-3-infected cells yet stain PAS negative. A twofold difference in the total accumulation of glycogen may represent a threshold for a positive reaction or other PAS-positive compounds are involved in the inclusions of infected cells. This speculation requires further investigation.

The results with strain MN indicate that a rapid glycogen depletion occurs toward the end of the infection. This observation is not seen in strain TW-3 infections. The differences in glycogen accumulation can serve as a marker to distinguish strain TW-3 from strain MN.

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