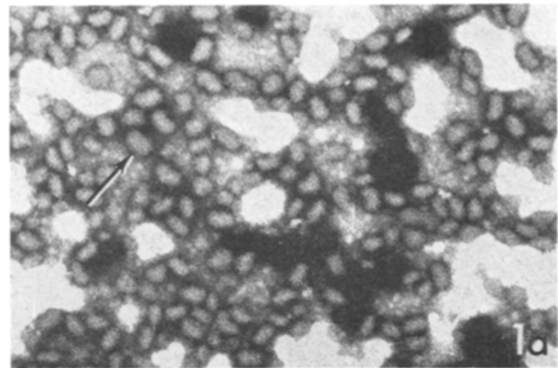
Glycogen in *Physarum polycephalum*

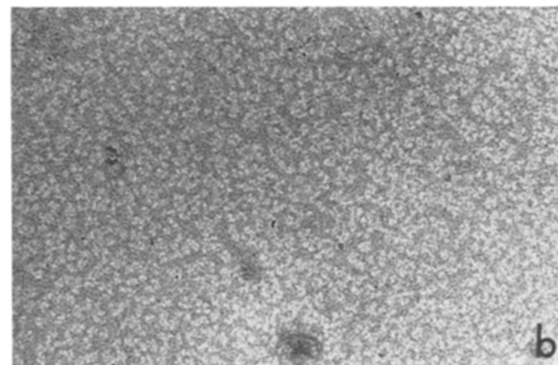
The presence of a glycogen-like polysaccharide during exponential growth of *Physarum plasmodia* in submerged shake cultures has been reported by DANIEL[1]. The present report shows definitively by direct isolation, biochemical analysis, and ultrastructure observations that this polysaccharide is indeed glycogen.

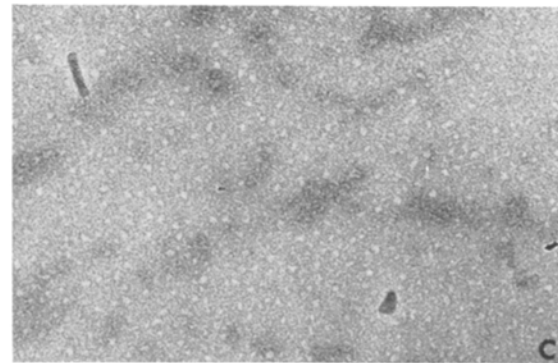
Plasmodia were grown in shake flasks according to MITTERMAYER et al.[2] and harvested during log growth. Following a wash in distilled water (0 °C), plasmodia were subjected to the procedure of ORRELL et al.[3] for the specific isolation of glycogen. The only modification was the substitution of amyl alcohol for octyl alcohol during deproteinization. Following isolation, the 'glycogen' was resuspended in distilled water, placed on carbon-coated grids, and stained with PTA (1% phosphotungstic acid, pH 7.0). Observations were made at 75 kV (kilovolt) on a Hitachi HU-11B-2 electron microscope. Susceptibility of 'glycogen' to degradative enzymes was studied by treatment with 1% α- or β-amylase (3 × crystallized from *Aspergillus oryzae*, Calbiochem., and type 11B from barley, Sigma) in distilled water for 1 h at 37 °C. The results of these enzymatic degradations were ascertained by placing a drop of the reaction mixture on a carbon-coated grid, staining with PTA, and observing with the electron microscope. Further characterization was obtained by measuring the amount of glucose released following the enzymatic treatment according to the glucose oxidase method (glucostat-special reagents, Worthington Biochem.). This reaction was allowed to proceed for 1 h at room temperature in 0.1 *M* phosphate buffer. In addition, a sample of commercial rabbit liver glycogen (Nutritional Biochem. Corp.) was compared with *Physarum* 'glycogen' by allowing each sample to react with an iodine solution (DOEZEMA and PHILIPS[4]) and then determining the absorption spectrum of each sample in a Beckman DB spectrophotometer.

The spherical particles obtained (Figure a) by the glycogen isolation procedure of ORRELL et al.[3] varied in size between 500 and 700 Å, and resembled the β-glycogen configuration described by REVEL[5]. After treatment with α- or β-amylase (Figures b and c), these particles measured approximately 300–350 Å in diameter. Glucostat analysis prior to amylase treatment revealed no free glucose, whereas after treatment approximately 1.0 mg of glucose per 2 ml sample was liberated. Both rabbit liver glycogen and the 'glycogen' obtained from *Physarum* gave similar absorption spectra, with maximum absorption at 480 nm for *Physarum* and at 460 nm for the rabbit liver glycogen.

The morphology of the isolated particles, their partial susceptibility to amylase treatment, and their absorption spectrum, which is similar to that of a known glycogen, indicates that one of the polysaccharides present in *Physarum* is glycogen[6].







(a) Isolated glycogen particles (arrow) stained with 1% PTA. × 60,500; (b) isolated glycogen particles after 1 h in 1% α-amylase. × 32,000; (c) isolated glycogen particles after 1 h in 1% β-amylase. × 32,000.

Zusammenfassung. Verschiedene biochemische und elektronenoptische Techniken wurden dazu benützt, eine partikuläre Speicherform für Glukose in *Physarum polycephalum* zu identifizieren. Die Ergebnisse zeigen, dass es sich um Glykogen handelt.

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