Table 1. Ratios of reaction rates for malate dehydrogenase from *Euglena gracilis* grown on autotrophic medium and on heterotrophic medium. *a*, 0.006*M*; *b*, 0.05*M*; *c*, 0.1*M*.

Coenzyme and analog ratios	Autotrophic	Heterotrophic
$PAAD_b/NAD_a$	0.21	0.30
$APAD_c/NAD_a$	4.4	3.1
$APAD_c/APAD_a$	1.1	0.82
$PAHxD_a/NAD_a$	0.08	0.00
$PAHxD_b/NAD_a$	0.13	0.05
$PAHxD_c/PAHxD_b$	1.4	2.4
$NHxD_b/NAD_a$	1.0	0.70

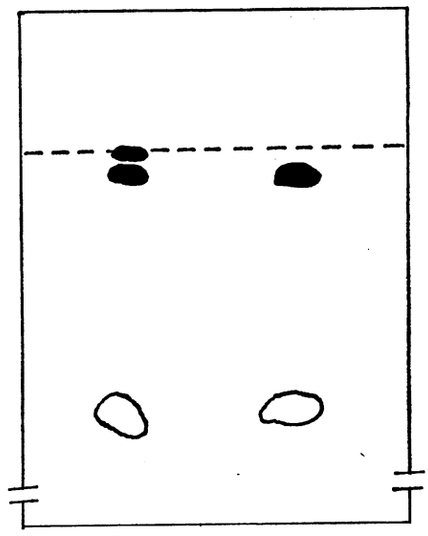

Fig. 1. Tracing of electrophoretic patterns of *Euglena* malate dehydrogenase on starch gel at *p*H 7.0. The cathode is at the top, the anode, at the bottom. The origin is indicated by the dotted line. The autotrophic extract is at the left, the heterotrophic, at the right. Solid bands, intense staining; clear bands, weak staining. The break in the tracing represents 10 cm.

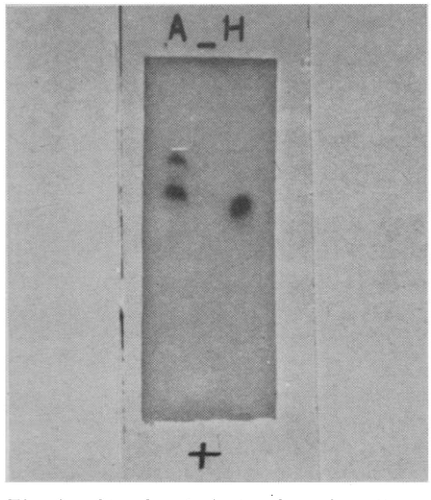

Fig. 2. Starch-gel electrophoresis patterns of *Euglena* malate dehydrogenase at *p*H 7.2.

in ratios for the enzyme from the two sources.

To exclude the possibility that the differences in ratios might be due to the presence of a "malic enzyme" capable of utilizing NAD, we determined the ratios of the rates when malate and NAD were the reactants and compared the rates when oxalacetate and NADH were the reactants. The ratio was 3.6 for both the autotrophic and heterotrophic *Euglena* extracts, indicating that the differences reported here are not due to variation in "malic enzyme" content.

These results have been confirmed by starch-gel electrophoresis experiments. Starch-gel electrophoresis of the malate dehydrogenase preparations was carried out at *p*H 7.0 as described for lactate dehydrogenase (*3*) except that, in the staining procedure, 2.0*M* sodium malate was substituted for 2.0*M* lithium lactate. A current of 25 ma was applied for 12 hours starting with 200 volts and ending with about 100 volts. The heterotrophic extract showed two spots, the autotrophic, three (Fig. 1). The heterotrophic extract had an intense spot which migrated only slightly toward the anode and a more rapidly moving faint spot which also migrated toward the anode. The autotrophic extract had these two spots, with the former somewhat less intense, plus an additional spot of slightly less intensity, which remained at the origin. Electrophoresis of the preparations at *p*H 7.2 was carried out under the aforementioned conditions for *p*H 7.0 except that the current was increased to 50 ma during the separation. Figure 2 shows the pattern at a *p*H of 7.2, where the additional autotrophic spot did migrate. The faint spots were absent on this gel as they had migrated off the end. Since these are not purified preparations of malate dehydrogenase, the slight difference in the migrations of the corresponding heterotrophic and autotrophic spots does not appear to be significant for it may be the effect of other substances in the preparations. We hope to purify the *Euglena* malate dehydrogenase in order to facilitate characterization of the different forms.

The additional form of malate dehydrogenase in the autotrophic extract may be due to its production by photosynthetic mechanisms. Alternatively, this additional form may be repressed in the heterotrophic extract by the presence of malate in the nutrient medium.

Both the analog data and the electrophoretic data show clearly that the molecular forms of malate dehydrogenase in *Euglena* depend in part upon the nutritional environment of the organism (*8*).

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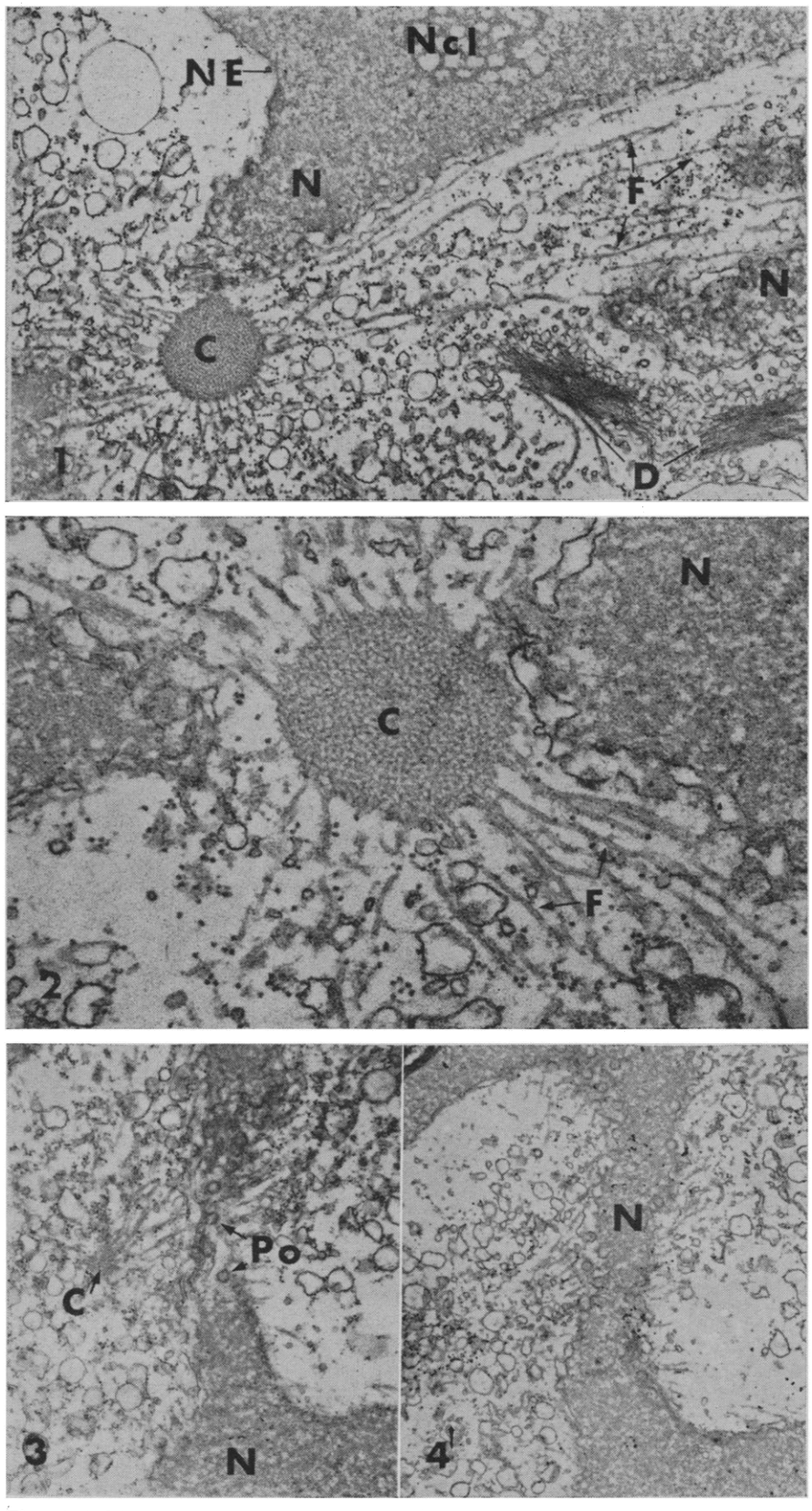
31 July 1963

Fine Structure of a Diatom Centrosome

Abstract. *The centrosome of the diatom* Surirella ovalis *is a dense spherical structure 500 mμ in diameter with filaments 15 mμ in diameter connected to it. The interior is filled with uniform granules but does not contain a typical centriole or clear area. The filaments emanating from it resemble the filaments of the mitotic apparatus, but they are present during interphase and appear to terminate at pores in the nuclear envelope.*

A centrosome has been observed in the diatom *Surirella ovalis*. It is a sphere about 500 mμ in diameter as determined from serial sections (Figs. 1 and 2). The centrosome (*C*) is located in a notch or hollow of the H-shaped nucleus (*N*) at the center of the cell. It persists during interphase, and only one such structure has been seen in each individual studied. The body is not limited by a membrane, and the interior is homogeneously filled with coarse uniform granules. Filaments (*F*) 15 mμ in diameter are attached to the body and extend toward pores (*Po*) in the nuclear envelope (*NE*).

The somatic division of another



Figs. 1–4. The diatom *Surirella ovalis*. *C*, centrosome; *N*, nucleus; *NE*, nuclear envelope; *Ncl*, nucleolus; *F*, filaments; *D*, dicytosomes; *Po*, pores. Fig. 1. Longitudinal section through the central cytoplasm. The centrosome (*C*) is the dense granular body with the filaments (*F*) attached to it. Two dicytosomes (*D*) at the lower right are grouped around a lobe of the nucleus (*N*) (×32,000). Fig. 2. The filaments merge with the centrosome (× 74,000). Fig. 3. Tangential section through the edge of the nuclear bridge near the center of the cell (×15,000). Fig. 4. The nucleus extends across the cell (×15,500).

member of the genus *Surirella* was extensively studied with a light microscope by Lauterborn (*1*), who described a granule located in a cavity or hollow of the nucleus as a centrosome. The structure we are describing is similarly located and sufficiently large to be seen in the light microscope; hence we are probably seeing the same structure Lauterborn saw. Lauterborn saw this structure give rise to another granule which in turn formed the mitotic spindle; he also showed that the nucleus rounds up and that the nucleoli disappear at the onset of mitosis. We consider the cells shown here to be at interphase because the nuclei are not rounded up, the reticulate nucleoli (*Ncl*) are often seen, and no areas in the remainder of the nuclei resemble condensed chromosomes.

As seen in the light microscope, a centrosome is generally considered to consist of a centriole and a surrounding clear area, often with filaments radiating from the outer margin of the clear area. The structure shown in Figs. 1 and 2 differs from this definition in that it does not contain either a typical centriole (*2*) or a clear area. However, 15-mμ filaments are continuous with its periphery. A similar relationship is seen between centrosomes and mitotic apparatus filaments in other cells. The filaments described here resemble those described by other workers (*3*) as filaments of the mitotic apparatus, but in *S. ovalis* they do not extend to the cell periphery as reported by Harris (*3*) for filaments in sea urchin eggs.

The specimens we used were cultured on agar as described previously (*4*). They were fixed at room temperature in 1-percent OsO_4 containing the divalent cation Ca^{++}, buffered at a *p*H of 6.1 (*5*). After dehydration in ethanol and infiltration with a mixture of propylene oxide and Epon, the specimens were embedded in Epon. Contrary to an earlier method (*4*), the siliceous cell walls of the diatoms were not removed with 10-percent HF before they were sectioned. The sections were cut with a diamond knife mounted in an LKB Ultrotome; they were picked up on copper grids covered with Formvar and carbon, stained with lead, and examined in an RCA EMU-3F electron microscope. All of the micrographs (Figs. 1–4) were taken of longitudinal sections cut nearly parallel to the girdle plane of the diatom frustule and perpendicular to the valve plane.

Since it does not contain either a

centriole or clear area, and is filled with dense uniform granules, the diatom structure we describe is not a typical centrosome. We have not seen it in other diatoms, either centric or pennate. Our observations may support the idea of Lauterborn that the spindle is formed outside the nucleus in some diatoms, perhaps in a manner analogous to that found in some flagellates (*6; 7*).

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5 August 1963

Invertebrate Ferritin: Occurrence in Mollusca

Abstract. *Ferritin, in both crystalline and paracrystalline forms, occurs in the columnar epithelial cells of the dorsal wall of the radula of the marine chiton* Cryptochiton stelleri, *order, Polyplacophora. The ferritin occurs in association with the magnetite of the radular teeth. It has been isolated and crystallized in the presence of cadmium sulfate.*

Ferritin is an iron-protein complex which acts physiologically as an iron storage mechanism. It has the approximate composition $(FeOOH)_8 \cdot (FeOPO_3H_2)$ (*1*), and contains about 20 percent of iron, dry weight. Ferritin was first isolated from horse spleen by Laufberger (*2*) in 1937 and subsequently has been the subject of intensive research (*1*, *3*). It has been reported to occur in a variety of organs and cells of many vertebrates. It is commonly found in the spleen, liver, bone marrow, and kidney, and has also been observed in the ameloblasts associated with maturing enamel in rat incisors (*4*). Among the invertebrates, Roche *et al.* (*5*) reported the occurrence of small amounts of ferritin in the chloragogen cells of the polychaete worm *Arenicola marina*. In flowering plants a similar protein, phytoferritin, has recently been identified and localized (*6*, *7*). We have recently isolated ferritin from the columnar epithelial cells of the radula of the marine chiton, *Cryptochiton stelleri*.

Iron was reported to occur in high concentrations in the denticles of the lateral radular teeth of the primitive molluscan chitons (Polyplacophora) (*8*). Lowenstam (*9*) used x-ray diffraction techniques to show that the iron in the mineralized denticles occurs, in some species of chitons, in the form of the mineral magnetite ($Fe_2O_3 \cdot FeO$), while in others it occurs as magnetite plus minor amounts of undetermined minerals. This was the first indication that iron is biologically precipitated in crystalline form as the ferric-ferrous compound, which is otherwise known as a common mineral formed by inorganic precipitation processes in igneous and metamorphic rocks.

In the radula of any individual chiton, the total number of denticles is considerable and, because of wear and loss, the anterior denticles have to be replaced very frequently. Hence there must be a continual supply of iron available for denticle formation (aside from that required for hemeprotein formation). According to most investigators, the radular teeth of molluscs are formed posteriorly in the radular sac by the odontoblast cells (*10*). When they have been formed and have moved anteriorly on the radular sheath, min-

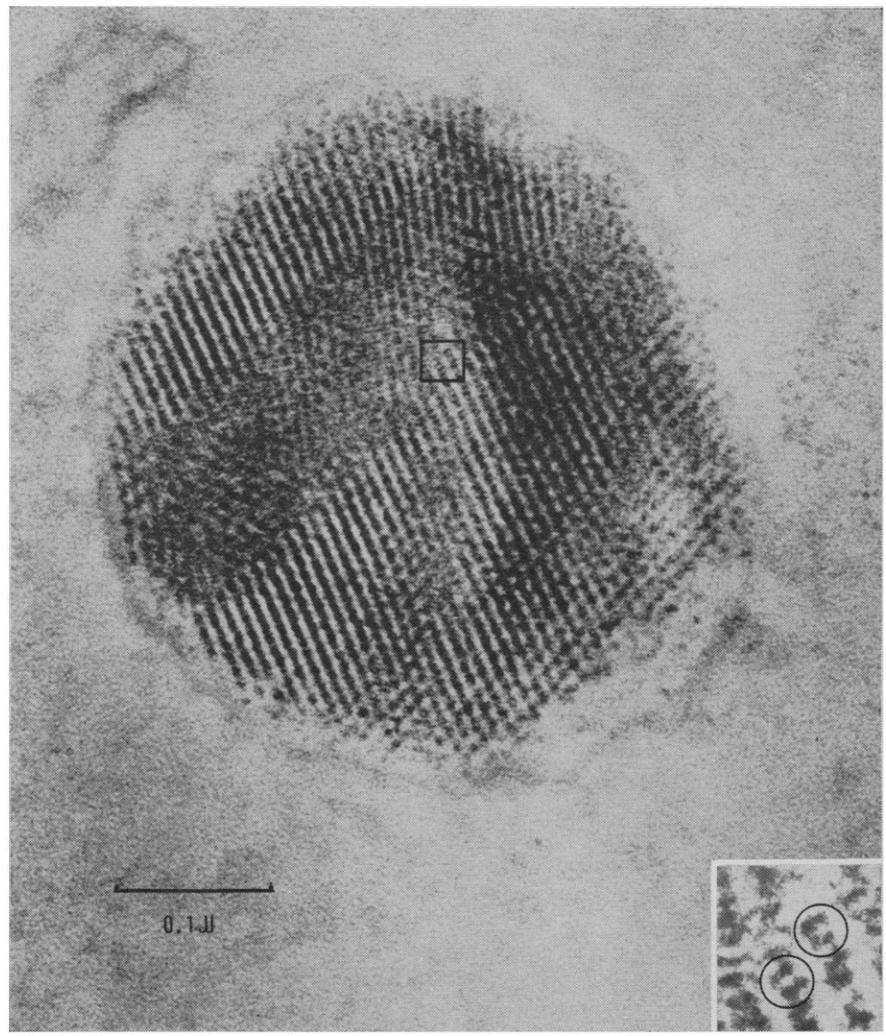

Fig. 1. Electron micrograph of a section of crystalline ferritin in the columnar epithelial cells of the dorsal wall of the radula of *Cryptochiton stelleri* (about × 206,000). Inset: Higher magnification of the crystal showing characteristic profiles of the individual iron micelles (about × 635,000).