10 to 15 years after recuperation from natural rubella. These findings suggest that neutralizing antibody persists at a relatively unchanged concentration for an extended period of time after natural infection. The amounts of antibody in human serum specimens (16 to 128) and those in standard commercial γ-globulin (256 to 2048) are consistent with the increase in concentration of γ-globulin (20 times) which occurs during the manufacturing process.

The variation in the protective value of the different lots of γ-globulin for rubella previously reported by several investigators (*2, 3, 4*) may be a reflection of the eight-fold difference in neutralizing antibody which was found in the lots of γ-globulin assayed in this investigation. Other factors, however, such as dosage and time of administration of γ-globulin, must be considered in this connection. Future studies of the protective value of lots of γ-globulin are necessary to establish the validity of the selection of specific lots for clinical use on the basis of antibody titrations.

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Molecular Heterogeneity of Enzymes: Malate Dehydrogenase of Euglena

Abstract. *Experiments with analogs of the coenzyme nicotinamide adenine dinucleotide demonstrate that the molecular forms of malate dehydrogenase in* Euglena *vary with the nutritional environment. Electrophoretic separations on starch gel show that* Euglena *grown on autotrophic medium has a malate dehydrogenase which is lacking in* Euglena *grown on heterotrophic medium.*

Analogs of the coenzyme, nicotinamide adenine dinucleotide (NAD), can be used to differentiate the molecular forms of dehydrogenases linked to pyridine nucleotides (*1–3*). The activities of a dehydrogenase are determined in the presence of the coenzyme and in the presence of individual analogs of the coenzyme. Differences in the ratios of activities of NAD and analogs with the same dehydrogenase from different sources indicate a difference in the molecular structure of the enzyme from the different sources. Kaplan and coworkers have used the technique to distinguish molecular forms of malate dehydrogenase (*1, 2*) and lactate dehydrogenase (*2, 3*). They found molecular heterogeneity of the same dehydrogenase from similar tissues of different species and from different tissues of the same species. The former phenomenon indicates that molecular heterogeneity of enzymes may be of value to the study of systematics. We studied malate dehydrogenase in *Euglena* to learn the effect of environment upon the molecular forms of dehydrogenase. This organism was chosen because of its controversial taxonomic position and because its nutritional environment can be varied by growing it on either an autotrophic or a heterotrophic medium.

Euglena gracilis variety *bacillaris* (*4*) was grown without aeration in erlenmeyer flasks at *p*H 3.5 on the heterotrophic medium described by Greenblatt and Schiff (*5*). Malate and glutamate serve as the sources of carbon for this medium. The cultures were exposed to continuous white fluorescent light and were maintained at 21°C to 24°C. The autotrophic cultures were grown in Fernbach flasks on a similar medium except that it lacked malate and glutamate and that 5-percent CO_2 in air was bubbled through it. Heterotrophic cultures were harvested at the end of 5 days and the slower-growing autotrophic cultures at the end of 10 days. After the cells were washed twice with phosphate buffer, *p*H 7.5, they were suspended in 0.2*M* phosphate buffer, *p*H 7.5, containing 0.001*M* disodium ethylenediaminetetraacetate (EDTA); the cells were then disrupted to form an extract, in a French pressure cell at 3 to 4 tons pressure (*6*). The extract was clarified by centrifugation at 35,000*g* for 30 minutes. To remove interfering endogenous activity, the protein was precipitated by addition of ammonium sulfate to 90-percent saturation and this precipitate, suspended in 0.1*M* phosphate buffer, *p*H 7.5, containing 0.001*M* EDTA, was used as the source of malate dehydrogenase.

Kinetic measurements of malate dehydrogenase activity were made in silica cuvettes with a Beckman DB spectrophotometer linked to a recorder. The reaction mixtures consisted of 0.09*M* bicarbonate-carbonate buffer (*p*H 10.2), 0.006*M* or 0.05*M* or 0.10*M* sodium malate, 0.00025*M* NAD or coenzyme analog—3-pyridinealdehyde adenine dinucleotide (PAAD), 3-acetylpyridine adenine dinucleotide (APAD), 3-pyridinealdehyde hypoxanthine dinucleotide (PAHxD), or nicotinamide hypoxanthine dinucleotide (NHxD)—and malate dehydrogenase suspension to make a total volume of 3.0 ml. The enzyme was added at zero time and the activity was related to the initial rate of increase in optical density at the absorption peak for the reduced coenzyme or analog (*1, 7*).

Ratios of reaction rates with NAD and its analogs were determined with a fixed amount of malate dehydrogenase. Data showing large differences in ratios for the enzyme from the two different types of *Euglena* cultures are reproduced in Table 1.

The data in Table 1 indicate that the malate dehydrogenase of *Euglena* grown on autotrophic medium is different from the malate dehydrogenase of *Euglena* grown on heterotrophic medium. For example, with the analog 3-acetylpyridine adenine dinucleotide, substrate inhibition was observed with the heterotrophic *Euglena* enzyme but not with the autotrophic *Euglena* enzyme. At low malate concentration the heterotrophic *Euglena* enzyme reacted only slightly with 3-pyridinealdehyde hypoxanthine dinucleotide in contrast to a low but measurable rate in the case of the autotrophic *Euglena* enzyme. The other data also show large differences

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

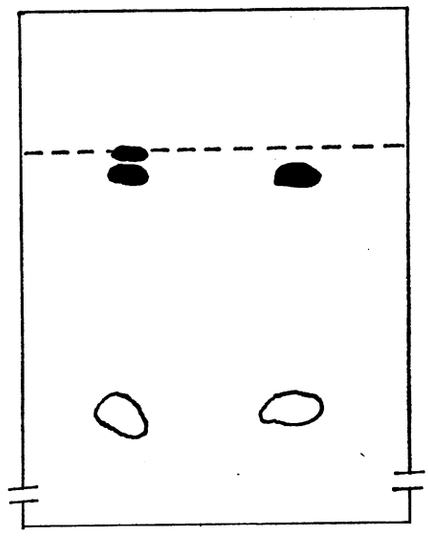

Fig. 1. Tracing of electrophoretic patterns of *Euglena* malate dehydrogenase on starch gel at *p*H 7.0. The cathode is at the top, the anode, at the bottom. The origin is indicated by the dotted line. The autotrophic extract is at the left, the heterotrophic, at the right. Solid bands, intense staining;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

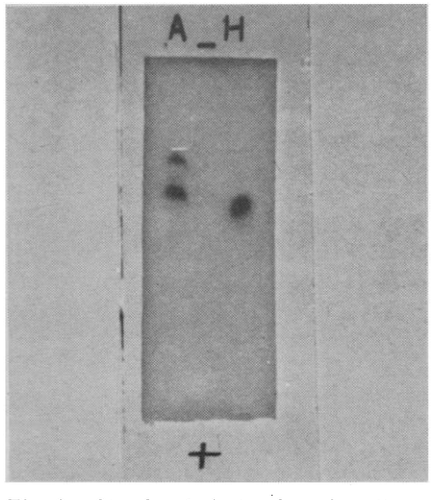

Fig. 2. Starch-gel electrophoresis patterns of *Euglena* malate dehydrogenase at *p*H 7.2.

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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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