positus is heterozygous for the abnormal hemoglobin gene and an alpha thalassemia gene.

Numerous siderocytes were observed on films of the peripheral blood, and vacuoles and inclusions similar to those described by Holroyde and Gardner (*1*) were seen with transmission electron microscopy (Fig. 1A). These vacuoles ("autophagic vacuoles") (*4*) contained ferritin, hemoglobin, or remnants of mitochondria and membranes. The vacuoles were seen not only deep within the red blood cell and near the surface, but also in communication with the cell surface. Continuity of the erythrocyte surface plasma membrane with the interior surface of the membrane of the inclusion-bearing vacuoles could be demonstrated (Fig. 1B). Scanning electron microscopy of the red cells revealed pits and craters (Fig. 2) comparable to those described by Holroyde and Gardner (*1*) and Preston and Shahani (*5*).

We believe that, as seen in transmission electron micrographs, vacuoles which are attached to the surface membrane are responsible for the pits seen in our scanning electron micrographs. The diameters of the openings of the pits seen in scanning electron micrographs and the diameters of vacuoles at the surface of the red cell in the transmission electron micrographs measured between 150 and 200 nm.

It is well recognized that the spleen is capable of "pitting" or removing various types of inclusions from red cells without destroying the entire cell (*6*). In patients without spleens (*1*) or in neonates (*7*) in whom splenic hypofunction may exist (*8*), vacuoles and inclusions in red cells are seen much more frequently than they are in adults with intact spleens. It is probable that normally the spleen also removes these vacuoles. In the absence of a functioning spleen, it is likely that these vacuoles fuse with the red cell membrane, open to the exterior, and expel their contents. This appears to be a mechanism whereby the red blood cell rids itself of solid material.

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Euglena gracilis: Formation of Giant Mitochondria

Abstract. *The addition of antimycin A (0.5 microgram per milliliter) to cultures of a bleached strain of* Euglena gracilis *in the logarithmic phase of growth on succinate as a carbon source results in (i) an interruption of growth for 24 hours and (ii) an increase in whole-cell respiration and the emergence of a novel succinoxidase activity within 2 to 4 hours. After 3 to 5 hours, the mitochondria enlarge, fuse, and form a sheathlike structure situated close to the periphery of the cell.*

The concept of plasticity of mitochondrial structure has received support from experiments in which mitochondrial profiles undergo extensive conformational changes in situ (*1*). In addition, the experiments of Luck have demonstrated that in *Neurospora crassa*, mitochondria can divide and subsequently grow by accretion of new material onto preexisting structures (*2*). These types of experiments provided the basis for the generally accepted notion that mitochondria exist within the cell as dynamic rather than static structures which are capable of continued fragmentation and fusion.

Recently, we described experiments with a bleached strain of *Euglena gracilis* demonstrating the effects of a variety of growth conditions on some biochemical properties of *Euglena* mitochondria (*3, 4*). In particular, addition of antimycin A, an inhibitor of mitochondrial electron transport, to the growth medium caused adaptive changes in the respiratory metabolism of these cells. We now describe the effects of antimycin on the morphological properties of *Euglena* mitochondria.

A Z strain of *E. gracilis* (B_8) bleached by heat was grown on succinate medium as previously described (*3*). Antimycin A (Sigma) was added to cultures at the logarithmic phase of growth (approximately 10^6 cells per milliliter) to a final concentration of 0.5 μg/ml. Samples of the culture were removed immediately before and at various times after the addition of antimycin for measurements of whole-cell respiration and succinoxidase activity in isolated mitochondria stimulated with adenosine 5′-monophosphate (AMP), and for electron microscopic analysis.

For measurements of whole-cell respiration, cells were harvested by centrifugation at 1200*g* for 5 minutes, washed once in buffer containing in final concentration 0.025*M* tris sulfate, *p*H 7.4; 0.15*M* NaCl; and 50 m*M* ethanol. Cells were then resuspended in the same medium at 10^7 to 5×10^7 cells per milliliter. Rates of oxygen consumption were determined at 28°C with a Clark-type oxygen electrode. Assays were carried out in buffer with 250-μl portions of a cell suspension in a final reaction volume of 1.4 ml. Protein was determined by the method of Stickland (*5*). Disruption of cells, isolation of mitochondria, and assay of the AMP-stimulated "alternate" succinoxidase activity which is insensitive to antimycin and cyanide was performed as previously described (*3, 4*).

The addition of antimycin (0.5 μg/ml) to logarithmically growing cultures of *E. gracilis* results in a number of profound physiological and biochemical alterations (Fig. 1). After the addition of antimycin, growth ceases and does not resume until approximately 24 hours later. (Strain B_8 displays a generation time of about 12 hours in the absence of antimycin.) Once adapted to antimycin, the growth response of cells is no longer sensitive to further additions of the inhibitor.

Almost immediately after the addition of antimycin, whole-cell respiration increases and achieves rates more than double the values observed prior to the addition of the inhibitor. Coincident with the increase in respiration is the appearance of a succinoxidase activity which displays novel properties and which can clearly be distinguished from

the classical mitochondrial succinoxidase activity (*4*). Briefly, the activity is insensitive to antimycin and cyanide and requires 1 to 2 m*M* 5′-AMP for activity; it also appears to involve the oxidation of succinate via a direct reduction of O_2 to H_2O at the succinic dehydrogenase flavoprotein or cytochrome b of the mitochondrial electron transport chain. What is clear from the data presented here is that the appearance of this activity, as well as the increase in whole-cell respiration, reaches a maximum well before the resumption of growth. A discussion regarding the possible physiological role of this terminal oxidase and its relationship to cellular respiration and energy conservation in *Euglena* has been given (*3*, *4*).

We have compared mitochondrial morphology in cells obtained from cultures of *Euglena* before and at various times after the addition of antimycin (Fig. 2). Before the addition of antimycin we observe numerous darkly staining ovoid mitochondria having diameters of between 0.4 and 1.5 μm and displaying typical cristae (Fig. 2A). From 3 to 5 hours after addition of antimycin, the mitochondria appear to enlarge and elongate; profiles about 2 μm long can be observed (Fig. 2B). After 5 hours, more profound changes in morphology become evident. The mitochondria appear to fuse with each other forming extensive anastomosing structures with fewer cristae than appear in mitochondria of cells grown in the absence of antimycin (Fig. 2C). A longitudinal section shown in Fig. 2D reveals two long mitochondrial profiles approximately 20 by 0.4 μm, extending the length of the cell. Such profiles typically appear near the cell periphery and give the appearance of long, filamentous structures. A more precise representation of the morphology of these mitochondria can be deduced from an examination of transverse sections (Fig. 2, E and F). Again, the mitochondrion appears as a long, filamentous structure near the periphery of the cell, with some branch points evident (Fig. 2E). However, the presence of essentially identical structures in transverse and longitudinal sections suggests a mitochondrial sheath rather than a filament which traverses much of the length of the cell.

Evidence has been obtained for the existence of a labile filamentous mitochondrial reticulum in *E. gracilis* grown on either ethanol or acetate as the sole carbon source. Furthermore, these mitochondrial filaments appear to be in a dynamic state in which there is considerable fusion and fragmentation. Certain conditions such as prolonged starvation appear to reduce the extent of this dynamic activity, and the mitochondria, although still filamentous, remain as essentially static structures within the cell (*6*). As shown here, *Euglena* mitochondria appear typical in cells grown on succinate as a carbon source in that small, ovoid profiles are observed. After the addition of antimycin to the culture, the appearance of the alternate succinoxidase and the augmentation of respiratory capacity are essentially complete before the ex-

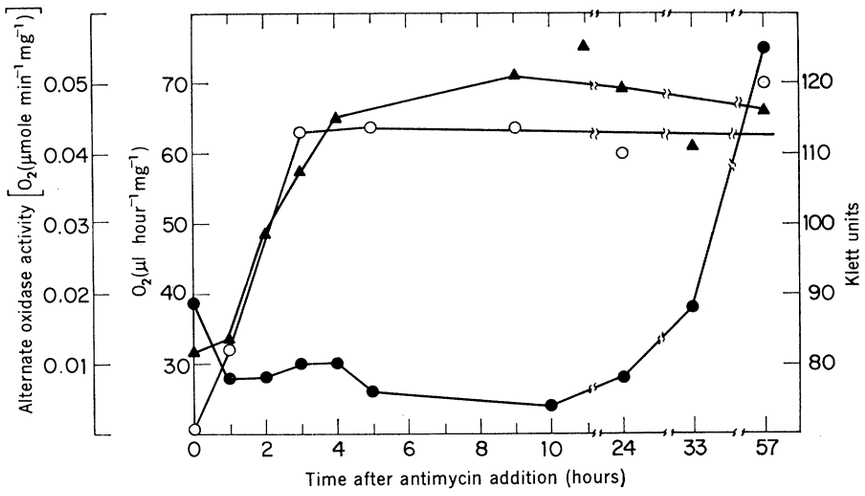

Fig. 1. Effect of antimycin on growth (●—●), whole-cell respiration (▲—▲), and alternate succinoxidase activity (○—○) of mitochondria isolated from *Euglena gracilis* B_S. Antimycin was dissolved in isopropanol and added at zero time to a final concentration of 0.5 μg/ml.

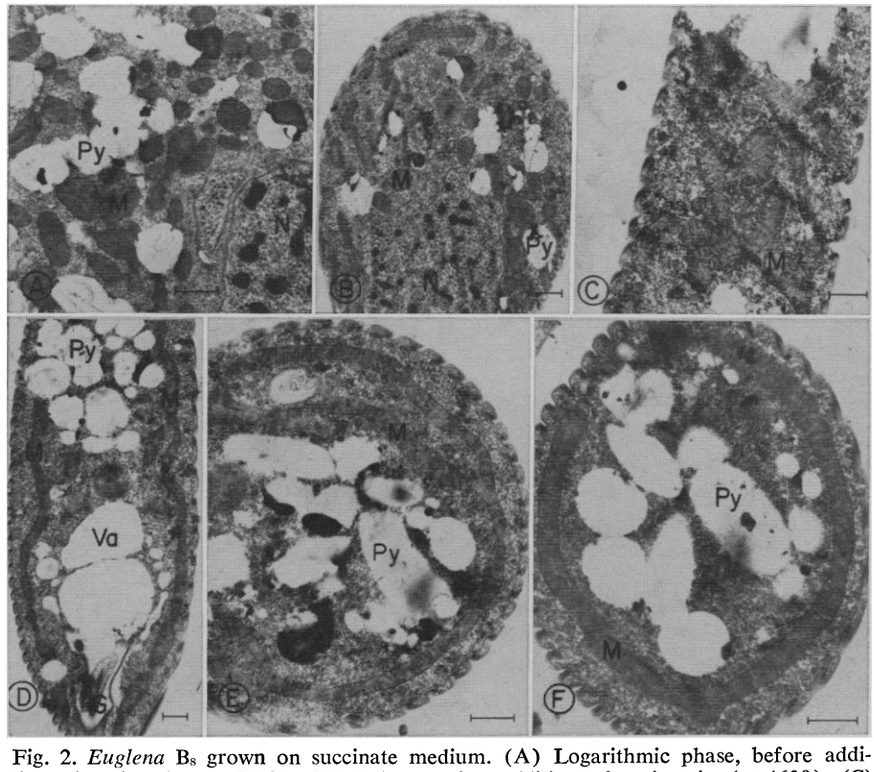

Fig. 2. *Euglena* B_S grown on succinate medium. (A) Logarithmic phase, before addition of antimycin (× 5670); (B) 3 hours after addition of antimycin (× 4620); (C) 25.5 hours after addition of antimycin (× 5040); (D) same as (C) (× 3530); (E) same as (C) (× 6300); (F) 34.5 hours after addition of antimycin (× 6800). *N*, nucleus; *M*, mitochondria; *Py*, paramylon; *Va*, vacuole; *G*, gullet. Cells were first fixed in 2 percent glutaraldehyde and then in 2 percent osmium tetroxide. Sections were stained with uranyl acetate and lead citrate. The bars indicate 1 μm.

tensive enlargement of the mitochondria occurs. The precise relationship between these activities and the formation of giant mitochondria is not clear.

Enlarged mitochondrial profiles, often with fused membranes and disoriented cristae, have been observed in mouse liver cells after the animal had been deprived of metals such as iron, copper, and manganese (*7*). The administration of cuprizone, a copper chelating agent, to mice also causes the formation of giant mitochondria, although their profiles do not appear as anastomosing structures (*8*). Similarly, riboflavin deficiency reportedly causes the production of abnormal mitochondria (*9*). Taken together, these results suggest that alterations of mitochondrial morphology may result from alterations in mitochondrial function.

Elson *et al.* (*10*) made the interesting observation that addition of antimycin to cultures of *Tetrahymena pyriformis* causes an increase in the rate of cell division and protein synthesis along with the formation of concentric lamellae of the endoplasmic reticulum; however, no effect on mitochondrial morphology was observed.

Since mitochondria in *Euglena* have been observed to continually fuse and fragment, the simplest interpretation of our findings is that antimycin directly or indirectly inhibits the process of division; alternatively, the rate of fusion of the organelles may be greatly enhanced. In this connection, some evidence for the alternating cycle of large and small mitochondria in relation to the cell cycle in *Euglena* has recently been obtained (*11*).

Our observations raise questions about the mechanisms, biochemical and mechanochemical, by which membrane structures fragment and fuse. In view of the semiautonomous existence of mitochondria within cells, the possible existence of precise signals which may initiate mitochondrial division, or perhaps fusion, is particularly interesting. Conditions which offer control over any of these processes as they may occur in the cell would be of obvious value in studies of the biochemical mechanisms which underlie the dynamic state of intracellular membranes.

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Connections of the Nurse Shark's Telencephalon

Abstract. *The extrinsic connections of the telencephalon of the nurse shark are very largely crossed and in this respect differ from those identified in all other vertebrate species so far examined. Studies with Nauta and Fink-Heimer methods have revealed telencephalic projections to the contralateral thalamus and optic tectum as well as to ipsilateral brainstem and rostral spinal cord.*

The Nauta method for tracing neuronal pathways has given more impetus for neuroanatomical studies than any other technique of this century. Only recently, however, has the method been applied to elasmobranch material (*1, 2*). The results of these initial studies have suggested that earlier workers greatly misunderstood the organization of connections in the shark brain, probably resulting from the use of inadequate techniques. The general notion that the selachian telencephalon is almost entirely an olfactory structure has remained unchallenged since the turn of this century (*3*); but recent experimental anatomical studies in the nurse shark revealed that the distribution of the olfactory tract is limited to a relatively modest volume of telencephalon (*2*).

This project is addressed to the question of what the nature could be of the vast territories of the shark's telencephalon which appear not to be primary recipients of olfactory input. The findings reported here do not permit a definite answer to this question, but their relevance to the problem of selachian forebrain organization appeared clear enough to warrant their publication. Our present findings indicate that the nurse shark telencephalon is more comparable to other vertebrate brains

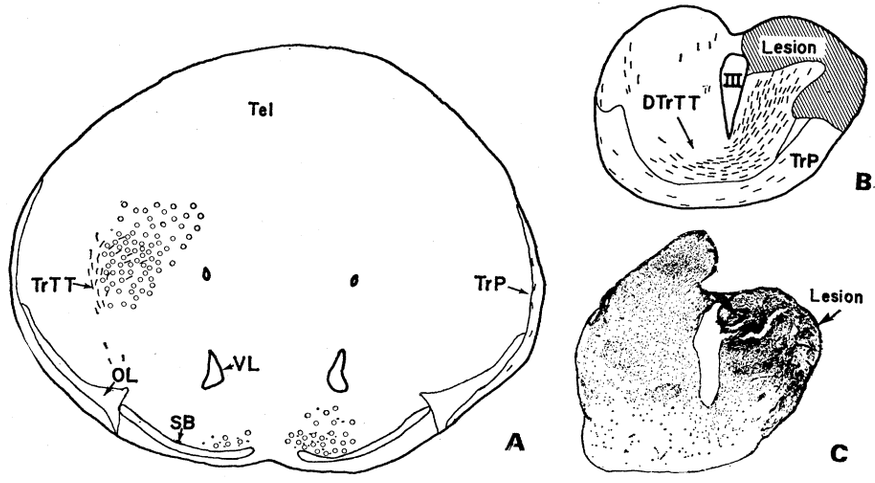

Fig. 1. Transverse sections through the caudal telencephalon (A), rostral diencephalon (B), and caudal diencephalon (C) of a nurse shark brain with a large diencephalic lesion; (C) is a photograph of a Nissl preparation. The interrupted lines in (A) and (B) indicate degenerating fibers; and the open circles in (A), loci of apparent termination of such fibers. Abbreviations: *DTrTT*, decussation of the thalamo-telencephalic tract; *OL*, lateral olfactory area; *SB*, superficial basal area of Johnston; *Tel*, telencephalon; *TrP*, tractus pallii of Edinger; *TrTT*, thalamo-telencephalic tract; *VL*, lateral ventricle; and *III*, third ventricle.