sine triphosphatase have been suggested, we have no new information on the question "which enzyme?" Our experiments do demonstrate that there is a prominent and reversible accentuation of the incidence and severity of audiogenic seizure coincident with transitory inhibition of protein synthesis by acetoxycycloheximide.

H. D. JAMESON
P. FALACE
A. PREROST
G. CLEMONS

Departments of Neurology and Physiology and Biophysics, University of Kentucky, Lexington 40506

References and Notes

1. M. Hamburgh and E. Vicari, *J. Neuropathol. Exp. Neurol.* **19**, 461 (1960).
2. A. W. Castellion, E. A. Swinyard, L. S. Goodman, *Exp. Neurol.* **13**, 206 (1965); J. L. Fuller and F. H. Sjursen, *J. Hered.* **58**, 135 (1967).
3. T. Kobayashi, O. Inman, W. Buño, H. E. Himwich, *Recent Advan. Biol. Psychiat.* **5**, 293 (1962).
4. D. L. Coleman, *Arch. Biochem. Biophys.* **91**, 300 (1960).
5. ——— and K. Schlesinger, *Proc. Soc. Exp. Biol. Med.* **119**, 264 (1965).
6. J. W. MacInnes, W. O. Boggan, K. Schlesinger, *Behav. Genet.* **1**, 35 (1970).
7. K. Schlesinger, W. Boggan, D. X. Freedman, *Life Sci.* **7**, 437 (1968); K. Schlesinger, R. A. Schreiber, B. J. Griek, K. R. Henry, *J. Comp. Physiol. Psychol.* **67**, 149 (1969); A. Lehmann, *Life Sci.* **6**, 1423 (1967).
8. L. G. Abood and R. W. Gerard, in *Biochemistry of the Developing Nervous System*, H. Waelsch, Ed. (Academic Press, New York, 1955), p. 467.
9. L. B. Flexner and J. B. Flexner, *Proc. Nat. Acad. Sci. U.S.* **55**, 369 (1966).
10. DBA/2J animals were obtained from the Jackson Laboratory, Bar Harbor, Maine.
11. The acetoxycycloheximide (NSC 32743) was supplied through the John L. Smith Memorial for Cancer Research, Chas. Pfizer and Company, Incorporated, Maywood, New Jersey. Supported by NIH contract PH-43-64-50. The drug with advice on its use was given to us by D. E. Knapp.
12. K. R. Henry and R. E. Bowman, *Proc. Soc. Exp. Biol. Med.* **128**, 635 (1968).
13. S. D. Huff and J. L. Fuller, *Science* **144**, 304 (1964).
14. L. B. Flexner, J. B. Flexner, R. B. Roberts, *Proc. Nat. Acad. Sci. U.S.* **56**, 730 (1966).
15. B. W. Agranoff, R. E. Davis, J. J. Brink, *Brain Res.* **1**, 303 (1966).
16. D. E. Knapp and S. Mejia, *Anesth. Analg. (Cleveland)* **48**, 189 (1969).
17. M. R. Siegel and H. D. Sisler, *Nature* **200**, 675 (1963); A. C. Trakatellis, M. Montjar, A. E. Axelrod, *Biochemistry* **4**, 2065 (1965).
18. Supported in part by General Research Support Branch Division of Research Facilities and Resources, NIH.

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Erythrocytes: Pits and Vacuoles as Seen with Transmission and Scanning Electron Microscopy

Abstract. *Vacuoles containing inclusions were observed by transmission electron microscopy in erythrocytes of a splenectomized patient with hemoglobin Ann Arbor. The membranes of these vacuoles became fused with the surface membrane of the red cell, thus opening the vacuoles and exposing their contents to the outside. These vacuoles when they have become thus attached to the cell membrane of the erythrocyte are responsible for the pits observed with scanning electron microscopy.*

Holroyde and Gardner (*1*) have reported that crater-like indentations of the erythrocyte surface membrane when viewed by means of interference-contrast microscopy are largely optical illusions. These authors present evidence that many of these indentations actually reflect the presence of vacuoles of low optical density lying beneath the plasma membrane. Scanning electron microscopy, however, showed a number of membrane indentations to be true pits or craters. We now present evidence that the pits and craters in red blood cells observed by scanning electron microscopy represent vacuoles, or vacuoles containing inclusions, which have reached the surface plasma membrane of the red cell and fused with it, as seen by transmission electron microscopy.

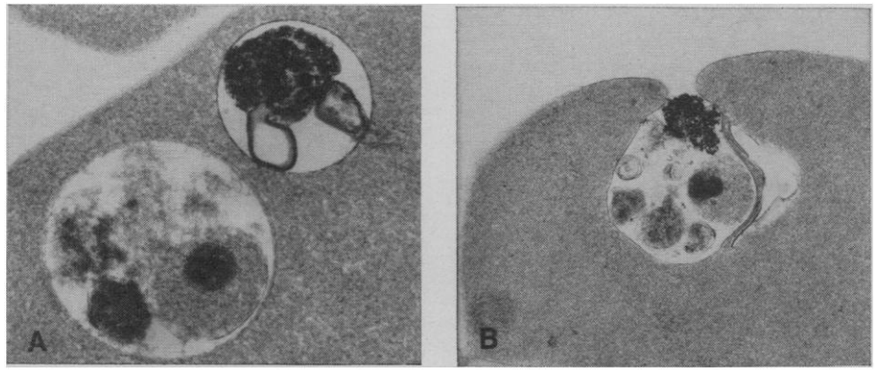

Fig. 1. A transmission electron micrograph of two inclusion-bearing vacuoles within red blood cell. Ferritin, hemoglobin, membranes, and remnants of mitochondria are present in the vacuoles (× 17,820). (B) Opening of the vacuole at the surface of the red cell (× 25,000).

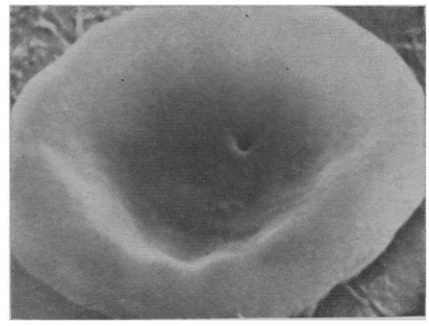

Fig. 2. A scanning electron micrograph of a red blood cell with a pit on the surface (× 9000).

We obtained red blood cells from a splenectomized patient. Blood for transmission electron microscopy was fixed at room temperature for 1 hour in 1.5 percent glutaraldehyde containing 2.5 percent sucrose and buffered with 0.05*M* sodium cacodylate (*p*H 7.4), treated with osmium tetroxide, dehydrated through a series of graded alcohols and propylene oxide, and embedded in Epon 812. Sections were cut with a Du Pont diamond knife, stained with uranyl acetate and lead citrate (*2*), and examined in a Siemens Elmiskop 101 or an RCA EMU-3H electron microscope. For scanning electron microscopy, the cells were washed three times in physiologic saline and then fixed for 1 hour in 1.5 percent glutaraldehyde containing 2.5 percent sucrose and buffered to a *p*H of 7.4 with 0.05*M* sodium cacodylate (300 milliosmoles). The red blood cells were washed three times in distilled water. A drop of the cell suspension was placed on an aluminum stub, freeze-dried, coated with palladium-gold, and examined in a scanning electron microscope (Materials Analysis Company, model 700).

The patient is the propositus of a family with hemoglobin Ann Arbor in which the 80th residue of the α-polypeptide chain, normally leucine, has been substituted by arginine (*3*). This abnormal hemoglobin is one of a number of heat-unstable inherited variants which cause hemolytic anemia. The pro-

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.

BERTRAM SCHNITZER
DOANLD L. RUCKNAGEL
HERBERT H. SPENCER
Departments of Pathology, Human Genetics, and *Internal Medicine, University of Michigan, Ann Arbor 48104,* and *Veterans Administration Hospital, Ann Arbor, Michigan 48105*
MASAMICHI AIKAWA
Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

References

1. C. P. Holroyde and F. H. Gardner, *Blood* **36**, 566 (1970).
2. E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
3. D. L. Rucknagel, N. J. Brandt, H. H. Spencer, in *Genetical, Functional and Physical Studies of Hemoglobin*, T. Arends, Ed. (Karger, Basel, in press).
4. G. Kent, O. T. Minick, F. I. Volini, E. Orfei, *Am. J. Pathol.* **48**, 831 (1966).
5. F. E. Preston and R. T. Shahani, *Lancet* **1970-I**, 1177 (1970).
6. W. H. Crosby, *Blood* **12**, 165 (1957); R. A. Rifkind, *ibid.* **26**, 435 (1965); N. S. Lawson, B. Schnitzer, E. B. Smith, *Arch. Pathol.* **87**, 491 (1969).
7. C. P. Holroyde, F. A. Oski, F. H. Gardner, *N. Engl. J. Med.* **281**, 516 (1969).
8. G. Acevedo and A. M. Mauer, *J. Pediat.* **63**, 61 (1963).

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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