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Anderson W A & Ellis R A. Ultrastructure of *Trypanosoma lewisi*: flagellum, microtubules, and the kinetoplast. *J. Protozoology* 12:483-99, 1965.
[Department of Biology, Brown University, Providence, RI]

This paper reexamined the ultrastructure of the trypanosome with particular reference to the flagellum, centrioles, and other organelles Microtubules located adjacent to the cell membrane were considered important in the maintenance of the cytoskeleton and movement of the cell, while the kinetoplast with its high DNA content was considered important in the ontogeny of mitochondria [The *SCI*® indicates that this paper has been cited in over 225 publications since 1965]

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I am indeed pleased that after 20 years our paper has become a *Citation Classic*. In 1965, I was a graduate student in the Division of Biological Sciences at Brown University, having recently received a master's degree in zoology from Howard University, Washington. A combination of events led to our interest in the structure and functions of trypanosomes. First, my mentor at Howard, Charles Brown, and his associate, Martin Clarke, were interested in sub-pellicular filaments and mitochondria of peritrich protozoa. Second, Harold Finley's research on the ultrastructure of *Vorticella* and other peritrichs gave incentive to young graduate students to pursue research in the area. Third, David Lincicome conducted a high-quality parasitology program and made available several strains of rodent and human trypanosomes for biochemical and ultrastructural studies. In this very active research environment, we were literally "probing in the dark" at the variability and functions of subcellular structures that were being "rediscovered." For instance, as early as 1960, we completed a study of a peculiar mitochondrion that contained about 10 percent of the total cellular DNA, yet we persisted in calling this mitochondrion the kinetoplast. Similarly, an organized array of "hollow filaments" immediately beneath the cell membrane of trypanosomes resembled filaments in the "pellicle" of *Spirostomum, Stentor,* and other free-living protozoa, so we persisted in calling them "subpellicular filaments or fibers."

Electron microscopy changed rapidly in the early 1960s, a period when descriptive morphology was being replaced by functional morphology—the new cell biology. Glutaraldehyde treatment followed by osmium tetroxide fixation yielded better preservation of subcellular organelles. Epoxy resins replaced the methacrylate polymers for embedding specimens, and diamond knives made better sections than glass knives. There was a virtual explosion of new and more accurate descriptions of cellular components, and ultrastructure took on a new and exciting significance. Ellis and I took advantage of these new technologies and decided to re-examine the ultrastructure of *Trypanosoma lewisi*, which was generously provided by my friend Roy Watkins, Lincicome's graduate student at that time.

We examined the trypanosome not just as a parasite, but as a dynamic, protein-synthesizing and secretory machine. We described the Golgi apparatus and its possible involvement in the packaging of lysosomes and secretory granules. We recognized a variation in cell coat, a characteristic that today is being emphasized as important in the organism's ability to evade the host's immune system. Subpellicular filaments seen in methacrylate-embedded tissues were now called microtubules, and these structures had a special relationship to each other and to the plasmalemma. Most certainly, we speculated, these microtubules were associated with the "maintenance of the cytoskeleton" and change of shape and motility of the organism; and ATP was in some way involved. These subpellicular microtubules were similar to microtubules in cilia and flagella, and interconnections between them certainly have ATPase activity.

In the early 1960s, there was much controversy as to whether the kinetoplast of free-living and parasitic protozoa was an endosymbiont or a *bona fide* organelle. In the late 1950s we recognized the kinetoplast of *T lewisi* to be a DNA-containing mitochondrion But after our re-examination in the 1960s, we were convinced that "large mitochondrial extensions from the kinetoplast" suggested that this organelle was concerned with the "ontogenesis" of mitochondria. We, therefore, joined others interested in the mitochondrial genome and ontogenesis. This initial study led George Hill and me to conduct further studies on the effect of acriflavine on kinetoplast structure and function and on DNA synthesis and division of the kinetoplast.[1,2] Indeed, the trypanosome provided us with the opportunity to study a vast array of significant organelles and test a variety of hypotheses that were relevant to the field of cell biology in the early 1960s and are still relevant today.

1 **Hill G C & Anderson W A.** Effect of acriflavine on the mitochondria and kinetoplast of *Crithidia fasciculata* Correlation of fine structure changes with decreased mitochondrial enzyme activity *J Cell Biol* 41 547-61, 1969 (Cited 45 times)

2 **Anderson W & Hill G C.** Division and DNA synthesis in the kinetoplast of *Crithidia fasciculata* *J Cell Sci* 4 611-20, 1969 (Cited 25 times)