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Schnaitman C & Greenawalt J W. Enzymatic properties of the inner and outer membranes of rat liver mitochondria. *J. Cell Biol.* 38:158-75, 1968.
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The distribution of 16 different mitochondrial enzymes was examined after digitonin fractionation of the mitochondria. The release of adenylate kinase at very low digitonin concentration indicated that this enzyme is found in the compartment between the inner and outer membranes. [The *SCI*® indicates that this paper has been cited in over 1,000 publications.]

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When my postdoctoral mentor, Jack Greenawalt, and I began approaching the problem of separating the inner and outer membranes of rat liver mitochondria, it quickly became apparent that we needed a specific enzymatic marker for the outer membrane. The surface of the inner membrane is much greater than that of the outer membrane. Thus, the absence of respiratory enzymes thought to be in the inner membrane could not be used to identify the outer membrane. In addition, mitochondria are always contaminated by microsomal membranes, which are derived from other subcellular organelles, and there was no satisfactory way to correct for this contamination in fractions enriched for outer membrane.

The solution to this problem was provided by Gene Erwin, who suggested that monoamine oxidase might be a marker for the outer mitochondrial membrane. It was known that this enzyme was present only in mitochondria and not other subcellular organelles and that the rate of oxidation of substrates was the same whether the mitochondria were assayed intact or after disruption.

Experiments with osmotically disrupted mitochondria showed that Erwin's suggestion was correct. The idea of using a digitonin titration to provide a much cleaner separation of the inner and outer membranes came from a study by Lévy and colleagues[1] that showed this detergent removed the outer membrane. The first paper describing our studies from a morphological point of view came out in 1967.[2] Our second, highly cited paper described improvements in the digitonin fractionation technique and also provided the first comprehensive study of enzyme distribution in mitochondria. We identified the localization of 16 different enzymes in submitochondrial fractions. This was not an easy task, since Jack and I wanted to do assays on all of these enzymes from a single batch of fractionated mitochondria. Working around the clock, it required almost 24 hours to complete the assays.

This paper is often cited because of its comprehensive nature, or because it described a fractionation technique that was widely used.[3] However, I think the most important observations in the paper are that adenylate kinase and nucleoside diphosphokinase are located in the space between the inner and outer membranes and that these enzymes can be removed by digitonin without damaging the inner membrane or its capability to carry out oxidative phosphorylation. This was important, since it allowed the direct study of the specificity of the adenine nucleotide transporter in the inner membrane. This demonstrated that the specificity of oxidative phosphorylation lay in the adenine nucleotide transporter and not in the ATPase, and thus provided the final proof that ADP was the primary phosphate acceptor in oxidative phosphorylation.

I am sorry that I can't share this honor with my coauthor, Jack Greenawalt, who died unexpectedly about a decade ago from a heart attack suffered during a tennis match.

1. **Lévy M, Toury R & André J.** Séparation des deux membranes mitochondriales. *CR Acad. Sci. D* 262:1593-6, 1966.
2. **Schnaitman C, Erwin V G & Greenawalt J.** The submitochondrial localization of monoamine oxidase, an enzymatic marker for the outer membrane of rat liver mitochondria. *J. Cell Biol.* 32:719-35, 1967. (Cited 725 times.)
3. **Tzagoloff A.** *Mitochondria.* New York: Plenum Press, 1982. p. 25-9.

1-19