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This Week's Citation Classic®

Schneider W C & Hogeboom G H. Intracellular distribution of enzymes. V. Further studies of the distribution of cytochrome *c* in rat liver homogenates. *J. Biol. Chem.* 183:123-8, 1950.
[National Cancer Institute, National Institutes of Health, Bethesda, MD]

This paper showed that a small soluble respiratory protein, cytochrome *c*, was concentrated in the mitochondrial fraction isolated from rat liver homogenates and was active in the oxidation of succinate by the succinic dehydrogenase that was also localized in the liver mitochondria. [The *SCI*® indicates that this paper has been cited in more than 1,810 publications.]

Intracellular Cytochrome *c*

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The end of World War II witnessed the onset of rapid developments in many fields of basic research. One was the isolation and biochemical study of subcellular components from animal tissues. R.R. Bensley and N. Hoerr[1] and A. Claude[2] reported the isolation of particulate large granules from guinea pig liver but disagreed as to whether they were mitochondria or secretory granules. Van R. Potter was instrumental in interesting me in this field, and, after completing my doctorate in 1945, I isolated the large granule fractions from both rat liver and kidney and showed that they contained almost 75 percent of the succinic dehydrogenase and cytochrome oxidase activities of the tissues.[3]

Impressed by these results, Potter arranged for me to spend a year with Claude as a postdoctoral fellow. On arriving in New York in 1946, it became apparent that Claude's other interests and his preference for working in the late afternoon and evening would leave me with free time. As a result, George H. Hogeboom, a young staff member, and I, together with George H. Palade, a young Rumanian histologist who had fled the Nazis and was also visiting Claude, began talking of another project. Since previous work[2,3] had showed that the large granules present in liver suspensions were strongly aggregated in isotonic saline and enormously swollen in distilled water, we decided to look for a medium that would prevent aggregation and yet maintain the morphological and cytological integrity of the mitochondria.

It was Palade's idea to try nonelectrolytes for this purpose, and we found sucrose to produce the desired results.[4] Homogenates of liver prepared in aqueous hypertonic sucrose solution showed the presence of many unaggregated rod-shaped granules that were also observed within the few unbroken cells present in the homogenates. Through testing, the granules were identified as mitochondria. Isolation of morphologically intact mitochondria was obtained by differential centrifugation of the liver homogenates.

These findings attracted considerable attention, and Hogeboom and I were invited to join the staff of the National Cancer Institute in 1948 to develop this work further. This *Classic* paper was one of the first results of that move. It was an extension of the work we had done in New York[5] showing that cytochrome *c* was associated with the large granules isolated from rat liver extracts made in either isotonic saline or distilled water. Present work uses either 0.88M or 0.25M sucrose.

In both media, approximately 50 percent of the cytochrome *c* and 60 percent of the succinoxidase activity was recovered in the mitochondrial fraction. Furthermore, the mitochondrial cytochrome *c* was highly active in the oxidation of succinate since the succinoxidase activity of the mitochondria was only doubled by excess added cytochrome *c*, whereas in the earlier work with water homogenates,[5] the succinoxidase activity of the large granules was increased 20-fold.

These findings were important in establishing that mitochondria were sites of respiratory activity and led to an explosion of experiments that defined the role of mitochondria as power plants of the cell as well as the seat of many other functions.[6] In addition, this paper served as a model for the complete fractionation of disrupted cells and led to the development of methods for the isolation of other subcellular structures. I can only speculate that this paper was probably cited more for its description of the fractionation of rat liver rather than for the findings on cytochrome *c*. I am happy to have had a part in developing this field and to have experienced the excitement that pervaded biochemistry then and continues even today.

1 **Bensley R R & Hoerr N.** The preparation and properties of mitochondria *Anat Rec* 60 449-55, 1934 (Cited 120 times since 1945)
2 **Claude A.** Particulate components of cytoplasma *Cold Spring Harbor Symp* 9 263-70, 1941 (Cited 130 times since 1945)
3 **Schneider W C.** Intracellular distribution of enzymes I The distribution of succinic dehydrogenase, cytochrome oxidase, adenosine triphosphatase, and phosphorus compounds in normal rat tissues *J Biol Chem* 165 585-93, 1946 (Cited 215 times)
4 **Hogeboom G H, Schneider W C & Palade G H.** Cytochemical studies of mammalian tissues 1 Isolation of intact mitochondria from rat liver, some biochemical properties of mitochondria and submicroscopic particulate material *J Biol Chem* 172 619-35, 1948 (Cited 1,150 times)
5 **Schneider W C, Claude A & Hogeboom G H.** The distribution of cytochrome *c* and succinoxidase activity in rat liver fractions *J Biol Chem.* 172 451-8, 1948 (Cited 150 times)
6 **Schneider W C & Kuff E L.** Centrifugal isolation of subcellular components (Bourne G, ed.) *Cytology and cell physiology* New York Academic Press, 1964 p 19-98

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