Intracellular Localization of Succinoxidase and Cytochrome Oxidase of the Kidney Cortex.* (20079)

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New concepts of intracellular localization have resulted from the chemical analysis of particles isolated by differential centrifugation of tissue homogenates. Thus, "large granules" from kidney isolated in alkaline water contain 66% of the total succinoxidase and cytochrome oxidase activity(1). Technics utilizing 0.88 M sucrose solution as the dispersion medium have since then been introduced(2) in order to preserve the morphologic integrity of mitochondria. The present study utilizes these newer methods in a complete fractionation of the renal cortex and the determination of these oxidative enzymes.

Experimental. Sherman strain rats weighing 175-225 g maintained on Purina chow, were used as a source of the material for the determination of the normal renal cortex partition. In the fasting experiments the weights ranged from 125-175 g. Animals were killed after the intraperitoneal injection of veterinary nembutal (0.1 ml/200 g) and the kidneys were rapidly removed and stripped of the perirenal fat, fascia, and capsule. The kidneys were blotted on filter paper to remove the extraneous blood, chilled, and the cortices separated from the medulla. The weight of this tissue was then obtained. The tissue was placed in an evaporating dish which was kept in an ice-bath. A quarter of each kidney was put in Zenkers solution for histologic examination. The cortical tissue was then cut into small pieces and transferred to a Potter-Elvehjem homogenizing tube. Homogenization of the tissue was done with a steel or glass grinder for 5 minutes, and 0.88 M sucrose solution added to the preparation so as to obtain a final volume 10× the wet weight of the tissue, so that the final concentration was 100 mg tissue per 1 ml of sucrose solution. All these procedures were performed in a cold room at temperature 1°-4°C. The total homogenate (H) was spun according to the procedure of Schneider, Hogeboom, and Palade(2). The first sediment (Pel 1) was removed at 600 × g for 2 minutes on an International Clinical Centrifuge and consisted of nuclei, unbroken cells, red blood cells and mitochondria (or "large granules"); the supernatant (Sup 1) consisted of mitochondria and microsomes but no nuclei, red cells or unbroken cells. This supernatant (Sup 1) was spun at 20,000 × g for 20 minutes and a tan-brown pellet (B-Pel) was obtained which contained mitochondria and some microsomes. This pellet was then washed a number of times with 0.88 M sucrose. The supernatant from which the pellet was obtained (Sup 2) was then centrifuged at 20,000 × g for 120 minutes at which time a red, translucent pellet was obtained which consisted of microsomes (R-Pel). This pellet was also washed repeatedly. The final supernatant (F-Sup) was a translucent reddish yellow fluid. All of the stages in the fractionation were followed with observations by phase microscopy. All fractions were brought to the volume of the original homogenate from which they were derived prior to removing aliquots for biochemical and microscopic analysis. Succinoxidase and cytochrome oxidase activities were determined manometrically(3). "Endogenous" cytochrome C was determined in a system wherein tissue, buffer, electrolytes but no cytochrome C were added. Nitrogen was determined according to the Ma-Zuzaga modification of the micro-Kjeldahl method(4).

Results. The succinoxidase, cytochrome oxidase and the "endogenous" cytochrome C of the renal cortex have been determined. All the results are reported in terms of 1.0 ml of the original 10% homogenate. In accordance with previous reports(1,2) the greatest per cent enzyme activity is present in the cytoplasm (Sup-1) and, within the cytoplasm, in

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the mitochondrial fraction (B-Pel). On the basis of wet weight, we find that 43% of the total succinoxidase activity and 49% of cytochrome oxidase activity is localized in the mitochondrial or "large granule" fraction of the renal cortex. On the basis of nitrogen, succinoxidase has an activity of 220 μl O_2/10′/mg N in the original homogenate and an activity of 497 μl O_2/10′/mg N in the mitochondria. The activity of cytochrome oxidase in the original homogenate and the mitochondria is 590 μl O_2/10′/mg N and 1656 μl O_2/10′/mg N respectively.

Discrepancies in recovery values of our findings with the earlier figures(1,2) led to a further investigation of the effects of repeated centrifugal washings on the mitochondrial fraction and the microsomal fraction. It was found (Table I) that in contrast to the reported lack of effect of repeated washings of the mitochondria of liver, mitochondria of the renal cortex lose a great deal of succinoxidase and cytochrome oxidase activity. After 2 washings there is a 58% decrease in succinoxidase activity per wet weight and 45% decrease in cytochrome oxidase activity per wet weight, whereas on the basis of nitrogen there was a 40% decrease in succinoxidase activity and no decrease in the cytochrome oxidase activity of the washed kidney mitochondria. These data show that succinoxidase activity decreases as rapidly as the nitrogen decreases while cytochrome oxidase activity decreases at a much slower rate than nitrogen after 2 washings.

In order to account for the relative decrease in enzymatic activity of the mitochondrial fraction noted after repeated washing, other fractions were combined with the mitochondria and the succinoxidase activity was determined (Table II). Although additions of Pel-1 and R-Pel resulted in no increase in succinoxidase activity, small amounts of the final supernatant fluid (F-Sup) raised the total activity of the mitochondria to 85% of the activity of the total homogenate. The succinoxidase activity of the added final supernatant fluid was so small that it contributed no inherent activity to the end result.

50% of the total "endogenous" cytochrome C of the cytoplasmic extract (Sup-1) was spun down with the mitochondria; whereas 40% of the "endogenous" cytochrome C of the total homogenate remained in the "nuclear" fraction (Pel-1). Since no unbroken cells were found this could indicate adsorption of "endogenous" cytochrome C to nuclear material. Previous determinations(5,6) report cytochrome C primarily localized in "large granules."

TABLE I. Effect of Washing on Succinoxidase Activity of Mitochondria.

Fraction	μl O_2/10′/mg wet	μl O_2/10′/mg N
B-Pel	241 (162–291)	497 (304–615)
" , W_1*	165 (115–210)	419 (361–489)
" , W_2*	99 (66–120)	300 (251–330)
" , W_3*	87 (78–100)	310 (300–317)
" , W_4*	88 (68– 94)	367 (350–410)

* W, with subscript, refers to No. of washings.

TABLE II. Effect on Succinoxidase of Adding Small Amounts of "Nuclei," Microsomes, and Final Supernatant Fluid to the Mitochondrial Fraction.*

Fraction	μl O_2/10′ /mg wet	% of H
B-Pel	206	37
" + Pel-1	210	38
" + R-Pel	220	40
" + .04 ml F-Sup	320	57
" + .12 ml "	430	76
" + .24 ml "	480	85

* In all cases amounts added were so small as to contribute negligible inherent activity. Thus any increase in activity was due to some "activator" contained in the added fraction.

Differences in the effect of homogenization with the use of metal and ground glass homogenizers were noted. The nuclear fraction (Pel-1) accounted for 24% of the total succinoxidase activity or 134 μl O_2/10′/mg wet weight, when metal was used, and for 8% or 47 μl O_2/10′ mg, when ground glass was used. The difference can probably be accounted for by the greater number of unbroken cells present after metal grinding, a finding which, however could not be definitely confirmed by phase microscopy.

In accord with previous reports(7) there was little decrease in the activity of succin-

oxidase and cytochrome oxidase of the total homogenate or the fractions after 16-140 hours of starvation.

Discussion. These experiments indicate that much less of the total succinoxidase and cytochrome oxidase activities were recovered with the renal cortex mitochondria isolated in sucrose than in whole kidney mitochondria isolated in alkaline water(1). This may be due to differences between cortical and medullary mitochondria or to differences induced by the suspending medium. In addition, less succinoxidase activity can be demonstrated in the renal cortex mitochondria than in mitochondria from other tissues. Thus, although there is qualitatively a tissue and species biochemical similarity of mitochondria, quantitatively there may exist tissue and possibly cellular differences.

Summary. 1. The results with 0.88 M sucrose solution as the dispersion medium agree essentially with those obtained with alkaline water medium. 2. Succinoxidase activity of the mitochondria accounts for 43% of the total homogenate activity and cytochrome oxidase 47% on a wet weight basis. 3. On nitrogen basis the activities are 2-3 times greater than that of the homogenate. 4. After 2 washings the activity of both enzymes on a wet weight basis is reduced; on a nitrogen basis succinoxidase activity is reduced to 33% but the cytochrome oxidase activity is the same as in the original mitochondria. 5. Small amounts of the final supernatant fluid added to mitochondria increase the succinoxidase activity to 85% of the original homogenate. This effect does not represent an addition of the activities of the 2 fractions. 6. There is no change in intracellular succinoxidase and cytochrome oxidase activities after a period of starvation of 16 to 140 hours.

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Anti-Gonadal Hormone Activity of 11α-Hydroxyprogesterone. (20080)

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The antagonism which one gonadal hormone may show for another is frequently brought about by the hormones' decreasing the secretion of gonadotrophins by the pituitary, and, therefore, secondarily diminishing the production of the endogenous sex hormones. On the other hand, there is evidence that a gonadal hormone may inhibit the effects of another sex hormone directly. Early investigations by de Jongh(1-3) revealed that androgens are capable of preventing certain responses in estrogen-treated animals. These observations were confirmed and extended by Zuckerman and Parkes(4), Robson(5), Gardner and Pfeiffer(6) and many others. The anti-androgen effects of estrogens were reported about the same time by Gley and Delor(7) and Mühlbock(8). Early experiments by Macht and Stickels(9), Hisaw and Lendrum(10) and Dessau(11) brought out the inhibitory effect which progesterone may exert on the biological activity of estrogen. Conversely, estrogen has been shown to be antagonistic to the development of certain progestational effects. The inhibition of the placentoma reaction by estrogen has been demonstrated by Courrier(12), Brouha(13), Votquenne(14) and others. Although both estrogen and progesterone are