# PROTEIN SYNTHESIS IN TWO MITOCHONDRIAL POPULATIONS ISOLATED BY ISOPYCNIC DENSITY CENTRIFUGATION FROM NORMAL AND RENOPRIVAL KIDNEY.

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Summary:

During the 48 hours following mononephrectomy, there is a proliferation of mitochondria in the remaining kidney of the rat. Mitochondria from both normal and renoprival kidneys were separated by isopycnic density centrifugation into two distinct bands with mean densities of 1.178($M_1$) and 1.162 ($M_2$). Using a dual-label procedure which reduces technical errors, the rates of mitochondrial protein synthesis of the fractions were compared. At 24, 48 and 72 hours post-mononephrectomy incorporation in vivo of leucine into $M_1$ was increased 35%, 58% and 29% and into $M_2$ 50%, 85% and 14% over control. The results indicate a gradual shift in the rate of amino acid incorporation from $M_2$ to $M_1$ during mitochondrial formation.

## Introduction:

Evidence for a proliferation of mitochondria in the renoprival rat kidney 24 to 48 hrs following unilateral nephrectomy has been reported (1-3). With the renoprival kidney, there is a higher rate of amino acid incorporation into mitochondrial protein both in vivo and in vitro and an increased yield of mitochondrial protein (2,3). Mitochondria isolated from microorganisms and mammalian tissues under normal and various physiological conditions have been shown to differ with respect to biochemicalproperties and buoyant density (4,5). The results reported here demonstrate the presence of two distinct mitochondrial populations in normal and renoprival kidney. The relative rates of protein synthesis during mitochondrial proliferation in these two populations were evaluated employing a dual-label procedure. This type of procedure has been used to reduce technical errors during isolation and assay and has proven valuable in quantitating changes in the rate of synthesis of cellular macromolecules (6,7).

## Methods:

Unilateral nephrectomies were performed on male Sprague Dawley rats

(200 ± 20 g), under ether anesthesia, by the dorsal peritoneal approach; the right kidney was removed routinely. At 24, 48, and 72 hrs post-mononephrectomy, animals were sacrificed by decapitation, the kidneys pooled and mitochondria isolated essentially by the procedure of Hogeboom et al (8). Mitochondrial fractions were separated by layering 1 ml (15-20 mg mitochondrial protein) over a 30-50% continuous sucrose density gradient containing 1 mM EDTA and 2 mM Tricine (N-tris-(hydroxymethyl) methylglycine), pH 7.4, and centrifuged at 25,000 rpm for 5 1/2 hrs in a Spinco SW-25.1 rotor. At the end of the run 8 drop fractions (0.15-0.18 ml) were collected. Succinate dehydrogenase was determined as described by King (9) and protein by the method of Lowry et al (10).

Labeled amino acid (20 μCi per 100 g body weight) was injected intravenously one hour prior to sacrifice of the animal. The experimental design involved two sets of animals each containing three control and three mononephrectomized rats. In Set I, the control animals were injected with $^{14}$C-leucine and the mononephrectomized animals with $^{3}$H-leucine, whereas in Set II, the control animals were with $^{3}$H-leucine and the mononephrectomized animals with $^{14}$C-leucine. At the end of the labeling period the animals were sacrificed and the kidneys from animals in each Set were pooled and mitochondria isolated.

Samples containing radioactivity were precipitated with 10% trichloroacetic acid and collected on 0.45 μ pore size cellulose acetate membranes. Radioactivities were determined with a Packard-Tricarb Scintillation Spectrometer, Model 3375. Counts of $^{3}$H and $^{14}$C were recorded simultaneously and corrected to dpm with the necessary corrections for channel spillover, counting efficiency and quenching, which was determined by external standardization.

For individual samples recovered from the gradients, the ratios of $^{3}$H (dpm)/$^{14}$C(dpm) for experiments in Set I and the ratios of $^{14}$C(dpm)/$^{3}$H(dpm) for Set II were calculated. The calculation of the relative rates of incorpo

ation of leucine into a given mitochondrial fraction from the renoprival as compared to normal kidney depends only on the $^{3}H/^{14}C$ and $^{14}C/^{3}H$ ratios, and was calculated as:

$$\text{Relative Rate} = 100 \times \sqrt{\left[\frac{^{3}H}{^{14}C}\right]_{\text{Set I}} \times \left[\frac{^{14}C}{^{3}H}\right]_{\text{Set II}}} \qquad (1)$$

This provides a value analagous to a geometric mean of the two separately analysed Sets (I and II).

Results and Discussion:

Utilizing isopycnic density centrifugation, the presence of two mitochondrial populations in normal and renoprival rat kidney has been observed. Figure 1 presents the results for normal kidney mitochondria where two distinct bands with mean densities of 1.178 ($M_1$) and 1.162 ($M_2$) were found. Both bands consisted of mitochondria as established by enzymic analysis; the specific activities of succinic dehydrogenase at the peaks of the bands were iden-

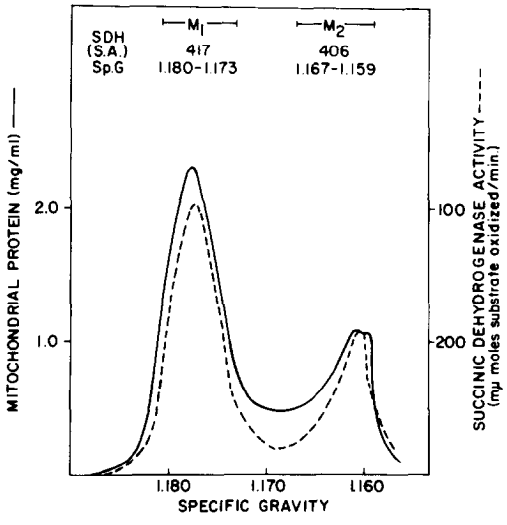


Fig. 1. Profile of Mitochondrial Protein and Succinic Dehydrogenase after Isopycnic Sucrose Density Gradient Centrifugation. Mitochondria in 0.2M mannitol were layered on top of a continuous sucrose gradient containing 1 mM EDTA and 2 mM Tricine, pH 7.4; the specific gravity extended from 1.1513 to 1.2296 g/cc. The sample was centrifuged at 25,000 rpm for 5 1/2 hrs in a Spinco SW 25.1 rotor. 170 fractions were collected; specific gravities were determined directly from the refractive indices of each fraction and plotted on the abscissa. Protein concentration (mg/ml) solid line, and succinic dehydrogenase activity (mumoles substrate oxidized/min) dashed line, were determined as described in Methods. $M_1$ and $M_2$ fractions consisted of fractions with specific gravity from 1.180-1.173 and 1.167-1.159 respectively.

tical. The ratio of protein content in $M_1/M_2$ varied with time after mononephrectomy and was 2.10, 1.52, 1.68 and 2.20, for kidney preparations at zero, 24, 48 and 72 hours post-mononephrectomy respectively.

Previous studies (2,3) had demonstrated a greater rate of amino acid incorporation into total mitochondrial protein from the renoprival as compared to normal kidney. The results were derived from single labeled isotope experiments, which are not conducive to determining quantitative changes in specific populations of mitochondria. A dual-label procedure, involving the same precursor but labeled with $^{14}C$ or $^{3}H$ and then used in two sets of experiments either for the control or treated animal, permits quantitation of the rate of synthesis of specific samples of mitochondria. The mixing of control and treated kidneys followed by the isolation and assay of mitochondria eliminates possible errors due to variations in these procedures. The rates of synthesis may be quantitated if the square root of the products of the ratios $^{3}H/^{14}C$ and $^{14}C/^{3}H$ are calculated as described in Methods. In Table I the $^{3}H/^{14}C$ and $^{14}C/^{3}H$ ratios are presented for $M_1$ (specific gravity between 1.172-1.180) and $M_2$ (specific gravity between 1.159-1.167) for both sets of experiments.

TABLE I.

RATES OF MITOCHONDRIAL PROTEIN SYNTHESIS IN RENOPRIVAL KIDNEY AS COMPARED TO CONTROL[1]

| Hours | $M_1$ FRACTION | | | $M_2$ FRACTION | | |
|---|---|---|---|---|---|---|
| | SET I ($^{3}H/C^{14}$) | SET II ($C^{14}/H^{3}$) | Relative Rate | SET I ($^{3}H/C^{14}$) | SET II ($C^{14}/H^{3}$) | Relative Rate |
| 24 | 1.21 | 1.51 | 135 | 1.29 | 1.74 | 150 |
| 48 | 1.37 | 1.84 | 158 | 1.60 | 2.14 | 185 |
| 72 | 1.40 | 1.19 | 129 | 1.08 | 1.21 | 114 |

(1) $M_1$ fraction contains all samples between specific gravity 1.172 and 1.180 and $M_2$ fraction between 1.159 and 1.167. In Set I, $^{3}H$-leucine, and in Set II, $^{14}C$-leucine, was administered to the mononephrectomized animal. Relative Rates as calculated in Methods, equation 1.

The ratios demonstrate that regardless which isotope was given to the treated animal, there was a higher rate of incorporation of leucine into mitochondria from the renoprival than normal kidney. At 24, 48 and 72 hours post-mononephrectomy incorporation in vivo of leucine into $M_1$ was increased 35%, 58% and 29% and into $M_2$ 50%, 85% and 14% over control when the synthetic rate was calculated from the results of both Sets.

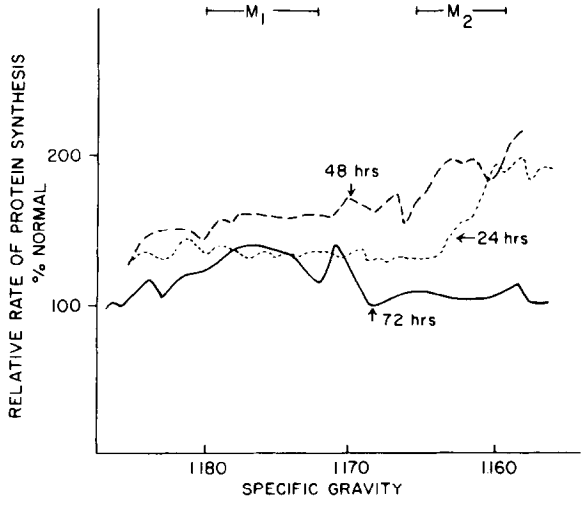


Fig. 2. Relative Rate of Mitochondrial Protein Synthesis In Vivo By Renoprival Rat Kidney. Experimental conditions and the calculations of protein synthesis rate as described in Methods. Time after unilateral nephrectomy was 24 hours, (----------) 48 hours (– – – – –) and 72 hours, (————————).

In Figure 2 the results of the calculation of the relative rates of mitochondrial amino acid incorporation, as calculated from equation 1, for all fractions collected from the sucrose gradient are presented. The results demonstrate that the $M_2$ fraction had a greater ability to incorporate labeled leucine at 24 and 48 hrs post-mononephrectomy and that there is a gradual shifting of the ability of leucine incorporation from the lower density fractions to the higher density fraction. It is our opinion that the $M_2$ band contains the newly formed mitochondria because of the higher rate of amino acid incorporation and that there is then a shift of this population into the $M_2$ band.

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