THE INCORPORATION OF GLYCEROL INTO MITOCHONDRIAL LIPIDS DURING NEONATAL RENAL COMPENSATORY GROWTH

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SUMMARY. [^{14}C]glycerol incorporation into isolated inner and outer mitochondrial membrane is enhanced in the remaining kidney after unilateral nephrectomy. Serum from neonatal rabbits taken 24 hours after unilateral nephrectomy and added to tissue slice incubations appears to stimulate incorporation of [^{14}C]glycerol into mitochondrial lipids of normal kidney cortex. Post-nephrectomy serum, however, depresses incorporation of [^{14}C] glycerol and [^{3}H]leucine into mitochondria when added to kidney cortex from animals in which uninephrectomy was performed 24 or 48 hours previously.

Phospholipid synthesis is rapid in the young animal (1), and would seem to be a manifestation of the rapid tissue growth occurring at this time normally, necessarily encompassing rapid new membrane proliferation. In early neonatal life, liver microsomal phospholipid- and protein-labeling from radioactive glycerol and leucine, respectively, show different kinetics of incorporation (2). Data in adult rat liver mitochondria, on the other hand, suggest a simultaneous pattern of label incorporation (3). The question of synchrony of lipid with protein synthesis is not established, however, particularly in this period of rapid growth and maturation.

One promising system for study of this problem in mammals is that of renal compensatory growth in very young animals, in which early mitochondrial proliferation in the contralateral kidney appears to occur relative to an increase in overall cell population, shortly after unilateral nephrectomy (4-6). The present investigation was undertaken in this system to evaluate the incorporation of labeled precursors of protein, and particularly of phospholipid, into mitochondria and mitochondrial membranes, as a means of approaching inducible mitochondrial biogenesis in mammalian systems. The effect of post-

uninephrectomy serum on this incorporation was also investigated.

METHODS Right nephrectomies or sham-operations were performed in single litters of neonatal New Zealand white rabbits (age 3-4 weeks) under sterile conditions (6). In paired animals from the experimental and sham groups at selected intervals thereafter, in vitro incorporation of radiolabeled precursors was studied in tissue slices from left kidney cortex. Slices (75μ thick, total 1-2 gm) were incubated with [^{3}H] leucine (20μc) and/or [^{14}C] glycerol (2μc) under O_2 with gentle shaking (37°, 1 hr). Cold sorbitol buffer containing 10 mM glycerol and/or 10 mM leucine was added, and mitochondria were isolated from homogenates prepared from minced slices (7). Outer and inner membrane fractions were prepared from mitochondria according to Schnaitman and Greenawalt (8). Protein was determined by the Lowry method (9). Radioactivity was determined after dissolution of the tissue materials in Beckman Biosolve BBS-2 and subsequent addition of liquid scintillation medium containing 0.4% PPO and 0.02% POPOP prior to double-label counting in a Nuclear-Chicago Mark I, utilizing an external standard for estimation of quench correction.

Total lipids were extracted from subcellular fractions with 25 volumes of chloroform:methanol, 2:1 (v/v), per volume of cellular fraction, washed with 0.2 volumes of 1: 2000 (v/v) H_2SO_4 and the chloroform phase concentrated under nitrogen (27-40°). Separation of lipids into subclasses was done by TLC[a] on Silica Gel-G, using hexane-diethyl ether-acetic acid (80:20:2) as developing solvent (10). Individual phospholipids were separated by two-dimensional TLC on Silica Gel-H (11), and identified by R_f values and staining properties, using commercial reference standards TLC-purified in our laboratory (12). Lipid phosphorous was determined to estimate the phospholipid content of the fractions or individual phospholipids (13).

[a] TLC, thin-layer chromatography.

RESULTS AND DISCUSSION Table I shows an inhibition of leucine incorporation into mitochondria by serum from animals unilaterally nephrectomized 24

Table I

INCORPORATION OF [^{3}H]LEUCINE INTO INTACT MITOCHONDRIA ISOLATED FROM RABBIT KIDNEY CORTEX TISSUE SLICES: EFFECT OF POST-UNILATERAL NEPHRECTOMY SERUM

(dpm/mg mitochondrial protein/10^7 dpm per flask)*

Status	Sham Serum+	Post-Nephrectomy Serum+
I. Control Kidneys		
a. Zero-Day	3270	4871
b. 1-Day Sham	5267	2161
c. 2-Day Sham	7022	4536
II. Renoprival Kidneys		
a. 1-Day Post-Nephrectomy	7760	3703
b. 2-Day Post-Nephrectomy	7607	6453

* Mitochondria were isolated directly from renal cortex tissue slices after incubation with the appropriate serum, according to Methods.

+ 0.8 ml serum added to each incubation flask, pooled from eight rabbits (age 3 weeks) obtained via cardiac puncture 24 hours following sham-operation or unilateral nephrectomy, respectively.

hours previously, when the serum is added to tissue slices from kidney cortex of either sham-operated or uninephrectomized animals. The effect is most pronounced at 1 day post-surgery in either group, although the inhibitory effect is still evident in the 2-day animals.

Glycerol incorporation in the renoprival (or remaining contralateral) kidney appears to be stimulated by post-uninephrectomy serum in sham animals, but inhibited by post-uninephrectomized serum in the renoprival kidney, being most evident at 24 hours also (Table II). Chloramphenicol has little effect on glycerol incorporation in sham animals, but it does seem to blunt the observed stimulation in sham animals by post-uninephrectomy serum. It appears to have little consistent effect in the renoprival kidneys of this experiment.

Table III demonstrates that [^{14}C] glycerol is incorporated into phospho-

Table II

INCORPORATION OF [^{14}C]GLYCEROL INTO MITOCHONDRIAL LIPID OF RABBIT KIDNEY CORTEX TISSUE SLICES: Effect of Serum Obtained from Animals 24 Hours Post Unilateral Nephrectomy*

	Effect of Added Chloramphenicol (dpm/μmol lipid phosphorous)	
	(-) Chloramphenicol	(+) Chloramphenicol
I. Sham Control Kidneys		
a. (+) Sham Serum	2305	2909
	3123	2724
b. (+) Nephrectomy Serum‡	4731	3485
	4538	3298
II. Renoprival Kidneys		
a. 1-day + Nephrectomy Serum	1994	2581
	1917	2353
b. 2-day + Nephrectomy Serum	2379	2157
	2395	1850

* Each data point represents a separate animal of a single litter of eight rabbits (age 3 weeks). Tissue slices were distributed from each animal for incubation with or without added chloramphenicol (0.32 mg/ml final concentration).

‡ 0.8 ml serum added to each incubation flask, and pooled from eight rabbits obtained via cardiac puncture 24 hours following unilateral nephrectomy.

lipids of both inner and outer mitochondrial membranes. There is essentially no difference in the 0-day control or 1-day sham animals in the specific activity of the total mitochondrial phospholipids of either membrane fraction. There is approximately 30% higher specific activity in the inner membrane in the 24-hour renoprival kidneys, comparing the appropriate age sham and nephrectomy rabbits. The higher incorporation activities seen in Table III can most likely be explained by the use of higher specific activity precursor in

Table III

INCORPORATION OF [^{14}C]GLYCEROL INTO MITOCHONDRIAL PHOSPHOLIPID OF RABBIT KIDNEY CORTEX TISSUE SLICES*

Animal Status	Inner Membrane (dpm/μmol lipid phosphorous)	Outer Membrane (dpm/μmol lipid phosphorous)
0-day Control	29,964[a]	23,015
	24,500[a]	16,000
	23,000[a]	8,500
1-day Sham	25,000[a]	13,625
	22,650[b]	20,000
1-day Nephrectomy	34,579[a]	27,053
	28,776[b]	8,905

* The results derive from rabbits of two litters (a = age 10 days; b = age 5 weeks).

these experiments.

Table IV demonstrates that the label from [^{14}C] glycerol is incorporated into the individual phospholipid subclasses. There is clearly differential incorporation into these phospholipid subgroups. The highest activities among these subclasses accord reasonably well with their relative percentages by lipid phosphorous analysis (14); that no label was detected in phosphatidyl serine was somewhat unexpected, even though it constitutes normally only a small proportion of total phospholipids. The rapid incorporation into phosphatidyl choline is similar to that observed in whole rat kidney (15). Chloramphenicol treatment appears to be associated with a lower specific activity of each of the subclasses after uninephrectomy compared to zero-day controls. In earlier studies chloramphenicol was found to cause 15-30% inhibition of [^{14}C]glycerol incorporation into intact mitochondria (6).

It is not clear whether these phospholipid components remain discretely associated with inner and outer mitochondrial membranes, in view of exchanges of phospholipids betwen microsomes and both these membranes (16). Consequently, interpretation of specific activity data may be difficult in assessing

Table IV

IN VITRO INCORPORATION OF [^{14}C] GLYCEROL INTO MITOCHONDRIAL PHOSPHOLIPID CLASSES OF RABBIT KIDNEY CORTEX TISSUE SLICES:

Effect of Chloramphenicol

	(dpm/μmol lipid phosphorous)	
	0-Day	1-Day Nephrectomy*
Phosphatidyl Inositol	11,149	7,615
Phosphatidyl Serine	0	0
Phosphatidyl Choline	83,074	33,539
Lysophosphatidyl Choline	11,248	2,990
Phosphatidyl Ethanolamine	38,851	19,737
Glycerophosphatides	42,814	18,850

* Intraperitoneal injection of chloramphenicol 200 mg/kg/day in 4 divided doses (every 6 hours), initiated at the time of removal of the respective right kidneys (time zero).

biosynthesis of phospholipids which are synthesized in the endoplasmic reticulum (17). The role of protein synthesized by the mitochondrial genome in mitochondrial membrane lipid-protein integration is also not clear. The present data suggest that chloramphenicol acts to suppress partially the stimulated incorporation of [^{14}C] glycerol into mitochondrial lipid induced by uninephrectomy serum. If proteins synthesized by the mitochondria were required for active integration of membrane phospholipid components into the membrane, chloramphenicol would expectedly exert some degree of inhibition of phospholipid precursor incorporation into the respective membranes. Thus, these data would appear to support the thesis that some element of the mitochondrial protein-synthesizing system is involved in mammalian mitochondrial phospholipid synthesis or incorporation during mitochondrial proliferation.

The mechanism of the compensatory growth and the associated biochemical changes is not yet understood. Whether the initial triggering mechanism is a

humoral agent (18) or the accumulation of metabolites causing more 'work' on the remaining kidney tissue (19), the initiating events at the cellular level must reflect the biochemical changes. Dicker has recently reported an inhibition of compensatory growth in rats by renal cortex microsomes and/or supernatant fluid (20). Our data suggest that a humoral factor or factors can both stimulate and inhibit biochemical changes early in compensatory growth. Additional data are needed to clarify these relationships, but it seems quite likely that a more complex mechanism than either a simple humoral agent or metabolite accumulation will be ultimately responsible for compensatory renal growth.

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