

Phospholipid Metabolism in the Initiation of Renal Compensatory Growth after Acute Reduction of Renal Mass

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ABSTRACT Membrane metabolism was studied during the initiation of compensatory growth after acute reduction in renal mass. The rate of [^{14}C]choline incorporation into phospholipid in renal cortical slices was increased by 37% at 5 min of compensatory growth in mice. The rate increased to the maximal value of 68% by 20 min and remained there for 3 h. The rate then remained increased at 28–34% above normal for 2 days and returned to normal by the 6th day.

The increase in rate of choline incorporation into renal phospholipid was independent of choline uptake. [^{14}C]Choline was found to be a specific precursor of the three renal phospholipids, phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin, which comprise over half the amount of the phospholipids. The relative distribution of the label in each of the three phospholipid classes did not change with compensatory growth. An increased rate of choline incorporation was also observed in kidneys of rats during compensatory growth and in the compensating kidneys of mice treated with indomethacin before uninephrectomy. The rate was increased 24% at 3 h after uninephrectomy *in vivo*. The increase appeared to be specific for the kidney, since it did not occur in the livers of these mice.

The results indicate that the onset of renal compensatory growth is associated with a specific enhancement of the synthesis of renal choline-containing phospholipids. Since the phospholipids largely occur in the cell membrane, early alterations in cell membrane metabolism may thus play a role in the initiation of cell growth.

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INTRODUCTION

After contralateral nephrectomy, the remaining kidney undergoes compensatory growth and function (1). After 2 days of compensatory growth, increased amounts of cellular membranes appear (2), as indicated by the presence of new organelles in the proximal tubular cells. New mitochondria (3, 4) and whorls of membranes, which morphologically resemble new smooth endoplasmic reticulum (5), are seen histologically. In addition, phospholipid, a major component of membranes, is accumulated in the cells. This results in a 35–48% increase in renal lipid phosphorus by the 4th day of growth (6).

These changes suggest that phospholipid metabolism might be altered early in compensatory growth. Although the mechanism of the initiation of renal compensatory growth is unknown, an early alteration in phospholipid metabolism could play a role, since in mouse tissue culture cells, a fivefold increase in phospholipid metabolism occurred within 15 min of growth stimulation by serum. This change, one of the earliest noted during the onset of growth, was independent of new protein, RNA, or DNA synthesis (7).

We therefore studied the metabolism of mouse renal phospholipids during initiation of renal compensatory growth by using [^{14}C]choline as a specific precursor of the three choline-containing phospholipids. The results indicate that an increased rate in choline incorporation into phospholipid occurred by 5 min after uninephrectomy, with an equal increase of choline incorporation into each of these phospholipids.

METHODS

Incubation procedure. 355 male Charles River mice weighing 27–41 g and 12 male Sprague-Dawley rate weigh-

ing 120–220 g (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were fed Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.). The animals underwent left or sham-left nephrectomy with light ether anesthesia. The left resting kidney was decapsulated immediately after uninephrectomy and sliced with a Stadie-Riggs microtome into cortical slices 0.4-mm thick for the mouse and into inner cortical slices, which extended 0.4–0.8 mm from the renal surface, for the rat. At various times later, the animals were killed by decapitation, and slices were obtained from the remaining right compensating kidney and from the kidneys of sham-operated animals. Within 2 min of slicing, a cortical slice (20–40 mg) was placed into a 25-ml Erlenmeyer flask with 2 ml Krebs-Ringer-bicarbonate medium, pH 7.4, containing 20 μ M [*methyl*- 14 C]choline chloride (sp act 49.9 mCi/mmol, 111 dpm/pmol) (New England Nuclear, Boston, Mass.). Flasks were gassed with 95% O₂-5% CO₂ for 30 s and incubated at 37.5°C for 30 min in a Dubnoff metabolic shaking incubator. The slice was then removed and dipped quickly five times in 10 ml of 72 mM [14 C]choline chloride (Sigma Chemical Co., St. Louis, Mo.) in saline to remove any surface radioactivity. The slice was blotted, weighed, homogenized in 4 ml iced 10% trichloroacetic acid (TCA) containing 72 mM choline, and centrifuged at 1,000 *g* at 2°C for 15 min. The pellet was washed three times by resuspending it in 3 ml TCA-choline and centrifuging the suspension. All supernatants were combined to form the acid-soluble fraction. More than 99.95% of the radioactivity in the pellet was in the lipid fraction, since it was extracted by chloroform:methanol (2:1, vol/vol). 0.2 ml aliquots of the acid-soluble fraction and the entire acid-insoluble fraction were individually dissolved in 10 ml of a toluene-based scintillant (Liquifluor, New England Nuclear) with 17% Bio-Solv (Beckman Instruments, Inc., Fullerton, Calif.) and counted to under an 0.8% error in a Beckman LS-250 scintillation spectrometer. The counting efficiency for the experiments was 66%, using automatic quench correction and external standardization.

The rate of [14 C]choline incorporation into phospholipid was measured at 10-min intervals, and the values are expressed as dpm [14 C]choline incorporated into the acid-insoluble fraction/mg wet wt/30 min of incubation. [14 C]-Choline uptake into the acid-soluble fraction was also measured at 10-min intervals, and the values are expressed as dpm 14 C radioactivity/mg wet wt/30 min.

The water spaces of cortical slices were determined by the difference in wet and dry tissue weight. The extracellular fluid space was calculated by using the distribution of [14 C]inulin, and the intracellular fluid space was calculated as the difference between the tissue water and extracellular fluid space (8).

Distribution of [14 C]choline in renal cortical lipids. Cortical slices from the kidneys removed at uninephrectomy, kidneys after 60 min of compensatory growth, and kidneys from sham-operated mice were incubated as described above. The rinsed weighed slices were immediately homogenized in 1 ml iced chloroform:methanol (2:1) to extract the [14 C]choline-labeled tissue lipids. The homogenate was transferred with 3 ml chloroform:methanol (2:1), and 1 ml water was added to extract any aqueous-soluble radioactivity. After centrifugation as before, the lipid fraction was saved, and the aqueous fraction and pellet were re-extracted with 1 ml chloroform. The organic fractions were then combined, evaporated to dryness with N₂, resuspended in 100 μ l of chloroform, and analyzed by thin-layer chromatography (9) and autoradiography. 40- μ l aliquots were placed on two silica gel H plates (E.

Merck AG, Darmstadt, W. Germany) and run in chloroform:methanol:acetic acid:water (65:25:4:1) and in chloroform:methanol:7N NH₄OH (60:35:5). The plates were dried, and an autoradiogram was prepared by overlaying the plate with Kodak NS-54T X-ray film (Eastman Kodak Co., Rochester, N. Y.). The films were exposed for 18 h, developed, and used to locate the specific radioactive lipid fractions on the plate. The fractions were then scraped into counting vials and counted in 20 ml Liquifluor. Values were expressed as the percent of the total radioactivity of the sample contained in each identified lipid fraction. After spraying the plates with 50% H₂SO₄ and charring at 200°C, tissue lipid fractions were identified by comparison with each of the following phospholipids: phosphatidylcholine, lysophosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine (Applied Science Labs, Inc., State College, Pa.). Identification of specific [14 C]phospholipids in tissue was enhanced by running the standards and sample fractions in two different solvent systems.

Since prostaglandins—which originate from tissue phospholipids (10, 11)—might mediate renal compensatory growth, their production was inhibited by injecting 0.3 ml indomethacin (Sigma Chemical Co.) 10 mg/kg (12, 13) in 1% aqueous methylcellulose (Fisher Scientific Co., Pittsburgh, Pa.) intraperitoneally 1 h before left uninephrectomy. Controls were injected with 0.3 ml of 1% methylcellulose.

When inactin anesthesia (Promonta, Hamburg, West Germany) was used instead of ether, mice were injected intraperitoneally with 200 mg/kg 10 min before surgery.

The rate of renal [14 C]choline incorporation was measured *in vivo* after intraperitoneal injections of 10 μ Ci (200 nmol) of [14 C]choline in 0.3 ml of water into uninephrectomized or sham-nephrectomized mice 50 min before decapitation. The radioactivity in the acid-insoluble and -soluble fractions of the kidney and liver was measured as described above.

In some experiments, 10 μ Ci [14 C]choline was injected intraperitoneally to label the renal phospholipid. 20 h later, the radioactivity in the resting kidney was determined; 3 h afterwards, that in the compensating kidney was determined, and the values were compared. A control group of mice underwent sham nephrectomy 20 h after the [14 C]choline was administered. Both kidneys were removed at decapitation 3 h afterwards. Blood for the determination of plasma radioactivity was obtained at decapitation of animals from both groups. These studies were performed to measure the disappearance of prelabeled choline with time.

Calculations. In the *in vitro* studies, values for each mouse were calculated as the mean rate of [14 C]choline incorporation in two slices from each kidney. Ratios were determined for each mouse and expressed as the CK (compensating kidney)/RK (resting kidney) ratio \pm SE. *P* values for the ratios were determined by pair analysis of the compensating and resting kidneys for each animal (14). Statistical significance was defined as a level of probability less than 0.05.

RESULTS

Fluid space of mouse cortical slices. Fluid spaces of the resting kidney and the compensating kidney were similar (Table I). Therefore, values for the rates of uptake and incorporation of [14 C]choline into the various tissue fractions were expressed per milligram wet

TABLE I
Fluid Spaces of Mouse Renal Cortical Slices after 60 Min
of Compensatory Growth

	RK	CK
	% of wet tissue weight	
Total tissue water	75.5±0.6*	74.7±0.6
Extracellular fluid space	31.2±0.8	32.4±1.6
Intracellular fluid space	43.1±0.8	41.9±1.6

Cortical slices from the kidney removed at uninephrectomy (RK) and from the animal's remaining compensating kidney removed 60 min later (CK) were incubated in Krebs-Ringer-bicarbonate medium for 30 min. Differences between RK and CK are not significant.

* Each value is the mean±SE for six slices.

tissue weight. The data also indicate that the initiation of renal compensatory growth was not associated with cellular imbibition of water.

Incubation of mouse cortical slices. The rate of uptake of [¹⁴C]choline into the acid-soluble fraction reached a peak at 20–30 min of incubation; the incorporation of [¹⁴C]choline into the acid-insoluble fraction of cortical slices continued at a constant rate for 60 min. As the extracellular concentration of choline was increased from 10 to 100 μM, incorporation of ¹⁴C radioactivity into the acid-soluble and -insoluble fractions also increased. Thus, the rate of [¹⁴C]choline incorporation into the acid-insoluble fraction increased from 24±1.8 pmol/mg/30 min at 10 μM extracellular choline to 73±2.5, at 100 μM.

[¹⁴C]Choline incorporation into renal phospholipid during compensatory growth. [¹⁴C]Choline was a specific precursor of renal phospholipids, since more than 99.95% of the radioactivity of the acid-insoluble fraction was extracted by chloroform:methanol. Over 99.7% of the extractable radioactivity was distributed in three phospholipid fractions: phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin (Table II). The relative distribution of [¹⁴C]choline into each of these fractions was similar in all the kidneys tested: the resting kidneys, the 30- and 60-min compensating kidneys, and the kidneys from sham-operated animals. Thus, the rate of [¹⁴C]choline incorporation into the acid-insoluble fraction was used as an index of the rate of incorporation of choline into the three renal choline-containing phospholipids. Since the rate varied markedly from mouse to mouse (Fig. 1), measurements of the rates of [¹⁴C]choline incorporation into phospholipid in resting and compensating kidneys were compared by pair analysis.

An increased rate of [¹⁴C]choline incorporation into renal phospholipid was observed as early as 5 min after uninephrectomy ($P < 0.01$) ($n = 7$) (Fig. 2). The rate increased to a mean maximal value 68±7% ($P <$

TABLE II
[¹⁴C]Choline Incorporation into Renal Cortical Lipid
Fractions after 60 Min of Compensatory Growth

Phospholipid fraction	RK	CK
	% of total radioactive lipid	
Phosphatidylcholine	87.8±2.7*	86.9±1.3
Lysophosphatidylcholine	10.4±2.5	10.0±1.0
Sphingomyelin	1.7±0.5	2.9±0.3
Phosphatidylethanolamine + neutral lipids	0.1±0.03	0.2±0.07

Renal cortical slices were incubated for 30 min in Krebs-Ringer-bicarbonate medium containing [¹⁴C]choline. The lipid fraction of each slice was isolated and the radioactivity of specific lipid fractions determined by thin-layer chromatography and autoradiography. RK = resting kidney, CK = compensating kidney, 60 min after nephrectomy. Differences between RK and CK are not significant.

* Each value is the mean±SE for three mice.

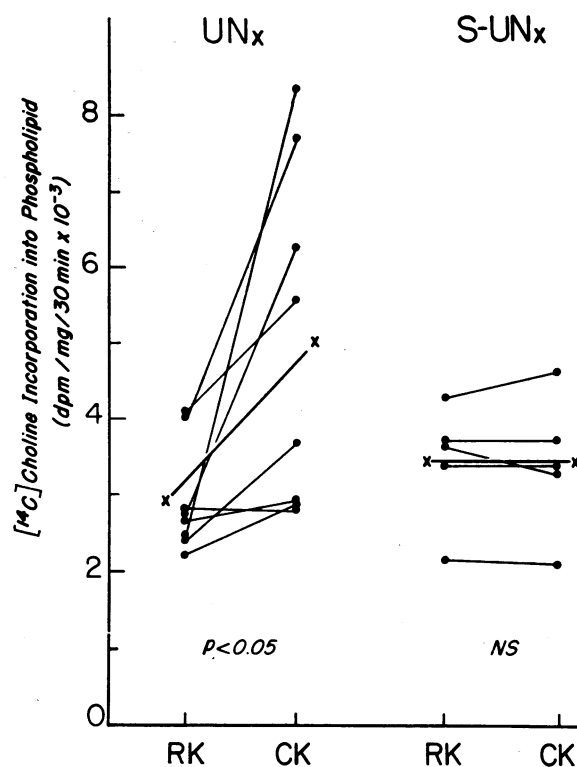


FIGURE 1 Incorporation of [¹⁴C]choline into phospholipid after 60 min of renal compensatory growth. The rate of incorporation was measured in two renal cortical slices from the kidney removed at nephrectomy (RK) and in two slices from the same animal's compensating kidney (CK). An increased rate was observed in the CK of uninephrectomized (UNx), but not in sham-uninephrectomized (S-UNx), mice. Points connected by a line are mean values for each kidney of one mouse. Heavy lines with crosses are the means of the individual values shown. P values were determined by pair analysis.

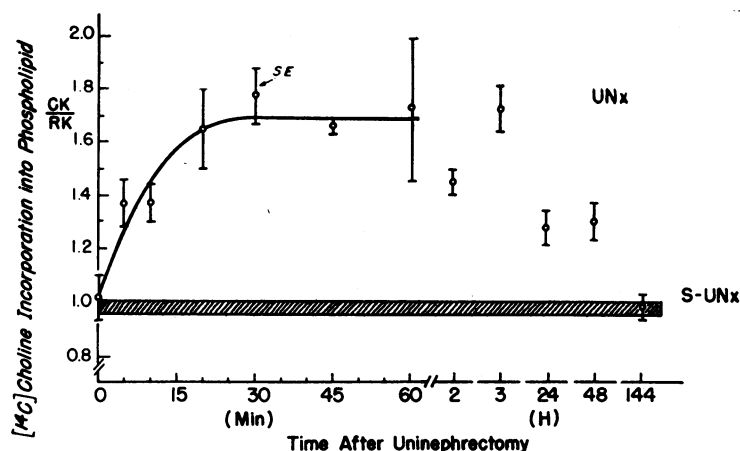


FIGURE 2 Incorporation of [^{14}C]choline into renal phospholipid during the initiation of renal compensatory growth. The rate of incorporation was measured in 2 renal cortical slices from the kidney removed at nephrectomy (RK) and in 2 slices from the compensating kidney (CK) of 45 mice. Each value on the ordinate is the ratio (CK/RK) of the rates \pm SE of 3–13 mice. P values were determined by pair analysis. The hatched area is the mean ratio \pm SE of the right to left kidney of 45 sham-operated (S-UNx) mice assayed at times similar to those for the experimental animals. The rate of incorporation in the CK was significantly greater than in the RK ($P < 0.01$) by 5 min after uninephrectomy (UNx), and remained so until 6 days later.

0.001) ($n = 42$) by 20 min and was maintained for at least 3 h. Table III shows the values at 1 h. The rate was 28–34% ($P < 0.01$) ($n = 31$) greater than normal at 1–2 days but returned to normal by the 6th day after uninephrectomy. At various times after the sham operation, the rate was similar in the 2 kidneys of 45 mice.

[^{14}C]Choline uptake into cortical slices. The ^{14}C radioactivity in the acid-soluble fraction was similar in slices from the resting and compensating kidneys when expressed as disintegrations per minute per milligram wet tissue weight or as percent of the medium [^{14}C]choline which was taken up. In addition, [^{14}C]choline radioactivity in the acid-soluble fractions was similar in slices of both kidneys from 43 uninephrectomized and 27 sham-operated mice, measured at each of 9 different times during the 24 h after surgery. Therefore, the in-

creased rate of incorporation of [^{14}C]choline into phospholipid during compensatory growth was not the result of increased choline uptake.

In vivo studies. The rate of renal [^{14}C]choline incorporation in vivo was increased 25% ($P < 0.02$) at 3 h after uninephrectomy (Table IV). The increased rate appeared to be specific for the kidney, since the rate of incorporation in livers from the uninephrectomized mice was the same as in sham-operated mice. Plasma radioactivity was similar in both groups of mice at 2 and 3 h after surgery.

The effect of 3 h of compensatory growth was measured in kidneys of 10 mice which had been labeled with [^{14}C]choline for 20 h in vivo (Table V). No differences in acid-insoluble or -soluble radioactivity were detected between 3-h compensating kidneys and resting kidneys,

TABLE III
[^{14}C]Choline Incorporation into Phospholipid and Acid-Soluble Fractions of Mouse Renal
Cortical Slices 60 Min after Uninephrectomy

	RK	CK	CK/RK	P
[^{14}C]Choline incorporation into phospholipid, dpm/mg/30 min	$2,930 \pm 250$	$5,030 \pm 800$	1.74 ± 0.28	< 0.05
Medium [^{14}C]choline incorporated, %	1.52 ± 0.12	2.95 ± 0.37	1.95 ± 0.22	< 0.005
[^{14}C]Choline uptake into acid-soluble fraction, dpm/mg/30 min	$33,080 \pm 1,620$	$31,860 \pm 1,620$	0.98 ± 0.08	NS
Medium [^{14}C]choline incorporated, %	17.2 ± 1.1	18.3 ± 1.0	1.08 ± 0.07	NS

Renal cortical slices from each mouse were incubated for 30 min in Krebs-Ringer-bicarbonate medium containing [^{14}C]choline. The radioactivity in the phospholipid and acid-soluble fractions of the slices were determined. RK = resting kidney. CK = compensating kidney, 60 min after uninephrectomy. P values were determined by paired data analysis. Each value is the mean \pm SE for eight mice.

TABLE IV
[¹⁴C]Choline Incorporation into Kidney and Liver In Vivo during Compensatory Renal Growth

Hafter UNx or S-UNx	S-UNx	UNx	UNx/S-UNx	P
<i>dpm/mg/50 min</i>				
Kidney				
2	1,760±130* (6)	1,940±100 (8)	1.10	NS
3	1,590±80 (9)	1,980±130 (8)	1.25	<0.02
4	1,720±170 (4)	2,190±90 (5)	1.27	<0.05
Liver				
4	2,540±190 (4)	2,410±290 (5)	0.95	NS

Rats underwent uninephrectomy (UNx) or sham uninephrectomy (S-UNx) and were decapitated at the times listed in the table. 50 min before decapitation, the mice were injected with [¹⁴C]choline. [¹⁴C]choline incorporation into the acid-insoluble fractions of both renal cortex and liver was measured. Kidney and liver values at 4 h were obtained from the same animals. Number of mice in parentheses. *P* was determined by group analysis.

* Mean±SE.

or between each of the two kidneys in seven sham-operated animals.

[¹⁴C]Choline incorporation during compensatory growth: (a) in another species, (b) with indomethacin treatment, or (c) after inactin anesthesia. (a) The rate of [¹⁴C]choline incorporation was significantly increased by 31±5% (*P* < 0.001) 60 min after uninephrectomy in eight rats but not in four sham-operated rats (Table VI). [¹⁴C]choline uptake into the acid-soluble fraction was not different in the two kidneys of the uninephrectomized animals.

(b) Since prostaglandins—which originate from tissue phospholipids—might mediate renal compensatory growth, a dose of indomethacin (10 mg/kg), which inhibits prostaglandin production in mice and rats (12, 13), was administered 1 h before uninephrectomy. Mice treated with indomethacin (*n* = 7) had the same increase in the rate of [¹⁴C]choline incorporation 60 min after uninephrectomy as did mice injected with the vehicle alone (*n* = 5), although injection of the vehicle appeared to increase the rate of incorporation over the rate seen in untreated mice.

(c) The increased rate of [¹⁴C]choline incorporation was not a specific effect of ether anesthesia, since, when anesthetization with inactin was employed, the

TABLE V
[¹⁴C]Phospholipid Turnover In Vivo during Renal Compensatory Growth

Protocol	RK	CK	CK/RK	Plasma ¹⁴ C radio- activity
<i>dpm/mg</i>				<i>dpm/ml/ 23 h × 10³</i>
UNx (10)	1,900±110*	1,810±100	0.95±0.03	274±20 (5)
S-UNx (7)	1,550±100	1,540±110	0.99±0.01	260±14 (6)

UNx = uninephrectomy. S-UNx = Sham uninephrectomy. RK = Resting kidney. CK = compensating kidney. Number of mice in parenthesis. Mice were injected with [¹⁴C]choline. The resting kidney was removed 20 h later by nephrectomy, and the other kidneys removed after 3 subsequent h of compensatory or "sham compensatory" growth. Duplicate determinations of radioactivity in the acid-insoluble fraction of the cortex of each kidney was determined. The CK/RK ratios are not significantly different from 1.

* Mean±SE.

rate was also increased in the compensating kidney 30 min after uninephrectomy (*P* < 0.01) in four mice but not in five sham-operated mice.

DISCUSSION

The results of this study demonstrate that increased metabolism of phospholipids in renal cortical cells occurs during the initiation of renal compensatory growth. The increased rate of choline incorporation is associated with equal increases of choline incorporation into each of the three choline-containing phospholipids.

Cellular membranes are composed largely of phospholipids and proteins. 99% of cellular phosphatidylcholine, the major phospholipid of cellular membranes, is found in membranes (15, 16). Incorporation of choline into phosphatidylcholine occurs via the Kennedy pathway, which employs cytidine diphosphocholine as precursor (17), the only significant pathway of phosphatidylcholine synthesis in mammalian kidney cells (18). The enzymes for the synthesis are located on the endo-

TABLE VI
[¹⁴C]Choline Incorporation into Phospholipid in Rat and Mouse Kidney Cortical Slices

Animal	RK	CK	CK/RK	P
<i>dpm/mg/30 min</i>				
Rats* (8)	3,490±310§	4,530±390	1.31±0.05	<0.001
Mice‡ (7)	4,800±450	7,010±700	1.47±0.09	<0.005

RK = resting kidney. CK = compensating kidney. Cortical slices of the kidneys removed at nephrectomy and of the remaining compensating kidney 60 min after uninephrectomy were incubated in media containing tracer amounts of [¹⁴C]choline. Number of animals in parenthesis. *P* determined by paired data analysis.

* Compensating kidneys were assayed after 60 min of compensatory growth. ‡ 60 min of indomethacin treatment was followed by 60 min of compensatory growth.

§ Mean±SE.

plasmic reticulum (19, 20). The phospholipids are then transported to other locations of the cell by phospholipid exchange proteins found in the cytosol (21, 22).

By isotopic analysis, newly synthesized phosphatidylcholine is found only in cellular membranes (23, 24). Thus, the rate of incorporation of radioactive choline into phosphatidylcholine has been used as an estimate of the synthesis of total cellular membrane phosphatidylcholine (25, 26). Methyl-labeled choline has been used, since its methyl groups are not transferred to other cell components at a significant rate (23). Neither the sequential N methylation of phosphatidylethanolamine (18, 27) nor the exchange of free choline with that in phosphatidylcholine (28, 29) appears to be significant in most mammalian cells. As long as the degradation of phospholipids remains constant, the rate of radioactive choline incorporation is an index of total cellular phosphatidylcholine synthesis, and an increased rate should correlate with membrane proliferation.

In the present study, the increased rate of choline incorporation into phospholipids began 5 min after uninephrectomy, reached a peak 15 min later, and remained elevated for at least 48 h. This increased rate represents net synthesis (Table IV), since the turnover of ^{14}C -labeled renal phospholipids appeared unchanged after 3 h of compensatory growth, while the rate of incorporation was increased at this time (Table V); this occurred both in vivo and in vitro. Previous work in this laboratory and by others into the initiation of compensatory growth indicated that an increase occurred in the pool of intracellular uracil nucleotides (30) and in the turnover of nuclear heterogeneous RNA (31) within 60 min after uninephrectomy. These observations indicated that alterations in RNA metabolism might initiate renal compensatory growth. The results of the present study suggest that altered membrane phospholipid metabolism precedes the known changes in RNA metabolism and occurs at the onset of renal compensatory growth.

The new membranes synthesized in renal cells during compensatory growth could be employed by the cell for new surface area for the exchange of extracellular compounds and intracellular transport and as a store of membranous components available to daughter cells in mitosis. Increased numbers of membrane-containing organelles do appear in renal cortical cells after contralateral nephrectomy. A 40% increase in the number of mitochondria (3) and large quantities of endoplasmic reticulum in whorl-like configurations appear at 2 days after uninephrectomy (5). In addition, a 35–48% increase in total renal lipid phosphorus occurs by the 4th day (6). Palmitate, which is both a precursor of membrane lipids and a major substrate for renal cortical cells, was found to be incorporated into phospholipid

at a rate 17% above normal 1 day after uninephrectomy (32). Thus, the increased rate of choline incorporation reported in the present study may be a prelude to the proliferation of renal membranes during compensatory growth.

Phospholipid accumulation appears to be independent of increased protein synthesis, since neither an increased protein content nor an increased rate of protein synthesis occurs before 14 h of compensatory growth (33). Since renal growth by cell division does not occur until the 2nd day after uninephrectomy (34), the observed increased metabolism of phospholipid in an index of renal cellular hypertrophy by the accumulation of cellular membranes, probably mainly in smooth endoplasmic reticulum and mitochondria. Thus, the results of this study lend support to the hypothesis that the cell membrane may be involved in the control of cell growth (7, 35, 36) and suggest that renal compensatory growth may be initiated by altered metabolism of cellular membranes.

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