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Contributions of nuclear magnetic resonance to renal biochemistry

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^{31}Phosphorus nuclear magnetic resonance (NMR) spectroscopy, ^{1}H (proton) NMR imaging, and spectroscopy of other nuclei, especially ^{19}fluorine, ^{23}sodium, and ^{13}carbon have progressed rapidly to the stage at which clinical interest can be justified. This has come about through improved magnetic technology and the availability of high-field spectrometers of 20 and 60 cm bore sufficient to permit examination of human organs and tissues in vivo. While ^{1}H magnetic resonance imaging has had its major clinical impact in brain imaging [1], we have yet to see the widespread application of ^{31}P NMR or other forms of spectroscopy in medicine. There has been some limited success in the diagnosis and monitoring of rare metabolic myopathies using ^{31}P NMR [2]. However, in many animal studies, the kidney has proved useful in practical developments of the technique. As a consequence, it seems highly likely that clinical nephrology may be one of the early beneficiaries of the introduction of whole-body, high-field magnets.

Since reviews in 1981 [3, 4] on NMR in renal physiology and metabolism, some 40 published reports have related directly to NMR of the kidney. In this report we summarize the most recent information which has accrued from ^{31}P NMR spectroscopy of the kidney and to indicate possible future applications in medicine and renal biochemistry. In particular, we focus on the ways in which NMR spectroscopy might assist in understanding renal function in health and disease. To avoid repetition of the principles of an NMR study, readers interested in the technical aspects should refer to [4, 5] because we intend to give only a brief introduction of the technique here.

Table 1 summarizes what we feel represents the significant literature in NMR related to renal metabolism arranged in terms of increasing biological complexity. Thus, the work with tissue extracts is considered before isolated tubules, while whole intact kidney and the kidney in vivo follow although this was not the chronological order of the work.

Principles of NMR spectroscopy

Applying a radiofrequency pulse to a population of nuclei 'aligned' in a powerful, homogeneous magnetic field induces a new orientation of nuclei, which can be observed as a nuclear magnetic resonance signal, itself of radiofrequency. The frequency used to excite the nuclei is specific for the molecular species, for example, the naturally occurring isotope ^{31}P. The frequency of emission, the signal observed, is measurably different for each phosphorus nucleus (chemical shift), and hence distinguishes Pi, phosphocreatine (PCr), ATP (α, β, and γ), glycerophosphorylcholine, and another as yet unknown phosphorus compound in the kidney. This chemical shift information is the basis of much of the chemical analysis by NMR. The technique is nondestructive and can be noninvasive. Chemical shift of Pi itself is a unique measurement of intracellular pH, probably that of the cytoplasm.

Quantitation of ^{31}P NMR analysis is inherently straightforward but involves determination of another magnetic property of the sample, the spin lattice relaxation time, T_1. T_1 is a property of great biological interest because it is greatly prolonged in tumor tissue. In proton NMR tomography (imaging) the T_1 property, which is shared by ^{31}P, has been exploited in identifying and localizing tumors in humans. This application of T_1 information is outside the scope of the present discussion. However, knowledge of T_1 for Pi and other metabolites permits accurate quantitation and furthermore opens the way to measurement of reaction rates in living tissues by saturation transfer NMR. Comparison of quantitative ^{31}P NMR with standard chemical or enzymatic analysis of kidney extracts has been of great interest [30, 34] and has important lessons for those applying NMR to other tissues. This review therefore begins with a discussion of quantitation by NMR in renal extracts.

^{31}P NMR in kidney extracts

ATP has 3-phosphorous NMR signals, but only its β-phosphate is unique to it; the frequencies of the α- and β-phosphates of ADP are so close to those of the α- and γ-phosphate of ATP, respectively, as to be indistinguishable in NMR experiments in vivo (see spectrum of control kidney in Fig. 1). Therefore, it is the usual practice for ADP (and nicotinamide nucleotides) to be quantified by subtraction of one peak area from another, that is, ADP equals $\gamma - \beta$; NAD^+ equals $\alpha - \gamma$. In control kidneys, when ATP is 6 μmoles/g dry wt, ADP was effectively zero ($\gamma - \beta = 0.07 \pm 0.14$), while chemical extraction reveals 2.5 μmoles ADP/g dry wt. This discrepancy is exaggerated during renal ischemia (Fig. 1, ischemia spectrum). Ischemia destroys renal ATP, but ADP is still undetectable by NMR: The enzymatic assay reveals an increase in [ADP] to 7.2 μmoles/g dry wt. When this kidney is extracted into perchloric acid, ADP is detectable to the extent of 5.4 μmoles/g dry kidney using NMR. Thus, within the limits of error of the experiment, the two

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Table 1. Summary of published work on renal NMR

Preparation/species	Study [reference number]
PCA extracts	
Rat	'Visible' ADP [6]
	Quantitation ATP, ADP, Pi [7]
	Identity AMP [8]
	Identity of mystery peak [9]
Rabbit	Identity of GPC (^{14}N NMR) [10]
Renal tubules	
Rabbit	Observed ADP [4]
	pH gradients [11]
Skin	
Frog	Identity of metabolites [12]
Bladder	
Toad	Thin film techniques [13]
Rectal gland	
Shark	PCA and effects of PC_r [14]
Intact (ischemic) kidney	
Rabbit	Loss of ATP [15]
	Preservation [16]
Canine	Loss of ATP (Marshall, Ross, and Smith, unpublished work)
Human	Loss of ATP [17]
	Preservation [17]
	Tumour [18]
Intact perfused kidney	
Rabbit	Preservation of ATP with blood [16]
Rat	pH determination:
	metabolic acidosis [19]
	respiratory acidosis [8]
	ATP etc quantitation [7]
	Saturation transfer Na : ATP [7]
	Thyrotoxicosis:
	saturation transfer [20]
Rat (^{23}Na)	Intra-renal Na etc [21]
Human	ATP and pH
	Ischaemia/preservation [22]
Intact perfused renal tumor	
Human	Tumour
	Chemotherapy/specific peak [22]
Intact kidney in vivo	
Rat	Preservation [23, 15]
	Ischaemia: quantitation [24]
	ATN/ARF [25, 24, 26]
	Focussing [27]
	Quantitation [25, 29, 30]
	Saturation transfer (O_2 consumption) [29]
Intact kidney in vivo	
Rat	Hypotension, quantitative saturation transfer [28, (Ratcliffe, Moonen, Holloway, Leingham, and Radda, unpublished work)]
	Hypotension, qualitative [31]
	Thyrotoxicosis, quantitative saturation transfer [31]
Human	(Proton) imaging, normal and abnormal [32, 33]
Canine	(Proton) imaging, normal and abnormal [31]

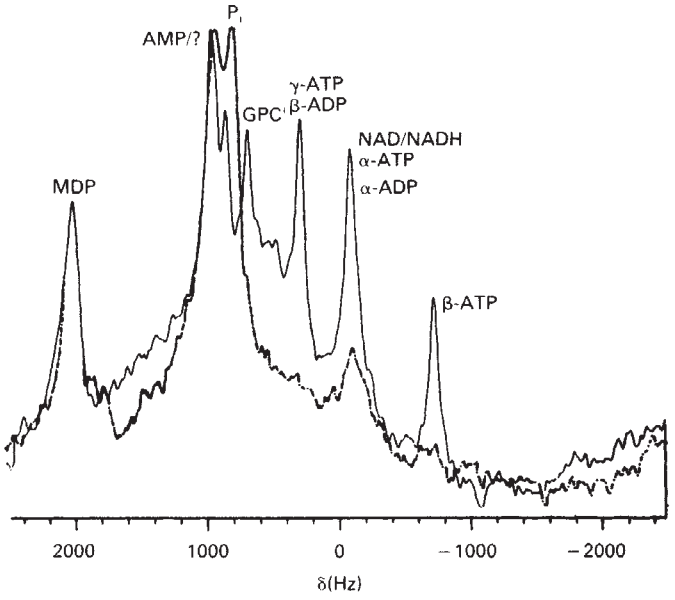

Fig. 1. *^{31}Phosphate NMR spectra of kidney in vivo.* Spectra were obtained with a 2-turn solenoid coil surrounding the kidney in an intact, anesthetized rat, within a 4.2 Tesla superconducting magnet (Oxford Instruments). Ischemia was produced by ligation of the renal pedicle. Spectra which were obtained under fully aerobic conditions (——) and during ischemia (------) have been superimposed. Abbreviations are: MDP, standard methylene diphosphaonate; AMP/?, unknown peak; Pi, inorganic phosphate; GPC, glycerophosphorylcholine; ATP, adenosine triphosphate (α, β, and γ phosphorus atoms); ADP, adenosine diphosphate (α and β phorphorus atoms); NAD/NADH, all intrarenal pyridine nucleotides.

extraction methods give identical results [6]. From these results it may be concluded that ADP must be 'free' to be seen in an NMR spectrum. Clearly, the concentration of free ADP is finely controlled in the kidney: Even a threefold rise in intracellular [ADP], which is in the micromolar range, may control the adenine-nucleotide translocator, the enzyme which, in turn, controls the rate of ATP synthesis. By extension, free [AMP] may also be present at very low, micromolar concentrations in the kidney. Because AMP is an important regulator of phosphofructokinase, there are implications for the regulation of the rate of renal glycolysis.

An essential prerequisite of ^{31}P NMR in biochemistry is that its quantitation should have a firm basis. Theory determines that amplitude of the Fourier transformed (FT) peak will have a fixed relationship to chemical quantity; this was tested for ATP, ADP, and Pi in perchloric acid extracts. ATP, ADP, and Pi were assayed by both NMR and currently accepted standard enzymatic and chemical assays. Methylene diphosphonate (MDP), the internal standard used for NMR comparisons, was calibrated against Pi standard solutions. The results agreed well with ±7% errors for each metabolite. Using the 4.2 Tesla magnet, however, the lowest metabolite concentration which could be accurately assayed by NMR was 0.1 mM. There was a marked discrepancy in "AMP" determinations: NMR determined AMP with a chemical shift of 12 ppm exceeded by three to four times the AMP assayed enzymatically [8]. With better coil design (Brindle, unpublished observations), it is possible to discriminate at least two components in this peak which must be assumed to include a variety of sugar phosphates of similar chemical shift. In a simple experiment, ischemic kidneys (in which AMP is known to increase to levels detectable by NMR) were extracted, assayed enzymatically and by NMR, and then subjected to progressive enzymatic degradation. AMP was removed stepwise by the addition of PEP, myokinase, and pyruvate kinase with a progressive drop in the 12 ppm peak.

Nevertheless, when enzymatically determined AMP has been removed entirely from the extract, a large peak at 12 ppm remained—clearly this is not AMP. It should be considered that this study was only possible with ischemic kidney, because the amount of AMP present (enzymatically) in normal kidney is below the limits of detection by NMR. It is likely that in intact kidney, even less AMP than enzymatically measured would be observed with NMR, because much of it is likely to be bound. Thus, the 12 ppm peak observed in spectra of whole kidney, or extracts of normoxic kidney probably receives no contribution from AMP and only 10 to 20% from sugar phosphates. Studies into the nature of the 12 ppm peak of renal extracts are incomplete [9]; it is a stable, water-soluble compound, of which more is found in cortex than medulla.

More success has been obtained in identifying glycerophosphorylcholine as the constituent of the renal medulla which appears in all renal spectra from all species of kidney examined to date [10, 35]. ^{14}N NMR was used to demonstrate that it contained not only phosphorus but nitrogen, and this makes it likely to be glycerophosphorylcholine. This is supported by introducing pure glycerophosphorylcholine into renal extracts [9]. Ullrich and Pehling [36] isolated and identified this compound in guinea pig papilla and showed that its concentration may alter in response to water-deprivation. This peak in ^{31}P NMR spectra of kidney may be useful to study the energetics of the renal medulla.

For thoroughness, we mention preliminary studies of ^{13}C NMR in which ^{13}C-1-acetate was added to the medium perfusing rat kidneys. Extracts of both perfusion medium and the kidney show metabolic interconversion of acetate (Stubbs, unpublished). More complete and detailed studies such as reported in the liver [11, 37] will be a potentially useful application of NMR.

^{31}P NMR in isolated renal tubules

Methods used to oxygenate and stir sufficient renal tubules for ^{31}P NMR studies were developed by Balaban et al [10] and refined by Adler, Shoubridge, and Radda [19]. The special advantages of renal tubules, biochemical homogeneity, and their ability to establish transmembrane gradients have been exploited in these two NMR studies. Ability to obtain viable renal fragments enabled Balaban et al [10] to detect a heterogeneity of intracellular pH between cortex and medulla, and between medulla and papilla, as reviewed previously [4]. This finding helps explain the very wide (40 Hz) Pi resonance which is characteristic of the intact kidney; it is derived from resonances of Pi at a variety of pHs [22]. This method also permitted the identification of the then 'mystery-peak' of renal spectra [22, 38], since it is confined to papilla and identical to the waterproofing substance of Ullrich and Pehling [36]. In addition, from these separate spectra, it is possible to detect ADP in outer medulla, possibly in papilla, but not in cortical tubules. While this may be a species difference (these studies are done in rabbit), it may also throw light on the nature of ADP binding which appears to exist in some parts of the kidney and not in others.

A central issue in renal metabolism for many years has been the regulation of ammoniagenesis by H^+. Simpson and Hager [39] stress the importance of dicarboxylic acid transport down a cytoplasmic:mitochondrial pH gradient. Krebs and Vinay [40] and Ross and Tannen [41] have attempted to calculate intramitochondrial pH from the glutamate dehydrogenase equilibrium. However, using ^{31}P NMR in the perfused kidney [19], Ackerman et al [22] conclude that intracellular pH changes were insufficient to account for the changes in ammoniagenesis and the anticipated alteration in the glutamatae dehydrogenase equilibrium. Activation of 2-oxoglutarate dehydrogenase by H^+, rather than altered glutamate dehydrogenase equilibrium was therefore assumed to be responsible for the metabolic adaptation to acidosis [42]. A better explanation for the discrepancy in NMR determined pH is that this measures not total intracellular pH, but pH of the cytoplasmic compartment alone [7]. Recent studies show that "visible" Pi by NMR represents only a small fraction of the whole cell Pi, thus, the pH determinations by NMR from the chemical shift of Pi must refer only to cell compartments in which Pi is observed with NMR, viz the cytosol.

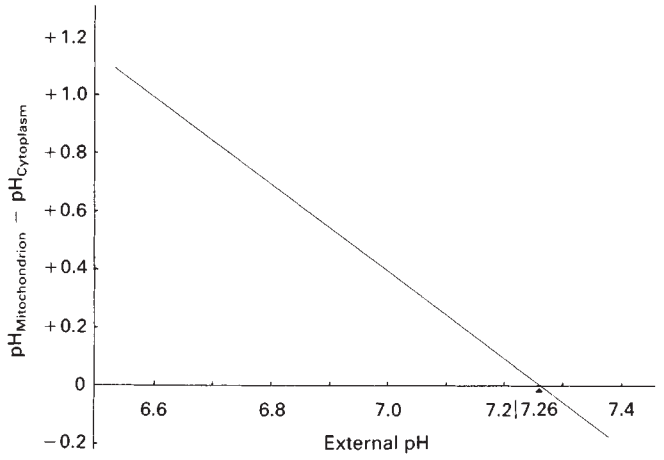

Fig. 2. *Transmitochondrial pH gradient in intact renal tubules of the rabbit.* Tubules were incubated under aerobic conditions with radioactive DMO, at various pH. Extra-cellular pH was determined electrometrically, cytoplasmic pH from the chemical shift of Pi, and intramitochondrial pH was calculated from the difference between pH determined by DMO distribution and that by ^{31}P NMR.

Combining ^{31}P NMR techniques with classical DMO pH determination in isolated rabbit cortical tubules, Adler, Shoebridge, and Radda [19] have demonstrated: *(1)* that NMR pH applies to the cytosol; *(2)* that a constant relationship exists between pH determined with DMO and by NMR; *(3)* that the difference between the results obtained by these two techniques permits determination of intramitochondrial and cytosolic pH in the same cells over a wide range of extrarenal pH (Fig. 2). At physiological external pH, no transmitochondrial gradient was detectable. In acute "acidosis" with external pH reduced to 6.9 to 7.0, a transmitochondrial pH gradient of 0.5 to 0.6 pH U (mitochondrion, alkaline) developed. Similar measurements in renal tubules or an intact rat kidney which adapts better to acidosis are anxiously awaited.

^{31}P NMR of other transporting epithelia

^{31}P NMR can be an effective means of monitoring energetic events in bladder and other transporting epithelia which could be a model for renal tubular function. Various technical im-

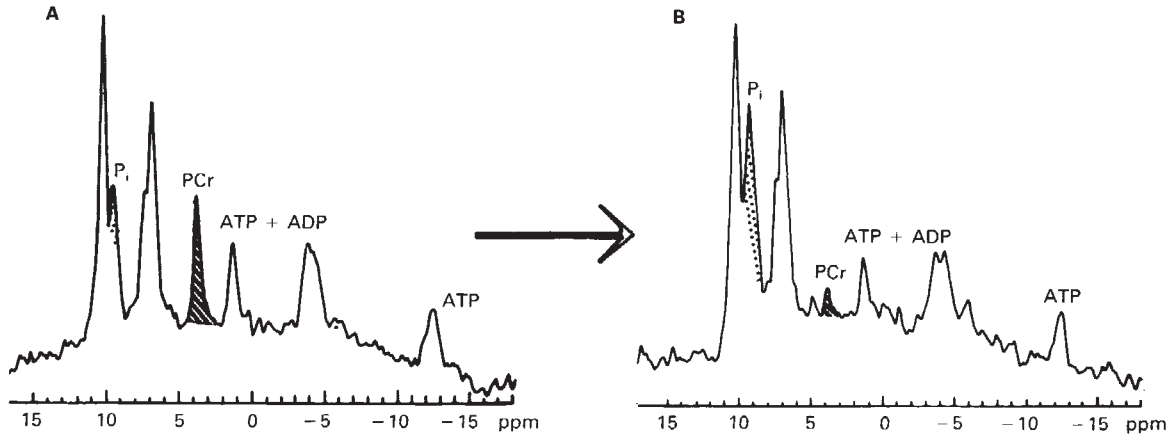

Fig. 3. *^{31}Phosphorus NMR spectra of perfused rectal gland of the shark* (Squalus acanthus). The gland was perfused with phosphate-free saline within a solenoid coil. Spectra were collected (**A**) before and (**B**) during stimulation of the gland to secrete by the addition of cyclic AMP (10^{-8} M) to the bathing medium. Note the increase in Pi at the expense of both PCr and ATP.

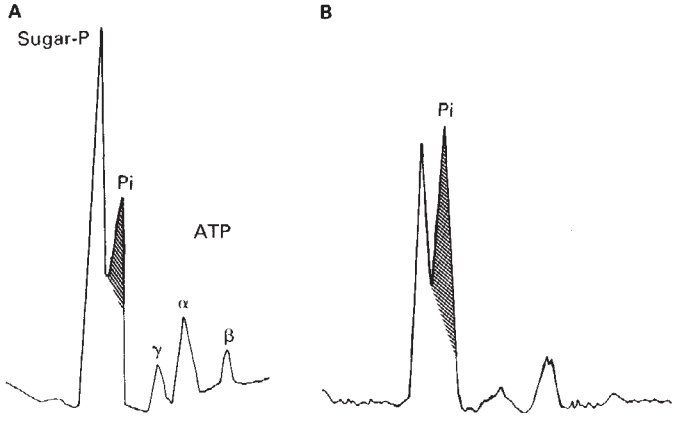

Fig. 4. *Hypothermic perfusion (10°C) of intact human kidney.* Partial recovery of renal metabolic activity was observed using a surface coil during perfusion of the kidney with standard cold-preservation medium. Spectrum **A** represents the normal, Spectrum **B** the abnormal (not perfused) pole of the same kidney. The entire kidney was maintained within the bore of a 20-cm 1.9 Tesla magnet.

provements have been reported [13]. Kokko [12] and Civan et al [43] reported satisfactory ^{31}P NMR spectra obtained from frog skin "rolled" into a spectrometer tube. The shark rectal gland, perfused through its vascular bed, differs from kidney in having a significant PCr peak, which falls sharply when the chloride pump is activated by exogenous cyclic AMP [14] (Fig. 3).

^{31}P NMR of ischemic kidney

Largely for reasons of expediency, earlier NMR studies of intact rat kidney were performed in an in vitro system without attempts to maintain oxygen delivery. Nevertheless, this crude method has an important relevance to monitoring viability of organ storage, and hence, is of interest to the transplant surgeons. The early ^{31}P NMR experiments in isolated rat kidneys were concerned with establishing conditions under which one could follow the depletion of metabolites during warm and cold ischemia. As expected, ATP is lost almost totally from ^{31}P NMR spectra as the excised rat kidney can be inserted in the NMR spectrometer. To prevent rapid degradation of tissue metabolites, the rat kidney was flushed with Perfudex® buffer at 0°C immediately after the clamping of the renal artery and stored in the same buffer prior to the study [23]. ATP falls to undetectable levels within 30 min of the onset of cold ischemia. The accumulated Pi is readily visible and continues to accumulate; pH falls for 20 to 30 min. This could be an assay of various flushing media used in normal organ preservation, because the fall in pH can be minimized by using a suitable buffer [44].

Similar approaches were used for kidneys removed from rabbits. In this model, the changes in ATP and pH were similar to those seen in isolated rat kidney [16]. Preliminary studies indicated that ATP could be restored by perfusing the kidney with blood from a second "supporting" animal [16].

The availability of magnets with a 20 cm bore makes it possible to extend the method to whole human kidney in vitro. Human kidneys removed for transplantation and other surgical indications have been studied [17, 18, 45]. In contrast to earlier results with rat and rabbit kidneys in which ATP signals disappear within minutes of the onset of ischemia, the human kidney retains some ATP for up to 12 hr in the cold; dog kidneys maintained intermediate ATP concentration. This may be a reflection of the inverse relationship between O_2 consumption and body size as pointed out by Krebs [46]; high residual O_2 consumption would lead to an accelerated ATP-turnover and hence more rapid disappearance of ATP after arresting blood supply. This difference should be considered in designing renal preservation studies and highlights the advantages of the non-invasive ^{31}P NMR assay for intrarenal pH and ATP concentration.

A significant preservation effect has been observed in the human kidneys by flushing with a hypertonic citrate solution prior to cold storage (Fig. 4) . Using ^{31}P NMR, this flushing solution is seen to exert two effects on the kidney. Firstly, ATP is preserved for a much longer period of time, and the change is in concert with sugar phosphate (including AMP) and Pi. These findings were validated by chemical assays of kidneys that were not used for transplantation. Secondly, the pH_i of citrate-flushed kidney drops considerably more slowly than the saline-flushed kidney [44].

Correlations between the metabolic state of the kidney prior to

transplantation and subsequent graft function were assessed using early graft function and the need for dialysis in week 1 after transplantation. In addition to the NMR changes of ATP, the accumulation of Pi relative to the sugar phosphate peak and the degree of intracellular acidosis provide some prognostic index to post-transplant acute tubular necrosis and the early outcome of the subsequent transplant in 40 kidneys [17]. However, the results do not show sufficient differentiation of 'good' from 'bad' kidneys to be useful for clinical purpose. Nevertheless, it has contributed to a better understanding of the effect of warm and cold ischemia in organ preservation and may provide new impetus to the study of organ preservation. For example, using NMR the damaged pole of one kidney was shown unable to regenerate ATP despite adequate perfusion (Fig. 4). These studies further demonstrate the feasibility of NMR to monitor metabolic changes in kidneys during preservation.

Isolated perfused kidney

In vitro perfusion of isolated kidney whereby nearly normal renal function and acceptable oxygen delivery can be assured, has been used in ^{31}P NMR studies. Apart from the technical achievement of obtaining acceptable ^{31}P spectra of rabbit kidney by supporting renal blood flow from a second animal, available data suggest that blood-free perfused kidney produced 'physiological' ^{31}P NMR spectra [16].

Systematic monitoring of intrarenal pH became possible with ^{31}P NMR in 1978 and 1979 [19]. An hypothesis relating equilibrium in the mitochondrial glutamate dehydrogenase to increased ammoniagenesis in acidosis was tested using ^{31}P NMR in the isolated perfused rat kidney. From the equilibrium equation:

$$\text{Keq} = \frac{[\text{glutamate}][\text{NAD}^+]}{[\text{2-oxogluatarate}][\text{NADH}][\text{NH}_3][\text{H}^+]}$$

and enzymatic assays of freeze-clamped kidneys, the change in H^+ in metabolic acidosis can be predicted. Using ^{31}P NMR the intrarenal pH change during metabolic acidosis was 0.4 pH U, significantly less than the predicted value of 0.8 pH U. This discrepancy was not fully understood initially [19], but led to the subsequent realization that pH by NMR represents cytoplasmic pH [7, 11]. Intramitochondrial pH has been determined subsequently in isolated rabbit tubules by a composite technique involving NMR and DMO indicator as described above.

The differential effects of H^+ and HCO_3^- on renal ammoniagenesis and urinary acidification have been tested using ^{31}P NMR in isolated perfused rat kidney [8]. Increased pCO_2 to produce acidosis in the external perfusing medium caused a rapid drop in tissue pH from 7.2 to 6.7. The changes were readily reversible when the gas mixture was restored to 5% CO_2. The extent of the pH change was indistinguishable from that observed in acute metabolic acidosis [19], but there was a major difference in the extent of urinary acidification. Whereas urine pH as low as 5.5 can be achieved by the perfused kidney in metabolic acidosis, it can be lowered to only 6.6 during respiratory acidosis. Calculation of intrarenal concentrations of bicarbonate based on pCO_2 and the intracellular pH determined by ^{31}P NMR yields 7 mM in metabolic and 37 mM in respiratory acidosis. This major difference in the transluminal membrane gradient for bicarbonate can explain the difficulty in generating an acid urine in respiratory acidosis.

Table 2. Comparison of metabolite content of perfused rat kidney[a]

	^{31}P NMR	Enzymatic assay	% by NMR	*P* value
ATP	4.7 ± 0.8[b] (4)	4.9 ± 0.3 (15)	95%	*NS*
ADP	0.9 ± 0.7 (4)	3.6 ± 0.3 (15)	~25%	< 0.01
Pi	2.4 ± 0.3 (4)	8.8 ± 0.6 (19)	27%	< 0.01

[a] The number of experiments is in parentheses.
[b] Value is mmoles/kg weight wt.

Table 3. High energy phosphates and metabolic rate in kidney of thyrotoxic rats[a]

	[ATP]	[ADP]	[Pi]	ATP synthesis *μmoles/min/g*
Thyrotoxicosis (*N* = 4)	3.6 ± 0.3	0.3 ± 0.1	2.0 ± 0.03	33.2 ± 12.7
Control (*N* = 10)	2.1 ± 0.2	0.3 ± 0.1	0.7 ± 0.1	16.1 ± 1.2
P	< 0.001	*NS*	< 0.001	< 0.05

[a] Perfused kidneys were taken from rats made thyrotoxic for 3-day injection of thyroxine and examined by continuous quantitative and saturation transfer NMR. Results are in mmoles/kg wet wt; the metabolic rate was calculated from the transfer of magnetization between γ-ATP and Pi observed during saturation of the γ-ATP (see [9]).

Quantitative ^{31}P NMR of isolated perfused kidney (Tables 2 and 3) [7,9]. As discussed in detail already, application of ^{31}P NMR to the isolated perfused kidney system proved ideal for an accurate comparison with earlier, enzymatic analyses of renal metabolites. ATP values were comparable by the two methods, but the major important differences were observed for both Pi and ADP. Values obtained by ^{31}P NMR were only 25% of those obtained by enzymatic or chemical assay (Table 2). The "extra" Pi observed in extracts could not be due to the breakdown of PCr during extraction as has been postulated in other tissues, because virtually no PCr can be found in renal spectra. Furthermore, insignificant amounts of ATP were broken down during extraction, so that neither the discrepancy in Pi determinations nor that of ADP could arise from this source. The differences appear to arise from a genuine difference in the metabolic pools 'observed' by the two methods; classical biochemical methods presumably determine all of the metabolite, while ^{31}P NMR determination is restricted to that fraction of the metabolic pool which is chemically "free." A number of important consequences can follow from this suggestion: *(1)* intracellular Pi = 0.7 mM, hence, discussion of Pi transport across the luminal membrane should be concerned with a different transmembrane Pi gradient from that usually assumed; *(2)* intracellular ADP is less than 0.2 mM, hence, regulation of the adenine nucleotide translocase may exist in the intact cell (with a K_m for ADP in the millimolar range), thus controlling oxidative phosphorylation in the intact kidney; *(3)* phosphorylation potential [ATP]/[ADP] × [Pi] = 12.2 × 10^3 M^{-1}, a value at least tenfold higher than that obtained by classical techniques [7]. Free energy of hydrolysis of ATP is controlled by this relation-

ship, so that control of Na^+ transport in the intact kidney may differ from the presently offered interpretation.

Thus, when Na-K-ATPase, the major active component of the Na pump, is considered to be an enzyme of fixed stoichiometry, transporting 3 Na ions per ATP hydrolyzed to ADP, insufficient energy is available to account for all, or even for the 50 to 70% of active Na transport. The higher phosphorylation potential now believed to exist in kidney would overcome this objection. The total energy required for Na^+ transport, calculated from the Nernst equation, could be met by the energy derived from the ATP hydrolyzed in the same time. This would require modification of our view of the Na pump [7] into one in which a continuously variable energy provision could be harnessed to transport Na not in the fixed proportion of 3 Na^+ to 1 ATP, but to the extent of free energy available from the ATPase reaction, which clearly contradicts the widely accepted model. Further evidence supporting this argument requires knowledge of the actual rate of ATP turnover in the functioning kidney. This can be provided by the method of saturation transfer discussed below.

ATP synthesis and the energy cost of Na^+ transport—saturation transfer NMR (STNMR) measurements [7]. One of the most interesting new developments with ^{31}P NMR is the exploration of techniques for the determination of metabolic reaction rates in an intact tissue or organ. Magnetic labelling of one reactant is possible; the rate at which this reactant is converted into another can be observed by the change of magnetization in the second metabolite. This method, described as "magnetization transfer" by its inventors, Forsen and Hoffman, is more commonly known as "saturation transfer" or STNMR. Saturation (the labelling process) is achieved by rapid, or continuous pulsing, with a second radiofrequency source, at the frequency of the chosen metabolic species, which results in its disappearance from Fourier transform spectra. Transfer is observed in the usual NMR experiment by quantifying the peak of interest in separate spectra obtained with and without "saturation." When the saturated species is γ-ATP, and that observed is Pi, the rate determined is the metabolic rate, ATP-synthesis. Freeman et al [7] systematically developed and validated this technique for the intact perfused rat kidney. Remarkably good agreement was obtained with the renal metabolic rate determined by recording oxygen consumption at the same time. The ATP:oxygen ratio of 2.45 speaks for the validity of the STNMR measurement of ATP synthesis.

Detailed discussion of the energy costs of Na transport of the perfused kidney has been presented [7]. ATP synthesis of 14 μmoles/min/g dry wt, only 30 to 50% of which is available, and net Na transport of 67 μmoles/min/g dry wt found in this study permit a comparison between Na per ATP and the relative energy required, 325 kJ/min, that is available from ATP hydrolysis (270 to 400 kJ/min). These authors conclude that only a pump of the latter type can account for active Na transport in the kidney.

The metabolic rate of kidney is known to vary with blood flow and with Na reabsorption, both of which may vary minute-by-minute in vivo. But a persistent increase in metabolic rate is likely to accompany the metabolic adaptation of thyrotoxicosis. This formed the basis of a preliminary study [47] to assess the validity of STNMR in T_3-induced thyrotoxicosis. In the perfused kidney, metabolic rate determined by STNMR increased from 12.9 ± 4.2 (SE) to 25.6 ± 6.1 in thyrotoxicosis. Oxygen consumption also doubled in kidneys from thyrotoxic rats, so that the ATP:oxygen ratio was unaltered in this metabolic adaptation. The increased metabolic rate might be linked to the observed increase in intracellular free-Pi (Table 3). GFR and Na transport increased in the isolated kidneys of thyrotoxic rats [47] and the energy cost of Na transport and overall contribution of Na transport to the respiratory rate of the kidney could be calculated. Because phosphorylation potential was lower in thyrotoxicosis (Table 3), it appears unlikely that this contributes to the extra Na transport. Instead, a higher overall metabolic rate, driven possibly by the elevated intracellular Pi, accounts for the extra energy required for the increased rate of Na transport in thyrotoxicosis: Which comes first is not clear from this study.

^{23}Na NMR and chemical shift reagents. One published study exploits the high signal intensity of ^{23}Na to follow the reversible increase in intracellular [Na^+] during episodes of ischemia [21]. Inhibition of Na,K-ATPase by ouabain produced a similar change. Dysprosium polyphosphate, a shift-reagent [20] which does not enter the renal cell, altered the chemical shift of extracellular Na so that two peaks for Na appear in spectra. Changes in Na_i^+ and Na_e^+ can then be observed at the same time. The perfused kidney was used in these studies because dysprosium is toxic to the animal; alternative "shift" reagents will be required for extension of this interesting study in vivo.

Isolated perfused human kidney

Human studies with ^{31}P NMR became possible with wide-bore, high-field magnets. Forearm muscle, lower limb, the brain of infants (the head small enough to enter a 20-cm bore magnet), and in the past year, adult brain and adult liver have all produced the ^{31}P NMR spectra which were anticipated from preceding animal studies. The human kidney was the first internal organ subjected to systematic ^{31}P NMR studies [17, 18, 38] and promises a rich seam of novel findings when the technique is applied in vivo.

The general appearance of ^{31}P NMR spectra of human kidney is identical to those now so familiar from the rat kidney in vivo or perfused in vitro. Six principal resonances are observed as shown in Figure 5. The kidney has an intracellular pH of 7.0 to 7.2. Among the predictable applications of ^{31}P NMR to clinical nephrology are the ability to detect acute ischemic or hypotensive changes prior to acute tubular necrosis. Reversible loss of ATP, accumulation of Pi, and tissue acidification have been demonstrated readily in the blood-perfused human kidney (Fig. 6). Recovery from acute ischemia is rapid (within 5 min). Recovery of renal ATP and Pi to normal is equally rapid after a period of warm ischemia, cold flushing with renal preservative, and storage in the cold for 2 hr. However, if storage in the cold is prolonged for 48 hr before recirculation of blood, recovery of ATP is both delayed and incomplete. This pattern is familiar to transplant surgeons and indicates the future usefulness of ^{31}P NMR in in vivo monitoring of the transplanted kidney.

^{31}P NMR of renal carcinoma. The isolated perfused human kidney, in which a tumor, either hypernephroma or Wilms' tumor is present, usually shares a single blood supply, and thereby establishes a unique control of normal and cancerous tissue for comparison in NMR studies [18, 38, 45]. A number of interesting features include: *(1)* If the tumor has a unique

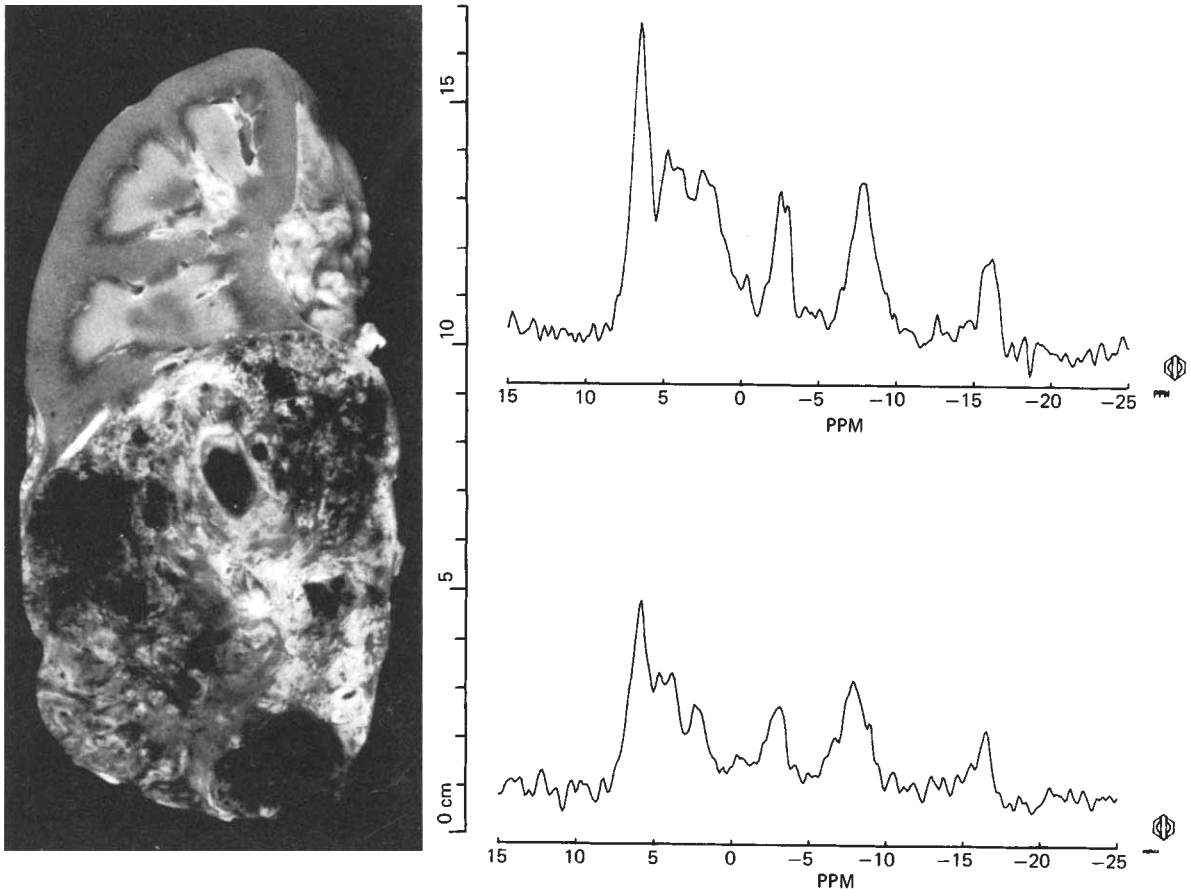

Fig. 5. *Hypernephroma of human kidney.* (*Left*) The appearance of the kidney after 6 hr of hormothermic perfusion. (*Right*) ^{31}Phosphorus NMR spectra obtained with two surface coils within a TMR 32 spectrometer. *Upper spectrum* represents the normal kidney; lower spectrum represents the hypernephroma. Note the complete profile of renal metabolites and an additional peak at +4.2 ppm in the renal cell carcinoma. For identification of peaks see Figure 6.

'phosphate' peak, it is probably indicative of Pi within cells of highly acidic environment; *(2)* during hypoxia anaerobic glycolysis maintains tumor ATP, while as ATP rapidly disappears from the normal pole of the same kidney; *(3)* omission of substrate glucose or pyruvate from the perfusing blood leads to the loss of the unique "tumor" peak and the addition of pyruvate in high concentration restores the peak. These observations are consistent with a Pi peak generated during glycolytic activity in the tumor and not in normal kidney. Evelhoch et al [48] have observed a similar tumor peak in mouse osteosarcoma. In one hypernephroma and one Wilms' tumor, the addition of chemotherapeutic agents to the perfusing blood dramatically altered the appearance of the tumor peak; in the hypernephroma it increased three- to fourfold in concentration at the expense of ATP. If this indicates drug sensitivity of this normally highly resistant tumor, then application of ^{31}P NMR to tumor monitoring in vivo has an important place in oncology in the future.

^{31}P NMR of intact kidney in vivo

Noninvasive study. Thus far, only a single study [27] in the rat demonstrates the capability of ^{31}P NMR to be an entirely noninvasive tool for studying the kidney. Using a surface coil and field profiling ("TMR") [27], the PCr peak of overlying muscle could be eliminated gradually from spectra until the characteristic renal spectrum revealed that focusing had occurred. This was confirmed by the loss of ATP from this spectrum when the renal pedicle was occluded. Most other published in vivo studies have been far from "non-invasive." Despite this, the true potential of ^{31}P NMR in clinical nephrology can be assessed readily, which will be briefly discussed below.

Intact kidney in vivo: renal preservation [*16, 25, 44, 49*]. Observations made on isolated tissues or organs gave the impetus to the development of new techniques for obtaining high resolution in vivo NMR measurements. The first approach was to introduce the radiofrequency coil by surgery around the organ of interest in anesthetized animals. This method was used to obtain ^{31}P NMR spectra of rat kidney in vivo [16] to study the mechanisms of cold and warm ischemic injury during kidney preservation for transplantation.

A typical ^{31}P NMR spectrum of kidney in vivo shows three signals corresponding to the α, β, and γ phosphates of ATP and signals for sugar phosphate, Pi, and glycerophosphorylcholine. An additional resonance of blood, 2,3-diphosphoglycerate distinguishes this spectrum from the blood-free perfused kidney. Ischemia caused the disappearance of the ATP signals and increase in the intensity and a shift in the frequency of the Pi

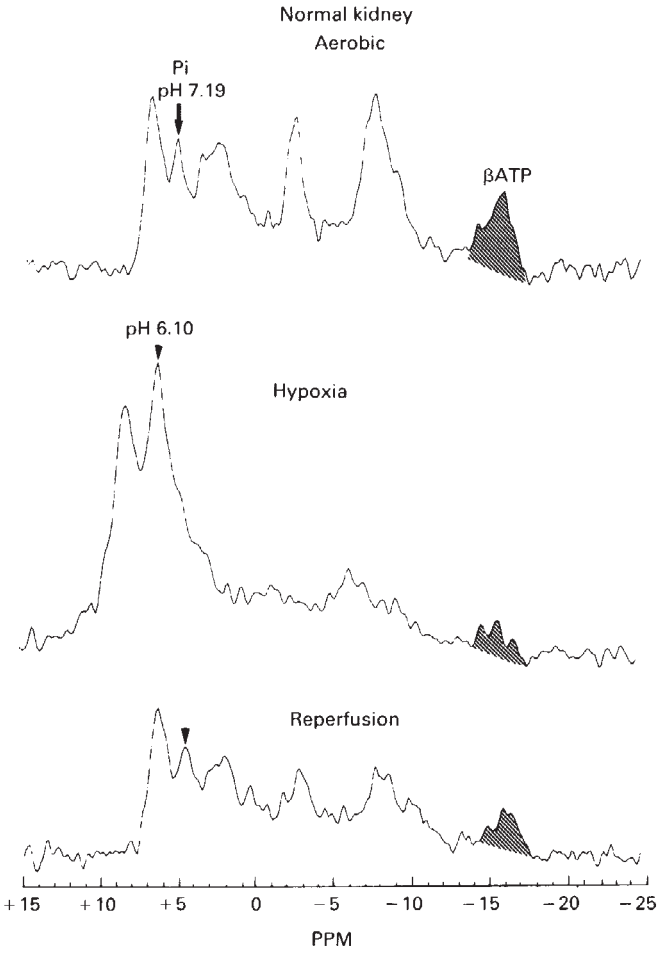

Fig. 6. *Normothermic perfusion (37°) of intact human kidney.* Full metabolic activity was restored to the kidney when perfused with blood, substrate, and oxygenated medium. Spectrum in the *top* may be compared with Figure 1. Hypoxia resulted in the loss of high energy metabolites (*middle panel*), which were almost fully restored with the resumption of oxygen supply (*bottom panel*). Peaks from *left* to *right* are: unknown (including AMP and sugar phosphate), Pi, ?glycerophosphorylcholine, (PCr), γ-ATP, α-ATP, and β-ATP. See Figure 1 for further details.

signal. The intracellular pH after 60 min of ischemia at 37°C was 6.65 ± 0.07 (N = 7). As expected, even at a low temperature (0°C), ATP was depleted by 15 min of cold ischemia. Sequential changes were obtained every 15 min for 2 hr, and then hourly for 24 hr postischemia. At 37°C the pH decreased by 0.45 U in 60 to 70 min, while at 0°C a decrease in pH of 0.45 U was measured only after some 20 to 24 hr [16].

The fall in pH with prolonged ischemia could be blunted by flushing the kidney with a solution of suitable pK and capacity of numerous buffers, agents used for kidney preservation in clinical practice [23, 49]; the most effective was the divalent buffer, Bis-tris propane (100 mmoles/liter, pH 7.6 at 37°C and 7.8 at 0°C). Introduced at the onset of ischemia and a pH which optimized its buffering capacity, the buffer prevented a fall in pH from 0.7 U in control animals to 0.4 U. It has subsequently been demonstrated that decreasing the fall in pH by prior buffer flushing preserves function in the isolated kidney [23].

Effects of renal ischemia. Occlusion of the renal artery has the predictable effect of reducing [ATP] to zero, increasing [Pi], and reducing intrarenal pH to 6.5 or lower. These changes have been observed with indwelling solenoid coils, implanted chronic coils, and with surface coils [24–26, 49, 50]. ADP was not observed in NMR spectra of the ischemic kidneys. A quantitative ^{31}P NMR study by Stubbs, Freeman, and Ross [6] documents the fate of ATP hydrolyzed during renal ischemia: ATP (6.3 μmoles) hydrolyzed yielded 24 μmoles Pi and no ADP. However, when these findings were compared with chemical assay, 5.4 μmoles ATP hydrolyzed yielded 3.2 μmoles of ADP and 44 μmoles Pi. It is clear that NMR detects only a fraction (about 50%) of the Pi within the ischemic kidney and virtually none of the ADP. Furthermore, between 13 to 20 μmoles of NMR-visible Pi are released from NMR-invisible percursor(s) during ischemia [6].

^{31}P NMR in diagnosis and management of acute renal failure. The systematic attempt to identify a lesion of energy metabolism in experimental acute renal failure in rats was discussed in earlier reviews [3, 4]. Studies in seven different models of acute renal failure indicated that acute changes in ATP and intrarenal pH could be expected in any model which used hypoxia or ischemia to induce acute renal failure [25]. Other models of acute renal failure produced by injection of nephrotoxic chemicals did not have this effect. A more careful analysis of the data [24, 25] indicates that even these nephrotoxic agents (mercuric chloride, folate, or uranyl nitrate) lead to depletion of renal ATP over the course of 3 to 4 hr. Hypotension developing over this time in the experimental animal seems to be the most likely explanation. However, it cannot be excluded that even in these purely "toxic" models of acute tubular necrosis, depletion of renal ATP may play a causative role. If depletion of ATP is important in this condition, then ^{31}P NMR lends itself as a method of monitoring therapeutic interventions. This has been exploited by Siegal et al [26] who used it to follow the increase in intrarenal ATP which occurs when rats are treated with intravenous infusion of $MgCl_2$-ATP.

An alternative theory of renal damage in acute renal failure implicates the severe intracellular acidosis which has been observed in those models in which ischemia was used to produce the syndrome [25, 50]. Indeed, renal tubular function appears to suffer severely as extracellular pH falls in the experimental setting of the perfused kidney [51]. Dissociating the effects of ATP depletion from those of H^+ accumulation has been difficult. NMR studies, however, suggest that depletion of ATP, by fructose infusion for example, can be severe, but does not cause acute tubular necrosis [25]. There is little doubt that ^{31}P NMR will have an important place in the identification of some forms of clinical acute tubular necrosis.

Quantitative renal ^{31}P NMR studies have been conducted in a semiquantitative manner, but relative concentrations of metabolites during the course of a study in vivo can be quite adequate. However, there is much to be learned concerning renal metabolism from a more rigorous quantitative approach. As discussed earlier, this requires an external standard and a pulse interval which avoids saturating the NMR signal. In consequence, such studies are almost inevitably much slower. However, because they form the basis of saturation transfer studies, this drawback has been acceptable.

Although [ATP] by NMR is equivalent to enzymatic assays

on the freeze-clamped kidney from a normal rat, there appears to be a discrepancy in thyrotoxic rats [9] in which much lower ATP values are obtained in freeze-clamped tissue. It is possible that at the much increased metabolic rate, ATP is lost even with rapid freezing of the tissue, in which case in vivo ^{31}P NMR may have special advantages.

Pi and ADP yield very different results quantitatively as has been discussed for the perfused rat kidney. While some 25% of Pi remains visible to NMR, virtually no ADP can be detected by NMR in the intact kidney in vivo. Whether the ADP which is observed in spectra of perfused kidney [7] is an artefact of relatively hypoxic perfusion, or whether it reflects differences in coil design such that a greater proportion of renal medulla is observed, remains to be established. Overall, phosphorylation potential of the kidney is even greater in vivo than that observed in the perfused kidney. Accordingly, free energy of ATP hydrolysis is yet higher than has been discussed.

Application(s) of STNMR to kidney in vivo [28–30, 50, 52]. In the long-term aim of determining renal metabolic rate in humans, the technique of STNMR has been developed first in the perfused rat kidney and recently in rat kidneys in vivo. Irradiation of γ-P of ATP results in vivo as in the perfused kidney, in a systematic and reproducible transfer of magnetization to Pi. With this technique, the rate of ATP synthesis in vivo has been calculated as 8.9 μmoles/min/g, a value which is only about 60% of that found in the isolated perfused kidney by the same technique. However, the oxygen consumption of the kidney determined in vivo was also somewhat lower with an ATP:oxygen ratio of 1.7. Within the limits of error, this represents satisfactory measurements of ATP synthesis by STNMR with a tissue in which the major respiratory fuel in vivo is lipid (ATP:oxygen = 2), which is thought to be the case for the kidney.

Renal metabolism in hypotension. Freeman et al [52] extended the study of renal metabolic rate in vivo to a fundamental question of renal energy metabolism. If renal oxygen consumption is comprised of energy required for the maintenance of nontransport functions and for Na reabsorption [53], then what rate of ATP synthesis is required to maintain a normal ATP in the absence of Na reabsorption? Because a fall in GFR secondary to a fall in renal blood flow is a common event in hypotensive shock, an answer to this question would give a clue as to whether the basal oxygen consumption can protect the kidney from ATP depletion. When systemic blood pressure was reduced by hemorrhage from 120 to 65 mm Hg in the rat, STNMR detected a 70% fall in the rate of ATP synthesis (8.9 to 3.0 μmoles/min). This is approximately the difference between normal and basal oxygen consumption discussed by Kramer and Deetjen [53]. Nevertheless, in the hypotensive rat, ATP concentration fell significantly from 2.2 to 0.9 μmoles/g. It appears that under the conditions of this study, "basal" oxygen consumption is insufficient to maintain a "normal" ATP concentration in the presence of markedly reduced GFR and hence Na reabsorption. Not only is this finding consistent with the view of the kidney as an organ "at the brink of anoxia" [27], but, since a drop in ATP concentration may itself result in renal damage, these results may indicate the existence of a prodromal phase to acute renal failure. If so, then the determination of renal metabolic rate in vivo by STNMR may offer a very early acute renal failure detection.

In more recent studies designed to examine this point further (Ratcliffe, Moonen, Holloway, Leingham, and Radda, unpublished observations), anesthetized rats were examined by ^{31}P NMR in the 20-cm bore TMR magnet. This permits the animals to be held in a more "physiological" horizontal posture. Not surprisingly, blood pressure could be lowered further without changing [ATP]. However, no measurements of renal metabolic rate were available to correlate the early drop in GFR, Na reabsorption, and unchanged energy state during hemorrhagic hypotension in the system. A complete answer to the question requires in vivo STNMR.

Proton and sodium imaging by NMR in vivo

For completeness, the now well developed use of proton NMR for clinical imaging of the kidney [32, 33] should be mentioned in this review, although biochemical information from this direction has been slight. There is a clear difference in T_1 relaxation properties between renal cortex and medulla. This changes during renal venous occlusion [31] but not during renal ischemia. Many anatomical lesions can be detected readily by this imaging technique.

While spectroscopic methods permit the enormous "water" and lipid peaks to be obliterated leaving the chemically important resonances of organic chemicals [54], novel pulse sequences and decoupling techniques have been applied recently to the rat brain in vivo [55] and muscle [56]. These techniques permit nondestructive determination of tissue lactate, amino acids, and other organic molecules; their applications to renal biochemistry can then be anticipated.

A further nucleus, the concentration of which is adequate to allow mapping by NMR, is sodium. To date, only the brain has been studied, but there would be a good reason to expect that corticomedullary sodium gradients would lend themselves to analysis in vivo by this technique.

Summary

^{31}P NMR as a descriptive technique is of interest to nephrologists. Particular contributions of ^{31}P NMR to our understanding of renal function may be enumerated.:

- "Free" metabolite levels are different from those classically accepted; in particular, ADP and Pi are low with implications for the control of renal metabolism and Pi transport, and, via the phosphorylation potential, for Na^+ transport.
- Renal pH is heterogeneous; between cortex, outer medulla, and papilla, and between cell and lumen, a large pH gradient exists. Also, quantitation between cytosol and mitochondrion of the pH gradient is now feasible.
- In acute renal failure of either ischemic or nonischemic origin, both ATP depletion and acidification of the renal cell result in damage, with increasing evidence for the importance of the latter. Measurements of renal metabolic rate in vivo suggest the existence of a prodromal phase of acute renal failure, which could lead to its detection at an earlier and possibly reversible stage.
- Human renal cancers show a unique ^{31}P NMR spectrum and a very acidic environment. Cancer chemotherapy may alter this and detection of such changes with NMR offers a method of therapeutic monitoring with significance beyond nephrology.
- Renal cortex and medulla have a different T_1 relaxation time, possibly due to differences in lipid composition.

• It seems that NMR spectroscopy has much to offer to the future understanding of the relationship between renal biochemistry and function.

Acknowledgments

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