Acute Intestinal Ischemia Studies by Phosphorus Nuclear Magnetic Resonance Spectroscopy

HAYWOOD BLUM, M.D., PH.D.
JAMES J. SUMMERS
MITCHELL D. SCHNALL, B.S.*
CLYDE BARLOW, PH.D.
J. S. LEIGH, JR., PHD.
BRITTON CHANCE, SC.D.
GORDON P. BUZBY, M.D.

^{31}P nuclear magnetic resonance (NMR) spectroscopy has been used to follow the metabolism of acutely ischemic rat small intestine and its recovery after reversal of ischemia. Loops of small intestine were subjected to occlusive external pressure for up to 60 minutes, followed by a recovery period. The depletion of PCr and ATP is rapid and complete within 20 minutes. Recovery from ischemia is also rapid but with recovery ATP levels lower than initial values after prolonged ischemic periods. Intestinal shock was avoided. Clinical recovery correlated with shorter ischemic periods. ^{31}P NMR spectroscopy thus appears to be a suitable technique for studying the effects of pharmacological agents and other treatments for amelioration of ischemic effects on the bowel.

From the Department of Surgery and Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, and Philadelphia VAMC, Philadelphia, Pennsylvania

ACUTE MESENTERIC ISCHEMIA is a significant cause of mortality and morbidity in the often infirm and elderly population usually affected.[1-4] With aggressive diagnostic evaluation, Boley et al.[5] reduced mortality from the typical 70–80% to less than 50%. To achieve these results, early angiography was employed. The use of this relatively invasive albeit potentially life-saving technique has been limited, however, possibly because of the age and associated complicating conditions in these patients, as well as the relative paucity of signs and symptoms. A less invasive technique may be better tolerated in these severely ill patients and perhaps employed with less reluctance.

Supported in part by VA Medical Research, NIH Grants 5T32 CA09430 and RR02305, an Educational Grant from Kendall McGaw Laboratories, Irvine, CA, and funds from the John Rhea Barton Surgical Research Foundation, the Ben Franklin Partnership, and the Advanced Technology Center of Southeastern Pennsylvania.

* MSTP trainee: NIH Grant 5T32 GM07170.

Reprint requests: Haywood Blum, Department of Biophysics and Biochemistry and Department of Surgery, Richards Building G4, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Submitted for publication: December 10, 1985.

Phosphorus nuclear magnetic resonance (NMR) spectroscopy, a potentially noninvasive technique, has been employed in the study of metabolic response to ischemia in cardiac tissue,[6] skeletal muscle,[7] kidney,[8] liver,[9] and brain.[10] A number of reviews have been published recently.[11-14] Characteristic changes are found in the concentrations of various phosphorylated metabolites. Their recovery after relief from ischemia can also be measured.[15-17]

The utility of phosphorus NMR in evaluation of ischemic insult to a variety of viscera suggested the possibility of monitoring mesenteric ischemia using phosphorus NMR, despite the fact that no prior report of intestinal ^{31}P NMR had been published. This may be attributable to the relatively low average density of intestine as well as the lower concentrations of phosphorus metabolites compared to other tissue (liver, heart, or kidney). As a result of this low density, the signal-to-noise ratio from intestine will be lower than that from high density tissues; thus, longer data collection times are needed to achieve results of comparable equality. An additional technical complication is associated with the lack of a definite specific location and shape for this viscus compared to the solid organs. Although spatial resolution for ^{31}P NMR cannot equal proton magnetic resonance imaging (MRI) because of concentration and magnetic moment differences, a variety of techniques are being developed[18-26] that will eventually allow *in vivo* bowel ^{31}P NMR spectroscopy.

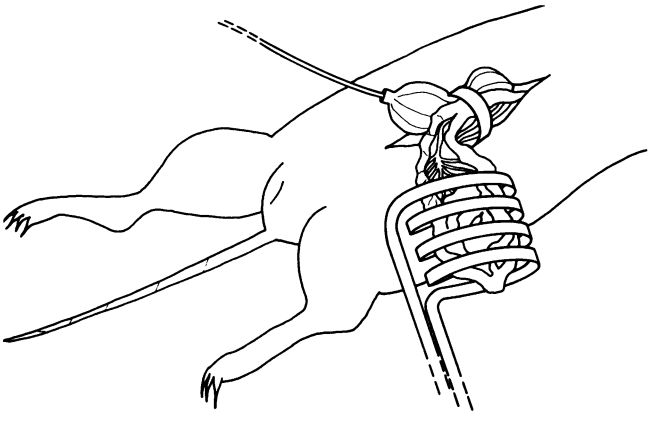


FIG. 1. Diagram of rat with exteriorized intestine placed in NMR solenoidal coil, positioned for application of hydrostatic compression to produce ischemia. The balloon is connected *via* arterial tubing to a calibrated manometer.

The purpose of this study was to develop and validate a model using ^{31}P NMR to detect acute ischemia in intestinal tissue. To this end, we have employed a rat model similar to that developed and characterized by Haglind et al.[27] for a different purpose. In this model, hydrostatic pressure is applied to the exteriorized root of the mesentery, occluding mesenteric circulation. Depending on the magnitude of the pressure applied, this will lead to irreversible shock and death. Although we compressed only two loops of small intestine, carefully excluding compression of the cecum, we used a hydrostatic pressure of 100 mmHg, which, in the model of Haglind et al., invariably leads to irreversible shock and death within hours. This was done to ensure a compressive pressure more closely approximating the mean arterial pressure. In this study, we delineate some of the parameters associated with this model, with the purpose of continuing its use in therapeutic attempts.

Methods

Twenty-six, 200–250 g healthy Wistar female rats (Ace, Boyertown, PA) were employed. Animals were maintained on standard rat chow and water *ad lib* until 2–4 plays prior to use in these experiments, at which time rat chow was withheld; water was continued. Pentobarbital sodium 5 mg/100 g was administered intraperitoneally for anesthesia. This dose was sufficient to ensure adequate sedation for at least 2–3 hours. Only one death on induction of anesthesia was encountered in this series.

A femoral arterial line was placed by cutdown for continuous monitoring of blood pressure and administration of fluids. At least 10 ml/kg/hr of physiological saline was given through this line during the experimental procedure to ensure adequate hydration and avoid hypovolemic shock.

A midline abdominal incision 2 cm long was made, and the bowel was gently exteriorized. The bowel was placed within a rubber sheath to minimize fluid loss. A compression device consisting of a plastic ring and a balloon, shown in Figure 1, was placed over two loops of small bowel, which was then inserted into a solenoidal NMR coil of four turns. The balloon was connected *via* arterial tubing to a manometer. The applied compressive pressure could be altered and continuously monitored without disturbing the position of the intestines in the NMR coil.

The entire assembly was placed in the position of maximum magnetic field uniformity in a 2.1 T Oxford Associates superconducting magnet (Oxford, England) with a 27 cm room temperature bore. Magnetic resonance spectra were obtained with a Phospho-Energetics Model 250-80 spectrometer (Philadelphia, PA). Proton magnetic resonance of water was used for adjusting the field uniformity. The frequency was then shifted to observe ^{31}P NMR at approximately 35.8 MHz. Scans were taken every 4 seconds. Pulse width was adjusted for maximum free induction decay (FID) signal. Adequate signal-to-noise could be obtained in under 5 minutes. On some occasions, a tiny sample of methyl phosphonate, in a capillary tube, within the NMR coil but external to the intestinal sheath, was employed as a calibrating standard. The spectrum from this sample lies well away from the magnetic resonance spectra of biological interest with a chemical shift of approximately 15 ppm downfield from phosphocreatine (PCr).

After obtaining a control baseline spectrum for each rat, the balloon was inflated to 100 mmHg. The bowel quickly became pale and then cyanotic. Ischemia was maintained for a variable time (up to 60 minutes). During this interval ^{31}P spectra were collected. At the end of the ischemic interval, the balloon pressure was released and a recovery interval followed. ^{31}P recovery spectra were collected during this time. After a 90-minute total evisceration time, the intestine was visually inspected and then returned to the abdominal cavity, which was closed in two layers. The femoral line was removed, and the rat was observed for survival for up to 1 week, at which time it was killed and the abdominal cavity inspected for evidence of stricture, abscess, peritoneal fluid, pus, or gangrenous bowel. Postlaparotomy, water was allowed *ad lib,* but rat chow was withheld for an additional 48 hours.

In order to compare this model of acute ischemia with that described by Haglind et al.,[27] additional rats were subjected to an equivalent regimen, without, however, being placed in the NMR spectrometer. This provided more accurate survival data. Figure 2 shows a typical

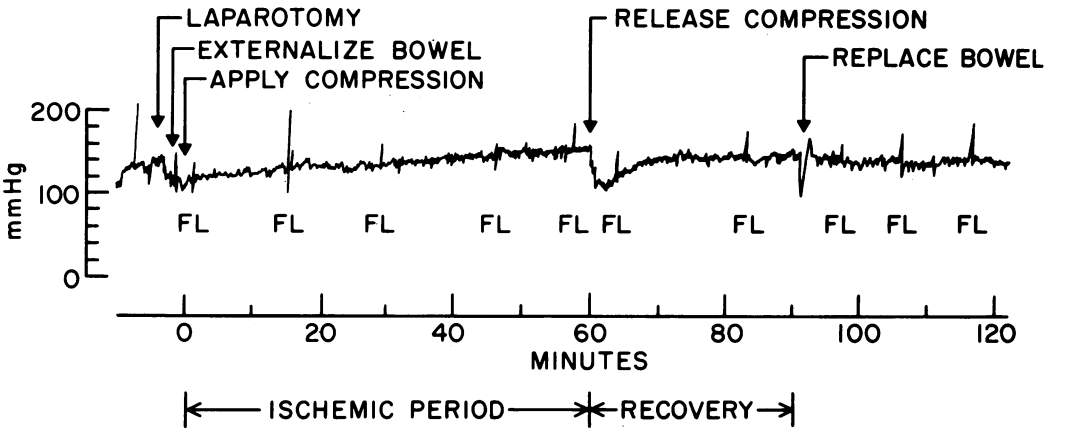


FIG. 2. Typical tracing of mean arterial pressure monitored during 60 min ischemic period and 30 min recovery period. Arterial line is placed in the femoral artery. FL = flush arterial line.

tracing of the mean arterial blood pressure (MAP) during the ischemic and recovery phases. There is a drop in MAP at the time of release of compression as the bowel is reperfused. This momentary hypovolemic pressure drop ends in about 10 minutes. With a compression of 100 mmHg, Haglind et al. found that the MAP falls throughout the ischemic period and continues to remain low in the recovery period. None of our experimental animals showed a decline in pressure during the ischemic period, possibly because of fluid replacement given continuously throughout the experimental period. Mortality rates, where comparable, are similar, however. These are shown in Table 1.

Results

The abdominal cavity of all the rats was inspected when the rats died or were killed. In the case of rats dying overnight, the inspection took place within hours of death. In no case was perforation, abscess, pus, or frankly gangrenous bowel observed. Of the rats surviving until they were killed, a few had developed adhesions, some of which were massive, but in no case was there stricture or obstruction. These rats all regained and surpassed their original weight.

A typical ^{31}P NMR spectrum of normal small intestine is shown in Figure 3A. Figure 3B shows a similar spectrum of the normal cecum. The lumen has been flushed with normal saline. The peaks have been labeled corresponding to their position as determined by comparison with high resolution spectra of known phosphorus compounds. As noted, there is some overlap in the position of some of the adenosine triphosphate (ATP), adenosine diphosphate (ADP), and nicotinamide adenine dinucleotide (NAD) peaks. The phosphomonoester (PME) peak often represents sugar phosphates that are located in the region shifted between 7 and 8 ppm to the left of PCr.[28] A methanol-hydrochloric acid extraction of the rat small intestine was performed and a high resolution ^{31}P NMR spectrum was obtained. In the PME region, two main peaks were found with chemical shifts of 6.95 and 6.40 relative to PCr (extract pH 8.9). These shifts are consistent with peak identifications of phosphorylethanolamine and phosphorylcholine, respectively.[20,29] Other, small peaks were seen in the extract consistent with phosphorylated sugars.

Figure 4 demonstrates typical ^{31}P NMR spectra taken before, during, and after acute segmental ischemia. Figure

TABLE 1. *Mortality Rates after Various Periods of Ischemia*

Total Ischemic Time		
Control	30 Minutes	60 Minutes
0/5*	3/6	5/6

* Number died/number subjected to treatment.

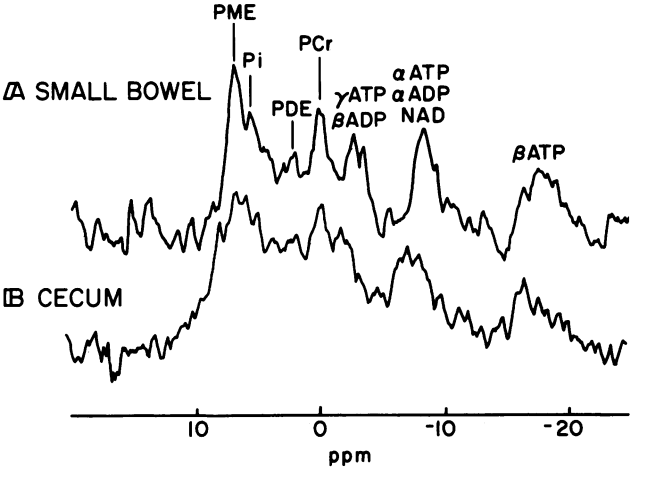
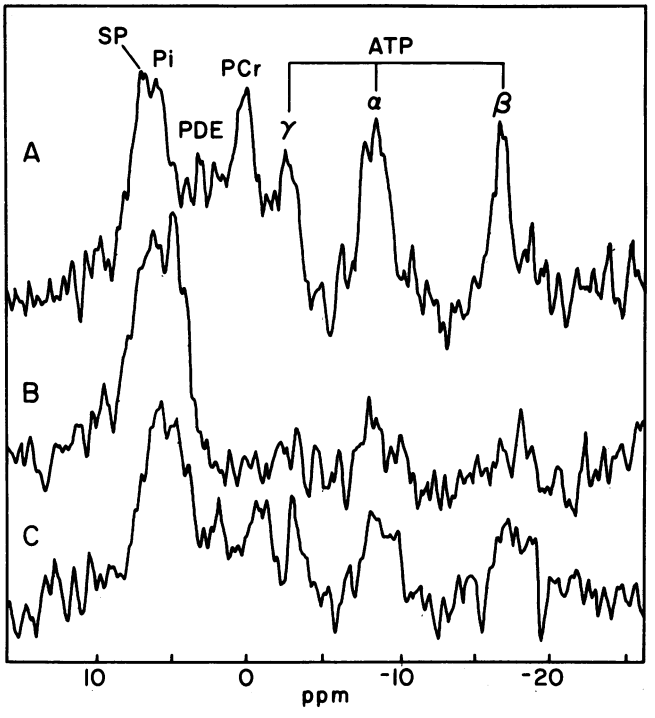


FIG. 3. Typical ^{31}P NMR spectrum of rat intestine at 35.8 MHz. The lumen has been flushed with normal saline. The labeled peaks are phosphomonester (PME), inorganic phosphate (P_i), phosphodiester (PDE), the α, β, γ peaks of adenosine 5′ triphosphate (ATP), the α and β peaks of adenosine 5′ diphosphate (ADP), nicotinamide adenine dinucleotide (NAD). Pulse width adjusted to 0.04 msec for maximum FID amplitude. The repetition time was 4 seconds. Each spectrum represents an average of over 240 scans with a total collection time of 16 minutes. A 15 Hz Lorentzian line filter was employed for improved signal-to-noise.



FIGS. 4A–C. Rat small intestine ^{31}P NMR. (*A*) control, (*B*) after 15 minutes of ischemia, (*C*) during recovery from ischemia. Peaks are identified as in the caption to Figure 3. Spectra each represent 75 scans averaged over 5 minutes. Other conditions are as in the caption to Figure 3.

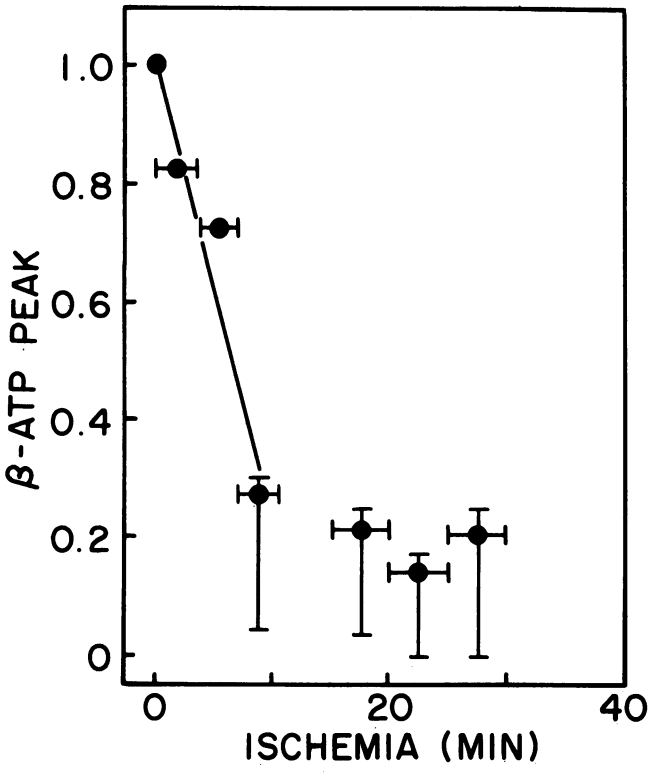


FIG. 5. Typical fall-off in amplitude of the ATP β peak relative to PME peak after onset of ischemia. Horizontal bar indicates time average of signal over this period.

4B was collected over the time period between 15 and 20 minutes after onset of ischemia. Figure 4C is averaged over the 5 minutes recovery period starting 5 minutes after release of compression. We note that the phosphocreatine peak, easily identified in Figure 4A, has disappeared in Figure 4B, as has the bulk of the peaks assigned to ATP. The inorganic phosphate peak has increased and moved toward the right (lower pH). As noted in Figure 3, only the beta peak of ATP is solitary. It has also moved to the right (lower pH). The PME peak appears essentially unchanged. In Figure 4C, the ATP peaks and the PCr peak have partially recovered. The recovery did not advance beyond this point when followed to 60 minutes (data not shown).

The PCr peak could not be accurately followed for position and intensity because of the overlap by the PME peaks. We therefore chose to use the ATP beta peak, which is spectrally isolated, although somewhat broader than PCr. Within our time resolution, which is a function of the signal-to-noise of the ^{31}P free induction decay, itself dependent on the concentration of the phosphorylated compounds, and the relaxation time of the ^{31}P, we found the PCr fall-off with ischemia to parallel that of the ATP peaks. In Figure 5, the decrease of the ATP beta peak with ischemia is shown. Its magnitude is shown relative to that of the PME peak, which does not appear to change with ischemia. Below a value of 0.2, it is difficult to distinguish the peak from noise. We see that the ATP beta peak falls to half its original value in about 5 minutes of ischemia.

During the recovery phase, we found that the ATP peaks and PCr seemed to return simultaneously. The recovery of the ATP beta peak following release of compression is shown in Figure 6. The ATP beta peak quickly reaches a plateau whose relative magnitude depends on the total ischemic time. The peak value does not increase thereafter, even with a 60-minute observation time. In Figure 7, we show the percentage of recovery to this plateau as a function of the total ischemic period. The curve is approximated by a straight line.

Discussion

The small intestinal mucosa is especially sensitive to ischemic insult. In the rat, only 30 to 60 minutes of ischemia are needed to produce prolonged functional and structural changes,[24,30] although eventual recovery can be complete.[31,32] Clinical recovery of the animal depends in addition on numerous factors such as the state of hydration, nutrition, acidosis, etc., during this period. A confounding phenomenon affecting recovery is intestinal shock,[27] partly on the basis of endotoxin release from the ischemic bowel.[33–35] Thus, clinical recovery, *per se,* may not be an appropriate indicator of ischemic damage, at

least in experimental animals. In the present study, we found increased mortality with prolongation of ischemia. This mortality was not associated with shock during the experimental period. Most of the animals evaluated for survival lived until they were killed (9/17) or died within 24 hours of the experiment (7/17). One died 3 days later, with no evidence of abscess, perforation, or gangrenous bowel. We also found that visual inspection of the bowel immediately postexperiment did not give any clear idea of survivability.

On a molecular level, PCr and ATP were rapidly depleted in this metabolically active tissue, with short-term recovery progressively limited with increasing ischemic periods. This appears to be a very typical pattern that has been seen in brain,[36] kidney,[15] and heart[17] but not in unexercised ischemic skeletal muscle.[7] In the latter tissue, ischemia occurring at rest allows for a very gradual depletion of PCr followed eventually by decreases in ATP.

There have been numerous pharmacological attempts to ameliorate the devastating effects of ischemia on the bowel with vasodilators,[5,37–40] free radical scavengers,[41] inotropic agents,[42–44] and substrate.[45] In other organs, $MgCl_2$-ATP has been successfully used to enhance recovery.[15,16,46,47] It is logical to enhance these types of studies with ^{31}P NMR spectroscopy with its possibility of repetitive nondestructive metabolic sampling.

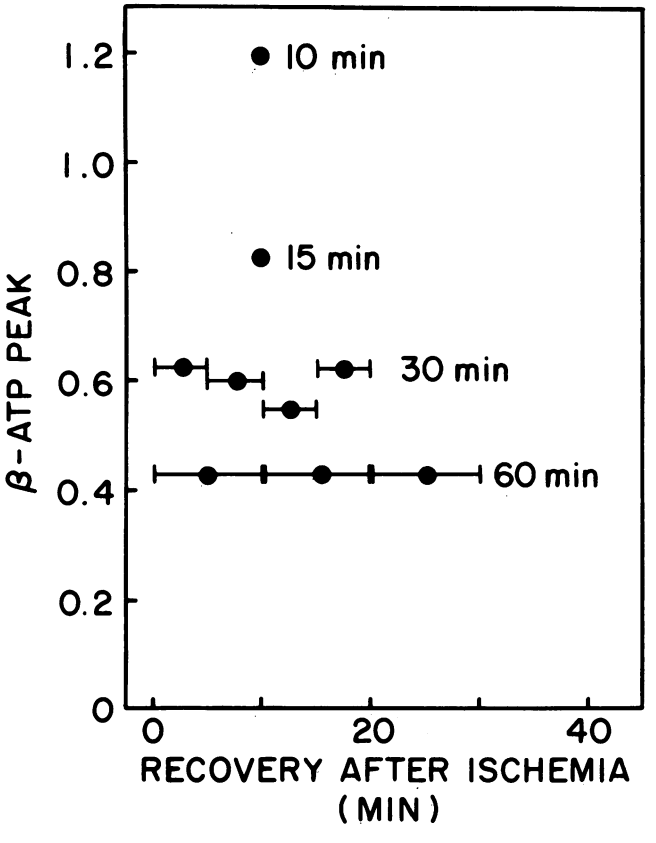


FIG. 6. Typical amplitude of the ATP β peak relative to PME after termination of ischemia.

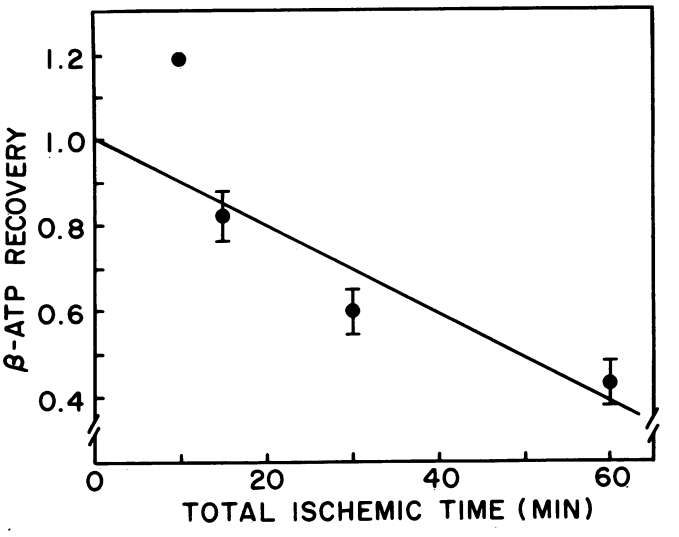


FIG. 7. Recovery amplitude of the ATP β peak relative to PME *versus* ischemic period. Vertical bars are ± S.D.

The present study demonstrates that intestinal ischemia can be easily detected by magnetic resonance spectroscopy. Further, the intestine is metabolically very sensitive to ischemic insult but can recover more or less fully if the period of ischemia is less than 15 minutes.

References

1. Andersson R, Parsson H, Isaksson B, Norgren L. Acute intestinal ischemia. Acta Chir Scand 1984; 150:217–221.
2. Ottinger L, Austen WG. A study of 136 patients with mesenteric infarction. Surg Gynecol Obstet 1967; 124:251–261.
3. Singh RP, Lee STJ. Acute mesenteric vascular occlusion: a review of 40 cases. Int Surg 1980; 65:231–234.
4. Krausz MM, Manny J. Acute superior mesenteric arterial occlusion: a plea for early diagnosis. Surgery 1978; 83:482–485.
5. Boley SJ, Sprayregan S, Siegelman SS, Veith FJ. Initial results from an aggressive roentgenological and surgical approach to acute mesenteric ischemia. Surgery 1977; 82:848–855.
6. Hollis DP, Nunnally RL, Jacobus WE, Taylor GJ. Detection of regional ischemia in perfused beating hearts by phosphorus nuclear magnetic resonance. Biochim Biophys Res Commun 1977; 75:1086–1091.
7. Osterman AL, Heppenstall RB, Sapega A, et al. Muscle ischemia and hypothermia: a bioenergetic study using ^{31}phosphorus nuclear magnetic resonance spectroscopy. J Trauma 1984; 24:811–817.
8. Sehr PA, Bore PJ, Papatheofanis J, Radda GK. Nondestructive measurement of metabolites and tissue pH in the kidney by ^{31}P NMR. J Exp Pathol 1979; 60:632–639.
9. Quistorff B, Engkagul A, Chance B. 31-P NMR in the study of liver metabolism in vivo. Pharmacol Biochem Behav 1983; 18(suppl 1):241–244.
10. Ligeti L, Barlow C, Chance B, et al. ^{31}P NMR spectroscopy of brain and heart. *In* Bicker HI, Bruley DF, eds., Oxygen Transport to Tissue IV. New York: Plenum Publishing Corp, 1983; 281–292.
11. Whitman GJR, Harkin AH. Applications of nuclear magnetic resonance to surgical disease: a collective review. J Surg Res 1985; 38:187–199.
12. Roberts JKM, Jardetzky O. Monitoring of cellular metabolism by NMR. Biochim Biophys Acta 1981; 639:53–76.

13. Gadian DG, Radda GK. NMR studies of tissue metabolism. Ann Rev Biochem 1981; 50:69–83.
14. Ingwall JS. Phosphorus nuclear magnetic resonance spectroscopy of cardiac and skeletal muscles. Am J Physiol 1982; 242:H729–H744.
15. Siegel NJ, Avison MJ, Reilly HF, et al. Enhanced recovery of renal ATP with postischemic infusion of ATP-$MgCl_2$ determined by ^{31}P-NMR. Am J Physiol 1983; 245:F530–F534.
16. Sumpio BE, Chaudry IH, Baue AE. Adenosine triphosphate–magnesium chloride ameliorates reperfusion injury following ischemia as determined by phosphorus nuclear magnetic resonance. Arch Surg 1985; 120:233–240.
17. Brooks WM, Willis RJ. ^{31}P nuclear magnetic resonance study of the recovery characteristics of high energy phosphate compounds and intracellular pH after global ischaemia in the perfused guinea-pig heart. Molecular and Cellular Cardiology 1983; 15:495–502.
18. Bendel P, Lai C, Lauterbur PC. ^{31}P spectroscopic zeugmatography of phosphorus metabolites. Journal of Magnetic Resonance 1980; 38:343–356.
19. Gordon RE, Hanely PE, Shaw D, et al. Localization of metabolites in animals using topical magnetic resonance. Nature 1980; 287: 736–738.
20. Maris JM, Evans AE, McLaughlin AC, et al. ^{31}P nuclear magnetic resonance spectroscopic investigation of human neuroblastoma in situ. N Engl J Med 1985; 312:1500–1504.
21. Cox SJ, Styles P. Towards biochemical imaging. Journal of Magnetic Resonance 1980; 40:209–212.
22. Maudsley AA, Hilal SK, Simon HE, Wittekoel S. *In vivo* MR spectroscopic imaging with P-31. Radiology 1984; 153:745–750.
23. Haselgrove JC, Subramanian VH, Leigh JS, et al. In vivo one-dimensional imaging of phosphorus metabolites by phosphorus-31 nuclear magnetic resonance. Science 1983; 220:1170–1173.
24. Budinger T, Lauterbur P. Nuclear magnetic resonance technology for medical studies. Science 1984; 226:288–298.
25. Styles P, Scott CA, Radda GK. A method for localizing high-resolution NMR spectra from human subjects. Magnetic Resonance in Medicine 1985; 2:402–409.
26. Bottomley PA, Foster TH, Darrow RD. Depth-resolved surface-coil spectroscopy (DRESS) for *in Vivo* ^{1}H, ^{31}P, and ^{13}C NMR. Journal of Magnetic Resonance 1984; 59:338–342.
27. Haglind E, Haglund U, Lundgren O, et al. Graded intestinal vascular obstruction: 1. Description of an experimental shock model in the rat. Circulatory Shock 1980; 7:83–91.
28. Barany M, Glonek T. Chemical shifts of muscle phosphates at about pH 7. Methods in Enzymology 1982; 85:653–654.
29. Bolinger L, Seeholzer S, Kofron J, Leigh JS. A guide to chemical shifts of 31-P, 1-H, and 13-C. News of Metabolic Research 1984; 1(2):32–45.
30. Robinson JWL, Mirkovitch V, Winistorfer B, Saegesser F. Response of the intestinal mucosa to ischaemia. Gut 1981; 22:512–527.
31. Cameron GR, Khanna SD. Regeneration of intestinal villi after extensive mucosal infarction. J Path Bact 1959; 77:505–510.
32. Pitha J. The fine structure of regenerating epithelium in the small intestine. Virchows Arch [Cell Pathol] 1971; 7:314–341.
33. Zanotti AM, Gaffin SL. Prophylaxis of superior mesenteric artery occlusion shock in rabbits by antilipopolysaccharide (anti-LPS) antibodies. J Surg Res 1985; 38:113–115.
34. Haglund U, Myrvoid H, Lundgren O. Cardiac and pulmonary function in regional intestinal shock. Arch Surg 1978; 113:963–969.
35. Bennion RS, Wilson SE, Serota AI, Williams RA. The role of gastrointestinal microflora in the pathogenesis of complications of mesenteric ischemia. Rev Infect Dis 1984; 6:S132–S138.
36. Schnall MD, Subramanian VH, Leigh JS, et al. A technique of simultaneous ^{1}H and ^{31}P NMR at 2.2 T *in vivo*. Journal of Magnetic Resonance 1985; 63:401–405.
37. Shapiro DM, Cronenwett JL, Lindenauer SM, et al. Effects of glucagon and prostacyclin in acute occlusive and postocclusive canine mesenteric ischemia. J Surg Res 1984; 36:535–546.
38. Cronenwett JL, Ayad M, Kazmers A. Effect of intravenous glucagon on the survival of rats after acute occlusive mesenteric ischemia. J Surg Res 1985; 38:446–452.
39. Fondacaro JD, Walus KM, Schwaiger M, Jacobson ED. Vasodilation of the normal and ischemic canine mesenteric circulation. Gastroenterology 1981; 80:1542–1549.
40. Seeman W-R, Mathias K, Roeren TH, et al. Adenosine and diltiazem: a new therapeutic concept in the treatment of intestinal ischemia. Invest Radiol 1985; 20:166–170.
41. Demetriou AA, Kagoma PK, Kaiser S, et al. Effect of dimethyl sulfoxide and glycerol on acute bowel ischemia in the rat. Am J Surg 1985; 149:91–94.
42. Gustafsson G. Effects of dopamine in segmental intestinal ischaemia studied with dopamine receptor blockers. Acta Chir Scand 1984; 150:145–151.
43. Gustafsson G, Norlen K, Rentzhog L, Wikstrom S. Delayed dopamine treatment of segmental small intestinal ischaemia in the rat. Acta Chir Scand 1984; 150:153–158.
44. Gustafsson G, Norlen K, Rentzhog L, Wikstrom S. Haemodynamic effects of phenoxybenzamine and dopamine in segmental ischaemia of the rat small intestine. Acta Chir Scand 1984; 150:159–163.
45. Ricci JL, Sloviter HA, Ziegler MM. Intestinal ischemia: reduction of mortality utilizing intraluminal perfluorochemical. Am J Surg 1985; 149:84–90.
46. Sumpio BE, Chaudry IH, Clemens MG, Baue AE. Accelerated functional recovery of isolated rat kidney with ATP-$MgCl_2$ after warm ischemia. Am J Physiol 1984; 247:R1047–R1053.
47. Ohkawa M, Chaudry IH, Clemens MG, Baue AE. ATP-$MgCl_2$ produces sustained improvement in hepatic mitochondrial function and blood flow after hepatic ischemia. J Surg Res 1984; 37:226–234.