Gut, 1992, 33, 762–766

Determination of a critical level of tissue oxygenation in acute intestinal ischaemia

W G Sheridan, R H Lowndes, G T Williams, H L Young

Abstract

Tissue oxygen tension (PtO_2) was measured using a miniaturised polarographic oxygen electrode in 134 segments of rat small intestine of varying degrees of ischaemia. Without knowledge of the PtO_2 levels, the viability of each segment was scored using clinical parameters and tissue damage scored by independent histological examination. Histologically non-viable bowel had significantly impaired tissue oxygenation when compared with viable bowel (*t* test, $p<0.001$). Marked degrees of tissue hypoxia were frequently tolerated before major histological damage became apparent, a critical PtO_2 level of 1·9 mmHg being identified. The overall accuracy rate of PtO_2 measurement in the operative prediction of intestinal viability was 92·5%, which contrasts with a rate of only 57·7% for clinical criteria alone.

University Departments of Surgery,
W G Sheridan
R H Lowndes
H L Young

and Pathology, University of Wales College of Medicine, Heath Park, Cardiff
G T Williams

Correspondence to:
Mr H L Young, FRCS, University Department of Surgery, University Hospital of Wales, Heath Park, Cardiff CF4 4XN.

Accepted for publication
7 October 1991

Intuition says that an adequate supply of oxygen is the most important determinant of tissue viability. The technology to measure intestinal tissue oxygen tension (PtO_2) per operatively is now available, having been developed from the original Clark type polarographic oxygen electrodes,[1] designed for use in cardiopulmonary bypass machines. This has been used in experimental animals[2-5] and, more recently, in man,[6,7] principally to evaluate the levels of tissue oxygenation necessary to allow intestinal anastomotic healing. Patterns of tissue oxygenation in ischaemic bowel and the relationship of oxygenation to tissue damage and therefore subsequent viability have, however, never been established in a systematic study.

In many cases of acute intestinal ischaemia, resection of the ischaemic bowel is the only treatment option. The assessment of intestinal viability in such cases remains an ongoing problem, one of the main difficulties being the unreliability of clinical parameters at operation.[8-10] The result is a tendency to err on the cautious side, leading to the resection of excessive amounts of potentially viable bowel, with the familiar gastroenterological and nutritional sequelae of short bowel syndrome. Attempts at bowel preservation, on the other hand, frequently necessitate the performance of a 'second look' laparotomy 24–48 hours later. The desire to avoid such 'second look' laparotomies, often in very ill patients, has been the stimulus to the evaluation in the past of a large number of techniques for the objective assessment of bowel viability, most of which have not been found to be of practical value in patients.

If measured tissue oxygenation, however, could be related to subsequent viability, then knowledge of per operative PtO_2 levels may allow greater conservation of potentially viable bowel. The aim of this study, therefore, was to investigate the relationship between PtO_2 and histological tissue damage in segments of intestine of varying clinical viability.

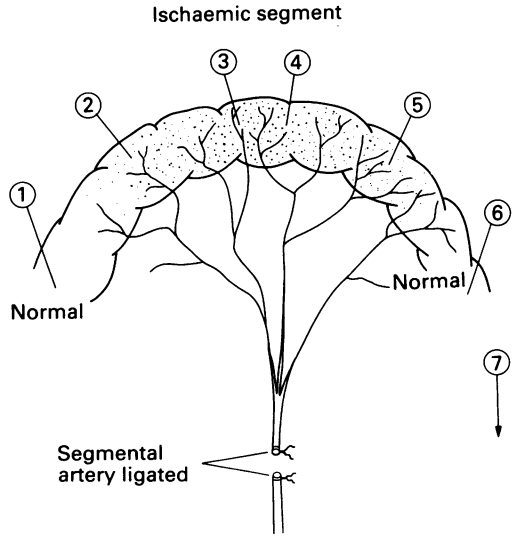

Figure 1: Schematic diagram of ischaemic segment of intestine after arterial ligation illustrating measurement sites for PtO_2.

Methods

ANIMALS

Twenty five male Wistar rats (weight 150–200 g) were used for this study. General anaesthesia was induced by ether inhalation and, through a short midline laparotomy incision, a segment of ileum was rendered ischaemic by ligating and dividing the main mesenteric artery branch to that segment (Fig 1). The ileum was returned to the peritoneal cavity, which was then closed, and an injection of 10 ml normal saline was given subcutaneously into the back, after which the animal was allowed to recover. By ligating the arterial supply in this way, it was hoped to create a segment of intestine of variable viability, ideally consisting of a central markedly ischaemic area, bounded at the proximal and distal margins by areas of intestine of questionable viability, the remaining bowel being normal.

Twenty four hours later, during a second laparotomy, the relevant segment of ileum was identified and a series of PtO_2 and surface temperature measurements were taken from the serosal surface. PtO_2 was measured using a specially built miniaturised Clark type oxygen probe (Fig 2). This 2·4 mm diameter probe consisted of a central platinum cathode (diameter 0·12 mm) and a concentric silver anode, cast in an epoxy resin body. The tip of the probe was covered with a Teflon membrane, which was

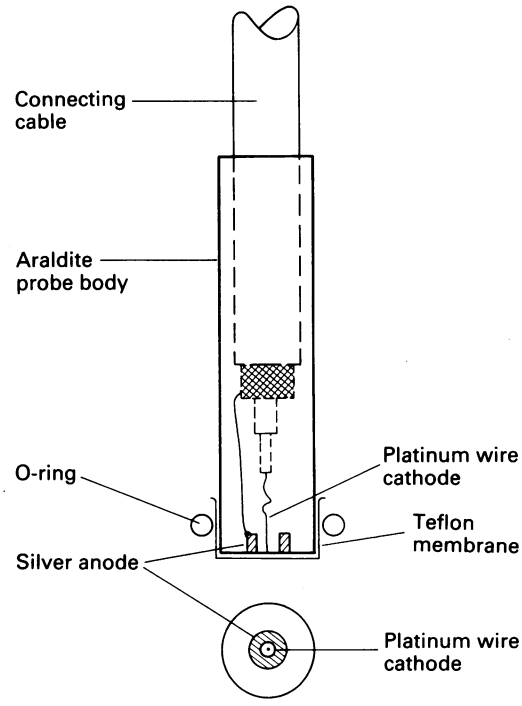

Figure 2: Diagram of miniaturised Clark type polarographic oxygen electrode.

separated from the electrodes by a very thin film of 0·75% (w/v) potassium chloride electrolyte (more extensive construction details for such probes are available elsewhere).[11] This probe produces a minute current which is directly proportional to the oxygen tension in the tissue to which it is applied. The current produced was detected by a specially constructed picoammeter and a calibration curve was used to convert the reading to PO_2 in mmHg. Surface temperature was measured using a bead thermistor applied to the intestine, adjacent to the oxygen probe. The measurement system was comprehensively validated in vitro before use.[11]

In each animal a single PtO_2 measurement was taken from each of the following sites as identified macroscopically (Fig 1):

(1) and (6) Control small intestine (unequivocally normal) proximal and distal to the devascularised segment. (2) and (5) Intestine of uncertain clinical viability at the proximal and distal margins of the devascularised segment. (3) and (4) The most ischaemic areas within the devascularised segment. (7) Stomach (distant control).

The clinical appearance of the intestine at each site of measurement was assessed using the standard clinical criteria of colour, the presence of arterial pulsation and peristalsis. This was scored as shown in Table I, the scoring being carried out without knowledge of the relevant PtO_2.

Histological specimens were then taken from sites 1 to 6, coded for subsequent examination and the animals killed. Examination was carried out by an experienced gastrointestinal pathologist without knowledge of the code or PtO_2 levels. Each specimen was scored according to the degree of histological damage observed (Table II), using a histological scoring method derived from the standard Chiu system (omitting the Grunhagen space)[12]. Scores of 0 or 1 were regarded as being representative of viable bowel and scores of 2, 3, or 4 of potentially non-viable bowel.

TABLE I *Scoring system for clinical assessment of viability*

Score	*Clinical impression*
0	Normal
1	Uncertain, probably viable
2	Uncertain, probably non-viable
3	Ischaemic
4	Necrotic, gangrenous or perforated

TABLE II *Scoring system for histological damage*

Score	*Histological appearance*
0	Normal
1	Ischaemic necrosis of mucosal villus tips only
2	Ischaemic necrosis of mucosal villi and crypts
3	Ischaemic necrosis extending into submucosa and inner muscle layers, but not full thickness muscle necrosis
4	Full thickness necrosis of bowel wall

Results

Two rats died shortly after induction of anaesthesia for the second laparotomy and so were excluded from the study. From the remaining 23 rats, a total of 134 histological specimens were examined and scored for macroscopic appearance and histological damage.

Although on clinical assessment the 134 specimens were fairly evenly distributed between viable (n=40), uncertain viability (n=56), and non-viable (n=38), histological examination revealed that the majority or the specimens (n=111) were potentially viable, as evidenced by no (grade 0) or minimal (grade 1) tissue damage. The mean (SD) stomach PtO_2 of the 23 rats was 38·4 (7·8) mmHg, which was significantly higher (t test, $p<0·05$) than the mean PtO_2 of the control small intestinal segments (33·4 (11·3) mmHg). PtO_2 levels recorded within the devascularised segment (14 (13·7) mmHg), however, were significantly lower than either stomach or control small intestine (t test, both $p<0·001$). PtO_2 changes across the ischaemic segments in the 23 animals are shown in Figure 3.

The use of clinical criteria alone correctly identified all 23 of the histologically compromised segments (grades 2–4). Of the 111 histologically non-compromised segments (grades 0 and 1), however, only 40 (36%) were felt to be viable using clinical parameters, giving an overall accuracy rate for clinical assessment. of 57·7%.

The relationship between PtO_2 and the degree of histological damage was assessed (Fig 4). Histologically compromised tissue (grades 2–4)

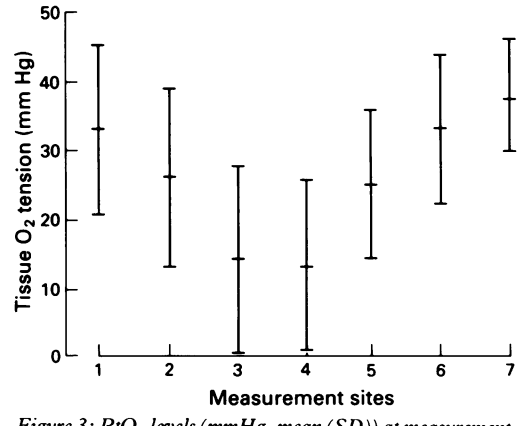

Figure 3: PtO_2 levels (mmHg, mean (SD)) at measurement sites on ischaemic bowel model.

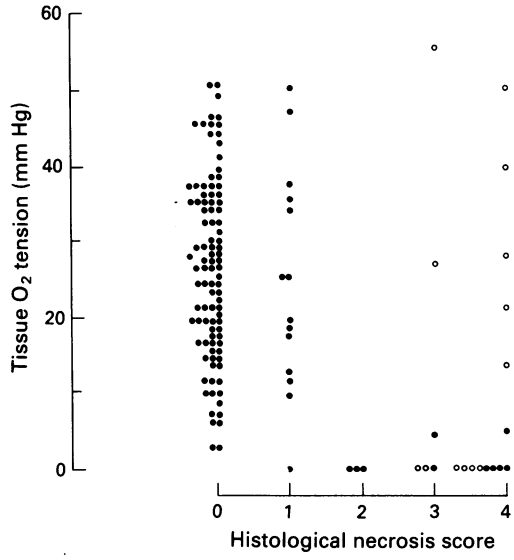

Figure 4: Relationship of PtO_2 and tissue necrosis as assessed histologically. (0=clinically gangrenous segment.)

had significantly poorer tissue oxygenation (*t* test, $p<0{\cdot}001$) than non-compromised tissue (grades 0 and 1). The critical PtO_2 (defined as being two standard deviations below the mean PtO_2 of the histologically non-compromised segments) was calculated to be 1·9 mmHg. Using this value, tissue viability was correctly predicted in 110 of the 111 histologically non-compromised segments (99·1%). Of the 23 compromised segments, seven (30·5%) would have been incorrectly predicted by PtO_2 measurement alone as being viable. Most of these false positive results, however, occurred in tissue which was grossly necrotic and the possible reasons for this will be considered in the discussion. The overall accuracy rate of PtO_2 measurement in the prediction of intestinal viability was therefore 92·5%, although if obviously gangrenous or necrotic tissue is excluded, this approaches 100% (Table III).

Discussion

Reliance on clinical parameters alone in the assessment of intestinal tissue perfusion at operation has been shown to be unreliable.[8-10] This has resulted in the development of a considerable number of techniques designed to objectively assess perfusion in this situation. These include Doppler ultrasound[10 13 14] and laser Doppler flowmetry,[15] fluorescein fluorescence,[13 16 17] radioisotope clearance,[18] intra-arterial dye injection,[19] radioactive microspheres,[20] pH measurement,[9] electromagnetic flowmeters,[21] surface temperature measurement,[8 19] and electromyography.[8 9] Most of these techniques are not applicable to routine use in the clinical situation, however, many requiring additional invasive procedures,[8 9 13 16 17 19-21] complex, expensive or cumbersome equipment,[8 9 15 18 20 21] or are difficult to interpret,[8 9 19-21] although the Doppler, laser Doppler and fluoroscein techniques have shown some promise. Even these, however, have drawbacks – Doppler ultrasound and fluoroscein fluoroscopy cannot give quantitative information and the laser Doppler is expensive and prone to movement artifacts.[15]

TABLE III *Accuracy of tissue oxygen tension measurement in the assessment of small intestinal viability (134 segments)*

PtO_2‡	*n*	*Viable*	*Non-viable*	*Accuracy*
<2†	15	1	14	93·3%
2–10	13	11	2	84·6%
11–15	11	10	1*	90·9%
16–20	18	18	0	100%
>20	77	71	6*	92·2%

*Gangrenous; †tissue with a PtO_2 of <2 mmHg considered to be non-viable; ‡mmHg.

The presence of adequate levels of oxygen in the tissues is a major determinant of viability and intuition says that its measurement should allow accurate assessment of the adequacy of tissue perfusion. The drawbacks noted above are minimised in tissue oximetry and the methodology of trans serosal tissue oxygen tension measurements in the gastrointestinal tract is now well established in animals and man.[2-7] Although applied to the serosal surface, such probes have been shown to measure oxygen from a diffusion zone, which has been calculated mathematically to be of the order of six times the diameter of the cathode tip.[22 23] With a 0·12 mm diameter cathode, such as was used in this study, the predicted diffusion zone should extend through all layers of the thin rat intestinal wall and therefore allow measurement of the mean transmural PtO_2. Variations in this should reflect oxygenation of the mucosal layer, which, because of its high oxygen requirements, is more prone to ischaemic damage than the other layers. Only one PtO_2 measurement was taken at each site, as these probes have been shown to provide highly reproducible readings with an average variability of only (2·9) mmHg.[7] The apparently large standard deviations in Figure 3 simply reflect variation in oxygenation between individual animals.

Although this technique has been used to effect in individual patients with ischaemic bowel,[24] an animal model was chosen for this study because of the practical difficulties in organising a systematic study in patients presenting with this condition. These difficulties arise from the sporadic nature of the problem combined with considerable variability in its surgical management (many patients with extensive advanced ischaemia have no procedure performed at the initial laparotomy, while others may have resection, revascularisation or 'second look' procedures or indeed any combination of these). Furthermore, histological specimens may not always have been obtainable.

Cases of ischaemic bowel usually do not come to surgery until many hours after the initial event and often after irreversible changes have taken place in the wall of the intestine. It is common clinical practice not to attempt revascularisation/reperfusion, either because it is not felt to be feasible or because the damage is not considered to be reversible. The animal model used in this study was devised to simulate this common clinical situation, with no attempt at intestinal reperfusion being made. It is acknowledged that a different experimental model would be required to assess the ischaemia/reperfusion process.

The use of histological appearance to assess

viability, although obviously it cannot be 100% accurate, is an objective and widely accepted technique.[13 14 16] An ischaemic time of 24 hours was chosen because preliminary studies revealed that shorter periods were frequently insufficient for the full spectrum of histological changes to develop and longer periods were associated with the development of shock states in many animals, as evidenced by control PtO_2 readings.

The results of this study suggest that by applying strict clinical criteria, potentially non-viable bowel is unlikely to be overlooked. In achieving this undoubtedly desirable result, however, much potentially normal intestine may be needlessly excised, as shown by the fact that 64% of the histologically non-compromised segments in this study were mistakenly designated as non-viable by standard clinical parameters. Hence the need for more accurate and objective methods.

Although PtO_2 measurement provides a more accurate overall assessment of non-reversible damage (92·5%), the occurrence of relatively high PtO_2 levels in some segments of both clinically and histologically gangrenous tissue was unexpected. Although it is not immediately clear why this should occur, it is suggested that it is artefactual and is probably caused by necrosis of cells lining the serosal surface of the intestine. It is possible that a metabolite or metabolites released from the necrotic cells may have crossed the oxygen probe membrane and undergone electrochemical reduction at the cathode surface, such as has been reported when these electrodes have been exposed to certain anaesthetic gases.[25 26]

From this study, it appears that small intestine can tolerate marked degrees of ischaemia for 24 hours before evidence of significant histological damage becomes apparent. Indeed, the calculated critical PtO_2 of 1·9 mmHg was considerably lower than expected. In other studies evaluating intestinal PtO_2, the critical level for anastomotic healing was found to lie between 20 and 25 mmHg in animals[3 5] and patients.[6] It must be emphasised, however, that the intestine in the present study was not subject to a wound which was required to heal, such as an anastomosis, unlike the studies referred to above. Presumably, in the circumstances of the present study, the requirement for oxygen for collagen synthesis is much less than in a healing anastomosis and, in the short term, oxygen may only be needed to maintain basal metabolism.

No comparable studies looking at the relationship between intestinal tissue oxygenation and histological damage exist. Workers using microelectrodes, however, have shown that resting intracellular PO_2 is low, 4 mmHg or less[27] and that the critical PO_2 for cellular function may be as low as 1–2 mmHg.[28] More recent studies, where the mitochondrial PO_2 has been measured, suggested that the critical PO_2 for this vital organ may be as low as 2 mmHg.[29] Critical levels of this magnitude would be supported by the findings of our study, with very low PtO_2 levels being found in some segments of intestine without any significant associated histological damage. It may be, however, that more prolonged exposure to such degrees of hypoxia would lead to significant damage – for example, strictures – in the long term.

Although further studies are required to examine the relationship of PtO_2 and tissue viability with differing ischaemic times, and also to evaluate changes in tissue oxygenation after intestinal reperfusion, the potential application of this technique in clinical practice can be appreciated. It may allow more intestinal conservation and reduce considerably the requirement for second look laparotomies. The occurrence of false positive indications of viability in obviously gangrenous tissue would not be a problem from a practical viewpoint, as the need for resection of such tissues would be clinically indisputable. In tissues where the clinical viability remains in doubt, however, PtO_2 measurement should be of considerable practical benefit.

1 Clark LC. Monitor and control of blood and tissue oxygen tensions. *Trans Am Soc Artif Intern Organs* 1956; **2**: 41–8.
2 Piasecki C. A new method for the assessment of gut viability. *Br J Surg* 1981; **68**: 319–22.
3 Locke R, Hauser CJ, Shoemaker WC. The use of surface-oximetry to assess bowel viability. *Arch Surg* 1984; **119**: 1252–6.
4 Foster ME, Laycock JRD, Silver IA, Leaper DJ. Hypovolaemia and healing in colonic anastomoses. *Br J Surg* 1985; **72**: 831–4.
5 Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. *Br J Surg* 1985; **72**: 606–9.
6 Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension measurement as a predictor of colonic anastomotic healing. *Dis Colon Rectum* 1987; **30**: 867–71.
7 Sheridan WG, Lowndes RH, Young HL. Intraoperative tissue oximetry in the human gastrointestinal tract. *Am J Surg* 1990; **159**: 314–9.
8 Bussemaker JB, Lindeman J. Comparison of methods to determine viability of small intestine. *Ann Surg* 1972; **176**: 97–101.
9 Katz S, Wohab A, Murray W, Williams LF. New parameters of viability in ischaemic bowel disease. *Am J Surg* 1974; **127**: 136–41.
10 Wright CB, Hobson RW. Prediction of intestinal viability using doppler ultrasound techniques. *Am J Surg* 1975; **129**: 642–5.
11 Sheridan WG, Lowndes RH, Young HL. Tissue oximetry as an assessment of perfusion in the human gastrointestinal tract. In: Price R, Evans JA, eds. *Blood flow measurement in clinical diagnosis*. London: Biological Engineering Society, 1988: 130–7.
12 Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesions in low flow states. A morphological, hemodynamic and metabolic appraisal. *Arch Surg* 1970; **101**: 478–83.
13 Mann A, Fazio VW, Lucas FV. A comparative study of the use of fluorescein and the Doppler device in the determination of intestinal viability. *Surg Gynecol Obstet* 1982; **154**: 53–5.
14 Cooperman M, Martin EW, Evans WE, Carey LC. Assessment of anastomotic blood supply by Doppler ultrasound in operations upon the colon. *Surg Gynecol Obstet* 1979; **149**: 15–6.
15 Ahn H, Lindhagen J, Nilsson GE, Salerud EG, Jodal M, Lundgren O. Evaluation of laser Doppler flowmetry in the assessment of intestinal blood flow in the cat. *Gastroenterology* 1985; **88**: 951–7.
16 Marfuggi RA, Greenspan M. Reliable intraoperative prediction of intestinal viability using a fluorescent indicator. *Surg Gynecol Obstet* 1981; **152**: 33–5.
17 Carter MS, Fantini GA, Sammartano RJ, Mitsudo S, Silverman DG, Boley SJ. Qualitative and quantitative fluoroscein fluorescence in determining intestinal viability. *Am J Surg* 1984; **147**: 117–23.
18 Hulten L, Jodal M, Lindhagen J, Lundgren O. Colonic blood flow in cat and man analysed by an inert gas washout technique. *Gastroenterology* 1976; **70**: 36–44.
19 Papachristou D, Fortner JG. Prediction of intestinal viability by intra-arterial dye injection: a simple test. *Am J Surg* 1976; **132**: 572–4.
20 Moosa AR, Skinner DB, Stark Violet, Hoffner P. Assessment of bowel viability using 99m Technetium-tagged albumin microspheres. *J Surg Res* 1974; **16**: 466–72.
21 Nxumalo JL, Teranaka M, Shenk WG. Hepatic blood flow measurement III: total hepatic blood flow measured by ICG clearance and electromagnetic flowmeters in a canine septic shock model. *Ann Surg* 1978; **187**: 299–302.
22 Fatt I. An ultramicro oxygen electrode. *J Appl Physiol* 1964; **19**: 326–9.
23 Silver IA. Problems in the investigation of tissue oxygen microenvironment. In: Gould RF, ed. *Chemical engineering in medicine*. Washington DC: American Chemical Society, 1973: 343–51.
24 Gillett ER, Sheridan WG, Mansel RE. Acute intestinal failure secondary to simultaneous occlusion of the coeliac axis and

the superior mesenteric artery. *J R Coll Surg Edinb* 1989; **34**: 166–7.
25 Bates Mary Louise, Feingold A, Gold MI. The effects of anaesthetics on an in-vivo oxygen electrode. *Am J Clin Pathol* 1975; **64**: 448–51.
26 Eberhard P, Mindt W. Interference of aneasthetic gases at skin surface sensors for oxygen and carbon dioxide. *Crit Care Med* 1981; **9**: 717–20.
27 Whalen WJ, Riley J, Nair P. A microelectrode for measuring intracellular PO_2. *J Appl Physiol* 1967; **23**: 798–801.
28 Hill DK. Oxygen tension and the respiration of resting frog's muscle. *J Physiol (Lond)* 1948; **107**: 479–95.
29 Whalen WJ. Recent experience with an intracellular oxygen electrode. In: Kreuzer F, ed. *Progress in respiration Research*. Vol 3. *Oxygen pressure recording in gases, fluids and tissues*. Basel: Karger, 1989: 158–60.