Effect of intraluminal oxygen on experimental ischaemia of the intestine

K. SHUTE

From the Department of Surgery, St. Thomas' Hospital, London

SUMMARY The small bowel of 44 rats has been made ischaemic for 120 minutes by occluding the superior mesenteric artery. Gaseous oxygen was introduced into the small bowel lumen of 18 of the rats, and gaseous nitrogen into the bowel lumen of a further eight, 30 minutes after the start of the ischaemia. The mortality rate of the control group of 18 rats was 89%, significantly higher than the 39% mortality of the oxygen-treated rats ($P < 0.005$). None of the eight rats treated with intraluminal nitrogen survived. Histological changes caused by ischaemia were much reduced in the intestine of the oxygen treated rats. It is suggested that intraluminal oxygen might be a useful additional form of therapy for the treatment of the partially ischaemic bowel that may be retained after the resection of definitely non-viable ischaemic bowel at initial laparotomy.

Acute massive ischaemia of the small bowel accounts for approximately one in every 1000 admissions to general hospitals (Laufman and Scheinberg, 1942; Ottinger and Austen, 1967). It is usually caused by occlusion of the superior mesenteric artery (SMA) from thrombosis on atheroma or by embolism, but 10% of cases follow venous thrombosis (Britt and Cheek, 1969) and, in at least 30% of cases, no major vessel occlusion can be found (Williams *et al.*, 1967; Renton, 1972). These cases of non-occlusive intestinal ischaemia are usually associated with low flow states, particularly cardiac failure (Pierce and Brockenbrough, 1970).

The mortality of massive intestinal ischaemia remains above 80% (Ottinger and Austen, 1967; Hadded, 1974), and, as stated by Marston (1964) 'occlusion of the main artery to the small bowel leads to death more certainly, and often more quickly than occlusion of a main coronary artery'.

It has been shown experimentally (Shapiro *et al.*, 1958; Milliken *et al.*, 1965) that, after a critical period of SMA occlusion, restoration of the blood supply to the intestine leads to death more quickly than does continuation of the occlusion. This apparently paradoxical behaviour is probably caused by a number of factors, including haemorrhage from the damaged mucosa (Marston, 1963), gross metabolic acidosis (Robertson *et al.*, 1969), and flooding of the systemic circulation with vasoactive agents as a result of an increased permeability of the damaged mucosal cells (Nelson and Noyes, 1952; Lillehei and

Maclean, 1958; Kobold and Thal, 1963; Gurd, 1965; Lefer and Martin, 1970; Manohar and Tyagi, 1973).

It is extremely difficult to diagnose acute intestinal ischaemia in the early stages, and, by the time patients present for surgery, the condition is well advanced (Bergan, 1967; Boley *et al.*, 1973). Thus, if an experiment involving treatment is to be analogous to the human situation, therapy should be instituted only when ischaemia is well established, and be aimed not only at saving bowel, but also at reducing the mortality produced by the revascularisation.

Methods

Forty-four adult male Wistar rats weighing 300-400 g and fed on Spratts Rodent Feeding Diet (Lab diet No. 1) were anaesthetised with intramuscular droperidol (2 mg) and fentanyl (0.05 mg). Midline laparotomy was performed and the base of the superior mesenteric artery (SMA) was isolated, preserving the splanchnic nerves, and occluded with a small bulldog clamp (Fig. 1). The animals were divided into two groups.

CONTROL GROUP

In 18 rats the arterial clamp was left in place for 120 minutes and then released.

TEST GROUP A

In another 18 rats 5 ml 100% gaseous oxygen in a plastic syringe was introduced through the wall of the small intestine with a 25 gauge needle at four roughly

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peared in the distal blood vessels. Distension of the intestine was not marked, and much less haemorrhage was seen from the mucosa of the bowel which was opened before revascularisation in four representative animals.

MICROSCOPIC CHANGES

THIRTY MINUTES AFTER SMA OCCLUSION—BOTH GROUPS (Fig. 2)

The tips of the villi were lost but the bases of the villi and crypts were intact. There was minor haemorrhage into the mucosa. The muscular and serous coats were intact.

FIVE MINUTES AFTER RELEASING THE SMA OCCLUSION

Control group (Fig. 3)

There was total loss of the normal architecture of the villi and crypts, many of which had sloughed. The structureless mucosa and submucosa were invaded by leucocytes and gross haemorrhage had occurred into both layers and into the lumen. The muscular and serosal layers were oedematous and minor haemorrhages had occurred into these layers.

Test group A (Fig. 4)

The changes were the same as those seen after only 30 minutes of SMA occlusion.

RESPIRATION (Fig. 5)

Control group

The rate of respiration increased gradually during the period of SMA occlusion. After releasing the arterial clamp the respiratory rate rose sharply initially, then more gradually until shortly before the death of the animal. At this time respiration became increasingly shallow, slower and often periodic.

Test group A

A gradual rise in the rate of respiration similar to that seen in the untreated group occurred, until the injection of the oxygen. Thereafter the rate of increase diminished. When the arterial clamp was released there was a further increase in the rate of respiration, but this was less than that of the untreated rats. After one hour the rate of respiration gradually returned to normal, except in those rats which died.

Statistical comparison of the rates of respiration (Student's *t* test) does not show any significant difference between the two groups until immediately before the release of the SMA clamp, when the mean rates of respiration were 51 per minute in the control

Fig. 2 *The microscopic appearance of the intestine after 30 minutes' SMA occlusion. The villi have been lost but the crypts are intact. Minor haemorrhage has occurred into the mucosa.*

Fig. 3 *The microscopic appearance of control rat intestine after release of the SMA clamp at 120 minutes. The crypts have disappeared and the mucosa and submucosal layers are without structure. Gross haemorrhage has occurred into both layers and into the lumen.*

group and 44 per minute in the test group ($P = 0.02$). Five minutes and one hour after releasing the arterial clamp the difference between the groups is more significant ($P = 0.002$ and 0.005 respectively).

MORTALITY RATE AT 48 HOURS (Table)

Sixteen of the untreated rats (89%) and seven of the oxygen treated rats (39%) were dead 48 hours after releasing the SMA occlusion. Statistical comparison of these results (χ^2 test) shows that this is a significant difference— $P = 0.005$.

The eight rats treated with the intraluminal nitrogen all died within 48 hours of releasing the SMA occlusion.

Fig. 4 Oxygen treated intestine after release of the SMA clamp at 120 minutes. The crypts have been preserved and little further change has occurred from that seen in Fig. 2.

Fig. 5 Respiratory changes during and after occlusion.

DURATION OF SURVIVAL (Fig. 6)

Control group

Sixteen rats died within 48 hours at times varying from 10 minutes to six hours after releasing the arterial clamp. The mean survival period for all animals was 2.7 ± 1.8 hours.

Test group A

Seven rats treated with intraluminal oxygen died from 4.5 to 16 hours after release of the arterial

Table Mortality at 48 hours after occlusion

	Rats (no.)	Deaths		P
		(no.)	(%)	
Control group Untreated	18	16	89	< 0.005
Test group A Oxygen treated	18	7	39	
Test group B Nitrogen treated	8	8	100	< 0.005

Fig. 6 Duration of survival after release of clamp of animals dying before 48 hours.

clamp, with a mean survival period of 9.25 ± 4.5 hours.

The difference between the control and test groups is highly significant— $P < 0.005$ (Student's *t* test).

NECROPSY RESULTS

The findings in all animals from both groups which died before 48 hours were similar. There was sero-sanguineous fluid present in the peritoneal cavity. Most of the small intestine was plum coloured and the lower ileum was clearly gangrenous. The lumen of the small bowel was filled with altered blood.

In the 13 animals which survived to 48 hours after releasing the SMA occlusion, there were fibrous adhesions between the loops of the bowel and the laparotomy incision. The intestine appeared otherwise normal, but in all survivors there were occasional small abscesses between adjacent loops of bowel.

Discussion

Many attempts have been made to decrease the mortality of acute intestinal ischaemia but, although some successes have been claimed in the experimental animal, there has been little improvement in the human situation (Boley *et al.*, 1973; Haddad, 1974). Bounos *et al.* (1965) have shown that ischaemia of the small intestine causes necrosis of the bowel which begins at the tips of the villi and gradually progresses toward the serosal surface. Chiu *et al.* (1970) instilled 10% glucose into ischaemic sacs of canine small intestine and not only prevented further necrosis of the mucosa, but possibly also reversed established damage. These workers showed that, although the cellular integrity could be maintained by anaerobic glycolysis, it led to an accumulation of metabolites and an undesirable lactic acidosis. Little attention had been given to the possibility of giving oxygen directly to the cells until 1973 when Ahren and Haglund perfused isolated loops of ischaemic cat small intestine with oxygenated saline and showed that this reduced the histological changes produced by ischaemia.

During a study of the effect on intestinal blood flow of distension of the intestine produced by injecting air into the lumen, Boley *et al.* (1969) noticed that, with intraluminal pressures high enough to reduce the total bowel blood flow by 80%, the bowel remained pink. They claimed that this appearance was caused by continued blood flow to the serosa together with a raised oxygen content in the venous blood, but it is equally possible that the oxygen in the injected air was diffusing into the tissues directly supporting metabolism and oxygenating the haemoglobin in the blood vessels.

The experiments described in this paper demonstrate that, when oxygen is introduced into the lumen of ischaemic rat small intestine, it reduces the mortality by 50%. Histological evidence of ischaemia is also diminished. It is likely that the high concentration of oxygen in the lumen sustains the metabolism and integrity of the mucosal cells, thus decreasing both fluid and blood loss into the lumen and the absorption of toxic agents after revascularisation. Studies are continuing into the exact mechanism by which intraluminal oxygen exerts this beneficial effect.

A major difficulty facing surgeons dealing with acute ischaemia of the small intestine is the inability to predict accurately the viability of revascularised intestine (Bussemaker *et al.*, 1972; Skinner *et al.*, 1974). Intestine which is pink with visible pulsation in its walls may become gangrenous after its return to the abdomen. It has been suggested (Jacobson, 1971; Boley *et al.*, 1973) that this is due to a pro-

longed vasoconstriction after the absorption of toxic vasoactive materials from the lumen. If it were possible to keep the mucosa alive until this vasoconstriction had passed, or, in the case of non-occlusive intestinal ischaemia, until the general circulation were restored to normal limits, the bowel might be saved from irreparable damage. It is suggested that the administration of intraluminal oxygen, to maintain the mucosal integrity in these situations until an adequate blood supply has returned, might improve the survival rate in man.

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References

- Ahren, K., and Haglund, U. (1973). Mucosal lesions in the small intestine of the cat during low flow. *Acta Physiologica Scandinavica*, **88**, 541-550.
- Bergan, J. J. (1967). Recognition and treatment of intestinal ischemia. *Surgical Clinics of North America*, **47**, 109-126.
- Boley, S. J., Agrawal, G., Warren, A., Veith, F., Levowitz, B., Treiber, W., Dougherty, J., Schwartz, S., and Gliedman, M. (1969). Pathophysiologic effects of bowel distension on intestinal blood flow. *American Journal of Surgery*, **117**, 228-234.
- Boley, S. J., Sprayregen, S., Veith, F., and Siegelman, S. (1973). An aggressive roentgenologic and surgical approach to acute mesenteric ischemia. *Surgery Annual*, **5**, 355-378.
- Bounos, G., Brown, R., Mulder, D., Hampson, L., and Gurd, F. N. (1965). Abolition of 'tryptic enteritis' in the shocked dog. *Archives of Surgery (Chic.)*, **91**, 371-375.
- Britt, L., and Cheek, R. (1969). Nonocclusive mesenteric vascular disease: Clinical and experimental observations. *Annals of Surgery*, **169**, 704-711.
- Bussemaker, J., and Lindeman, J. (1972). Comparison of methods to determine viability of the small intestine. *Annals of Surgery*, **176**, 97-101.
- Chiu, C. J., Scott, H. J., and Gurd, F. N. (1970). Intestinal mucosal lesions in low flow states II. The protective effects of intraluminal glucose as energy substrate. *Archives of Surgery*, **101**, 484-488.
- Gurd, F. N. (1965). Metabolic and functional changes in the intestine in shock. *American Journal of Surgery*, **110**, 333-336.
- Haddad, H. (1974). Clinical features of ischemic bowel disease. *Canadian Journal of Surgery*, **17**, 435-437.
- Jacobson, E. D. (1971). Physiologic aspects of the intestinal circulation. In *Vascular Disorders of the Intestine*, pp. 19-24. Edited by S. J. Boley, S. S. Schwartz, and L. F. Williams Jr. Appleton-Century-Crofts: New York.
- Kobold, E., and Thal, A. (1963). Quantitation and identification of vasoactive substances liberated during various types of experimental and clinical intestinal ischemia. *Surgery, Gynecology and Obstetrics*, **117**, 315-322.
- Laufman, H., and Scheinberg, S. (1942). Arterial and venous mesenteric occlusion. Analysis of 44 cases. *American Journal of Surgery*, **58**, 84-92.
- Lefer, A., and Martin, J. (1970). Origin of myocardial depressant factor in shock. *American Journal of Physiology*, **218**, 1423-1427.
- Lillehei, R. C., and Maclean, L. D. (1958). Intestinal factor

- in irreversible endotoxic shock. *Annals of Surgery*, **148**, 513-525.
- Manohar, M., and Tyagi, R. (1973). Experimental intestinal ischemia shock in dogs. *American Journal of Physiology*, **225**, 887-892.
- Marston, A. (1963). Causes of death in mesenteric artery occlusion. *Annals of Surgery*, **158**, 952-959.
- Marston, A. (1964). Patterns of intestinal ischaemia. *Annals of the Royal College of Surgeons of England*, **35**, 151-181.
- Milliken, J., Nahor, A., and Fine, J. (1965). A study of the factors involved in the development of peripheral vascular collapse following release of the occluded superior mesenteric artery. *British Journal of Surgery*, **52**, 699-704.
- Nelson, R. M., and Noyes, H. E. (1952). Permeability of the intestine to bacterial toxins in hemorrhagic shock. *Surgical Forum*, **3**, 474-480.
- Ottinger, L., and Austen, W. G. (1967). A study of 136 patients with mesenteric infarction. *Surgery, Gynecology and Obstetrics*, **124**, 251-261.
- Pierce, G., and Brockenbrough, E. (1970). The spectrum of mesenteric infarction. *American Journal of Surgery*, **119**, 233-239.
- Renton, C. J. (1972). Nonocclusive intestinal infarction. *Clinical Gastroenterology*, **1**, 655-661.
- Robertson, G., Lyall, A., and Macrae, J. (1969). Acid-base disturbances in mesenteric occlusion. *Surgery, Gynecology and Obstetrics*, **128**, 15-20.
- Shapiro, P. B., Bronshter, B., Frank, E., and Fine, J. (1958). Host resistance to hemorrhagic shock. XI. Role of deficient flow through intestine in development of irreversibility. *Proceedings of the Society of Experimental Biology and Medicine*, **97**, 372-376.
- Skinner, D., Zarins, C., and Moossa, A. (1974). Mesenteric vascular disease. *American Journal of Surgery*, **128**, 835-839.
- Williams, L. F., Anastasia, L. F., Hasiotis, C. A., Bosniak, M. A., and Byrne, J. J. (1967). Nonocclusive mesenteric infarction. *American Journal of Surgery*, **114**, 376-381.