

## Sodium metabisulphite causes epithelial damage and increases sheep tracheal blood flow and permeability

U.M. Wells, Z. Hanafi, J.G. Widdicombe

*Sodium metabisulphite causes epithelial damage and increases sheep tracheal blood flow and permeability. U.M. Wells, Z. Hanafi, J.G. Widdicombe. ©ERS Journals Ltd 1996.*

**ABSTRACT:** Inhaled sodium metabisulphite (MBS) causes bronchoconstriction, cough and microvascular leakage. We have studied its effects on tracheal blood flow, potential difference (PD) and the permeability from tracheal lumen to venous blood of a low molecular weight hydrophilic tracer, <sup>99m</sup>Tc-labelled diethylenetriamine penta-acetic acid (<sup>99m</sup>Tc-DTPA) in anaesthetized sheep.

Flow was measured in a tracheal artery and blood from a cannulated tracheal vein collected for 5 min periods. The tracheal lumen was filled with Krebs-Henseleit solution (KH) containing <sup>99m</sup>Tc-DTPA for six to eight 15 min periods. During the third or fourth period, MBS (1, 20 or 100 mM) was washed into the tracheal lumen for 15 min.

MBS increased tracheal blood flow (venous flow ( $Q_v$ ), 5-10 min MBS exposure period: 1 mM -9±18% (n=3); 20 mM +16±5% (n=5; p<0.05); 100 mM +43±13% (n=5; p<0.05). It decreased PD in a concentration-dependent way. Venous <sup>99m</sup>Tc-DTPA concentration increased progressively to +266±176 and +958±321% 25-30 min after exposure to 20 and 100 mM MBS, respectively (p<0.05 for both). These effects were not blocked by luminal frusemide (3-7 mM) or flurbiprofen (100-500 μM). Histological sections showed changes to the epithelial cells and large intercellular spaces.

Thus, luminal sodium metabisulphite increases tracheal blood flow, reduces transmural potential difference and causes tracheal epithelial damage, leading to an increase in <sup>99m</sup>Tc-labelled diethylenetriamine penta-acetic acid permeability.

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Sodium metabisulphite (MBS) is a widely-used food preservative which, when ingested, causes bronchoconstriction in some asthmatics [1]. On inhalation it causes bronchoconstriction in man - asthmatics, atopics [2], and normals [3] - and also in animals [4, 5].

The mechanism of MBS-induced bronchoconstriction is still uncertain. In solution it forms the bisulphite ion and SO<sub>2</sub> [6]. Some [7], but not all [4, 8], studies in man have shown that the bronchoconstriction is blocked by muscarinic antagonists. Its effect is also inhibited by drugs that inhibit sensory nerves, *e.g.* nedocromil sodium [9, 10] and cromoglycate [9], and in the guinea-pig its action is reduced by capsaicin pretreatment [4] and inhibited by neurokinin 1 and 2 (NK<sub>1</sub> and NK<sub>2</sub>) receptor antagonists [11], suggesting the involvement of tachykinins released from sensory nerve endings.

MBS-induced bronchoconstriction is also blocked by frusemide [12, 13]. Frusemide is effective against a variety of other indirectly acting bronchoconstrictor stimuli, including distilled water aerosols [14], exercise [15] and allergen [16]. The mechanism appears to be unrelated to its diuretic action. The airway effects could be due to the release of the bronchodilator prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) [17]. However, more recent evidence has shown that flurbiprofen, a cyclo-oxygenase inhibitor, partly blocks

the effects of MBS on bronchoconstriction and also enhances the effect of frusemide [18]. This implies that MBS may cause the release of bronchoconstrictor prostaglandins.

Despite much research on inhaled MBS, relatively little is known about its other actions in the airway. It causes cough [8, 9], microvascular leakage in guinea-pigs *via* NK<sub>1</sub> receptors [19], and a short-lived increase in bronchial blood velocity in conscious sheep [20]. We have, therefore, studied the effects of MBS on tracheal blood flow, potential difference (PD) across the tracheal wall and on epithelial permeability to <sup>99m</sup>Tc-labelled diethylenetriamine penta-acetic acid (<sup>99m</sup>Tc-DTPA) in anaesthetized sheep. In separate experiments, the effects of either frusemide or flurbiprofen, given intraluminally, on the responses to MBS were investigated.

### Methods

Twenty six sheep were used in this study. The tracheae of the eight sheep used in preliminary experiments to test the effects of 1 and 20 mM MBS had first been exposed to hyperosmolar and hyposmolar Krebs-Henseleit solution (KH) in the same experiment [21] (see Protocol

section). Sheep used in the remaining 18 experiments and for the histological examination had not been exposed to osmotic stimuli.

### Experimental preparation

The experimental preparation has been described previously [21, 22]. Female sheep (25–30 kg) were anaesthetized with sodium pentobarbitone (initially 20 mg·kg<sup>-1</sup>, *i.v.*). Additional doses were given as required to maintain surgical anaesthesia [22]. The animals were positioned supine. The right external jugular vein was catheterized (8 FG; Portex) for administration of drugs. All animals were paralysed with an intravenous injection of gallamine triethiodide (1 mg·kg<sup>-1</sup> initially), and ventilated *via* a tracheal cannula inserted in the lower cervical trachea. The tidal volume was 12–16 ml·kg<sup>-1</sup> and the respiratory frequency 28 breaths·min<sup>-1</sup>. Additional neuromuscular blocking agent was given as necessary to suppress spontaneous breathing movements, and always in combination with additional anaesthetic. The right femoral artery was catheterized (8 FG; Portex) and the catheter was connected to a pressure transducer (P231D; Gould) for measurement of systemic arterial blood pressure.

A tracheal branch from the left carotid artery was isolated, and the left carotid artery was ligated cranial to this so that blood would only perfuse the trachea (fig. 1). Branches perfusing other tissues were tied off. To ensure an intact blood supply to the brain and a normal pressure in the carotid sinuses, the occluded segment of the left carotid artery was by-passed from the right carotid artery using a plastic Y-tube connection.

An electromagnetic flow-probe was inserted between two catheters into the left carotid artery supplying the isolated region of perfusion. The flow-probe was connected to a square-wave electromagnetic flow meter (Carolina Medical Electronics) and tracheal arterial flow

was recorded continuously throughout the experiment. The mean flow for each 5 min period was later calculated, using measurements taken at 1 min intervals.

A vein on the left side and draining the perfused region of the trachea was isolated and catheterized (3 FG; Portex). Venous blood was collected for 5 min periods continuously throughout the experiment. The distribution of perfused tracheal circulation was tested by close-arterial injection of Evans blue dye diluted in 0.15 M NaCl.

The cervical trachea was isolated by inserting two balloon-cuffed tracheostomy tubes into the trachea, one below the larynx, the other just above the low cervical tracheostomy supplying airflow to the lungs. The enclosed segment was usually 10–15 cm long and included the area perfused by the isolated tracheal artery.

### Protocols

**MBS concentration-response study.** In each experiment, the tracheal lumen was filled with a known volume of KH containing <sup>99m</sup>Tc-DTPA. At the end of each 15 min period, the KH was withdrawn and replaced. After two or three control periods, MBS (either 1 mM (n=3), 20 mM (n=5) or 100 mM (n=6)) was washed into the tracheal lumen for 15 min in KH containing <sup>99m</sup>Tc-DTPA. Two or three control periods then followed. Each concentration of MBS was given only once per experiment and each sheep received only one concentration of MBS. PD across the tracheal wall was measured in the 100 mM MBS experiments.

In the preliminary experiments using 1 and 20 mM MBS, the trachea was first exposed to hyperosmolar and hyposmolar KH, given in a random order with two control periods between each stimulus. These results with non-isosmolar solutions have been published previously [21]. The tracheae were left for 30 min after the second osmotic stimulus before MBS was applied. Exposure to the osmotic stimuli did not appear to affect the integrity of the epithelium, since the permeability coefficient for <sup>99m</sup>Tc-DTPA during the control period prior to MBS ( $-10.0 \pm 4.6 \times 10^{-7}$  cm·s<sup>-1</sup>; n=8) was not significantly different to that during the control prior to the initial osmotic stimulus in these experiments ( $-5.9 \pm 1.8 \times 10^{-7}$  cm·s<sup>-1</sup>). This value is similar to the baseline permeability coefficient ( $-11.3 \times 10^{-7}$  cm·s<sup>-1</sup>) in the original study using <sup>99m</sup>Tc-DTPA in this model [22]. In addition, histological specimens from other experiments in which the trachea was exposed only to osmotic stimuli revealed an intact epithelium. We therefore feel justified in treating these results in the same way as those from tracheae exposed to 100 mM MBS.

In two further experiments, the trachea was exposed to KH, containing <sup>99m</sup>Tc-DTPA, acidified with hydrochloric acid to a pH of 5.6–5.7. In other respects, the protocol was similar to that of the 100 mM MBS experiments.

**Frusemide and flurbiprofen experiments.** The effects of 3–7 mM luminal frusemide (n=3) and of 100–500 μM flurbiprofen (n=3) on the response to 100 mM MBS were tested in separate experiments. The protocol was identical to the 100 mM MBS experiments, except that either frusemide or flurbiprofen (in KH containing <sup>99m</sup>Tc-DTPA) was present in the tracheal lumen, starting 30 min before addition of the MBS.

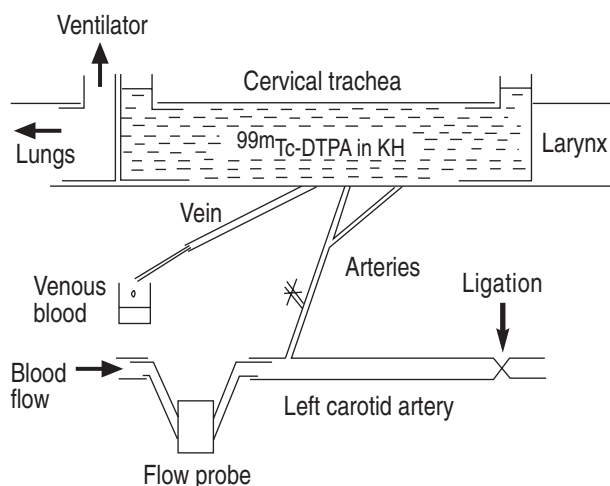


Fig. 1. — Experiment set-up. The tracheal lumen was filled with <sup>99m</sup>Tc-DTPA in Krebs-Henseleit solution (KH). Flow through a single tracheal artery was measured using a flow probe in the left carotid artery. Blood from a single tracheal vein was collected continuously for 5 min periods. The weight of each venous sample and the venous <sup>99m</sup>Tc-DTPA concentration were measured. <sup>99m</sup>Tc-DTPA: <sup>99m</sup>technetium-labelled diethylenetriamine penta-acetic acid.

### Measurement of $^{99m}\text{Tc}$ -DTPA output

Venous flow per 5 min was determined by weighing the venous blood samples. The venous  $^{99m}\text{Tc}$ -DTPA concentration (counts $\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ ) in each sample was measured using a gamma counter (Beckman Gamma 5500). The  $^{99m}\text{Tc}$ -DTPA concentration was multiplied by venous flow to obtain the total  $^{99m}\text{Tc}$ -DTPA output (counts $\cdot\text{min}^{-2}$ ) in the catheterized vein for each 5 min period. Thus, three measurements of venous  $^{99m}\text{Tc}$ -DTPA output were obtained during each 15 min exposure to KH. The background level of  $^{99m}\text{Tc}$ -DTPA in systemic arterial blood was measured in femoral arterial samples taken at 15 or 30 min intervals throughout the experiment.

### Measurement of potential difference

The electrical PD across the tracheal wall was measured in some experiments using two calomel reference electrodes. These were filled with 3.8 M KCl and placed in separate beakers of the same solution. Electrical contact was made with the preparation using two agar bridges constructed from polyethylene tubing (0.5 mm internal diameter) filled with 3.8 M KCl in 2.5% (w/v) agar solution. The end of one agar bridge was inserted into the lumen of the isolated segment of trachea *via* the lower cannula; the end of the other bridge was placed in a pool of saline touching the external tracheal wall. Output from the two electrodes was *via* a high input impedance buffer amplifier ( $>10^9 \text{ M}\Omega$ ) and displayed on a digital voltmeter.

### Measurement of osmolality and pH of MBS solutions

The osmolality of KH containing MBS at different concentrations was measured using a Wescor 5100C vapour pressure osmometer. pH was measured using a Jenway 3020 pH meter. The pH and osmolality of solutions containing 1, 20 and 100 mM are shown in table 1.

### Histology

Sections of the trachea were taken at the end of three of the 100 mM MBS experiments, 60 min after removal of the MBS from the tracheal lumen. Sections were also

taken from two sheep exposed to 20 mM MBS (following two 15 min periods with KH in the tracheal lumen). One trachea was removed immediately after the 15 min exposure period. The other trachea was taken 30 min after removal of the MBS, the lumen having been filled with KH during this time. Finally, sections were taken from one control trachea which was exposed to KH only for 90 min. Only a single KH control was carried out, since similar controls have been performed in earlier studies, *e.g.* [23], and have shown the epithelium to be normal and intact.

All sections were taken immediately postmortem and were fixed in 20% formal saline for standard paraffin processing. They were then stained using a routine haematoxylin and eosin stain. Photomicrographs were obtained using a Zeiss photomicroscope and Ilford Pan F 50 black and white film.

### Analysis of results

The results for tracheal arterial flow, venous flow,  $^{99m}\text{Tc}$ -DTPA concentration and  $^{99m}\text{Tc}$ -DTPA output during each 5 min of the 15 min test and post-test periods were calculated as mean ( $\pm\text{SEM}$ ) percentage changes from the first 5 min of the pretest controls. The statistical significance of changes was tested using Student's two-tailed paired t-tests by comparison with the values of the final 5 min of the 15 min pretest controls. In addition, the effects of MBS on  $^{99m}\text{Tc}$ -DTPA concentration and output were tested using the Wilcoxon test by comparison with the values of the final 5 min of the preceding control. The magnitude of the effects of 20 and 100 mM MBS on blood flow were compared using Student's two-tailed unpaired t-test.

PD results were expressed as the mean percentage of the baseline value during the first 5 min of the control period preceding MBS delivery. The maximum change measured during each 5 min period was used.

The permeability coefficients for  $^{99m}\text{Tc}$ -DTPA were calculated during test periods and also during the control period immediately preceding the first test period in each experiment. The permeability coefficient,  $P = - (dQ/dt) / (S \times \Delta C)$ , where  $dQ/dt$  is the output of  $^{99m}\text{Tc}$ -DTPA in counts $\cdot\text{min}^{-2}$ ,  $\Delta C$  is the concentration gradient of  $^{99m}\text{Tc}$ -DTPA from tracheal lumen to venous blood in counts $\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$ , and  $S$  is that part of the surface area of the isolated trachea that is perfused. Using Evans blue injections, the perfused surface area was shown in an earlier study, using similar variety and weights of sheep, to be 30  $\text{cm}^2$ . This value, which is slightly smaller than the total surface area of the tracheal segment [22], has been used here. Because only part of the arterial blood is collected in the cannulated vein, it is assumed that total  $^{99m}\text{Tc}$ -DTPA uptake is the measured output times the ratio of arterial blood inflow to venous outflow [22]. The mean ( $\pm\text{SEM}$ ) permeability coefficients for each stimulus were compared using the Student's t-test.

### Drugs

Evans blue dye was obtained from Aldrich Chemical Corp.; heparin sodium from CP Pharmaceuticals; galamine triethiodide and sodium pentobarbitone (Sagatal)

Table 1. — pH and osmolality of MBS solutions

	n	pH	Osmolality $\text{mOsm}\cdot\text{kg}^{-1}$
1 mM MBS	3	7.3 $\pm$ 0.1	284 $\pm$ 1
20 mM MBS	4	6.4 $\pm$ 0.1	331 $\pm$ 3
100 mM MBS	4	5.6 $\pm$ 0.3	489 $\pm$ 4
100 mM MBS + 500 $\mu\text{m}$ flurbiprofen	3	5.5 $\pm$ 0.1	-
100 mM MBS + 7 mM frusemide	1	5.2	-
Acidified KH	2	5.6 and 5.7	280 and 284

Data are presented as mean $\pm$ SEM. MBS: sodium metabisulphite; KH: Krebs-Henseleit solution.

from May & Baker; sodium metabisulphite from BDH Chemicals; flurbiprofen (2-fluoro- $\alpha$ -methyl-4-biphenyl acetic acid) from Sigma; and frusemide from both Sigma and Antigen Pharmaceuticals. The composition (mM) of the KH was: NaCl 120.8; KCl 4.7;  $\text{KH}_2\text{PO}_4$ , 1.2;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.2;  $\text{NaHCO}_3$  24.9;  $\text{CaCl}_2$ , 2.4; and glucose 5.6.

**Results**

*MBS concentration-response study*

**Baseline blood pressure and tracheal blood flow.** Mean systemic arterial pressure was  $107 \pm 4$  mmHg at the start of the experiment and  $101 \pm 6$  mmHg at the end ( $n=14$ ). Mean baseline tracheal arterial flow in the 15 min before MBS exposure was  $5.3 \pm 0.7$  mL $\cdot$ min $^{-1}$  ( $n=10$ , since arterial flow was not measured in all 100 mM MBS experiments (see below)). Mean baseline tracheal venous flow was  $0.58 \pm 0.09$  mL $\cdot$ min $^{-1}$  ( $n=14$ ).

**Tracheal blood flow.** One millimolar MBS in the tracheal lumen had no effect on arterial (fig. 2) or venous flows (fig. 3). Twenty millimolar MBS significantly increased both arterial and venous flows, with the maximum effect occurring during the middle 5 min of the exposure period (figs. 2 and 3). A vasoconstriction occurred in the final 5 min of the following control period. One hundred millimolar MBS produced a significantly greater increase than 20 mM MBS in arterial flow (fig. 2) and in venous flow during the first 5 min of exposure (fig. 3).

**Potential difference.** PD was not measured in the 1 mM MBS experiments. In the remaining experiments, mean

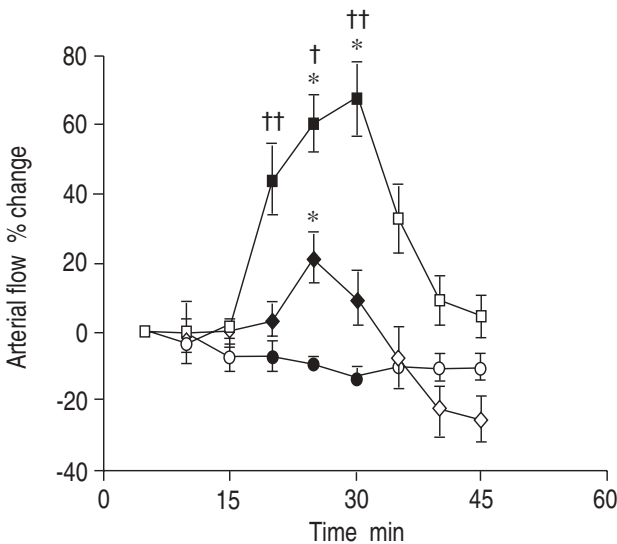


Fig. 2. — Percentage change in tracheal arterial flow in response to 1, 20 and 100 mM sodium metabisulphite (MBS) in the tracheal lumen in separate experiments. Filled symbols represent period of MBS exposure. Results are normalized against value of first 5 min of control preceding MBS. Each point represents mean value of a 5 min period. \*:  $p < 0.05$  compared with 10–15 min of control preceding MBS exposure (paired t-test). †:  $p < 0.05$ ; ††:  $p < 0.01$  (100 mM vs 20 mM, unpaired t-test). —□—: 100 mM MBS ( $n=3$ ); —◇—: 20 mM MBS ( $n=5$ ); —○—: 1 mM MBS ( $n=3$ ).

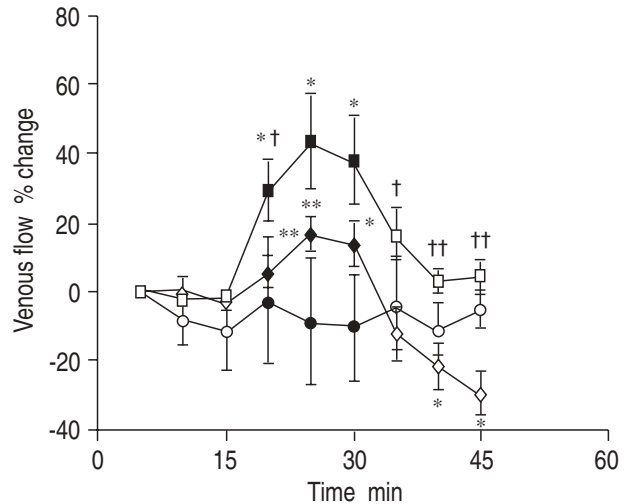


Fig. 3. — Percentage change in tracheal venous flow in response to 1, 20 or 100 mM sodium metabisulphite (MBS) in the tracheal lumen. Filled symbols represent period of MBS exposure. Results are normalized against value of first 5 min of control preceding MBS. Each point represents mean value of a 5 min period. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$  compared with 10–15 min of control preceding MBS exposure (paired t-test); †:  $p < 0.05$ ; ††:  $p < 0.01$  (20 mM vs 100 mM MBS, unpaired t-test). —□—: 100 mM MBS ( $n=6$ ); —◇—: 20 mM MBS ( $n=5$ ); —○—: 1 mM MBS ( $n=3$ ).

baseline PD in the control preceding MBS exposure was  $-26.6 \pm 2.4$  mV ( $n=10$ ). Twenty millimolar MBS significantly reduced PD during the exposure period (fig. 4); measurements were not made after exposure. One hundred millimolar MBS reduced mean PD to close to zero (fig. 4). No significant change in PD occurred during the subsequent 30 min.

**$^{99m}\text{Tc-DTPA}$  concentration and output.** One millimolar MBS had no effect on venous  $^{99m}\text{Tc-DTPA}$  concentration (fig. 5a). Twenty millimolar MBS caused a small reduction in the  $^{99m}\text{Tc-DTPA}$  concentration of  $-52 \pm 8\%$  during the middle 5 min of the exposure period (fig. 5).

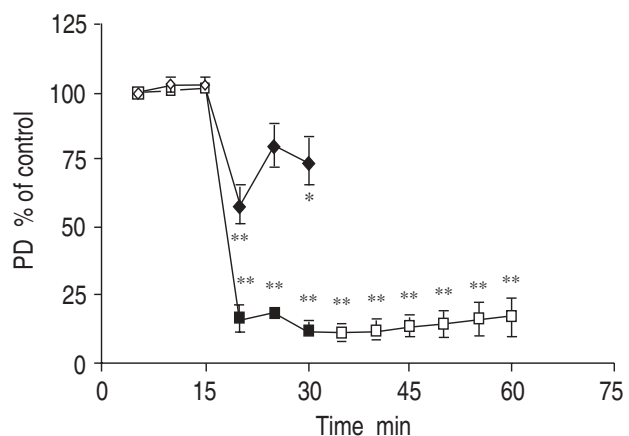


Fig. 4. — Potential difference (PD) across the tracheal wall, expressed as percentage of control, in response to luminal 20 or 100 mM sodium metabisulphite (MBS). Filled symbols represent period of MBS exposure. Results are normalized against value of first 5 min of preceding 15 min control. Each point represents maximum change during 5 min period; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$  compared with 0–5 min of control preceding stimulus (paired t-test). —□—: 100 mM MBS ( $n=6$ ); —◇—: 20 mM MBS ( $n=4$ ).

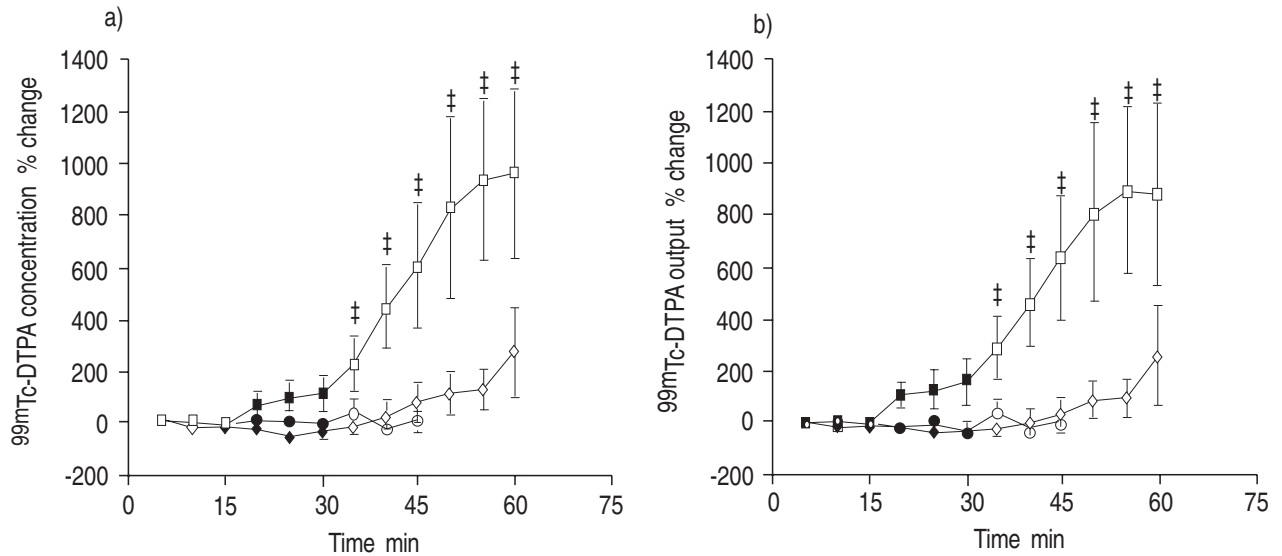


Fig. 5. – Percentage change in a) venous <sup>99m</sup>Tc-DTPA concentration; b) venous <sup>99m</sup>Tc-DTPA output in response to 1, 20 or 100 mM MBS in the tracheal lumen. Filled symbols represent period of MBS exposure. Results are normalized against value of first 5 min of control preceding MBS. Each point represents mean concentration over a 5 min period. ‡: p<0.05 compared with 10–15 min of control preceding MBS exposure (Wilcoxon test). —□— : 100 mM MBS (n=6); —◇— : 20 mM MBS (n=5); —○— : 1 mM MBS (n=3). <sup>99m</sup>Tc-DTPA: <sup>99m</sup>technetium-labelled diethylenetriamine penta-acetic acid; MBS: sodium metabisulphite.

This was followed after removal of MBS by a slow onset increase in <sup>99m</sup>Tc-DTPA concentration in three out of five experiments; 30 min after removal of the MBS the concentration had increased to a mean of +265±176% (n=5). None of these changes was statistically significant.

During exposure to 100 mM MBS a mean increase both in venous <sup>99m</sup>Tc-DTPA concentration and output occurred (fig. 5a and b). These continued to rise in all experiments after removal of the MBS; 30 min after removal of the MBS the mean increase was 958±321% for <sup>99m</sup>Tc-DTPA concentration and 865±346% for <sup>99m</sup>Tc-DTPA output (n=6; p<0.05 for both, Wilcoxon test).

Table 2. – Effect of flurbiprofen and frusemide on MBS-induced changes in tracheal venous flow

	n	Venous flow % change <sup>#</sup>		
		0–5 min	5–10 min	10–15 min
100 mM MBS	6	29±9*	43±13*	37±13*
MBS + flurbiprofen	3	30±11	59±16	34±6*
MBS + frusemide	3	41±13	37±6**	43±3**

Values are presented as mean±SEM. #: % increases from first 5 min of preceding 15 min control. \*: p<0.05; \*\*: p<0.01, paired t-test (vs 10–15 min control). MBS: sodium metabisulphite.

Table 3. – Effect of flurbiprofen and frusemide on MBS-induced changes in tracheal PD

	n	PD % control <sup>#</sup>								
		Control			100 mM MBS			Control		
		0–5 min	5–10 min	10–15 min	0–5 min	5–10 min	10–15 min	0–5 min	5–10 min	10–15 min
100 mM MBS	6	100	101±1	101±5	16±4**	16±2**	11±3**	10±2**	10±3**	12±3**
MBS + flurbiprofen	3	100	97±3	97±3	13±1**	10±3**	7±2**	3±1**	4±2**	4±2**
MBS + frusemide	3	100	99±1	93±5	20±9*	14±7*	13±7*	20±17	20±19	16±15

Values are presented as mean±SEM. #: % changes from first 5 min of 15 min control preceding MBS. \*: p<0.05; \*\*: p<0.01, paired t-test (vs 10–15 min control preceding MBS). MBS: sodium metabisulphite.

**Acidified KH.** In two experiments, KH acidified with hydrochloric acid to 5.6–5.7 (equivalent to the pH of 100 mM MBS) was placed in the tracheal lumen (table 1). It reduced PD by 7% and 19%, respectively, during exposure, and PD returned to baseline in the following control period. It did not increase arterial or venous flows. Increases in <sup>99m</sup>Tc-DTPA concentration and output similar to that in response to MBS did not occur.

*Effects of frusemide and flurbiprofen*

Neither luminal frusemide (3–7 mM; n=3) nor luminal flurbiprofen (100–500 µM; n=3) altered the effects of 100 mM MBS on tracheal venous flow (table 2) or PD (table 3). Furthermore, neither drug significantly altered baseline blood flow or PD (results not shown).

*Histology*

In sections taken from a control trachea exposed only to KH for 45 min, a well-ordered epithelium was apparent (fig. 6a). The layer of basal cells was clearly visible as were mucous cells within the epithelium. Submucosal glands and blood vessels appeared normal.

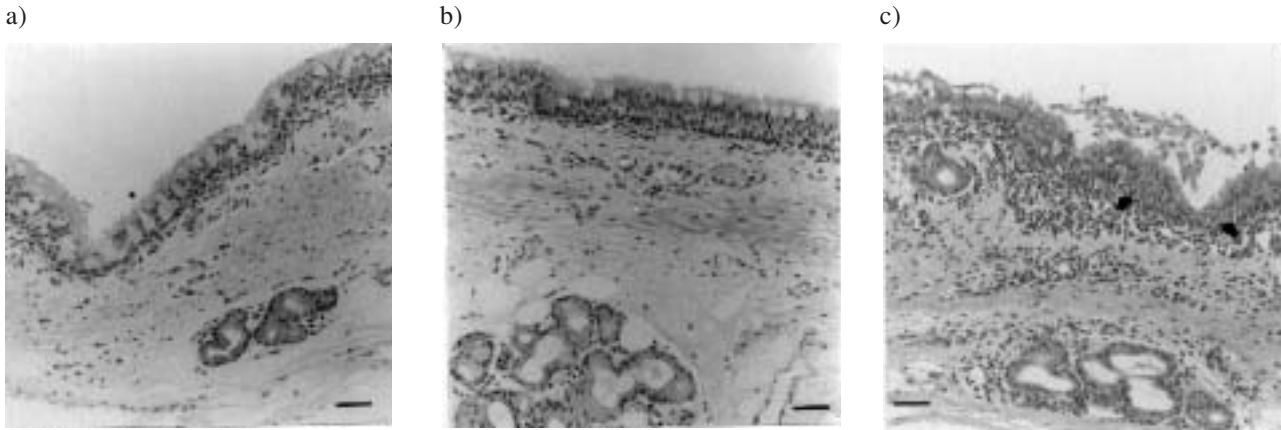


Fig. 6. — Light microscope sections showing epithelium of: a) control trachea exposure only to KH; b) trachea exposed to 20 mM MBS for 15 min followed by KH for 30 min; and c) trachea exposed to 100 mM MBS for 15 min. Arrows show intercellular spaces. The tracheae were exposed to KH for 30 min before addition of MBS. MBS: sodium metabisulphite; KH: Krebs-Henseleit solution. (Internal scale bar = 50  $\mu\text{m}$ ).

In sections from two tracheae exposed to 20 mM MBS (fig. 6b) the epithelium appeared slightly abnormal. The deeper half of the epithelium contained numerous cell nuclei. In one trachea these were well ordered, but in the other trachea the arrangement of the nuclei was more irregular. The upper half of the epithelium appeared amorphous and stained intensely pink. Although individual cell outlines could not be distinguished, a few mucous cells were present in this layer. Cilia were visible overlying the surface of the epithelium.

One of the tracheae exposed to 100 mM MBS had an appearance similar to that described for the 20 mM specimens. Sections from the other two tracheae showed a greater degree of epithelial damage. The epithelia contained cells with an elongated, shrunken appearance (fig. 6c), and numerous enlarged intercellular spaces could be seen. Cilia were present on the surface of the epithelium. In a few areas the epithelium was completely eroded. As with the 20 mM specimens, the submucosal glands and blood vessels appeared normal. In three tracheae (one exposed to 20 mM MBS, the other two to 100 mM MBS) there were numerous subepithelial inflammatory cells. These were present in smaller numbers in sections from the other tracheae exposed to MBS.

### Discussion

Luminal MBS produced a rapid onset concentration-dependent increase in tracheal arterial and venous flows. A short-lived increase in bronchial blood velocity in response to inhaled MBS has also been observed in conscious sheep [20]. In addition, MBS produced epithelial damage, a reduction in PD and a large increase in  $^{99\text{m}}\text{Tc}$ -DTPA permeability.

The concentrations of MBS (1–100 mM) tested are low compared with those used in many studies, *e.g.* 130 mM–2.1 M [20], 20–200 mM [19], 32–526 mM [10]. However, our method using a fluid-filled trachea may provide a more sustained stimulus than studies which deliver a small volume of MBS at high concentration by aerosol. Small volumes will be diluted by mixing with airway surface liquid and perhaps also by water movement into the lumen as a result of the hyperosmolarity of the solution. Nevertheless, this may also be true in

our study, where, for these reasons, the concentration of MBS in the solution at the interface with the epithelium may be less than that in bulk solution.

The PD results suggest that the highest concentration of MBS disrupts tight junctions between epithelial cells and may cause shedding of cells. These results contrast with the lack of effect of aerosolized MBS at bronchoconstrictor doses on nasal PD in asthmatics [12]. It is not clear whether the large numbers of inflammatory cells observed in some sections are due to MBS exposure or to a chronic infection. However, the large intercellular gaps in the epithelium were only seen in sections from MBS-treated tracheae.

The sustained increases in venous  $^{99\text{m}}\text{Tc}$ -DTPA concentration after removal of the MBS are the result of loss of the barrier effect of the epithelium and also occur with other agents which cause epithelial damage, *e.g.* Triton X-100, a detergent that causes erosion of the tracheal epithelium leaving only a layer of basal cells [23], and hydrogen peroxide (U.M. Wells and J.G. Widdicombe, unpublished results). The magnitude of the percentage increases was highly variable; this reflects the variation in baseline  $^{99\text{m}}\text{Tc}$ -DTPA permeability and possibly also the degree of epithelial damage. The mean increases 25–30 min after removal of the MBS were  $958 \pm 321\%$  for  $^{99\text{m}}\text{Tc}$ -DTPA concentration and  $865 \pm 346\%$  for  $^{99\text{m}}\text{Tc}$ -DTPA output. These increases were significant using the Wilcoxon test.

The prolonged increases in venous  $^{99\text{m}}\text{Tc}$ -DTPA concentration caused by MBS cannot be explained by a "squeezing" of luminal fluid into the interstitium during MBS-induced bronchoconstriction, because the tracheal segment was open at both ends and the luminal pressure would increase only slightly. Furthermore, increased pressure on the luminal side has little effect on the movement of low molecular weight solutes across the epithelium towards the submucosa [24].

Because epithelial damage changes the concentration gradient of  $^{99\text{m}}\text{Tc}$ -DTPA between lumen and venous blood, the  $^{99\text{m}}\text{Tc}$ -DTPA results are more usefully expressed as permeability coefficients (PDTPA). PDTPA in response to 20 mM MBS was not significantly increased (control:  $-2.8 \pm 1.6 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$ ; 30 min post-MBS:  $-3.1 \pm 0.8 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$  ( $n=5$ )). PDTPA could only be calculated for two 100 mM MBS experiments; the baseline values

increased approximately fourfold and 20 fold, respectively, to give values of  $-37 \times 10^{-7}$  and  $-149 \times 10^{-7}$  cm·s<sup>-1</sup> 30 min after MBS exposure. Corresponding increases in <sup>99m</sup>Tc-DTPA output in these two experiments were 281 and 1,607%. These changes are smaller than the mean change produced by Triton X-100 ( $-240 \times 10^{-7}$  cm·s<sup>-1</sup>) [23].

Twenty millimolar MBS initially caused a reduction in venous <sup>99m</sup>Tc-DTPA concentration (fig. 5). This was probably a consequence of the increase in blood flow, a negative relationship between blood flow and <sup>99m</sup>Tc-DTPA concentration and output having been demonstrated previously [21, 22]. The mechanism is not established. This initial decrease was not seen in response to 100 mM MBS, possibly because it caused epithelial damage more rapidly, so that any reduction in venous <sup>99m</sup>Tc-DTPA concentration due to the increased blood flow may be masked by the greater penetration of <sup>99m</sup>Tc-DTPA across the epithelium.

### Mechanisms

The mechanism of the changes in blood flow and epithelial permeability are unknown. They are unlikely to be due only to the pH of the MBS solutions, since KH acidified to pH 5.6–5.7 did not increase blood flow or <sup>99m</sup>Tc-DTPA permeability. Acidified KH also produced a much smaller change in PD (which was also reversible) than 100 mM MBS.

*Sulphur dioxide.* MBS in solution probably forms bisulphite ions and SO<sub>2</sub>, with the release of some H<sup>+</sup> ions [6]. SO<sub>2</sub> stimulates sensory nerves [25], so luminal MBS may increase blood flow *via* local axon reflexes by release of tachykinins. This is speculative, although MBS-induced bronchoconstriction in the guinea-pig is partly mediated *via* NK<sub>1</sub> and NK<sub>2</sub> receptors [11]. In addition to its effects on sensory nerves, SO<sub>2</sub> at high concentrations (500 ppm, 75 min) causes focal loss of ciliated cells, a decrease in PD, and an increase in permeability of solutes up to the size of inulin across dog tracheal epithelium [26]. Our results are consistent, therefore, with the known actions of SO<sub>2</sub>.

*Osmolarity.* Another possible cause of the increase in blood flow (but not of the increase in <sup>99m</sup>Tc-DTPA permeability) in response to MBS is the osmolarity of the MBS solutions (table 1), since hyperosmolarity increases tracheal blood flow [21]. This may be due to degranulation of mast cells [27], or to stimulation of sensory fibres [28]. However, neither bronchial blood velocity [20] nor tracheobronchial blood flow [29] is increased by inhalation of hypertonic aerosols. Furthermore, the MBS-induced increase in bronchial blood velocity is not blocked by histamine antagonists or nedocromil [20]. The apparent discrepancy between these studies may result from a difference in response of the vasculature between trachea and bronchi, or a difference in the total osmotic load imposed in the studies (see above). The increase in <sup>99m</sup>Tc-DTPA permeability is not due to the osmolarity of the MBS solutions because luminal hyperosmolarity reversibly decreases <sup>99m</sup>Tc-DTPA permeability [21]. The hyperosmolarity due to MBS in the

present results was far smaller than that used in the studies quoted above.

*Frusemide.* Luminal frusemide (4–7 mM) did not alter the effects of 100 mM MBS on blood flow nor did it change baseline flow. A similar lack of effect of frusemide delivered by aerosol on MBS-induced bronchial blood velocity was found by NICHOL *et al.* [20]. For reasons discussed above, direct comparison of the luminal concentration of frusemide used in our study with that given by aerosol is difficult but the concentration we used is greater than those used in studies *in vitro* [30, 31]. This suggests that the increase in blood flow may not be due to a central reflex, since inhaled frusemide blocks bronchoconstriction not only in response to MBS [13, 20, 32] but also to a variety of other stimuli which act indirectly, for example fog, exercise, allergen and adenosine monophosphate (see [33] for references). Bronchial blood velocity in response to MBS is also unaffected by cholinergic antagonists [20]. Frusemide also failed to block the effects of 100 mM MBS on PD or <sup>99m</sup>Tc-DTPA permeability.

*Flurbiprofen.* MBS may release prostaglandins from epithelium [18], as the cyclo-oxygenase inhibitor flurbiprofen taken orally partly inhibited MBS-induced bronchoconstriction in asthmatics [18]. However, in our study, luminal flurbiprofen had no effect either on MBS-induced changes in blood flow, PD or <sup>99m</sup>Tc-DTPA permeability. We used a concentration greater than that which blocks PGE<sub>2</sub> production in airway smooth muscle cells [34]. MBS may have other actions on epithelial cells, since slow-onset changes in <sup>99m</sup>Tc-DTPA permeability in response to 20 mM MBS have also been seen *in vitro* using cultured sheep tracheal cell sheets [35] (although whether this was an effect of pH alone was not tested). The mechanism is unknown.

### Summary

In summary, tracheal exposure to sodium metabisulphite increased arterial and venous blood flows, caused epithelial damage and reduced potential difference across the tracheal wall. The flux of <sup>99m</sup>Tc-diethylenetriamine penta-acetic acid was greatly increased by 100 mM sodium metabisulphite. These changes were not blocked by frusemide or by flurbiprofen. The mechanism of the effects of MBS is uncertain but is consistent with the known actions of SO<sub>2</sub>.

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