

# Peritoneal mesothelial hypoxia during pneumoperitoneum is a cofactor in adhesion formation in a laparoscopic mouse model

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**Objective:** To develop a laparoscopic mouse model to evaluate the hypothesis that mesothelial hypoxia during pneumoperitoneum is a cofactor in adhesion formation.

**Design:** Prospective randomized trials.

**Setting:** Academic research center.

**Animal(s):** One hundred thirty female Naval Medical Research Institute (NMRI) mice.

**Intervention(s):** Adhesions were induced by opposing monopolar lesions in uterine horns and pelvic side walls during laparoscopy and evaluated after 7 or 28 days under microscopic vision during laparotomy. The following pneumoperitoneum variables were assessed: duration (10 or 60 minutes), insufflation pressure (5 or 15 cm of water), insufflation gas (CO<sub>2</sub> or helium), and addition of oxygen (0–12%).

**Main Outcome Measure(s):** Adhesions were scored quantitatively and qualitatively for extent, type, and tenacity.

**Result(s):** Scoring of adhesions 7 or 28 days after laparoscopic surgery was comparable. Adhesions increased with duration of pneumoperitoneum and with insufflation pressure and decreased with the addition of oxygen. Half-maximal reduction of adhesions was obtained at 1.5% oxygen, whereas a maximal reduction required only 2%–3%. The effect of CO<sub>2</sub> and helium was similar.

**Conclusion(s):** These data demonstrate the feasibility of the intubated laparoscopic mouse model and confirm previous observations in rabbits, indicating that mesothelial hypoxia plays a key role in adhesion formation. (Fertil Steril® 2001;76:560–7. ©2001 by American Society for Reproductive Medicine.)

**Key Words:** Adhesions, mesothelial hypoxia, laparoscopy, pneumoperitoneum, insufflation pressure, CO<sub>2</sub>, helium, oxygen, endotracheal intubation, mice

Adhesions can be congenital or postinflammatory, but in most cases they are the result of a previous surgery (1). Postoperative adhesion formation is a major clinical problem because of the complications, such as intestinal obstruction (1), chronic pelvic pain (2), female infertility (3), and difficulties at the time of reoperation (4). In addition, the economical impact is huge for patients and the healthcare system (5).

Laparoscopic surgery was claimed to be less adhesiogenic than laparotomy. This, however, was poorly substantiated with controversial results. Some animal data demonstrated less adhesion formation, less de novo adhesion for-

mation, and less adhesion reformation (6, 7) after laparoscopy in comparison with laparotomy. Other studies failed, however, to show differences in adhesion formation and in de novo adhesion formation, but these investigations were not designed to address adhesion reformation (8, 9). In women with an ectopic pregnancy, a prospective randomized trial demonstrated less adhesion formation and less de novo adhesion formation after laparoscopy (10). Other human nonrandomized trials supported this observation but failed to show significant differences in terms of adhesion reformation (11, 12).

Laparoscopy has advantages that may lead

to less postoperative adhesions, such as gentle tissue handling, meticulous hemostasis, constant irrigation, no intraabdominally talc powder exposure, the use of microsurgical instruments, and a reduced and more precise operative field. Laparoscopy, however, has specific effects as it is performed in a different environment, the pneumoperitoneum, which could be deleterious. Carbon dioxide, the most common gas used for pneumoperitoneum, induces local changes such as acidosis (13–15), desiccation (16, 17), hypothermia (16), and adverse effects upon microcirculation (18, 19), possibly inducing hypoxia.

Furthermore, we recently demonstrated that CO<sub>2</sub> pneumoperitoneum is a cofactor in adhesion formation. Indeed, adhesions increased in rabbits with duration of pneumoperitoneum (20, 21) and with higher insufflation pressures (22) and decreased with the addition of 4% oxygen to CO<sub>2</sub> pneumoperitoneum (21). Similar effects were observed for helium pneumoperitoneum; adhesions increased with duration of pneumoperitoneum and decreased with the addition of oxygen, whereas no differences were found between CO<sub>2</sub> and helium (21). In the mouse model it was already demonstrated that duration of pneumoperitoneum increased adhesion formation using heated and humidified CO<sub>2</sub> (23). This study, however, was performed at only 2.5 cm of water of insufflation pressure because a high mortality rate was found at higher pressures.

These data lead to the hypothesis that, because of the compression of the capillary flow in the superficial peritoneal layers during pneumoperitoneum, mesothelial hypoxia plays a role in adhesion formation. To investigate in detail the intrinsic mechanisms involved, a model suitable for laparoscopies at high insufflation pressures and for the evaluation of cytokines and growth factors expression is required. Therefore, this study was performed to develop a laparoscopic mouse model at high insufflation pressures, to confirm the known effects of pneumoperitoneum, and to investigate more specifically the effect of the addition from 0.5% up to 12% oxygen to CO<sub>2</sub> pneumoperitoneum upon adhesion formation.

## MATERIALS AND METHODS

### Animals

One hundred thirty female NMRI mice that were 6–8 months old and that weighed between 45 and 55 g were used. Animals were kept under standard laboratory conditions (temperature 20°–25°C, relative humidity 40–70%, 14 hours light and 10 hours dark) at the Center for Laboratory Animal Care of the Katholieke Universiteit Leuven. They were fed using a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with free access to water and food before and after the laparoscopic procedure. The study was approved by the Institutional Review Animal Care Committee.

### Anesthesia and Endotracheal Intubation

After the IM anesthesia with pentobarbital (Nembutal, Sanofi Sante Animale, Brussels, Belgium; 0.06 mg/g) the abdomen was shaved and the animal was secured to the table in supine position. For laparoscopic surgeries, endotracheal intubation was necessary as insufflation pressures higher than 5 cm of water limit the spontaneous expansion of the diaphragm with subsequent death of the mouse (23). The animal was placed with the neck under a light source; the tongue was grasped with a hemostatic clamp and pulled out visualizing the vocal cords by transillumination. A 20-gauge catheter with a blunt guide wire was inserted into the trachea. After removal of the wire, the mouse was connected to a mechanical respirator (Rodent Ventilator, Harvard Apparatus, Holliston, MA) using 1.5 mL of air per stroke and 85 strokes/min.

### Surgical Procedure for Induction of Adhesions

Laparoscopic surgery was performed under aseptic conditions. After disinfection, a 3.5-mm abdominal incision was made caudal to the xyphoides appendix. A 0 degree, 2.1-mm endoscope held at 0 degrees, with a outer sheath for protection and insufflation of 3.3 mm (Karl Storz, Tuttlingen, Germany), connected to a single chip video camera (Karl Storz) and light source (Karl Storz), was introduced into the abdominal cavity. The endoscope was secured in a holder and the incision was sutured around the endoscope with 6-0 polyglycolic acid suture (Dexon II, Davis+Geck, Gosport, UK) to avoid any gas leakage.

An insufflator (Termoflator Plus, Karl Storz) capable of adding 0–12% oxygen to either CO<sub>2</sub> or helium was used for the pneumoperitoneum. In addition, the insufflation mixture was heated at 37°C (Optitherm, Karl Storz) and humidified with vapor (Dräger, Lübeck, Germany). Because most insufflators have an intermittent delivery of gas, a water valve with a free escape of gas was used to have a continuous flow and a constant pressure. An elastic balloon was placed next to the water valve to eliminate virtually all pressure changes in the tiny abdominal cavity of the animal. Because the peritoneum has a high exchange capacity, continuous removal of any oxygen that could diffuse from the circulation is required to maintain the predefined concentration of gas inside the abdominal cavity. Therefore, a 26-gauge needle (Insyte, Vialon, Becton Dickinson, Madrid, Spain) was inserted next to the endoscope to obtain a continuous flow of 10 mL/min of gas.

After the establishment of the pneumoperitoneum, two 14-gauge catheters (Insyte, Vialon, Becton Dickinson) were inserted under direct laparoscopic vision in both right and left flanks for the working instruments. Using a 1.5-mm grasper, the bicorn uterus was individualized by removal of the surrounding fat tissue and grasped in the midline. Using a homemade 1.5-mm ball electrode, a linear monopolar coagulation lesion of 10 mm was performed in the antimes-

enteric border of one uterine horn for 3–5 seconds with a power of 10 W in the standard coagulation mode (Autocon 350, Karl Storz). An identical lesion was performed in the ipsilateral pelvic side wall. The lesions were done randomly, either in the left or in the right side. The procedure took 3–4 minutes, but the pneumoperitoneum was maintained for different time periods according to the experimental design. At the end of the surgery, the incisions were sutured with 6-0 polyglycolic acid suture (Dexon II, Davis+Geck) in single layers.

### **Surgical Procedure for Evaluation of Adhesions**

Seven or 28 days after laparotomy was performed the adhesions were evaluated using an operative microscope (Zeiss, Hallbergmoos, Germany). The entire abdominal cavity was visualized using a xyphopubic midline and a bilateral subcostal incision. After the evaluation of ports sites and viscera for de novo adhesions, the fat tissue surrounding the uterus was carefully removed. The length of the visceral and parietal lesions and adhesions were measured. Adhesions, when present, were lysed to evaluate their type and tenacity. The animal was sacrificed immediately afterward.

### **Experimental Design**

All experiments were performed using block randomization by days. Therefore, a block of animals comprising one animal of each group was always operated the same day, avoiding day-to-day variability. In addition, within a block, experiments were performed in random order.

In the first study, survival after 1 hour of pneumoperitoneum was evaluated in six groups ( $n = 5$  in each group) with insufflation pressures of 5, 10, 15, 20, 25, and 30 cm of water, respectively. Survival was evaluated maintaining the pneumoperitoneum up to 3 hours at 15 cm of water ( $n = 5$ ).

In the second study, a 2 by 2 factorial design was used to evaluate the effect of duration of pneumoperitoneum (10 and 60 minutes) and insufflation pressure (5 and 15 cm of water) on adhesion formation. Pneumoperitoneum at 5 cm of water was maintained for 10 minutes (group 1;  $n = 7$ ) and 60 minutes (group 2;  $n = 7$ ). Similarly, pneumoperitoneum at 15 cm of water was maintained for 10 minutes (group 3;  $n = 7$ ) and 60 minutes (group 4;  $n = 7$ ). In addition, evaluation of adhesions after 7 days (group 4;  $n = 7$ ) and 28 days (group 5;  $n = 7$ ) was compared. Therefore, in group 5, pneumoperitoneum was maintained for 60 minutes at 15 cm of water.

In the third study, a 2 by 2 factorial design was used to evaluate the effect of insufflation of gas ( $\text{CO}_2$  and helium) and addition of oxygen (0 and 3%) on adhesion formation. Carbon dioxide pneumoperitoneum was used with 0% oxygen (group I;  $n = 5$ ) and with 3% oxygen (group II;  $n = 5$ ). Similarly, helium pneumoperitoneum was used with 0% oxygen (group III;  $n = 5$ ) and with 3% oxygen (group IV;  $n = 5$ ). The effect of adding different proportions of oxygen

to  $\text{CO}_2$  pneumoperitoneum was assessed more specifically in eight additional groups ( $n = 5$  in each group). The concentration of oxygen was 0.5%, 1%, 1.5%, 2%, 2.5%, 6%, 9%, and 12% in groups V, VI, VII, VIII, IX, X, XI, and XII, respectively. The pneumoperitoneum was maintained in all groups for 60 minutes at 15 cm of water.

Adhesions were evaluated blindly by two observers, one being the surgeon (RM) and the other an independent observer (AP), the score given being the common evaluation. This was done in random order using block randomization by day. The codes of the intervention order were broken only at the end of the experiment. The qualitative and quantitative evaluation was performed under microscopic vision during laparotomy. The qualitative evaluation was done assessing extent (0: no adhesions; 1: 1%–25%; 2: 26%–50%; 3: 51%–75%; 4: 76%–100%), type (0: no adhesions; 1: filmy; 2: dense; 3: capillaries present), tenacity (0: no adhesions; 1: essentially fall apart; 2: require traction; 3: require sharp dissection), and total (extent + type + tenacity). For the quantitative evaluation, the proportion of adhesions was calculated by measuring the length of the line covered by adhesions and the length of the lesion. Calculation was made according to the formula: adhesions (%) = (sum of the length of the individual attachments/length of the lesion)  $\times$  100 (24). Adhesions in the visceral and parietal peritoneum were evaluated separately.

### **Statistics**

Statistical analysis was performed with the SAS system (SAS Institute, Cary, NC) using Wilcoxon analysis and two-way analysis of variance (Proc logistic). The advantage of the 2 by 2 factorial design is that to achieve the same statistical precision, as with the one at a time approach, twice as many observations would have been needed. This increase in power of the factorial design is only valid when the effects of the two factors are additive (i.e., when no interaction between the two factors is present). The possibility of detecting an interaction (i.e., a different effect of one factor at different levels of the other factor) can, however, also be considered an advantage of the factorial design as this effect could otherwise easily be missed. One should be aware that a positive interaction (with subsequent reduction of power to demonstrate the effect of the two factors) can be missed when the number of observations is low, especially when the between-subject variability is high (25).

## **RESULTS**

Intubation was a safe and quick procedure taking less than 1 minute without injury or mortality. All animals tolerated the intubation and the laparoscopic procedures and none of them died during the observation period (7 or 28 days), except for two mice in the first study. In the second study, one mouse in group 5 was excluded because there was no remaining uterine horn at the time of the evaluation and

**TABLE 1**

Effect of the duration of pneumoperitoneum (60 vs. 10 min) and of the insufflation pressure (15 vs. 5 cm of water) upon adhesion formation.

Duration (min)	Pressure (cm of water)	Adhesions scores			
		Extent	Type	Tenacity	Total
10	5	0.8 ± 0.2	1.1 ± 0.4	0.9 ± 0.2	2.8 ± 0.8
60	5	1.1 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	3.7 ± 0.8
10	15	0.9 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	3.4 ± 0.3
60	15	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	5.4 ± 0.4

Values are means ± SEM.

After 7 days of laparoscopic surgery, adhesions were scored qualitatively for extent (0–4), type (0–3), tenacity (0–3), and total (0–10).

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consequently, six instead of seven animals were evaluated in this group. None of the mice showed signs of intestinal obstruction or other serious complications. Adhesions consistently formed to the injured parietal and visceral sites attaching the pelvic fat. Two mice presented filmy adhesions of the omentum to the laparoscopic ports and no de novo adhesions were found.

All data presented are the sum of visceral and parietal scores divided by two (means ± standard error of the mean). Because group 4 (n = 7) in the second study and group 1 (n = 5) in the third study were identical (100% CO<sub>2</sub> pneumoperitoneum, 60 minutes, 15 cm of water, evaluation of adhesions after 7 days) and as adhesion scores and the proportion of adhesions were similar, both groups were combined for further analysis (n = 7 + 5 = 12).

Insufflation pressures of 5, 10, 15, 20, 25, and 30 cm of water for 1 hour caused mortality during surgery in 0, 0, 0, 0, 1, and 1 animal, respectively, whereas all animals survived when the pneumoperitoneum was maintained for 3 hours at 15 cm of water. All mice surviving the surgery survived after 1 week, indicating that both intubation and laparoscopy did not have long-term complications in terms of mortality.

Total, extent, type, and tenacity adhesions scores were 5.4 ± 0.4, 1.8 ± 0.2, 1.8 ± 0.1, and 1.8 ± 0.1 after 7 days (group 4) and 5.8 ± 0.7, 1.8 ± 0.3, 2.0 ± 0.2, and 2.0 ± 0.3 after 28 days (group 5) (Wilcoxon test, P=not significant [NS]), respectively. The proportion of adhesion was 38.3% ± 5.1% after 7 days (group 4) and 40.3% ± 6.3% after 28 days (group 5) (Wilcoxon test, P=NS).

By two-way analysis of variance, adhesions increased with duration of pneumoperitoneum and with insufflation pressure without interaction between both. Duration of pneumoperitoneum (60 vs. 10 minutes) increased total (P=.002), extent (P=.004), type (P=.001), and tenacity (P=NS) of adhesion scores (Table 1) and the proportion of adhesions (P=.001) (Fig. 1). Also a higher insufflation pressure (15 vs. 5 cm of water) increased total (P=.02), extent (P=.03), type

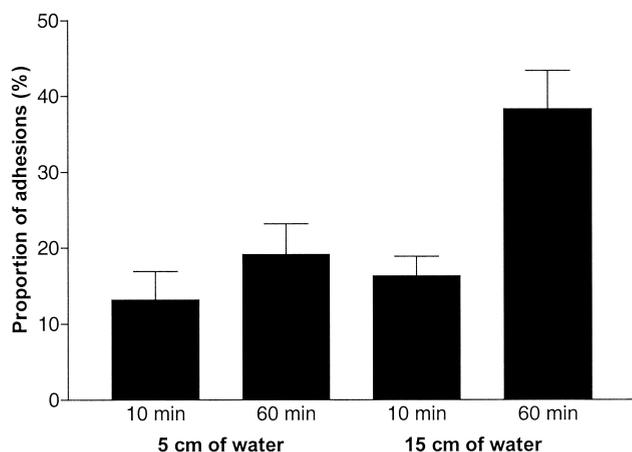
(P=NS), and tenacity (P=.008) of adhesion scores (Table 1) and the proportion of adhesions (P=.005) (Fig. 1).

Comparing groups individually (Wilcoxon test), longer duration of pneumoperitoneum (60 vs. 10 minutes) at 15 cm of water increased total (P=.005), extent (P=.005), and type (P=.002) of adhesion scores and the proportion of adhesions (P=.002), whereas a higher insufflation pressure (15 vs. 5 cm of water) during 60 minutes increased extent score (P=.03) and the proportion of adhesions (P=.004). Most differences, however, were not or only marginally significant.

By two-way analysis of variance, the addition of 3% oxygen to CO<sub>2</sub> and helium pneumoperitoneum decreased

**FIGURE 1**

Effect of the duration of pneumoperitoneum and of the insufflation pressure upon adhesion formation. After 7 days of laparoscopic surgery, extent of adhesions was scored quantitatively taking into account the area of lesion covered by adhesions (60 vs. 10 minutes: P=.001; 15 vs. 5 cm of water: P=.005).



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**TABLE 2**

Effect of the type of the insufflation gas (CO<sub>2</sub> vs. helium) and of the addition of oxygen (3% vs. 0%) upon adhesion formation.

Type of gas	Proportion of oxygen (%)	Adhesions scores			
		Extent	Type	Tenacity	Total
CO <sub>2</sub>	0	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	5.4 ± 0.4
CO <sub>2</sub>	3	0.7 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	2.3 ± 0.7
Helium	0	2.1 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	7.0 ± 0.2
Helium	3	1.1 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	3.8 ± 0.6

Values are means ± SEM.

After 7 days of laparoscopic surgery, adhesions were scored qualitatively for extent (0–4), type (0–3), tenacity (0–3), and total (0–10).

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adhesion formation, whereas some differences were observed between CO<sub>2</sub> and helium. The addition of 3% oxygen decreased total ( $P=.0001$ ), extent ( $P=.0003$ ), type ( $P=.0003$ ), and tenacity ( $P=.0002$ ) of adhesion scores (Table 2) and the proportion of adhesions ( $P=.0001$ ) (Fig. 2). No differences between CO<sub>2</sub> and helium pneumoperitoneum were observed concerning the proportion of adhesions (Fig. 2). Helium, however, induced higher extent ( $P=.05$ ), type ( $P=.01$ ), tenacity ( $P=.004$ ), and total ( $P=.008$ ) of adhesion scores in comparison with CO<sub>2</sub> (Table 2).

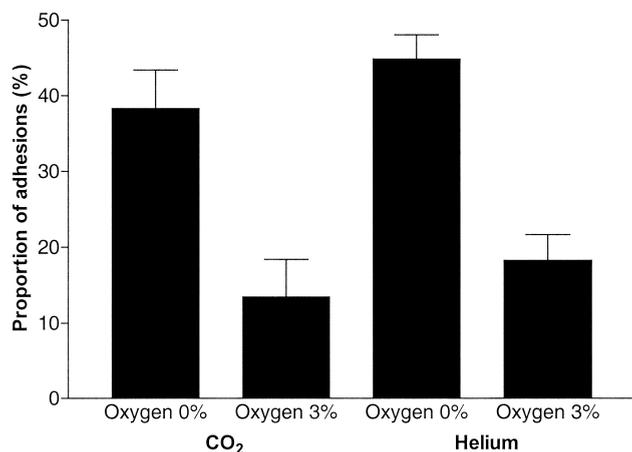
Comparing groups individually (Wilcoxon test), for CO<sub>2</sub> pneumoperitoneum, the addition of 3% oxygen decreased total ( $P=.003$ ), extent ( $P=.005$ ), type ( $P=.01$ ), and tenacity

( $P=.004$ ) of adhesion scores and the proportion of adhesions ( $P=.004$ ). Also for helium pneumoperitoneum the addition of 3% oxygen decreased total ( $P=.01$ ), extent ( $P=.01$ ), type ( $P=.01$ ), and tenacity ( $P=.02$ ) of adhesion scores and the proportion of adhesions ( $P=.01$ ). No differences between 100% CO<sub>2</sub> and 100% helium were found for extent score and for the proportion of adhesions. Helium, however, induced higher type ( $P=.02$ ), tenacity ( $P=.04$ ), and total ( $P=.02$ ) of adhesion scores. By adding 3% oxygen to CO<sub>2</sub> and helium, no differences were found between the two gases for extent, type, tenacity, and total adhesion scores nor for the proportion of adhesions.

The effect of the addition of different proportions of oxygen to CO<sub>2</sub> was investigated more specifically. In comparison with group 1 (0% oxygen), total, extent, type, and tenacity adhesion scores (Table 3) and the proportion of adhesions (Fig. 3) decreased (Wilcoxon test) with the addition of 12% ( $P=.04$ ,  $P=NS$ ,  $P=.05$ ,  $P=NS$ ,  $P=NS$ ), 9% ( $P=.005$ ,  $P=.04$ ,  $P=.009$ ,  $P=.006$ ,  $P=.05$ ), 6% ( $P=NS$ ,  $P=.05$ ,  $P=NS$ ,  $P=NS$ ,  $P=.03$ ), 2.5% ( $P=NS$ ,  $P=NS$ ,  $P=NS$ ,  $P=NS$ ,  $P=.01$ ), and 2% ( $P=.02$ ,  $P=.009$ ,  $P=.01$ ,  $P=NS$ ,  $P=.003$ ), but not with 1.5%, 1%, and 0.5% oxygen. No differences were found between the different groups.

**FIGURE 2**

Effect of the type of the insufflation gas and of the addition of oxygen upon adhesion formation. After 7 days of laparoscopic surgery, extent of adhesions was scored quantitatively taking into account the area of lesion covered by adhesions (CO<sub>2</sub> vs. helium:  $P=NS$ ; 3% vs. 0% oxygen:  $P=.0001$ ).



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## DISCUSSION

The mouse model is well suited to investigate adhesion formation after laparoscopic surgery. First, the mouse is a well-established model for the study of intraabdominal adhesion formation after laparotomy (26). Second, the mouse model is one of the best-developed animal models with many specific assays available. In addition, the availability of nude, severe combined immunodeficient and knockout mice permit detailed investigations. Therefore, it is not surprising that laparoscopic surgery in mice was recently developed to study postoperative tumor growth (27), port-site metastases (28), immune response (29), gastrointestinal transit (30), and morphologic alterations of the mesothelium (31). These procedures were, however, all done in nonintu-

**TABLE 3**

Effect of the addition of different proportions of oxygen (0–12%) to CO<sub>2</sub> pneumoperitoneum upon adhesion formation.

Proportion of oxygen (%)	Adhesions scores			
	Extent	Type	Tenacity	Total
0	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	5.4 ± 0.4
0.5	1.7 ± 0.4	1.4 ± 0.3	1.5 ± 0.3	4.4 ± 0.9
1	1.6 ± 0.2	1.3 ± 0.3	1.5 ± 0.3	4.6 ± 0.8
1.5	1.4 ± 0.2	1.9 ± 0.2	2.0 ± 0.0	5.3 ± 0.3
2	0.7 ± 0.3	0.9 ± 0.3	1.3 ± 0.4	2.9 ± 0.9
2.5	1.0 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	3.8 ± 0.1
3	0.7 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	2.3 ± 0.7
6	0.9 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	3.4 ± 1.1
9	1.1 ± 1.2	1.1 ± 0.1	1.0 ± 0.0	3.2 ± 0.3
12	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	3.8 ± 0.4

Values are means ± SEM.

After 7 days of laparoscopic surgery, adhesions were scored qualitatively for extent (0–4), type (0–3), tenacity (0–3), and total (0–10).

Molinas. *Role of pneumoperitoneum in adhesions. Fertil Steril 2001.*

bated mice at a low insufflation pressure. In a previous study (23), we found a high mortality when insufflation pressures were greater than 5 cm of water, making surgical procedures virtually impossible.

Our model with intubated mice is, to the best of our knowledge, the first model permitting insufflation pressures up to 15 mm Hg (i.e., comparable to surgery on humans) and surgical procedures. This model permits 1–3 hours of surgery without anesthetic mortality. This is extremely impor-

tant to investigate the effect of laparoscopy on adhesion formation as we know from rabbit experiments that adhesions increase with insufflation pressure (22). It should be stressed that in this mouse model humidification was close to 100% because of the low flow rates (22, 23). In addition, using the Termoflator Plus, insufflation can be achieved with gas heated at 37°C because of the distant heating device close to the animal, whereas this setup permits the use of an oxygen concentration between 0 and 12%. A water valve and an elastic balloon were introduced to prevent any overpressure, which is critical in mice.

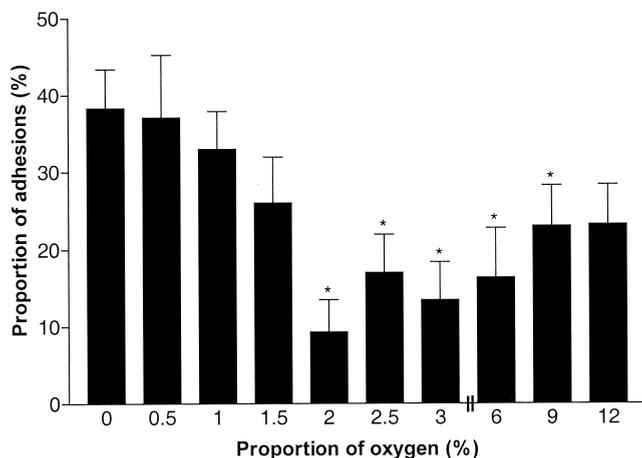
Adhesions evaluated after 7 and 28 days were almost identical, confirming previous observations that adhesion formation did not vary significantly after 7 days (24). Evaluation after 7 days is advantageous for research purposes because it permits one to induce adhesions during 1 week and to evaluate them the following week. Results from the first experiment can be obtained before beginning the second experiment.

Our data are consistent with the following hypothesis: CO<sub>2</sub> or helium pneumoperitoneum, especially at higher insufflation pressures, will compress the capillary flow in the superficial peritoneal layers inducing local ischemia, whereas diffusion of pure CO<sub>2</sub> or helium from the abdominal cavity to the bloodstream will create a gradient of hypoxia, the superficial layers being exposed to virtually 100% CO<sub>2</sub> or helium without oxygen. This hypothesis is supported by the observation that adhesions increase with duration and pressure of pneumoperitoneum and decrease by the addition of oxygen, 3% having a maximal effect and 1.5% a half-maximal effect.

Our hypothesis of mesothelial hypoxia as a driving mechanism in adhesion formation is consistent with the intracellular partial pressure of oxygen, being 5 to 40 mm Hg

**FIGURE 3**

Effect of the addition of different proportions of oxygen to CO<sub>2</sub> pneumoperitoneum on adhesion formation. After 7 days of laparoscopic surgery, extent of adhesions was scored quantitatively taking into account the area of lesion covered by adhesions (\**P*<.05 compared with 0% oxygen).



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(average, 23 mm Hg), in contrast with the arterial, interstitial, and venous partial pressure of oxygen, which are 95, 40, and 40 mm Hg, respectively (32). Compression of the capillary flow in the superficial peritoneal layers by the pneumoperitoneum will reduce the partial pressure of oxygen in the mesothelial cells. A partial pressure of oxygen less than 5 mm Hg, which causes hypoxic changes, thus could be reached. A mixture of 3% oxygen in 97% CO<sub>2</sub>, insufflated at 15 cm of water (~12 mm Hg), results in a partial pressure of oxygen of 23 mm Hg (3% of 760 mm Hg atmospheric pressure + 12 mm Hg insufflation pressure). This partial pressure of oxygen is obviously sufficient to increase the mesothelial partial pressure of oxygen to normal levels, thus abolishing any local hypoxia.

The half-maximal reduction in adhesion formation around 1.5% oxygen (partial pressure of oxygen of 11.4 mm Hg) is similar to the half-maximal up-regulation of hypoxia inducible factor-1. Hypoxia inducible factor is a protein implicated in the transcriptional activation of genes encoding vascular endothelial growth factor in hypoxic mammalian cells (33). Hypoxia inducible factor-1 levels vary exponentially according to the intracellular oxygen tension. Indeed, in human cervical carcinoma HeLa S3 cultured cells, a half-maximal response was obtained between 1.5% and 2% oxygen (partial pressure of oxygen of 11.4 mm Hg and 15.2 mm Hg) and a maximal response at 0.5% oxygen (partial pressure of oxygen of 3.8 mm Hg) (34). Because of this similarity we speculate that the driving mechanism of the increase in adhesion formation is a strong hypoxia, by an environment with virtually no oxygen or at least less than 0.5% to 1%, with subsequent hypoxia inducible factor and vascular endothelial growth factor induction.

Hypoxia is the main stimulus of vascular endothelial growth factor (35) and a few studies have already reported its role in adhesion formation. A polyclonal rabbit antibody to vascular endothelial growth factor limited adhesions after laparotomy in mice (36) and vascular endothelial growth factor was found in peritoneal adhesions by immunohistochemistry (37), enzyme-linked immunosorbent assay (38), and reverse transcriptase-polymerase chain reaction (39).

Other effects of hypoxia cannot be ruled out. Previous reports indicate that hypoxic conditions (2% oxygen) in cultured mesothelial cells up-regulate the expression of transforming growth factor- $\beta$ 1 and - $\beta$ 2 (40) and tissue inhibitors of metalloproteinases, possibly through a transforming growth factor- $\beta$ 1-dependent mechanism (41). Transforming growth factor- $\beta$  up-regulates plasminogen activator inhibitor-1 and down-regulates tissue plasminogen activator, decreasing plasmin and thus, inhibiting the lysis of fibrin increasing adhesion formation (42). Transforming growth factor- $\beta$  decreases the expression of matrix metalloproteinases and increases the expression of tissue inhibitors of metalloproteinases and therefore, decreases matrix degradation increasing fibrous adhesions (43). Further studies are

required to clarify the intrinsic mechanism whereby mesothelial hypoxia induces adhesions and the laparoscopic mouse model is well suited for these investigations.

The similarity between CO<sub>2</sub> and helium, which is chemically, pharmacologically, and physiologically inert and does not produce hypercarbia and acidosis (44, 45), showed at least that these two effects—hypercarbia and acidosis—were less pronounced in adhesion formation, thus pointing to the hypoxia hypothesis. It is unclear, however, why helium pneumoperitoneum induced slightly more adhesions than CO<sub>2</sub> in mice, an observation that was not made in rabbits (21). We only can speculate that hypoxia with a less soluble gas was more pronounced, whereas the lower interanimal variability of the mouse model permitted us to detect this. This observation is another argument that hypoxia, rather than pH changes, is the driving mechanism whereby CO<sub>2</sub> pneumoperitoneum induces adhesion formation.

In conclusion, these experiments demonstrate the feasibility of the laparoscopic mouse model at high insufflation pressures and confirm the effect of pneumoperitoneum on adhesion formation. They are consistent with the hypothesis of mesothelial hypoxia and suggest a role for vascular endothelial growth factor by way of hypoxia inducible factor up-regulation.

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