

Original Article

Effect of Upper Abdomen Tissue Manipulation on Adhesion Formation between Injured Areas in a Laparoscopic Mouse Model

Ron Schonman, MD*, Roberta Corona, MD, Adriana Bastidas, MD, MSc, Carlo De Cicco, MD, and Philippe Robert Koninckx, MD, PhD

From the Departments of Obstetrics and Gynecology at the University Hospital Gasthuisberg, Leuven, Belgium (all authors) and the Chaim Sheba Medical Center, Tel-Hashomer, Israel (Dr. Schonman).

ABSTRACT **Study Objective:** These experiments were designed to examine the effect of manipulation during surgery as a cofactor in adhesion formation at trauma sites.

Design: Randomized, controlled trial. Canadian Task Force Classification—class 1.

Setting: University laboratory research center.

Subjects: A standardized laparoscopic mouse model (Balb/c mice 9–10 weeks old) for adhesion formation after opposing bipolar lesions and 60 minutes of carbon-dioxide pneumoperitoneum. In this model adhesions are known to decrease after the addition of 3% of oxygen, dexamethasone, or both. In addition, adhesions decrease with experience (i.e., with a decreasing amount of manipulation during the learning curve).

Interventions: A factorial design was used to evaluate the effects of dexamethasone and of adding 3% of oxygen on manipulation-enhanced adhesion formation during a learning curve. Blocks of 4 animals were thus randomized as controls (carbon-dioxide pneumoperitoneum only) or received an additional 3% of oxygen, dexamethasone, or both. In a second experiment, the effects of manipulation on adhesion formation were quantified. In a third experiment we evaluated whether dexamethasone had a specific effect on manipulation-enhanced adhesion formation.

Measurements and Main Results: Qualitative and quantitative adhesion scoring 7 days after the intervention. The first experiment confirmed that adhesion formation decreased during the learning curve ($p < .0001$) and after the addition of dexamethasone whether assessed as the total adhesion score ($p < .0001$ and $p = .0009$, respectively) or a quantitative score ($p < .0001$ and $p < .0001$, respectively). The second experiment showed that adhesion formation increased by standardized touching and grasping of omentum and bowels (proportion score $p = .0059$ and $p = .0003$, respectively) and this effect increased with duration of touching ($p = .0301$). In the third experiment, dexamethasone was confirmed to decrease adhesion formation ($p = .0001$) but this effect was not specific for manipulation-enhanced adhesion formation.

Conclusion: Manipulation of intraperitoneal organs in the upper abdomen enhances adhesion formation at trauma sites, confirming that the peritoneal cavity is a cofactor in adhesion formation. Dexamethasone decreases adhesion formation but the effect is not specific for manipulation-enhanced adhesion formation. *Journal of Minimally Invasive Gynecology* (2009) 16, 307–12 © 2009 AAGL. All rights reserved.

Keywords: Adhesion formation; Tissue manipulation; Learning curve; Dexamethasone; Laparoscopy; Animal model

Postoperative adhesion formation remains an important clinical problem causing small bowel obstructions, chronic pain, and decreased fertility [1]. Previous abdominal surgeries were reported to be the cause of 33% to 50% and 79% of

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Corresponding author: Ron Schonman, MD, Department of Obstetrics and Gynecology, University Gasthuisberg, Herestraat 49 B-3000 Leuven, Belgium.

E-mail: ronschonman@gmail.com

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large and small bowel obstructions, respectively [2,3]. Adhesions are believed to be an important cause of pelvic pain but the efficacy of adhesiolysis [4] remains unclear [5]. Intraperitoneal (IP) adhesions are considered to be a cause of female infertility [6] and are in 15% of the cases the only factor found [7]. At repeated surgeries, adhesions increase difficulty and duration of surgery and complication rates.

The widely accepted pathophysiology of adhesion formation after surgical injury to the peritoneal surfaces involves exudation [8], inflammation, and fibrin deposition [9]. Fibrin is removed by fibrinolysis and simultaneously the mesothelium is repaired within a few days by proliferation of mesothelial

cell islands on the injured surface [10]. If the repair process fails, adhesions can be formed. Adhesions increase with prolonged inflammation (i.e., infection or suture resorption) whereas it is believed that the amount of necrotic tissue increases inflammation and adhesions. Adhesion formation thus was considered as a local phenomenon. It was shown that adhesions increase with the duration of carbon-dioxide (CO₂) pneumoperitoneum, with the insufflation pressure, and with desiccation whereas adhesions decrease with cooling or after adding 3% to 4% of oxygen to the CO₂ pneumoperitoneum [11–13]. These data show that the peritoneal cavity is a cofactor in adhesion formation at the injured site. The exact molecular or cellular mechanisms involved are still unknown.

Good surgical practice is widely believed to reduce adhesion formation [14] and microsurgery has claimed a reduction of adhesions through minimizing surgical trauma and ischemia and by avoiding desiccation [15]. The effect of desiccation was shown unequivocally in our mouse model [13]. The reduction of adhesion formation by gentle tissue handling was not yet investigated directly. Indirect evidence is the decrease of adhesion formation by surgical expertise, as revealed in the rabbit nephrectomy model. Surgical expertise, however, always associates a decrease in duration of surgery with an increase in quality as shown in numerous animal and human training curves such as the pig model for paraaortic lymph node dissection [16], pig model for nephrectomy [17], rabbit nephrectomy model [18], and human training curves [19].

In our mouse model of adhesion formation it is well known that the amount and the variability of adhesion formation decreases exponentially with experience during the first 50 to 80 experiments and a training curve was standard practice for each new researcher before starting experiments. Because our mouse model duration of CO₂ pneumoperitoneum was standardized to 60 minutes, the decrease in adhesion formation with expertise should be the consequence of a decrease in tissue manipulation. These experiments were designed to specifically investigate the effect of tissue manipulation on adhesion formation. Because the effect of tissue manipulation might be mediated by inflammation, we evaluated whether dexamethasone, known to have anti-inflammatory effects, specifically could reduce manipulation-enhanced adhesion formation.

Materials and Methods

The Laparoscopic Mouse Model for Adhesion Formation

The experimental setup (i.e., animals, anesthesia and ventilation, laparoscopic surgery, and induction and scoring of IP adhesions) was described in detail previously [11,12,20–24]. Briefly, the model consisted of pneumoperitoneum-enhanced adhesions induced during laparoscopy by creation of opposing mechanical lesions. The pneumoperitoneum was maintained for 60 minutes using humidified CO₂ at an insufflation pressure of 15 mm Hg. Gas and body temperature were kept strictly at 37°C using a heated chamber. Female

Balb/c mice, 9 to 10 weeks old and weighing 19 to 24 g, were used because in this inbred strain adhesion formation was high with low interanimal variability [25]. Animals were kept under standard laboratory conditions and they were fed with a standard laboratory diet with free access to food and water. The study was approved by the institutional review animal care committee.

Mice were anesthetized with IP 0.08 mg/g of pentobarbital, intubated with a 20-gauge catheter and mechanically ventilated (Mouse Ventilator MiniVent, type 845, Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) using humidified room air with a tidal volume of 250 µL at 160 strokes/min to prevent cooling. To avoid variability introduced by any other procedures as anesthetizing and intubating the Balb/c mice, which are very small, the investigator was trained in this before the start of the study. The investigator, moreover, was an experienced endoscopist.

A midline incision was performed caudal to the xyphoids, a 2-mm endoscope with a 3.3-mm external sheath for insufflation (Karl Storz, Tuttlingen, Germany) was introduced into the abdominal cavity, and the incision was closed gas tight around the endoscope to avoid leakage. The pneumoperitoneum was created (Thermoflator Plus, Karl Storz) using humidified insufflation gas [13,26]. After the establishment of the pneumoperitoneum, 2 14-gauge catheters were inserted under laparoscopic vision. Standardized 10- by 1.6-mm lesions were made in the antimesenteric border of both right and left uterine horns and pelvic sidewalls with bipolar coagulation, BICAP bipolar hemostasis probe BP-5200A 5F 200 cm (IMMED Benelux, Linkebeek, Belgium) at 20 W (standard coagulation mode, Autocon 200, Karl Storz, Tuttlingen, Germany).

Adhesions were scored blindly (the investigator was not informed of the group being evaluated) both qualitatively and quantitatively under stereomicroscopic vision during laparotomy 7 days later. The qualitative scoring system assessed extent (0 = no adhesions; 1 = 1%–25%; 2 = 26%–50%; 3 = 51%–75%; 4 = 76%–100% of the injured surface involved), type (0 = no adhesions; 1 = filmy; 2 = dense; 3 = capillaries present), and tenacity (0 = no adhesions; 1 = easily fall apart; 2 = require traction; 3 = require sharp dissection) of adhesions, from which a total score was calculated (extent + type + tenacity). The quantitative scoring system assessed the proportion of the lesions covered by adhesions using the following formula: adhesion (%) = (sum of the length of the individual attachments/length of the lesion) × 100. The results are presented as the average of the adhesions formed at the 4 sites (right and left visceral and parietal peritoneum), which were individually scored.

Setup and Design of the Experiments

To control temperature, animals and equipment (i.e., insufflator, humidifier, water valve, ventilator, and tubing) were placed in a closed chamber maintained at 37°C with a heated air patient warming system (WarmTouch, model

5700, Mallinckrodt Medical, Hazelwood, Mo). The insufflation gas temperature was determined by the environmental temperature (i.e., at 37°C). Because anesthesia and ventilation can influence body temperature and body temperature can influence adhesion formation [13], the timing and temperature were strictly controlled. Mice temperature was measured by rectal probe before anesthesia and was between 35°C and 37.7°C for all mice. The time of the anesthesia injection was considered time 0. The animal preparation and ventilation started after exactly 10 minutes. The pneumoperitoneum started at 20 minutes and was maintained for 60 minutes for a total of 80 minutes. In all experiments, animals were block randomized by day (i.e., 1 animal of each group was operated on the same day in random order) to eliminate eventual variability caused by the day or surgery.

Experiment I ($n = 80$) was performed to confirm the decrease in adhesion formation during sequential experiments even when performed by an experienced endoscopist. Because duration of surgery was strictly standardized at 60 minutes, the only variable was decreasing manipulation with experience. Using a factorial design, we specifically evaluated in each sequential block of 4 animals the effect of dexamethasone and the effect of adding 3% of oxygen, because both were proved previously to reduce adhesion formation in this model [12,27]. In each block, mice thus were randomized to no additional treatment (group I); treatment with 40 mg of dexamethasone twice, the first after surgery and the second after 24 hours (group II); the addition of 3% oxygen to the CO₂ pneumoperitoneum (group III); or both dexamethasone and oxygen treatment (group IV).

Experiment II ($n = 32$) was designed to evaluate the effect of touching and grasping in the upper abdomen on adhesion formation in the lower abdomen (i.e., at the site of the injury). Group I ($n = 8$) was the control group. In groups II ($n = 8$) and IV ($n = 8$) the omentum and large and small bowels were moved gently up and down across the abdomen for 5 and 10 minutes, respectively, with the shafts of 2 graspers (i.e., tissues were moved around without grasping). In group III ($n = 8$), to check whether grasping was more traumatic thus more adhesiogenic, the omentum and small and large bowels were grasped and manipulated repetitively for 5 minutes with a 1.5-mm atraumatic grasper.

Experiment III ($n = 32$) was designed specifically to evaluate whether dexamethasone, known to reduce CO₂ pneumoperitoneum-enhanced adhesions, had a specific effect on manipulation-enhanced adhesions. A factorial design ($n = 8$ animals/group) was used in which group I consisted of the control group (i.e., CO₂ pneumoperitoneum-enhanced adhesions without any treatment). Group II received 2 doses of 40 µg of dexamethasone, one immediately after the end of pneumoperitoneum and another 24 hours later. In group III a similar manipulation as in experiment II of the omentum and large and small bowels was performed for 5 minutes with a 1.5-mm nontraumatic grasper. Group IV received similar manipulation for 5 minutes as group III but also received dexamethasone in the same fashion as in group II.

Statistics

Software (Graph Pad Prism, Graph Pad Software Inc, San Diego, Calif), was used for curve fitting. Statistical analyses were performed with software (SAS System, SAS Institute, Cary, NC) using nonparametric tests, because the numbers were too small to confirm reliably a normal distribution. For the first experiment a 3-way analysis of variance (ANOVA) with the block number, dexamethasone, and oxygen as independent variables (Proc GLM [general linear methods]) was used to evaluate simultaneously the effects of experience/manipulation, dexamethasone, and oxygen addition. This 3-way ANOVA was used as a simplification of a 2-way ANOVA (factorial design of oxygen and dexamethasone treatment) within a repetitive measurement (sequential blocks). In the second experiment differences in adhesions between 2 specific groups were evaluated by Wilcoxon rank sum test. For the third experiment a 2-way ANOVA (Proc GLM) evaluated the effects of manipulation and of dexamethasone. Although nonparametric tests were used for statistical analysis, means and SD (instead of medians, 10th–90th percentiles) are given in text and figures because they are easier to read and interpret.

Power analysis was based on the low interanimal variability in inbred Balb/c mice (<5%) resulting in a power of 90% to detect a difference of 4%, 7%, and 9% when comparing 2 groups of 40, 12, or 8 mice, respectively. Indeed a factorial design, permitting a 2- or 3-way ANOVA, has almost the same power for each variable as if the experiment were conducted sequentially for each variable with the same number of animals. The factorial design, moreover, permits the exclusion of interaction between the factors investigated [28].

In the first experiment 3 mice died immediately after surgery in the first 3 blocks. In these blocks, adhesion formation was, moreover, erratically high. Because this obviously was a confounding factor caused by the surgeon's limited laboratory experience and because statistical results did not vary with or without these 3 blocks, the first 3 blocks were not included in the graph or statistical analysis in this article.

Results

In experiment I, an obvious learning curve was observed in the control group with a logarithmic decline in adhesion formation, whether evaluated as proportion or as total amount of adhesions (Proc GLM, $p < .0001$ for both). Dexamethasone decreased adhesion formation whether evaluated as adhesion proportion, total adhesion score, extension, type, or tenacity (Proc GLM, $p = .0001$, $p = .0009$, $p < .0001$, $p = .0063$, and $p = .007$, respectively). Of interest, the effect of the learning curve almost disappeared with dexamethasone treatment (Fig. 1). The decrease in adhesion formation with the addition of 3% of oxygen was not significant (Proc GLM, $p = .0658$) probably as a consequence of the high variability during the learning curve.

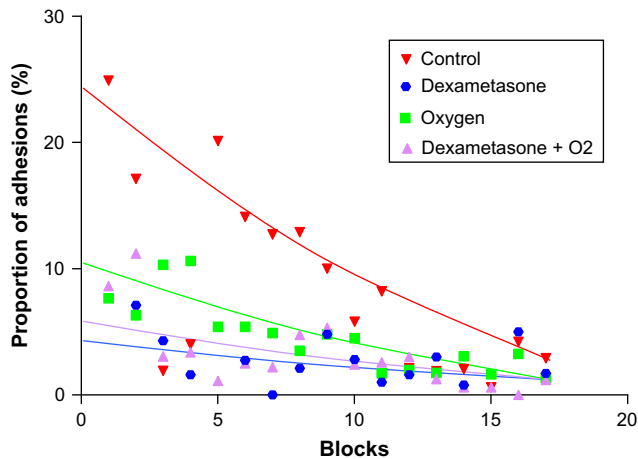


Fig. 1. Adhesion formation during learning curve (i.e., first 80 animals). Using block, randomization of 4 sequential mice to 60 minutes of CO₂ pneumoperitoneum only, addition of 3% of oxygen, addition of dexamethasone, or both show that adhesions decrease with experience ($p < .0001$), with addition of dexamethasone ($p < .0001$ for adhesion proportion), and with addition of oxygen (not significant, $p = .06$ for adhesion proportion).

In experiment II, manipulation of omentum and bowels increased adhesion formation and this effect increased with time. Groups II, III, and IV, had higher adhesion proportion in comparison with the control group (Wilcoxon test, $p = .0059$, $p = .0003$, and $p = .0003$, respectively). The effect of manipulation increased with duration (i.e., 5 vs 10 minutes) for adhesion proportion and adhesion extension (Proc GLM, $p = .0301$ and $p = .0241$, respectively). In addition, grasping increased adhesions but the effect was not more pronounced than manipulation (Fig. 2). In none of the mice was adhesion found in the upper abdomen.

Experiment III confirmed that manipulation increased adhesion formation and that dexamethasone reduced adhesions whether evaluated quantitatively (2-way ANOVA, $p = .0001$ for both) (Fig. 3) or qualitatively. The adhesion scores for extension, type, tenacity, and total score were lower under dexamethasone treatment (Proc GLM, $p < .0001$, $p < .0001$, $p < .0001$, and $p < .0001$, respectively) and were significantly higher with grasping manipulation for 5 minutes (Proc GLM, $p < .0001$, $p < .0001$, $p < .0001$, and $p < .0001$, respectively). Dexamethasone decreased adhesions similarly in the control and manipulation-enhanced adhesion groups. Manipulation also enhanced quantitative adhesion scores when comparing the 2 groups receiving dexamethasone (Wilcoxon, $p = .008$).

Discussion

These results revealed that tissue manipulation in the upper abdomen by itself enhances adhesion formation at the site of lesions. The effect is seen with different types of tissue manipulation such as touching and grasping, and is dose dependent. In addition, the higher adhesion formation at the beginning of the learning curve is consistent with more manipulation by the inexperienced surgeon (Fig. 1)

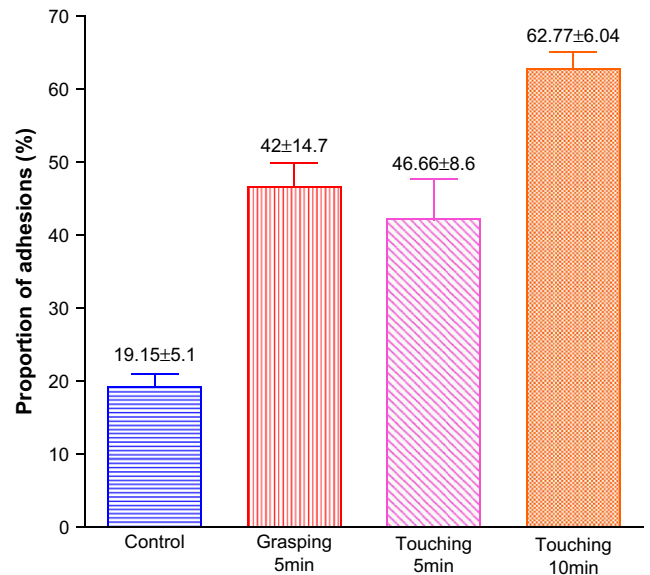


Fig. 2. Increase in adhesion formation in laparoscopic mouse model by manipulation of tissue either touching ($p = .0059$ for adhesion proportion) or grasping ($p = .0003$ for adhesion proportion) for 5 minutes. Effect of touching is dose dependent (5 vs 10 minutes, $p = .0301$ for adhesion proportion). Bars present means and SD of each group.

because this was the only variable, as in these experiments the duration of the pneumoperitoneum and of the anesthesia was kept constant at 60 minutes. This effect of trauma and manipulation strongly supports the concept of gentle tissue handling as one of the important factors in adhesion prevention.

That manipulation alone has such a profound effect on adhesion formation (Fig. 2) could have far reaching clinical importance. Indeed, although during surgery mobilization cannot be avoided for exposition of structures and during dissection, the amount of manipulation will decrease with experience of the surgeon, as reflected also in shorter operating times. It, moreover, suggests that during learning curves not only is the duration and quality of surgery affected, but

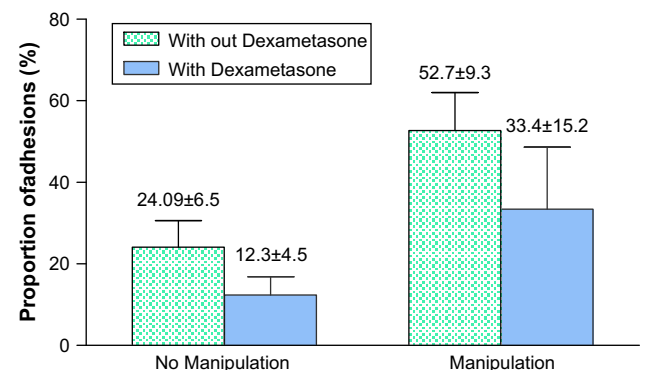


Fig. 3. Manipulation increases ($p = .0001$ for adhesion proportion) and dexamethasone decreases ($p = .0001$ for adhesion proportion) adhesion formation in laparoscopic mouse model (2-way ANOVA; proportion). Bars present means and SD of each group.

also the overall tissue trauma and manipulation and possibly also human adhesion formation.

These experiments confirm and extend the model that the peritoneal cavity is a cofactor in adhesion formation between traumatized peritoneal areas. Although the adhesions occur only at the lesion sites (i.e., the uterus and the abdominal wall), the amount of adhesion formation depends on factors in the peritoneal cavity or fluid. These experiments showing that manipulation outside the lesions will affect adhesion formation unequivocally reveal that the effect has to be mediated through the peritoneal cavity or fluid. The mechanism and the factors involved are still unknown, although they seem to be a consequence of the trauma caused by manipulation, desiccation, hypoxia, or reactive oxygen species (ROS). Obviously this is not the peritoneal macroscopic injury, because no adhesion formed between the organs manipulated, but only at the area of injury.

The model that the peritoneal cavity is a cofactor in adhesion formation at the level of peritoneal trauma is bound to influence our clinical concept of adhesion prevention. First, the clinical wisdom of gentle tissue handling and careful and bloodless surgery becomes experimentally supported. The importance of experience and training gains another dimension, because in addition to reducing complication rates and operation time it also reduced tissue manipulation. We thus begin to understand why training and experience might be key factors in adhesion prevention.

Clinical adhesion prevention today is limited to barriers and flotation agents, both of which are effective. The mechanism of their action, however, could be interpreted differently, because barriers could also prevent peritoneal factors from reaching the trauma sites whereas flotation agents could attenuate these factors by massive dilution. The concept of the peritoneal cavity as a cofactor in adhesion formation has other far-reaching implications for adhesion prevention, because therapy should also be oriented to minimizing or preventing these peritoneal factors. In addition to quality of surgery (i.e., gentle tissue handling and duration of surgery); minimizing the mesothelial trauma of ROS, hypoxia, and desiccation by humidification; and adding small amounts of oxygen and cooling, other factors should be considered (e.g., dexamethasone). In these experiments we specifically used dexamethasone because it proved efficacious in previous experiments using this model [27], and because we anticipated some inflammatory reaction after manipulation. Moreover, in the first experiment the effect of dexamethasone was so pronounced that we wanted to test whether the effect might be specific for manipulation-enhanced adhesion formation. Dexamethasone was confirmed to attenuate effectively the adhesiogenic effect of CO₂ pneumoperitoneum plus manipulation although not more than the effect of CO₂ pneumoperitoneum only. This effect thus seems not specific for manipulation-enhanced adhesions, because the reduction was only proportional to the control group (i.e., adhesions after manipulation and dexamethasone treatment remaining more important than after dexamethasone only).

To estimate the clinical implications of these findings the model used should be understood. The mouse model combines minimal surgical trauma (enough to induce reliably albeit minimal adhesions) together with factors affecting the entire peritoneal cavity and enhancing this posttraumatic adhesion formation. In human surgery the surgical trauma is generally much more pronounced whereas the intensity of the other factors enhancing adhesions is variable. Today we do not have clinical evidence of adhesion reduction by reducing these adhesion-enhancing factors (i.e., CO₂ pneumoperitoneum, ROS, desiccation, and tissue manipulation). The importance of the latter 2 factors, however, has been postulated clinically for decades. In addition, the adhesion-reducing effect of dexamethasone should be considered carefully. Although effective in rabbits [29], efficacy was not consistently confirmed in human beings [30] or monkey models [31]. In our mouse model dexamethasone was shown to be highly effective in reducing adhesions in a pure surgical trauma model. When used in the adhesion enhancement model (trauma or CO₂ pneumoperitoneum) the adhesions reduction remains statistically significant but the effect of dexamethasone clearly is not specific for this adhesion enhancement. Absence of significant adhesion reduction in human and primate models thus is not surprising because the enhancement factors involved in adhesion formation were poorly controlled, whereas variability in non-inbred strains is much higher. This interindividual variability, together with ethical constraints limiting standardization of human surgery, hampers clinical investigation. Understanding the underlying mechanisms, however, could be important in designing effective adhesion reduction regimens.

In conclusion, adhesion formation between traumatized areas is enhanced by manipulation of pelvic organs. This further supports the concept that the entire peritoneal cavity is a cofactor in adhesion formation. Indeed, because hypoxia, addition of oxygen, and desiccation affect both traumatized areas and the peritoneal cavity it has been difficult to conclude unequivocally that the effect was through the peritoneal cavity. These experiments, to the contrary, by revealing the effect of manipulation in the upper abdomen at distance from the trauma sites, show that the peritoneal cavity produces cofactor or cofactors modulating adhesion formation. Clinically the concept of gentle tissue handling is supported, emphasizing the importance of training and skill development not only to reduce operating time and complications but also to minimize unnecessary tissue manipulation.

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