We have reported previously that there is a high incidence of hemothorax and substantial mortality in rabbits that are given tetracycline derivatives intrapleurally. However, such complications have not been reported in humans when pleurodesis is attempted with tetracycline derivatives. One primary difference in the two situations is that a chest tube is placed only in humans. The objective of this study was to evaluate the hypothesis that chest tube placement would prevent the development of hemothoraces and lead to better pleurodesis in rabbits given doxycycline intrapleurally. Eighty New Zealand White male rabbits received doxycycline, 20 mg/kg, in a total volume of 2 mL. One half of the rabbits were randomized to receive a chest tube at the time of the injection and were subjected to pleural fluid aspiration twice daily. The remaining rabbits (control group) received no chest tube and no aspiration. Ten rabbits from each group were killed on days 4, 7, 14, and 28. The intrapleural injection of doxycycline induced the production of large exudative effusions. The insertion of chest tubes prevented the development of hemothorax (0/20 in chest tube group, 15/20 in control group, p<0.001). The insertion of chest tubes was also associated with a significant reduction in mortality and a significant improvement in pleurodesis. When pleurodesis is attempted in rabbits with intrapleural doxycycline, the insertion of a chest tube will prevent hemothorax and lead to a better pleurodesis. 

**Key words:** chest tube; doxycycline; hemothorax; pleural fluid; pleurodesis

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**Chemical pleurodesis is frequently employed to obliterate the pleural space, most commonly for the treatment of recurrent pleural effusion or spontaneous pneumothorax.** Tetracycline or its derivatives are commonly used to induce a pleurodesis.1,2 When tetracycline or its derivatives are used to induce a pleurodesis in rabbits, there is a high incidence of hemothorax3,4 and considerable mortality.3 However, when tetracycline derivatives are administered to patients with pneumothorax or pleural effusion, such complications are rarely if ever seen. One of the main differences between the pleurodesis procedure in rabbits and in humans is that chest tubes were not used in the rabbits. The purpose of the present study was to assess the hypothesis that placement of a chest tube in the injected hemothorax will prevent the development of the hemothorax and decrease mortality in rabbits. Our reasoning is as follows: the intrapleural injection of a tetracycline derivative induces severe pleural inflammation with the production of a large pleural effusion. The inflammation of the visceral pleura of the lung underlying the effusion produces neovascularization and fibrosis. This fibrosis prevents the lung from reexpanding when the effusion resolves. Accordingly, the pleural pressure becomes quite negative and the fragile capillaries associated with the neovascularization in pleura may rupture producing a hemothorax.

**Materials and Methods**

New Zealand white male rabbits weighing 2.0 to 3.0 kg were utilized in this study. The protocol was approved by the animal...
were included that

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7 mg/kg. The doxycycline was injected directly
through the chest tube. The tube was secured in place with a purse string suture and was attached to a Heimlich valve with a three-way stopcock in-line between the chest tube and the valve. The rabbits were fitted with a vest to which the chest tubes were attached.

Following the doxycycline injection, the chest tube was closed via the stopcock for the following 2 h. The stopcock was opened then so that the fluid could drain spontaneously through the Heimlich valve. In addition, the chest tube was aspirated twice daily using the three-way stopcock; the amount of fluid recovered at each aspiration was recorded. The chest tube was left in place for at least 3 days. If the amount of pleural fluid aspirated was > 1 mL, the chest tube was left in place a maximum of 6 days. To remove the chest tube, the rabbits were lightly anesthetized with ketamine hydrochloride, 17 mg/kg, plus xylazine hydrochloride, 2.5 mg/kg.

The rabbits without chest tubes, the control group, had the doxycycline injected directly into the right pleural space. A 16-gauge IV catheter placement unit (Deseret Medical Inc; Becton Dickinson and Company; Sandy, Utah) was inserted into the pleural space. The catheter plug was then removed so that the right lung collapsed, thereby preventing damage to the lung by the catheter's needle. The needle was removed and the plastic catheter left in place. A three-way stopcock was attached to the end of the catheter. All air was evacuated from the pleural space via the stopcock. Verification that the tip of the catheter was in the pleural space was obtained by documenting inspiratory pressure drops with a pressure transducer. The position of the catheter in the pleural space was verified by observing the characteristic pleural pressure tracing on an oscilloscope prior to injection of the doxycycline. The catheter was removed immediately after the doxycycline was injected.

All rabbits were given 150 mL of normal saline solution with 5% glucose subcutaneously immediately after surgery. Rabbits that appeared lethargic or that had diarrhea were given the same solution every 12 h for the first 48 h. All rabbits received huprenorphine hydrochloride (Reckitt & Colman Products; Hull, England) 0.05 mg/kg, subcutaneously, immediately upon awakening and 12 h later. Rabbits that appeared to have any distress over the following 36 h were given the same medication. Rabbits that appeared distressed after this time were killed.

The protocol called for 10 rabbits in each group to be killed 4, 7, 14, and 28 days after the injection. Rabbits that died or were killed due to distress within the first 96 h were replaced and are not included in the analysis. Rabbits that died after the first 96 h were included in the analysis. The rabbits were killed by the injection of 40 mg/kg pentobarbital solution (Abbott Laboratories; North Chicago, Ill) into the marginal ear vein. The necropsy was done carefully by two of the investigators (L.R.T. and W.W.). Immediately after the rabbits were killed, attempts were made to aspirate all fluid from the peritoneal cavity. Organomegaly of the liver or spleen was recorded. In addition, attempts were made to aspirate all fluid from both pleural spaces using a posterior transdiaphragmatic approach. The volume of aspirated pleural fluid was recorded. Small incisions were made in the diaphragm to allow better access of the fixative (10% formaldehyde solution) to the pleural cavities. Attempts were made to expand the lung with the intratracheal injection of 10% formaldehyde solution through a plastic catheter (6-mm diameter) that had been inserted. After the trachea was ligated with silk, the entire thorax was submerged in 10% formaldehyde solution for at least 48 h.

The macroscopic evaluation was performed by two of the investigators (L.R.T. and R.W.L.) who were blinded as to the treatment the animal had received and the day of killing. Each pleural cavity was exposed following our method as previously described. Each pleural cavity was carefully exposed by making bilateral incisions through the diaphragms and through all the ribs at approximately the midclavicular line. In this manner, the sternum and the medial portions of the anterior ribs were removed so that the lung and pleural cavities could be evaluated. In particular, we evaluated and recorded the presence of adhesions, hemothorax, pleural effusion, and atelectasis.

The degree of gross adhesions was graded according to the following scheme: 0, normal pleural space; 1, one to three small adhesions in the pleural space; 2, more than three scattered adhesions but the lung is easily separated from chest wall; 3, generalized scattered adhesions with areas where the lung can be separated from the chest wall only with difficulty; and 4, complete obliteration of the pleural space by adhesions.

The presence of effusion and hemothorax was recorded on a zero- to 4-point scale with 0 indicating no effusion or hemothorax, 1 indicating an effusion or hemothorax that involved <15% of the hemothorax, 2 indicating involvement from 15 to 33%, 3 indicating involvement from 33 to 75%, and 4 indicating involvement of >75% of the hemothorax. At gross examination, the diagnosis of hemothorax was made if there were blood clots in the pleural space. Atelectasis was classified as none (0), partial (1), or complete (2).

The effectiveness of the pleurodesis was assessed according to the following scheme: 1, poor, presence of one or more of the following: score <2 for adhesions; hemothorax or effusion score of 3 or 4; complete atelectasis; 2, acceptable, presence of the following: gross adhesion score of 3 or 4; hemothorax or effusion score of >2; none or partial atelectasis; and 3, excellent, presence of both the following: gross adhesion score of 4; and no hemothorax, pleural effusion, or atelectasis.

At the time that the pleura was evaluated grossly, samples of the visceral pleura and lung from each hemothorax were obtained from the anterior lower lobes and placed in neutral buffered 10% formaldehyde solution. These tissue samples for histologic examination were processed routinely and stained with hematoxylin and eosin. The microscopic slides were evaluated blindly by two of the investigators (R.W.L. and L.R.T.) for the presence of inflammation and fibrosis in the visceral pleura. The degree of microscopic inflammation and fibrosis was graded none (0), equivocal (1), mild (2), moderate (3), or marked (4) as we have previously described. The degree of atelectasis in the underlying lung was graded with the same scale. The degrees of congestion, vascularization, and necrosis in the livers were also graded with the same scale.

Laboratory and Animal Investigations

564
anesthetic when the chest tube was placed, two died of diarrhea despite saline solution infusions, and three died of unknown causes). One additional rabbit was killed because it was distressed from scrotal edema. All 10 of the deaths occurred in rabbits in the chest tube group. These 10 rabbits were replaced and are not included in the analysis. Most of these deaths occurred with the early rabbits while we were perfecting the anesthesia and our operative methods.

Twelve rabbits died >1 week after receiving the doxycycline intrapleurally. Ten of the 12 deaths were in the control group while only 2 were in the chest tube group (X² = 5.83, p < 0.05). There were eight deaths in the 28-day control group and these occurred between 10 and 17 days. There were two deaths in the 14-day control group (10 and 13 days). There were two deaths in the 28-day chest tube group (10 and 12 days). All the rabbits that died in the control group had large pleural effusions and ascites and six had a hemothorax. In contrast, the rabbits that died in the chest tube group did not have pleural effusions, ascites, or hemothorax. The results from these 12 rabbits are included in the analysis at the time point when they were originally scheduled to be killed.

The intrapleural injection of doxycycline resulted in the formation of large amounts of pleural fluid. In the rabbits with chest tubes, pleural fluid could be aspirated from almost every rabbit from 2 h to 4 days postinjection (Fig 1). The mean amount of fluid aspirated after 2 h was 2.6 ± 0.3 mL and this increased to 8.6 ± 0.8 mL 1 day after injection. Relatively large amounts of fluid continued to be formed between day 1 and day 2. Thereafter, the amount of pleural fluid obtained each day became progressively less. The amount of fluid aspirated on each day after the second day was significantly (p < 0.01) less than that aspirated on the previous day.

There were substantial amounts of pleural fluid present in the right hemithorax at the time of killing.

### Table 1—Amount of Fluid* Aspirated at Necropsy

| Groups | Right Side | | Left Side | | | Ascites | | |
|--------|------------|----------------|------------|----------------|----------------|----------------|----------------|
|        | Chest Tube | Control       | Chest Tube | Control       | Chest Tube | Control       |                |
| 4 d    | 4.9±1.7    | 19.4±3.5†     | 1.0±0.0    | 5.3±0.9†      | 0.0±0.0    | 0.0±0.0       |                |
| 7 d    | 5.3±1.7    | 19.7±3.5†     | 0.0±0.0    | 0.8±0.5       | 0.0±0.0    | 2.3±1.5       |                |
| 14 d   | 1.8±1.1    | 38±4.0†       | 0.0±0.0    | 8.7±4.0†      | 0.0±0.0    | 55.5±9.8†     |                |
| 28 d   | 0.4±0.4    | 47±6.9†       | 0.0±0.0    | 11.0±4.3      | 0.0±0.0    | 74±20.4†      |                |

*In milliliters, mean ± SEM.
†p<0.01.
‡p<0.001 compared with chest tube group.
§p<0.05.

**Statistical Analysis**

All data are expressed as the mean ± SEM. The results with the two different treatments (chest tube or no chest tube) on the different days were compared using the unpaired t test. If the data failed the normality test, the results were then compared using the nonparametric Mann-Whitney rank sum test (SigmaStat; Jandel Scientific; San Rafael, Calif). Differences in the treatment results were considered significant when p<0.05. χ² tests were used for statistical analysis when incidences in different groups were compared.

**RESULTS**

In this study, nine deaths occurred in the first 96 h after injection (four never recovered from the
The rabbits without chest tube placement also tended to have more atelectasis at necropsy. In the control group, 11 of the 20 rabbits had complete atelectasis and the remaining 9 rabbits all had partial atelectasis. In comparison, in the chest tube group, none of the rabbits had complete atelectasis, and 14 had no atelectasis ($\chi^2=25.6, p<0.0001$).

The results with pleurodesis were much better in the chest tube group than in the control group (Fig 3). Fifteen of the 20 rabbits in the chest tube group had an excellent pleurodesis result while none of the 20 control group rabbits had an excellent result and only one had an acceptable result ($\chi^2=36.7, p<0.0001$). The primary reason that the rabbits in the control group had a poor result was that they had significant amounts of hemothorax, pleural effusion, and/or atelectasis that prevented adhesions between the visceral and parietal pleura. The rabbits in the control group did have gross adhesions between the visceral and the parietal pleura when these two surfaces were not separated by blood or fluid. The mean adhesion score did not differ significantly between the two groups.

When the right pleura was examined microscopically, the degrees of inflammation and fibrosis were comparable in the chest tube and the control group (Table 3). The pleural inflammation was moderate in both groups at all study times. The degree of inflammation was significantly ($p<0.05$) greater in the control group on day 28. At 4 days, the degree of pleural fibrosis was low, but it subsequently increased and there were no significant differences between the two groups. There was significantly more atelectasis in the underlying lung in the control group at 14 days and at 28 days.

At the time of killing, many of the rabbits were

![Graph showing the mean amount of pleural fluid aspirated from right hemithorax after killing in the two different groups. Solid bars = chest tube group; hatched bars = control group. Asterisk (*): p<0.01 when the chest tube group and the control group were compared; double asterisk (**): p<0.001 when the chest tube group and the control group were compared.](image)

in the control group of rabbits (Table 1 and Fig 2). The mean amount of fluid tended to increase with time after the intrapleural injection. The largest amount of fluid in any rabbit was 70 mL and this occurred in a rabbit in the 28-day control group that died prematurely on the 14th day. The mean amount of fluid in the right pleural space was significantly greater ($p<0.05$) in the rabbits that died prematurely than in those that were killed at the scheduled time (Table 2). The rabbits with chest tubes had significantly less fluid in their right pleural space at each time of death (Table 1).

At autopsy, the rabbits in the control group also tended to have substantial amounts of fluid in their left pleural space and in their peritoneal cavity while the rabbits in the chest tube group tended to have fluid in neither of these compartments (Table 1). The amount of pleural fluid in the left side was significantly less than that in the right side in both groups. Interestingly, the rabbits that died prematurely tended to have more fluid in the left pleural space and in the peritoneal cavity than did those that did not die prematurely (Table 2).

The incidence and the magnitude of hemothorax were much less in the rabbits that had chest tubes. Hemothorax indicates the presence of blood clots in the pleural space at the time of postmortem examination. Fifteen of the 20 control rabbits that were scheduled for killing on day 14 or 28 had a hemothorax while none of the 20 rabbits with a chest tube had a hemothorax ($\chi^2=20.9, p<0.0001$). The hemothorax occupied $>33\%$ of the hemothorax in 12 of these 20 rabbits.

![Table 2—Amount of Pleural Effusion Aspirated at Necropsy.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of Death</th>
<th>n</th>
<th>Amount of Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right pleural space</td>
<td>28 d</td>
<td>8</td>
<td>51.2±6.9†</td>
</tr>
<tr>
<td>28 d</td>
<td>10.5±9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>55.0±5.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>33.5±3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left pleural space</td>
<td>28 d</td>
<td>8</td>
<td>22.0±4.9†</td>
</tr>
<tr>
<td>28 d</td>
<td>10.0±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>30.0±10.0‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>3.4±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritoneal</td>
<td>28 d</td>
<td>8</td>
<td>92.5±20.6‡</td>
</tr>
<tr>
<td>28 d</td>
<td>0.0±0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>50.0±5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 d</td>
<td>56.0±12.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In milliliters, mean±SEM.
†Comparison between the rabbits with premature death and the rabbits that were killed at predetermined time.
‡p<0.05 when compared with killing at groups 28 and 14 days.
noted to have hepatomegaly. When the livers were examined microscopically, those in the control group tended to have more congestion than did the livers in the chest tube group (Fig 4). The hepatomegaly appeared to be due mostly to congestion since there was only minimal evidence of vacuolization or necrosis.

**DISCUSSION**

The results from the present study demonstrate that the insertion of a chest tube for the first few days after the intrapleural injection of doxycycline prevents the development of hemothorax. The pleurodesis produced after the combination of doxycycline and chest tube is superior to that which we have observed after doxycycline alone. The chest tube removes the large amount of pleural fluid that is formed in response to the doxycycline intrapleurally and results in a closer approximation of the visceral and parietal pleura such that pleurodesis can occur.

The present study confirms observations in previous studies\(^3,4\) that hemothorax occurs after the intrapleural administration of tetracycline derivatives in rabbits. In the present study, the incidence of hemothorax in the animals that did not receive chest tubes was 75% at 14 days or later. This incidence is somewhat higher than the 50% incidence that we previously reported after the intrapleural administration of 20 or 40 mg/kg minocycline or 35 mg/kg tetracycline. Hurewitz and coworkers\(^4\) observed bloody pleural fluid 14 days after the intrapleural injection of tetracycline, 35 mg/kg, and doxycycline, 10 or 35 mg/kg.

The mechanism for the development of hemothorax in rabbits that are given high doses of tetracycline derivatives intrapleurally remains unclear. Nevertheless, several observations might provide clues as to the mechanism. First, the intrapleural injection of tetracycline derivatives induces severe intrapleural inflammation with formation of exudative pleural effusion as shown in this and previous studies.\(^4,8\) The intrapleural injection of tale as a slurry also produces an acute exudative effusion, but the inflammation is less intense and animals given tale intrapleurally usually do not develop a hemothorax.\(^9\) Second, after the intrapleural injection of tetracycline derivatives, the pleural fluid tends to become more hemorrhagic with time. For example, in the present study, none of the effusions were hemorrhagic after 4 or 7 days, but by 14 days, 75% of the animals had hemothorax. Hurewitz and associates\(^4\) reported that hemorrhagic fluid filled the injected left pleural cavity in all animals that had received tetracycline or doxycycline 2 weeks previously. Third, the intrapleural inflammatory reaction continues at a high level for at least 4 weeks (Table 3). As the inflammation continues, neovascularization develops and becomes prominent on the surfaces of the visceral and parietal pleura.\(^4\) It

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**Table 3—Mean Degree of Microscopic Fibrosis and Inflammation of Right Visceral Pleura and Microscopic Atelectasis of Underlying Right Lung***

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pleural Inflammation</th>
<th>Pleural Fibrosis</th>
<th>Atelectasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chest Tube</td>
<td>Control</td>
<td>Chest Tube</td>
</tr>
<tr>
<td>4 d</td>
<td>2.8±0.2</td>
<td>3.3±0.3</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>7 d</td>
<td>2.9±0.2</td>
<td>2.9±0.2</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>14 d</td>
<td>2.4±0.4</td>
<td>2.8±0.2</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>28 d</td>
<td>2.2±0.3</td>
<td>3.1±0.2</td>
<td>2.9±0.3</td>
</tr>
</tbody>
</table>

*\(N=10\) in each group.
**\(p<0.05\).
***\(p<0.01\) when compared with chest tube group.
is likely that rupture of one or more of these newly formed capillaries on the surface of the pleura transforms the exudative serous effusion into an exudative hemorrhagic pleural effusion. The transformation of the serous effusion to the hemorrhagic effusion between 7 and 14 days lends support to this. Fourth, we have observed at autopsy, 28 days after minocycline or doxycycline intrapleurally, that the mediastinum is shifted markedly toward the side that received the injection and that the underlying lung is usually completely atelectatic. It is likely that the intrapleural pressure is quite negative given the shift of the mediastinum. Accordingly, the negative intrapleural pressure could rupture the fragile vessels in the neovascularized tissue, which in turn would produce a hemothorax.

Why does chest tube placement prevent the development of the hemothorax? If an animal has a large exudative pleural effusion, the underlying lung must be atelectatic. If pleural inflammation persists, an inflammatory peel will form on the surface of the atelectatic lung. If the lung remains atelectatic, the inflammatory peel will prevent the underlying lung from expanding. When the fluid is eventually reabsorbed, the lung cannot expand, creating a negative pleural pressure. Placement of the chest tube removes the inflammatory fluid. Hence, the inflammation occurs with the lung fully inflated so that there is much less underlying atelectasis. Pleural inflammation with the lung expanded will result in a good pleurodesis and markedly negative pleural pressures will not develop.

Another possible explanation for the chest tube preventing the hemothorax is that the chest tube could have possibly removed cells, cytokines, or other factors that are responsible for the development of the hemothorax.

In the present study, the effectiveness of pleurodesis was much better when the animals also had a chest tube (Fig 3). We believe that this is due to the fact that the chest tube prevented the atelectasis in the initial period after the injection. Therefore, the lung did not become trapped by the inflammatory peel. The inflammatory process in the closely approximated visceral and parietal pleura led to fusion of the surfaces with excellent pleurodesis.

There was a substantial mortality rate in the animals in the control doxycycline group after the first 10 days. We suggest that this mortality was due to right heart failure since most rabbits that died had bilateral pleural effusions, ascites, and hepatomegaly. Microscopic examination of the liver revealed primarily congestion with only minimal vacuolization or necrosis. Unfortunately, we did not measure the level of protein in the contralateral hemithorax or the peritoneal cavity. We hypothesize that the hemothorax and atelectasis produce severe hypoxia, which in turn leads to pulmonary hypertension and right heart failure, although we have no direct proof of this.

From this study, we conclude that the placement of a chest tube when tetracycline derivatives are used intrapleurally in rabbits will prevent the development of hemothorax with its associated high mortality. The primary clinical implication from the present study is that pleurodesis should not be attempted with tetracycline derivatives unless a functional chest tube is in place.

REFERENCES

FIGURE 4. Mean degree (±SEM) of liver congestion at various times postinjection. Asterisk (*): p<0.05 when the chest tube group and the control group were compared.