# STUDY OF LUNG SURFACTANT ACTIVITY IN GUINEA PIGS AFTER VAGOTOMY

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**Summary:** Vagotomy in guinea-pigs reduces the pulmonary surfactant activity. Unilateral vagotomy brings about a similar reduction in the lung on the same side. The findings indicate that surfactant reduction may be an important causative factor in the initiation of pulmonary edema in vagotomised guinea-pigs. It is suggested that surfactant synthesis or release may be affected by alterations in the pulmonary blood flow or removal of the effect of neuro-secretory fibres, following vagotomy.

**Key words:** bilateral vagotomy, surfactant activity, pulmonary edema, surface tension, unilateral vagotomy

## INTRODUCTION

The importance of surfactant material at the alveolar lining layer in maintaining the structural stability of the terminal lung units during respiration is well recognised. Abnormal surfactant activity was demonstrated in vagotomised animals by Tooley *et al.* (10) and Said *et al.* (9). On the other hand, Bolande and Klaus (1) have shown that there is no change in minimal surface tension of the lung extracts following vagotomy, but suggest that surface tension is seriously influenced by the presence of pulmonary edema. Whether the formation of edema is a cause or effect of inhibition of lung surfactant, remains controversial. Hence the present work was undertaken to study systematically the pulmonary surfactant activity with time following vagotomy in guinea pigs.

## MATERIALS AND METHODS

Healthy guinea-pigs of either sex (380-500 g) were divided into several groups for the study.

Under intraperitoneal sodium pentobarbital anaesthesia, (35-40 *mg/kg*) trachea was cannulated with a T shaped glass cannula. One end of the 'T' tube was utilised for recording respirations by connecting it to Marey's tambour. Vagal nerve/nerves were sectioned in the midcervical region. Care was taken to maintain the patency of the tracheal tube throughout the duration of the experiment.

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In the groups III, IV, V, VII and VIII subjected to bilateral vagotomy, the animals were sacrificed immediately, 1/2, 1, 2, 3 and 4 hr after section of the vagi respectively. The control animals, (Group II), subjected to the same surgical procedure except vagotomy were sacrificed 5 hr after tracheostomy. In other series, following unilateral vagotomy the animals were sacrificed at the end of 5 hr.

The lungs were carefully excised, rinsed in saline, blotted between filter paper and weighed separately nearest to 10 *mg* and their gross morphologic features noted.

**Surface tension measurement of lung extract:**

0.5 g of lung tissue was cut into 1 mm pieces with scissors and minced in 0.9% saline. The filtrate, made up to 10 *ml*, was kept for aging for 1/2 hr in the trough of modified Whilhelmy surface tension balance (4). The surface film was compressed and expanded at 10 cycles per min for one hr, at the end of which, maximum surface tension (T max, at 100% expansion) and minimum surface tension (T min, at 20% compression) were recorded. Extract stability Index (SI) was calculated, using the formula

$$S.I. = \frac{2\ (T\ max - T\ min)}{T\ max + T\ min}$$

**Assessment of pulmonary edema:**

Pulmonary edema was assessed in each lung separately by (i) Lung Body Index (L.B.I.) expressed as lung weight (LW) to body weight (BW) percentage ratio; (ii) ratio of wet weight to dry weight (WDR) of the lung. For dry weight, lung slices were oven dried at 60°C until the weight remained constant for 48 hrs and (iii) routine histopathological examination. In bilateral vagotomy the LBI was determined for both the lungs together.

For statistical analysis, the findings in experimental groups were compared with those in the control group. The lung on the side of intact vagus formed the control in unilateral vagotomy experiments.

## RESULTS

Bilateral vagotomy always produces lethal pulmonary edema in guinea pigs (8). Survival time after bilateral vagotomy determined separately in the beginning of this study ranges from 5-7 hrs. The external manifestations of frank edema were not observed before the end of 4 hrs after vagotomy.

The respiratory rate in control animals ranged from 50-70 per min. Unilateral vagotomy on either side did not produce change in rate or depth of the respiration. Bilateral vago-

tomy however, resulted in slowing of the respiration. After a lapse of about 4 hrs, slow, apneustic breathing accompanied by generalized convulsions with apneusis, was observed in most of these animals. The pulmonary surfactant activity of the groups III and IV (5 animals in each) after bilateral vagotomy was not different from that of the controls (6 animals) (Table I). However, significant increase in T/min, T/max and decrease in S.I. in group V (6 animals) shows a definite change in surfactant activity. The surface tension measurements of lung extracts in groups VI, VII and VIII (6 animals in each group), bilateral vagotomy showed further increse in T/min and T/max.

TABLE I : Surfactant activity of the lungs and L.B.I. after bilateral vagotomy in guinea pigs. (Values are Mean ± S.D.)

| Group | L.B.I. | Surface tension (Dynes/cm) | | Stability Index |
|---|---|---|---|---|
| | | T Min | T Max | |
| I Sham operated (6) | 0.60±0.05 | 27.73±1.34 | 51.23±1.20 | 0.59±0.04 |
| II Control (6) | 0.70±0.11 | 27.04±1.42 | 51.60±0.89 | 0.62±0.05 |
| III Immediately Vx (5) | 0.59±0.04 | 27.04±1.42 | 51.60±0.89 | 0.62±0.05 |
| IV 1/2 hr Vx (5) | 0.61±0.05 | 27.56±1.42 | 50.68±1.23 | 0.58±0.06 |
| V 1 hr Vx (6) | 0.75±0.15 | 36.15±0.30* | 54.30±0.35* | 0.40±0.00* |
| VI 2 hr Vx (6) | 1.26±0.21* | 39.90±1.68* | 55.75±1.70* | 0.33±0.01* |
| VII 3 hr Vx (6) | 1.55±0.03* | 42.90±1.50* | 54.70±3.12* | 0.24±0.02* |
| VIII 4 hr Vx (6) | 1.83±0.34* | 42.20±2.15* | 53.92±2.51* | 0.25±0.05* |

Number in the paranthesis indicates the number of animals.
*P< 0.001 Vx = After vagotomy Control : Sacrificed 5 hr after tracheostomy

Unilateral vagotomy (Table II) increases the T/min and T/max of the lung on the side of vagotomy whereas, the other lung of the animal has shown normal surfactant acitvity.

The pulmonary edema as assessed by LBI, (Table I) WDR and histopathology was detected in all groups sacrificed after 2 hr of bilateral vagotomy. The edema increases with time as seen by progressive increase in LBI. None of the lungs in unilateral vagotomy showed edema on histological studies. In control animals, WDR ratio was 4.37 ± 0.44. But in pulmonary edema this ratio increased to 10.47 ± 2.00.

In histopathological examinations pulmonary congestion was observed in lungs before the interstitial or alveolar edema was detected.

## DISCUSSION

Lung surfactant activity and LBI are not significantly altered by the experimental procedure like tracheostomy or anaesthesia, even after 5 hrs. This is evident from the findings in the sham-operated and control groups (Table I).

TABLE II : Effect of unilateral vagotomy on lung surfactant and L.B.I., calculated for each lung. (Values are Mean±S.D.)

| Group | L.B.I. | Surface tension (Dynes/cm) | | Stability Index |
|---|---|---|---|---|
| | | T Min | T Max | |
| *LEFT VAGOTOMY* (6) | | | | |
| Left lung | 0.35±0.01 | 42.12±2.84* | 55.60±1.66* | 0.28±0.03* |
| Right lung | 0.24±0.01 | 27.96±2.15 | 51.40±2.27 | 0.56±0.04 |
| *RIGHT VAGOTOMY* (5) | | | | |
| Right lung | 0.32±0.02 | 41.60±1.80* | 55.00±1.60* | 0.27±0.02* |
| Left lung | 0.30±0.02 | 27.50±2.00 | 55.10±2.20 | 0.66±0.05 |

Number in the paranthesis indicates the number of animals.
*P <0.001

The observation that lung surfactant activity in groups III and IV following bilateral vagotomy was comparable to those of control shows that surfactant activity was not affected for half an hr after vagotomy. Increase in the T/max and T/min in the group sacrificed 1 hr after vagotomy definitely indicates a decrease in surfactant activity. However, this group did not show pulmonary edema. This excludes pulmonary edema as the cause of reduction in surfactant activity in this group and shows that surfactant activity is decreased before the edema is evident.

In other groups, with severe pulmonary edema, besides vagotomy, the edema itself may have contributed to the reduction of surfactant activity. Such observation were recorded by Bolande and Klaus, 4 hrs after vagotomy.

Decreased surfactant activity confined to the lung on the side of unilateral vagotomy after 24 hrs is reported by Tooley *et al.* (10). In our studies, this reduction was observed eve after 5 hrs following unilateral vagotomy. In this group, pulmonary edema was absent, though there was slight increase in the weight of the lung on the side of vagotomy.

Vagotomy is likely to change the cardiac performance and thereby affect pulmonary circulation (11). This alteration in circulation should change the surfactant production in both lungs, (11) but the unilateral vagotomy experiments have shown that the surfactant activity is decreased in the lung on the side of vagotomy.

Denervation of afferent fibres following vagotomy may affect lung expansion reflexly. Alterations in respiratory activity was not observed following unilateral vagotomy. Hence the surfactnat reduction may not be due to such respiratory effects. But removal of afferent activity in vagal and sympathetic fibres may reduce the pulmonary blood flow, which is important for surfactant synthesis by alveolar type II cells.

Few studies have shown that alveolar epithelial cells are supplied by nerve fibres but their role has not yet been clearly defined (3, 5). The prominent effects of cholinergic agents (2, 6)

and efferent vagal stimulation (7) on surfactant release suggest that they may be neuro-secretary in nature and involved in some way with the control of surfactant secretion. The sectioning of vagus may remove this secretary activity, thereby reducing surfactant production or its release.

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