

# EXPERIMENTAL STUDY OF PULMONARY MICROEMBOLISM IN RABBITS

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## INTRODUCTION

The influences of intrapulmonary reflexes and local responses upon changes of respiration and circulation have long been studied. The Bezold-Jarisch-like reflex<sup>1,2)</sup>, which is also observed in the acute stage of pulmonary embolism, is one of the typical patterns composed of those reflexes and responses. This reflex includes brady-cardia, systemic hypotension, pulmonary hypertension and hypoventilation<sup>1,3)</sup>. Comroe<sup>3)</sup> regarded this reflex as an expression of composite reactions which follow excitation of pulmonary depress- or chemo-reflex, coronary chemoreflex and pulmonary respiratory chemoreflex<sup>1)</sup>. This idea is supported by many investigators<sup>4,5)</sup> and they presumed that this reflex is triggered by the stimulation of sensory nerve endings at the area of the pulmonary vessels and coronary vessels.

In the first step to reveal the mechanism of reflex, the author observed the disturbances in respiration and circulation during the acute atage of pulmonary microembolism induced by rapid injection of barium sulfate emulsion into the right heart.

In the second step, autonomic nerves were blocked prior to the procedures to produce pulmonary hypertension, such as injection of barium sulfate emulsion, histamine, and so forth in order to reveal the effects of nervous control upon the pulmonary hemodynamics and respiration.

## MATERIAL AND METHODS

Rabbits (1.8 to 2.4 kg) were anesthetized with intravenous nembutal sodium 3.5 mg/kg. Respiration was recorded through the tracheal cannula on the kymographin via a tambour, and during thoracotomy it was maintained with positive pressure respirator, supplied with 2 l of pure oxygen per minute. Positive pressure ranged from 2 to 6 cm H<sub>2</sub>O per cycle.

Heparin 1000 I.U./kg was used as an anticoagulant. The catheter for injection

of barium sulfate solution was introduced through the right jugular vein into the right atrium. Pulmonary embolization was brought about by rapid injection of barium sulfate solution of 5.5 volume per cent suspended in saline. Systemic pressure was measured by a catheter connected to Statham transducer for recording, which was inserted via a left carotid artery into aortic arch. The thorax was opened by a median sternotomy and the heart was exposed for insertion of catheters to measure the pressures of main pulmonary artery and right ventricle. Since there was an essential difference between responses to those receiving more than 2.0 cc of the solution and those receiving less solution, rabbits were divided into two groups, that is, A and B. Group A received 0.6 to 1.0 cc/kg of the solution and Group B 1.1 to 1.4 cc/kg, respectively.

As procedures for autonomic nervous blockade, imidaline 2 mg/kg was injected for sympathetic blockade and both cervical vagi were cut off for parasympathetic blockade.

## RESULTS

### (1) Changes in hemodynamics

#### A. Changes in pulmonary artery pressure (PAP)

Systolic PAP of both groups began to increase in 1.5 to 2.0 sec after injection of the solution and attained its maximum value in 4 to 10 sec, which suggested that the initial response of PAP to embolization was mediated by an intrapulmonary reflex. After 10 sec it began to decline gradually (Fig. 1,2). Grades of increase were more prominent in Group B. The systolic PAP in group A returned nearly to the level of pre-embolization after 2 min, and thereafter maintained a slightly higher value, but in group B it decreased further and the rabbits died in several minutes (Fig. 2).

Diastolic PAP changed almost parallel to systolic PAP. Pulse pressure decreased prominently in group B, indicating right heart failure.

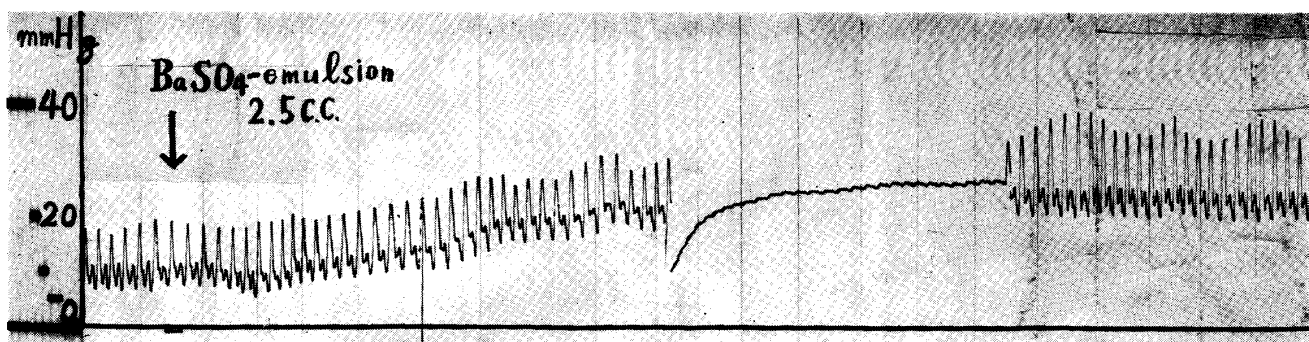


Fig. 1 Pulmonary artery pressure (PAP) began to increase within 2 to 3 sec of injection of barium sulfate emulsion into the right atrium. Both systolic and diastolic pressure increased. After 10 sec they began to decline gradually.

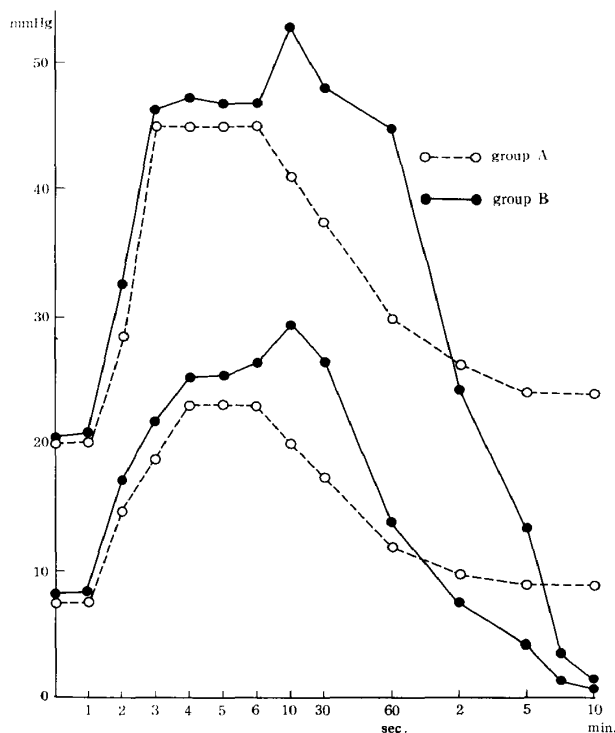


Fig. 2 Both systolic and diastolic PAP began to increase within 3 sec of pulmonary embolization. In group A after they attained stable maximum of PAP (mean value of 5 rabbits), they began to decline and reached a slightly higher level than that of pre-embolization. In group B, (mean value of 5 rabbits), PAP continued to decline further and rabbits died in several minutes.

B. Changes in right ventricular pressure (RVP)

Both systolic and diastolic RVP increased transiently 1 sec after injection, and then soon returned to the level of pre-injection. After 3 sec they began to increase again (Fig. 3). The response of RVP appeared later than that of PAP and this suggested that changes in RVP were secondary to changes in PAP. In group A systolic RVP changed almost parallel to that of PAP and returned to the pre-injection level after 2 min, but in group B the decrease in pressure after 10 sec

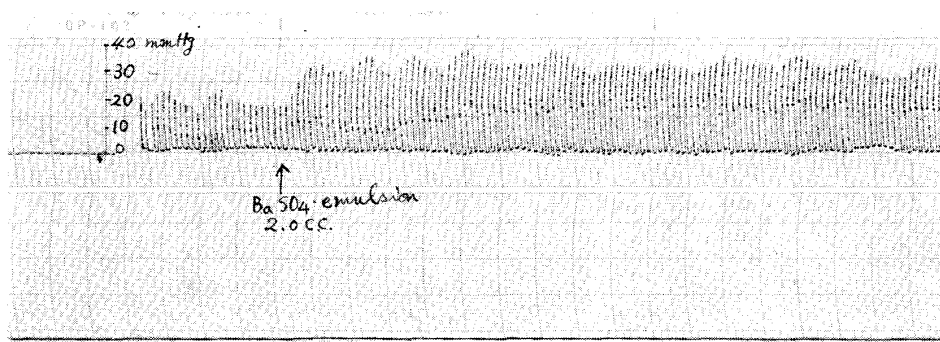


Fig. 3 Right ventricular pressure (RVP) of rabbits belonging to group A. Both systolic and diastolic RVP began to increase 3 to 4 sec after injection of barium sulfate emulsion.

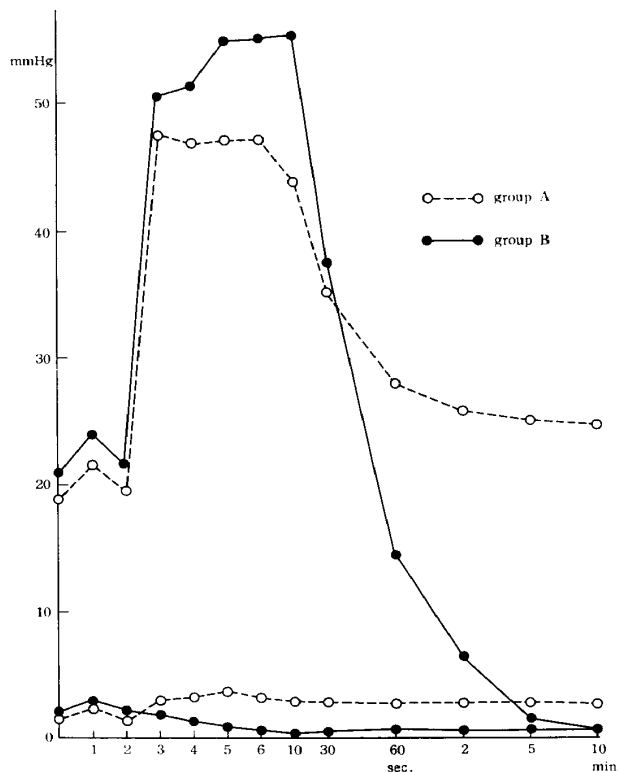


Fig. 4 Both systolic and diastolic RVP began to increase 3 to 4 sec after injection of barium sulfate emulsion, ensuing about one sec later than that of PAP. Changes of systolic RVP were almost parallel to those of systolic PAP. (mean value of 5 and 6 rabbits in group A and group B, respectively)

was more abrupt than that of group A, and rabbits died in several minutes (Fig. 4).

C. Changes in left atrial pressure (LAP)

LAP slightly decreased or stayed unchanged after pulmonary embolization. It was probably due to decrease in pulmonary blood flow (Fig. 5).

D. Changes in carotid artery pressure (CAP)

Both systolic and diastolic CAP began to decrease 1.5 to 2.0 sec after injection of the solution.

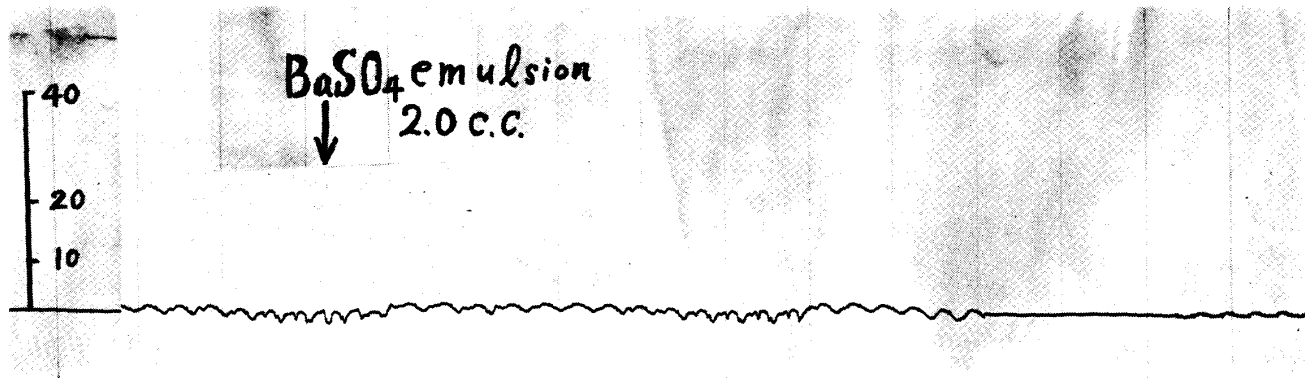


Fig. 5 Left atrial pressure (LAP) decreased slightly or stayed unchanged after pulmonary embolization.

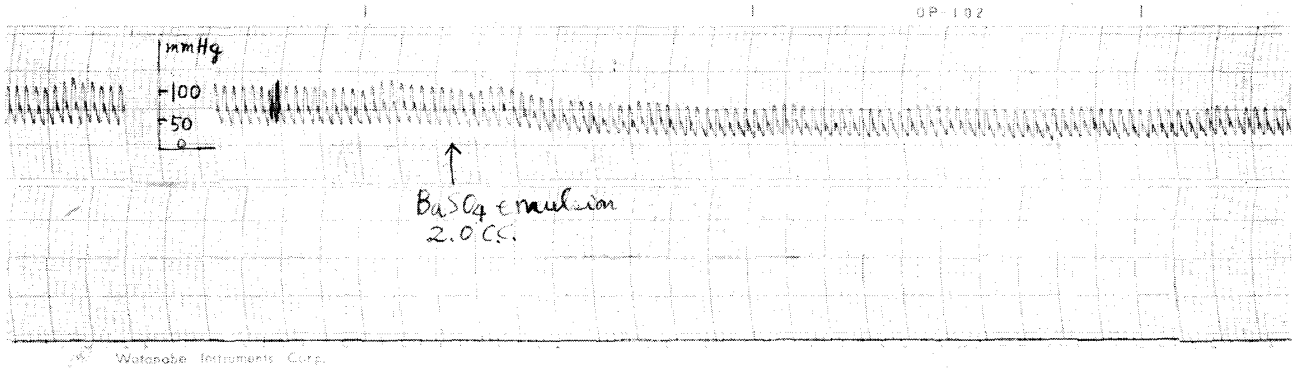


Fig. 6 Carotid artery pressure (CAP) began to decrease 1.5 to 2.0 sec after injection of the solution, suggesting that the changes were initiated by reflex. And then CAP gradually recovered to a somewhat lower level than that of pre-injection in 2 min in group A.

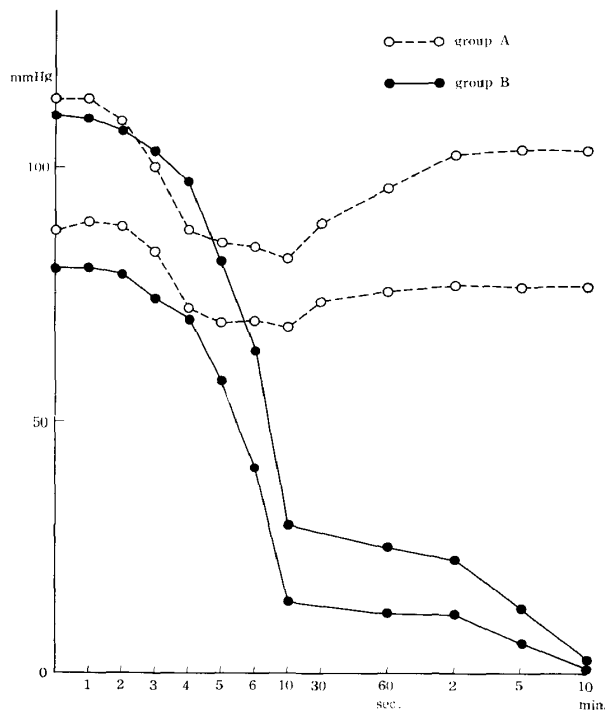


Fig. 7 Both systolic and diastolic CAP began to decrease 1.5 to 2.0 sec following injection of the solution. In group A, CAP gradually returned near the pre-injection level after 2 min, but in group B it continued to decrease until rabbits died. (Mean value of 5 rabbits, respectively)

In group A CAP reached the minimum value after 10 sec and then gradually recovered in 2 min to a stable, somewhat lower level than that of pre-injection (Fig. 6, Fig. 7).

They continued as they were for a considerable period, presenting a mirror image of PAP changes in group A.

Simultaneous response of CAP with that of PAP to pulmonary embolization suggested that response of CAP (hypotension) was provoked by an intrapulmonary nervous reflex. The ensuing hypotension was probably due to decrease in cardiac

output.

In group B, CAP continued to decline until the rabbits died.

**(II) Changes in respiration**

In both groups, changes in respiration appeared later by 3 to 4 sec than those of hemodynamic changes. This means that somewhat different mechanisms from those of hemodynamic changes are acting in respiration following microembolism.

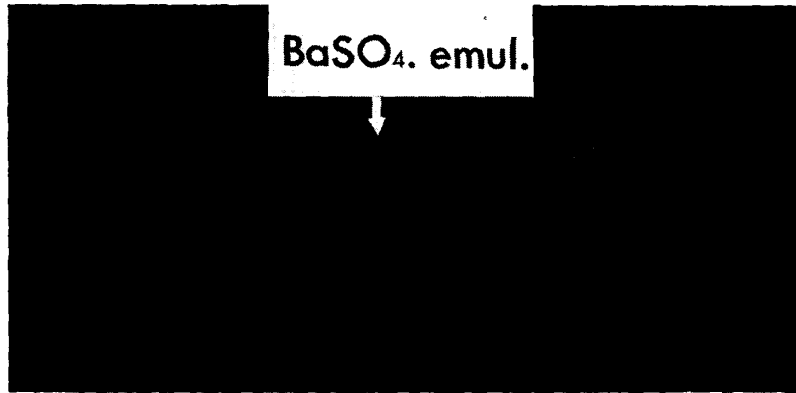


Fig. 8 Changes in respiration appeared later by 3 to 4 sec than those of hemodynamic changes. They showed hypoventilation suddenly 4.5 to 6 sec after embolization. In group A hyperventilation gradually appeared thereafter.

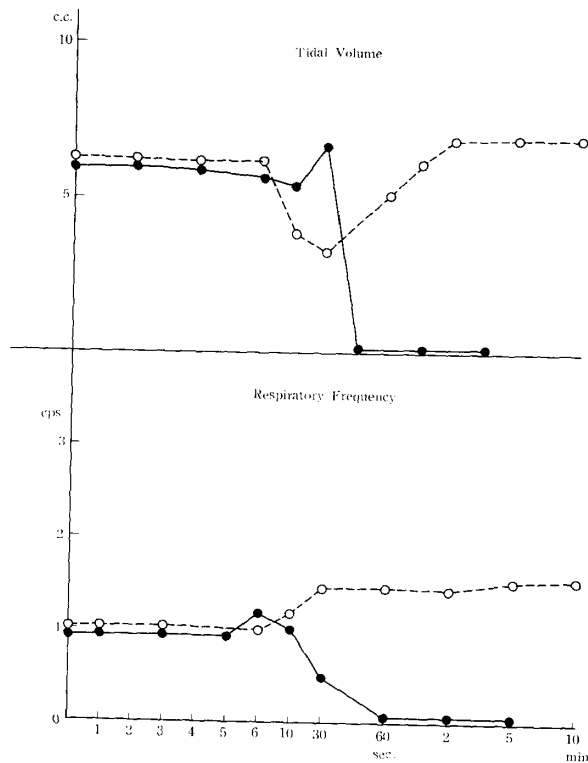


Fig. 9 In both groups, respiratory changes appeared later by 3 to 4 sec than those of hemodynamic changes. After initial sudden bradypnea, tachypnea/or hyperventilation developed in group A. But in group B bradypnea progressed to apnea.

They showed hypoventilation suddenly 4.5 to 6 sec after embolization; respiratory rate and tidal volume decreased transiently, presenting hypoventilation (Fig. 8).

In group B hypoventilation continued to worsen until apnea appeared within 1 min.

In group A hyperventilation gradually appeared after 40 sec of embolization and continued for a considerable period (Fig. 9).

**(III) Changes in pulmonary hemodynamics and ventilation after the procedures to induce pulmonary hypertension in the state of autonomic nervous blockades.**

In this experiment, all rabbits belong to group A, that is they survived the procedures to induce pulmonary hypertension.

Besides barium sulfate emulsion, histamine 50  $\gamma$ /kg, adrenaline 0.5 mg/kg, noradrenaline 5 mg/kg, and serotonin 50  $\gamma$ /kg were used as test solutions. Interruption of the right main pulmonary artery by ligation with silk thread was also done to observe the effects of decrease in pulmonary vascular beds. In addition, large blood clots were used as emboli. Histamine was especially used because it brought about very similar effects to pulmonary emboli upon hemodynamics and respiration in rabbits.

**(A) PAP**

Both systolic and diastolic PAP started to increase in 2 sec after injection of barium sulfate emulsion or other procedures, and systolic PAP reached a stable maximum within 10 sec as shown in Fig. 10.

It also holds true with cases of sudden decrease in pulmonary vascular beds, such as interruption of right main pulmonary artery by ligation and pulmonary embolization with multiple large clots. In cases of rapid injection of adrenaline and noradrenaline, PAP began to increase (slower than in above cases by 10 sec or so) and reached a stable maximum within 30 sec of injection, with ratio of maximum increase of systolic pressure, 50 % and 40 %, respectively. (Fig. 11).

In these cases, diastolic PAP decreased or continued unchanged in the early period, which was probably secondary to activated cardiac function.

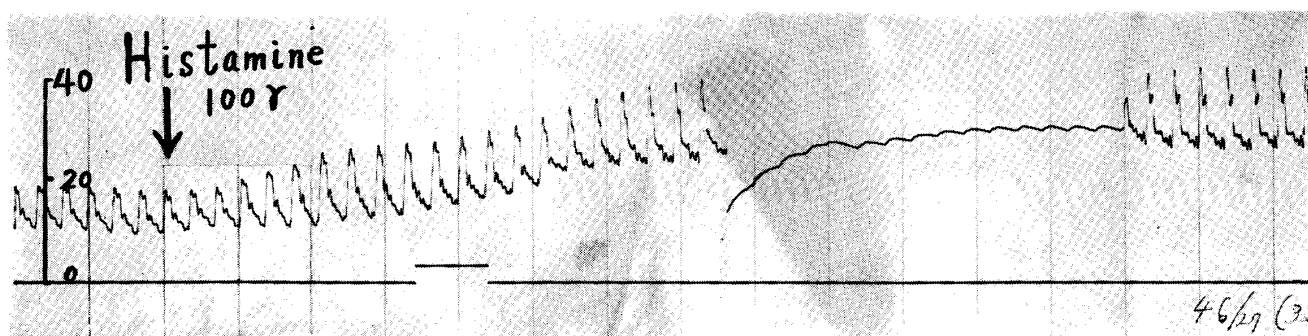


Fig. 10 Pulmonary hypertension following injection of histamine into the right heart of a rabbit. PAP began to increase 2 or 3 sec after injection. A stable maximum was attained within 10 sec of injection.

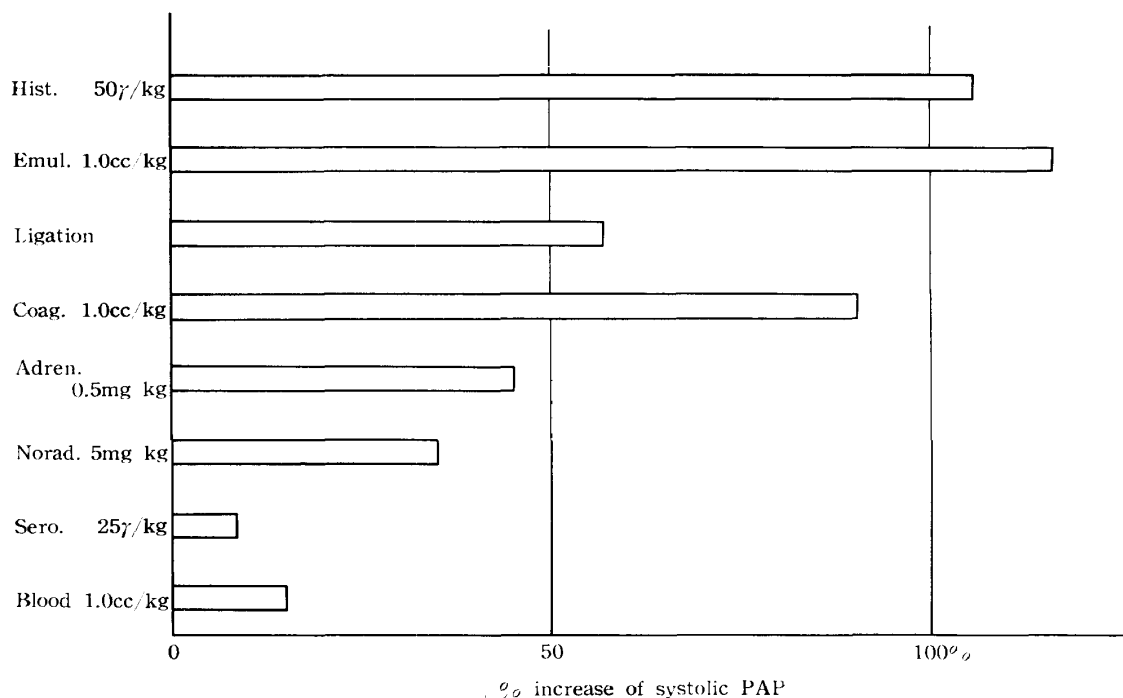


Fig. 11 Per cent increase of systolic PAP above normal average in rabbits. (average values were obtained from 4 to 7 rabbits.) Hist. denotes histamine dihydrochloride suspended in saline; Adren. denotes adrenaline hydrochloride; Norad. denotes noradrenaline hydrochloride; Ligation means ligation of right pulmonary artery by silk thread; Serot. denotes serotonin creatinine sulfate suspended in saline; Emul. denotes pulmonary embolization caused by intravenous injection of 5.5% barium sulfate emulsion. Blood clots denotes pulmonary embolization by injection of 2 cc of blood clots. All the procedures except injection of serotonin caused increase in systolic PAP.

Injection of serotonin solution caused no change in PAP (Fig. 11).

Then, autonomic nerves were blocked by the methods mentioned above. Respiratory undulation became conspicuous in either sympathectomy or vagotomy, and PAP slightly decreased after sympathectomy, but not after vagotomy.

In vagotomized rabbits, when the chemical agents mentioned above were injected,

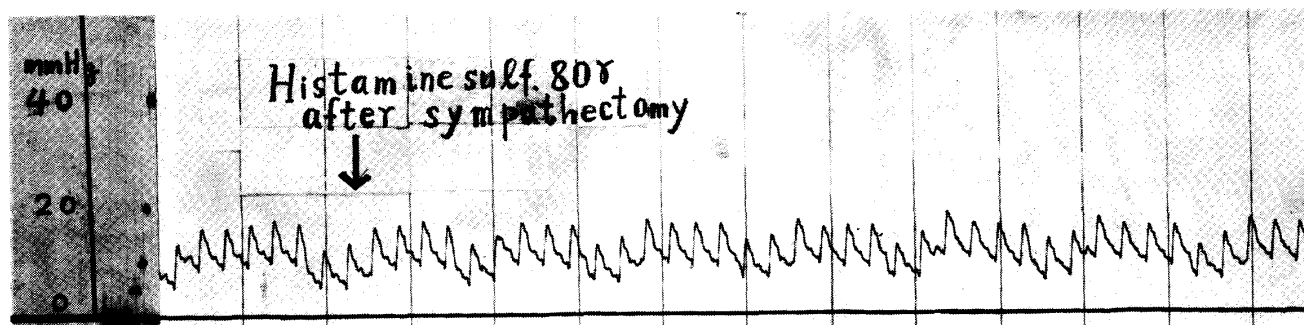


Fig. 12 Changes in PAP following intravenous injection of histamine in rabbits, sympathectomized by injection of imidaline 2 mg/kg. Increase in PAP was severely inhibited.



changes in PAP were the same as those in intact rabbits. In sympathectomized rabbits, however, the effects of chemical agents to PAP were strongly inhibited (Fig. 12). But in cases of sudden decrease in the pulmonary vascular bed, such as in pulmonary embolization and interruption of right main pulmonary artery, inhibitive effects of sympathectomy upon pulmonary hypertension were small. (Fig. 13, 14).

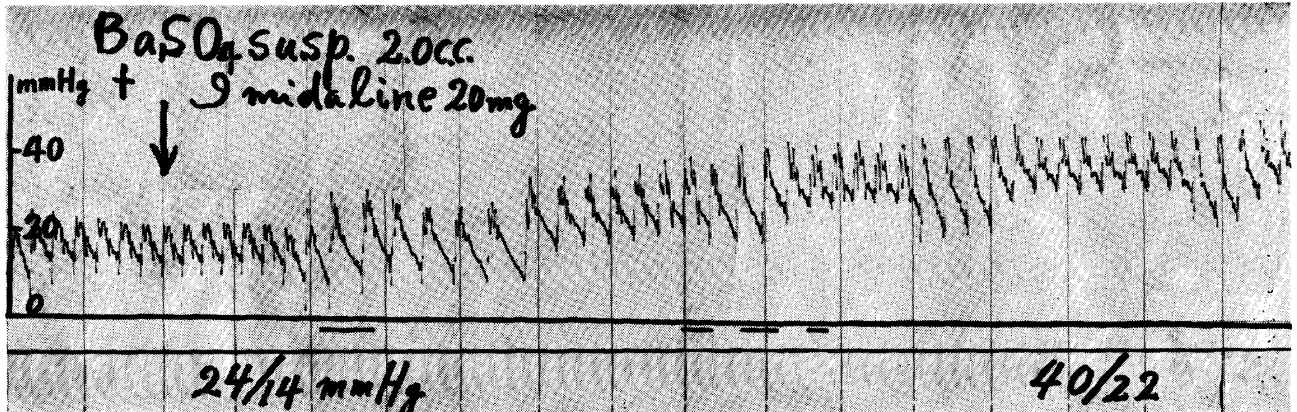


Fig. 13 Changes in PAP following pulmonary embolization in rabbits sympathectomized by injection of imidaline 2 mg/kg. Increase in PAP was slightly less as compared with those in intact rabbits.

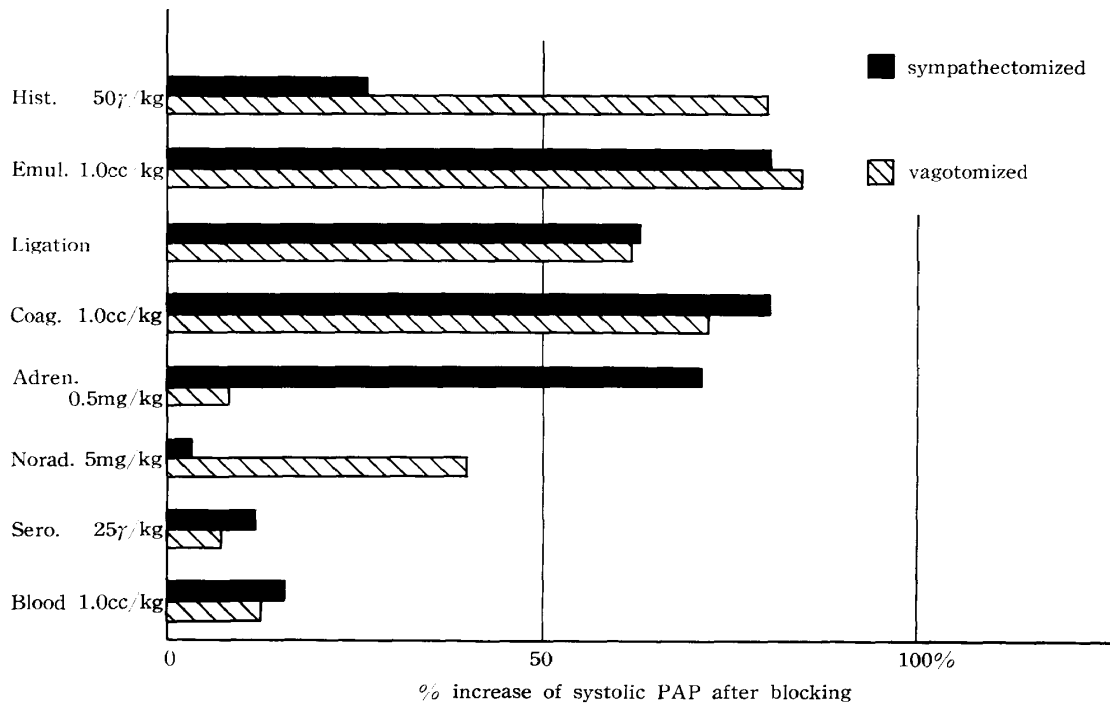


Fig. 14 Per cent increase of systolic PAP after the procedures above mentioned in sympathectomized and vagotomized rabbits. In sympathectomized rabbits, increases in systolic PAP following injection of histamine, adrenaline and noradrenaline were severely inhibited, but only moderately inhibited in cases of embolization and ligation of right pulmonary artery. In vagotomized rabbits, increases in systolic PAP were not altered at all.

(B) LAP

Left atrial hypertension was observed only in cases of injection of catecholamines, such as adrenaline and noradrenaline, but was not seen at all in cases of pulmonary embolization and injection of histamine (Fig. 15,16). In vagotomized rabbits it was the same as in intact cases. But in sympathectomized rabbits, increase in LAP following injection of adrenaline or noradrenaline was inhibited completely (Fig. 17).

(C) Respiration

After pulmonary embolization and injection of histamine, respiration changed from transient hypoventilation to hyperventilation after a latent period of 5 to 6 sec as mentioned before.

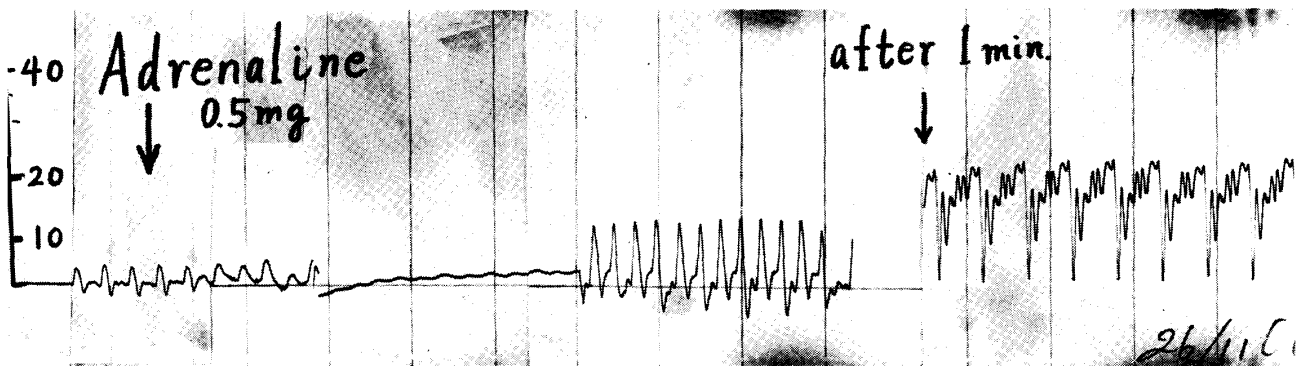


Fig. 15 Left atrial hypertension was observed in cases of injection of catecholamines, such as adrenaline. In cases of pulmonary embolization and injection of histamine it was not seen at all as in Fig. 5.

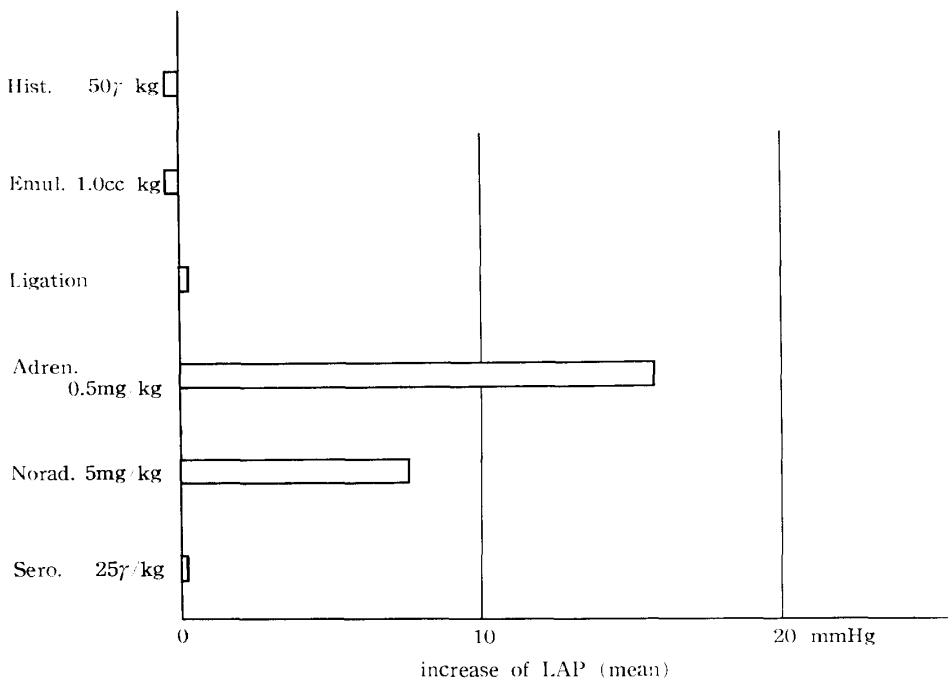


Fig. 16 Per cent increase in left atrial pressure after the procedures. Only catecholamines such as adrenaline and noradrenaline produced left atrial hypertension.

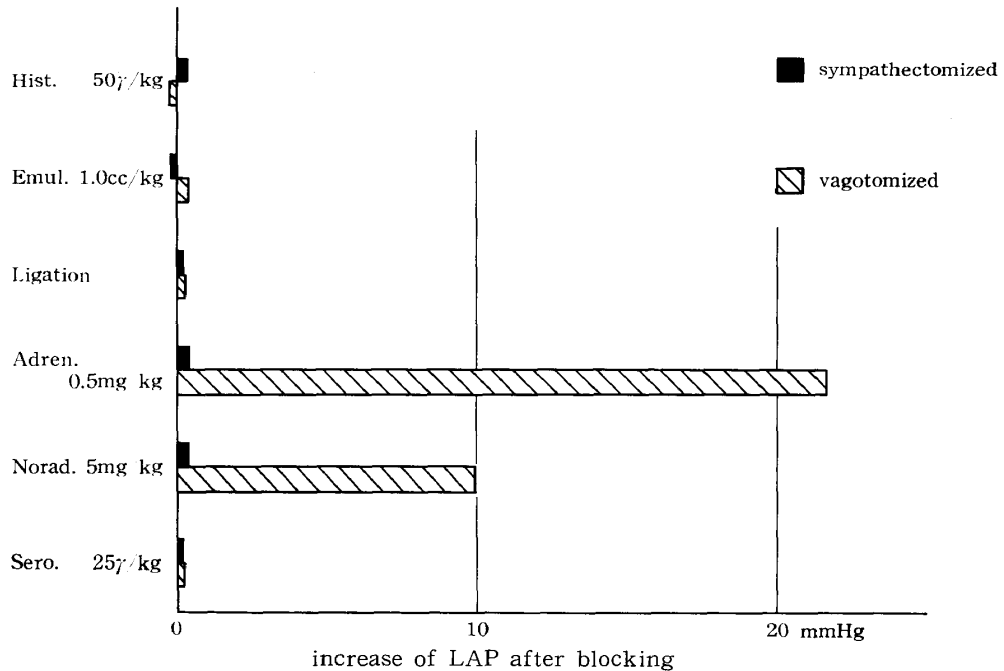


Fig. 17 Left atrial hypertension observed following injection of catecholamines were completely inhibited in sympathectomized rabbits, but not in vagotomized rabbits.

It was almost the same with the procedure of sympathectomy but following vagotomy respiratory changes issued immediately (Fig. 18, 19). In either sympathectomized rabbits or vagotomized ones, effects of both embolization and histamine-injection were strengthened to a considerable degree (Fig. 20, 21). Therefore, it cannot be said with certainty whether the sympathetic nerve or vagal nerve is dominant in the control of respiration.

### DISCUSSION

In this study, the net effects of pulmonary microembolization were investigated in intact rabbits with closed chest in the first step. It was clearly shown in this

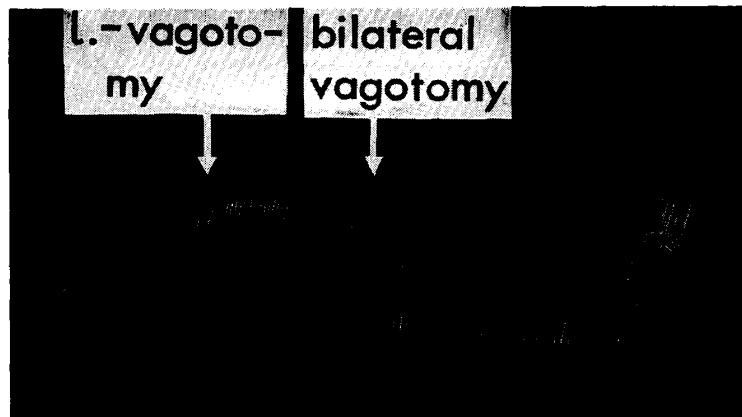


Fig. 18 Respiratory changes after bilateral cervical vagotomy. Hyperpnea or hyperventilation issued immediately after bilateral vagotomy.

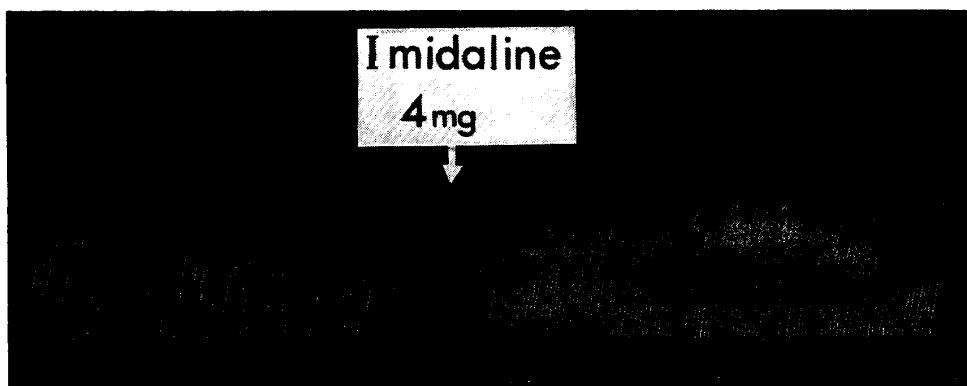


Fig. 19 Respiratory changes following sympathectomy ; 5-6 sec after injection of imidaline 2 mg/kg, hypopnea appeared and then it gradually changed to hyperpnea.

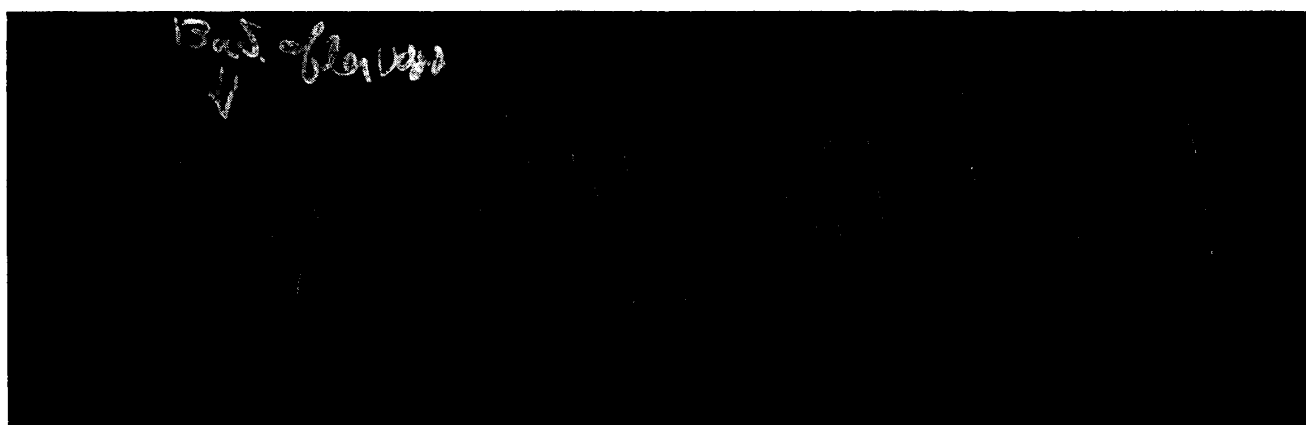


Fig. 20 Respiratory changes following embolization in vagotomized rabbits. Further increase in ventilatory volume (both in respiratory rate and in tidal volume) was observed.

experiment that pulmonary hypertension following injection of barium sulfate emulsion occurred within 2 to 3 sec, concomitantly with systemic hypotension, suggesting that pulmonary hypertension due to emboli may be provoked in part by the nervous reflex.

The important factors responsible for elevation of pulmonary hypertension are ; (1) elevation of pulmonary capillary pressure, (2) decrease in overall cross-sectional area of the pulmonary vascular bed, (3) significant increase in pulmonary artery flow, and (4) increase in pulmonary vascular resistance.

In the acute stage of pulmonary emboli, cross-sectional area of pulmonary vascular bed decreases significantly due to mechanical obstruction of pulmonary vessels and the pulmonary capillary pressure remains unchanged, considering the fact that left atrial pressure showed no change or slight decrease probably due to decrease in pulmonary blood flow.

It was in fact, observed that to obtain a measurable increase in pulmonary artery pressure, its circumference must be reduced by 50 to 60 per cent or else<sup>14-16)</sup> the extent of the pulmonary vascular bed must be reduced by 52 to to 60 per cent<sup>17,18)</sup>. And in this experiment, more than 0.6 cc/kg of 5% barium sulfate

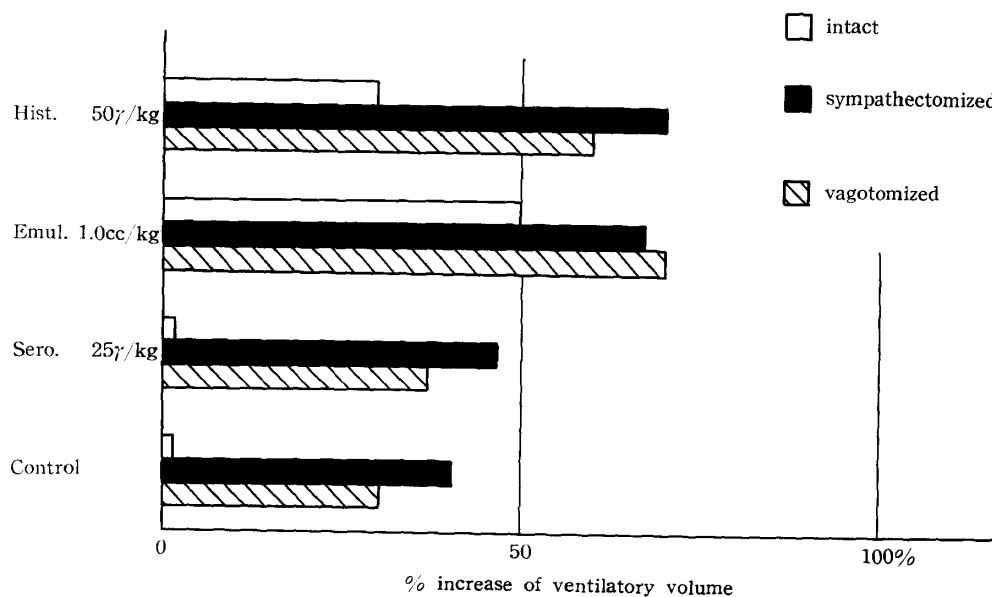


Fig. 21 Comparison of changes in ventilatory volume (tidal volume x resp. rate) in the stable states of hyperventilation following embolization among the intact, sympathectomized and vagotomized rabbits. Changes in ventilatory volume were similar both in vagotomized and in sympathectomized rabbits.

emulsion was used to obtain an effective pulmonay hypertension. It is possible that active pulmonary vasoconstriction occurred reflexively, contributing to pulmonary hypertension following emboli<sup>10,11,13</sup>.

Ventilatory changes are somewhat different from those of circulatory changes. The initial hypoventilation corresponds to the so-called Hering-Breuer reflex or response to the slowly adapting pulmonary stretch receptors<sup>1,6</sup>, the afferent nerves of which originate from the bronchial wall and run in the vagi.

The late hyperventilation might be brought about by sensitization of the slowly adapting pulmonary stretch receptors<sup>1,7</sup>, which are stimulated by histamine-like substance released at the site of emboli<sup>8,9</sup>. There are several explanations for the latent period of 5 to 6 sec between injection of barium sulfate emulsion and the onset of hypoventilation, among which is the possibility that the pulmonary stretch receptors are not immediately accessible to the pulmonary circulation<sup>1</sup>.

In the second step, autonomic nervous influences upon pulmonary hypertension following embolism and other procedures were investigated in rabbits which were sympathectomized or vagotomized prior to the procedures. As compared with increase ratio of systolic pulmonary artery pressure in intact rabbits following emboli and other procedures, those in sympathectomized rabbits were reduced distinctly, especially with injection of histamine, adrenaline and noradrenaline, and also with embolization to a moderate degree. Considering the relation between PAP and LAP (Fig. 14 Fig. 17), sympathectomy acted to inhibit increase in pulmonary vascular resistance produced by injection of histamine and barium sulfate emulsion.

Dexter showed in 1964<sup>14)</sup> that small emboli with diameter of 100 to 1000  $\mu$  could produce active vasoconstriction, in contrast to those emboli larger than 1000  $\mu$  in diameter. He concluded that there exists precapillary active vasoconstriction by way of autonomic nerves after microembolism. The factors which possibly may stimulate the pulmonary receptors for active vasoconstriction are increase in arteriolar pressure, mechanical irritation in vessels, hypotension distal to the segment of artery occluded by the embolus, and release of chemical substances. Among the latter, serotonin<sup>20,21)</sup>, histamine<sup>8,22)</sup>, and other unknown substances, have been either recognized or hypothesized. Halmagyi in his ingenious experiment in sheep<sup>23)</sup> showed that some humoral substance released at the site of emboli certainly caused pulmonary hypertension.

From my experiment in the second step, this was supposed to be a histamine-like substance in rabbits, since histamine produced very similar effects on hemodynamics and respiration and serotonin brought about no discernible changes in pulmonary artery pressure and other indices. Respiratory changes were estimated by changes in ventilatory volume and were compared among intact rabbits, sympathectomized ones, and vagotomized one. After sympathectomy and vagotomy, ventilatory volume increased moderately in both cases. Injection of histamine and barium sulfate emulsion into the right heart produced further increase from the control deviation. This shows that both sympathetic and vagal nerves act to inhibit hyperventilation following injection of histamine, and barium-sulfate embolism.

### SUMMARY

1. Pulmonary microembolism induced by injection of barium sulfate emulsion produced Bezold-Jarisch-like reflex immediately after the procedure, presenting bradycardia, systemic hypotension, hypo-to-hyperventilation and pulmonary hypertension.
2. Hemodynamic changes began almost simultaneously following embolism, suggesting that they were reflex in origin.
3. Respiratory changes began later by 4 sec than those of circulatory changes, disclosing that different mechanisms exist in respiration.
4. Sympathetic nervous blockade markedly inhibited the increase ratio in transpulmonary vascular pressure difference (PAP minus LAP) in cases of injection of histamine and pulmonary emboli, indicating that sympathetic nerves contributed to pulmonary vascular tone in pulmonary emboli.
5. Vagal blockade had no distinct effect upon pulmonary vascular tone.
6. Ventilation increased following either vagotomy or sympathectomy, and in addition was moderately promoted by each.
7. Histamine displayed quite similar effects upon hemodynamics and ventilation to those of pulmonary emboli.

### ACKNOWLEDGEMENT

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