Acta Med. Nagasaki 31 : 242—252

Healing at the tracheal anastomosis with special reference to tension load

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Received for publication, June 30, 1986

The healing process at the cervical tracheal anastomosis was assessed in dogs by means of microangiography, breaking strength test and histologic examination in terms of tension load added to the anastomosis.

The results are as follows:

1) Neovascularity around the anastomosis was evident on day 7 after tracheal anastomosis by microangiography. The development of neovascularity limited to the area of upper and lower 2 cartilaginous rings at the anastomosis. Thereafter, newly growing vessels were no longer observed on day 21.
2) Based on the results of breaking strength test, tensile strength at the anastomosis was enforced by the addition of tension load. Of interest was the fact that proper tension load at the anastomosis was adequate for promoting the healing process.
3) Regeneration of the epithelium at the anastomosis was jeopardized before at least 21 days following anastomosis.
4) The fibrocollagenous tissues to make the anastomosis tighten developed histologically in proportion to the tension load ranging from 400 to 1000g.
5) An appearance of densely staining spot on day 7 in the submucosal and adventitial layers at anastomosis by Alcian-Blue staining method was characteristic despite disappearance on day 21, demonstrating high activity in the biochemical active zone at the anastomosis.

INTRODUCTION

Great strides in surgery for the tracheobronchial tree had been achieved and the reconstructive surgery of choice had become accepted widely in the treatment of traumatic rupture, stricture by scar formation and/or benign and malignant tumors arising from the

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tracheobronchial tree since GRILLO[1] had reported that a 6.4cm resection of the trachea was feasible to repair.

Postoperative complication, however, did not infrequently occur and it was not so reduced with time as indicated by ISHIHARA[2] from a nationwide survey. According to his survey, the frequency of anastomosis insufficiency was 9 in 195 (4.6%) and that of stenosis was 21 (10.8%) respectively. MAEDA[3] also reported that the frequency of anastomosis insufficiency occurred in 6.1% and that of stenosis was 7.1%.

To our knowledge, there were various etiologic factors for anastomosis insufficiency in relation to suture material, operative technique, tension load at anastomosis, poor blood flow and so on.

Very little information was available for etiology of anastomosis insufficiency, especially with regard to tension load at the anastomosis.

This study was experimentally undertaken to clarify a jeopardy of healing process at tracheal anastomosis in terms of tension load added at the anastomotic site.

MATERIALS AND METHODS

Adult mongrel dogs weighing 5 to 12kg were anesthetized with 25mg/kg of sodium pentobarbital, intubated with a cuffed endotracheal tube and ventilated with room air using a volume respirator (*HARVARD*).

In the supine position, the cervical trachea was exposed through mid-cervical skin incision. The trachea from the 7th cartilage to 3 rings was circumferentially removed. The tension added at both cut edges of the trachea separated each other was measured by using the spring tension metories. In group A containing 10 dogs, this was adjusted to a 100g tension by a resection of 3 tracheal cartilaginous rings. Thereafter, the trachea was anastomosed end to end using continuous sutures of 4-0 nylone.

In group B containing 10 dogs, the trachea was removed from the 7th cartilage to 8 to 12 rings so as to adjust to a 400g tension.

In group C containing 4 dogs, the trachea was removed from the 4th cartilge to 12 to 17 rings so as to adjust to a 1000g tension of the separated tracheal edges. In this group it was difficult to get survivors due to the complication of anastomosis. These dogs were given cephalosporin as a dosis of 100mg/kg on the day of operation.

Postmortem examination was made on day 7 and day 21 in group A and B, and also on day 7 in group C. These examinations included postmortem tracheal arteriography,

measurement of breaking strength and evaluation of morphologic alteration by Hematoxylin-Eosin, Alcian-Blue and Elastica-van Gieson staining methods.

Postmortem tracheal angiography was performed as follows. The dogs was sacrified by infusion of 20ml KCL after giving a 5ml heparin iv.

The common carotid arteries were exposed bilaterally via midsternotomy.

A 8 Fr cut down tube was inserted and also cannulation to the superior vena cava was done to aspirate the blood and almost the total blood of a dog was replaced with 1000 ml of saline, infusing it from the carotid artery via the catheter.[4)]

The contrast media was composed of 60ml of 30% $BaSO_4$ heated at 40°C and 90ml of $BaSO_4$ containing 1% gelatin agar heated at 40°C. These were separately given via the carotid artery.

The neck and the thoracic cage of dogs were cooled with sterilized ice slush to make the constrast media infused harden for 30 min. Thereafter, the trachea and the both lungs were removed out of the thorax.

Postmortem roentgenogram was taken by Softex Type EMB (*Softex Co.*) with use of Fuji Softex film on condition with 32 KVp 3mA 60 sec for the trachea and 32 KVp 3mA 6 sec for the extended preparations of the trachea longitudinally cut.

Breaking strength of the trachea at anastomosis was measured with use of the tensile meter, DSC 500 (*SHIMAZU Co.*) at a cross head speed of 0.5cm/min (Fig. 1). The anastomosed trachea was cut into the strips perpendicular to the suture line of the trachea, which was made 1cm wide and 8cm long, each 2cm away from the anastomotic line upwards and downwards.

RESULTS

All of the dogs in this study were sacrified. The healing at the tracheal anastomosis was assessed by means of microangiography, breaking strength test and histologic examination.

The degree development of neovascular communication across the anastomotic site was graded 1～3 according to the microangiographic findings as follows (Fig. 2).

Grade 1 : There were a few communications of visible arteries across the anastomotic site on the microangiogram.

Grade 2 : There were well communications of visible arteries beyond the anastomosis, like normal tracheal vascurality.

Grade 3 : Many communications were visible around the anastomosis.

On the basis of the findings of microangiogram, the degree of neovascularity was assessed among group A, B and C.

In group A, most showed well developed vascularity on day 7, 2 were grade 3, 1 grade 2 and 2 grade 1. In general, neovessels grew with a range of 2 cartilages distally and proximally at the anastomosis as shown in Fig 3. On day 21, two showed grade 2 and 3 grade 1, some of them showed only network of neovessels so that the anastomotic site could not be identified as indicated in Fig 4.

In group B, the neovascular pattern on day 7 was better than in group A. Two showed grade 3 and three grade 2. On day 21, it was similar to that in group A, new vessel tended to diminish, 3 were grade 2, and 2 grade 1 (Fig. 5).

In group C, the neovascularity well developed, grade 3 was in 2, grade 2 in 2 respectively (Fig. 6).

The breaking strength values among the three groups were also compared as shown in Fig 7, 8 and 9.

In group A, the breaking strength values were 220 ± 135g on day 7 and 986 ± 399 on day 21. In group B, these were 409 ± 173g on day 7 and 1596 ± 343 on day 21. These were superior to those in group A. In group C, this was 575 ± 276 on day 7 and was superior to those in group A and B.

These results indicated that the greater the tension against the tracheal anastomotic site, the stronger the tensile strength.

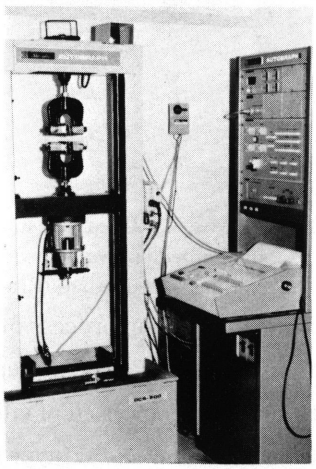

Fig. 1. Showing tensil meter, DSC 500 (SHIMAZU CO.) Left half measuring the tensil strength and right half was recording parts.

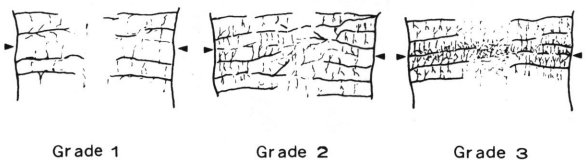

Fig. 2. Showing microangiographic graduation at the anastomotic site.

Grade 1 : There was a few communication across the anastomotic site

Grade 2 : There was well communications beyond the anastomosis, like normal tracheal vascularity

Grade 3 : Many communications were visible around the anastomosis

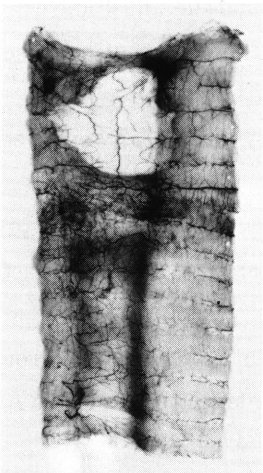

Fig. 3. This tracheal microangiography showed good anastomotic neovascularity at day 7 in group A (Grade 3).

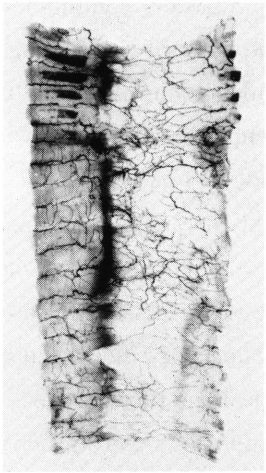

Fig. 4. Anastomotic neovascularity was disappeared at 21th post operative day in A group (Grade 2). It was difficult to identify the anastomotic site.

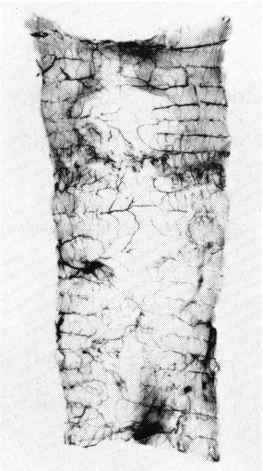

Fig. 5. In this case, Grade 2 neovascularity but it was easy to identify the anastomotic site, at day 21 in B group.

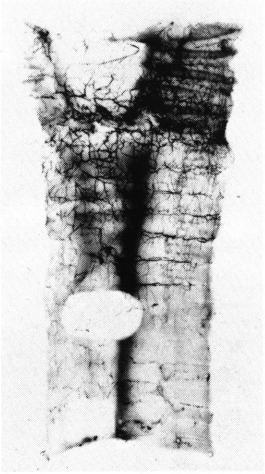

Fig. 6. Neovascularity at anastomotic site limited upper and lower two caltilages in C group (Grade 3).

On the basis of the findings of histologic examination the healing process of tracheal anastomosis was evaluated. In HE-staining preparation regeneration of the epithelium was shown on day 7 in group A. The regenerated epithelium was composed of ciliated low cuboidal epithelium with infiltration of the lymphocytes in the submucosal layer (Fig. 10).

In group A the fibrocollagenous tissue did not develop in proportion to the regeneration of the epithelium which was completed on day 21. Some changed from cuboidal epithelium to cylindrical one with cilia. Histologic dearrangement was not seen at that

time.(Fig. 11a, b) In group B, progress in regeneration of the epithelium was similar to that seen in group A. However, no complete regeneration of the epithelium was seen even on day 21 despite the fact that the regenerated epithelial cells at 21 days became taller. In contrast, the fibrocollagenous tissues well developed and these were densely arranged as compared to those in group A (Fig. 12).

In comparison with histologic healing process between group A and B, the regeneration of the epithelium in group A was much more facilitated than that in group B although

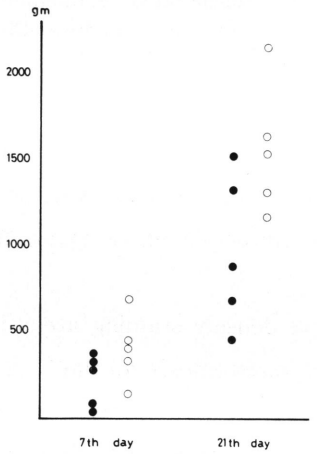

Fig. 7. Breaking strengths of a 1cm strip of the wall of the trachea taken perpendicular to the anastomosis at intervals, Group A it showed closed circle and Group B, open circle.

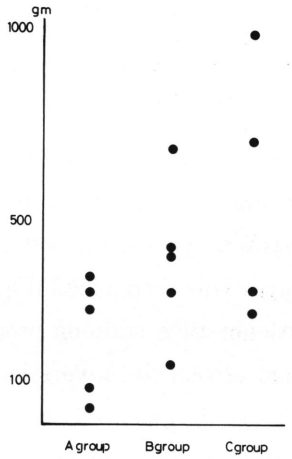

Fig. 8. Breaking strengths was compared among the three groups. It showed that wound healing a higher a tension over the anastomosis led to an increased tracheal anastomotic breaking strength in short time.

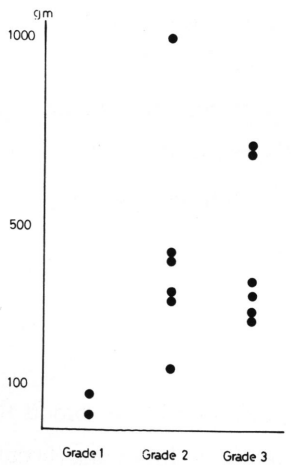

Fig. 9. Relationship between breaking strengths and neovascularity in anastomotic site, at 7th day.

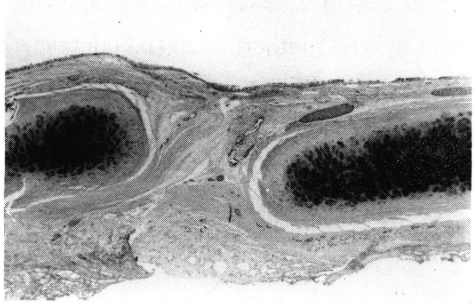

Fig. 10. Inflammatory granulation tissue was appeared in submucosal layer and regenerated epithelium was incompletely covered at the portion of also the anastomosis in Group A.

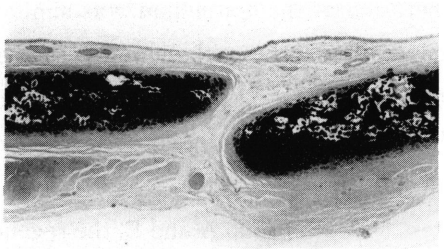

Fig. 11a. Regenerated epithelium was almost covered at the portion of the anastomosis and inflammatory alternation reduced in submucosal layer at day 21 in Group A.

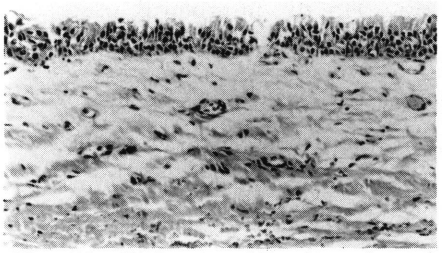

Fig. 11b. High magnification showed regenerated epithelium was tufty and cyliadrical the same as Fig lla.

fibrocollagenous connective tissues in group B developed still more than that in group A.

In group C, the degree of healing at anastomosis varied and some were covered with the regenerated epithelium, and proliferation of the fibrocollagenous tissue in the submucosal layer was recognized (Fig. 13).

In Alcian-Blue staining preparation, there were densely staining areas in the submucosal and adventitial layers in the vicinity of the anastomosis on day 7 in group A. However, these disappeared on day 21.

Group B showed almost the same finding as group A. In group C, there was nothing densely stained.

In Elastica-van Gieson staining preparation, a presence of the collagenous fiber was apparent around the cartilage and it crossed over in part on day 7. In contrast, that in group B had become stained in the submucosal layer on day 21 and it arranged crossing over each other and losing the order in comparison with that in group A.

In group C, a growing collagenous tissue in the submucosal layer was markedly stained by HE method. On the contrary, this was faintly stained by Elastica-van Gieson method. Based on the finding that the cartilages are dispersed, it is not too much to say that the contribution of the cartilaginous membrane to healing is uncertain.

DISCUSSION

It is important to say that wound healing is facilitated by better blood flow. KAWAHARA[5] and KIMINO[6] reported as to restoration of blood flow following bronchoplastic operation on dogs by means of microangiography. KIKUCHI[7] also studied on neovascular connection in the tracheal anastomotic site by using the silicon rubber infusion technique.

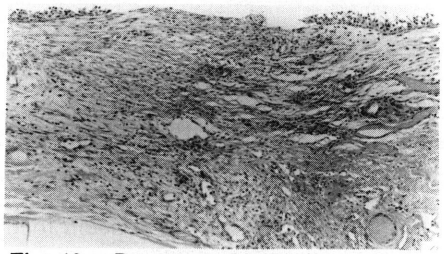

Fig. 12. Regenerated epithelium was incompletely covered, but submucosal fibrocollagenous alternation was already developed, as compared with the same day in Group A.

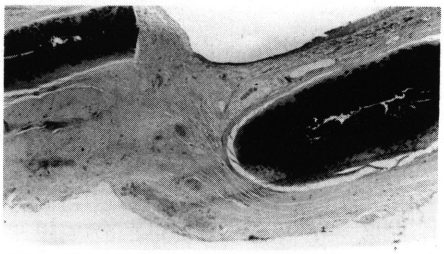

Fig. 13. Fibrocollagenous tissues of Group C developed densely rather than that of Group A and B.

They confirmed that a 7 day duration would be required for neovessel connection across the anastomotic site.

In the present study, the small vessel structure at the anastomosis of the cervical trachea was assessed[8)-10)] by microangiography previously used by KAWAHARA.

The results indicated that neovascularity seen around the anastomosis still remains within a range of the distal and proximal 2 cartilages. This is in accordance with the area of the biochemical active zone[11)] in which new vessels usually develop.

On day 21 following anastomosis the network of newly developed vessels had become disappeared, losing a sharp contrast to the healthy tissues. It seemed to be a consequence of the completion of healing process at 21 days. It is clear that tension added to the anastomotic site is one of the most influential factors on its healing. MAEDA[12)] identified that the limit of tension load to ensure the healing of tracheal anastomosis was 1000g in puppies and 1750g in adult dogs. CANTREL[13)] also reported that its limit was a 1700g load. He gave warning that when tension load exceeded 1850g, separation of the anastomotic site would have taken place.

In this series, tension load against the anastomosis was adjusted by the resected tracheal length. A preliminary study showed that to make the tension load 100g against anastomosis, a resection of 3 cartilaginous rings was needed. In 400g load, 8 to 12 cartilages and in 1000g load, 12 to 17 cartilages respectively.

These were within the limitation of occurring separation of the anastomosis. It is concluded that the tension load to allow for separation of the anastomosis is a resection of over 16 cartilagious rings for the cervical trachea in dogs. In the anastomotic site, the tensile strength has become increased with time against tension load. KIKUCHI reported that tensile strength following anastomosis gradually increased to 35g on day 3, 157g on day 7, 631g on day 14, and 1240g on day 28 and it reached a level of satisfactory tensile strength

on day 28.

LIMA[14] and GOLDBERG[15] also confirmed that tensile strength was less than 100g on day 4, 600 to 800g on day 9 and 1232g on day 23 respectively.

In the present study, tensile strength was measured on day 7 and 21. In group A with a 100g tension load at anastomosis the tensile strength was 220 ± 135g on day 7 and 986 ± 399g on day 21 respectively. In contrast, in group B with a 400g tension load this was 409 ± 173g on day 7, 1596 ± 343g on day 21 respectively, showing enforced tesile strength than those in group A. Meanwhile, in group C with a 1000g tension load a maximum of tensile strength of 575 ± 276g on day 7 was provided.

Of interest is the fact that tension load added to anastomosis is still facilitating to make tensile strength greater. However, it is doubt as to whether tension load against anastomosis is of benefit in achieving satisfactory healing or not.

VALSKY[16] also compared the tensile strength following anastomosis between the patients with tracheal resection and non-resection and/or absorbable and non-absorbable suture materials used. He concluded that tensile strength was much greater in performing tracheal resection and in utilizing absorbable suture material.

Based on the results in this series, tensile strength was at least accelerated by growth of the fibrocollagenous tissue around anastomosis. However, this may by chance contribute to the ensuing stenosis by overgrowth of granulation tissue.

From the standpoint of histologic examination with respect to wound healing, it is generally believed that wound healing first begins at the site of abundant distribution of the vessels. Much has been said that the membrane of the cartilage might play a key role in healing.[17][18] In view of histology, there was no clue suggestive of a role of the cartilagious membrane in healing.

Interestingly enough, when a tension load against anastomosis is increasing, the development of the fibrocollagenous tissue has become much more pronounced in the submucosal layer, separating between the cartilages. As for the time when regeneration of the epithelium is completed following tracheal anastomosis, it is not consistent by many investigators.[6][16][19] It varies from 5 to 56 days. The author detected a presence of mucopolysaccharide in the submucosal and adventitial layers at anastomosis by Alcian Blue staining technique. It seems to be a reflection of activity in biochemical active zone.

In contrast, the elastic fiber of the basement membrane in the newly regenerated epithelium is not seen by Elastica-van Ginson staining even on day 21. This fact is demonstrating that a new epithelium is not yet functioning even on day 21.[20]

ACKNOWLEDGEMENT

The author would like to express his gratitude to Professor M. TOMITA for his valuable advice and encouragement and to the cooperators in the 1st Surgical Department for skillful technical assistants.

REFERENCES

1) GRILLO HC, DIGMAN EF, MIURA T: Extensive resection and reconstruction of mediastinal trachea without prosthesis or graft: As anatomical study in man. *J. Thorac. Cardiovasc Surg.*48: 741-749, 1964.

2) ISHIHARA T., *et al.*: Current clinical data concerning tracheobronchoplasty in Japan. *Nippn Kyobu Geka Gakkai Zasshi* 26: 1007-1012, 1978 (in Japanese with English Abstruct).

3) MAEDA M, NARUKE T: Chairman's comments. *Nippon Kikanshi Gakkai Zasshi* 6: 418-420, 1984 (in Japanese).

4) EVANS HE, CHRISTENSEN GC: Miller's anatomy of the dog. WB Saunders Co Philadelphia 2nd., 652-657.

5) KAWAHARA K: An Experimental Study on Bronchial Recirculation following Bronchial Reconstruction-Especially the Correlation of Pulmonary Artery Stenosis with Bronchial Arterial Regeneration. *Nagasaki Igakkai Zasshi* 55: 199-214 1980 (in Japanese with English Abstract).

6) KIMINO K: Experimental Study on Healing Process in Bronchial Anastomosis.-Especially Availability of Omental Wrapping and Pericardial Covering-*The Acta Medica Nagasakiensia* 30: 34-46, 1985.

7) KIKUCHI K: Morphological Biochemical Study to Investigate Healing Process at The Site of Tracheal Anastomosis in The Dog. *Nippon Kyobu Geka Gakkai Zashi* 34: 357-364, 1986 (in Japanese with English Abstract).

8) FUKAI S, ISHIHARA T, KIKUCHI K, NEMOTO E: The Blood Supply and Microcirculation of Canine Trachea. *J. Jpn. Bronchoesophagol. Soc. Society* 35: 229-232, 1984 (in Japanese with English Abstruct).

9) SALASSA JR, PEARSON BW, PAYNE WS: Gross and Microscopical Blood Supply of the Trachea. *Ann Thorac Surg* 24: 100-107, 1977.

10) MIURA T, GRILLO HC: The contribution of the inferior thyroid artery to the blood supply of the human trachea. *Surg Gynecol Obstet* 123: 99-102, 1966.

11) ADAMSONS RJ, MUSCO F, ENQUIST IF: The chemical dimensions of a healing incision. *Surg Gynecol Obstet* 123: 515-521, 1966.

12) MAEDA M, GRILLO HC: Effect of tension on tracheal growth after resection and anastomosis in puppies. *J Thorac Cardiovasc Surg* 65: 658-668, 1973.

13) CANTRELL JR, FOLSE JR: The repair of circumferetial defects of the trachea by direct anastomosis: Experimental evaluation *J. Thorac Cardiovasc Surg* 42: 589-598, 1961.

14) LIMA O, COOPER JD, PETERS WJ, AYABE H, TOWSEND E, LUK SC, GOLDBERG M: Effects of

methylpredonisolone and azathioprine on bronchial healing following lung autoplantation. *J Thorac Cardiovasc Surg* **82**: 211-215, 1981.

15) GOLDBERG M, LIMA O, MORGAN E, AYABE H, LUK SC, FERDMAN A, PETERS WJ, COOPER JD: A comparison between cyclosporin A and methylpredonisolone plus azathioprine on bronchial healing following canine lung autoplantation. *J Thorac Cardiovasc Surg* **85**: 821-826, 1983.

16) VALESKY A, LEHNHARD FJ, SCHILDBERG FW: Wandheilung von Trachealanastomosen. *Der Chirurg* **52**: 648-653, 1981.

17) ISHIHARA T, KOBAYASHI K, KIKUCHI K, NEMOTO E, KATO R: Reconstruction of the Trachea and Bronchus-recent topics- *The Japanese Journal of Chest Disease* **44**: 9-15, 1986 (in Japanese).

18) TADA H *et al.*: Tracheal Anastomosis and Absorbable Suture. *Nippon Kikanshi Gakkai Zasshi* **6**: 421-426, 1984 (in Japanese with English Abstruct).

19) FERGUSON D. J., WILCL JJ, WANGEN Steen OH: Experimental Resection of Trachea. *Surgery* **28**: 597-619, 1950.

20) GIORCLANA A, HOLSCLAW DS: Tracheal resection and mucociliary clearance. *Ann Otol* **85**: 631-639, 1976.