SUBGLOTTIC STENOSIS AND CRICOID GROWTH

an experimental study

(SUBGLOTTISCHE STENOSE EN GROEI VAN HET CRICOID

een experimenteel onderzoek)

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam.
Op gezag van de rector magnificus
Prof. Dr. A.H.G. Rinnooy Kan
en volgens besluit van het College van Dekanen.
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This study is part of the project Airway Stenosis.
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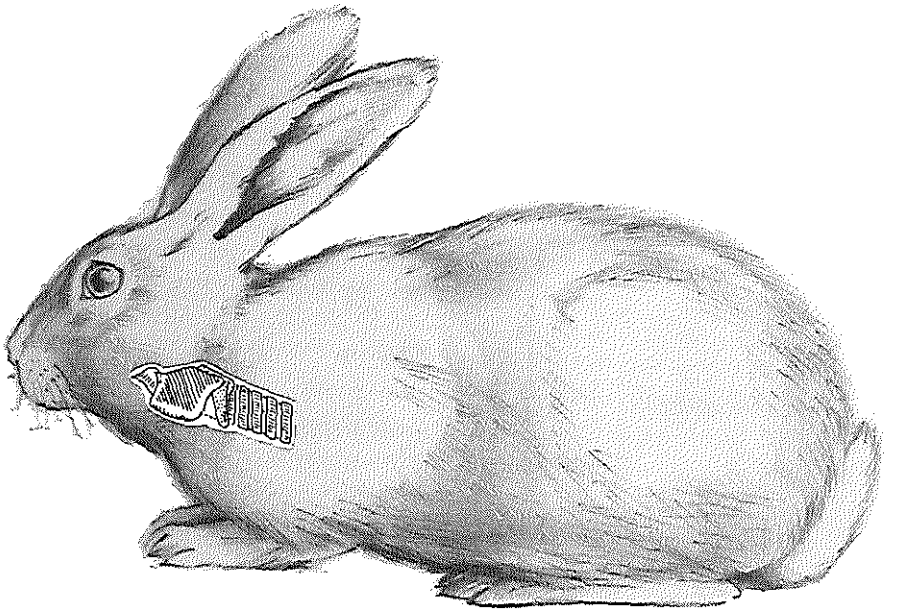
AAN MONIQUE EN MIJN OUDERS



VOORWOORD

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CONTENTS

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CHAPTER 1

INTRODUCTION

The otorhinolaryngologist frequently meets problems involving the patency of a tubular organ. Some of these diseases merely cause discomfort, while others imply severe disstress. Among the latter laryngotracheal stenosis presents a difficult condition to handle. In the larynx cicatricial stenosis is usually a sequela of prolonged intubation for ventilatory support. Other causes can be external trauma, endoscopic procedures, tracheotomy, caustic ingestion and inhalation injury. Especially in infants and children, the treatment of acquired stenosis in the subglottic region is not always successful, even in an era of great advances in medical skills and techniques.

In adults the purpose of treatment of an acquired laryngeal stenosis is the restoration of the airway lumen. In infants and children therapy has a second goal: re-establishment of conditions necessary for further growth. Only by growth the airway can fulfil the increasing respiratory demands of the growing individual. Therefore, negative effects of treatment upon growth are to be prevented. In 1932 Jackson already warned for such effects of surgical treatment and preferred a conservative approach: tracheotomy awaiting normal growth of the larynx to solve the problem (15). On the other hand, infants and children with severe subglottic stenosis and prolonged tracheotomy were reported to have a high mortality rate, ranging from 5,3% to a startling 24% (12,14). So, Cotton and Evans, among others, advocated the (more aggressive) open surgical approach (10).

A review of literature illustrates a variety of methods, developed to manage patients with an acquired subglottic stenosis. In general, 2 categories of therapy can be distinguished:

A. internal (endolaryngeal) methods; one or a combination of the following 3 methods have been recommended:

   1. (traditional) conservative treatment by tracheotomy and intermittent dilatation of the stenosis (15);
   2. prolonged dilatation with nasotracheal tubes or stents (7);
   3. microsurgical, cryosurgical or laser resection of the narrowing tissues (13).

B. external (open surgical) methods; 5 types of airway reconstruction have been developed:

   1. anterior cricoid split, especially for difficult decannulation in infants (9);
   2. augmentation of the subglottic circumference by interposition of a graft after splitting the cricoid arch in the midline (12);
   3. castellated incision of the larynx and the first tracheal rings followed by the insertion of a stent (11);

4. anterior and posterior incision of the cricoid with placement of a stent (17);
5. resection of the stenotic part of the airway followed by end-to-end anastomosis (8).

In the Academic Hospital Rotterdam - Dijkzigt and Sofia Children's Hospital - the hazards of therapy in patients with a laryngotracheal stenosis have instigated substantial experimental and clinical research during the last 25 years. Berkovits (1,2,3,4,5,7) inspired a continuous interest in the treatment of airway stenosis. He strongly emphasized that stents and tubes should not damage the epithelium of the airway, even in case of prolonged contact. He described qualities of intubation material required for application in the human airway. An interesting innovation was the introduction of special siliconized silicon rubber tubes and stents. The silicon oil creates "boundary and weeping lubrication". The material was demonstrated to have no detrimental effects on the in vitro growth of fibroblasts and on respiratory epithelium in clinical situations. Many patients were treated by Berkovits in cooperation with Bos and later Van der Pot, Van der Schans and Hoeve in Rotterdam, Edens and Van Overbeeke from the University E.N.T. Department in Groningen and colleagues of the intensive care units. In their hands prolonged intubation or stenting appeared to be a very useful tool in the management of laryngotracheal stenosis, even in cases in which a series of previous treatments had proven to be unsuccessful.

In a number of cases, especially in infants and children, both conservative and surgical treatment fail to restore an adequate airway. Even among patients with the same type of stenosis, the results can vary from poor to excellent. It could be that routine diagnostic procedures do not discriminate factors which determine a favourable or infavourable prognosis.

Factual consideration raised the following questions:

1. is the diagnosis of subglottic stenosis in fact applied for different conditions with a different etiology, pathogenesis and prognosis, demanding different treatment modalities?
2. do different types of trauma to the subglottis induce different types of stenosis?
3. are the effects of trauma to the subglottis different in growing and adult individuals?

Holinger proposed a classification of subglottic stenosis (Table), based on his own observations (14) and on reports from others (16,18). He pointed to the importance of a correct histopathologic diagnosis before therapy is started: e.g. a cartilaginous stenosis can not be responsive to dilatation and urges for an open surgical therapy; a soft tissue stenosis can be readily dilated, but needs additional therapy to prevent collapse after withdrawal of the dilator. Holinger's classification actually is a catalogue of anomalies observed and does not refer to the etiology and pathogenesis of the stenosis. Systematic investigations of the development of subglottic stenosis after various types of trauma have not been published. Nevertheless, some preliminary experimental studies, in particular by Borowiecki (6) encouraged a series of complementary experiments.

Elucidating some aspects of the above mentioned questions will contribute to the knowledge of subglottic stenosis.

Table: Holinger's classification of histopathology of subglottic stenosis (1982)

Cartilaginous stenosis

1. Cricoid cartilage deformity (stenosis)
   - Normal shape
     - small for infant's size
   - Abnormal shape
     - large anterior lamina
     - oval (elliptical) shape
     - large posterior lamina
     - generalized thickening
     - submucous (occult) cleft
     - other congenital cricoid stenosis

2. Trapped first tracheal ring

Soft tissue stenosis

1. Submucosal fibrosis (fibrous connective tissue)

2. Submucosal mucinous gland hyperplasia

3. Granulation tissue

The investigations, reported in this thesis, are restricted to the subglottic part of the larynx of young rabbits as a growing "model". In these small animals prolonged endolaryngeal intubation was not possible to induce subglottic stenosis.

The effects of some well defined types of trauma of the subglottis in 4-week-old rabbits were studied 20 weeks later in the adult stage:

1. internal (endolaryngeal) trauma:
   a. circular injury to the soft tissue lining (epithelium and subepithelial layer) of the subglottis and the perichondrium and inner zone of the cricoid cartilage (chapter 2 and 3);
   b. circular injury to the subglottic soft tissue lining only (chapter 6).

2. external (open surgical) trauma:
   a. interruption of the circularity of the cricoid by an anterior midline split and

resection of the anterior third or half of the ring, preserving the soft tissue lining (chapter 4 and 5);

b. interruption of the circularity of the cricoid by resection of the anterior third or half of the cricoid with resection of the adjacent soft tissue lining (chapter 4 and 5);

c. interruption and repair of the circularity by resection of the anterior third of the cricoid ring without injury to the soft tissue lining; both the effects of repair with an autogenous and an alloplastic graft were studied (chapter 7 and 8).

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CHAPTER 2

A MORPHOMETRIC STUDY OF THE GROWTH OF THE SUBGLOTTIS AFTER ENDOLARYNGEAL TRAUMA

Abstract

In young rabbits the growth of the subglottis was studied over a period of 20 weeks following an endolaryngeal trauma. This lesion of the larynx at the age of 4 weeks resulted in the development of a stenosis in the adult stage. The stenosis appeared to be caused by a specific change in the pattern of growth leading up to an anterior narrowing ("pear"-like) of the cricoid ring and a conspicuous thickening of the subepithelial layer.

Introduction

Despite medical progress in the intensive care of critically ill infants and children and recent advances in techniques of airway management, the improved survival rate in this group of young patients unfortunately coincides with a raised incidence of acquired subglottic stenosis (2,3,9,13,15,21). In the last decade this complication following prolonged endotracheal intubation is reported to range from 2.0 to 8.3% (4,6,13,14,19). The crux of the problem seems to be the cricoid, being the narrowest cartilaginous part of the airway skeleton and the only circular structure in the larynx. The rigid ring does not allow expansion if too wide a tube is inserted to support ventilation; the resultant pressure can lead to lesions of the inner soft tissue lining of the airway and damage to the perichondrium and cartilage of the cricoid, resulting afterwards in laryngeal stenosis at the level of the cricoid (5,12,13,17). To date the treatment of subglottic stenosis in infants and children remains a controversial issue. Both conservative - prolonged stenting or intubation - and surgical methods have been recommended, but none offer a definite solution, the reason for therapy failure often being unknown.

Especially in infants and children, growth is assumed to be an important adjuvant factor in the treatment of subglottic stenosis. According to Skolnik (16) conservative treatment should be preferred as long as the full growth potential of the larynx has not been realized. Baker (1) thinks subglottic edema in difficult decannulation is best treated by allowing the larynx to grow. Strome (18) asserts that laryngeal soft tissue injury in children will respond to conservative therapy, since potential growth will ensure an adequate airway. Holinger et al. (9) conclude that in their patients decannulation can be possible after one or two years "due to expected growth of the larynx". Gates et al. (6) state that in hard cicatrical subglottic stenosis operative correction is usually indicated only if normal growth fails to result in a sufficient airway. Goode et al. (7) think "time (=growth) to be the treatment of choice" in airway stenosis in children. Papsidero (13) et

et al. also postulate that "growth may contribute significantly to decannulation" in their patients after a treatment period of 100 weeks or more.

However, growth of the larynx after an endolaryngeal injury has never been subjected to a systematic study, neither in young patients nor in experimental animals. It is not known to what extent later growth can "normalise" a stenosis, acquired at a younger age.

This study aims to investigate the growth of the subglottis in growing rabbits after a standardized endolaryngeal trauma in comparison with normal growth.

Materials and methods

53 Female New Zealand white rabbits were used. The subglottis was studied in 13 non operated animals 4 weeks old (*series I*), 28 non operated adult animals 24 weeks old (*series II*) and 11 animals (24 weeks old) in which an endolaryngeal trauma was performed at the age of 4 weeks (*series III*). This is the youngest age at which the animals, weighing between 400 and 500 grams, can be weaned and survive surgery. During the following 8 weeks the growth rate is very high to decrease later to a minimum at 24 weeks after birth (20). The anaesthetic consisted of intramuscular xylazin (Rompun®) 0.1 ml/100 mg. and ketaminchlorid (Ketaset®) 0.1 ml/100 mg. The larynx and trachea were exposed by a ventral midline incision through the skin and soft tissues. The subglottic lumen was reached by a transversal incision of the ventral half of the crico-tracheal ligament. A ring of epithelial and subepithelial tissue, covering the cricoid was excised. The perichondrium and innermost layers of the cricoid cartilage were damaged circumferentially using a burr drill. Rags of tissue were removed by suction. The transversal incision was closed with a Prolene 6-0 suture. The subcutaneous tissues and the skin were suture tagged with catgut 6-0. No antibiotics were administered in the postoperative period. After surgery no signs of wound or respiratory tract infection were observed: 5 out of 16 animals died hemorrhagic enteritis 2 animals.

After sacrificing the animals, the larynx of 11 specimens could be studied. The subglottic part of the airway was excised and filled with liquid paraffin. This material was left to coagulate in order to prevent shrinkage during subsequent fixation in a formaldehyde 10% solution. After histological processing, 5 μm transversal sections were obtained from a circumscript subglottic segment confined by the crico-thyroid joint cranially and the inferior margin of the cricoid lamina on the caudal side.

In these sections 6 landmarks could be identified on the outer surface of the cricoid (fig. 1): 1) A_1 and P_1:midventrally and middorsally. 2) B_1, C_1, D_1, and E_1 on the tangents connecting the lateral prominent points of the cricoid; perpendicular lines from these points to the inner cricoid border give A_2, P_2, B_2, C_2,D_2 and E_2 and extended to the epithelial lining A_3, P_3, B_3, C_3, D_3 and E_3.

The lumen surface area was measured by a Zeiss Videoplan computer. Before measuring the diameters and the thickness of the cricoid and the subepithelial layer with a metric ruler, the sections were enlarged 10 times. The following measurements were

carried out:

1. lumen surface area (mm^2) of the subglottic airway;
2. inner diameters of the cricoid ring (mm): median A_2P_2, transversal B_2C_2, transversal D_2E_2;
3. thickness of the cricoid cartilage (mm):

$$A_1A_2,\ P_1P_2,\ \frac{(B_1B_2+C_1C_2)}{2},\ \frac{(D_1D_2+E_1E_2)}{2};$$

4. thickness of the subepithelial layer (mm):

$$A_2A_3,\ P_2P_3,\ \frac{(B_2B_3+C_2C_3)}{2},\ \frac{(D_2D_3+C_2C_3)}{2}.$$

In some specimens not all measuring points could be identified in the same histologic section as a result of small variations in the direction of the transversal sectioning through the subglottis. For measurements one section was selected in which the greatest number of landmarks was present. The number of measurements in each specimen is listed in table 1-4. Within the studied segment of the airway the cricoid and the lumen of series I and II showed very small variations. However, in series III the subglottic lumen area appeared to exhibit marked variances. Therefore, the surface area was measured in a number of sections; the smallest area was used for further calculations.

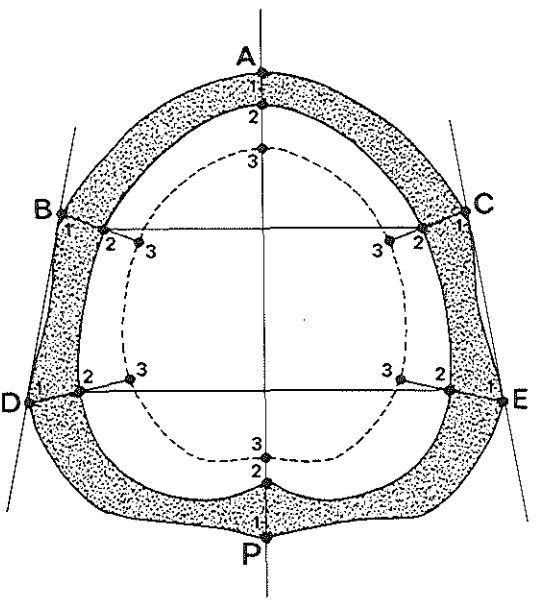

fig.1 Schematic transversal section of the cricoid ring and the epithelial lining.

Means and standard deviations in each series were calculated. Student's two tailed paired t-test was applied for statistical analysis. Due to a small number of specimens a normal distribution of the population could not be demonstrated accurately. Analysis was performed assuming unequal variances. A p-value of 0,05 or less was considered to indicate a significant difference between the series.

Results

The morphometrical observations are presented in table 1-4.

Table 1: Subglottic lumen area (mm^2)

SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARGING TRAUMA (n)	P I/II	P I/III	P II/III
8.6 ± 1.4 (13)	21.1 ± 2.9 (28)	11.5 ± 4.3 (11)	0	0.04	< 0.01

significance $p \leq 0.05$
n = number of measurements

Table 2: Inner diameters (mm)

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG TRAUMA (n)	P I/II	P I/III	P II/III
A_2P_2	4.43 ± 0.21 (10)	7.24 ± 0.46 (25)	7.46 ± 0.42 (9)	0	0	0.21
B_2C_2	3.54 ± 0.39 (13)	5.19 ± 0.43 (27)	3.70 ± 0.85 (11)	0	0.57	< 0.01
D_2E_2	4.02 ± 0.30 (13)	6.07 ± 0.39 (26)	4.94 ± 0.68 (11)	0	< 0.01	< 0.01

significance $p \leq 0.05$
n = number of measurements

Table 3: Thickness of cricoid cartilage (mm)

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG TRAUMA (n)	P I/II	P I/III	P II/III
A_1A_2	0.55 ± 0.06 (10)	0.58 ± 0.09 (24)	0.81 ± 0.17 (9)	0.28	< 0.01	< 0.01
$\frac{B_1B_2 + C_1C_2}{2}$	0.76 ± 0.08 (13)	0.75 ± 0.08 (27)	0.89 ± 0.12 (11)	0.71	< 0.01	< 0.01
$\frac{D_1D_2 + E_1E_2}{2}$	0.75 ± 0.08 (13)	0.87 ± 0.12 (26)	1.15 ± 0.26 (11)	< 0.01	< 0.01	< 0.01
P_1P_2	0.69 ± 0.15 (13)	0.71 ± 0.13 (24)	0.76 ± 0.15 (10)	0.69	0.28	0.37

significance $p \leq 0.05$
n = number of measurements

Table 4: Thickness of subepithelial layer

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG TRAUMA (n)	P I/II	P I/III	P II/III
A_2A_3	0.52 ± 0.11 (10)	0.66 ± 0.25 (25)	0.96 ± 0.27 (9)	0.03	< 0.01	0.01
$\frac{B_2B_3 + C_2C_3}{2}$	0.46 ± 0.11 (13)	0.71 ± 0.14 (27)	0.97 ± 0.73 (11)	< 0.01	0.04	0.27
$\frac{D_2D_3 + E_2E_3}{2}$	0.65 ± 0.10 (13)	1.09 ± 0.27 (26)	1.11 ± 0.44 (11)	0	< 0.01	0.89
P_2P_3	0.31 ± 0.13 (13)	0.50 ± 0.17 (24)	0.90 ± 0.52 (10)	< 0.01	< 0.01	0.04

significance $p \leq 0.05$
n = number of measurements

Series I: 4-week-old non operated rabbits (fig.2).

The lumen of the subglottis is oval with the longest axis in dorsoventral direction. For the whole series the surface area is ± 8,6 mm^2. The inner surface of the cricoid ring has an oval shape with a sagittal diameter of ± 4,43 mm and transversal dimensions from ± 3,54 mm in the ventral half to ± 4,02 mm in the dorsal half. The linear measurements of the cartilage thickness are ± 0,55 mm for the ventral side and ± 0,69 mm for the dorsal side. The lateral thickness varies from ± 0.75 mm to ± 0,76 mm. The subepithelial layer has a diameter between ± 0,31 mm on the dorsal side and ± 0,65 mm on the dorsolateral side.

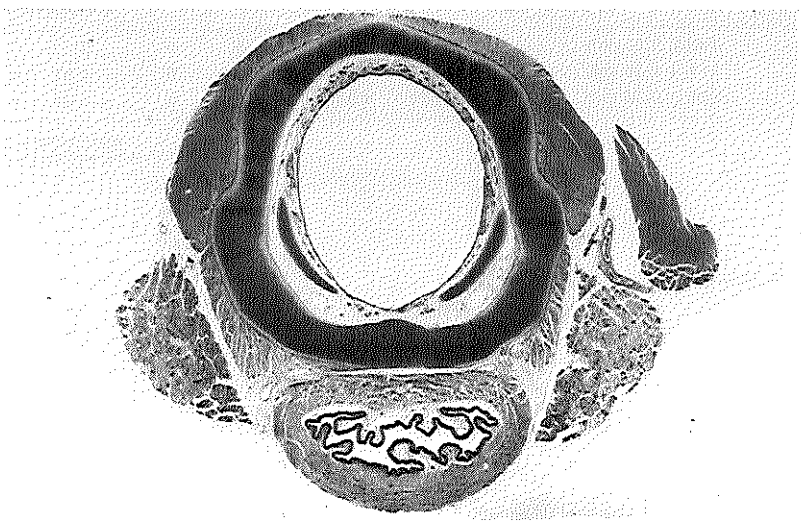

fig.2 Transversal section of normal subglottis in 4-week-old rabbit (series I) with more or less round shape; dorsolateral in subepithelial layer upper part of first tracheal ring. (haematoxylin-azophloxin, magn. 10x)

Series II: 24-week-old normal rabbits (fig.3).

The oval lumen of the subglottis in this series has increased to a surface area of ± 21,1 mm^2. The sagittal diameter of the cricoid ring is ± 7,24 mm and the transverse measurements are ± 5,19 mm and ± 6,07 mm in the ventral and dorsal half respectively. The thickness of the cartilage midventrally and middorsally measures ± 0,58 mm and ±0,71 mm respectively. The lateral side varies between ± 0,75 mm and ± 0,87 mm. The subepithelial soft tissue has a thickness between ± 0,50 mm middorsally and ± 1,09 mm dorsolaterally.

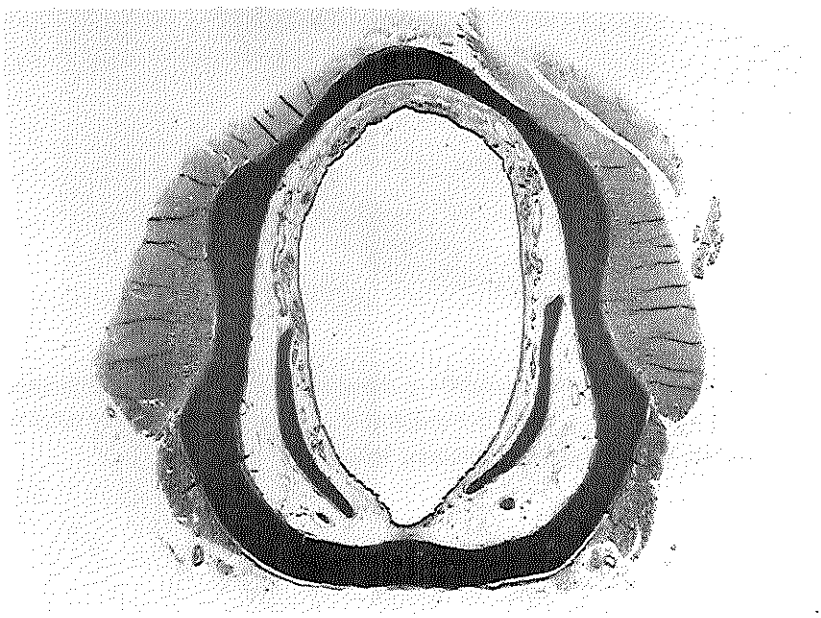

fig.3 Transversal section of normal subglottis in 24-week-old rabbit (series II) with oval shape of cricoid ring and subglottic airway lumen; largest diameter in sagittal direction. (haematoxylin-azophloxin, magn. 10x)

Series III: 24-week-old rabbits with endolaryngeal trauma at the age of 4 weeks (fig.4).

The subglottic airway is obviously stenotic. The surface area of the lumen measures ± 11,5 mm^2. In all specimens anomalies of the cricoid ring are present, but none of the cricoids show any interrupting defect. However, the shape is found to have been changed in a characteristic way. The normal cricoid obtains a marked oval shape during growth from 4 to 24 weeks, whereas in most specimens from series III the cartilage develops a "pear"-like shape due to a smaller transversal diameter especially in the ventral half (fig.5). The midsagittal diameter does not differ from those in the non operated specimens of the same age and measures ± 7,46 mm. The transversal diameters are

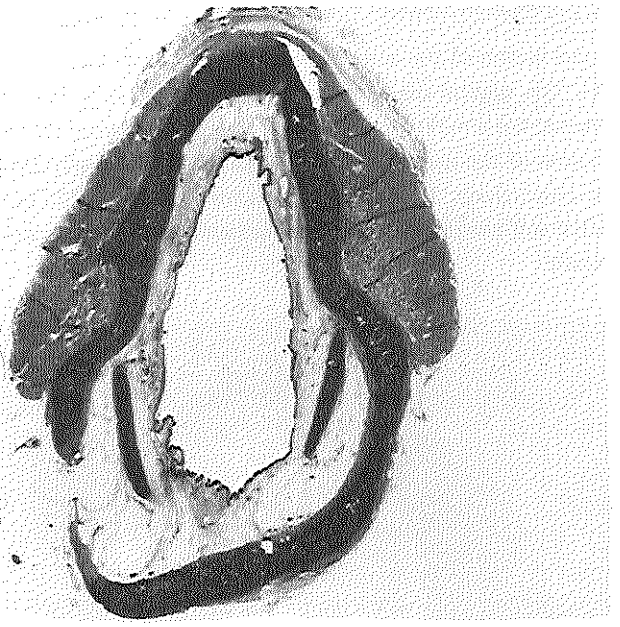

fig.4 Transversal section of the subglottis of 24-week-old rabbit with endolaryngeal trauma at 4 weeks of age; "pear"-like shape of cricoid ring with stenosis of subglottic airway lumen. (haematoxylin-azophloxin, magn. 10x)

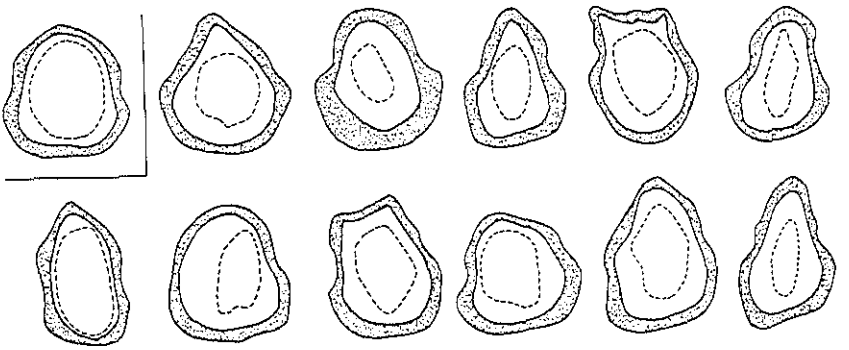

fig.5 Schematic drawings of cricoid rings of adult rabbits; one control specimen of series II (upper left) and all specimens of series III.

proportionally smaller and measure ± 3,70 mm and ± 4,94 mm. The thickness of the cartilage at the medioventral side has increased to ± 0,81 mm against ± 0,58 mm in series II and at the lateral sides to ± 0,89 mm and ± 1,15 mm respectively against ± 0,75 and ±

0,89 mm in the control series. The subepithelial layer has augmented remarkably. Midventrally the diameter is ± 0,96 mm against ± 0,66 mm in the adult control specimens and middorsally ± 0,90 mm against ± 0,50 mm. At the lateral sides the soft tissues did not increase in thickness.

Discussion

The growth of the subglottic part of the larynx in rabbits between the 4th (series I) and 24th week after birth (series II) resulted in an increase of the lumen surface area in serial sections of about 150%. In this period the growing cricoid ring developed a slightly more oval shape. The dorsoventral diameter increased about 65%. The transverse dimensions augmented between 45% and 50%. It is interesting to note that the thickness of the cricoid cartilage only changed slightly in the dorsolateral region (± 15%). The mean thickness of the soft tissue lining increased between 30% and 65%.

In humans the cricoid cartilage was also demonstrated to keep approximately the same shape during growth from prepuberty to adulthood (11): the inner sagittal and transversal diameters were calculated to increase with about 60% in males and with about 35% in females (10).

After endolaryngeal trauma at the age of 4 weeks 10 out of 11 animals developed a subglottic stenosis. In transversal sections the mean surface lumen area increased only from 8,5 mm^2 to 11,5 mm^2 compared to 21,1 mm^2 in adult control rabbits.

The first and most important anomaly was a characteristic deformity of the cricoid ring. The overall transversal growth was seriously impaired especially in the ventral region. The inner contour changed from slightly oval to a "pear"-like shape with the narrowest part on the ventral side. The growth in dorsoventral direction was not hampered. This observation seems to correspond with clinical data: Cotton (3) found in cases of severe subglottic stenosis in young patients the greatest extent of narrowing on the ventral side of the subglottis.

Thinking about the cause of this abnormal growth pattern some considerations emerge from these experiments: 1. Local differences in the degree of traumatization of the inner wall of the subglottis. However, the whole area was within easy reach of the burr drill and the operation could be well controlled. 2. Regional differences in the growth activity and, therefore, the vulnerability of the cricoid ring or soft tissues. No data concerning postnatal differences in growth potential of various parts of the cricoid ring have been described. Further autoradiographic studies are performed to elucidate this point. 3. In case of a developing stenosis the dorsal ends of the first tracheal ring could act as a natural stent. These ends extending to a level just inside the cricoid cartilage could promote the transverse growth in the dorsal half of the airway leading to a "pear"-like shape.

Extra thickening of the subepithelial layer between ± 85% (ventral) and ± 190% (dorsal), compared with ± 30% and ± 65% in the control animals,is the second reason for subglottic stenosis in rabbits. The thickening of the cricoid cartilage itself seems

hardly to contribute to the narrowing of the airway lumen. These observations do not concur with data on the thickening of the cricoid ring in cases of acquired subglottic stenosis in infants and children. Crysdale (4) observed the cricoid lamina to be thickened three to four times in three young patients; Grahne (8) reported the lamina to be extremely thickened in all of seven children operated upon. The authors postulate that the process of cricoid thickening is due to perichondritis, although histologic evidence is not presented.

In conclusion, the experimental results lend no support to the idea that an acquired subglottic stenosis in infants and children is "normalized" during later growth. To gain further insight in the pathogenesis of the anomalies discussed and to understand the difference in opinion on the significance of cartilage thickening for stenosis formation, histologic examination will be performed.

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CHAPTER 3

A HISTOLOGIC STUDY OF THE GROWTH OF THE SUBGLOTTIS AFTER ENDOLARYNGEAL TRAUMA.

Abstract

In young rabbits the histologic features of the growth of the subglottis were studied after an endolaryngeal trauma. This lesion of the larynx leading to a subglottic stenosis, caused specific pathologic changes of the cricoid cartilage and the subepithelial tissues. Activation of chondrocytes resulted in proliferation and regeneration of destructed cartilage. The normal subepithelial structures were replaced by granulation tissue differentiating into a thick fibrous scar and into cartilage. This ectopic cartilage together with the repaired cricoid formed a composite thickened "ring"-like cartilaginous structure in the adult stage.

Introduction

In a previous morphologic study (1), it was demonstrated that an endolaryngeal trauma in growing rabbits results in a combined cartilaginous and soft tissue type of subglottic stenosis. After trauma the shape of the cartilaginous ring of the cricoid in the transversal plane changed from oval to "pear"-like. The subepithelial soft tissue layer showed marked thickening. On the other hand, considerable thickening of the cricoid cartilage has been reported by Cotton (4,5), Crysdale (7) and Grahne (10) to be an important factor in the development of an acquired subglottic stenosis in infants and children.

For a better understanding of the disturbed growth pattern of the subglottis and the differing observations concerning the cricoid thickening in young patients and growing rabbits, further histologic investigations were performed.

Literature

Subglottic histopathology after endolaryngeal trauma was previously described in experimental animals by Borowiecki et.al (2,6) investigating the larynx of dogs upto 12 weeks after endolaryngeal trauma and by Marshak (16) and Supance (20) studying growing puppies upto 4-5 weeks after an endolaryngeal injury. They reported a loss of soft tissue lining of the laryngeal lumen, followed by epithelial regeneration with foci of squamous epithelium and the formation of subepithelial granulation tissue and subsequent fibrosis causing various degrees of airway obstruction. In severely injured

animals partial destruction of the cricoid cartilage was observed.

After intubation, regressive changes of the soft tissue lining of the subglottic airway have been described. Prolonged intubation and infection can lead to ongoing ulceration (3,9,13,15), penetrating into the inner perichondrial layer of the cricoid cartilage (11,12,14) and causing perichondritis (8,19). Necrosis of cartilage seems not to be a prominent feature; Donnely (8) observed necrosis of the innermost layer; only Quiney (17) found full thickness necrosis of the cartilage in three infants. Further reparative mechanisms, such as the formation of abundant granulation tissue (19) and subepithelial fibrosis (18), were reported as well as re-epithelialization in part by squamous epithelium.

The data from experimental and clinical studies are still too fragmentary to give a clear picture of the subglottic growth after endolaryngeal trauma and in particular the mechanism of the abnormal cricoid growth and nature of the subepithelial thickening.

Experimental animals and methods

The same series of female, New Zealand white rabbits were used in the previous morphologic study (1) and in the present histologic study:

Series I : 13 non operated rabbits, 4 weeks old.

Series II : 28 non operated rabbits, 24 weeks old.

Series III : 16 rabbits with an endolaryngeal trauma the age of 4 weeks: 11 rabbits sacrificed 20 weeks after trauma and 5 rabbits which died of haemorrhagic enteritis 3 days (3 animals), 10 days (1 animal) and 30 days (1 animal) after trauma.

In series III at the age of 4 weeks the soft tissue lining of the subglottis and the inner surface of the cricoid were damaged circularly (1). In all series, the subglottic part of the airway was histologically processed to obtain 5 μm transversal sections, which were stained with haematoxylin - azophloxin and elastica-Van Gieson. Every tenth section was mounted on slides.

Results

Series I: 4-week-old non operated rabbits (fig.1).

The subglottic airway is lined with normal respiratory epithelium. The subepithelial layer consists of loose connective tissue, glands, blood vessels and fatty tissue. Furthermore, this layer contains a network of elastic fibers which predominantly run in a longitudinal direction creating a subepithelial elastic tube. Middorsally, the fibers of this tube are attached to the perichondrium of the cricoid.

The cricoid cartilage is composed of a basophilic intercellular substance and

chondrocytes with a centrally positioned, prominent nucleus. The cells have an oval shape in the central zone of the cartilage and a spindle-like shape near the perichondrium. The chondrocytes are grouped in chondrons. Mitosis is scarcely found; a number of lacunae appear to contain two cells, whereas some cells have two nuclei, suggesting mitotic activity. The inner and outer perichondrial layer is equal in thickness. No regional differences in the histology and mitotic activity of the cricoid can be observed.

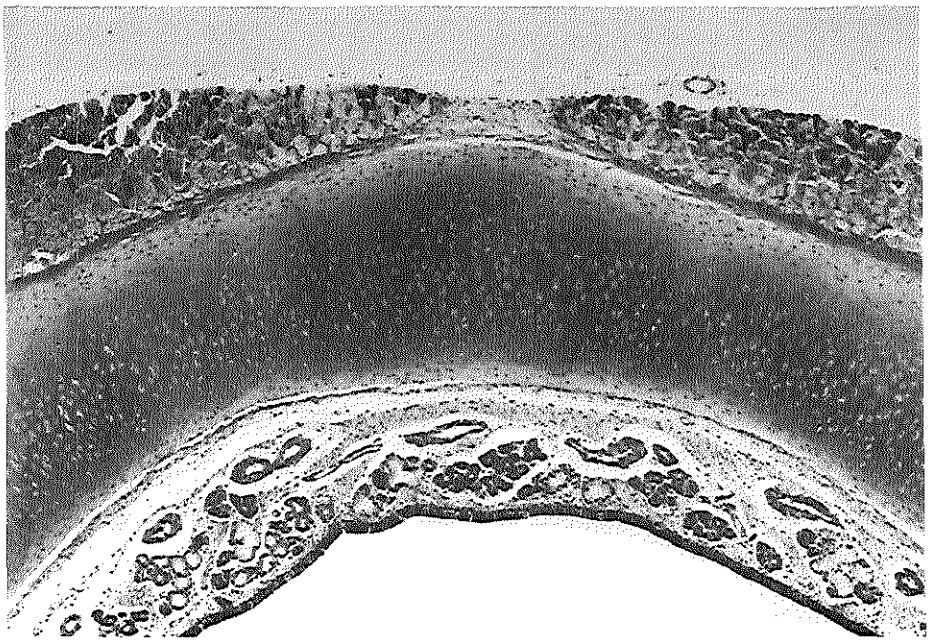


fig.1 Midventral part of subglottis in 4-week-old rabbit; small chondrocytes with prominent nucleus in centre of cell; subepithelial layer with loose connective tissue, blood vessels and glands; respiratory epithelium.(haematoxylin-azophloxin, magn.40x)

Series II: 24-week-old non operated rabbits (fig.2).

The epithelial lining of the subglottic airway and the subepithelial soft tissues are similar to those in the 4-week-old stage.

The cricoid cartilage has a fully differentiated appearance characterized by a proportionally small amount of intercellular substance and large, rounded, hypertrophied chondrocytes with a flattened, excentrally located nucleus. No signs of calcification are observed. The inner and outer perichondrial layer do not differ in morphology. The histologic characteristics of the cartilage are the same throughout all parts of the cricoid ring.

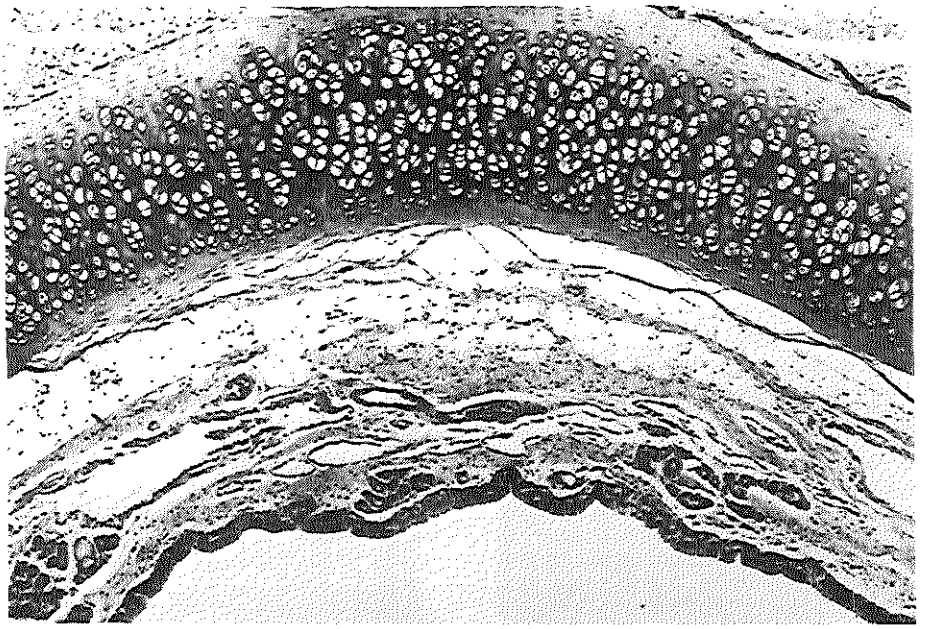
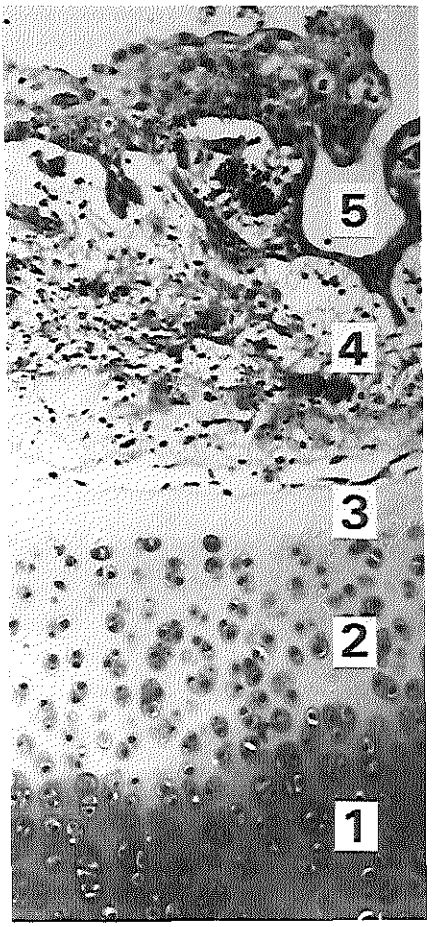


fig.2 Midventral part of subglottis in 24-week-old rabbit; hypertrophied chondrocytes with flattened, excentrally located nucleus; subepithelial soft tissues and epithelium similar to 4-week-old stage.(haematoxylin-azophloxin, magn.40x)

Series III: rabbits with an endolaryngeal trauma at the age of 4 weeks.

a. Three days after trauma (3 animals); (fig.3). In 2 animals the wound area is closed for the greater part by simple or stratified squamous epithelium. In 1 animal, no signs of re-epitheliazation are observed. The cricoid cartilage in all animals is covered with a layer of granulation tissue, rich of capillary loops and fibroblasts with a variable number of inflammatory cells.

The circular structure of the cricoid is preserved in all cases and has still a more or less round form. The inner perichondrium appears to be totally absent. The cartilage shows distinct reactive changes: three layers can be distinguished from the inside to the outside. The innermost layer is characterized by a poor staining of the intercellular substance and necrosis of nearly all cells. The second, intermediate layer demonstrates a gradient of increasing pathology to the inner side. The staining of the intercellular substance gradually becomes poor; a number of cells is necrotic, whereas the remaining chondrocytes show progressive swelling of the nucleus with signs of increased mitotic activity. The third layer has the same histologic features as the normal cartilage in the 4 weeks old control animals. The thickness of the three layers shows local differences without a recognizable pattern. In 1 animal, the reactive changes even reach the outer perichondrium at some sites; here, the third layer is missing.



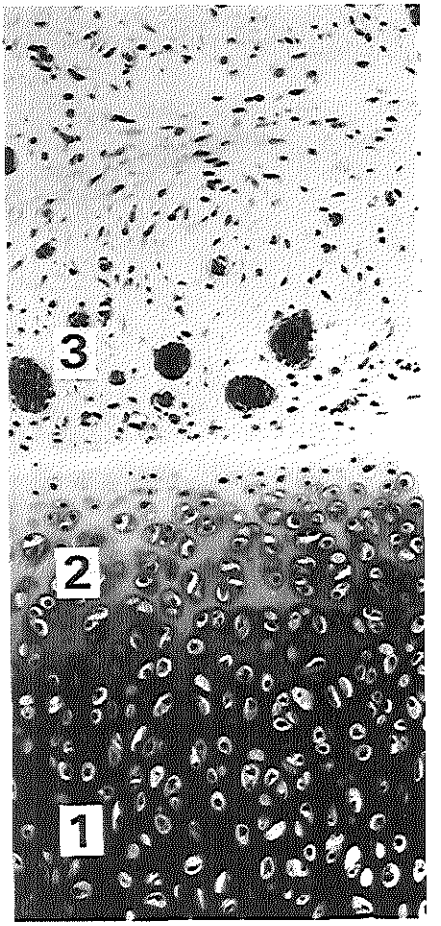


fig.3 *Middorsal part of subglottis 3 days after endolaryngeal trauma; cricoid consisting of 3 layers: normal cartilage (1); poorly staining cartilage with necrotic cells and chondrocytes with swollen nucleus showing increased mitotic activity (2); poorly staining cartilage with necrosis of all cells (3); perichondrium absent; subepithelial granulation tissue with many inflammatory cells (4); simple and stratified squamous epithelium (5).(haematoxylin-azophloxin, magn.100x)*

fig.4 *Middorsal part of subglottis 30 days after endolaryngeal trauma; cricoid consisting of 2 layers: normal cartilage (1); poorly staining cartilage with cells showing increased mitotic activity and only few necrotic chondrocytes (2); perichondrium absent; thick subepithelial layer of granulation tissue with small number of inflammatory cells (3).(haematoxylin-azophloxin, magn.100x)*

b. Ten days after trauma (1 animal). The lumen of the airway is almost completely lined with a stratified squamous epithelium. The subepithelial layer still consists of granulation tissue. A first sign of cartilage formation is noticed within the subepithelial tissue: irregularly arranged chondrocytes within a fibrous, basophilic matrix.

The circular structure of the cricoid has maintained a round form. In the cartilage, only two layers can be distinguished: an outer, normal staining zone and an inner, poor staining zone showing the characteristics of the intermediate layer as observed three days after trauma. Necrotic cells are still present, but their number has decreased. Furthermore, fibroblast-like cells are found in this innermost layer, whereas some of these cells also line the inner cricoid surface; normal perichondrium is still absent.

c. Thirty days after trauma (1 animal); (fig.4). The epithelium is of the simple or stratified squamous type. The subepithelial layer can still be characterized as granulation tissue without any glandular structures or elastic fibers. Cartilaginous differentiation of subepithelial tissues is not observed.

The round form of the non interrupted cricoid ring has not changed. The cartilage is still composed of two zones. The inner, poorly staining layer contains many young cells with a dense, prominent nucleus in the centre; a few necrotic cells are left. The perichondrium has not been regenerated. The outer layer reveals the aspect of normal cartilage of this age.

d. Twenty weeks after trauma (11 animals); (fig.5,6). Normal respiratory epithelium has recovered and is lining the subglottic airway. Only occasionally, the epithelium is not of a fully differentiated respiratory type, but consists of ciliated, cuboidal cells and a few goblet cells. In most cases the subepithelial layer is thickened. It is composed of fibrous scar tissue with collagen fibers, predominantly running circularly. Only thin and inorderly arranged elastic fibers can be observed as well as fatty tissue. Dilatated blood vessels are often seen near the inner surface of the cricoid which had an irregular aspect. The rest of the subepithelial layer contains few blood vessels.

In 10 animals, areas of young cartilage are found in the subepithelial tissue and once even a piece of bone was observed. The ectopic cartilage resembles either a hyaline type or a fibro-cartilaginous type. In 6 animals, this cartilage has direct contact with the cricoid. At these sites no intervening perichondrial layer can be distinguished. The transition between the fibro-cartilaginous type and the surrounding fibrous tissue is gradual; collagen fibers are seen to continue their course, the main difference being the intercellular deposition of a basophilic material specific for cartilage. Elsewhere normal perichondrium borders the inner surface of the cricoid.

In all specimens the circular continuity of the cricoid is not interrupted. In most cases the cricoid has developed a characteristic "pear"-like shape.

Discussion

During the 20 weeks of the experiment - from the 4th week (series I) after birth to the adult stage (series II) - the epithelium and subepithelial layer in the control animals

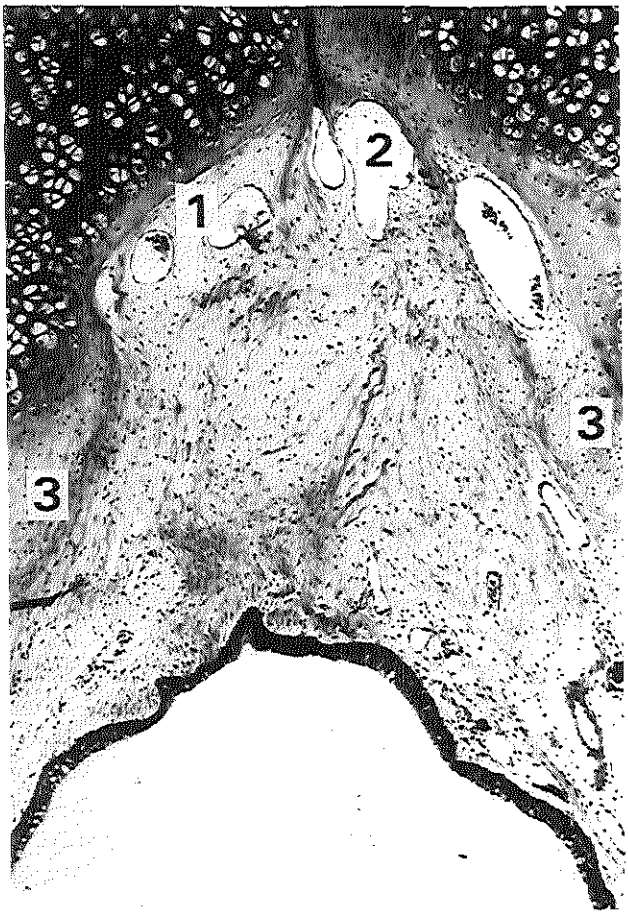


fig.5 Midventral part of subglottis 20 weeks after endolaryngeal trauma; normal cricoid cartilage with irregular surface and partly regenerated perichondrium (1); subepithelial fibrous scar with ectatic bloodvessels (2) and ectopic cartilage in direct contact with the cricoid (3); regenerated respiratory epithelium.(haematoxylin-azophloxin, magn.40x)

do not demonstrate any histologic changes. The cricoid cartilage shows signs of maturation: progressive hypertrophy of the cells accompanied by an increased cell/intercellular substance ratio and a flattening and migration of the nucleus to the periphery of the cell. Growth centres are not observed in the postnatal cricoid. The cricoid has a uniform histologic aspect all round. Changes in form and size have been reported previously (1).

The endolaryngeal trauma in 4-weeks-old rabbits leads up to a subglottic stenosis in the adult stage in 10 out of 11 animals (series III). This stenosis is mainly caused by a ventral narrowing of the cricoid ("pear"-like shape) and a pronounced thickening of the

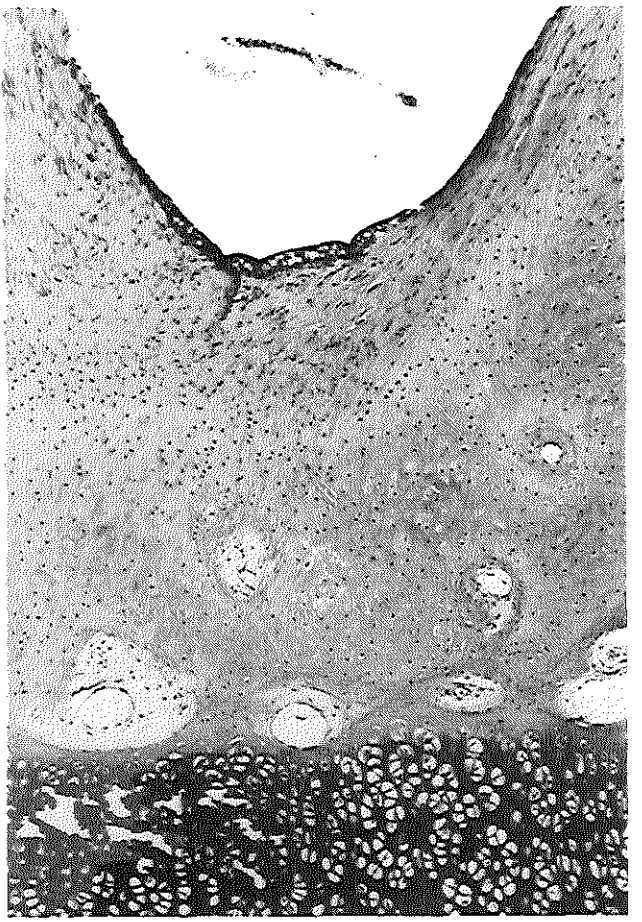


fig.6 Middorsal part of subglottis 20 weeks after endolaryngeal trauma; abundant formation of ectopic cartilage in mekedly thickened subepithelial layer; epithelium partly atypical with cuboidal ciliated cells.(haematoxylin-azophloxin, magn.40x)

subepithelial layer. The thickening of the cricoid does not substantially contribute to the narrowing of the airway lumen.

The findings in the animals which died before the end of the experiment provide valuable information about the evolution of the subglottic stenosis and will be discussed together with the data of the adult specimens.

3 Days after abrasion of the epithelium, subepithelial soft tissues, inner perichondrium and a thin layer of cartilage (series III), the remaining cricoid is covered with granulation tissue partly lined with simple and stratified squamous epithelium. In the following 4 weeks the aspect of the epithelium does not change. In the the adult stage most of the epithelium is of the normal respiratory type. An interesting feature is

that the glands do not regenerate.

Initially (after 3 and 10 days) the granulation tissue contains many inflammatory cells which have disappeared 30 days after trauma. Then, most cells have acquired the morphology of fibroblasts with spindle shaped nuclei. The thickness of this layer of fibroblasts shows marked variations. Most of the blood vessels are found in the deepest zone of the subepithelial layer neighbouring upon the cricoid cartilage. In the adult stage this thick subepithelial layer shows further differentiation into fibrous scar tissue, fatty tissue and cartilage, resembling either a hyaline or a fibro-cartilaginous type. The first sign of this ectopic chondroneogenesis is already noticed 10 days after trauma. The system of elastic fibers in the subepithelial layer is obviously not restored.

The removal of the inner perichondrium and damage to the adjacent cartilage results in a specific wound reaction of the cricoid cartilage. The innermost layer is necrotic and the adjacent, intermediate zone contains necrotic cells as well as cells with a swollen nucleus, the last as a sign of activation. This process leads to enhanced mitotic activity (3 and 10 days). The resulting proliferation of chondroblasts is likely to contribute to the regeneration of the innermost layer (10 and 30 days) and the partial thickening of the cricoid. 30 Days after trauma most of the necrotic cells have been replaced by viable chondrocytes. In the adult stage the cartilage has regained a normal histologic differentiation. Abnormal is the absence of perichondrium at various sites. This might play a role in the cartilage formation of the adjacent granulation tissue. newly formed ectopic cartilage in the subepithelial layer. The ventral narrowing ("pear"-like shape) as a rather late phenomenon, becomes manifest after the 30th day post trauma. The experimental results do not give further information about the mechanisms of this disturbed growth pattern.

A markedly thickened cricoid cartilage in infants and children with an acquired subglottic stenosis was ascribed to perichondritis (4,5,7,10). Histologic evidence for this hypothesis was not presented. The experimental results clearly demonstrate that the thick layer of cartilage can be formed within the granulation tissue and be added to the original cricoid, forming together a thick irregular cartilaginous structure. So, a composite thickened "ring"-like structure is produced without a prolonged perichondritis.

From these observations the hypothesis emerges that the cartilage thickening in acquired subglottic stenosis in infants and children is also of a composite nature mainly caused by the formation of ectopic cartilage. Investigations of human stenotic larynges can supply evidence of the supposed chondroneogenesis in the subepithelial layer.

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CHAPTER 4

A MORPHOMETRIC STUDY OF THE GROWTH OF THE SUBGLOTTIS AFTER INTERRUPTION OF THE CIRCULAR STRUCTURE OF THE CRICOID

Abstract

In young rabbits the growth of the subglottis was studied over a period of 20 weeks after interruption of the circularity of the cricoid on the ventral side. In all cases the cricoid developed into a U-like structure which did not cause an airway narrowing. However, a cricoid lesion combined with an injury to the soft tissue lining led up to a subglottic stenosis. It was concluded that when the soft tissue layer is undamaged, an intact circular structure of the cricoid is not compulsory for the normal development of the subglottis.

Introduction

The treatment of acquired subglottic stenosis in infants and children is a challenging problem. A wide variety of both conservative (4,5,12) and surgical (7,10,16) methods have been recommended. To date it has not been possible to cure all patients, whereas the reasons for failure of therapy are still scarcely understood. Therefore, it is necessary to investigate systematically the factors which can give rise to poor results.

The long-term effects of an endolaryngeal trauma on the development of the subglottis have been investigated previously in growing rabbits (1,2). The growth of the cricoid ring was demonstrated to be markedly impaired after endolaryngeal traumatization, whereas the soft tissue lining showed considerable thickening due to formation of scar tissue and ectopic cartilage. The resulting stenosis was not compensated during further growth, contrary to the hypothesis that an acquired subglottic stenosis in infants and children is "normalized" during later development.

Another problem in laryngeal traumatology is the interruption of the cricoid ring. In patients this can be the result of :

1. full thickness necrosis of parts of the cricoid after traumatic intubation (15);
2. fracturing of the cricoid in external laryngeal trauma; the cricoid arch is reported to be prone to fracturing (3);
3. surgery: high tracheotomy, erroneously involving the cricoid (11) or anterior midline cricoid split (6,8,9,13).

An anterior midline split is advocated by some authors as a method of treatment for children with extubation problems. On the other hand, Jackson stated that anterior

division of the cricoid cartilage is a frequent cause of subglottic stenosis (11). Also, Nelson demonstrated in a clinical and experimental study that anterior division of the cricoid was more likely to result in an airway stenosis than incisions of the larynx and trachea at other sites (14). Wright reported arrest of cricoid growth after incision of the cricoid ring during emergency tracheotomy (17).

Opinions, based on clinical experience, apparently diverge as far as the effects of interruption of the cricoid ring on the subglottic airway lumen are concerned. Therefore, an experimental study was performed to investigate morphologically in growing rabbits:

1. the growth of the subglottis after an anterior midline cricoid split and after resection of the anterior 1/3 and 1/2 of the cricoid ring.
2. the growth of the subglottis after resection of the anterior 1/3 and 1/2 of the cricoid ring including the adjacent soft tissue lining of the airway.

Materials and methods

Seven series of female New Zealand white rabbits were included in this study:

Series I : 13 non operated rabbits, 4 weeks of age.

Series II : 28 non operated adult rabbits, 24 weeks of age.

Series III : 10 rabbits (24-week-old) in which the cricoid cartilage was split anteriorly in the midline at the age of 4 weeks without damaging the subepithelial tissues and the epithelium.

Series IV : 10 rabbits (24-week-old) in which the anterior 1/3 of the cricoid ring was excised at the age of 4 weeks, preserving the adjacent soft tissue lining of the airway.

Series V : 10 rabbits (24-week-old) in which the anterior 1/2 of the cricoid was excised at the age of 4 weeks, also preserving the inner soft tissue layer.

Series VI : 12 rabbits (24-week-old) in which the anterior 1/3 of the cricoid was resected at the age of 4 weeks, including the adjacent subepithelial tissues and the epithelium.

Series VII : 10 rabbits (24-week-old) in which the anterior 1/2 of the cricoid ring was resected at the age of 4 weeks, including the inner soft tissue lining of the airway.

The animals of series I and II were used as controls. In the operated animals xylazin (Rompun ®) 0,1 ml/100mg and ketaminchlorid (Ketaset ®) 0,1 ml/100mg have been used for anaesthesia. The subglottis was reached by a longitudinal incision of the skin and subcutis; these were sutured with catgut 6-0 after laryngeal surgery. No antibiotics were administered postoperatively. Some animals of series VI and VII developed a transient subcutaneous emphysema without dyspnoea. No signs of respiratory tract or wound infection have been observed.

After sacrifice the subglottic part of the airway was histologically processed to obtain 5 μm transversal sections. The methods to select and to measure one section from a circumscript subglottic segment of each specimen have been described earlier (1). The following measurements were carried out (fig.1):

1. lumen surface area of the subglottic airway (mm^2);
2. inner diameters of the cricoid ring (mm): A_2P_2, B_2C_2, D_2E_2;
3 thickness of the cricoid cartilage (mm):

$$A_1A_2,\ P_1P_2,\ \frac{B_1B_2+C_1C_2}{2},\ \frac{D_1D_2+E_1E_2}{2}$$

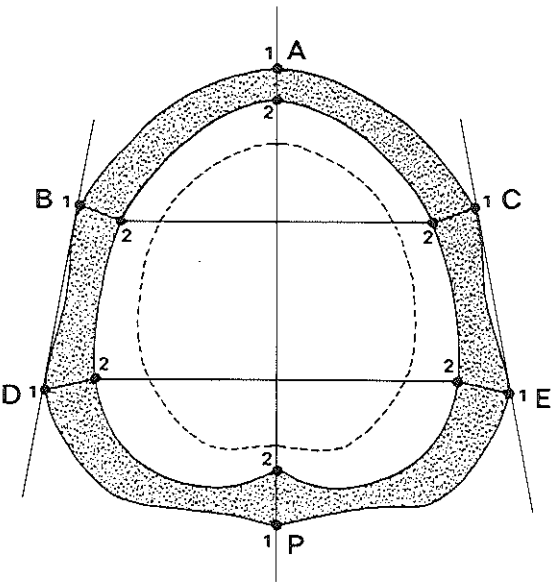

fig.1 Schematic transversal section of cricoid ring and epithelial lining (A-P = antero-posterior direction).

Results

The morphometric observations are presented in Tables I-III.

Series I and II: 4- and 24-week-old non-operated rabbits (fig. 2 and 3).

The measurements of the control specimens (Table I) show a considerable augmentation of the lumen surface area during growth to adulthood, accompanied by an increase of the diameters of the cricoid ring in both the sagittal and the transversal plane. The cricoid ring has developed a more oval shape, whereas the cartilage exhibits only a slight thickening on the dorsolateral side. In a previous paper information has been presented in more detail (1). Figure 4a shows a sagittal schematization of the larynx. The first tracheal ring runs in an oblique direction with the posterior side at a higher level extending into the subepithelial layer of the subglottis, enclosed by the cricoid.

Table I: Comparison of means of series I, II, III

		SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ANTERIOR SPLIT (n)	P I/II	P I/III	P II/III
Subglottic lumen area (mm^2)		8.6 ± 1.4 (13)	21.1 ± 2.9 (28)	24.3 ± 4.2 (10)	0	0	0.05
Diameters (mm)	A_2P_2	4.43 ± 0.21 (10)	7.24 ± 0.46 (25)	–	0	–	–
	B_2C_2	3.54 ± 0.39 (13)	5.19 ± 0.43 (27)	5.82 ± 0.74 (10)	0	< 0.01	0.03
	D_2E_2	4.02 ± 0.30 (13)	6.07 ± 0.39 (26)	6.34 ± 0.76 (10)	0	< 0.01	0.31
Thickness of cicroid (mm)	A_1A_3	0.55 ± 0.06 (10)	0.58 ± 0.09 (24)	–	0.28	–	–
	$\frac{B_1B_2 + C_1C_2}{2}$	0.76 ± 0.08 (13)	0.75 ± 0.08 (27)	0.79 ± 0.08 (10)	0.71	0.38	0.20
	$\frac{D_1D_2 + E_1E_2}{2}$	0.75 ± 0.08 (13)	0.87 ± 0.12 (26)	0.85 ± 0.12 (10)	< 0.01	0.04	0.66
	P_1P_2	0.69 ± 0.15 (13)	0.71 ± 0.13 (24)	0.70 ± 0.12 (10)	0.69	0.86	0.83

significance $p \leq 0.05$
n = number of measurements

Series III: 24-week-old rabbits with an anterior midline cricoid split (fig.5).

After splitting the cricoid in the midline the ends receded immediately, leaving a gap of ± 0,5 mm. In the adult specimens the subglottic lumen has obtained an oval shape like in the 24-week-old control animals (series II). The surface area has increased to ± 24,3 mm^2. The lateral inclination of the ends of the cricoid leads to increased transversal dimensions of ± 5,82 mm and ± 6,34 mm. The thickness of the cartilage in the dorsal midplane is ± 0,70 mm and varies between ± 0,79 mm and ± 0,85 mm on the lateral sides.

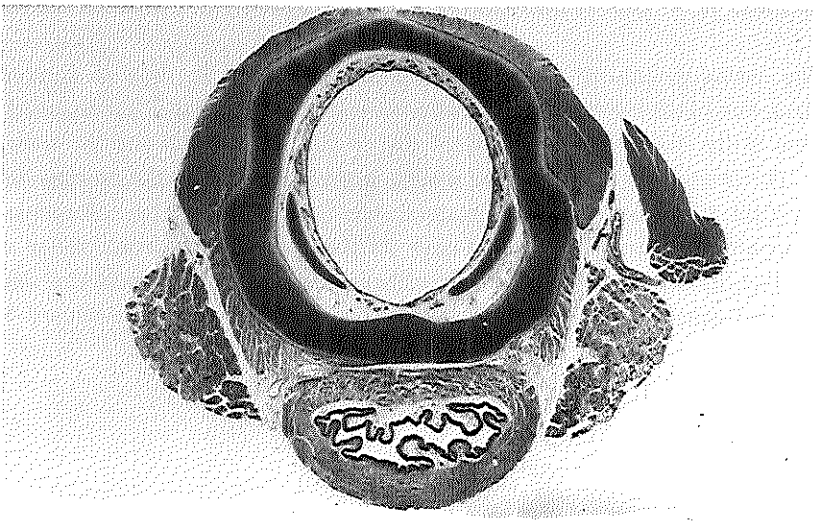

fig.2 Transversal section of normal subglottis in 4-week-old rabbit (series I) with "egg"-like shape of cricoid and oval subglottic airway lumen; dorsolaterally in subepithelial layer upper part of first tracheal ring. (haematoxylin-azophloxin, magn. 10x)

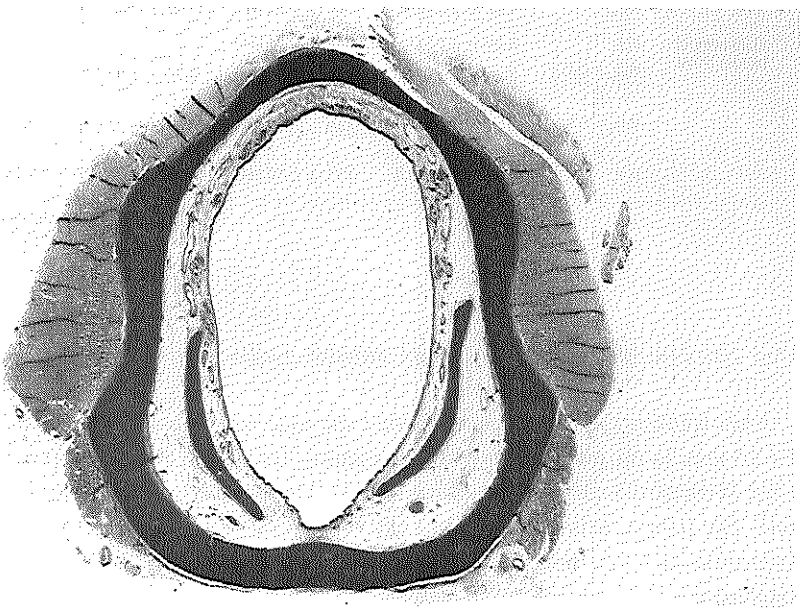

fig.3 Transversal section of normal subglottis in 24-week-old rabbit (series II) with "egg"-like shaped cricoid ring and marked oval subglottic airway lumen. (haematoxylin-azophloxin, magn. 10x)

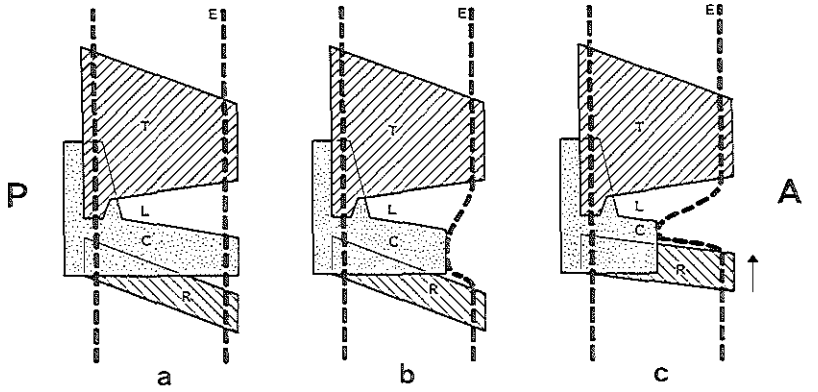

fig.4 *Sagittal scheme of the larynx; A = anterior, P = posterior, T = thyroid, C = cricoid, R = first tracheal ring, E = epithelium, L = airway lumen:*

a. *adult stage in non-operated rabbit (series II); first tracheal ring is running in oblique direction; dorsal half enclosed by cricoid;*
b. *adult stage in rabbit after resection of anterior 1/3 of cricoid and adjacent inner soft tissues (series VI); subglottic stenosis bounded by ends of cricoid;*
c. *adult stage in rabbit after resection of anterior 1/2 of cricoid and adjacent inner soft tissues (series VII); cranial displacement of first tracheal ring; subglottic stenosis limited by stubs of cricoid (at the level of crico-thyroid joint) and confined by tracheal ring (at the level of distal margin of the cricoid).*

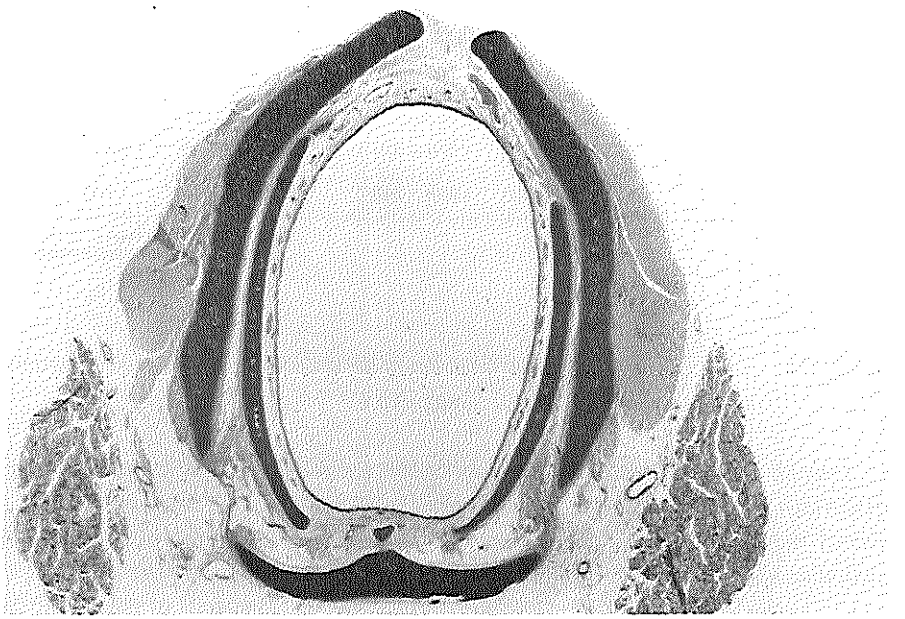

fig.5 *Transversal section of subglottis of 24-week-old rabbit with anterior cricoid split (series III). (haematoxylin-azophloxin, magn. 10x)*

Table II: Comparison of means of series I, II, IV, V

		SERIES IV 24 WEEKS RESECTION 1/3 (n)	P I/IV	P II/IV	SERIES V 24 WEEKS RESECTION 1/2 (n)	P I/V	P II/V
Subglottic lumen area (mm^2)		20.0 ± 5.0 (10)	0	0.50	19.9 ± 2.6 (10)	0	0.21
Diameters (mm)	A_2P_2	–	–	–	–	–	–
	B_2C_2	7.52 ± 1.18 (10)	< 0.01	< 0.01	–	–	–
	D_2E_2	7.14 ± 1.30 (10)	< 0.01	0.03	7.00 ± 0.91 (10)	0	0.01
Thickness of cicroid (mm)	A_1A_2	–	–	–	–	–	–
	$\frac{B_1B_2 + C_1C_2}{2}$	1.16 ± 0.36 (10)	< 0.01	< 0.01	–	–	–
	$\frac{D_1D_2 + E_1E_2}{2}$	1.11 ± 0.33 (10)	< 0.01	0.05	1.31 ± 0.30 (10)	< 0.01	< 0.01
	P_1P_2	0.82 ± 0.21 (10)	0.12	0.15	0.78 ± 0.14 (10)	0.15	0.19

significance $p \leq 0.05$
n = number of measurements

Table III: Comparison of means of series I, II, VI, VII

		SERIES VI 24 WEEKS RESECTION 1/3 + SOFT TISSUES (n)	P I/VI	P II/VI	SERIES VII 24 WEEKS RESECTION 1/2 + SOFT TISSUES (n)	P I/VII	P II/VII
Subglottic lumen area (mm^2)		13.5 ± 4.7 (12)	< 0.01	< 0.01	9.7 ± 3.5 (10)	0.36	0
Diameters (mm)	A_2P_2	–	–	–	–	–	–
	B_2C_2	7.09 ± 0.93 (12)	0	< 0.01	–	–	–
	D_2E_2	6.86 ± 1.07 (11)	< 0.01	0.04	7.09 ± 0.98 (10)	< 0.01	< 0.01
Thickness of cicroid (mm)	A_1A_2	–	–	–	–	–	–
	$\frac{B_1B_2 + C_1C_2}{2}$	1.10 ± 0.21 (12)	< 0.01	< 0.01	–	–	–
	$\frac{D_1D_2 + E_1E_2}{2}$	1.21 ± 0.22 (11)	< 0.01	< 0.01	1.17 ± 0.24 (10)	< 0.01	< 0.01
	P_1P_2	0.87 ± 0.25 (12)	0.04	0.06	0.81 ± 0.14 (10)	0.06	0.07

significance $p \leq 0.05$
n = number of measurements

Series IV: *24-week-old rabbits after resection of the anterior 1/3 of the cricoid, preserving the underlying soft tissue lining (fig.6).*

Despite the missing ventral part of the cricoid the subglottic lumen has developed an oval shape as in the adult control specimens (series II). Ventrally the lumen appears to exceed the stubs of the cricoid. The surface area is ± 20,0 mm^2 with considerable individual variations. In all specimens the cricoid has grown into a U-like structure with large transversal diameters of ± 7,52 mm and ± 7,14 mm. The stubs of the cricoid have increased in thickness at the level BC, measuring ± 1,16 mm ; at the level DE and in the dorsal midplane the thickness measures ± 1,11 mm and ± 0,82 mm respectively.

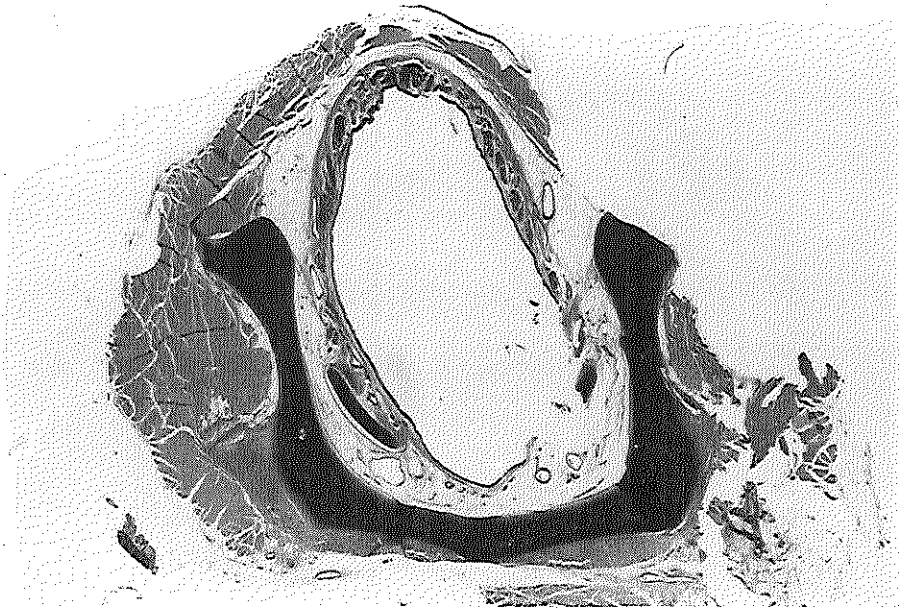

fig.6 *Transversal section of subglottis of 24-week-old rabbit after resection of anterior 1/3 of cricoid ring (series IV); U-like form of cricoid; normal size and shape of subglottic airway lumen with arcade of soft tissues on ventral side. (haematoxylin-azophloxin, magn. 10x)*

Series V: *24-week-old rabbits after resection of the anterior 1/2 of the cricoid, preserving the adjacent soft tissue layer (fig.7).*

Since the resected part of the cricoid has increased from 1/3 to 1/2, not only the subglottic lumen, but also the first tracheal ring is reaching more anteriorly than the cricoid ends. The form of the lumen has changed from oval to more or less round and measures ±19,9 mm^2. The soft tissue lining has retained its arch-like configuration on the ventral side. The remaining cricoid has flattened and developed into a shallow U-like structure with a large transversal diameter of ± 7,00 mm at the level DE. Furthermore, in contrast to the normal anatomy, the complete first tracheal ring can be seen in the subglottic subepithelial tissues in 8 out of 10 specimens, indicating an upward displacement of the tracheal ring on the anterior side. The thickness of the cartilage is middorsally ± 0,78 mm and laterally ± 1,31 mm.

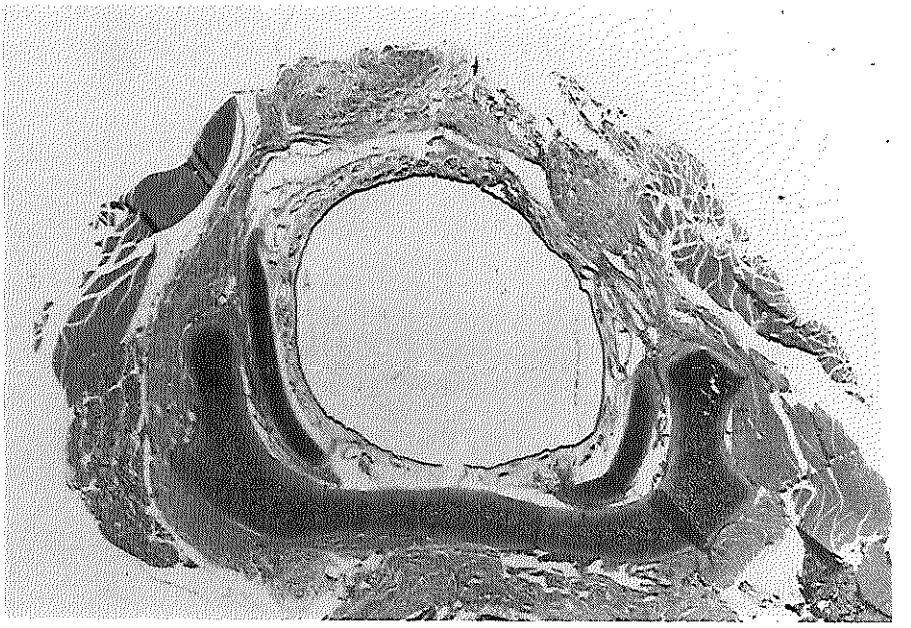

fig.7 Transversal section of subglottis of 24-week-old rabbit after resection of anterior 1/2 of cricoid ring (series V); shallow U-like shape of cricoid; no stenosis of subglottic airway; round shape of lumen with anterior arcade of soft tissues. (haematoxylin-azophloxin, magn. 10x)

Series VI: 24-week-old rabbits after resection of the anterior 1/3 of the cricoid, including the adjacent subepithelial tissues and epithelium (fig. 8)

In none of the specimens the ventral side of the subglottic lumen shows an arcade of soft tissues as observed in series IV and V. Instead, the airway exhibits a flattening at the level of the cricoid stubs. The surface area measures ± 13,5 mm^2. The cricoid has grown into a U-like shape with transversal diameters of ± 7,09 mm and ± 6,86 mm. The thickness of the cartilage middorsally is ± 0,87 mm and varies laterally from ± 1,10 mm at the level BC to ± 1,21 mm at the level DE. A sagittal schematization of the larynx (fig.4b) outlines the anterior stenosis caused by the collaps of the soft tissues reaching up to the level of the cricoid stubs.

Series VII: 24-week-old rabbits after resection of the anterior 1/2 of the cricoid, including the inner soft tissue lining of the airway (fig.9).

Due to the extent of the cricoid resection the part of the first tracheal ring within the subglottic subepithelial tissues is exceeding the ends of the cricoid in anterior direction. In all specimens the remaining cricoid has developed into a flattened U-like structure

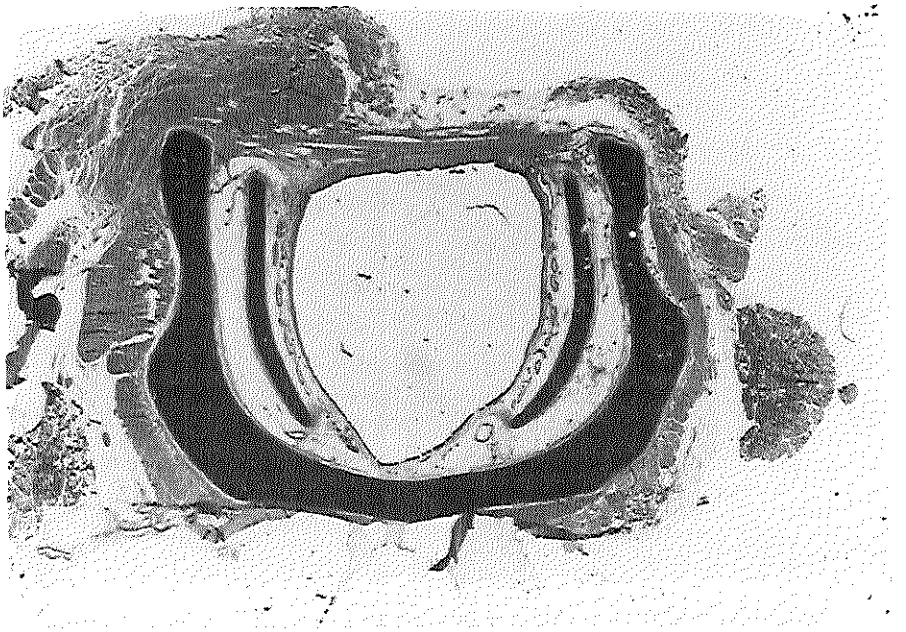

fig.8 Transversal section of subglottis of 24-week-old rabbit after resection of anterior 1/3 of cricoid ring with adjacent subepithelial layer and epithelial lining (series VI); stenosis of subglottic airway lumen caused by flattening of ventral side. (haematoxylin-azophloxin, magn. 10x)

with small lateral parts and a transversal diameter of ± 7,09 mm. In 9 out of 10 specimens the subglottic lumen exhibits an anterior flattening; the mean surface area only runs to ± 9,7 mm^2. In 6 out of 10 specimens the complete first tracheal ring can be seen within the subglottic subepithelial layer as observed in series V. Furthermore, in 2 specimens the first tracheal ring has a distorted appearance with an abnormal small transversal diameter. The thickness of the cricoid cartilage middorsally measures ± 0,81 mm and is ± 1,17 mm on the lateral side. A sagittal schematization of the larynx (fig.4c) illustrates the subglottic stenosis. The collapse of the anterior soft tissues reaches the cricoid ends at the level of the crico-thyroid joint; at the level of the lower margin of the cricoid the extent of airway stenosis diminishes because of the presence of the first tracheal ring.

Discussion

During the experimental period of 20 weeks (between the 4th week after birth and the adult stage of 24 weeks) the diameters of the cricoid ring in the control specimens (series I and II) showed an increase of ± 65% in sagittal direction (A_2P_2) and between ± 45% (B_2C_2) and ± 50% (D_2E_2) in transversal direction. The subglottic lumen changed

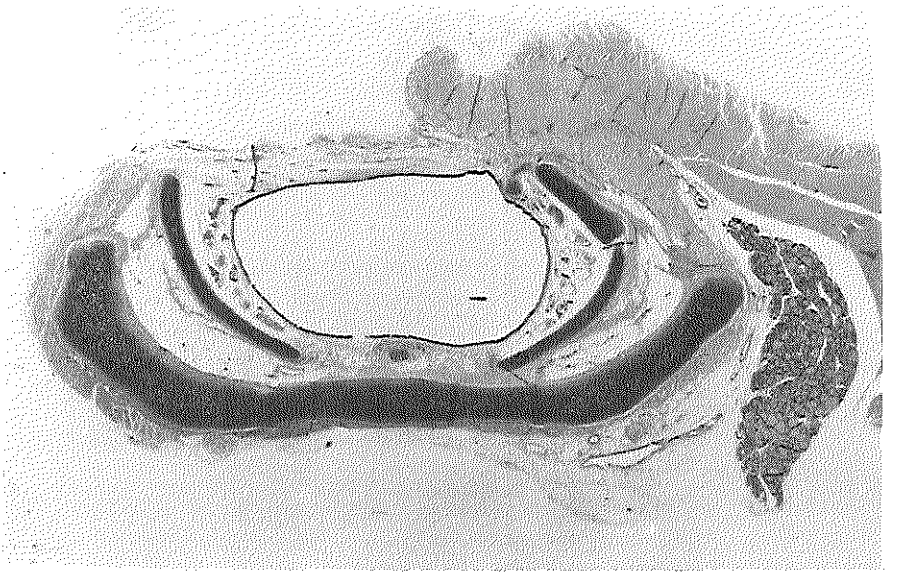

fig.9 Transversal section of subglottis of 24-week-old rabbit after resection of anterior 1/2 of cricoid ring with adjacent subepithelial layer and epithelial lining (series VII); marked stenosis of subglottic airway lumen caused by flattening of ventral side; distortion of first tracheal ring. (haematoxylin-azophloxin, magn. 10x)

from slightly to marked oval; the surface area augmented with ± 150% (from ±8,6 mm^2 to ±21,1 mm^2). The growth of the cricoid and the airway lumen in rabbits between the age of 4 and 24 weeks correspond proportionally to human laryngeal development from prepuberty to adulthood (1).

In the animals operated at the age of 4 weeks (series III-VII) interruption of the cricoid ring by an anterior midline split or partial resection of the arch affected the subsequent development of the remaining cricoid. First, the lateral parts of the cricoid tended to incline laterally (U-like shape), leading up to an increase of the transversal diameters with a maximum of ± 110% (from ±3,54 mm^2 to ±7,52 mm^2) at the level BC (series IV) and ±80% (from ±4,02 mm^2 to ±7,14 mm^2) at the level DE (series IV). The cause of these specific changes in the development could be a (physiologic) tension within the cricoid ring. The existence of such a circular tension is also suggested by the gap observed to occur between the cut ends of the cricoid immediately after an anterior midline split. The latter phenomenon was also reported to occur in human neonates (6). In most cases the second consequence of the interruption of the cricoid ring was an increased thickness of the cartilage on the lateral sides (Table II) compared with the adult control specimens (series II); the origin of this feature is not yet understood.

An anterior split of the cricoid ring at the age of 4 weeks (series III) led up to an extra enlargement of the subglottic airway lumen with ±15% in the adult stage compared with the control specimens in series II (from ±8,6 mm^2 to ±24,3 mm^2). These

experimental findings support the rationale for an anterior cricoid split in the "difficult-to-extubate" premature child (6,8,9,13) and are not in agreement with the clinical observations of subglottic stenosis (11,14) and even arrest of cricoid development (17) after midventral incision.

An intact circular structure of the cricoid is not a prerequisite for the development of a normally shaped and sized subglottic airway as long as the soft tissue lining of the airway is undamaged (series IV and V). In the absence of the ventral 1/3 or 1/2 of the cricoid ring these soft tissues form an arcade which does not collapse. It should be noted that this conclusion is based on the situation found in laryngeal specimens fixed in the anatomic position; it remains to be studied to what extent collapse might occur in case of flexion or extension of the neck in the living animal.

Contrary to the findings in series III-V a subglottic stenosis was present in most specimens after resection of the cricoid and the adjacent soft tissue lining (series VI and VII). The airway narrowing in the adult stage appeared to be formed by a flat layer of fibrous tissue stretched between the cricoid stubs. In series VI the collapse of the anterior wall was bounded by the stumbs of the cricoid. The remaining cartilage seemed to act as a stent preventing further airway narrowing. Resection of the ventral 1/2 of the cricoid and the adjacent soft tissues (series VII) also caused a collapse of the wall limited by the cricoid stubs near the crico-thyroid joint; at a more caudal level the part of the first tracheal ring, exceeding the cricoid stumbs, appeared to "take over" the function of airway lumen keeper. These findings illustrate that damage to the soft tissue lining causes a stenosis due to collapse of the wall. To a certain degree the collapse is proportionate to the extent of cricoid resection; then, the first tracheal ring prevents further airway narrowing. Reconsidering the subglottic stenosis reported by Jackson (11), Nelson (14) and Wright (17), it may be concluded from the experimental results that in their patients the stenosis is probably not caused by the lesion of the cricoid, but by a combination of damage to the cartilage and the inner soft tissue lining.

The type of subglottic stenosis in the specimens of series VI and VII is essentially different from the airway narrowing in rabbits after an endolaryngeal trauma (1,2); the latter type was demonstrated to be determined by an impaired development of the cricoid ring in the transversal plane plus thickening of the subepithelial layer.

Resection of the ventral 1/2 of the cricoid (in contrast to removal of the ventral 1/3!) appeared to be followed by an upward displacement of the first tracheal ring (fig.4c), whether or not the inner soft tissues were preserved (series V and VII). The cause of this feature will be further investigated in sagittal sections. The experimental results permit the following conclusions:

1. an anterior interruption of the circular structure of the cricoid ring induces a specific pattern of abnormal growth of the remaining cricoid (development of a U-like structure and a slight thickening of the lateral parts);
2. an anterior midline split in young rabbits leads up to an enlargement of the subglottic airway compared with control animals of the same age;
3. an intact circular structure of the cricoid is not compulsory for the development of a normally shaped and sized subglottic airway lumen;

4. the combination of interruption of the cricoid ring and injury to the inner soft tissues causes the development of a stenosis of the subglottic airway;
5. in case of a missing ventral part of the cricoid the soft tissue lining - if undamaged - is capable of forming a non-collapsable arcade bordering the subglottic airway. Further histologic investigations have to elucidate the nature of this arcade.

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CHAPTER 5

A HISTOLOGIC STUDY OF THE GROWTH OF THE SUBGLOTTIS AFTER INTERRUPTION OF THE CIRCULAR STRUCTURE OF THE CRICOID

Abstract

In a previous study in growing rabbits (1) it was demonstrated that after resection of a ventral part of the cricoid, the lumen of the subglottic airway developed a normal size during further growth up to the adult stage. Histologic investigations of these cases suggest that a subepithelial network of elastic fibres, ventrally suspended to the thyroid and the tracheal rings, ensures the airway lumen and prevents the development of a subglottic stenosis. Resection of a ventral part of the cricoid including the adjacent soft tissue lining of the airway causes an irreversible loss of the supporting elastic layer and the formation of scar tissue resulting in a stenosis. It was concluded that the larynx and trachea can be considered as an inner, elastic tube (= conus elasticus) lined with epithelium, suspended to an outer, segmented cartilaginous tube. At the level of the subglottis an intact conus elasticus is of more importance for the normal development of the airway lumen in young rabbits than an intact ventral half of the cricoid ring.

Introduction

The cricoid is the only complete cartilaginous ring in the skeleton of the airway. This laryngeal cartilage is thought to function as an essential support for the airway lumen. Interruption of the circular structure has often been hold responsible for the development of subglottic stenosis (4,5,7,8). However, in a previous morphometric study in growing rabbits (1), it was demonstrated that an intact circularity of the cricoid is not compulsory for the normal growth of the subglottic airway as long as the inner soft tissue lining has not been damaged. To elucidate the role of the subepithelial tissues in preventing the development of a stenosis, histologic investigations were performed. Also, the effects of injury to the subepithelial layer leading up to an airway narrowing, have been studied.

Materials and methods

Seven series of female New Zealand white rabbits previously included in a morphometric study (1), were used for histologic examination:

Series I : 13 non operated rabbits, 4 weeks of age.
Series II : 28 non operated adult rabbits, 24 weeks of age.
Series III : 10 rabbits (24-week-old) in which the cricoid cartilage was split anteriorly in the midline at the age of 4 weeks without damaging the subepithelial tissues and the epithelium.
Series IV : 10 rabbits (24-week-old) in which the anterior 1/3 of the cricoid ring was excised at the age of 4 weeks, preserving the adjacent soft tissue lining of the airway.
Series V : 10 rabbits (24-week-old) in which the anterior 1/2 of the cricoid was excised at the age of 4 weeks, also preserving the inner soft tissues.
Series VI : 12 rabbits (24-week-old) in which the anterior 1/3 of the cricoid was resected at the age of 4 weeks, including the adjacent subepithelial tissues and the epithelium.
Series VII : 10 rabbits (24-week-old) in which the anterior 1/2 of the cricoid was resected at the age of 4 weeks, including the inner soft tissue lining of the airway.

After sacrificing the animals at the adult age of 24 weeks the subglottic part of the airway was processed histologically to obtain 5μm transversal sections. Every tenth section was mounted on slides and stained with haematoxylin-azophloxin and elastica-Van Gieson. In 2 extra non-operated animals, 4 and 24 weeks of age, the larynx and the upper part of the trachea were cut for serial sagittal sections.

Results

Series I-II (control animals)

In transversal sections the epithelium and the subepithelial layer show identical morphologic features in 4- and 24-week-old rabbits (fig. 1 and 2). The epithelium is of the respiratory type. The subepithelial layer contains a network of elastic fibres all round. In the ventral and lateral parts this network is composed of three layers of elastic fibres, predominantly running in longitudinal direction. The thin, innermost layer, located immediately underneath the epithelium, is separated from the intermediate layer by a zone of glandular tissue. Loose connective tissue, blood vessels, glands and fatty tissue are present between the intermediate and the thick, outer ply of elastic fibres. Dorsally the fibres run in a more oblique direction. Here, only one stratum of elastic tissue can be distinguished which is attached to the inner perichondrium of the cricoid (fig.3).

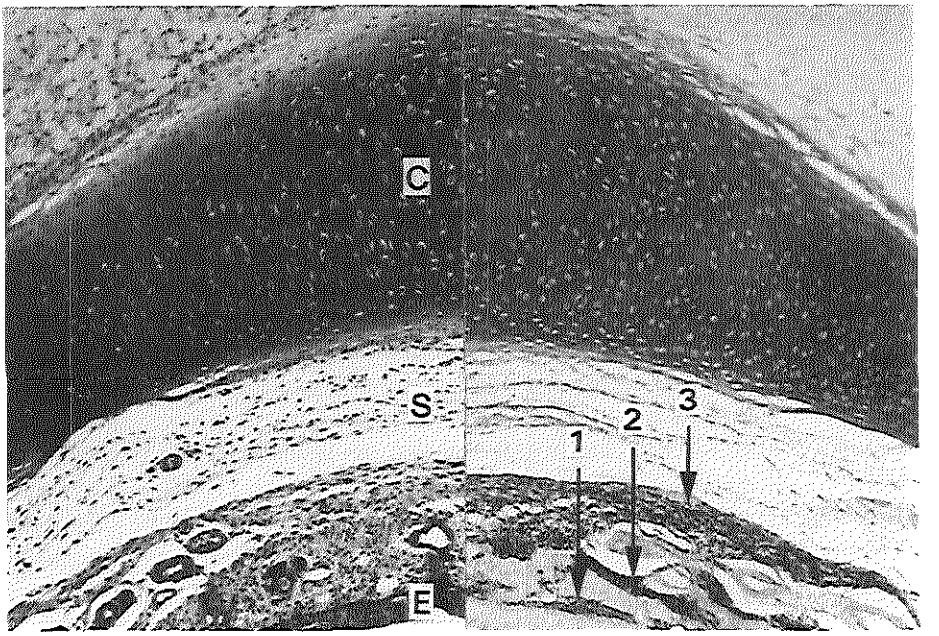

fig.1 *Midventral part of subglottis of 4-week-old rabbit (series I); left side, haematoxylin-azophloxin staining; right side, elastica-Van Gieson staining; cricoid cartilage (C) with small chondrocytes; prominent nuclei in centre of cells; subepithelial layer (S) with loose connective tissue, blood vessels, glands and three layers of elastic fibres (1,2,3) predominantly running in longitudinal direction (pointed cross-sections of fibres); respiratory epithelium (E). (magn. 40x)*

In sagittal sections (fig.4) the thick, outer layer is seen to be suspended to the thyroid. The fibres are running down into the subepithelial tissues to join the inner ply of elastic tissue at the level of the first tracheal ring. During their course thin lamellae, forming the intermediate layer as observed in the transverse sections, are going off to the inner stratum of elastic fibres. On the exterior side thin branches are running to the upper edge of the cricoid and the first tracheal ring. In the trachea the inner zone of elastic tissue is suspended to the tracheal cartilages by a system of elastic fibres which also is interconnecting the rings. On the dorsal side a single elastic layer is lying against the lamina of the cricoid and the tracheal rings.

Schematically the larynx and the trachea can be represented by an elastic tube (composed of various layers at the level of the larynx), suspended to an outer, segmented cartilaginous tube (fig.5a).

During the experimental period the cartilage of the cricoid shows progressive histologic maturation (1).

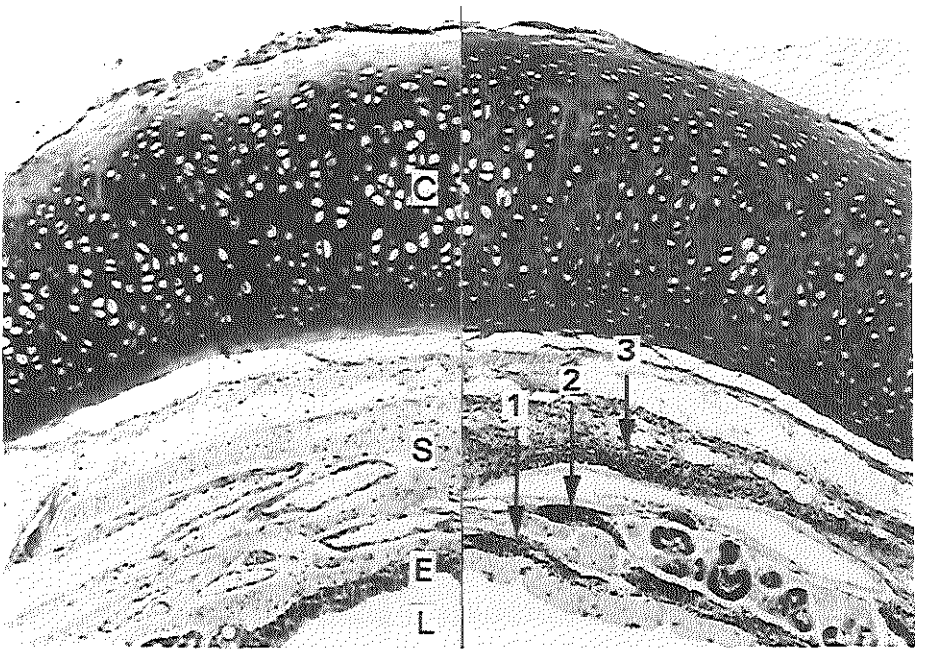

fig.2 Midventral part of subglottis of 24-week-old rabbit (series II); left side, haematoxylin-azophloxin staining; right side, elastica-Van Gieson staining; cricoid cartilage (C) with hypertrophied chondrocytes; flattened, excentrally located nuclei; subepithelial soft tissues (S) similar to 4-week-old stage with three layers of elastic fibres (1,2,3); respiratory epithelium (E); airway lumen (L). (magn. 40x)

Series III-V

In the adult stage the preserved subepithelial layer appears to be similar to that of the control series (fig.6). The longitudinal tube of elastic fibres is observed to be intact (fig.5b). The remaining cricoid cartilage shows a fully differentiated aspect. The cut ends demonstrate reactions to earlier traumatization. A few necrotic chondrocytes are still present, whereas in some animals a thin layer of new cartilage has been formed at the stumbs. In all cases the ends of the cricoid are covered with newly formed perichondrium.

Series VI-VII

In these series combined resection of the ventral part of the cricoid and the adjacent soft tissue lining results in a subglottic stenosis at the adult age. The flattened ventral side of the airway lumen is lined with a regenerated epithelium consisting of columnar

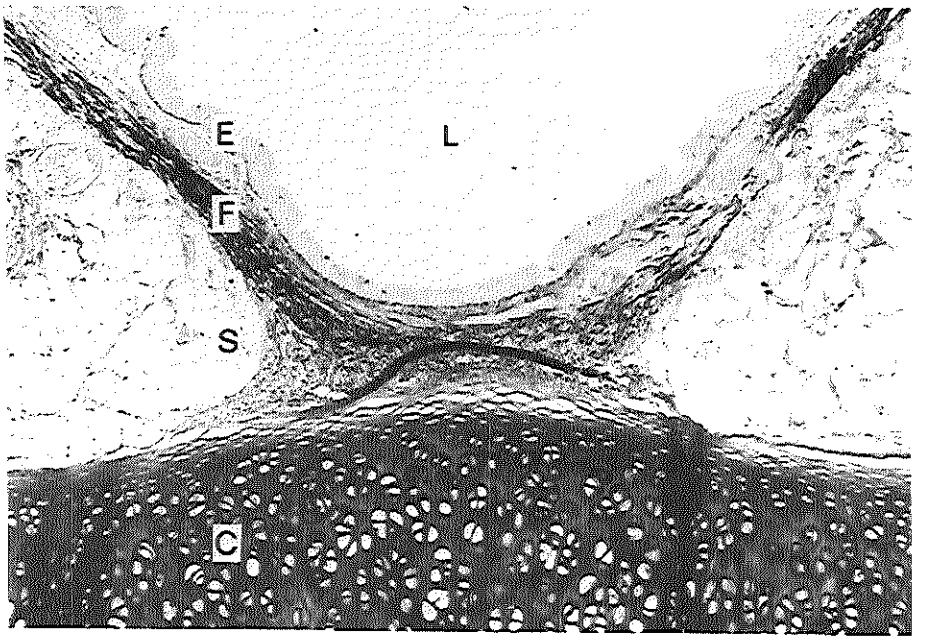

fig.3 Middorsal part of subglottis of 24-week-old rabbit (series II); elastic fibres (F) connected with inner perichondrium of cricoid (C); subepithelial layer (S); respiratory epithelium (E); airway lumen (L). (elastica-Van Gieson staining, magn.40x)

and cubic, ciliated cells and goblet cells (fig.7). The underlying subepithelial layer is composed of dense fibrous scar tissue between the stubs of the cricoid. The abundant collagen fibres are orientated in a predominantly transverse direction. Few blood vessels are present, whereas glands are totally missing. Only a few, inorderly arranged elastic fibres can be distinguished in the fibrous scar, demonstrating a discontinuity of the tube of elastic tissue in the regenerated area (fig.5c). On the undisturbed dorsal side the subepithelial layer including the elastic fibres is normal. The ventral scar tissue contains small areas of young, newly formed cartilage in half of the specimens of series VI and VII (fig.8). At some sites the ectopic cartilage resembles a hyaline, in other places a fibro-cartilaginous type. The histologic differentiation and the evolution of the cut ends of the cricoid is similar to those described for series III-V.

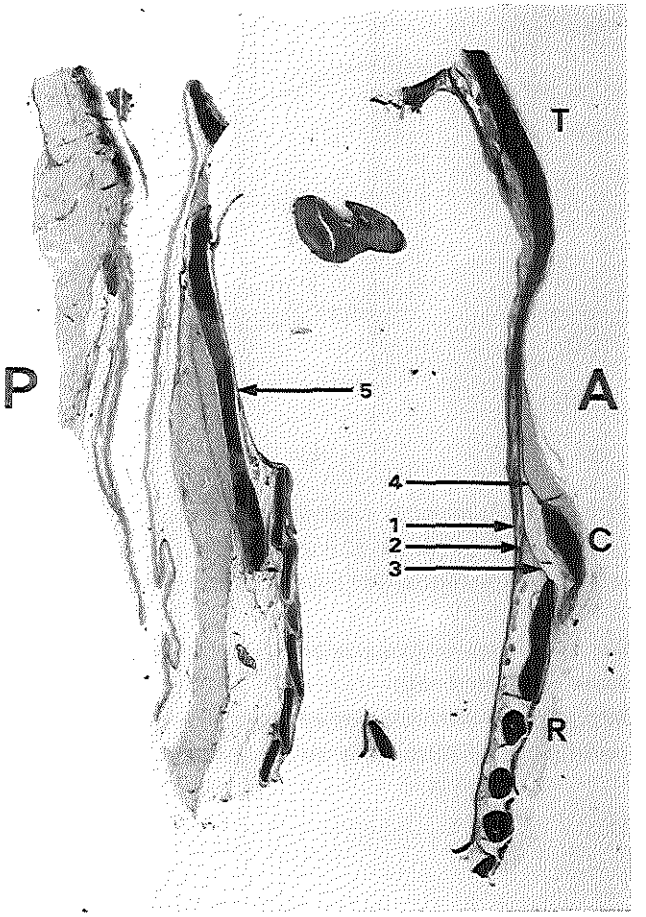

fig.4 *Paramedian sagittal section of the larynx and trachea of non-operated 24-week-old rabbit; A = anterior, P =posterior, T = thyroid, C = cricoid, R = tracheal rings; at level of cricoid arch three layers of elastic fibres: 1. inner layer immediately underneath epithelium; 2. intermediate layer of elastic fibres; 3. outer layer of fibres going to upper edge of first tracheal ring; lamella to cricoid arch (4); dorsally elastic layer lying against cricoid lamina (5). (elastica-Van Gieson staining, magn. 4x)*

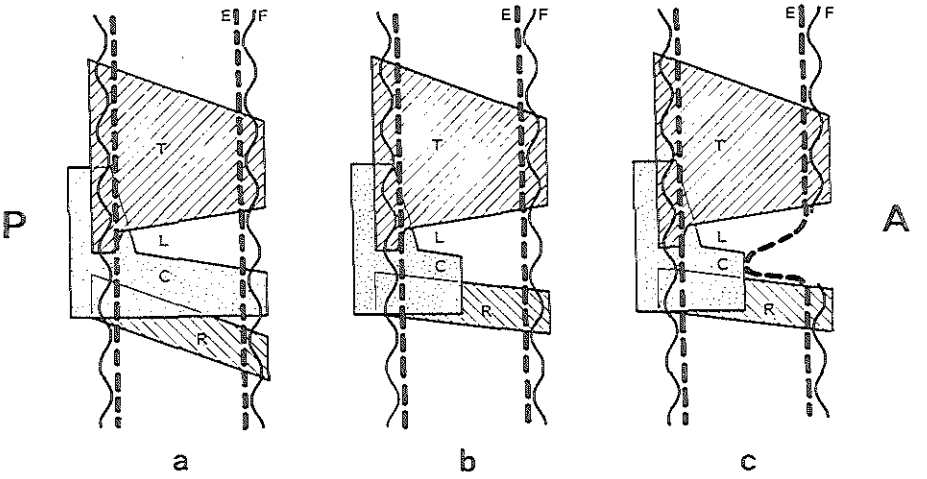

fig.5 *Sagittal scheme of the larynx; A = anterior, P = posterior, T = thyroid, C = cricoid, R = first tracheal ring, L = lumen, E = epithelium, F = elastic fibres:*

a. *adult stage in non-operated rabbit; longitudinal tube of elastic fibres in subepithelial layer;*
b. *adult stage in rabbit after resection of anterior part of cricoid; non-interrupted tube of elastic tissue on ventral side;*
c. *adult stage in rabbit after resection of anterior part of cricoid and adjacent soft tissues; interruption of elastic tube on ventral side.*

Discussion

The study of Van Gieson-stained histologic sections of the larynx demonstrated that the airway lumen in both 4- and 24-week-old rabbits (series I and II) is surrounded by epithelium supported by a subepithelial tube of elastic tissue. The latter is suspended to an outer, segmented cartilagenous framework, in particular to the inferior margin of the thyroid and the tracheal rings on the ventral and lateral sides and middorsally to the lamina of the cricoid. Only a thin bundle of elastic fibres is connecting the elastic tube with the cricoid arch. In this respect the system of elastic tissue in rabbits shows a striking similarity to the human conus elasticus (3).

Resection of an anterior part of the cricoid caused neither immediate collapse during surgery, nor irreversible damage to the subepithelial tissue including the lamina elastica (series IV and V). The subglottis appeared to develop a more or less normally shaped and sized lumen with an arch-like border on the ventral side (1). The elastic network is thought to maintain the normal shape of the subglottic lumen in absence of the cartilaginous skeleton on the ventral side. Obviously by the resection the conus elasticus is deprived of suspending fibres over too small an extent to cause a collapse of the airway. The effects of flexion or extension of the larynx upon the lumen in case of an interrupted cricoid ring need further investigation in the living animal.

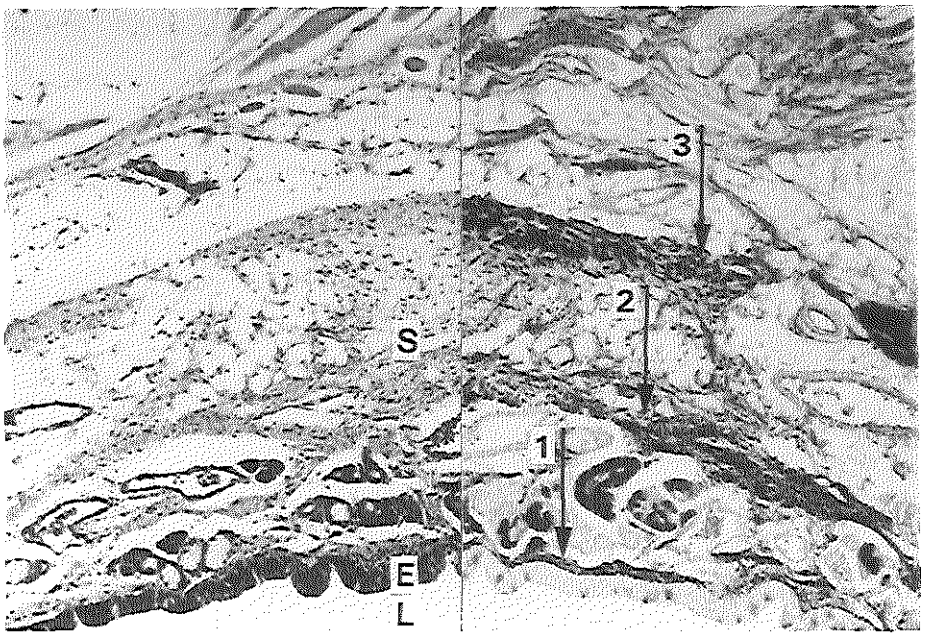

fig.6 Midventral part of subglottis of 24-week-old rabbit after resection of anterior 1/3 of cricoid ring (series IV); left side, haematoxylin-azophloxin staining; right side, elastica-Van Gieson staining; normal subepithelial structures (S) with glands and three layers of elastic fibres (1,2,3); normal respiratory epithelium (E); airway lumen (L). (magn. 40x)

Combined resection of a part of the cricoid with the underlying soft tissues (series VI and VII) resulted in a marked flattening of the ventral side with a pronounced airway narrowing (1). The subepithelial layer on the ventral side did not exhibit a "restitutio ad integrum" in the adult stage. It consisted of fibrous scar tissue between the cricoid stubs. Hence, formation of (retracting ?) scar tissue and loss of the continuity of the elastic tube are considered important factors in the flattening of the anterior wall resulting in a subglottic airway stenosis. In view of these findings interruption of the elastic tunica could also contribute to the development of a subglottic stenosis after endolaryngeal trauma (2).

Poor regeneration of elastic tissue in humans has been reported in healing of skin wounds (6). In this respect the elastic fibres in the wall of the airway in rabbits seem to behave in the same way. In literature on subglottic stenosis the elastic tunica got no attention till date. It should be recommended to focus on the conus elasticus in future studies of human specimens with subglottic stenosis.

In all operated animals (series III-VII) the cricoid was found to mature into normally differentiated cartilage. Only minimal post-traumatic changes could be distinguished at the cut ends. Resection of the subepithelial layer did not influence the

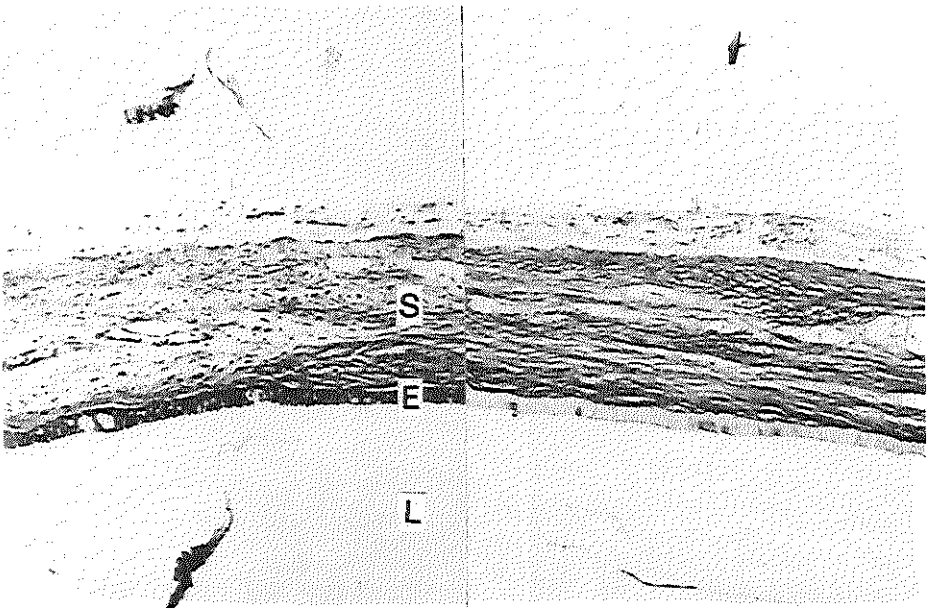

fig.7 Midventral part of subglottis of 24-week-old rabbit after resection of anterior 1/3 of cricoid ring and adjacent subepithelial tissues (series VI); left side, haematoxylin-azophloxin staining; right side, elastica-Van Gieson staining; subepithelial layer (S) consisting of fibrous scar tissue with many collagen fibres; glands and network of elastic fibres absent; regenerated respiratory epithelium (E); airway lumen (L). (magn. 40x)

histological reactions of the cricoid stubs. No histologic features were observed which could explain the slight thickening and outward deviation of the lateral parts of the cricoid after an anterior split and resection of the midventral part (1).

Newly formed cartilage within the regenerated subepithelial layer is probably the product of differentiation of granulation tissue. Ectopic cartilage formation was previously observed after endolaryngeal traumatization in growing rabbits (2).

In conclusion the results of this study demonstrated that:

1. in rabbits, as in humans, the subglottic airway is surrounded by a subepithelial mantle of elastic fibres (conus elasticus) suspended to an outer cartilaginous framework;
2. resection of the ventral half of the cricoid in growing rabbits causes no immediate or late collapse with stenosis of the airway, as this part of the cricoid is obviously of minor importance in the suspension of the elastic tunica;
3. the elastic tissue in the wall of the subglottic airway is not capable of regeneration;
4. the subglottic stenosis developing after resection of a part of the cricoid and the adjacent soft tissue lining is primarily due to an irreversible interruption of the elastic tunica and the formation of scar tissue.

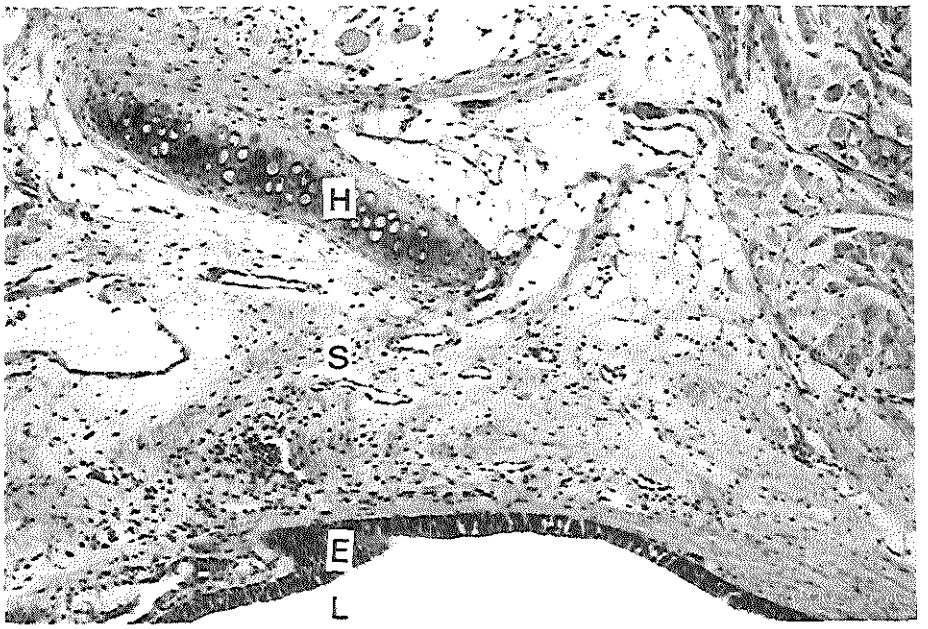

fig.8 Midventral part of subglottis of 24-week-old rabbit with regenerated subepithelial layer (S) (series VI); fibrous scar tissue containing newly formed hyaline cartilage (H); regenerated respiratory epithelium (E); airway lumen (L). (haematoxylin-azophloxin staining, magn. 40x)

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CHAPTER 6

DIFFERENTIAL EFFECTS OF ENDOLARYNGEAL TRAUMA UPON THE GROWTH OF THE SUBGLOTTIS

Abstract

In young rabbits (4-week-old) the growth of the subglottis was studied after an endolaryngeal soft tissue trauma. The injury induced the formation of a subglottic stenosis. In contrast to earlier observations on endolaryngeal trauma extending into the cricoid cartilage, the cricoid developed normally to the adult size and shape at 24 weeks. Similar to the earlier findings, the subepithelial layer showed considerable thickening as the result of formation of scar tissue, ectopic cartilage and fatty tissue with interruption of the elastic tunica (= conus elasticus). It was concluded that after endolaryngeal trauma in rabbits two types of subglottic stenosis can develop, determined by the depth of the injury.

Introduction

Histopathological studies of human post-mortem specimens with an endolaryngeal trauma revealed the lesions to vary in depth (5,6,7,8). In most cases only the soft tissue lining seemed to be affected. In a minority the injury included the cricoid cartilage as well. The effects of the latter type of trauma were previously studied in growing rabbits (1,2). Injuries involving both the soft tissue lining and the inner zone of the cricoid cartilage resulted in the formation of a subglottic stenosis due to a progressive deformation of the cricoid ring and a thickening of the subepithelial layer. The expansion of the cricoid in transverse direction appeared to be diminished in the ventral half. Consequently, the shape of the cricoid changed from "egg"- to "pear"-like. An interesting feature of the subepithelial thickening was the formation of ectopic cartilage. In the present experimental study the effects of the most frequent type of endolaryngeal trauma, confined to the epithelium and the subepithelial structures, were investigated.

Histologic observations in rabbits suggested the larynx to be composed of two concentric tubes (3,4). The inner one is formed by a network of elastic fibres, supporting the epithelium and suspended to the outer tube, formed by the laryngotracheal cartilages. Resection of a ventral part of the cricoid ring at a young age was found to induce no airway stenosis during further growth, as long as the subepithelial layer was preserved. The observations led to the conclusion that the inner tube (= conus elasticus) is an important structure in keeping up a normal airway lumen. Therefore, in this study special attention was paid to the fate of the conus elasticus after endolaryngeal traumatization.

Materials and methods

Female New Zealand white rabbits were used. The morphologic and histologic data of the control specimens of series I (13 non-operated rabbits, 4 weeks of age) and series II (28 non-operated adult rabbits, 24 weeks of age) were reported earlier (1,2). In series III an endolaryngeal trauma was performed at the age of 4 weeks (10 rabbits). The injury was strictly limited to the inner soft tissue lining of the airway. Care has been taken to leave the cricoid cartilage and its inner perichondrium undamaged.

After sacrificing the animals at the adult age, the subglottic part of the airway was processed histologically. 5 μm Transversal sections were stained with haematoxylin-azophloxin and elastica-Van Gieson. For details on the operative techniques and the methods of selecting and measuring sections of each specimen is referred to the above mentioned papers. The following measurements were carried out (fig.1):

1. lumen surface area of the subglottic airway (mm^2);
2. inner diameters of the cricoid ring (mm): median A_2P_2, transversal B_2C_2 and D_2E_2;
3. thickness of the cricoid cartilage (mm):

$$A_1A_2,\ P_1P_2,\quad \frac{(B_1B_2+C_1C_2)}{2},\ \frac{(D_1D_2+E_1E_2)}{2};$$

4. thickness of the subepithelial layer (mm):

$$A_2A_3,\ P_2P_3\quad \frac{(B_2B_3+C_2C_3)}{2},\ \frac{(D_2D_3+E_2E_3)}{2}$$

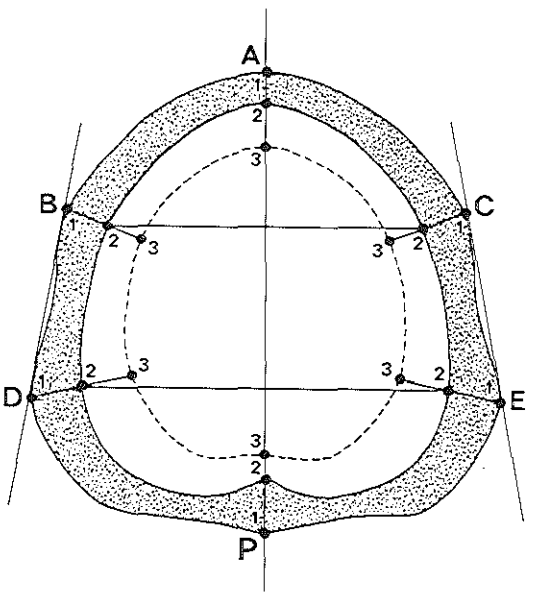

fig.1 Schematic transversal section of cricoid ring and epithelilial lining (A-P = antero-posterior direction).

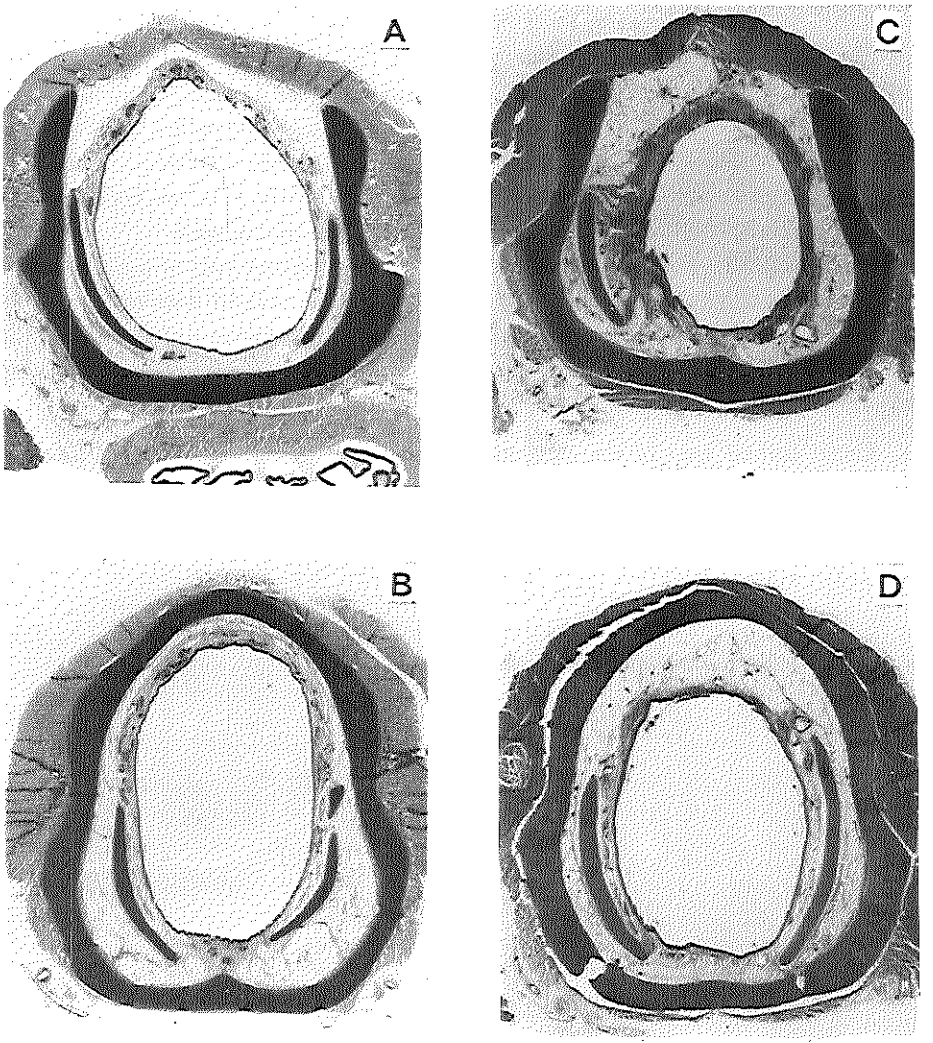

fig.2 *Transversal sections of subglottis of specimens of series II (a,b) and series III (c,d):*

- *a. normal shape and thickness of cricoid, cranial to level of arch; normal width of subepithelial layer; oval subglottic airway lumen;*
- *b. normal "egg"-like shape of cricoid ring with normal thickness; normal width of subepithelial layer; oval airway lumen;*
- *c. normal shape and thickness of cricoid, at the same level as a; stenosis of subglottic airway lumen; thickened subepithelial layer all round;*
- *d. normal shape and thickness of cricoid ring at the same level as b; narrowing of subglottic airway in antero-posterior direction; thickened subepithelial layer in ventral half. (haematoxylin-azophloxin, magn. 10x)*

Results

Morphology

Transversal sections of the subglottic area from series II and III are shown in figure 2. The morphometric observations are presented in tables 1 - 4.

Table 1: Subglottic lumen area (mm^2)

SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG. TRAUMA (n)	P I/II	P I/III	P II/III
8.6 ± 1.4 (13)	21.1 ± 2.9 (28)	14.4 ±4.2 (10)	0	< 0.01	< 0.01

significance $p \leq 0.05$
n = number of measurements

Table 2: Inner diameters (mm)

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG. TRAUMA (n)	P I/II	P I/III	P II/III
A_2P_2	4.43 ± 0.21 (10)	7.24 ± 0.46 (25)	6.87 ± 0.59 (9)	0	0	0.11
B_2C_2	3.54 ± 0.39 (13)	5.19 ± 0.43 (27)	5.17 ± 0.80 (10)	0	< 0.01	0.94
D_2E_2	4.02 ± 0.30 (13)	6.07 ± 0.39 (26)	6.35 ± 0.61 (10)	0	0	0.20

significance $p \leq 0.05$
n = number of measurements

Table 3: Thickness of cricoid cartilage (mm)

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG. TRAUMA (n)	P I/II	P I/III	P II/III
A_1A_2	0.55 ± 0.06 (10)	0.58 ± 0.09 (24)	0.64 ± 0.10 (10)	0.28	0.03	0.12
$\frac{B_1B_2 + C_1C_2}{2}$	0.76 ± 0.08 (13)	0.75 ± 0.08 (27)	0.80 ± 0.08 (10)	0.71	0.25	0.11
$\frac{D_1D_2 + E_1E_2}{2}$	0.75 ± 0.08 (13)	0.87 ± 0.12 (26)	0.85 ± 0.08 (10)	< 0.01	< 0.01	0.58
P_1P_2	0.69 ± 0.15 (13)	0.71 ± 0.13 (24)	0.70 ± 0.12 (10)	0.69	0.86	0.83

significance $p \leq 0.05$
n = number of measurements

Table 4: Thickness of subepithelial layer (mm)

	SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES III 24 WEEKS ENDOLARYNG. TRAUMA (n)	P I/II	P I/III	P II/III
A_3A_3	0.52 ± 0.11 (10)	0.66 ± 0.25 (25)	1.22 ± 0.32 (9)	0.03	< 0.01	< 0.01
$\frac{B_3B_3 + C_3C_3}{2}$	0.46 ± 0.11 (13)	0.71 ± 0.14 (27)	1.02 ± 0.25 (10)	< 0.01	< 0.01	< 0.01
$\frac{D_3D_3 + E_3E_3}{2}$	0.65 ± 0.10 (13)	1.09 ± 0.27 (26)	1.39 ± 0.43 (10)	0	< 0.01	0.06
P_3P_3	0.31 ± 0.13 (13)	0.50 ± 0.17 (24)	0.68 ± 0.21 (10)	< 0.01	< 0.01	0.03

significance $p \leq 0.05$
n = number of measurements

The mean surface area of the subglottic lumen in series III reaches ± 14,4 mm^2 in the transversal plane. In 6 out of 10 specimens the lumen area is smaller than 21,1 mm^2 (adult controls) minus twice the standard deviation.

The thickness of the cricoid cartilage is similar to that in the control specimens. It has a normal "egg"-like shape in all cases (fig.3). Accordingly, the inner diameters in both sagittal and transversal direction do not differ from those in the adult control animals.

In reaction to the trauma the mean thickness of the subepithelial layer has augmented considerably. Only one animal shows normal dimensions with regard to these measurements.

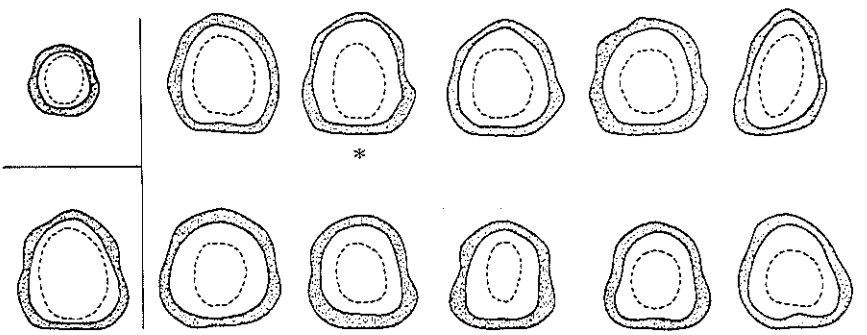

fig.3 Schematic drawings of cricoid ring of specimens of series III (= specimen shown in fig.4); upper left, control specimen of 4 weeks; lower left, control specimen of 24 weeks.*

Histology

The subglottic airway is lined with a regenerated respiratory epithelium consisting of cilindric, ciliated cells and goblet cells in all specimens. At some sites the epithelium does not have the normal differentiated histology, but includes cubic, ciliated cells and few goblet cells. Epithelial glandular structures are missing.

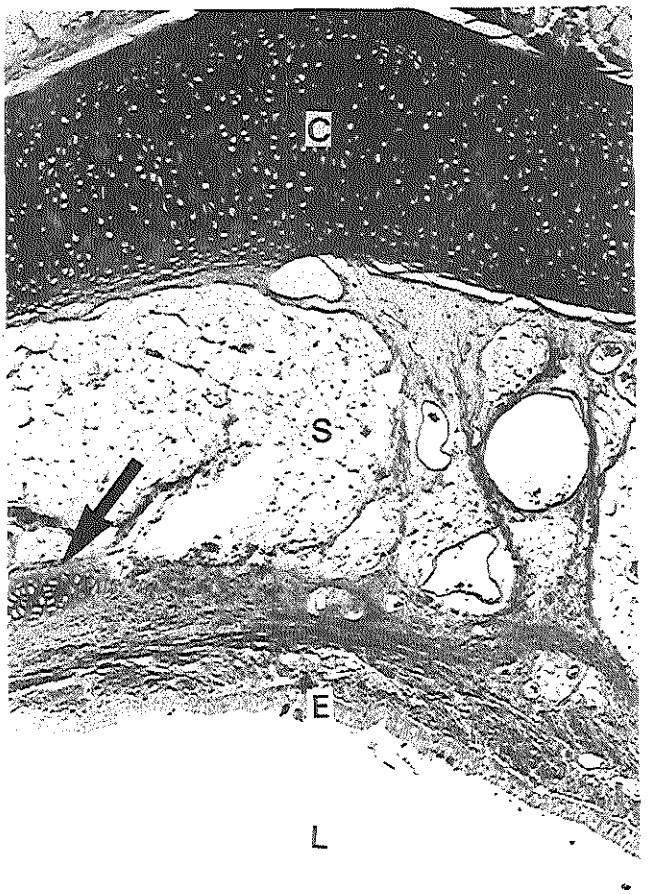

fig.4 Midventral part of subglottis in 24-week-old rabbit with endolaryngeal trauma at the age of 4 weeks (series III); normal cricoid cartilage (C) and perichondrium; thickened subepithelial layer (S) consisting of fibrous scar tissue without glands and elastic fibres; ectopic cartilage (arrow) in subepithelial tissue; normal respiratory epithelium (E); airway lumen (L). (elastica-Van Gieson, magn. 40x)

In all animals the regenerated subepithelial layer encloses fibrous scar tissue, neighbouring the epithelium, and fatty tissue, mainly situated between the scar tissue and the inner cricoid border (fig.4). Few blood vessels are present. The collagen fibres are orientated in a predominantly circular direction. The tunica elastica has not been regenerated. Only sporadic, inorderly arranged elastic fibres can be distinguished. In all specimens young cartilage is distributed randomly in the circular scar tissue (fig.4,5). This ectopic cartilage, separate from the cricoid margin, resembles either a hyaline or a fibro-cartilaginous type. In the former a perichondrial lining can be observed, whereas in

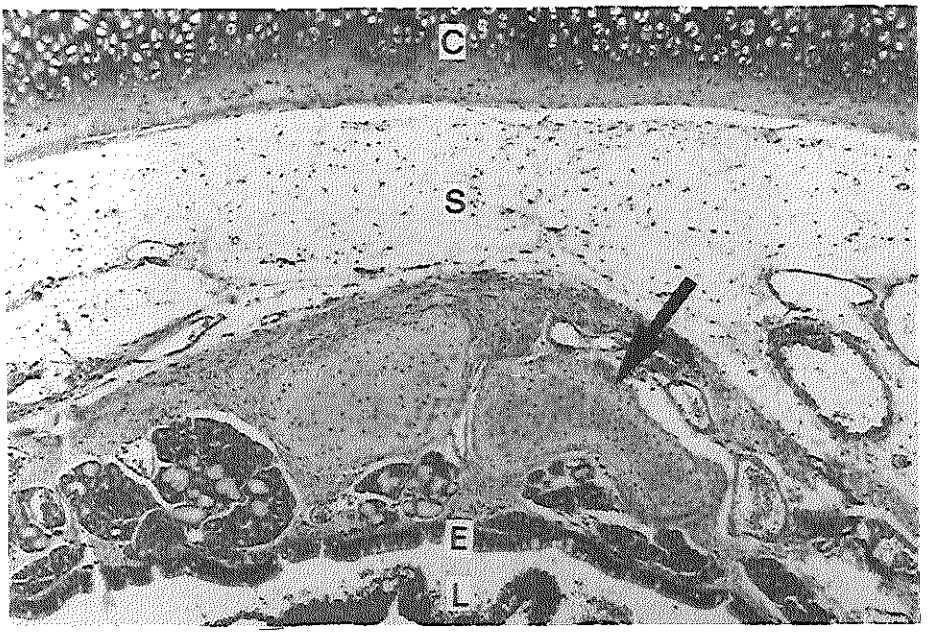

fig.5 Midventral part of subglottis in 24-week-old rabbit with endolaryngeal trauma at the age of 4 weeks (series III); cricoid cartilage (C) with normal perichondrium; newly formed cartilage (arrow) in "normal" subepithelial layer (S) (caudal to circular scar tissue); respiratory epithelium (E); airway lumen (L). (haematoxylin-azophloxin, magn.40x)

the latter the fibrous matrix, merging into the surrounding cicatricial tissue, is discerned. The newly formed cartilage is located in the wound area, continuing into the adjacent cranial and caudal zones of the subepithelial layer which holds normal structures such as glands and elastic fibers (fig.5).

The cartilage and the inner perichondrial layer display a normal adult histologic aspect in all but one animal. In this case the perichondrial lining is absent over a small length of the dorsolateral inner side of the cricoid. Here, an irregular contour is present, similar to that observed after endolaryngeal traumatization within the cricoid cartilage (2), indicating accidental injury to the cricoid during surgery.

Discussion

Different types of endolaryngeal trauma in young rabbits showed a striking discrepancy in growth of the cricoid. A lesion restricted to the soft tissues, described in this paper, did not affect cricoid development. Despite the formation of a circular scar around the airway lumen (soft tissue stenosis) the cricoid ring obtained a normal shape

and size. A deeper traumatization of the subglottis, extending into the cricoid cartilage caused a progressive cartilaginous deformation (1). The cricoid exhibited a diminished transversal expansion resulting in a "pear"-like shape, and reactive local thickening leading up to an irregular contour of the inner surface. Preservation of the inner perichondrium seems to be an important factor in determining normal or abnormal growth of the cricoid.

A common feature on both types of endolaryngeal traumatization is thickening of the subepithelial layer. A zone of scar tissue consisting of circularly orientated collagen fibres was lying immediately underneath the epithelium. The layer of fatty tissue between the cicatricial fibres and the inner contour of the cricoid was varying in width. In the present experiment, the expansion of this layer took place, in particular, on the ventral side. As no signs of conus elasticus fibres were found, this elastic tube supporting the laryngeal epithelium, is irreversibly lost at the level of the stenosis. A similar incapacity of the elastic tissue to regenerate was earlier reported after external laryngeal trauma (4) and in wound healing of the skin (9).

Ectopic cartilage formation was another common feature during regeneration of the subepithelial layer. The preservation of the inner perichondrium, observed in the present experiment, suggests that the newly formed cartilage did not originate from the cricoid or its perichondrium. Clearly, the subepithelial granulation tissue, formed after the trauma, can differentiate into cartilage. On the other hand, a contribution to the ectopic cartilage by an injured cricoid can not be excluded, considering the direct contact - without intervening perichondrium - of ectopic and cricoid cartilage as observed in the previous experiment. The cartilage found within normal tissue immediately cranial or caudal to the stenosis, can point to a transient inflammatory reaction with formation of granulation tissue differentiating into cartilage. The experimental results suggest that this ectopic cartilage should not be considered as a hamartoma, but as a product of trauma. Probably, investigations of human larynges with a stenosis can lend support to this theory.

It is concluded that in rabbits the depth of the endolaryngeal trauma determines the type of the later arising subglottic stenosis:

1. trauma limited to the inner soft tissue lining, does not impair the development of the cricoid ring, but induces the formation of a stenosis only due to thickening of the subepithelial layer with a deposit of ectopic cartilage;
2. traumatization of soft tissues and cricoid cartilage results in a stenosis due to a progressive deformation and thickening of the cricoid ring in combination with a thickening of the subepithelial layer.

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CHAPTER 7

POROUS HYDROXYLAPATITE-PERICHONDRIUM GRAFT IN CRICOID RECONSTRUCTION.

Abstract

Composite grafts of hydroxylapatite-perichondrium were used for reconstructing defects in the cricoid of young rabbits. The alloplastic material showed no signs of resorption; it was firmly attached to the surrounding tissues by ingrowing connective tissue; only a moderate foreign body reaction was observed. The perichondrium formed new cartilage around the hydroxylapatite.

Introduction

A surgical treatment for some cases of subglottic stenosis in both adults and children is the anterior split of the cricoid ring with interposition of a graft. Rib cartilage is frequently used for this purpose (1). Also cartilaginous implants from the nasal septum (2), thyroid ala (3), auricle (4) and epiglottis (5) have been reported.

Problems with these grafts are frequent (partial) resorption and inconveniences from the healing of the donorsite. Therefore, the use of alloplastic materials must be considered (6).

Some 20 years ago, various alloplastic materials were tested in reconstructing tracheal rings in experimental animals (7). None of these materials obtained a place in clinical practice. Later porous alloplastic materials were developed. Proplast and Plastipore were grafted to replace resected parts of the larynx and trachea in animal experiments (8,9,10,11). These grafts only "survived" when the soft tissue layers ,lining the airway lumen, were intact. Otherwise infection and ultimate extrusion were observed. Probably as a result of the small diameter of the pores the Plastipore appeared to be inadequately attached to the surrounding tissues (10), whereas Proplast was reported to have the disadvantage of being soft and fragile (9).

A recently developed alloplastic porous material is hydroxylapatite. It is a stable and inert ceramic material with good biocompatibility (12,13). The structure is nearly identical to the mineral matrix of bone. For several years now the material, in contact with bony structures, has been used succesfully in middle ear surgery (14 15,16). No observations have been reported about the behaviour of hydroxylapatite versus cartilage and perichondrium.

In this study hydroxylapatite was applied for reconstruction of the cricoid ring after resection of the anterior 1/3. The combination of this bone-like material and cartilage

was assumed to resemble the normal anatomic connection between bone and catilage as present in the rib and the epiphysis of long bones. The material has a macroporosity (pore size ca. 100 um) of 26% and a microporosity (pore size ca. 3 um) of less than 5%; the pores are interconnected. In surgery of the subglottic region, defects of the epithelium are likely to occur. The above mentioned experiments demonstrate the risks of these defects for chronic infection and extrusion of the implants. Therefore, in our study the hydroxylapatite graft was wrapped in an envelope of perichondrium.

Materials and methods

Two series of female rabbits (New Zealand white) were studied:

Series I : 28 non operated, adult rabbits, 24 weeks old (control animals)
Series II : 14 rabbits (24 weeks old) with a reconstruction of the cricoid by a composite porous hydroxylapatite-perichomdrium graft.

The rabbits of series II were operated upon at the age of 4 weeks, weighing 400 - 500 grams; anaesthesia was achieved by intramuscular injection of xylazin (Rompun ®) 0,1 ml/100 mg and ketaminchlorid (Ketaset ®) 0,1 ml/100 mg. The ventral part of the larynx was reached by a median incision through the skin and overlying soft tissues. The anterior 1/3 of the circular structure of the cricoid was resected, whereas the soft tissue lining of the subglottic airway was preserved. The resected part was replaced by a composite graft of porous hydroxylapatite-perichondrium, trimmed to the same size and shape. The perichondrium was taken from the inner side of the auricle. The graft was sutured to the remaining cricoid with Prolene 6-0. Postoperatively, an intramuscular injection of Penidural was given. No signs of respiratory and wound infection or dyspnoe were observed. Three animals died of haemorrhagic enteritis between 2 and 4 weeks after surgery. The other animals were sacrificed at the adult age of 24 weeks. For histologic study the subglottic part of the airway was excised and decalcified. In this process hydroxylapatite was dissolved; as a result only the outlines of the graft can be seen in histologic sections. 5 μm Transversal sections were stained with haematoxylin-azophloxin and elastica-Van Gieson.

Results

Series I: 24-week-old non operated rabbits (fig.1)

The cricoid cartilage is fully differentiated and consists of a basophilic intercellular substance and hypertrophied chondrocytes with a flattened and excentrally positioned nucleus. Mitotic activity is not present. The subglottic lumen is lined with columnar pseudostratified epithelium containing goblet cells. In the subepithelial layer loose

connective tissue with blood vessels, serous and mucous glands and fatty tissue was noted. A network of elastic fibers occurs, predominantly running in longitudinal direction.

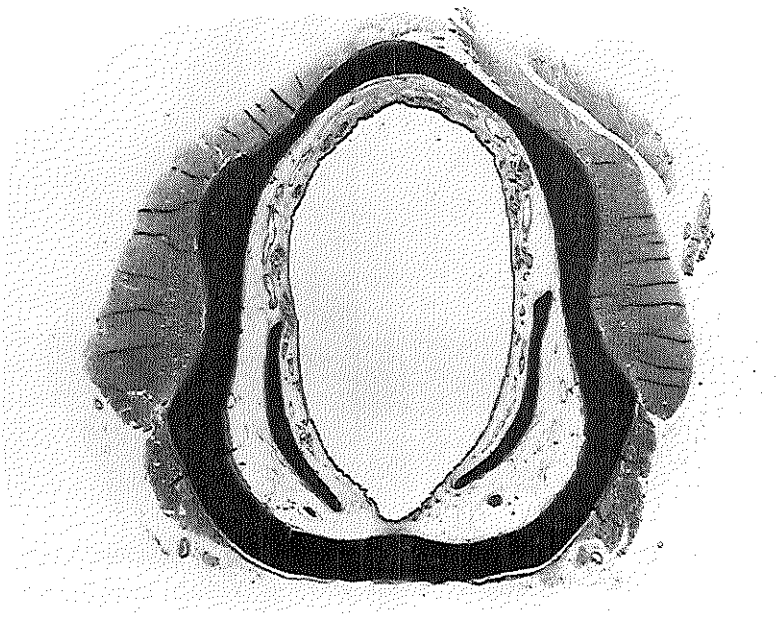

fig.1 Transversal section of normal subglottis in 24- week-old rabbit (series I); largest diameter of cricoid ring and subglottic lumen in sagittal direction; dorsolateral in subepithelial layer upper part of first tracheal ring. (haematoxylin-azophloxin, magn. 10x)

Series II: 24-week-old rabbits after resection of the ventral part of the cricoid and replacement by a composite graft of porous hydroxylapatite-perichondrium (fig.2)

No gross signs of infection and resorption of the graft were observed. In all cases a layer of fibrous tissue surrounded the hydroxylapatite implant. In five specimens the implant had direct contact with the viable cartilage of the cut ends of the cricoid. In the other 6 cases a thin layer of fibrous tissue was present between hydroxylapatite and the stubs of the cricoid (fig.3). In three specimens the graft had lost any contact with the in situ cartilage of the cricoid on one side. In ten adult specimens young cartilage was found within the fibrous capsule at various sites (fig. 4).
Most of the newly formed cartilage was of the hyaline type and sometimes continuous with the pre-existing cricoid. Pores in hydroxylapatite contained both dense fibrous tissue, rich of collagen, as well as loose connective tissue containing blood and lymphatic

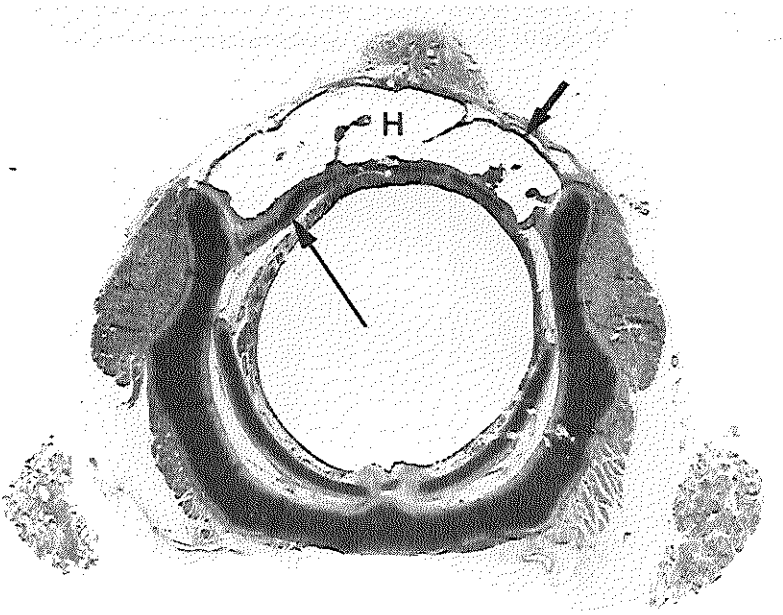

fig.2 Transversal section of reconstructed cricoid ring in 24-weeks-old animal (series II); hydroxylapatite graft (H) replaces ventral part of cricoid cartilage; macropores filled with ingrowing tissue; newly formed cartilage (thin arrow); layer of fibrous tissue around hydroxylapatite (thick arrow). (haematoxylin-azophloxin, magn. 10x)

vessels and fatty tissue; ingrowth of nerves was not observed. Some pores were partly filled with exudative fluid. Newly formed cartilage was even present in a pore in direct contact with hydroxylapatite in 3 animals (fig.5). Macrophages and multinuclear foreign body giant cells with multiple vacuoles were found at the interface between hydroxylapatite and the surrounding tissues (fig.6).

The cricoid cartilage showed the same fully differentiated appearance as the 24-week-old control specimens (series I). No signs of regression were observed. The subglottic airway was lined with normal respiratory epithelium; the subepithelial loose connective tissue contained normal glandular structures, blood vessels, fatty tissue and elastic fibers.

It may be added that in the specimens of 2 animals which died 4 weeks after operation already formation of new cartilage could be noticed within the fibrous layer around the hydroxylapatite implant, contrary to the findings 2 weeks after surgery.

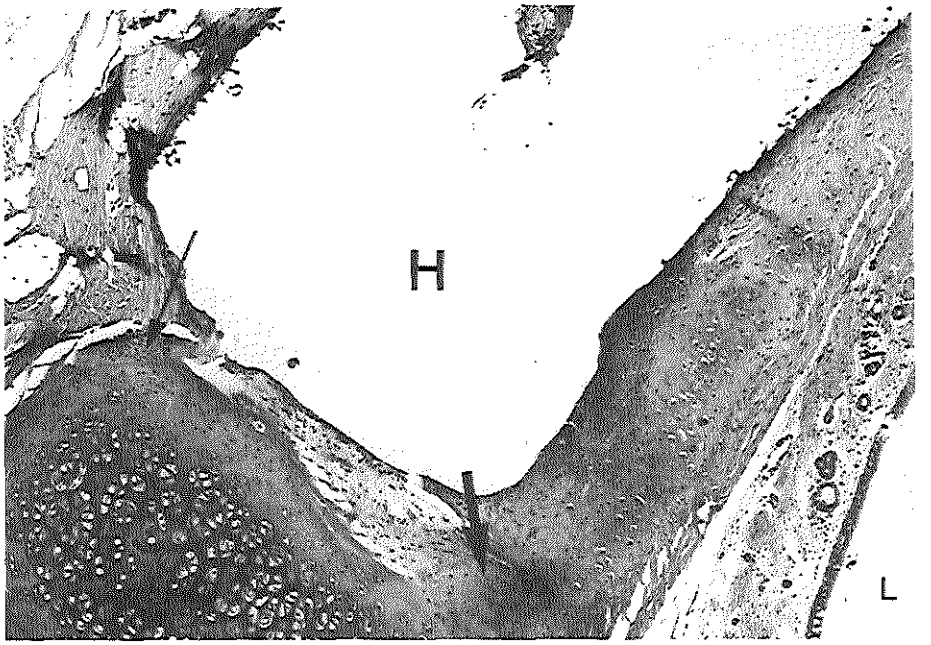

fig.3 Hydroxylapatite (H) in contact with cut end of cricoid cartilage (series II); fibrous capsule attached to perichondrium (thin arrow);newly formed cartilage connected with alloplastic graft and cricoid cartilage; airway lumen (L). (haematoxylin-azophloxin, magn. 40x)

Discussion

This study has demonstrated in growing rabbits that the use of a composite hydroxylapatite-perichondrium graft for the reconstruction of the cricoid was not complicated by extrusion and resorption of the graft or infection in the adjacent tissues. Previously exposure of hydroxylapatite to the airway lumen was shown to result in infection (17). Although defects of the epithelium during surgery could be expected in the present experiment, apparently the perichondrium and the preserved soft tissues gave sufficient protection to infection.

In the adult stage the perichondrial envelop could not be identified. In 12 animals the new cartilage indicated the presence of cells with chondrogenic potential, probably derived from the perichondrium. The chondroneogenesis occurred at various sites around the implant. On other sites a thin fibrous layer covered the implant, suggesting a loss of chondrogenic activity due to dedifferentiation or necrosis of the transplanted perichondrium. This fibrous layer could be derived from the perichondrium or from the in situ connective tissue. Newly formed cartilage was already present 4 weeks after surgery indicating chondroneogenesis to occur shortly after operation. In this process hydroxylapatite appeared to determine the shape of the new cartilage, as it was adapted

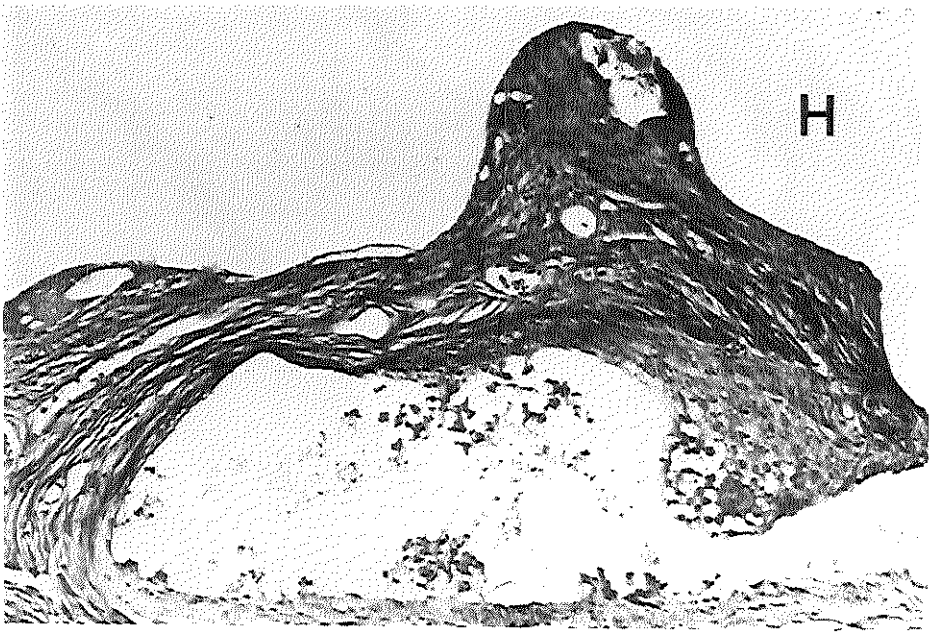

fig.4 Pores in hydroxylapatite (H) with dense fibrous tissue; collagen fibers anchor hydroxylapatite to surrounding tissues. (haematoxylin-azophloxin, magn. 100x)

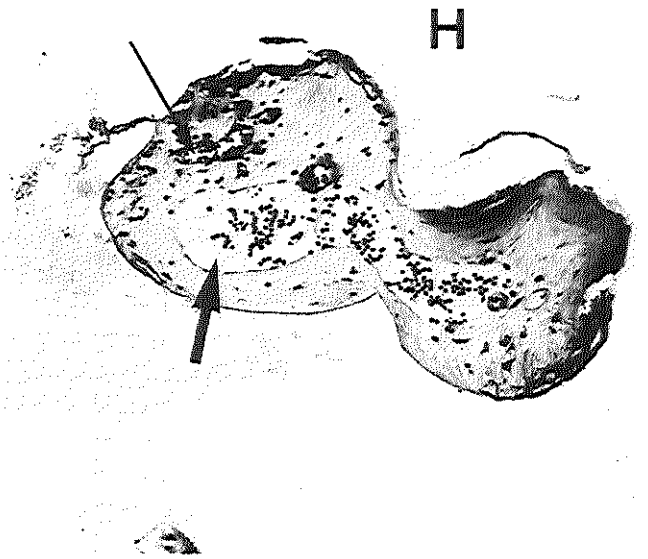

fig.5 Pore in hydroxylapatite (H) with loose connective tissue, blood vessels (thin arrow) and lymphatic vessel (thick arrow). (haematoxylin-azophloxin, magn. 100x)

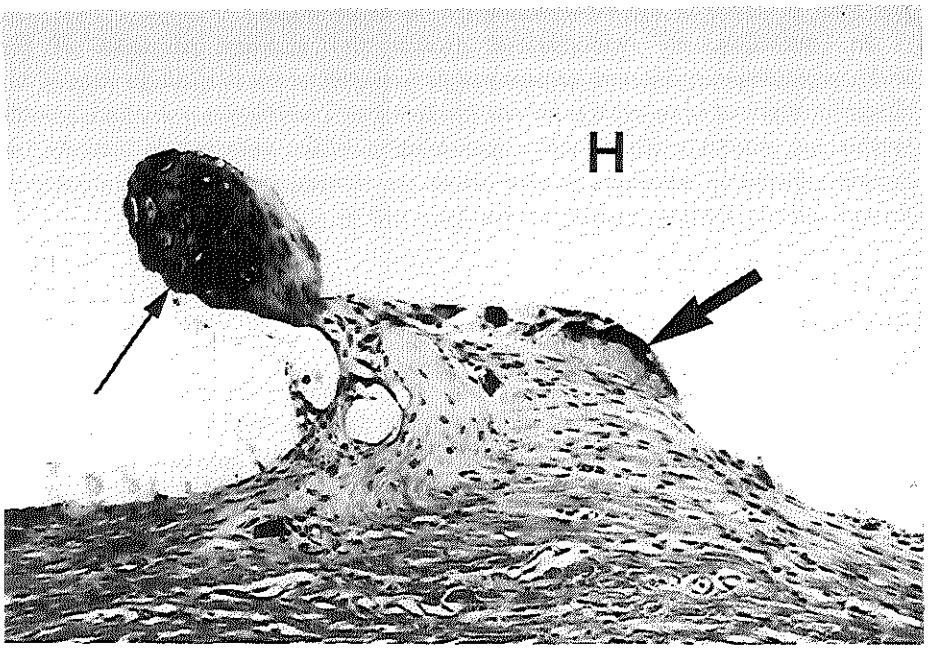

fig.6 Pore in hydroxylapatite (H) with dense fibrous tissue and newly formed cartilage (thin arrow); foreign body giant cell (thick arrow). (haematoxylin-azophloxin, magn. 100x)

to the curvature of the implant.

The results demonstrated a firm attachment of the alloplastic graft to cartilage by fibrous tissue and newly formed cartilage. Ingrowth of fibrous tissue into the pores assured a tight anchorage of the graft to the surrounding tissues. The exudative fluid present in some pores should be regarded as a remnant of the wound reaction after surgery; this fluid can be expected to give way to ingrowing fibrous tissue (18). Ingrowing tissues into the hydroxylapatite implant do not amount to 30% being the porosity of the material used; hence, a part of the pores is not yet filled with oinvading cells 20 weeks after implantation. The lost contact between the graft and one end of the cricoid in 3 animals was probably caused by technical inexperience, as it concerned the first 3 animals operated in this series.

In cases of direct contact between the implant and the cricoid, hydroxylapatite did not cause necrosis or reactive changes of cartilage. Also, no influences were observed on the physiological differentiation of the cricoid cartilage from the moment of operation up to the adult stage. However, a moderate foreign body reaction as indicated by the presence of macrophages and multinuclear giant cells appeared to be induced at the interface between hydroxylapatite and fibrous tissue. These cells indicate a slowly progressive biodegradation of hydroxylapatite (19). Actual resorption of the material has

not been observed in the experimental period of 20 weeks.

In conclusion, the demonstrated stability and infection resistance of the hydroxylapatite-perichondrium implant and its firm attachment to cartilage suggest this alloplastic material to be suitable for reconstruction of a cartilaginous structure like the cricoid ring, at least in adults! Before application to infants and children, however, the question should be answered to what extent the reconstructed cricoid is capable of further growth. Further analysis of this issue will be dealt with later.

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CHAPTER 8

GROWTH OF THE SUBGLOTTIS AFTER RECONSTRUCTION OF THE CRICOID WITH AUTOGENOUS AND ALLOPLASTIC GRAFTS

Abstract

In young rabbits the growth of the subglottis was studied after replacement of the anterior third of the cricoid ring by grafts of cartilage-perichondrium or hydroxylapatite-perichondrium. Half of the cartilaginous implants showed regressive changes. The alloplastic grafts were well accepted and firmly connected to the cricoid stumps without signs of resorption. The growth pattern of the reconstructed rings demonstrated specific differences, related to the type of graft. Irrespective of the type of implant, reconstruction induced the formation of various degrees of subglottic stenosis during growth up to the adult stage, whereas resection of the cricoid arch without subsequent repair, did not affect the development of the subglottic airway lumen.

Introduction

Autogenous cartilage transplants are usually applied to repair destructed parts of the cricoid (7). The surgical treatment does not always provide an adequate airway. Two factors might contribute to a poor outcome.

First, cartilage grafts show resorption or extrusion in a number of cases. Animal experiments confirmed the unpredictable behaviour of the grafts, ranging from complete viability to total resorption (11,12,13,16,18,26,27,30). Alloplastic implants have not been used for clinical reconstruction to date.

A second factor could be abnormal growth of the reconstructed larynx (20,22,25,29). No detailed information is available about the laryngeal growth after reconstruction. Most studies in patients are based on clinical inspection without quantitative data (6,8,9,10,19,23). The results of animal experiments are not conclusive considering the small number of specimens used and disputable morphometric methods (14,15,21,26).

In previous experiments in young rabbits it was demonstrated that resection of the ventral third of the cricoid ring induces specific changes in the growth pattern of the remaining two-third (1,2). The lateral parts showed an outward deviation resulting in a "U"-like shape. As long as the subepithelial tissues were preserved, no airway stenosis occurred, probably owing to the tension in the conus elasticus.

In this study the cricoid ring was reconstructed after resection of the anterior third by immediate replacement of the resected arch or by implantation of a porous hydroxylapatite "arch" in an envelope of perichondrium. The removed part of the cricoid

was used as an non-pediculed, isotopic cartilage-perichondrium graft of perfect size and shape. Hydroxylapatite is a calciumphosphate ceramic. Preceeding histologic studies already revealed this porous alloplastic material to have an excellent biocompatibility and to develop a firm attachment to cartilage and perichondrium (5,28).

The following items were investigated:

1. the behaviour of autogenous cartilaginous and hydroxylapatite-perichondrium implants;
2. the growth of the cricoid ring after reconstruction with either type of graft.

Materials and methods

Four series of female New Zealand white rabbits were included in this study. Details of the morphologic and histologic findings of the control animals of series I (13 non-operated rabbits, 4 weeks of age) and series II (28 non-operated, adult rabbits, 24 weeks of age) were reported earlier (3,4). Series III consisted of 10 adult rabbits in which the cricoid was reconstructed at the age of 4 weeks with a cartilage-perichondrium graft. Series IV enclosed 11 adult rabbits in which the cricoid was reconstructed at the age of 4 weeks with a hydroxylapatite-perichondrium graft.

In the operated animals anaesthesia was achieved by intramuscular injection of xylazin (Rompun ®) 0,1ml/100mg and ketaminchlorid (Ketaset ®) 0,1ml/100mg. After exposure of the larynx (3) the ventral third of the cricoid ring including the perichondrial lining was resected. The underlying subepithelial layer and epithelium were preserved. In series III the resected segment was reimplanted and fixed with two Prolene 6-0 sutures on either side. In series IV the circularity of the cricoid ring was restored with an arch-like implant of hydroxylapatite, trimmed to the same size and shape as the resected part. The ceramic material was wrapped in an envelope of perichondrium from the auricle and suture-tagged with a single Prolene 6-0 suture. Postoperatively the animals received an intramuscular injection of Penidural ®. No signs of dyspnoea and infection of the respiratory tract or the wound were observed. The animals were sacrificed at the adult age of 24 weeks.

For histologic study the subglottic part of the larynx was excised. 5 μm Transversal sections were stained with haematoxylin-azophloxin and elastica-Van Gieson. The methods of selecting and measuring sections from each specimen have been reported previously (3). The following measurements were carried out (fig.1):

1. lumen surface area of the subglottic airway (mm^2);
2. inner diameters of the (reconstructed) cricoid ring (mm): median A_2P_2, transversal B_2C_2 and D_2E_2.

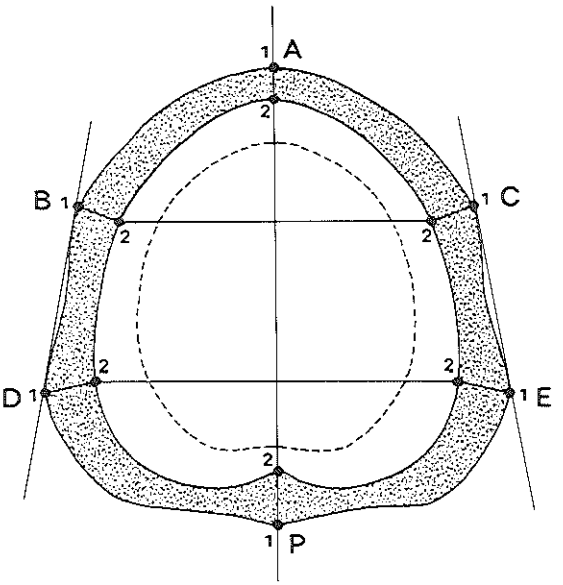

fig.1 Schematic transversal section of the cricoid ring and the epithelial lining (A-P = antero-posterior direction).

Table II: Comparison of means of series I, II, IV

		SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES V 24 WEEKS HYDROXYLAPATITE-PERICHONDRIUM GRAFT (n)	P I/II	P I/III	P II/III
Subglottic lumen area (mm^2)		8.6 ± 1.4 (13)	21.1 ± 2.9 (28)	14.5 ± 2.2 (11)	0	< 0.01	0
Diameters (mm)	A_2P_2	4.43 ± 0.21 (10)	7.24 ± 0.46 (25)	5.20 ± 0.63 (11)	0	< 0.01	0
	B_2C_2	3.54 ± 0.39 (13)	5.19 ± 0.43 (27)	5.22 ± 1.24 (11)	0	< 0.01	0.94
	D_2E_2	4.02 ± 0.30 (13)	6.07 ± 0.39 (26)	5.82 ± 0.62 (11)	0	0	0.24

significance $p \leq 0.05$
n = number of measurements

Table I: Comparison of means of series I, II, III

		SERIES I 4 WEEKS CONTROL (n)	SERIES II 24 WEEKS CONTROL (n)	SERIES IV 24 WEEKS CARTILAGE-PERICHONDRIUM GRAFT (n)	P I/II	P I/III	P II/III
Subglottic lumen area (mm^2)		8.6 ± 1.4 (13)	21.1 ± 2.9 (28)	17.9 ± 4.2 (10)	0	< 0.01	0.05
Diameters (mm)	A_2P_2	4.43 ± 0.21 (10)	7.24 ± 0.46 (25)	5.54 ± 0.73 (10)	0	< 0.01	< 0.01
	B_2C_2	3.54 ± 0.39 (13)	5.19 ± 0.43 (27)	7.82 ± 0.67 (10)	0	0	0
	D_2E_2	4.02 ± 0.30 (13)	6.07 ± 0.39 (26)	7.07 ± 1.25 (10)	0	< 0.01	0.03

significance $p \leq 0.05$
n = number of measurements

Reconstruction of the cricoid with a cartilage-perichondrium graft (series III)

The morphometric data are listed in Table I.

Compared with the shape of the cricoid in the adult control specimens (fig.2), the cricoid has developed a flattened contour on the ventral side (the transplant) and a "U"-like shape of the dorsal part (fig.3,4). None of the grafts has been extruded. Only 5 animals have a complete viable graft with a fully differentiated histologic aspect. In the other animals the implant shows signs of regression of cartilage, characterized by diminished basophilia of the intercellular substance, poor staining of the chondrocytes with cluttering of nuclear chromatine as an expression of cell death, ingrowth of fibrous tissue and blood vessels into the graft and the presence of fatty tissue (fig.5). The perichondrium seems normal in all specimens. The morphology of the regressive cartilage resembles the stage of differentiation at the age of 4 weeks (4).

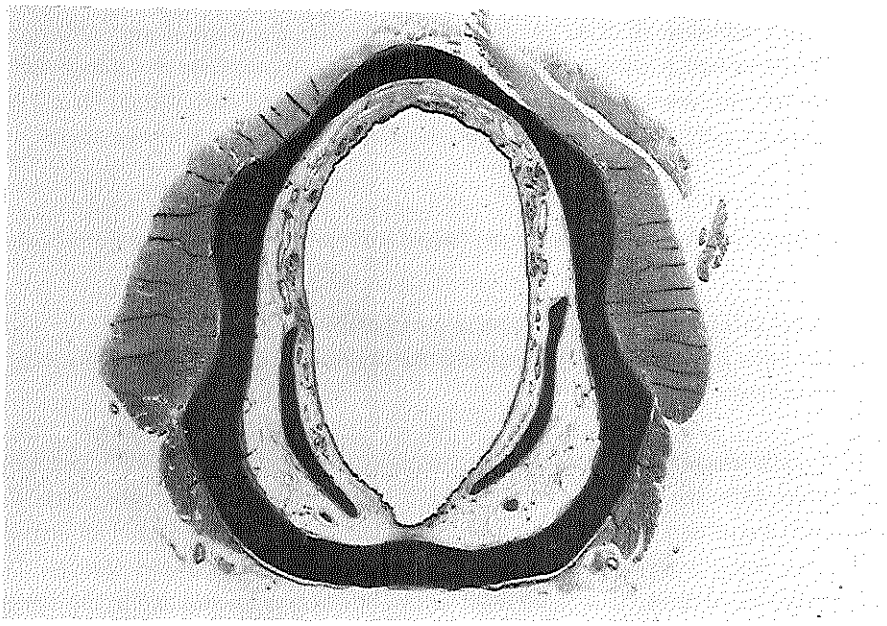

fig.2 Transversal section of normal subglottis in 24-week-old rabbit (series II); "egg"-like form of the cricoid and marked oval subglottic airway lumen. (haematoxylin-azophloxin, magn. 10x)

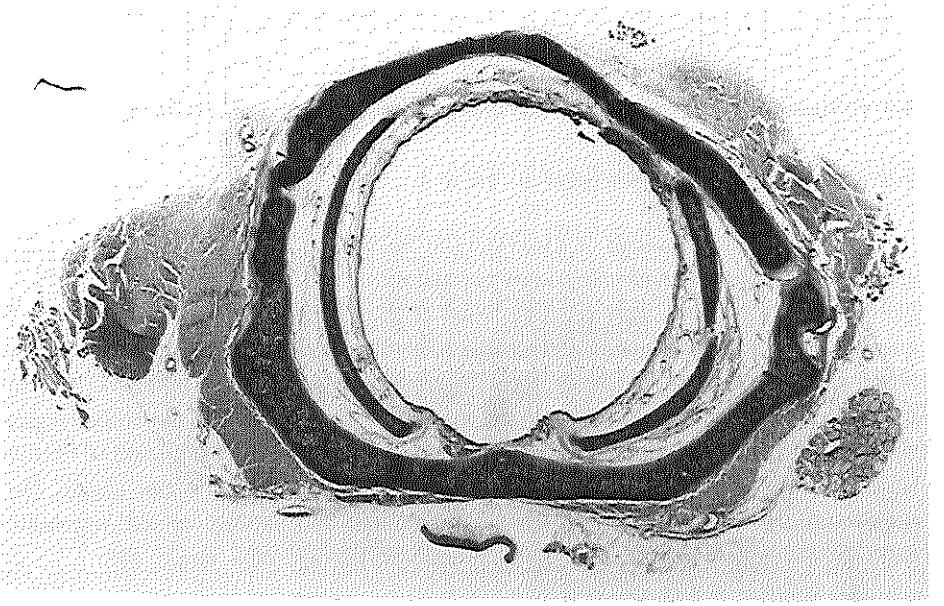

fig.3 Transversal section of subglottis of 24-week-old rabbit after resection of ventral 1/3 of cricoid and immediate reimplantation as composite autogenous cartilage-perichondrium graft at the age of 4 weeks (series III); flat shape of graft; U-like form of dorsal half of cricoid; normal staining cartilage of transplant and dorsal half of the cricoid; synchondrotic connection between graft and cricoid stubs on the left; "end to end" contact on the right. (haematoxylin-azophloxin, magn. 10x)

Two types of connection between the cricoid stubs and the graft can be distinguished (fig.6):

1. an "end to end" contact (8x) with a perichondrial layer in between;
2. a thin layer of young cartilage between the transplant and the cricoid, forming a synchondrosis (6x).

In 4 adult specimens with pronounced regression of the cartilage, the implant has lost contact with the cut ends of the cricoid on one (2x) or both sides (2x). The untouched, dorsal part of the cricoid has a normal adult histologic aspect in all animals. In 5 cases some regressively changed cartilage is found around the sutures. The subepithelial loose connective tissue holds normal structures as observed in the control specimens (series II): glands, blood vessels, fatty tissue and an intact network of longitudinally running elastic fibres (= conus elasticus) all round. The airway lumen is lined with normal respiratory epithelium.

Instead of oval, the subglottic airway lumen has become more or less round with a mean surface area of ± 17,9 mm^2. In 4 specimens with almost complete regression of the

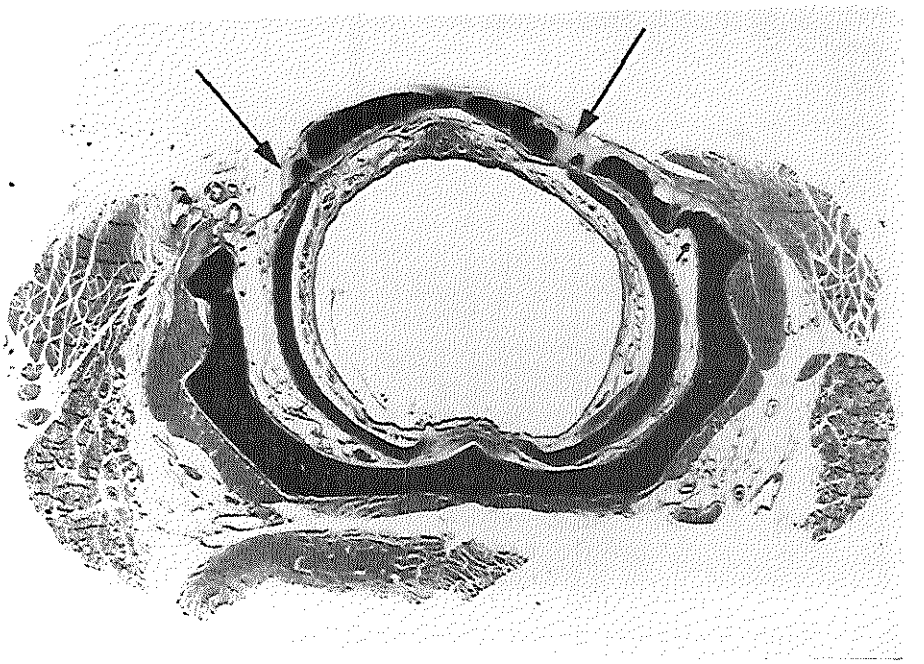

fig.4 Transversal section of subglottis of adult rabbit after resection of ventral 1/3 of cricoid and immediate reimplantation at the age of 4 weeks (series III); flat shape of the graft; U-like form of the dorsal half of cricoid; small subglottic airway lumen; pronounced regression of transplant (arrows); lost connection of graft with end of cricoid on the left; synchondrotic connection on the right; normal staining cartilage of dorsal half of cricoid. (haematoxylin-azophloxin, magn.10x)

graft the lumen area is even smaller than 15,3 mm^2 (21,1 mm^2 (adult controls) minus twice the standard deviation). On the other hand, in 3 animals with a viable graft the airway dimensions approximate the mean value of the controls. Accordingly, standard deviations in this series are high.

Reconstruction of the cricoid with a hydroxylapatite-perichondrium graft (series IV)

The morphometric data are listed in Table II.

The microscopic features of the implant have been subject of an earlier study (5,28). In all cases the subglottic airway is lined with respiratory epithelium covering the subepithelial layer which encloses the normal structures including an undamaged conus elasticus. The remaining cricoid consists of fully differentiated cartilage.

None of the grafts show signs of resorption or extrusion. However, in the first 3

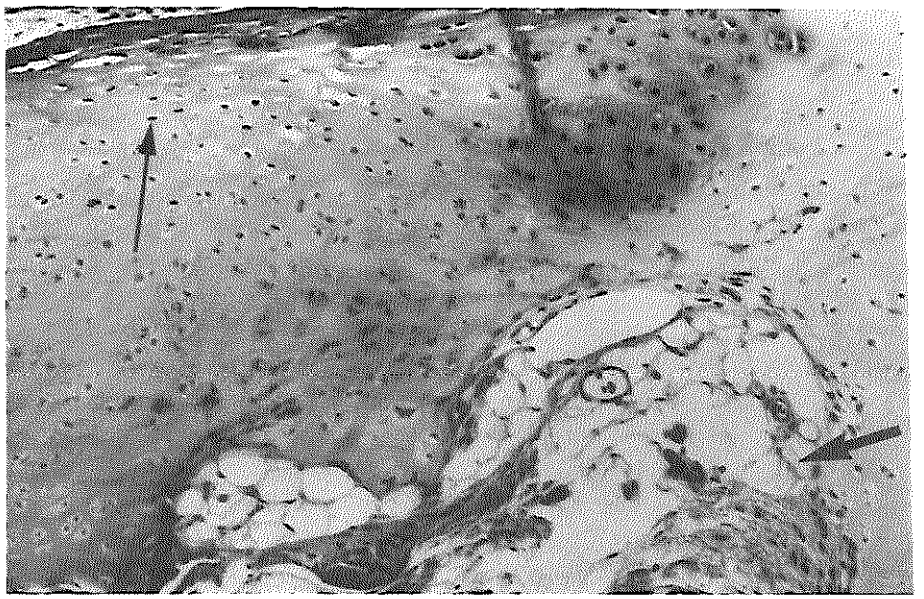

fig.5 Details of regression in graft in 24-week-old specimen (series III); decreased basophilia of intercellular substance and chondrocytes; cluttering of chromatine (thin arrow); ingrowth of fibrous tissue with blood vessels and fatty tissue (thick arrow); normal perichondrium. (haematoxylin-azophloxin, magn. 40x)

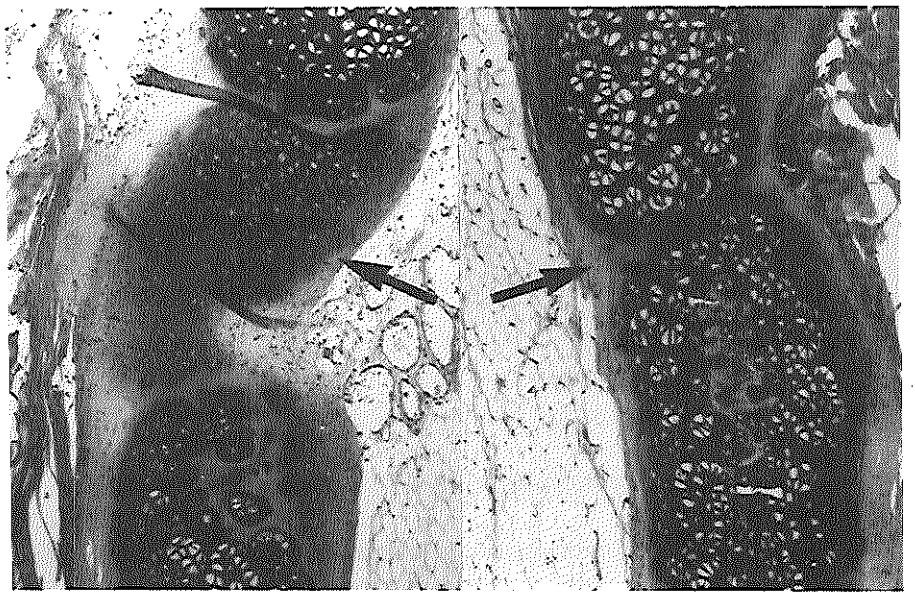

fig.6 Details of connection between graft and cricoid; young cartilage forming a synchondrosis on the left (arrow); "end to end" contact on the right (arrow). (haematoxylin-azophloxin, magn. 40x)

animals operated upon, the graft has lost contact with the cut end of the cricoid on one side. In these specimens the cricoid exhibits the same U-like structure as observed in series III. This abnormal growth pattern did not occur in the cricoid of the other specimens (fig.7).

The subglottic airway lumen has developed a more or less round form. The mean surface area measures ± 14,5 mm^2 with a low standard deviation. At the level BC a large standard deviation is found as the result of the "U"-like deformity of the cricoid in 3 cases.

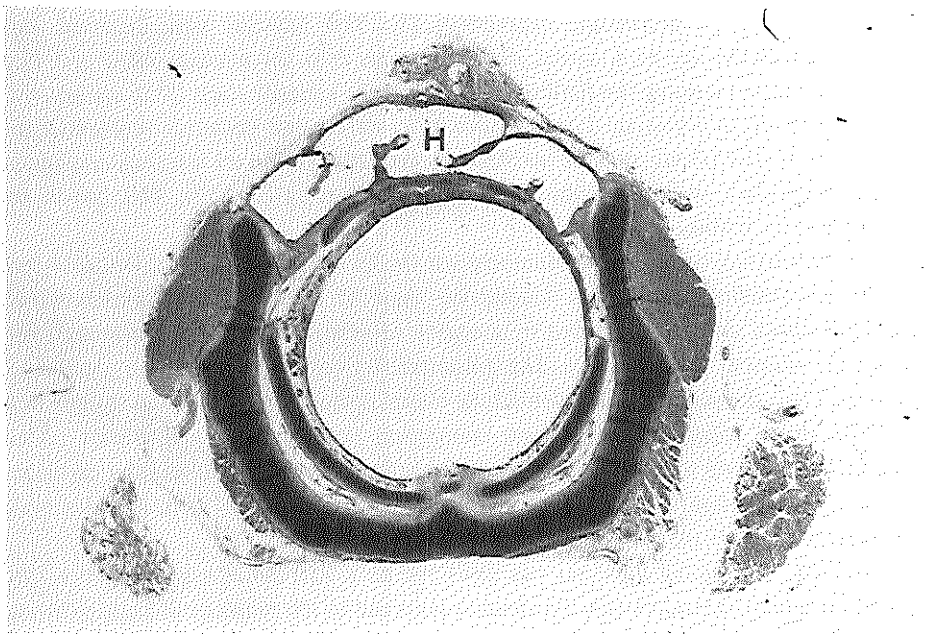

fig.7 Transversal section of subglottis in 24-week-old rabbit after replacement of anterior 1/3 of cricoid ring by a porous hydroxylapatite-perichondrium graft (H) (series IV); firm connection between implant and cricoid on both sides by fibrous tissue and newly formed cartilage; normal shaped posterior cricoid segment; small airway lumen. (haematoxylin-azophloxin, magn, 10x)

Discussion

The reimplanted cricoid arch (series III) showed the unpredictable behaviour, already described for other cartilaginous implants. It ranged from complete viability (5 animals) to various degrees of regression (5 animals). Observations in the adult stage demonstrated that the viable implants displayed further histologic differentiation after surgery at the age of 4 weeks and had contact with the posterior cricoid segment in all

cases. The considerable increase in width at the level BC in the specimens with a viable graft suggested the implant to grow after reimplantation.

In most cases of severe regression of the reimplanted cartilage the ends lost contact with the posterior cricoid segment. The extensive regression is probably caused by too long a period of disturbed blood circulation in the perichondrium and the neighbouring tissues. No signs of infection could be found.

The growth pattern of the reconstructed cricoid ring (series III) appeared to be abnormal in all specimens, irrespective of the presence of regressive changes in the graft. The posterior part always developed a "U"-like form, as observed after resection of the anterior third in a preceeding experiment (1). The anterior part, resected and reimplanted at the age of 4 weeks, did not change into the normal, adult arch-like configuration, maintaining the flattened contour of the younger stage (3). Probably, the development of an abnormal form of both the anterior and posterior segment is an expression of (disturbed) intrinsic mechanical properties of the cricoid ring, analogous to the interlocked stress, described for costal cartilage by Fry (17). This has to be investigated in further detail.

The hydroxylapatite-perichondrium implant (series IV) appeared to be well accepted by the surrounding tissues and showed no signs of resorption. The perichondrial envelope stayed viable as indicated by a mantle of newly formed cartilage around the alloplastic material in most cases. The reconstructed cricoid ring had two prominent features. First, the stable graft prevented the lateral deviation of the cricoid stumbs to a "U"-like shape, except in case of lost connection between graft and cricoid . This observation suggests a firm connection between the ceramic implant and the cartilage. Hence, the regulation of form of the growing posterior cricoid segment depends on a mechanical fixation to the alloplastic material.

Secondly, the sagittal diameter was rather small: ± 5,20 mm compared with ± 7,24 mm in the adult control animals. This feature is caused by the presence of a non-growing alloplastic anterior segment and the lack of compensatory -extra- growth of the viable cartilaginous segment in reaction to the increasing respiratory demands of the growing animal. Moss' concept of the "functional matrix" (24), controlling growth is obviously not valid for the cricoid ring. The formation of a stenosis in this series demonstrates the limitations of the use of non-growing grafts in a growing organ.

In a previous experiment (3) resection of the ventral third of the cricoid ring did not cause an airway narrowing. In these cases the intact conus elasticus in the subepithelial layer was considered to keep up a normal airway lumen. On the other hand, in the present study, reconstruction of the ring with either type of graft, induced various degrees of stenosis, although the conus elasticus was preserved. The impaired anterior outgrowth of the reconstructed ring might restrict the expansion in ventral direction of the conus elasticus and, consequently, the airway lumen.

In conclusion, the experimental results suggest that in young rabbits:

1. the altered growth pattern of the cricoid ring after interruption and repair of the circular structure is determined by intrinsic mechanical forces within the cricoid;
2. repair of the anteriorly interrupted cricoid ring is contra-indicated, if the conus

elasticus is undamaged.

3. of the two types of implants investigated the hydroxylapatite-perichondrium graft is the most promising, although the form has to be adapted to the demands in the growing larynx.

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CHAPTER 9

SUMMARY AND CONCLUDING REMARKS

The introduction of this thesis (chapter 1) ended in three questions, concerning stenosis of the airway and of the subglottis in particular. The first and second issue can be summarized as follows: do different types of trauma have different effects on the growth of the subglottis and is the condition of subglottic stenosis to be considered as the ultimate effect of different processes causing an airway narrowing?

These questions cannot be answered by clinical or post-mortem investigations. Holinger proposed a classification of histopathologic types of subglottic stenosis, but did not include factors regarding etiology and pathogenesis (9). Post-mortem specimens are not available in such numbers that an apt answer can be given. So, an experimental study was necessary. At first the investigations were confined to a growing "model", as in clinical practice most problems are met in infants and children. Young rabbits were used as experimental animals, because of their convenient size for laryngeal surgery at an early age (4 weeks after birth). The effects of various types of trauma were studied 20 weeks later in the adult stage.

Some of the results and conclusions will be summarized and shortly discussed in this chapter:

1. The normal growth of the subglottis in the rabbit during the experimental period of 20 weeks is characterized by an increase of the surface area of the airway lumen in transversal sections of 150% (from ± 8,6 mm^2 to ± 21,1 mm^2). Simultaneously the cricoid ring expands, slightly more in anteroposterior (± 65%) than in transversal (± 50%) direction. The thickness of the cricoid ring does not change in this period (chapter 2). The development of the cricoid and the airway lumen in rabbits between the age of 4 and 24 weeks appears to correspond roughly to the growth of the human subglottis from prepuberty to adulthood (in both sagittal and transversal direction with ± 60% in males and ± 35% in females) (10).

2. The larynx, and also the trachea, is not a simple tube-like organ with an inner epithelium, lining the airway lumen. At the subglottic level the airway must be regarded as composed of two concentric tubes (chapter 5). The inner tube is the conus elasticus, lying immediately under and sustaining the airway epithelium. This elastic mantle is suspended by elastic lamellae to the outer segmented cartilaginous tube, providing a stable and yet flexible support. This feature of subglottic anatomy has not been considered in literature on airway stenosis to date. Reviewing literature on human anatomy demonstrated the fairly adequate description of these subglottic structures already in 1925 by Schumacher (1) (chapter 5)!

3. The cricoid ring, the only circular cartilaginous structure in the upper airway, has always been thought by clinicians to be a compulsory element in the skeleton of the

airway for maintaining the airway lumen (4,12). However, resection of the anterior third or even half of the cricoid ring does not result in a subglottic stenosis, as long as the inner soft tissue layer, containing the conus elasticus, is preserved (chapter 4 and 5). The conus elasticus is considered to keep open the airway lumen, even when parts of the cartilaginous framework on the ventral side are missing. In clinical literature no similar observations are described. The consequences of an absent cricoid arch upon the airway lumen during flexion, rotation or extension of the neck remain to be studied. Also the influence on voice production has to be examined more closely.

4. The reaction of the soft tissue layer of the subglottis to endolaryngeal traumata is similar whether or not the cricoid cartilage is involved. A circular traumatization of the inner soft tissue lining results in the formation of a stenotic ring combined with a normally expanding cricoid (chapter 6). The histologic features consist of a thickening of the subepithelial layer due to the formation of fibrous scar tissue, ectopic cartilage and fatty tissue between the cricoid and the contracting fibrous tissue. Regeneration of the disrupted conus elasticus and of glandular structures did not occur. These features have not been reported in histologic studies of the stenotic infant larynx (7,8,11). However, it is possible that ectopic cartilage has been diagnosed as hamartoma. Case-histories of patients with a hamartoma of the larynx should be re-examined with special attention to previous laryngeal trauma.

5. The cricoid ring reacts in a specific way to different types of trauma. Damage to the inner perichondrium and the adjacent zone of cartilage does not hamper the increase of the anteroposterior diameter. On the other hand, the transverse diameter, especially in the ventral half, is far too small in the adult stage. The normal "egg"-like form appears to be "collapsed laterally" to a "pear"-like shape (chapter 2).

   Interruption of the cricoid ring -anterior midline split or resection of an anterior part- always results in a lateral deviation of the stumps of the remaining cricoid during growth up to the adult stage (chapter 4). Division of the cricoid ring in a small anterior part (arch) and a posterior segment leads to a "stretching" of the anterior segment, comparable to the "U"-like change of the posterior segment (chapter 8).

6. Resection of the anterior wall - soft tissues and cricoid arch - is followed by a "U"-like deformation of the remaining cricoid segment in combination with the formation of a flat layer of subepithelial fibrous tissue between the stumps. The resulting stenotic lumen is roughly proportional to the remaining part of the subglottic wall (chapter 4).

7. Malformations of the cricoid ring - without involvement of the soft tissue - can interfere with the normal cricoid expansion. So, too narrow a cricoid ring causes a cartilaginous stenosis (chapter 8).

8. The expression subglottic stenosis refers to different conditions, each with a specific pathogenesis. In this study in growing rabbits 4 types could be distinguished (fig.1):

   1. a pure soft tissue stenosis (chapter 6);
   2. a combined soft tissue-cartilaginous stenosis (chapter 2);
   3. a pure cartilaginous stenosis (chapter 8);
   4. a status post partial resection of the subglottic wall (chapter 4).

   Careful study of clinical cases has to reveal whether in human pathology similar specifications of subglottic stenosis can be found. Holinger's catalogue of subglottic airway narrowing already suggests some common features.

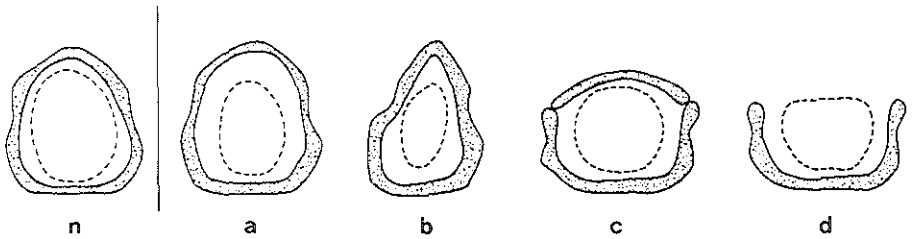

fig.1 Schematic drawings (cricoid and epithelial lining) of normal subglottis (n) and 4 types of subglottic stenosis:
a. pure soft tissue stenosis;
b. combined soft tissue-cartilaginous stenosis;
c. pure cartilaginous stenosis;
d. status post partial resection of subglottic wall.

9. An anterior midline split of the cricoid resulted in a subglottic airway lumen at the adult age with larger dimensions than in control animals (chapter 4). This observation seems to support the rationale for an anterior midline split as treatment for children with an acquired subglottic stenosis (5). However, in view of the different types of stenosis observed, the effects of an anterior midline cricotomy in animals with different types of stenosis have to be studied.

10. The function of the elastic tube in keeping open the airway lumen should be considered in surgery. As the elastic fibres have a predominantly longitudinal or slightly oblique course on the ventral side, transverse incisions will inevitably interrupt the conus elasticus. The cut ends are likely to retract, whereas regeneration of the elastic tissue is observed not to occur (chapter 4). Therefore, the use of transverse incisions or flap-door openings to open the laryngeal lumen has to be re-considered. An interesting topic for further research will be finding ways to repair an interrupted conus elasticus.

11. After identification of specific growth patterns of the cricoid ring ("U"-like shape, "pear"-like shape) following certain traumata, it remains to be analysed which morphogenetic factors are controlling the development of the cricoid shape during

post-natal growth. In chapter 8 is referred to the concept of interlocked stress proposed by Fry to explain (and predict) changes in form of nasal septal cartilage (6). In this concept the cartilage is a turgescent mass, hold together by a three-dimensional system of collagen fibres, converging at the surface to form a perichondrial envelope. Injury to the envelope on one side leads to a change in form of the whole cartilaginous structure. In this way the "inward collapse" of the lateral sides of the cricoid ring after damaging the inner perichondrium might be explained (chapter 2). Further investigations are performed to explore possibilities to manipulate the growth of the (abnormal) cricoid by surgery.

12. For the first time it is demonstrated that the ceramic material porous hydroxylapatite is accepted and -to a certain extent- integrated in the cricoid cartilage, opening the way to new techniques of reconstruction. On the other hand, the experimental results also show the limitations of an alloplastic implant in a growing organ. Form and size of the graft have to be adjusted to the desired later dimensions.

13. In the treatment of airway stenosis, as practised by Berkovits and colleagues, using siliconized silicon stents and tubes, the preservation of the airway epithelium is stressed (2,3). In view of their close anatomical relation, both epithelium and conus elasticus will share the benefits of this special material. The importance of the latter structure for keeping open the airway lumen, has already been emphasized in this study (chapter 5).

14. The various types of trauma in this study were both acute and mechanically induced. In patients subglottic impairment is usually the consequence of prolonged intubation. In this situation a long-term mechanical pressure exerted on the subglottic wall or toxicity of the tube-material can be of importance. The effects on subglottic growth of both factors should be topic of further experimental investigation.

15. Whether the reported reactions of cricoid and soft tissue lining to trauma are restricted to growing animals can only be determined by performing similar experiments in adult animals.

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CHAPTER 10

SAMENVATTING

De subglottis is het deel van de larynx (strottehoofd) juist onder de stembanden, opgebouwd uit het cricoid (ringkraakbeen) en de daarbinnen gelegen weke delen (slijmvlies). Onder een subglottische stenose wordt een vernauwing van dit gebied verstaan. Meestal ontstaat een dergelijke stenose als reactie op beschadiging van de wand van de luchtweg van binnen uit (door een tube ingebracht voor beademing) of van buiten af. Voor behandeling van subglottische stenosen zijn verschillende conservatieve en chirurgische methoden beschreven. In het Academisch Ziekenhuis Rotterdam - Dijkzigt en Sophia Kinderziekenhuis - heeft Berkovits zich meer dan 15 jaar toegelegd op de behandeling van patienten met luchtwegvernauwingen. Het door hem geintroduceerde gesiliconeerde silicon rubber bleek zo "weefsel-vriendelijk" voor het slijmvlies van de luchtweg, dat uit dit materiaal gemaakte inwendige "spalken" (tubes en stents) voor langere tijd in de luchtweg geplaatst kunnen worden om de vernauwing op te rekken. Door toepassing van deze conserverende werkwijze, eventueel gecombineerd met chirurgische ingrepen, werden bij vele patienten goede resultaten geboekt.

Uit de literatuur is bekend dat in alle centra voor behandeling van subglottische stenosen een kleine groep patienten, vooral kinderen, resteert waarbij geen enkele therapie het gewenste resultaat oplevert, zonder dat in alle gevallen de oorzaak duidelijk is. Deze constatering heeft geleid tot de formulering van de volgende vragen:

1. wordt de diagnose subglottische stenose gebruikt voor aandoeningen met verschillende etiologie (oorzaak) en pathogenese (ontstaanswijze), die daarom een verschillende therapie behoeven?
2. zijn verschillende typen van subglottische stenose het gevolg van verschillende vormen van trauma (beschadiging)?
3. zijn de effecten van een subglottisch trauma bij een volwassene anders dan bij een kind?

Om een bijdrage te leveren aan de beantwoording van de bovenstaande vragen is experimenteel onderzoek bij proefdieren verricht.

In dit proefschrift worden de resultaten gepresenteerd van een experimenteel onderzoek, dat zich beperkt tot het subglottische deel van de larynx bij groeiende konijnen. Na bestudering van de normale groei van de subglottis tussen het jonge stadium van 4 weken en de volwassen leeftijd van 24 weken werden bij konijnen in het jonge stadium een reeks verschillende subglottische letsels aan gebracht:

1. inwendig (endolaryngeaal) trauma:

   a. circulaire beschadiging van het epitheel en subepitheliale laag (slijmvlies) van de subglottis en het perichondrium (kraakbeenvlies) en de binnenste laag van het

cricoid (hoofdstuk 2 en 3);

b. circulaire beschadiging uitsluitend van de weke delen (hoofdstuk 6).

2. uitwendig (chirurgisch) trauma:

   a. onderbreking van de circulaire structuur van het cricoid door een anterieure mediane incisie (snede midden-voor) en door resectie van het voorste 1/3 of 1/2 deel van het cricoid, waarbij de weke delen gespaard werden (hoofdstuk 4 en 5);
   b. onderbreking van de circulaire structuur van het cricoid door een anterieure mediane incisie en door resectie van het voorste 1/3 of 1/2 deel van het cricoid met de aangrenzende weke delen (hoofdstuk 4 en 5);
   c. onderbreking van de circulaire structuur van het cricoid door resectie van het voorste 1/3 deel van het cricoid met behoud van de aangrenzende weke delen, gevolgd door herstel met behulp van autologe (uit het eigen lichaam) en alloplastische (kunststof) implantaten (hoofdstuk 7 en 8).

De effecten op de groei van de subglottis werden 20 weken later in het volwassen stadium onderzocht.

Tijdens de normale groei van het proefdier tussen 4 en 24 weken na de geboorte neemt de oppervlakte van het subglottische luchtweg in een dwarsdoorsnede met ± 150% toe. De diameters van het cricoid worden in voor-achterwaartse richting ± 65% groter en in zijdelingse richting ± 50%. De dikte van het cricoid neemt in deze periode niet toe (hoofdstuk 2). Verhoudingsgewijs komt de mate van groei overeen met die bij de mens gedurende de periode van prepuberteit tot volwassen leeftijd.

De experimentele resultaten tonen aan dat bij het konijn de larynx, evenals de trachea (luchtpijp), is opgebouwd uit 2 concentrische buisvormige structuren (hoofdstuk 5). De binnenste wordt gevormd door de conus elasticus, die het epitheel steunt. Deze structuur van voornamelijk in lengterichting verlopende elastische vezels is door aftakkende lamellae opgehangen aan de buitenste "buis", gevormd door gesegmenteerde kraakbenige elementen, die stevigheid bieden en toch flexibel zijn. Het blijkt dat op het niveau van de subglottis de ventrale helft van het kraakbenig skelet gemist kan worden, zolang de onderliggende weke delen onbeschadigd zijn (hoofdstuk 4 en 5). In deze gevallen lijkt de conus elasticus de luchtweg open te houden tijdens de verdere groei. Dit aspect van de anatomie van de subglottis heeft tot nu toe in de literatuur over subglottische stenose geen aandacht gekregen.

Beschadiging van een of beide buisvormige structuren in het jonge proefdier leidt tot een veranderde groei van de subglottis. Dit betekent niet in alle gevallen het ontstaan van een subglottische stenose.

Het cricoid als onderdeel van de buitenste "buis", reageert op een specifieke manier op verschillende vormen van trauma. Onderbreking van de circulariteit van het ringkraakbeen door een anterieure mediane incisie leidt tot een uiteenbuigen van de uiteinden van het cricoid met vergroting van de oppervlakte van de subglottische luchtweg in dwarsdoorsnede op volwassen leeftijd (hoofdstuk 4). Verdere onderbreking van de circulariteit door verwijdering van een voorste deel van het cricoid veroorzaakt

een "U"-vormige uitgroei van het resterende deel (hoofdstuk 4). Het ontstaan van deze abnormale vorm kan worden voorkomen, indien op de plaats van het gereseceerde deel een (niet groeiende) boog van hydroxyapatiet wordt geplaatst. Voor het eerst werd een stevige verbinding van kraakbeen en perichondrium met dit materiaal aangetoond (hoofdstuk 6 en 8). Na onderbreking van de circulariteit door verdeling van het cricoid in een klein anterieur (voorste) segment en een groter posterieur (achterste) segment treedt eveneens "U"-vorming van het achterste deel op, terwijl de normale vorming van een boog van het voortse deel tijdens de groei uitblijft (hoofdstuk 8), waardoor de voor-achterwaartse diameter van het cricoid klein is. In essentie is dit fenomeen identiek aan de "U"-vorming van het posterieure deel.

Een inwendig trauma waarbij het perichondrium en de binnenste laag van het cricoid worden beschadigd, leidt tot gestoorde uitgroei van het cricoid in zijdelingse richting, vooral van de voorste helft. In plaats van de normale "ei"-vorm ontstaat hierdoor meer een "peer"-vorm (hoofdstuk 2).

De weke delen van de subglottische luchtweg reageren eveneens op specifieke wijze op een laesie. Na inwendige circulaire beschadiging met of zonder letsel van het cricoid (hoofdstuk 4 en 6), ontstaat een verdikking van de subepitheliale laag door vorming van littekenweefsel, ectopisch (op abnormale plaats aanwezig) kraakbeen en vetweefsel tussen het contraherende littekenweefsel en het cricoid. De onderbroken conus elasticus, de binnenste "buis", regenereert niet. Uitwendig trauma, zoals resectie van het voorste deel van de wand van de subglottis leidt tot regeneratie van de subepitheliale laag door vorming van littekenweefsel zonder herstel van de onderbroken elastische vezels (hoofdstuk 4 en 5). Beschadiging van de conus elasticus en vorming van ectopisch kraakbeen zijn tot nu toe niet beschreven in histologische studies van de vernauwde larynx bij kinderen.

Met betrekking tot de in het begin van deze samenvatting gestelde vragen tonen de resultaten van de experimenten bij groeiende konijnen aan dat een subglottische stenose het gevolg kan zijn van verschillende aandoeningen van het cricoid en de weke delen die ieder op kenmerkende wijze zijn ontstaan na verschillende typen van trauma. Zo zijn te onderscheiden:

1. een weke delen stenose door verdikking van de subepitheliale laag (hoofdstuk 6);
2. een gecombineerde weke delen-kraakbenige stenose door verdikking van de subepitheliale laag en een te nauw, "peer"-vormig cricoid (hoofdstuk 2 en 3);
3. een stenose uitsluitend door een in voor-achterwaartse richting onvoldoende uitgegroeid ringkraakbeen (hoofdstuk 6,7 en 8);
4. een stenose na partiële resectie van de wand van de subglottis, waarbij de oppervlakte van de luchtweg in dwarsdoorsnede in verhouding staat tot de grootte van de resterende wand (hoofdstuk 4 en 5).

De vraag resteert of het cricoid en de weke delen bij volwassen dieren op dezelfde wijze reageren op beschadiging. Voor een antwoord zullen gelijksoortige experimenten herhaald moeten worden op de volwassen leeftijd. Zorgvuldige bestudering van klinische pathologie zal moeten leren in hoeverre dergelijke verschillende vormen van

subglottische stensen ook bij de mens voorkomen. Sporadische literatuurgegevens (Holinger, Cotton) wijzen zeker in deze richting.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 18 augustus 1956 geboren te Tilburg. In 1974 behaalde hij het eindexamen gymnasium ß aan het Mill Hill College in Goirle. In dat zelfde jaar werd begonnen met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Het artsexamen werd afgelegd in januari 1982.

Na beëindiging van de studie was de auteur gedurende een jaar werkzaam als arts-assistent op de afdeling heelkunde van het Ikazia ziekenhuis in Rotterdam. Vanaf maart 1983 is hij verbonden aan de afdeling Keel-, Neus-, en Oorheelkunde van het Academisch Ziekenhuis en de Erasmus Universiteit te Rotterdam: gedurende het eerste half jaar als arts-assistent, vervolgens voor een periode van drie jaar als wetenschappelijk medewerker. Thans is de auteur in opleiding tot keel-, neus-, en oorarts.