

## The Reduction of the Laminectomy Membrane in Dogs by the Synovial Fluid-Soaked Absorbable Gelatin Sponge

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**Abstract:** The reduction of the laminectomy membrane (LM) with synovial fluid soaked absorbable gelatin sponge (SSAGS) after laminectomy was investigated. Modified laminectomy of the first and second lumbar vertebra and concomitant full length durotomy were carried out in 16 mongrel dogs of different ages and sex. Two pieces of absorbable gelatin sponge (AGS-Gelfoam, Upjohn), soaked with 2 ml of synovial fluid taken from the shoulder joint, were implanted into the laminectomy defect in 8 dogs. Two pieces of AGS were implanted into the laminectomy defect in the control group of 8 dogs. Animals were allocated to 4 groups, each consisting of 4 dogs according to follow up examinations done in weeks 2, 5, 10 and 20. Myelography was performed at the end of the observation periods and the dogs were sacrificed. Gross and histologic evaluations of the operated region were done by an observer blinded to the groups. Modified laminectomy and full length durotomy were tolerated well by the dogs except for one dog, during both the early and the late postoperative observation periods. There was no noticeable difference between the two groups according to the clinical findings. Myelography indicated that the dural sac was reproduced in the SSAGS, but loss of the dorsal contrast column was noticeable in the control group at week 20. Gross and histological findings suggested that qualitative decreases in the inflammatory reaction at an early stage and LM in later periods were present in the SSAGS group. In conclusion SSAGS could be an alternative way of reducing the LM.

**Key Words:** Dorsal laminectomy, durotomy, laminectomy membrane, synovial fluid, absorbable gelatin sponge, dog

### Köpeklerde Sinovial Sıvı Emdirilen Absorbabl Jelatin Spanç ile Laminektomi Membranının Azaltılması

**Özet:** Bu çalışmada köpeklerde laminektomiden sonra sinovial sıvı emdirilen AGS ile laminektomi membranının azaltılması amaçlandı. Farklı yaş ve cinsiyette 16 melez köpekte lumbal 1 ve 2. vertebralarda modifiye laminektomi ve durotomi gerçekleştirildi. Sekiz köpekte, omuz ekleminden alınan 2 millilitre sinovial sıvı laminektomi defekti büyüklüğünde kesilen iki tabaka AGS ye emdirilerek operasyon yapılan bölgeye yerleştirildi. Geriye kalan 8 köpekte ise sadece iki tabaka AGS laminektomi defektlerine yerleştirilerek kontrol grubu oluşturuldu. Köpekler 2, 5, 10 ve 20 haftalık gözlem sürelerine göre 4 gruba bölündü. Gözlem sürelerinin sonunda tüm köpekler miyelografi gerçekleştirildikten sonra ötenazi yapıldı. Köpek gruplarından habersiz olan bir gözlemci tarafından operasyon yapılan bölgenin makroskopik ve histolojik değerlendirilmesi yapıldı. Modifiye laminektomi ve durotomi işleminin gerek erken, gerekse geç postoperatif gözlem sürelerinde köpekler tarafından iyi tolere edildiği gözlemlendi. Klinik bulgulara göre iki grup arasında önemli bir farklılık izlenmedi. Yirminci haftada alınan miyelografilerde, SSAGS grubunda dural kesenin yeniden oluştuğu, kontrol grubunda ise dorsal kontrast sütunun izlenmediği dikkat çekti. Makroskopik ve histolojik muayeneler sonucu; erken dönemde yangısal reaksiyonun geç dönemde ise laminektomi membranının SSAGS grubunda daha az olduğu belirlendi. Bu çalışmanın sonuçlarına göre, SSAGS' in laminektomi membranının azaltılmasında alternatif bir yaklaşım olabileceği kanaatine varılmıştır.

**Anahtar Sözcükler:** Dorsal laminektomi, durotomi, laminektomi membranı, sinovial sıvı, absorbabl jelatin spanç, köpek

### Introduction

The laminectomy membrane (LM) or epidural fibrosis is an obstacle after decompressive surgery of the thoracolumbar or lumbar spinal diseases in humans and dogs (1,2). Epidural fibrosis is a formation of extradural fibrotic tissue that results in the adherence of the duramater and nerve roots to the erector spinea muscle and disk and vertebral body (3). Excessive epineural fibrosis and scarring, dural adhesions, nerve root

attenuation, and spinal cord compression and degeneration may complicate the healing process. Nevertheless, such scar tissue formation is rarely recognized as a clinical problem (4,5). The biological events that occur in the laminectomy defect are similar to the healing of long bone fractures. The first step in the formation of LM is the invasion of the hematoma with fibroblasts filling the laminectomy defect. After the hematoma is organized and fills the defect, the formation

of fibrous callus with varying extents of metaplasia from cartilage to bone is observed. Even though the etiology of this physiopathological process is not well known, it has been suggested that the location and width of the defect in the spine, the homeostatic agent used, the height of the laminar bone left, the amount of removed epidural fat, the experience of the surgeon etc. are important factors that influence the healing of this complicated wound (6-8).

The majority of authors agree that the principal causes of the persistent or recurrent symptoms after spinal surgery are recurrence of the disk herniation and excessive epidural scar formation in humans (2,5,9). The second surgery for getting rid of compression might have a higher risk for damaging the neural tissue (3,10).

Many attempts have been made to prevent the invasion of the scar tissue into the laminectomy defect forming a physical barrier. Various materials, including autogenous free and pedicle fat graft (1,2,11,12), Zenoderm (13), omental pedicle graft (10), Kiel bone graft (14), Absorbable gelatin sponge (AGS) (1,11,15,16), vicryl (polyglactine 910) mesh (17,18), AGS embedded carbohydrate polymer (19), thrombin embedded collagen gel (20), polylactic acid membrane (21), polytetrafluoroethylene (22), carboxymethylcellulose (23), silastic sheet (14), methylmetacrylate (20), fibrin glue (24), sodium hyaluronate (25), urokinase (26) and glucocorticoids (1,15), have been used to prevent the invasion of scar tissue. Each of them has shown different degrees of positive effect.

Hyaluronic acid, which is present in synovial fluid (SF) and is a natural material of the body, has an anti-inflammatory effect and it decreases scar tissue formation by inhibiting the migration of fibroblasts. The purpose of this study was to investigate the longer term presence of SF in the laminectomy defect by the absorbable gelatin sponge (AGS) and to make a comparison between synovial fluids soaked absorbable gelatin sponge (SSAGS) and AGS with respect to clinic, radiologic and histopathologic findings.

## Materials and Methods

Sixteen mongrel dogs weighing 20-25 kg, 2-5 years old, and of different sex (11 male, 5 female) were studied with the permission of Ankara University Animal Rights Committee. The thoracolumbar and shoulder

region was prepared for surgery and modified laminectomy (removal of dorsal lamina and caudal articular process of L<sub>1</sub> with excavation of the lateral lamina and pedicle) was performed at L<sub>1</sub> - L<sub>2</sub>. Epidural fat was removed and full length durotomy was performed on the dorsal aspect of the dural sac at the laminectomy defect. Dogs were allocated into two groups, each consisting of 8 dogs. In the SSAGS group, two pieces of AGS soaked with 2 ml of SF taken from the shoulder joint were implanted. In the AGS group, the same size AGS as the laminectomy defect was implanted alone. The dogs were re-allocated into 4 groups with each one consisting of 4 dogs according to the observation period including the 2<sup>nd</sup>, 5<sup>th</sup>, 10<sup>th</sup> and 20<sup>th</sup> weeks postoperatively, and they were physically and neurologically examined every other day. Cysternal myelography was performed at the end of the observation periods under general anesthesia, induced with thiopentone sodium (Pentothal Sodium, Abbott) and maintained with halothane (Hoechst) in a semiclosed system. After myelography, the dogs were heparinized (Liquemin, Roche) and the left carotid artery and jugular vein were exposed. While the carotid artery had been excised and blood flow was allowed, the vein was catheterized and about 3 liters of saline (0.9% NaCl), followed by 3 liters 10% buffered formalin solution was infused. The pertinent section of the vertebral column was removed and immersed in buffered formalin solution. All segments of the spine were frozen and transversally sectioned in about 1 cm thickness and evaluated macroscopically. The sections underwent decalcification with 10% formic acid buffered with sodium citrate. After decalcification, the specimens were embedded in paraffin, sectioned into pieces of 5-6 mm thickness and stained by H&E (hematoxylin eosin). Histologic evaluation was performed qualitatively from 1-3+ (slight, moderate and severe) by an observer blinded to the groups.

## Results

**Neurologic Examination:** All dogs were ambulatory except for one, which could not bear the weight or use its hind limbs on the first day of the physical and neurological examination. Two ambulatory dogs had slight conscious proprioceptive deficits. On the 2<sup>nd</sup> day, none of the 13 remaining dogs had signs of neurological deficit. Neurological status did not get worse during the later period. Dogs with slight neurological deficit

improved but the nonambulatory dog was sacrificed during the second week.

**Myelography:** Complete blockage was not present in any of the dogs according to the myelographic evaluation, which was performed at the end of the observation period. On the other hand, irregularity of the dorsal contrast column appeared at the durotomy site in the early periods (2, 5 weeks) in both groups. However, the dural sac was remarkably restructured in the SSAGS implanted group (Figure 1). The same appearance was not observed in the AGS applied group and the dorsal contrast column had unclear borders in the 20 weeks (Figure 2).

**Necropsy:** The spinal cord was not located centrally in almost all of the dogs upon macroscopical examination of the transversal sections of the operation site. Hardening of the fibrous tissue that filled the laminectomy defect, over time, was detected by palpation. During the early periods of the SSAGS implanted group, the fibrous tissue was easily detached from the duramater. However, the duramater was more tightly attached to the fibrous tissue in later periods (Figure 3). In one dog from the AGS applied group, the piamater was attached to the fibrous tissue and the dorsal part of the spinal cord had moved upwards (Figure 4).

**Histologic Evaluation:** Cellular reaction, including macrophages, lymphocytes and neutrophils, was observed at the laminectomy defect in the 2nd week. The durotomy sites were bonded to fibrous tissue that was replaced between the duramater and paraspinal muscle.

Osteoblastic activity was seen at the border of the cut bone. Either cellular reaction or formation fibrosis was remarkably less in the SSAGS group than in the AGS group.

Cellular reaction was decreased in both groups and it was less noticeable in SSAGS in the 5<sup>th</sup> week. Hyalinized fibrous tissue, which was characterized by trabecular structure and osteoblastic activity, filled the laminectomy defect. A thin and dense fibrous tissue was observed to fill up the space between the dural edges. The cut ends of the duramater were more tightly attached to fibrous tissue in the AGS group than in the SSAGS group in both the 5<sup>th</sup> and 10<sup>th</sup> weeks.

LM, hyalinization and trabecular bone formation had reached a greater degree in both groups by the 20<sup>th</sup> week. The dural sac was almost recompleted without adhesion to the spinal cord in the SSAGS group (Figure 5). However, the spinal cord, the duramater and fibrous tissue were adhered to each other in one dog and the others had a lack of regular shape of dural sac in the AGS group (Figure 6). Mean fibrous tissue formation of the dogs is shown in Figure 7.

## Discussion

The source of the LM was attributed to the results of the damaged spinal muscle and the activated fibroblasts, which provide a key point or the first step in epidural fibrosis. Although there are many factors contributing to the healing process of this type of complicated wound,

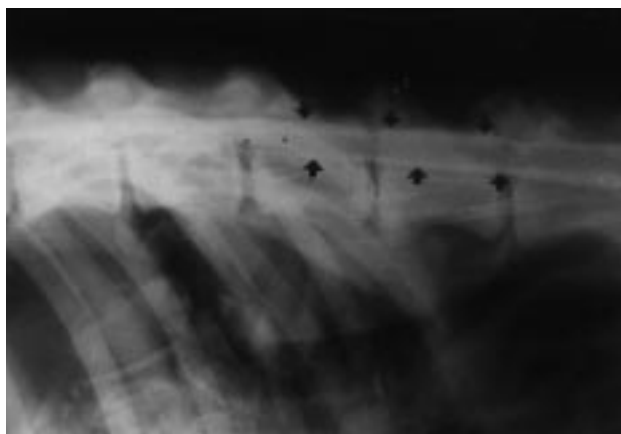


Figure 1. Regular appearance of the dorsal and ventral contrast column at the L1-L2 level in the myelography (Arrow) (20-week SSAGS group).

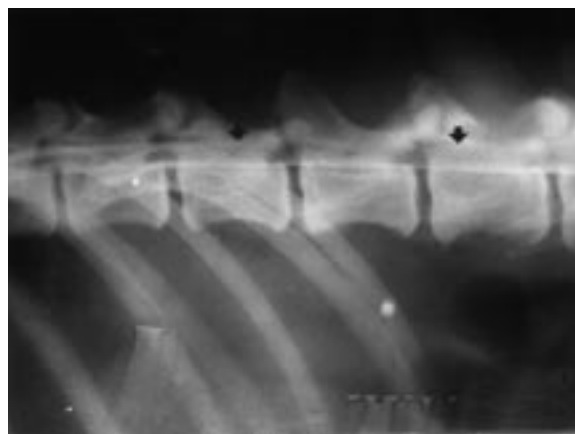


Figure 2. Blockage of the dorsal contrast column at the L1-L2 level in the myelography (Arrow) (20-week AGS group-necropsy).

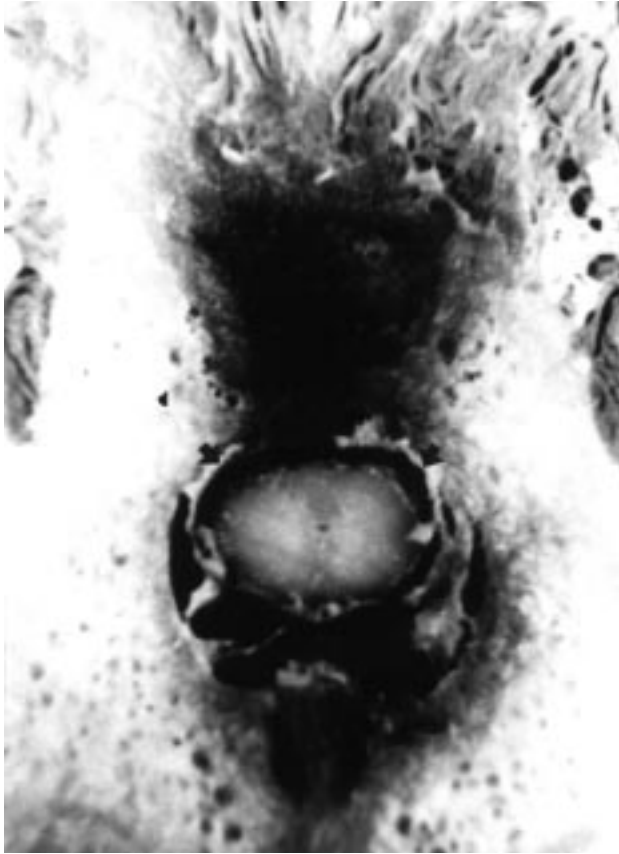


Figure 3. Slightly dislocated spinal cord from its central position of the vertebral canal and bonding duramater to the fibrous tissue in the operation site and reforming the subarachnoid space (20-week SSAGS group-necropsy).

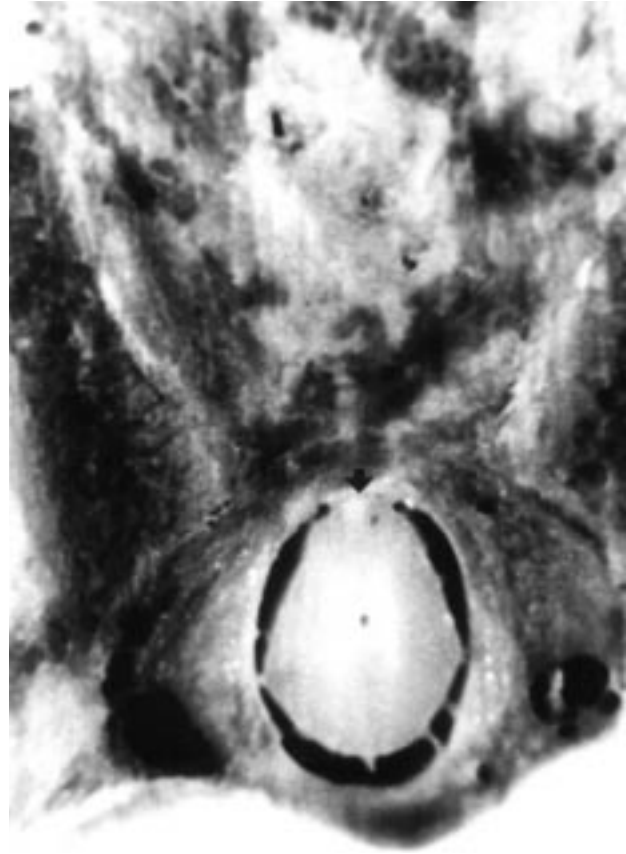


Figure 4. Displacement of the spinal cord due to the bonding of the duramater and fibrous tissue, and by the retracting fibrous tissue (20-week AGS group-necropsy).

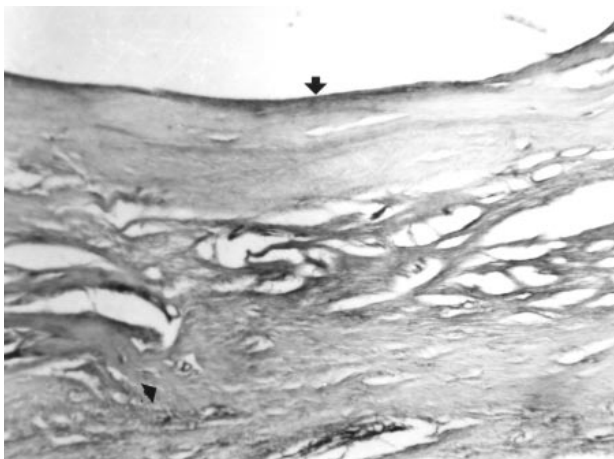


Figure 5. Fibrous band formation (Arrow) on the dural defects with hyalinized fibrous tissue and trabecular bone formation (Arrow head) (H&E x16), (20-week SSAGS group)

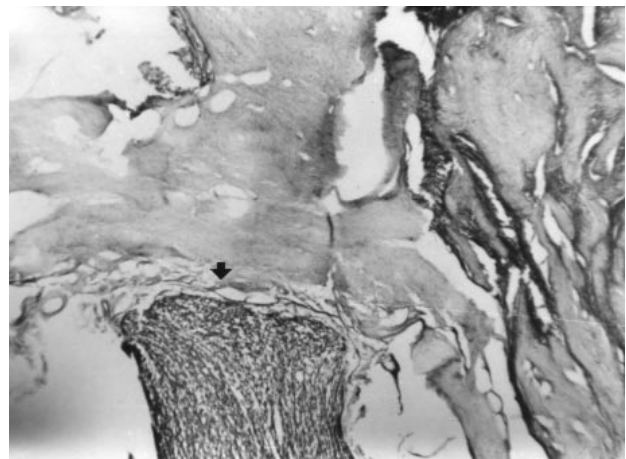


Figure 6. The bonding of dorsal part of spinal cord to the hyalinized fibrous tissue in the laminectomy defect (Arrow) (H&E x 16) (20-week AGS group).

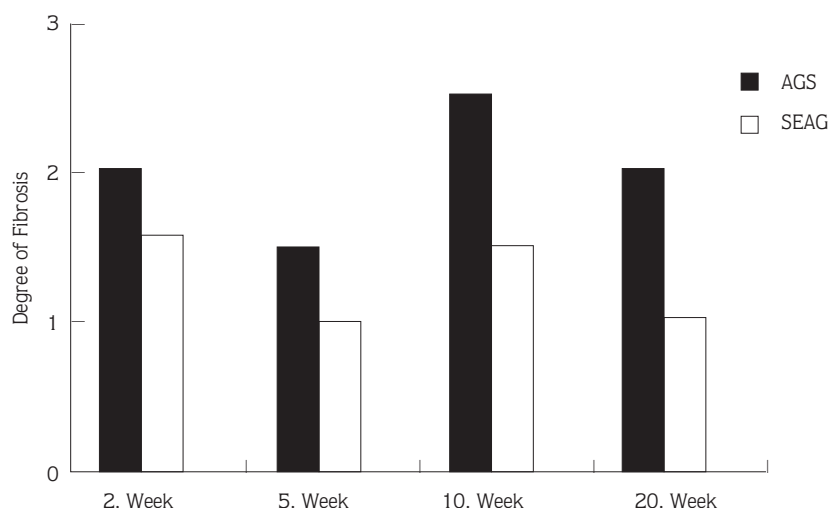


Figure 7. Mean fibrosis at the laminectomy defect of the dogs according to observations periods (+ : Slight, ++: Moderate, +++: Severe).

suppressing fibroblast activity and barrier formation are methods accepted by the majority of authors (1,4,16). Each attempt focused on either biological or nonbiological material for the purpose of making a barrier to fibrosis, and thus, reduction of fibroblastic activity. Although AGS has been used routinely by some authors (1,27), it is criticized due to its exaggerated and deleterious fibrous tissue response (1,15). Hyaluronic acid in SF serves as a lubricant, protecting articular cartilage and soft tissue surfaces during joint functions (28). It also has an important role in the extracellular regulation of the migration of inflammatory cells. Hyaluronic acid inhibits the migration of lymphocytes, macrophages and granulocytes in in-vitro studies (29). The movement and multiplication of the fibroblasts were also inhibited by the high molecular weight of hyaluronic acid. It has also been reported that hyaluronic acid decreases the adhesion in other areas such as around traumatized tendon (25). SF was preferred because it includes hyaluronan and extracellular matrix (collagen, proteoglycan, fibronectin etc.) and is cost effective. Controlled release of SF for longer activity was thought possible with soaked AGS. Another reason to prefer AGS was the quality, which does not vary, in contrast to a biological material such as fat tissue, which differs in quality in each dog.

Despite the fact that the dural tear results in meningitis and pseudocyst in humans (30,31), durotomy is suggested for diagnostic, prognostic and therapeutic purposes in veterinary neurosurgery (32). When durotomy is performed on a healthy dog, temporary neurologic deficit can be seen, and healing of the wound will be second intention (4). Postoperative neurological

deficits in our study were in correlation with those in the literature. In myelography, the irregular appearance of the dorsal column was concluded to be a result of the dural attachment to fibrous tissue and irregular enhancement radioopacity at the border of fibrous tissue. The dural flap was also seen to be adhered to the spinal cord and dorsally located fibrous tissue in some AGS applied dogs. However, restructuring of the dural tube was observed in SSAGS applied dogs in almost all periods. Both the macroscopic and histologic evaluations were consistent with the radiological findings. These findings supported the idea that reformation of the dural sac started with adhesion of the spinal cord to the fibrous tissue (4). The authors thought that the healing process continued at the dorsal part between dural flaps and fibrous tissue, and consequently in the durotomy site. During the healing process, the fibrous tissue was raised by the cerebrospinal fluid from the ventral to the dorsal aspect of the subarachnoid space and thus, adhesion between duramater and spinal cord was resolved. When the inflammatory process was severe and produced fibrous tissue compressed to the dural sac, adhesion developed between the duramater - spinal cord and fibrous tissue, which filled the laminectomy defect irreversibly. In light of this, it can be suggested that SSAGS can reduce fibrosis and establish the restructuring of the dural sac properly. Myelography was found to be a useful method for the interpretation of the epidural fibrosis in dogs.

Narrowing of the vertebral canal at the laminectomy defect and deviation of the spinal cord situation in the vertebral canal had been reported in AGS implanted dogs

(33). These results were in correlation with those of the previous study and, in the neurologically and physically normal dogs, spinal cord deviation from its normal location was present in both groups at the necropsy.

On histologic evaluation, a constant sequence of events will be found in the AGS implanted laminectomy defect. Initially it is filled with cellular elements of blood. Leukocyte infiltration start on the 3<sup>rd</sup> day, continuing until the 3<sup>rd</sup> week, during which some remnants of the AGS can be seen. AGS had disappeared by the 6<sup>th</sup> week (16). In the present study, some remaining AGS was observed in the 2<sup>nd</sup> week but there was no evidence of AGS in the

5<sup>th</sup> week in both groups. When both groups were compared histologically according to epidural fibrosis at the early stages, cellular activation and in the late period the amount of fibrosis were remarkably lower in the SSAGS group than in the AGS group.

In conclusion, according to radiologic and histologic findings, it can be suggested that SSAGS can decrease postlaminectomy fibrosis in dogs. These findings indicate that future studies in this field could explain the prevention of postlaminectomy membrane which causes recurrent low back pain after laminectomy.

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