The Influence of Experimentally Induced Osteoarthrosis on Articular Nerve Fibers of the Sheep Temporomandibular Joint

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Aims: *To study the effect of experimentally induced osteoarthrosis, or non-inflammatory degenerative changes, on the innervation of the sheep temporomandibular joint (TMJ) through the use of indirect immunohistochemistry and image analysis quantification.* **Methods:** *Bilateral condylar scarification was performed in 8 sheep, which were killed at 16 weeks post-operation; 3 unoperated sheep served as controls. Tissues from 8 osteoarthrotic joints and 4 control joints were processed for the immunostaining with antisera for protein gene product 9.5 (PGP 9.5), substance P (SP), calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and tyrosine hydroxylase (TH). An additional 10 joints were decalcified to study the morphologic changes induced by the condylar abrasion.* **Results:** *Osteoarthrotic changes were commonly seen in the anterior and lateral regions of the joint and included fibrosis, peripheral osteophyte formation, cysts, and erosion of articular surfaces. In the osteoarthrotic joints, the distribution of PGP 9.5-, CGRP-, and SP-immunoreactive (IR) nerve fibers was similar to that observed for control joints in the capsule, synovium, and capsule/disc junction. There were statistically detectable decreases in the percent surface area of IR nerve fibers in the capsule for both PGP 9.5 and CGRP in arthrotic joints compared with control joints. The lateral and anterior regions of the capsule had greater density of PGP 9.5- and CGRP-IR nerve fibers than other parts of the capsule in both control and arthrotic joints, and the medial capsule was poorly innervated in all joints. Immunostaining for substance P was always weaker.* **Conclusion:** *This study suggests that while inflammatory arthritis has a marked influence on the density of sensory and autonomic nerve fibers in synovium in a variety of joints in different species, experimentally induced non-inflammatory osteoarthrosis in the sheep TMJ also leads to a depletion of the density of nerve fibers in the capsule, especially in the lateral part of the joint. Further work is required to determine whether other parts of the joint, such as synovium and marrow, respond differently to experimentally induced osteoarthrosis.*

J OROFAC PAIN 2001;15:206–217.

Key words: calcitonin gene-related peptide, substance P, protein gene product 9.5, neuropeptide Y, nerve fibers, temporomandibular joint, osteoarthrosis

The role of the nervous system in joint diseases has become an important focus for arthritis research in the last 10 years. Recent developments in immunocytochemical methods have shown that neuropeptides within articular nerves play an important role in the physiology and metabolism of synovial joints both in normal and pathologic conditions. Decreased

nerve fiber immunoreactivity to antisera for substance P (SP), calcitonin gene-related peptide (CGRP), and protein gene product 9.5 (PGP 9.5) in inflammatory arthritis has been shown in a variety of joint tissues in humans and experimental animals.[1–4] Limited, contradictory data are available for the influence of osteoarthritis on joint innervation; for example, while degenerative changes associated with collagenase-induced arthritis have been shown to be associated with reduced SP and CGRP immunoreactivity in periosteum and synovium in mouse knee joints,[5] osteoarthritis did not deplete nerve fibers immunoreactive (IR) for SP or CGRP antisera in human synovium.[1]

Degenerative changes associated with osteoarthritis or rheumatoid arthritis are common in temporomandibular joint (TMJ) pathology and often feature pain along with disruption of normal joint movements. To gain a better understanding of TMJ pathology, the Japan/Australia TMJ research group at the University of Adelaide has used Merino sheep as an experimental model for TMJ dysfunction in humans.[6] Bosanquet and Goss[7] used sheep since this species has many advantages, including availability, ease of handling, and size similar to that of human TMJ, compared to other animal models used for studying TMJ pathology. Ishimaru and Goss[8] produced mild condylar scarification in sheep TMJs to induce changes characteristic of stage 3 osteoarthrosis in humans (eg, fibrosis, osteophyte formation, subcortical cysts, and articular surface erosion). In this model there is no inflammation and thus strictly is an osteoarthrosis rather than an osteoarthritis. This strict use of terminology is not, however, universally used, particularly by authors in the United States. To better understand the pathologic processes associated with arthritis of the TMJ, there is a need for detailed information on the innervation of TMJ tissues in normal as well as arthritic joints. The observations regarding innervation of the normal fetal and adult TMJ in the sheep were presented in Tahmasebi-Sarvestani et al.[9,10]

The effects of osteoarthritis on the innervation of TMJ are not known. In the present study, osteoarthrosis was surgically induced in the sheep by mild condylar scarification. The influence of osteoarthrotic changes on the innervation of the TMJ was investigated by immunohistochemistry and image analysis techniques.

Materials and Methods

Animals

The TMJs of adult male Australian Merino sheep (about 2 years of age and approximately 60 kg in weight) were studied (n = 11). All animals were provided by the Gilles Plains Agriculture Research Centre, Adelaide, and had been bred specifically for experimental research. Ethical approval for this study was granted by the Animal Ethics Committees of the University of Adelaide and Institute of Medical and Veterinary Science. The animals were divided into 3 groups. In group 1, 3 animals were used as normal controls, with no surgical intervention. In group 2, bilateral condylar scarification was produced in 4 animals to study the effects of osteoarthrosis on joint morphology. In group 3, bilateral condylar scarification was produced in 4 animals to study the effects of osteoarthrosis on nerve fibers in the TMJ.

Surgical Procedures

All surgical procedures were followed in accordance with previously described procedures[7,8] that have been used routinely to induce changes similar to the osteoarthritic changes found in the TMJs of humans, eg, fibrosis, osteophyte formation, subcortical cysts, and articular surface erosion.

Sample Collection

At 120 days following the initial operation, the sheep were reanesthetized and the TMJs were perfused by an intra-articular injection of Zamboni's fixative (to preserve neuropeptide integrity). Animals were then killed by intravenous overdose of sodium pentobarbital (Lethabarb, Arnolds of Reading). Immediately, the right and left TMJs were removed en bloc with a band saw and immersed in the same perfused fixative (Zamboni's fixative) for 24 hours.

Ten of the joint blocks (2 control and 8 experimental) were decalcified in a solution containing 9.5% hydrochloric acid and 1% sodium acetate in saturated ethylenediaminetetraacetate (Sigma Corporation) for 2 weeks. Conventional plain radiographs were used to assess decalcification. Decalcified blocks were sectioned in the sagittal plane into lateral, central, and medial parts. These were then embedded in paraffin, cut at 10 μm, and stained using hematoxylin and eosin, Masson's trichrome, alcian blue, or van Giesson's method for studying the effects of osteoarthrosis on the general morphology of the TMJ.

Table 1 Primary Antibody Characteristics

Antisera	Code number	Dilution	Host species	Source	References for specificity
PGP 9.5	2582	1/600	Rabbit	Ultraclone, Cambridge, United Kingdom	Gulbenkian et al[51]
SP	1657	1/400	Rabbit	Hammersmith Hospital	Hökfelt et al[52]
SP	RMSP-1	1/800	Rabbit	R. Murphy	Gibbins and Morris[53]
CGRP	1204	1/500	Rabbit	Hammsersmith Hospital	Gibson et al[54]
CGRP	6006-N	1/2,000	Rabbit	Peninsula	Kummer et al[55]
NPY	RMJ263	1/600	Rabbit	C. Maccarone and B. Jarrot	Morris et al[56]
TH	LNC-1	1/2,000	Rabbit	Incstar	Gibbins and Matthew[14]
VIP	F1/111	1/1,000	Rabbit	R. Murphy	Morris and Gibbins[57]

PGP 9.5 = Protein gene product 9.5; SP = substance P; CGRP = calcitonin gene-related peptide; NPY = neuropeptide Y; TH = tyrosine hydroxylase; VIP = vasoactive intestinal peptide.

Immunohistochemistry

Tissue Processing. After fixation, a total of 12 joints (4 control and 8 operated joints) were processed for the indirect immunofluorescence procedure according to Coons et al.[11] For each joint, the disc, capsule, and attached synovial membrane were removed intact and divided into anterior, posterior, lateral, and medial pieces. These pieces were washed in 3 changes of 80% ethanol to remove unbound picric acid and were then cleared in dimethyl sulfoxide before being stored in 0.1 mol/L phosphate-buffered saline (PBS) (pH 7.4) containing 20% sucrose as a cryoprotectant. Pieces of tissue were mounted in Tissue Tek (Miles Inc) on metal stubs and frozen at –30°C. Cryostat sections (30 µm thickness) were mounted on slides coated with 3-aminopropyl-triethoxy-silane (Sigma) and allowed to dry overnight at room temperature. After treatment with 0.3% Triton-X 100 (Rohmand Haas Co) in PBS, pH 7.4 for 30 minutes, the sections were incubated for 1 hour with diluted normal blocking serum, which was prepared from the same species in which the secondary antibody was raised.

During immunohistochemical procedures, control sections were processed in parallel except that they were incubated in normal serum instead of primary antisera. No immunoreactivity was observed in control sections.

Single Labeling. For the single-labeling method, after the excess serum was blotted, the sections were incubated overnight with antisera for PGP 9.5, CGRP, or SP with a dilution of 1:500 in a solution consisting of 1% bovine serum albumin and 0.05% sodium azide in 0.1 mol/L PBS. After 3 washings in PBS for 30 minutes each, the sections were incubated in the dark for 1 hour with goat fluorescein isothiocyanate- (FITC) labeled anti-rabbit gamma G immunoglobulin (Sigma) diluted to 1:60 in PBS at room temperature. Then the sections were rinsed thoroughly in PBS for 30 minutes and sealed with coverslips using PBS and glycerin. Sections were viewed with an epifluorescence microscope fitted with filters for FITC fluorescence, and images were captured and analyzed with the use of the Video-Image analysis system. Some details of the antisera, including the immunospecificity of the reaction and cross-reactivities, are presented in Table 1.

Double and Triple Labeling. In addition to single labeling, double- and triple-labeling immunofluorescence[12–14] was used to visualize more than 1 antigen simultaneously in a single tissue section. This technique was used to visualize the coexistence of SP, vasoactive intestinal peptide (VIP), tyrosine hydroxylase (TH), neuropeptide Y (NPY), CGRP, and PGP 9.5 immunoreactivities. After preincubation with normal blocking serum, the sections were incubated for 24 hours with a mixture of 2 primary antibodies for double labeling and a mixture of 3 primary antibodies for triple labeling, each raised in a different species. After being washed with PBS, the sections were incubated for 1 hour with 2 or 3 species-specific secondary antibodies, which were all raised in donkeys (Jackson Immunoresearch) and were conjugated with 7-amino-4-methylcoumarin-3-acetic acid (AMCA), dichlorotriazinylamino fluorescein (DTAF), or indocarbocyanine (Cy3). These antibodies were also diluted in hypertonic PBS. The sections were viewed with a fluorescence microscope fitted with filters selective for the red fluorescence of Cy3, the green fluorescence of DTAF, or the blue fluorescence of AMCA. All images were captured with image analysis and saved onto an optical disc.

Quantification and Statistical Analysis

The disc and attached capsule from each of 12 joints (4 control and 8 arthrotic) were cut into 4 blocks (anterior, posterior, medial, and lateral). The 3 consecutive sections from each block that had the cleanest immunolabeling were selected for quantification. From each section, 32 fields (chosen as a manageable number of fields) were systematically sampled by dividing the section into 32 regions and selecting 1 field of view from the center of each region at a 10× objective magnification. The fluorescent image was analyzed through the use of the Video-Image analysis system, which included a color Panasonic camera attached to a BH-2 Olympus microscope and a modified version of the software package Video Pro 32 (Leading Edge Australia). Immunostained nerve fibers exhibited a bright fluorescence, which the Video-Image analysis system was programmed to detect. The width and percentage surface area of IR nerve fibers were recorded.

The results for different regions of control and arthrotic TMJs were analyzed for statistical comparison with *t* tests for independent samples; a P value $< .05$ was accepted as significant. The Levene test using SPSS software was used to test for equality of variances. The Mann-Whitney nonparametric analysis of variance was used to test for differences between total arthrotic and control data for each stain.

Results

Morphology of the Osteoarthrotic Temporomandibular Joint

The articular surfaces of the temporal fossa and the articular condyles of normal, healthy TMJs had a smooth, uniform texture and even margins (Fig 1a). The arthrotic joints were characterized by abnormalities, mainly in the condylar surfaces (Figs 1b and 1c). Macroscopically, the articulating surface of the glenoid fossa appeared normal. Erosions and outgrowths were observed on all the condylar surfaces (Figs 1b and 1c), but these deformities were different from one animal to another, even between the left and right joints of the same animal. The osteoarthrotic changes were commonly seen in the anterior and lateral regions of the condyle. In only 1 joint had these deformities extended into the posterior part of the condyle.

Microscopically, erosions of the articular surface and cysts were obvious (Figs 1b and 1c). There was marked proliferation of the synovial connective tissue in the anterolateral parts of 2 joints, making the joint space narrower. In 1 joint, the space was completely obliterated by fibrous ankylosis. No disc perforation was present in all joints examined. However, the peripheral parts of the lateral and anterior parts of the discs were either folded or sharply thinned.

Immunocytochemical Observations

Normal Sheep TMJ Tissue. Table 2 presents an overview of the relative abundance of IR nerve fibers in the TMJs of sheep. In normal control sheep, the anterior part of the TMJ capsule was densely innervated by PGP 9.5- and CGRP-IR nerve fibers. Substance P-IR fibers were less frequently observed. Bundles of PGP 9.5-IR fibers were seen in the deep stroma and joint capsule. Immunoreactive nerve fibers contained CGRP and SP but not VIP in triple staining. No TH immunoreactivity could be found in the capsule, in double staining with NPY antisera. Nerve fibers IR to NPY and VIP antisera were mainly found perivascularly in the anterior part of the capsule.

In the synovium, fibers were located either as plexuses around blood vessels or as free fibers lying parallel to the synovial lining. Substance P-, CGRP-, and PGP 9.5-IR fibers were often seen immediately beneath the lining synovium, while some fibers even penetrated between the lining synoviocytes. No VIP-, NPY-, and TH-IR fibers could be located in the synovium with double- and triple-labeling methods.

Nerve fibers IR for PGP 9.5, CGRP, and SP antisera were found in the peripheral part of the disc, at the disc/capsule junction and mainly in the anterior side. Thin varicose fibers were present. No TH, VIP, or NPY immunoreactivity could be seen in any parts of the disc.

Arthrotic Joints. Immunoreactivity for PGP 9.5, SP, and CGRP antisera was found consistently in the nerve fibers of the anterior, posterior, lateral, and to a lesser degree in the medial part of the capsule; in the peripheral disc; and in the synovial tissue of all osteoarthrotic TMJs (Table 2). The anterior part of the joint capsule had dense networks, both free and perivascular, of nerve fibers that were strongly IR to antisera for PGP 9.5 or CGRP (Figs 2a to 2h). Substance P-IR fibers were seen less frequently. Nerve fibers originated mostly from the surrounding connective tissue and terminated at the site of the attachment of the capsule to the disc. Fibers related mostly to the blood vessels. Some arteries displayed dense neural plexuses (Figs 2b

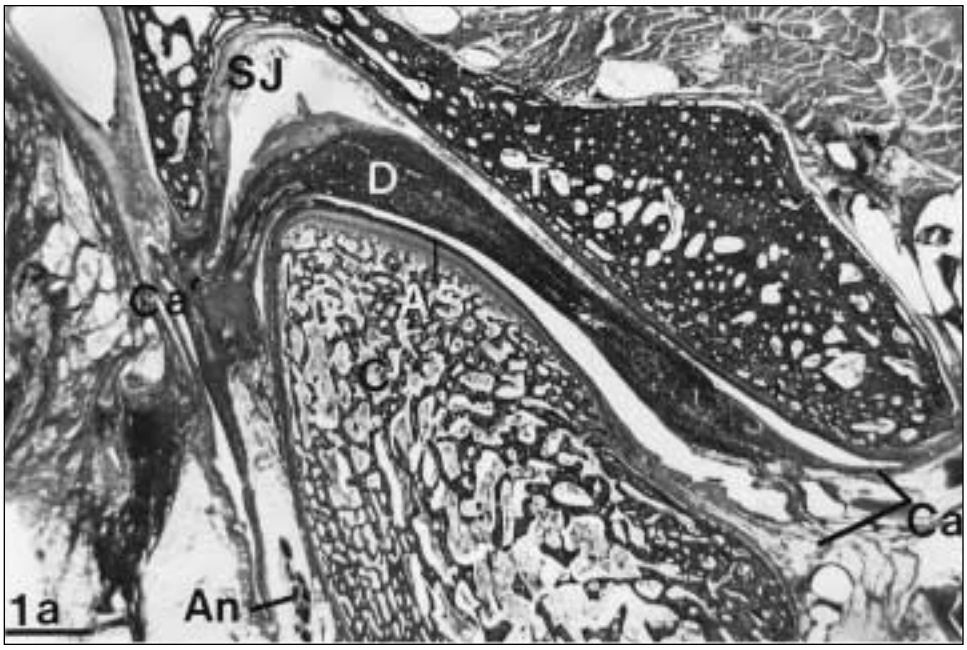


Fig 1a Sagittal section of decalcified, silver-impregnated right TMJ of an adult sheep. The section shows the articular surface (AS), disc (D), superior joint cavity (SJ), capsule (Ca), and auriculotemporal nerve (An) (bar = 2 mm).

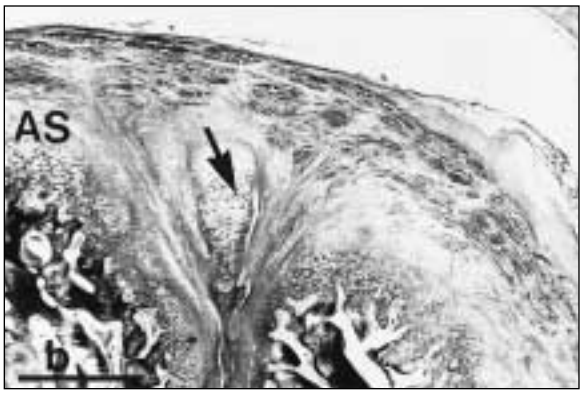


Fig 1b Light micrograph of arthrotic TMJ tissue stained with Masson's trichrome. The micrograph shows the articular surface (AS). The arrow indicates area of chrondrocyte proliferation in degenerated area of the articular surface (bar = 15 µm).

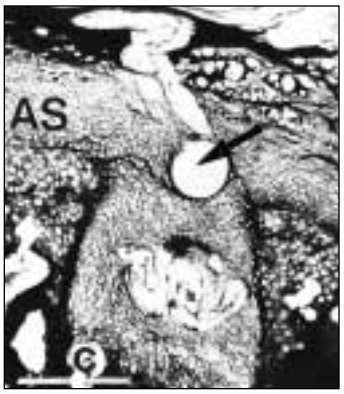
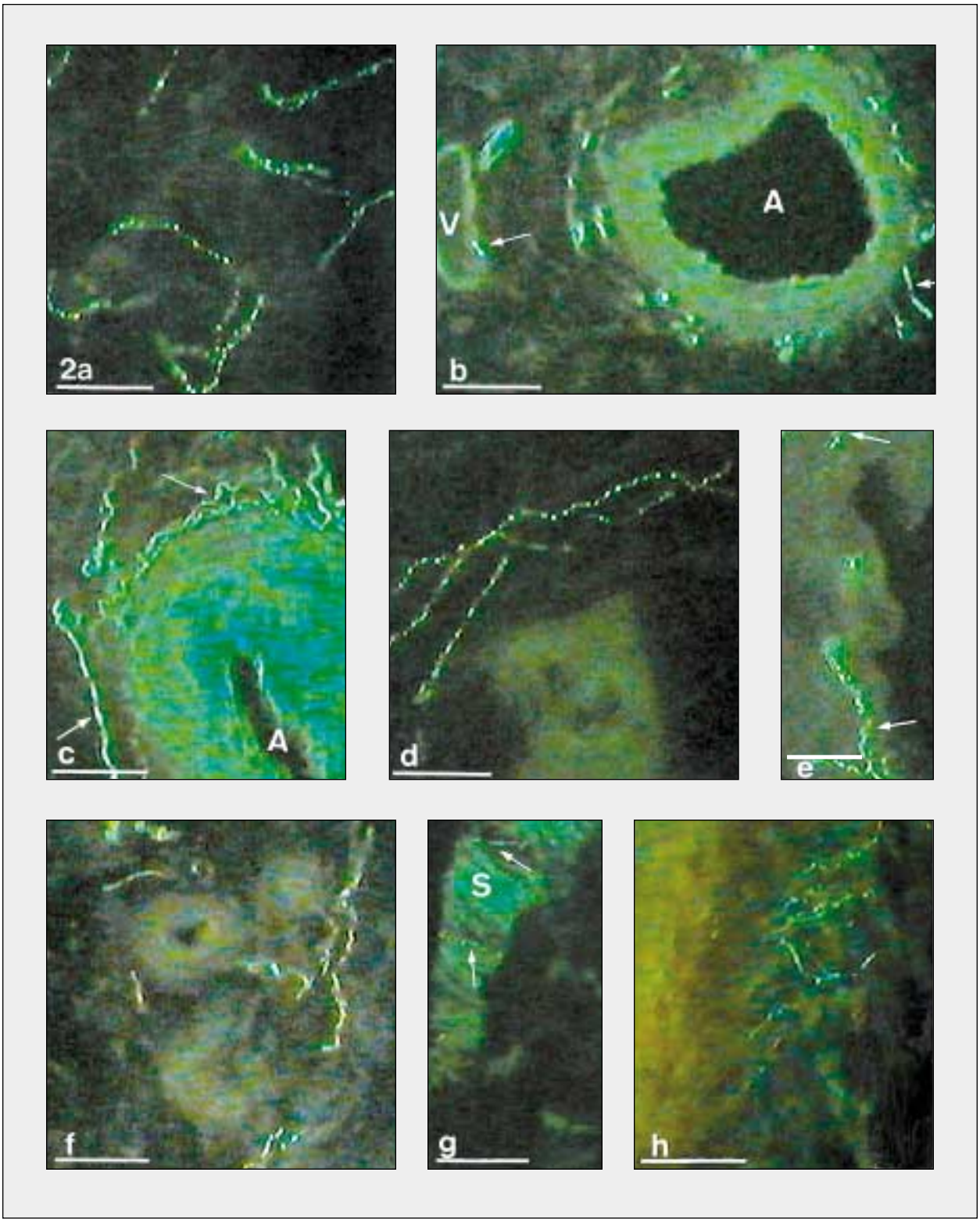


Fig 1c Light micrograph of arthrotic TMJ tissue stained with Masson's trichrome. The micrograph shows the articular surface (AS) of the condyle. *Arrow* = a cyst (bar = 15 µm).

Table 2 Relative Abundance of Immunoreactive Nerve Fibers in Different Tissues Within the Sheep Arthrotic TMJ

	PGP 9.5		CGRP		SP		TH		VIP		NPY	
Location	N	A	N	A	N	A	N	A	N	A	N	A
Capsule	+++	++	++	+	+	+	–	–	+	+	+	+
Synovium	+	+	+	+	+	+	–	–	–	–	–	–
Disc, periphery	++	++	+	+	+	+	–	–	–	–	–	–
Disc, central	–	–	–	–	–	–	–	–	–	–	–	–

PGP 9.5 = Protein gene product 9.5; CGRP = calcitonin gene-related peptide; SP = substance P; TH = tyrosine hydroxylase; VIP = vasoactive intestinal peptide; NPY = neuropeptide Y; N = normal control joints; A = arthrotic joints.



Figs 2a to 2h Immunofluorescence micrographs of arthrotic adult sheep TMJ capsules. The micrographs show PGP 9.5-IR fibers (2a to 2e) and CGRP-IR fibers (2f to 2h). S = synovium; A = artery; V = vein. Arrows indicate nerve fibers accompanying vessels (2b and 2c), nerve fibers in capsule near osteophytes (2e), and nerve fibers penetrating the synovium (2g) (bar = 20 μm).

Table 3 Percent Surface Area Measurements for Immunoreactive Nerve Fibers in the Sheep TMJ Capsule

Antisera	Region	Control	Arthrosis	Significance
CGRP	Anterior	0.47 ± 0.16 (64)	0.19 ± 0.05 (192)	$P < .05$
CGRP	Lateral	0.44 ± 0.24 (32)	0.10 ± 0.03 (160)	$P < .05$
CGRP	Posterior	0.09 ± 0.06 (32)	0.12 ± 0.04 (192)	NS
CGRP	Medial	0.10 ± 0.07 (32)	0.08 ± 0.03 (128)	NS
CGRP	Total	0.316 ± 0.08 (160)	0.127 ± 0.02 (672)	$P < .05$
PGP 9.5	Anterior	0.62 ± 0.20 (128)	0.48 ± 0.11 (192)	NS
PGP 9.5	Lateral	0.82 ± 0.46 (64)	0.14 ± 0.04 (192)	$P < .05$
PGP 9.5	Posterior	0.13 ± 0.06 (64)	0.19 ± 0.07 (192)	NS
PGP 9.5	Medial	0.16 ± 0.06 (64)	0.12 ± 0.04 (160)	NS
PGP 9.5	Total	0.47 ± 0.12 (320)	0.24 ± 0.04 (736)	$P < .05$

Comparisons were made between the control and arthrotic TMJs. Each value corresponds to mean ± standard error of the mean (number of observations). CGRP = calcitonin gene-related peptide; PGP = protein gene product.

and 2c). Veins had fewer fibers. The innervation of the lateral part of the osteoarthrotic joint was denser than that in the medial part. In the medial side of the joint, the few nerve fibers present showed PGP 9.5 and CGRP immunoreactivity, and most of these were perivascular.

The synovium had a sparse innervation compared to that of the capsule and the peripheral disc. Both varicose and non-varicose nerve fibers were observed. Substance P-, CGRP-, and PGP 9.5-IR fibers were seen in subintimal synovium, and some terminated between the lining cells (Figs 2g and 2h).

There seemed to be fewer nerve fibers in parts of the TMJ that were most affected by the degenerative changes, eg, the capsule near osteophytes. In these areas, there was an accumulation of blood vessels, and some PGP 9.5-IR nerve fibers were seen (Fig 2e). The density of PGP 9.5- and CGRP-IR nerve fibers appeared to be decreased in arthrotic joint capsules near the areas of bone that displayed osteoarthrotic changes compared to the control joints.

Immunoreactive nerve fibers showed CGRP and SP but not VIP or NPY in triple staining. No immunoreactivity for TH antiserum could be detected. The distribution pattern of nerve fibers showing immunoreactivity for VIP and NPY antisera resembled that seen for SP but was found exclusively in perivascular locations.

Quantitative Observations

There were insufficient data to quantify the SP immunoreactivities. It was not possible to get sufficient data for quantitative analysis of the synovium, so only data for the capsule are presented.

Percentage Surface Area. There was a significant decrease in the percentage surface area of PGP 9.5- and CGRP-IR nerve fibers in the capsule of TMJs from sheep with experimentally induced osteoarthrosis compared to control tissues (Table 3). The data also showed that the main influence of the osteoarthrosis was in the lateral region of the TMJ capsule. There were no significant differences in the percentage surface area of PGP 9.5- and CGRP-IR nerve fibers in the posterior or medial areas of the capsule. While there was also a significant decrease in the percentage surface area of CGRP-IR nerve fibers in the anterior region of the capsule, the decrease in PGP 9.5-IR nerve fibers in the anterior region was not statistically significant. In both control and arthrotic adult TMJs, the anterior and lateral regions of the capsule had the highest density of IR nerve fibers for both PGP 9.5 and CGRP antisera. In addition, the percentage surface area of PGP 9.5-IR fibers was higher than that for CGRP-IR fibers.

Width of Nerve Fibers. The PGP 9.5-IR fibers were significantly wider in arthrotic tissues compared to control tissues; however, there was no difference between the nerve fiber widths of control and arthrotic tissues for CGRP antisera (Table 4). The distribution of nerve fiber widths showed that 72% of nerve fibers were less than 6 μm in width and 12% were less than 2 μm in width.

Discussion

Many studies have induced osteoarthrosis in animal models but only a few have used the TMJ.[8,15] Silberman[16] used mice to study the osteoarthritic changes in TMJ by an intraarticular injection of steroid. Others, like Merjersjo and Kopp,[17] induced osteoarthritis in the guinea pig. Yaillen et

Table 4 Width Measurements of Immunoreactive Nerve Fibers in the Sheep TMJ Capsule

Antisera	Region	Control	Arthrosis	Significance
CGRP	Anterior	3.67 ± 0.43 (33)	5.42 ± 0.34 (44)	NS
CGRP	Lateral	5.13 ± 0.44 (12)	5.22 ± 0.52 (20)	NS
CGRP	Posterior	3.33 ± 0.74 (7)	5.87 ± 0.70 (13)	$P < .05$
CGRP	Medial	4.17 ± 0.74 (8)	6.12 ± 0.52 (9)	NS
CGRP	Total	4.84 ± 0.28 (21)	5.53 ± 0.29 (57)	NS
PGP 9.5	Anterior	3.67 ± 0.42 (33)	5.42 ± 0.34 (44)	NS
PGP 9.5	Lateral	6.06 ± 0.57 (13)	4.96 ± 0.51 (11)	$P < .05$
PGP 9.5	Posterior	4.57 ± 0.09 (3)	6.19 ± 0.53 (14)	$P < .05$
PGP 9.5	Medial	6.28 ± 0.09 (2)	4.89 ± 0.38 (8)	$P < .05$
PGP 9.5	Total	3.99 ± 0.28 (60)	5.51 ± 0.24 (86)	$P < .05$

Comparisons were made between the control and arthrotic TMJs. Each value corresponds to mean ± standard error of the mean (number of observations). CGRP = calcitonin gene-related peptide; PGP = protein gene product.

al[18] and Helmy et al[19] used surgical methods to study osteoarthritic changes in monkey TMJs, and Ishimaru and Goss[8] used sheep, since this species had many advantages over other animal models for studying TMJ pathology. These authors considered the sheep a good model for study of osteoarthrosis in the TMJ because the degenerative changes induced by surgery were characteristic of osteoarthritis in humans.

Degenerative changes associated with osteoarthrosis are common in human TMJ pathology and often involve pain as well as disruption of normal joint congruity and normal joint movements.[20,21] Synovial inflammation, fibrosis, osteophyte formation, and various cytoskeletal changes are features of these diseases.[22] The osteoarthrotic-type changes described in the present study (fibrosis, osteophyte formation, and subcortical cysts) resemble the changes reported by Ishimaru and Goss[8] using the same technique of mild condylar scarification in sheep. In both studies there were variations in the severity of these degenerative changes in different joints.

In the present study, qualitative assessments of the effect of experimentally induced degenerative joint disease (osteoarthrosis) on the sheep TMJ suggested that the density of nerve fibers IR to antisera for PGP 9.5, CGRP, and SP was less in areas of the arthrotic TMJ capsule, which showed marked degenerative changes, than in the normal capsule. When data from all areas of the capsule were examined, not just those areas that showed marked osteoarthrotic changes, there was a statistically significant effect of the arthrosis on the percent surface area of PGP 9.5- and CGRP-IR nerve fibers. This effect was most pronounced in the lateral region of the capsule.

Few comparable data are available regarding the effects of arthritis on peripheral nerve fibers. Several studies have reported increased levels of neuropeptides in synovial fluid from patients with rheumatoid arthritis compared to synovial fluid from individuals without rheumatoid arthritis.[23–25] Menkes et al[26] and Hernanz et al[27] reported much higher levels of SP immunoreactivity in synovial fluid from patients with rheumatoid arthritis than synovial fluid from patients with osteoarthritis. In addition, Menkes et al[26] found that the SP immunoreactivity levels in rheumatoid arthritic synovium were less than those in osteoarthritic synovium. These results confirmed a role of SP in joint inflammation and further supported the contention that active secretion of SP into the synovial fluid in the inflamed tissue depletes SP in the nerve fibers.[26]

Lundeberg et al[28] recorded an increase in the levels of SP, CGRP, neurokinin A, and NPY in the TMJ fluid of humans bilaterally following unilateral injection of carrageenan, which induced inflammation and cartilage changes similar to osteoarthritis in humans. This was interpreted to indicate that carrageenan-induced osteoarthritis activates both sensory and sympathetic nervous systems in the TMJ.[28] Collagenase-induced osteoarthritis in the mouse knee joint was characterized by sclerosis of the subchondral bone, osteophyte formation, synovial proliferation, and cartilage abrasion.[5] While no differences could be found in the innervation of the joint capsule and the medullary cavity in normal and arthritic joints, the innervation was depleted at locations where arthritic changes were severe, eg, the periosteum at some locations, and the synovium.[5] In the present study there were fewer nerve fibers IR for PGP 9.5,

CGRP, and SP in areas of the capsule near areas of the joint that showed marked osteoarthrotic change, such as near osteophytes. Grönblad et al[1] found no difference in the density of nerve fibers IR to SP and CGRP in the synovium of human patients with normal or osteoarthritic joints, but noted a decrease in immunoreactivity for these antisera in synovium from rheumatoid (inflammatory) arthritis. Hukkanen et al[4,29] observed a depletion of nerves IR for antisera to PGP 9.5, SP, and CGRP in the synovium of rat ankle joints with severe adjuvant-induced polyarthritis (inflammatory arthritis). Rheumatoid human synovium from knees and metatarsophalangeal joints has a reduced innervation, as determined by PGP 9.5, SP, CGRP, and NPY and its C terminal flanking peptide (C-PON) immunoreactivity, compared to normal synovia.[30] Similarly, Konttinen et al[2] noted that nerve fibers immunostained by antisera to PGP 9.5, SP, and CGRP were depleted in inflammatory arthritic synovium compared to normal synovium. Mapp et al[3,31] noted decreased density of perivascular fibers and weaker immunostaining for PGP 9.5, SP, CGRP, and C-PON antisera in the synovia of arthritic rats compared to synovia of normal rats.

The limited data suggest that while inflammatory arthritis has a marked influence on the density of sensory and autonomic nerve fibers in synovium, the influences on other areas of the joint are not clear. Osteoarthritis may induce some changes in the synovial innervation, but this is not consistent in the various studies reviewed. Some of these inconsistencies may be a result of the failure of any of these studies to consider the influence of nerve regeneration as part of the arthritic changes. In 1997 Imai et al[32] reported that CGRP-IR and PGP 9.5-IR fibers in the synovium of tarsal joints of rats undergo an initial phase of degeneration, followed by a phase of regeneration, within 3 weeks of inoculation with Freund's adjuvant to induce arthritis. The ultrastructural appearance of the regenerated axons differed from that characteristic of normal nerve fibers. Similarly, using the adjuvant arthritic rat model, da Silva et al[33] observed a progressive reinnervation of periarticular tissues, epiphysis, and synovium of the ankle joint once the clinical arthritis subsided. The reinnervation often exceeded the density of nerve fibers in control animals. Yoshida et al[34] also noted that synovitis of the human TMJ was associated with intense SP-IR in the synovium. While the influence of the osteoarthritic changes in the TMJ of the sheep on the innervation of the capsule of this joint, as outlined in the present work, is consistent with data reported in the literature, the present study highlights the variable effect of the arthritic changes in different parts of the joint capsule. Additional research is needed to determine the extent to which regeneration of nerve fibers occurs in the model of osteoarthrosis used in the present study.

Type IV receptors are noncorpuscular endings that belong to small, myelinated (< 6 μm diameter) A-delta or unmyelinated C-type nerve fibers.[35] They are the most numerous of the 4 categories of receptors found in the TMJ in humans,[36] cats,[35] rhesus monkeys,[37] mice,[38] rats, guinea pigs, and rabbits.[39] In the present study, the majority of nerve fibers (72%) located in the sheep TMJ capsule were less than 6 μm in width. Many of these probably have a nociceptive role and may also serve polymodal mechanoreceptive functions.[38] Some of these fibers would have been unmyelinated sympathetic efferents.

Antisera for SP and CGRP have been used to localize sensory nerve fibers in adult TMJ tissues from rats,[40,41] monkeys,[42] and sheep.[9] Antiserum for PGP 9.5 (a neuron-specific protein) has been used in many studies to detect sensory and autonomic fibers, small nerve endings, and corpuscles in a variety of tissues, including the TMJ.[43,44] In the present study of sheep TMJ capsules from control and arthrotic animals, more nerve fibers were revealed by PGP 9.5 immunoreactivity than by CGRP immunoreactivity. It is likely, therefore, that the additional nerve fibers stained by PGP 9.5 antiserum compared to the CGRP antiserum were autonomic fibers. Since the average width of PGP 9.5-IR nerve fibers in the present study was significantly larger in the arthrotic capsule than the normal capsule, the decrease in density of nerve fibers in arthrotic tissues may have been the result of loss of more of the smaller C-fibers. There was no significant difference in the average width of nerve fibers stained by CGRP antiserum; hence it is likely that the increase in average width of PGP 9.5-IR fibers in the arthritic capsule resulted from loss of the autonomic C-fibers. A review by Schaible and Grubb[45] noted that 80% of articular nerve fibers are unmyelinated and that 50% of these are sympathetic efferent fibers. Large nerve bundles (branches of the masseteric nerve) innervating the TMJ of the sheep do not contain autonomic nerve fibers.[9] Schmid[46] found that autonomic nerve fibers in a branch of the otic ganglion entered the medial side of the capsule in the human TMJ. He presumed that sympathetic fibers came from a sympathetic plexus of the internal maxillary artery that joined the otic ganglion.

Autonomic nerve fibers characteristically form plexuses in the walls of arteries and accompany arterioles and capillaries in joint capsules as well as form plexuses separate from vessels in the capsule and synovial membrane.[9] A retrograde tracing study by Widenfalk and Wiberg[47] revealed an "impressive" number of sympathetic efferents to the rat TMJ that originated from the superior cervical ganglion. These authors hypothesized that nociceptive input from the TMJ could modulate activity in sympathetic efferents, which normally have a vasomotor role.

Both PGP 9.5 and CGRP immunoreactivity demonstrated that the lateral and anterior regions of the capsule of the sheep TMJ were more densely innervated than other areas of the capsule in normal and arthrotic joints. When gold chloride, silver, and methylene blue techniques are used, the richest innervation occurs in the posterior/posterolateral part of the TMJ capsule in the cat,[48] mouse,[38] monkey,[37] and human.[49] Kido et al,[41] using immunocytochemical techniques for the peptides SP and CGRP, found the highest density of neural elements in the anterolateral part of the capsule of the rat TMJ. Johansson et al[42] found SP-IR nerve fibers to be sparser in the posterior capsule than in other parts of the monkey TMJ. Qualitative observations of normal sheep TMJ[9] showed the greatest density of CGRP-IR fibers (using an immunoperoxidase technique) in the anterior capsule. It seems that the asymmetric arrangement of nerve fibers in different parts of the joint is characteristic of the TMJ. As highlighted by Haeuchi et al,[50] there appear to be species differences in the distribution of nerve fibers in the TMJ.

Work by Ishimaru et al[8,15] has shown that the degenerative effects of the surgical procedures used to induce osteoarthrotic changes do differ from animal to animal and from side to side. In addition, the effects of the degenerative changes on the density of nerve fibers vary in different parts of each joint. For example, decreases in nerve fiber density were seen in the areas most affected by the osteoarthrosis, such as near osteophytes.

Ishimaru and Goss[8] established that the experimentally induced osteoarthrosis of the sheep TMJ is a good model for understanding arthritis in the TMJ of humans. However, there are insufficient studies of the peptidergic innervation of the TMJ of humans or other primates to make meaningful comparisons with the data collected in the present study in sheep. The innervation of the sheep TMJ reflects that seen by Johansson et al[42] in relation to the sparse innervation of the posterior capsule but resembles the human TMJ in the rich innervation in the anterolateral aspects of the capsule that has been described by Haeuchi et al.[50] As Morani et al[44] recognized, the existence of a rich innervation of the human TMJ capsule emphasizes the need for further characterization of the nerve fibers in regard to type, function, and neuropeptide content. Much work remains to be done to elucidate the distribution and function of the innervation of the TMJ, especially with respect to species variation.

This study suggests that while inflammatory arthritis has a marked influence on the density of sensory and autonomic nerve fibers in the synovium of a variety of joints in different species, experimentally induced non-inflammatory osteoarthrosis in the sheep TMJ also leads to a depletion of the density of nerve fibers in the capsule, especially in the lateral part of the joint.

Acknowledgments

The skillful technical assistance of Ms G. Hermanis, Mr C. Leigh, Ms S. E. Matthew, and Mr R. Murphy is gratefully acknowledged. The first author would like to thank the Shiraz Medical Science University and Ministry of Health and Medical Education, Tehran, Iran, for providing a scholarship, without which this work would not have been possible. We thank Dr M. Hukkanen and the Royal Postgraduate Medical School, London, England, for providing some of the antisera used for the immunostaining. We also thank Professor I. Gibbins, Department of Anatomy and Histology, Flinders University of South Australia, for allowing us to use the facilities of his laboratory and for providing antisera for the double- and triple-labeling work.

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