The Ultrastructure of the Human Spinal Nerve Root Cuff in the Lumbar Spine

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BACKGROUND: Spinal nerve root cuffs may be relevant in selective nerve root and epidural blockade.

METHODS: We examined the ultrastructural aspects of spinal nerve root cuffs, such as their cellular and fibrillar components, using special histological staining methods, transmission and scanning electron microscopy, from six human cadavers.

RESULTS: The morphology of the spinal nerve root cuff resembles that of the spinal subdural compartment. Cells gather together in compact layers due to specialized junctions. The thickness of its cellular layers is 5 to 8 microns; cells appear oriented parallel to the direction of their own nerve roots. The fibrillar component, made largely of collagen fibers, is found in the outer part of the spinal nerve root cuff and measures 100 to 150 microns. Numerous adipocytes separate dural laminas in concentric layers, extending from the dural sac to the spinal nerve root ganglia. However, adipocytes are not found within the thickness of the dural sac.

CONCLUSIONS: The presence of few capillaries and the short distance between fat and axons may affect the passage of epidurally injected substances towards nerve root axons.

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Spinal nerve root cuffs are lateral prolongations of the dura mater and arachnoid lamina enclosing spinal nerve roots in their way across the epidural space towards the vertebral foramina. Nerve root cuffs have previously been termed "dural sleeves," but this term may now be considered inaccurate because the arachnoid is also part of the root cuffs.[1,2] The contribution of spinal nerve root cuffs in the distribution of local anesthetics injected in the epidural space is of interest to anesthesiologists performing epidural blocks. The complex junction at which roots leave the subarachnoid space and enter the nerve root sheath and dorsal root ganglion is particularly relevant to epidural anesthesia. Bromage predicted that local anesthetics crossed arachnoid villi within spinal nerve root cuffs, reaching cerebrospinal fluid and nerve roots.[1] Such predictions have been challenged, and it is now suggested that local anesthetics diffuse across the arachnoid lamina to reach the subarachnoid space.[3] Spinal nerve root cuffs are also relevant in selective nerve root blockade. Here, the route of injection may be either outside or inside the vertebral foramina in order to block specific nerve roots.[4,5]

Epidural fat has been regarded as the store place for lipophilic drugs injected in the epidural space. However, it is unknown what influence epidural fat may exert over the kinetics of injected local anesthetics. Although the presence of variable amounts of adipocytes in the epineurium of peripheral nerve fascicles has been described,[6,7] similar investigations of the existence of fat in spinal nerve root cuffs have not been performed.[2,8,9] We studied the ultrastructural aspects of spinal nerve root cuffs such as their cellular and fibrillar components, with emphasis on fat deposits that are in direct contact with nerve roots.

METHODS

Approval from the research ethics committee, and family consent for the donation of organs for autopsy and procedures included in this research were obtained. The dural sac and its contents at thoracic and lumbar levels were extracted from six fresh human cadavers between 48 and 78-yr-of-age. Causes of death such as chronic medical or surgical illnesses were excluded. Time lapse from death to the fixing of samples was less than 1 h in the donor group and bodies were refrigerated between 8 to 12 h in the autopsy group. After organ donation in three cadavers and autopsy of the others, anterior laminectomies were performed; the dural sac and spinal nerve root cuffs were isolated. The lower thoracic and lumbar spinal dural sacs with their corresponding spinal nerve roots and cuffs (T_{11}-L5) were excised to a

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distance of 1 cm from the vertebral foramen. Samples were cut including portions of dural sac and nerve roots with their cuffs. Ventral attachments of the dura to the spinal canal wall at the level of the discs were surgically dissected to avoid damage of the dural laminas. Samples were labeled with sutures to identify their orientations outside the spinal canal. Longitudinal and transversal cuts were performed on spinal nerve root cuffs and their nerve roots from T_{12}-L4.

We studied the region of the spinal nerve root cuffs located between the dural sac and spinal ganglia. Histological staining techniques were used to identify structures under light microscopy and electron microscopy techniques were used to observe ultrastructural details.

Light Microscopy

Samples observed under light microscopy were fixed with 10% buffered formaldehyde. After fixation, cuts with a thickness of 4 microns were made with a microtome. Each spinal nerve root cuff component was identified using specific staining techniques under standard conditions. Masson's trichrome staining was used to identify collagen fibers and orcein staining identified elastic fibers. Fibroblasts were visualized with hematoxylin-eosin, and amorphous intracellular substance with periodic acid-Schiff combined with Alcian blue at a pH of 2.5.

Immune-Histochemistry: Leptomeningeal cells were identified with epithelial membrane antigen; adipose cells and myelin from Schwann cells, with anti-S100; and macrophages with anti-human macrophage. Immune staining was performed on paraffin-embedded tissue after the streptavidin-biotin-peroxidase technique. Sections with a thickness of 4 to 6 microns were cut, air-dried for 15 min, and heat-fixed at 58°C to 60°C overnight and, later, at 100°C for 10 min. Slides were deparaffined with xylene, dehydrated in decreasing concentrations of alcohol, and later washed in distilled water. Antigen retrieval was achieved in a pressure cooker for 90 s, treating the slides with H_2O_2 for 10 min eliminating endogenous peroxidase activity. Later, the slides were incubated for 30 min with diluted primary mouse antibodies, pre-diluted anti-S100 protein (Clona 4C4.9), pre-diluted epithelial membrane antigen (clona, ZCE, 113), and pre-diluted anti-human macrophage (CD68; clona KP1) (Master Diagnostica, Granada, Spain). The technique was performed in an automated immune histochemical Stainer (LAB Vision Corp., Master Diagnostica). Amino-ethyl-carbazole substrate solution was used to make the peroxidase reaction visible. Sections were counterstained with hematoxylin (Sigma) and wet mounted.

Scanning Electron Microscope

The samples were fixed by immersion for 4 h in 2.5% glutaraldehyde with a phosphate solution buffered at a pH of 7.28 to 7.32, and then dehydrated through repeated immersions in acetone 50%, 70%, 80%, 90%, 95% until a concentration of 100%. The acetone from the samples was exchanged with CO_2 in a closed pressurized chamber (Balzers CPD 030-Critical Point Dryer, Bal Tec AG, Fürstentum, Lichtenstein) at 31°C of temperature until the critical point of pressure of 73.8 bar was reached.

Tissue samples with a length of 20 mm were placed over slides 25 mm of diameter.

A carbon layer was then deposited on the samples to a thickness of <200 Amstrong with a Balzers MED 010 Mini Deposition System (Balzers, Bal Tec AG, Fürstentum, Lichtenstein). The evaporating carbon, upon passing an electrical current through a graphite electrode within a vacuum chamber, regulated to 10^{-5} millibars. The specimens were covered with a gold microfilm by circulating 20 amps electrical current through a gold electrode within a vaporization chamber SCD 004 Balzers Sputter Coater (Balzers, Bal Tec AG, Fürstentum, Lichtenstein) regulated to 0.1 millibars vacuum.

Afterwards, the specimens were studied with a JEOL JSM 6400 Scanning Electron Microscope (JEOL Corporation, Tokyo, Japan). Quality control for the fixation technique was checked against the fixation characteristics of biological cells of well-known morphology and size (e.g., erythrocytes), as these cells are present in the majority of the specimens of the meninges.

Transmission Electron Microscope

The specimens were fixed for 4 h in a solution of glutaraldehyde 2.5% and a buffer phosphate solution at a pH of 7.2 to 7.3. They were later fixed with a solution of 1% osmium tetroxide for 1 h. The specimens were dehydrated with increasing concentrations of acetone in water and soaked in resin epoxy, Epon 812. The resin was polymerized at 70°C for 72 h. Thin slides, 0.5 microns thick, were dyed with Richardson's methylene blue and observed by light microscopy. Ultra thin slides, 70 nanometers thick, were cut with an ultramicrotome and treated with 2% acetate of uranyl solution, as well as Reynold's lead citrate solution. These specimens were then observed under a Zeitz EM 902, transmission electron microscope (Carl Zeitz, Oberkochen, Germany).

RESULTS

Samples from donor cadavers were cut and fixed within an hour and compared with the samples obtained from other cadavers during autopsy. No lytic changes were identified in samples from bodies with longer postmortem intervals. Results obtained in both types of samples were similar. Ten lumbar spinal nerve roots and their cuffs were obtained from each body as follows: samples from 11 lumbar nerve roots and their cuffs were processed under standard staining and immune-histochemical techniques; samples from 21 roots and their cuffs were examined under scanning electron microscopy and another 17 were

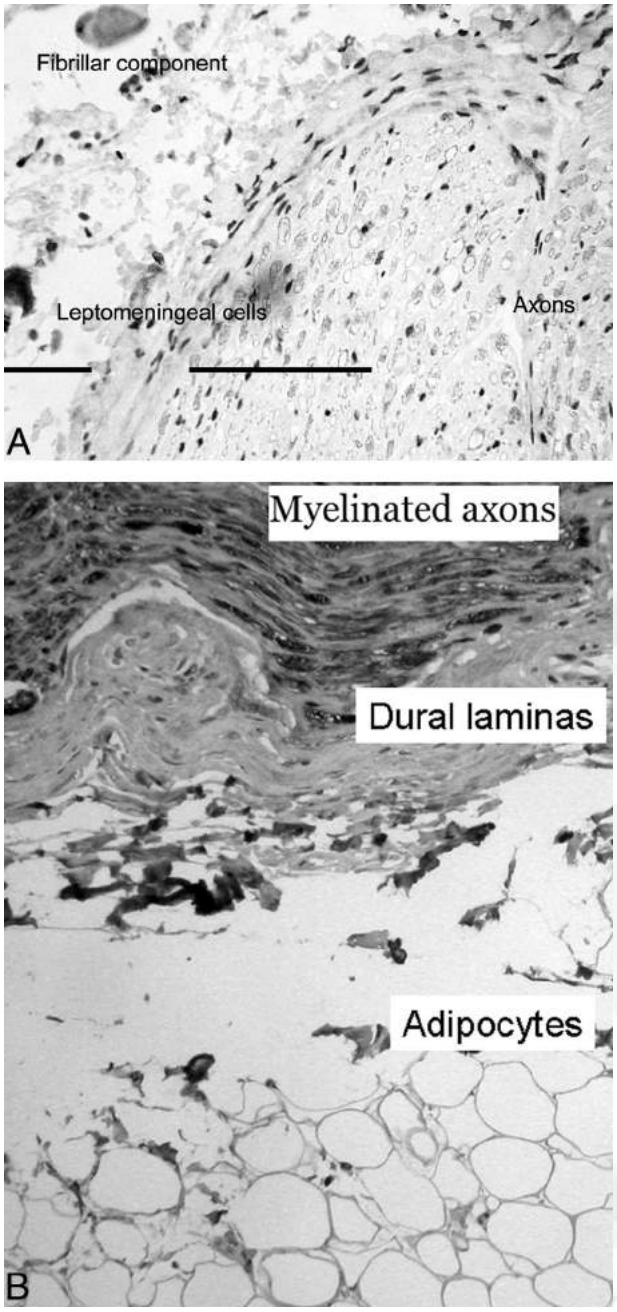

Figure 1. Spinal nerve root cuff. (A) and (B) Longitudinal cut dyed with immune histochemical technique. (A) Leptomeningeal cells with epithelial membrane antigen. (B) Adipocytes are seen inside the fibrillar component and myelin from Schwann cells with S-100 technique.

observed by transmission electron microscopy. Specific staining technique and/or type of microscope will be indicated in parentheses below for each specific structure obtained.

Only few adipocytes were seen in the outer surface of the dural sac since almost all the epidural fat and epidural veins remained attached to the periostium of the vertebral canal during dissection and extraction of the canal contents. The orifices giving exit to lumbar nerve roots are positioned in the anterior and lateral regions of the dural sac. Trabecular arachnoid tightly encloses anterior and posterior nerve roots; these arachnoid wrappings appear more compact near the orifices (scanning electron microscope).

With the immune-histochemistry technique, we observed that flattened leptomeningeal cells were brown-colored due to the presence of epithelial membrane antigen (Fig. 1A). Adipocytes inside the fibrillar component appeared magenta-colored, and myelin from Schwann cells appeared brown with S-100 technique (Fig. 1B).

The thickness of spinal nerve root cuffs cellular layers was 5 to 8 microns (transmission electron microscope). The cells appeared flattened and elongated, oriented parallel to the direction of their own nerve root and have a thickness 0.2 to 0.6 microns. Single cell layers joined together by specialized junctions, type desmosome, and tight junctions produced 8 to 14 cellular strata (Figs. 2A–C). Numerous vacuoles and highly developed rough endoplasmic reticulum were observed inside the cytoplasm and were very similar in all cellular layers. Also, there were small granules in their extracellular space (Figs. 2A–C). The inner cellular stratum was in contact with myelinated and nonmyelinated axons (transmission electron microscope, S-100) (Fig. 1B). A fibrillar component with a thickness of 100 to 150 microns was found in the outer portion of spinal nerve root cuffs. This was composed mainly of collagen fibers (Masson's trichrome) arranged in concentric laminas (scanning electron microscope) and contained scarce elastic fibers (orcein, scanning electron microscope). A large number of adipocytes (scanning electron microscope, S-100) could be seen dividing dural laminas in groups of 3 to 5 concentric layers (scanning electron microscope) (Figs. 3A–C). Adipocytes measure 50 to 70 microns, some were spherical, whereas others appeared flattened (Figs. 3D and 4). Adipocytes from longitudinal and transversal cuts were empty, displaying an image similar to a honey comb (Figs. 3A–D). Scanning electron microscopy showed the distribution of adipocytes, and these cells extended from the dural sac to the spinal nerve root ganglia. In the outer epidural surface of the spinal nerve root cuffs under scanning electron microscope, there were areas of collagen fibers belonging to dural laminas. Other areas showed spherical cells infiltrating dural laminas (Fig. 4). In two samples, few macrophages were seen between cells and collagen fibers (transmission electron microscope).

DISCUSSION

Using ultrastructural techniques, we studied the spinal nerve root cuff and identified leptomeningeal cells and fibrillar structure of dural laminas. Our examination reported findings that have not been observed in prior studies in humans, such as the presence of adipocytes inside spinal nerve root cuffs. In the cellular component, cells gather together in compact layers due to specialized membrane junctions, contributing to the barrier effect against the

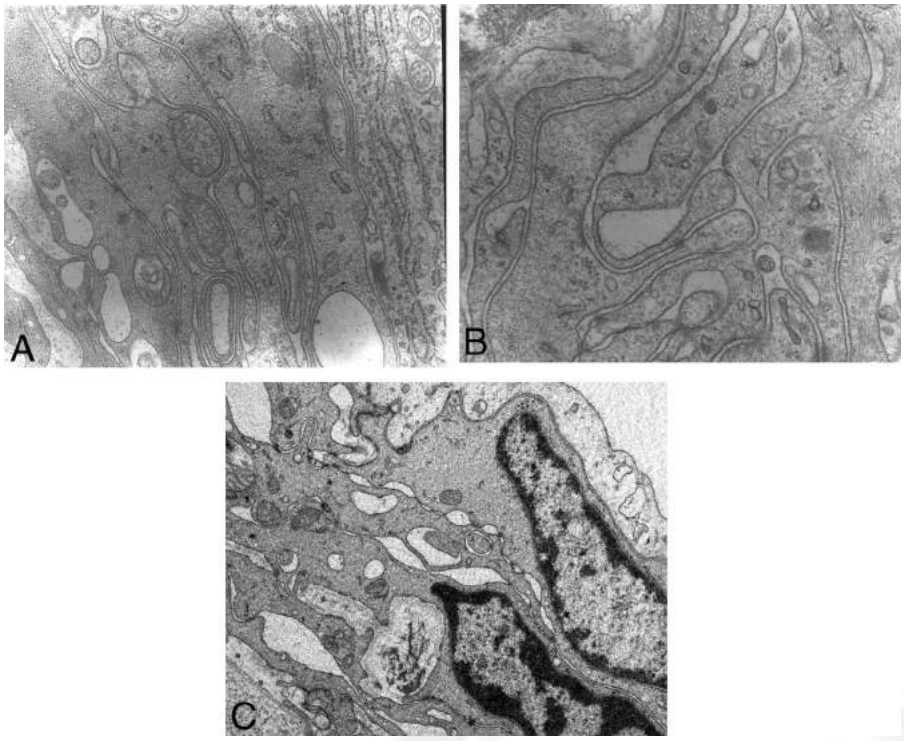

Figure 2. Cellular layers of spinal nerve root cuff. (A), (B), and (C) Flattened and elongated cells join together by specialized junctions in different samples. Transmission electron microscopy. Magnification: (A) and (C): ×20,000. (B) ×30,000.

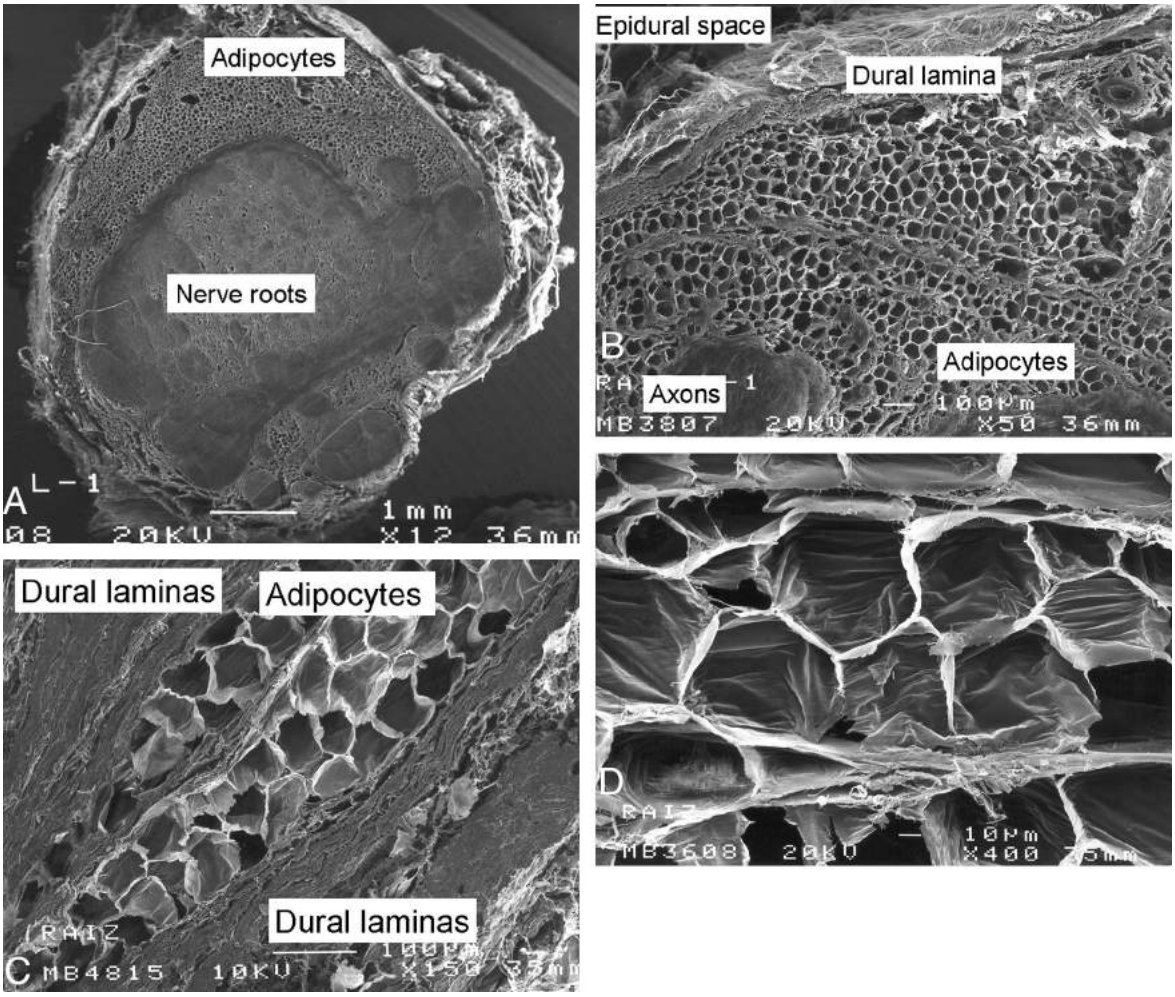

Figure 3. Spinal nerve root cuff. (A) Complete view of a nerve root cuff. Transversal cut. (B) and (C) Dural laminas and adipocytes. (D) Sample shows adipocytes cut in halves. Scanning electron microscopy. Magnification (A) ×12, (B) ×50, (C) ×150, and (D) ×400.

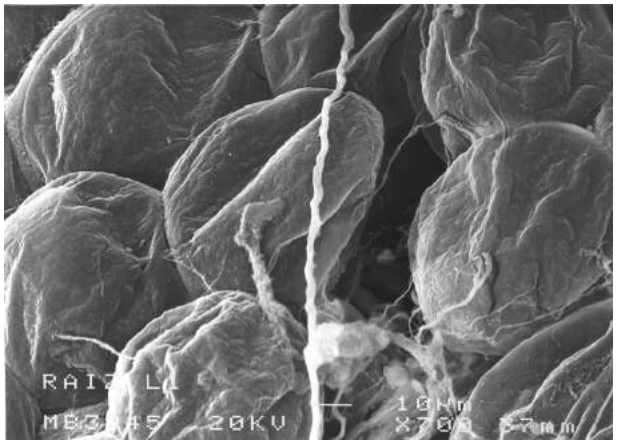

Figure 4. Spinal nerve root cuff. Adipocytes protruding through dural laminas, observed from the epidural space. Scanning electron microscopy. Magnification: ×700.

passage of substances towards nerve root axons. These elongated and flattened cells have cytoplasmic invaginations interposing with others from neighboring cells; thin granules and scanty collagen fibers occupy their interstitial space. Spinal nerve root cuffs cellular and interstitial morphology resemble that of the spinal subdural compartment in the dural sac. Such findings suggest the possibility of a transitional leptomeningeal cellular structure, probably due to their common embriogenic origins, shared also with neurothelial cells from the subdural compartment, arachnoid and pial cells.

In the dural sac, the fibrillar portion contains approximately 80 dural laminas,[10,11] with collagen fibers oriented in different directions[12,13] and few elastic fibers. Its thickness varies between 270 to 350 microns at the lumbar level.[14] However, adipocytes are not found within the thickness of the dural sac.[10,11] On the other hand, spinal nerve root cuffs fibrillar constituent has collagen fibers and adipocytes. Here, the fibrillar portion is thinner, about 100 to 150 microns of thickness; however, this difference may not account for changes in spinal nerve root cuffs barrier effect because the cellular component is exclusively responsible for such an effect. Adipocytes observed on a scanning electron microscope are similar to those found in peripheral nerve samples from human sciatic nerve[6]; the fact that they appear smaller and the absence of spherical shape in some of them is probably due to loss of fat from vacuoles during sample preparation. Fat inside spinal nerve root cuffs covers groups of nerve root axons, although no adipocytes are seen enclosing axons individually. This fat occupies either partially or totally the thickness of the spinal nerve root cuffs fibrillar component, depending on the zone studied.

Considering our findings about spinal nerve root cuffs ultrastructural aspects, we propose that if an accidental puncture occurs while performing transforaminal nerve root blockade,[15,16] the pressure exerted by injected substances could cause dissection of cellular planes, resulting in accidental migration of such substances into the spinal subdural compartment. In this case, the onset of subarachnoid effects would take several minutes, depending on its diffusion across the arachnoid lamina.

Hogan and Toth[17,18] studied the anatomy of soft tissue of the spinal canal and examined the epidural fat. Other authors[19,20] observed variability of distribution of epidural fat and changes produced by diseases. The concentration of epidural fat in the lumbar vertebral canal is not uniform, being greater in the posterior region and lesser in anterior and lateral zones.[19] Bernards et al.[21,22] reported that opioid drug kinetics depends on the drug partition coefficient in relation to the epidural fat. Fat located next to nerve root axons could influence the absorption and distribution of drugs injected in the epidural space. We support the hypothesis that, not only does the multiplicity of elements within a porous membrane affect local anesthetic action, so might the presence of fat inside spinal nerve root cuffs. Drug redistribution could be less marked at the level of the epidural space when compared with spinal nerve root cuffs because the latter structure has fewer capillaries and distances between fat and axons are shorter. Further research is needed to measure amounts of fat inside spinal nerve root cuffs, its distribution along the spinal canal, and its relationships with its neighboring root axons.

In conclusion, different histological techniques show ultrastructural aspects of cellular and fibrillar spinal nerve root cuffs constituents, as well as the presence of fat inside them.

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