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MORPHOLOGICAL STUDIES ON THE GUTTURAL POUCH OF DONKEY
II- THE SUBEPITHELIAL GLANDS

(With 12 Fig.)

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دراسات موفولوجيه على ردب الانبوب السمعى فى الحمار
٢ - الغدد تحت الطلائيه

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أجرى هذا البحث بغرض دراسة تركيب الغدد تحت الطلائيه لردب الانبوب السمعى فى الحمار باستخدام المجهر الضوئى والالكترونى .

أظهرت الدراسه أن الغدد تحت الطلائيه عباره عن غدد انبوبيه بسيطه متفرعه قليلة الالتفاف .

هذا وقد تميزت حبيباتها الافرازيه إلى نوعين ، نوع تفاعل بالايجاب مع معامل شيف ، يظهر مملؤ كلية أو جزئياً بماده متجانسه ذو عتامه الكترونيه مصحوبه بماده فاتحه الكترونياً .

أما النوع الثانى فقد اظهر تفاعلا ايجابياً مع كل من معامل شيف والالسين الازرق ويظهر كشبكه خيطيه بها وحدة أو وحدتين فرعيتين ذو عتامه الكترونيه .

هذا وقد احتوت الخلايا المفرزه على كميه غير معتاده من الشحميات فى الجزء السفلى منها ، هذا وقد لوحظت كريات الشحم المفرزه كامله فى القنيات الداخليه الخلويه وكذلك الوحشيه بين الخلويه مصاحبه للماده المفرزه أثناء عملية الافراز . وكذلك فى لمعات النهايات المفرزه والقنوات المفرغه .

وأظهرت الدراسه أيضاً أن الخلايا العضليه الطلائيه تحيط بالنهايات المفرزه وكذلك القنوات المفرغه .

هذا ويرى أن الغدد تحت الطلائيه لردب الانبوب السمعى فى الحمار تفرز كل من الشحم وعديد السكريات المخاطى .

SUMMARY

The subepithelial glands of the guttural pouch of the donkey were studied by light and electron microscopy. They were simple less coiled branched tubular glands. Their secretory granules were differentiated into two types: The PAS positive type which appeared ultrastructurally filled completely or partially with homogenous electron dense substance accompained by electron leucent material. The PAS- Alcian blue positive type which appeared ultrastructurally fillid with filamentous network with one or two electron dense subunites. The secretory cells were contained also unusual large amount of lipid which located mainly in their basal portion. The extruded fat globules have been observed as intact lipid globules either in the lateral intra- or inter cellular canaliculi accompaning the secretory substance in the process of exocytosis. They were also demonstrated within the lumina of the secretory end- pieces and excretory ducts. Myoepithelial cells were observed surrounding the secretory end- pieces and the excretory ducts. It was suggested that the subepithelial glands of the guttural pouch of the donkey secret both lipid and mucopolysaccharide.

Keywords: Subepithelial glands, guttural pouch, donkey.

INTRODUCTION

The strategical position of the guttural pouch and its numerous relations to the vital organs give it considerable importance. This is evident from the fact that even moderate disease processes of the pouch may lead to dysfunction of the related organs.

The mucosa of the guttural pouch is secretory (de *LAHNTA and HABEL, 1986).* The secretion may be derived from two sources, goblet cells which were scattered throughout the epithelium as previously described by *YOUSRIA* et al. *(1994b)* and the subepithelial glands which are seromucous *(BROWN, 1987)* or mucous and serous *(TRAUTMAN and FIEBIGER, 1957 and BANKS, 1993).*

From the available literatures, these subepitheial glands have received little attention about their histology and histochemistry. Therefore, the aim of this study is to

elucidate the histological, histochemical and ultrastructural features of subepithelial glands of the donkey guttural pouch.

MATERIAL and METHODS

The guttural pouches from 3 male and 3 female donkeys were used in this investigation.

For histochemical study, pieces of the guttural pouch were fixed in Bouin's fluid, sectioned after routine histological preparation and stained with alcian blue- PAS *(MOWRY, 1956)*.

For ultrastructural study, the tissue was cut into small pieces and fixed by immersion in 2.5% glutaraldehyde with 2.5% paraformaldehyde in 0.1M Na-cacodylate buffer (pH 7.3) for 2 hours. The pieces were washed in the same buffer, postfixed in buffered 1% osmium tetroxide, dehydrated in ascending grades of alcohol and embedded in Epon-Araldite. Semithin sections were cut on an LKB microtome and stained with toluidine blue and alcian blue- PAS *(BOECK, 1984)* for light microscopical examination. Ultrathin sections were doubly stained with uranyl acetate and lead citrate, examined and photographed with JEOL 100 CX II electron microscope.

RESULTS

The subepithelial glands of the donkey guttural pouch were simple less coiled branched tubular glands formed of secretory end-pieces and excretory ducts. The tubular end-pieces were composed of pyramidal shaped secretory cells, that were arranged around a wide irregular lumen and possessed large round or ovoid nuclei. The secretory cells were contained variable number of secretory granules as well as lipid droplets, that fill most of the cytoplasm of some cells (Fig. 1, 2).

The excetory ducts were exhibited a wider lumen and lined with cuboidal epithelial cells. Few of them were contained secretory granules as well as lipid droplets similar to those of the secretory end-pieces. Lipid was also observed within their lumina (Fig. 3). Myoepithelial cells have been observed surrounding both end-pieces and excretory ducts.

Histochemically, the granules of the secretory end-pieces as well as that of the excretory ducts were differentiated into 2 types; one was reacted positively with PAS that filling the apical portions of the cells. The other type was demonstrated positive reaction with both PAS and alcian blue and filling most of the cell cytoplasm (Fig. 4).

At the ultrastructural level, the secretory cells forming the end-pieces (Fig. 5 a,b) were generally contained abundant cisternae of rough endoplasmic reticulum either in the basal cytoplasm or distributed between the secretory granules and a well-developed supranuclearly located Golgi- apparatus, that consisted of variable number of cisternae and associated vesicles. Also moderate number of mitochondria, bundles of cytoplasmic microfilaments as well as polyribosomes were observed among the secretory granules.

These secretory granules were varied in their ultrastructural appearance. Two types of secretory granules were distinguished within the secretory cells. The first type (Fig. 5 & 6) was the more common one, that accumulated mainly in the apical portion of the cells. It was varied in size and form. Some were appeared filled completly with homogenous electron dense substance. Others were contained incomplete moon or elliptical formig homogenous electron dense substance accompained by electron leucent filamentous material locating on one or both sides respectively. The second type was seen in few cells (Fig. 7), their granules were of variable size and form consisting of filamentous network with one or two peripheral electron dense subunits. The secretory granules may coalesce with each other to form a secretory mass. Exocytosis of their content have been observed on the luminal surface or through the lateral intracellular canaliculi which extend deep into the cell cyloplasm (Fig. 8). In addition to the secretory granules, the cells of the end-pieces were contained also numerous lipid droplets as well as globules of variable size (Fig. 5, 7), that were accumulated mainly in the cell basal portion. Some cells were appeared distended with the lipid droplets, which fuse with each other forming large fat globules. The extruded fat globules have been observed either in the lateral intra- or intercellular canaliculi accompaning the secretory substance in the process of excretion (Fig. 8, 9) as well as within the lumina of the secretory end-pieces (Fig. 10) and excretory ducts.

The cell apical portions were provided with few short microvilli and joined together by tight junction (Fig. 10). Laterally, they were attached together with cytoplasmic interdigitations and desmosomes (Fig. 10). Basally, the plasma membrane of some cells showed basal folds that attached to the myoepithelial cell processes with desmosomes (Fig. 9) or to the basal lamina with hemidesmosomes. The basally located nuclei of the secretory cells were rounded or ovoid in shape with irregular contour. Sometimes they were compressed basally due

to the large accumulation of lipid globules. The nuclei were contained moderate amount of heterochromatin as well as one or two prominent nucleolei (Fig. 5).

The cuboidal epithelial cells lining the excretory ducts (Fig. 11, 12) were contained few number of rough endoplasmic reticulum cisternae and mitochondria. Supranuclearly located Golgi-apparatus and polysomes were also observed. Some cells were contained also secretory granules similar to that of the first type of the secretory end-pieces as well as lipid droplets. Others were completly distended with lipid globules which lead to deforming their nuclei (Fig. 12). The free surface of the lining cells was provided with few short microvilli (Fig. 12). Apico-latterally, they were joined together with tight junction. The lateral plasma membranes were attached together with cytoplasmic interdigitations and desmosomes. Basally they were attached to the basal lamina and to the myoepithelial cell processes with hemidesmosomes and desmosomes respectively.

Their basally located nuclei were large and rounded in shape with some identations. They were contained prominent nucleolei and large clumps of heterochromatin.

The myoepithelial cells were surrounded the secretory end-pieces (Fig. 9) as well as the excretory ducts (11, 12). They were attached to the basal lamina with hemidesmosomes and to the secretory cell processes with desmosomes. They were contained numerous myofilaments, few mitochondria and lipid droplets as well as flattened nuclei.

DISCUSSION

The present investigation revealed that the subepithelial glands of the guttural pouch of the donkey were simple less coiled branched tubular gland. Regarding to their histochemical characteristics, their granules were differentiated into PAS positive granules that located in the cell apical portion and PAS- Alcian blue positive granules filling most of the cell cytoplasm. Similar observations have been recorded in other glands as the submandibular gland *(SIRIGU et al., 1985)* and Harderian gland of one humped camel *(FATH ELBAB et al., 1990)*. These results indicate that the nature of the content of the subepithelial glands of the guttural pouch of donkey is composed of acid and neutral mucopolysaccharide. That the secretory granules within the secretory cells of the end-pieces as well as that of the excretory ducts were differentiated also into two types: The first type, which corresponds to the PAS positive granules appeared filled mostly with homogenous

electron dense substance similar to that described by *COWLEY and SHACKLEFORD (1970); TOYOSHIMA and TANDLER (1991) and NAGATO and NAGKI (1993)* in the submandibular gland of squirrel monkey, rodent and rat respectively. While the second type of granules that correspond to PAS- Alcian blue positive granules, appeared as network of filamentous material containing electron dense subunites resemble that recorded in the submandibular gland of pig, sheep, cow, rat *(GALLAGHER et al., 1969)* and cat *(SHACKLEFORD and WILBORN, 1970)* as well as esophageal gland of fowl *(YOUSRIA et al., 1994 a). SHACKLEFORD* and *WILBORN (1970)* have interpreted the filamentous structures of the granules as mucin molecules, while the electron dense core appear to be concentrated protein with highly ordered substructures *(LAVKER, 1969).*

In addition to the secretory granules, the subepithelial glands of the guttural pouch of the donkey contain also unusual amount of lipid which have been recorded not only as fat droplets or globules filling the cell basal portion but also as globules in the inter- or intracellular canaliculi accompanying the secretory substance in the process of excretion. They were also observed in the lumen of both end-pieces and excretory ducts. Concerning the presence of lipid within the secretory cells, *WOODHAUSE and RODIN (1963), HOFFMAN (1971), WOODING (1980) and ABUO ELMAGED et al. (1990)* have recorded certain amount of lipid within the Harderian glands of mouse, golden hamsters, rabbit and camel respectively. In contrast to the observation of *BOSHELL and WILBORN (1978)* in pig parotid gland that the lipid extrusion occurs in compination with the secretory substance. The unusual character of the guttural pouch subepithelial in donkey is the excretion of lipid as intact globules which may act as sobum reduce the possible entry of the microorganisms as well as keep the mucous membrane soft and pliable as mentioned by *TRAUTMAN and FIEBIGER, 1960 and BANKS, 1993.*

As obtained from the present study it is suggested that the subepithelial glands of the guttural pouch of the donkey secret a both lipid and mucopolysaccharide, which act as a protective coat on the surface epithelium of the guttural pouch.

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LEGENDS

Fig. 1,2: Semithin section of the subepithelial glands of the guttural pouch of the donkey showing the end-pieces contain secretory granules as well as lipid droplets (arrowheads). Myoepithelial cell (arrow). **X 25.**

Fig. 3: Semithin section of an excretory duct with wide lumen contains lipid (arrowhead). **X 25.**

Fig. 4: Semithin section of the subepithelial glands of the guttural pouch demonstrating secretory granules reacting positively with PAS- Alcian blue. **X 25.**

Fig. 5 a,b: Electron micrograph of a glandular end-piece of the subepithelial glands showing the first type of secretory granules (Sg) and lipid droplets (L) in the basal portion. Lumen (Lu), nucleus (N). **X 2700.**

Fig. 6: Higher magnification of the first type of secretory granules showing the incomplet moon of the electron dense substance accompained by electron leucent filamentous material. **X 8000.**

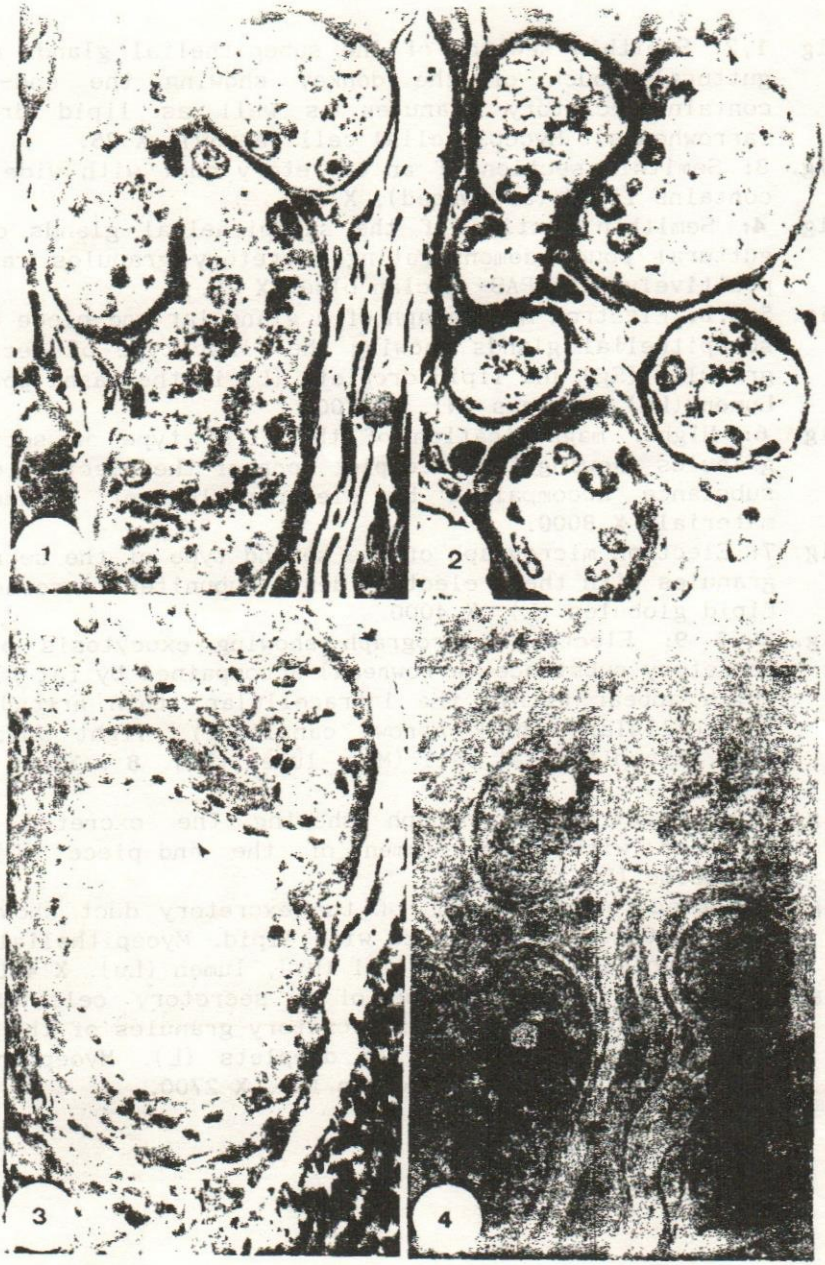
Fig. 7: Electron micrograph of the second type of the secretory granules with their electron dense subunites (arrowheads). Lipid globules (L). **X 4000.**

Fig. 8 & 9: Electron micrograph showing exocytosis of the secretory substance (arrowhead) accompained by fat globule which appear within the intracellular (thin arrow) or intercellular (thick arrow) canaliculi. Tight Junction (Tj), myoepithelial cell (My), lumen (Lu). **8 : X1000 ; 9: X 4000.**

Fig. 10: Electron micrograph showing the excreted lipid globules within the lumen of the end-piece (L). Desmosome (D). **X 10000.**

Fig. 11: Electron micrograph of the excretory duct showing a cell that completly filled with lipid. Myoepithelial cell (My), nucleus (N), microvilli (Mv), lumen (Lu). **X 4000.**

Fig. 12: Higher magnification of a secretory cell of the excretory duct filled with secretory granules of the first type (arrowheads) and lipid droplets (L). Myoepithelial cell (My), lumen (Lu), nucleus (N). **X 2700.**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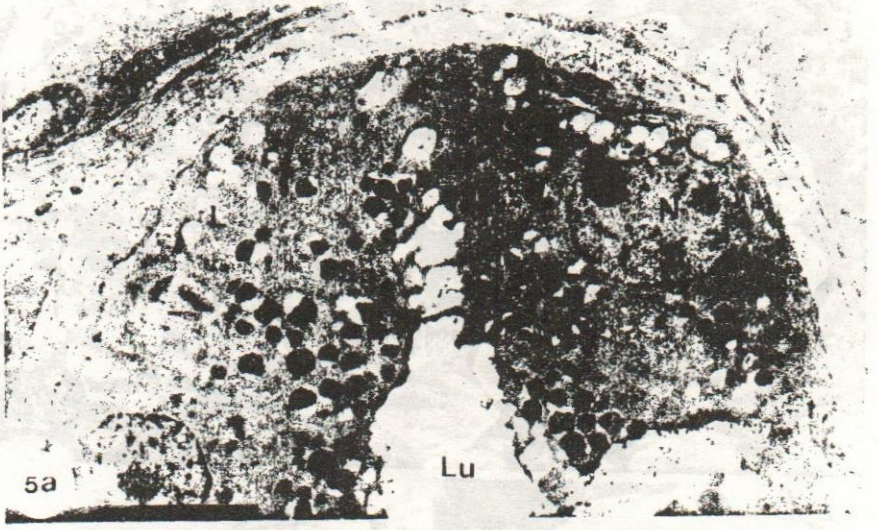

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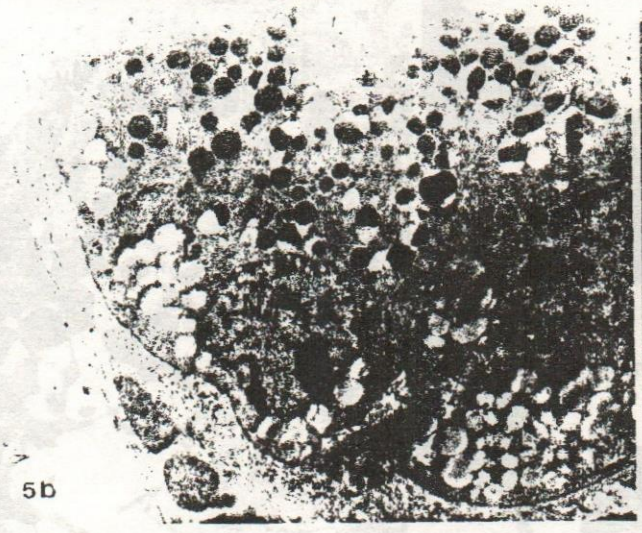

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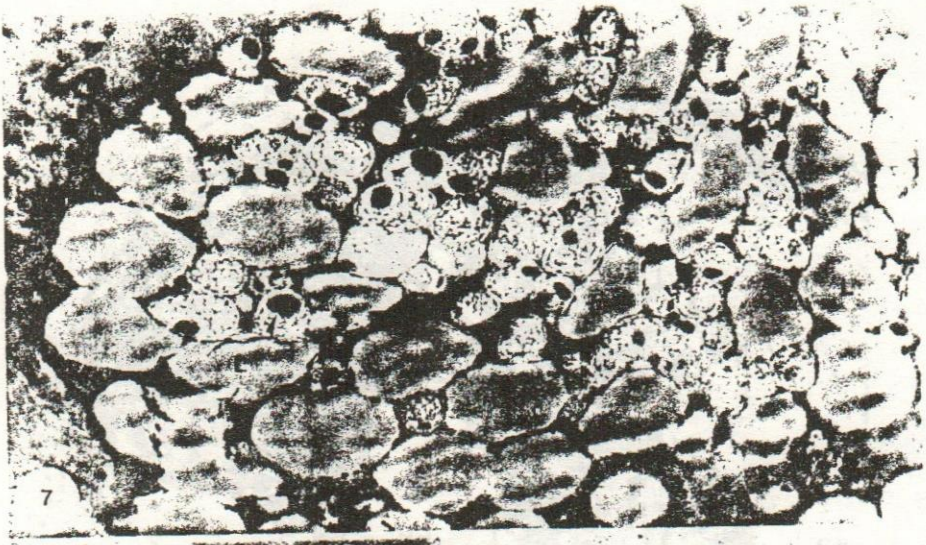
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Lu
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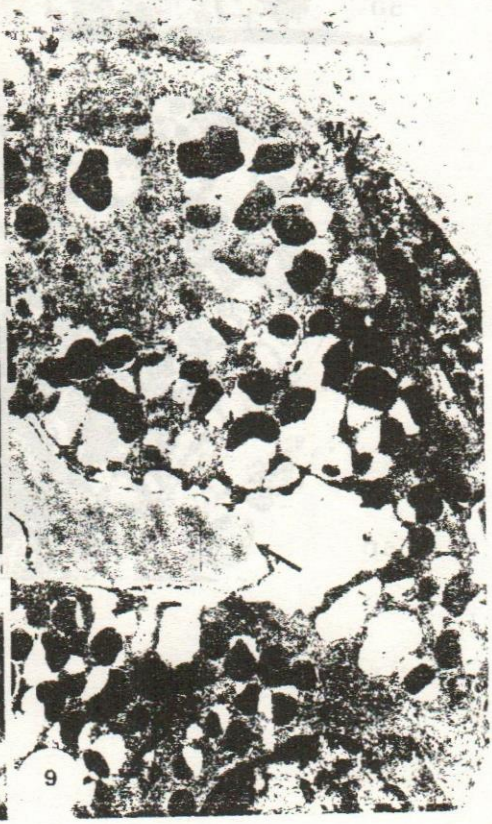
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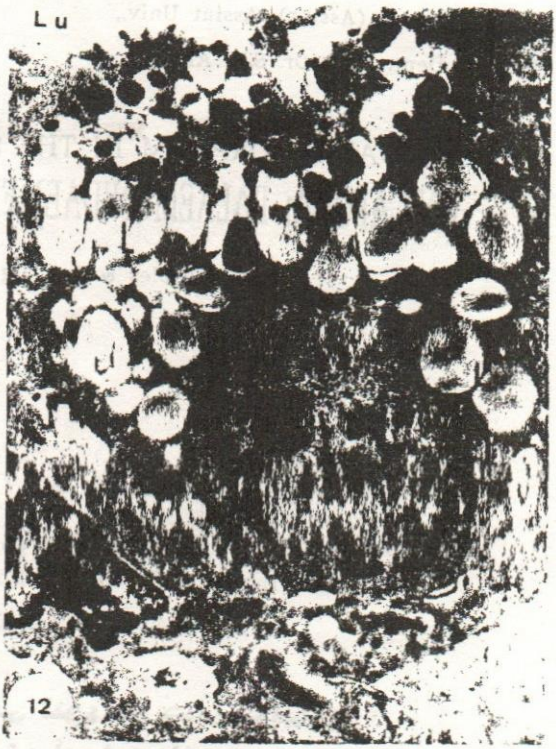
Lu
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Lu
Mv
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