Department of Veterinary Anatomy, Yamaguchi University, Yamaguchi, Japan;
Department of Anatomy, Université de Montréal and Department of
Experimental Surgery, St. Justine Hospital for Children,
Montréal, Canada

The Ultrastructure of the Goblet Cell in the Dog Ileum at the Later Stage of Secretion*

Takashi MAKITA, Sukechika KIWAKI, Edmund B. SANDBORN,
F. M. GUTTMAN and A. KHALESSI

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Summary. The globules in goblet cells in the epithelium of the dog ileum show a moderate contrast between the light and dark types. The globules appear to arise from the Golgi apparatus. Membranes encapsulate all of the globules in the earlier stages but a part of the mucosubstance undergoes a dispersion or, in some cases, possibly a fusion before their discharge into the intestinal lumen. There is little evidence of any escape of organelles into the lumen during secretion and the residual cytoplasm is always limited by a continuous membrane when the goblet is emptied. The residual portion of the cytoplasm appears to be functionally active at the stage when the goblet has been effectively emptied. Both the Golgi apparatus and the rough surfaced endoplasmic reticulum contain a substance similar to that in the light globules. The discharged mucus is distinctly different in appearance from the glycocalyx of the microvilli of nearby absorptive cells.

Most of the reports on goblet cell ultrastructure are from the intestine of the rat and mouse (PALAY, 1958; FLOREY, 1960; SHEARMAN and MUIR, 1960; HAMPTON, 1960; HOLLMANN and HAGUENAU, 1961; HOLLMANN and STAÜBLI, 1962; BIERRING, 1962; FLOREY, 1962; FREEMAN, 1962; HOLLMANN, 1963; RHODIN, 1963; FREEMAN, 1966; BERLIN, 1967; BUDD, 1968; TONER, 1968; SANDBORN, 1970). Studies made on materials from the human (BIERRING, 1962; TRIER, 1963; FREEMAN, 1966; TONER, 1968), guinea pig (FREEMAN, 1966, TAKEUCHI, 1967), swine (BERLIN, 1967), monkey (TAKEUCHI, 1967), bat (PORTER and BONNEVILLE, 1968), eel (FAWCETT, 1966) and fowl (MAKITA and NISHIDA, 1966; MAKITA and KIWAKI, 1968; KIWAKI, 1970) have been less systematic. Only two reports have dealt with the goblet cell of the dog under experimental conditions (FRASCA et al., 1968). Reserches on the goblet cell of the rat intestine mentioned above has been directed mainly to the intracellular mucus droplet formation (LANE et al., 1964, NEUTRA and LEBIOND, 1966; BEAMS and KESSEL, 1968). They have dealt mainly with the initial and intermediate phases of mucus synthesis, particularly in the Golgi apparatus of cells in the lower part of the intestinal crypt. To the contrary, the tips of the villi have been avoided intentionally (BEPLIN, 1967) because they are likely to be involved in cytoplasmic degeneration.

Whether the mucus droplets keep their individuality until they are discharged or whether they swell and coalesce prior to secretion has been an open question. As this is important in understanding the mechanism of secretion, we focused our interest primarily on this point in the present study.

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Materials and Methods

Biopsies were taken from exteriorized segments of ileum of fifteen dogs under pentobarbital anesthesia (GUTTMAN et al., 1969; MAKITA et al., 1971). The small pieces of tissue were immersed in cold glutaraldehyde solution (6.5 % glutaraldehyde and 5 % dimethyl sulfoxide in 0.1 m phosphate buffer at pH. 7.4) (SANDBORN, 1970) and were post-fixed for 2 hrs in 2 % veronal acetate buffered osmium tetroxide solution. For light microscopy, sections approximately 1μ in thickness of epon embedded tissues were stained with 1 % toluidine blue solution, followed by differentiation through a mixture of toluol, epoxy resin and cedar oil, three changes of toluol and xylol (SANDBORN, 1970).

Fig. 1. A light micrograph of a section of epon embedded dog ileal epithelium illustrates heavily stained mucin in goblet cells which has not yet opened into the intestinal lumen (white arrows). Those goblets, which are in the process of discharging their content, contain a much less densely stained material in which a few densities can be seen. The nucleus and cytoplasm of the goblet cells are more densely stained than those of the neighboring absorptive cells. $\times 560$

somes, both attached and free in type, in the cytoplasm of the goblet cell. The cisternae of the endoplasmic reticulum appear as lighter, irregular channels. The mitochondria in the apex of the goblet cell are larger and paler than those of the absorptive cells. The microvilli of the goblet cell are more slender and stain more darkly than do those of the absorptive cells (Fig. 2). An extensive Golgi complex is seen adjacent to the nucleus. Mucus droplets in the cell vary in electron density

Results

The incidence of goblet cells increases gradually from the duodenum to the colon. In light microscopy of toluidine blue-stained sections of epon embedded tissues the immature goblet cells in the epithelium appear quite dense in comparison to the neighboring absorptive cells. The supranuclear portion of the cells becomes distended with moderately dense content. At a later stage, with this type of fixation and stain, the content becomes less dense while the layer of cytoplasm surrounding it remains heavily stained. The mucus in the process of discharge from the cell can be followed into the lumen of the crypt and among the villi (Fig. 1).

In electron microscopy of the dog ileum, the goblet cell which does not contain droplets shows remarkable difference from the neighboring absorptive cells. Comparatively, the goblet cell cytoplasm appears dark with the exception of the very large mitochondria. This is mainly due to the greater concentration of ribo-

and in size. Between the intact droplets there appear either remnants of the dense cytoplasm or, more commonly, dispersed light mucoid content. Some droplets appear to have coalesced. The Golgi complex is large with a pale content in the sacs. Some very large vesicles can be seen on various aspects of the Golgi complex. The basal cytoplasm contains many ribosomes and a long tube of rough surfaced endoplasmic reticulum which is in continuity with the outer nuclear membrane (Fig. 3). In the development of the globules within the Golgi complex, which, in this case (Fig. 4), appear to be of the lighter variety, a distinct membrane of the Golgi sac surrounds the pale material with a fibrillar content. At a later stage within the goblet the evidence of a membrane on some of the globules is lacking. The fibrillar network within the globules appears to become more compact. The fibrillar network appears to contain membranous structures (Fig. 5).

Fig. 2. Compared with the adjacent absorptive cell (*Ab*), the goblet cell has more slender and darker microvilli, much darker cytoplasm due to a considerable number of ribosomes on a dense background, and larger paler mitochondria. $\times 21,000$

Fig. 3. A typical goblet cell in the dog ileum demonstrates a moderate contrast of the dark and light types of mucus globules within the goblet. Their size is variable, partly due to their plane of section. The lighter globules are distinct in the Golgi field. Rough surfaced endoplasmic reticulum is plentiful in the basal portion of the cell and some of it extends upward to lie among the globules of the goblet. One cistern of the endoplasmic reticulum is continuous with the nuclear envelope (arrow). $\times 23,000$

Fig. 4. Pale mucus globules appear to be developing within sacs of the Golgi apparatus. There is a fibrillar appearance to the content. $\times 70,000$

Fig. 5. The dark globules show a more regular fibrillar arrangement than do the paler globules. Part of the fibrillar networks contain membrane structure. $\times 60,000$

Fig. 6. As the goblet, in maturing, becomes distended, the Golgi complex becomes less distinct. Typically the apical portion of the goblet becomes filled with diffuse fibrillar material against

a light background similar to the appearance of the pale globules. Dark globules fill the lower portion and are scattered through the lighter content of the upper part of the goblet. The limiting membrane around the dark globules is preserved. Dense bodies, which are small collections of cytoplasm, are also seen between the globules. $\times 11,500$

The fusion of the lighter mucus droplets and an increase in the diffuse content becomes quite considerable in the apical part of the goblet cell wall before it opens into the lumen. The remaining droplets, mostly of the darker type and located in the basal portion of the goblet, retain their limiting membrane (Fig. 6).

On rupture of the free surface, some of the mucus escapes through a substantial opening into the lumen. Only a very thin cuff of distinct, heavily stained cytoplasm remains. The microvilli are still seen only at the border of the free surface of the cell. The secretion consists mainly of a background material with a fibrillar appearance distributed in the direction of its flow. The mucus flow from the cell is different in appearance and remains distinct from the fibrillar coat which covers the tips of the microvilli on the luminal surface of the cell. The mass of mucus, now in both the lumen and the goblet, contains some dense materials (Fig. 7). Within the mucus mass of the goblet cell which is in continuity with the lumen, dense globules may occur which still retain their membrane attached to some fragments of cytoplasm. The cytoplasm remains as a thin shell about the goblet (Fig. 8).

In cells which have been largely emptied of their secretory content, a few globules of mucus often remain attached to the thin periphery of the theca. The content of the crescentic nucleus appears quite unchanged during the process. In the

Fig. 7. By the time the free surface of the cell opens into the intestinal lumen the globules in the most apical portion of the goblet have lost their individuality and have changed into a mass of mucus. The mucus which flows into the lumen is distinctly different in appearance from the glycocalyx on the surface of the microvilli. Some denser residual cytoplasm is extruded with the mucus. The wall of the goblet appears as a very thin shell of densely stained cytoplasm. The residual cytoplasm is lined by a continuous membrane when the goblet is emptied. $\times 11,000$

lighter globules the membrane facing the lumen of the goblet appears to have disintegrated. A distinct Golgi complex and rough surfaced endoplasmic reticulum can be seen. The content of the Golgi sacs resembles that of the immature cell. There is a considerable amount of content in the long cistern of endoplasmic reticulum. Thus when the discharge of the mucus seems to be nearing completion the content of the endoplasmic reticulum suggests continued activity as does the Golgi complex (Fig. 9, 10).

Discussion

The characteristics of the goblet cells in the dog ileum are, in general, similar to those in other mammals. The shape of the goblet cells as reported by light microscopists (Моё, 1968) varies in accordance with subdivision of the intestine and also with the portion of the villi in which it is found. Thus it reflects the regional difference rather than specific difference in the cell. The height of the epithelium, the number of mucus globules and the activity of the goblet cell appear to be the dominant factors influencing its shape.

The fine structure of the mucus globules in the dog have some different characteristics from those of other animals. Those of the dog ileum show less contrast

Fig. 8. The mass of mucus discharged from the goblet cell contains a few membrane bound, irregularly shaped bits of the cytoplasm (arrows) attached to darker globules of mucus which also retain a surrounding membrane. Small vesicular profiles and a number of homogeneous densities are seen in the mucus. The background mucus demonstrates a loose network of very fine fibrillar material. $\times 13,000$

between dark and light types. Furthermore, the cells lack the dense vacuolated globules which were found in the human jejunum (FREEMAN, 1962) or the cored globules which were found in the avian oviduct (MAKITA and NISHIDA, 1966; MAKITA and KIWAKI, 1968). It has been reported that the mucus globules in the rat jejunum and guinea pig duodenum are homogeneous, dense, small and round while those in mouse colon (HAMPTON, 1960) and in the monkey (TAKEUCHI, 1967) are clear and large. A comparison of the ileal epithelium with that of the trachea (FRASCA et al., 1968) in the dog demonstrates a marked similarity of the mucus globules in the two locations.

Fig. 9. When the goblet has been emptied to the point where only a few globules of mucus are attached to the base, one can distinguish long cisternae of the rough surfaced endoplasmic reticulum (ER) in the whole length of the residual cytoplasm. The cytoplasm at this stage becomes much less densely stained. The mitochondria now are small and have a very dense matrix. The nucleus which is flattened along the base of the goblet appears quite normal. Globules with varying amounts of surrounding membrane are still attached to the base of the goblet. $\times 33,000$

Methods of tissue preparation may influence the appearance of the mucus globules. MOE (1968) suggested that some buffers resulted in a higher density of the globules. A much more important factor in this respect is the choice of fixatives. In particular, pre-fixation with glutaraldehyde is responsible for remarkable differences in the electron microscopic profiles. Without pre-fixation the globules are generally pale and the filamentous substructure is more distinct. Pre-fixation increases the density of the ground substance of the globule.

Several factors may influence the function of goblet cells. Functional variations

may occur during the "migration" from the depth of the crypts to the tips of the villi (BIZZORERO, 1892; MESSIER and LEBLOND, 1960; MERZEL and LEBLOND, 1969). The goblet cells in this study were from the villi and therefore were approaching the end of their migration. Secretory cycles have been postulated for the goblet cell. Generally these are divided into three phases: (a) the initial phase wherein the goblet cells are in the lower part of the crypt and the amount of stored mucin increases, as synthesis exceeds discharge; (b) the intermediate phase when the cells are in the upper part of the crypt and on the lower or middle of the villi where the mucus content is considerable; (c) the final phase of short duration in which the cells are located near the tips of the villi and the discharge exceeds synthesis (MOE, 1968). The present study describes the final stage of these cycles.

It has also been suggested that the secretory cycle of the individual goblet cell repeats itself (FREEMAN, 1962, 1966). Stage of recovery from the deformation with an increase in the residual cytoplasm after evacuation of the goblet are difficult to identify with certainty in sections of fixed tissue. However at the stage in which the goblet is practically evacuated, the nucleus, the Golgi complex, the mitochondria and the endoplasmic reticulum appear active.

Not only the Golgi complex which has been commented upon by almost all investigators, but similarly the mitochondria, the microvilli and the quantity of ribosomes and rough surfaced endoplasmic reticulum are distinctively different from those in other types of epithelial cells. The quantity of ribosomes contributes to the density of stain in the goblet cell cytoplasm but some other factors must also contribute to this since the density of the microvilli is also very high. But the dark appearance is also evident in the pre-secreting goblet cell which contains no visible mucus globules (Fig. 2) and thus the compression of the cytoplasm would not be the dominant factor.

The existence of the small amounts of mucopolysaccharides in the Golgi complex of the absorptive cells has prompted the suggestion that these may share the same function as the goblet cells (BERLIN, 1967). Although a study of the

Fig. 10. The Golgi complex (*Golgi*) of the emptied goblet cell demonstrates evidence of secretory material within its sacs. A dark globule appears to be in the process of separating from a much denser part of the near by residual cytoplasm. The cisternae of the rough surfaced endoplasmic reticulum (*ER*) have become moderately distended and all of the residual cytoplasm has diminished in its density. $\times 30,000$

ultrastructure of cells does not give any definite conclusions as to the origin or the fate of the goblet cells, their profiles at all stages of secretion suggest marked differences from the neighboring absorptive cells.

Whether the mucus globules keep their individuality until they are discharged, or whether they swell and coalesce prior to secretion has been of interest to investigators in this field. MOE (1968) was of the opinion that both situations occur, while TRIER (1963) seems to consider that limiting membranes of individual droplets or groups of coalesced droplets fuse one with another prior to the opening of the plasma membrane. On the other hand, there are investigators who consider the coalesced globules to be an artifact produced mainly by poor fixation. They had reasonable evidence in the earlier stage of electron microscopic research. They found little to suggest that ripe mucin globules coalesced immediately before discharge (FLOREY, 1962) and the commonly described process was one in which intact globules were discharged in to the lumen. In most of the cases described, the globules not only retained their limiting membrane but were still surrounded by the plasma membrane of the cell and might not have reached the maturity required for discharge.

Experimental stimulation to force the cell to evacuate the mucus seems to create the situation in which an intact globule is discharged into the lumen (FLOREY, 1960, 1962; FREEMAN, 1966). Despite the situations mentioned above, the findings of the present study suggest that the dispersion of the lighter type of intracellular globules commences prior to the rupture of the luminal surface of the goblet cell. It was also evident in the freeze etching study of the goblet cell of rats (MAKITA et al., 1971). It would appear that about the time that the goblet cell matures there is a disintegration of the membranes around the individual lighter globules. Whether the darker globules are distinctly different in nature from lighter ones is not known. It is possible that the enlargement and diminution of the density of the individual globules is the result of a simple dilution of its content.

The evidence presented indicates a normal residual cell after discharge of the goblet is consistent with published studies which suggested that the goblet cell is capable of repeating the secretory cycle.

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イヌの回腸の杯細胞の分泌末期の微細構造

牧田登之, 木脇祐順, E. B. SANDBORN, F. M. GUTTMAN, A. KHALESSI

従来, 杯細胞は主にラット、マウスなどについての研究が多く, イヌの報告例が少い。それも, 分泌物がゴルジ装置で産生されることに重点がおかれ, 分泌物の放出の段階についての記載に乏しい。そこで, イヌの回腸の杯細胞で分泌の最終段階にあるものを観察した。まず杯細胞の特徴は, 隣接する吸収細胞と比較して, リボゾームや粗面小胞体が多いので細胞質が暗く, 明るく大きいミトコンドリアをもち, 微絨毛が細くて長い。成熟した粘液顆粒は, 暗調のものと, やや明調のものがあるが, その差異は他の動物ほ

どではなく、さらにゴルジ野には未熟な明るい分泌物がみられる。いずれも細線維質の内容容をもっており、暗調顆粒では部分的に膜様の構造もみられる。杯細胞の自由表面の細胞膜が破れて粘液が放出されるに先立って、これらの粘液顆粒の限界膜が破れはじめる。この段階では、暗調のものほど基底部に多く、後まで残る傾向がある。細胞膜が破れて粘液が放出される段階に達しても、いまや細胞内と腸管腔とにある粘液内には、暗調な物質、おそらく細胞質の一部、が散見されるのみで、細胞小器官や限界膜につつまれた顆粒の流出は認められない。細胞の辺縁に残された細胞質は膜で境をされて粘液の集団と混ざることなく、細胞質の中に正常な核、粗面小胞体、ゴルジ装置などをみる。ミトコンドリアは小さく暗調になっていることが多い。杯細胞から放出された粘液は、もはや膜につつまれていないが、微絨毛をおおう物質とはすぐには混ざることなく、両者は容易に識別できる。

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牧田 登之
〒753 山口市吉田 1677-1
山口大学農学部
家畜解剖学教室

Dr. Takashi MAKITA
Department of Veterinary Anatomy
Yamaguchi University
Yamaguchi, 735 Japan