NOTE *Anatomy*

A Histochemical Study of the Camel (*Camelus bactrianus*) Duodenal Glands

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ABSTRACT. The complex carbohydrates in the camel duodenal glands were examined histochemically at light and electron microscopic levels. The duodenal glands of the camel were distributed in the submucosa 2 m caudal from the pylorus. These were branched tubuloalveolar glands. The terminal portion of each lobule was formed by only one type of mucous cell. The duodenal gland cells contained acidic and neutral carbohydrates. The mucous cells mainly contained sulfate and carboxyl carbohydrate with sialic acid, and they also contained a few neutral carbohydrates with different saccharide residues such as mannose, glucose, galactose, N-acetyl glucosamine and N-acetyl galactosamine. The results showed that the secretary granules of the duodenal glands in the camel contain mainly acidic carbohydrates. These findings seem to be the morphological characteristics of the duodenal glands in the camel.—KEY WORDS: camel, duodenal gland, histochemistry.

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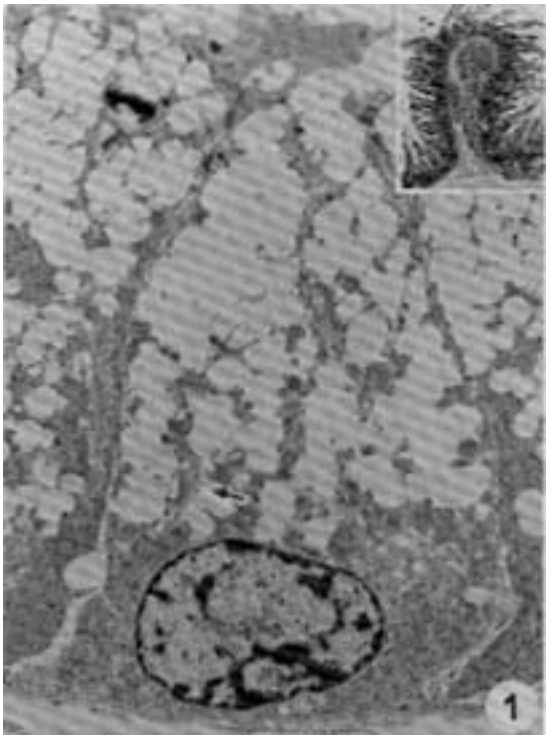
The duodenal glands, or glands of Brunner, are present only in mammals. The glands commence at the gastrointestinal junction and extend for a variable distance aborally in the intestinal tract. The cellular composition varies from species to species [3]. On the other hand, histochemical studies in various animals have shown that the duodenal glands consist mainly of neutral carbohydrates [1, 5, 8–11]. However, there have been no studies on the detailed structure of and secretory substances in the duodenal glands of the camel. The camel is a domestic animal of economic importance in some of the hotter and drier regions of the world. The camel is a typical ruminant, but its stomach differs morphologically from that of other ruminants [2]. The pH of the abomasal content in the camel was reported to be higher than that in the bovine [6]. Generally, the duodenal glands are believed to produce an alkaline, highly viscous mucus, which is thought to protect the duodenal mucosa from the acidity of the chyme. In this paper, cells in camel duodenal glands have been classified mainly on the basis of histological examination and composition of carbohydrates in mucin by using a histochemical technique.

Two female camels (*Camelus bactrianus*, 3 and 6 years old, 250–300 kg) were used in this study. Both animals appeared to be clinically normal. Tissues (0.1 m, 0.5 m, 1 m, 2 m and 3 m caudal from the pylorus) were collected after induction of deep anesthesia with sodium pentobarbital and exsanguination. To prepare tissues for light microscopic analysis, the duodenum was fixed in 10% formalin containing 2% calcium acetate for 48 hr, dehydrated through an ethanol-xylol series, and embedded in paraffin. The sections were cut at 5 μm in thickness, deparaffinized, and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), high iron diamin (HID), low iron diamin (LID), alcian blue (AB) (pH 1.0), AB (pH 2.5) and AB (pH 2.5)/PAS, and peroxides-labeled lectins diaminobenzidine (DAB) [7]. Lectins (E-Y Laboratories, San Mateo, U.S.A.), their acronyms, and specifies and inhibitors used for the control are summarized in Table 1. For control prodedures of lectin staining, sections were also treated with inhibitors. The sections were also treated only with DAB to detect the location of intrinsic perioxidase in the tissue. For analysis by electron microscopy, the specimens were prefixed at 4°C for 2 hr in 3% glutaraldehyde. The tissues were post-fixed in 1% osmium tetroxide at 4°C for 1 hr, dehydrated, embedded in Quetol 812, and sectioned with a diamond knife. The sections were stained first in uranyl acetate and then in lead citrate. For cytochemical analysis, the duodenal tissues were cut into small pieces and placed in 0.1 M phosphate buffer containing 4% paraformaldehyde (pH 7.4) at 4°C for approximately 2 hr. After washing with the same buffer and dehydration with graded N, N-dimethylformamide at 4°C, the tissues were embedded in glycol methacrylate (GMA) at −20°C [12]. The tissues

Table 1. Lectins, their specificities and control inhibitory sugars

Lectin	Carbohydrate specificity	Inhibitory sugars
Concanavalin A (con A)	Mannose, Glucose	Glucose
Grifforia simplicifolia I (GS-I)	Galactose	Galactose
Grifforia simplicifolia II (GS-II)	N-GluNAc	N-GluNA
Soy bean agglutinin (SBA)	D-GalNAc, Galactose	D-GalNac
Limulus polyhemus agglutinin (LPA)	Sialic acid	Sialic acid



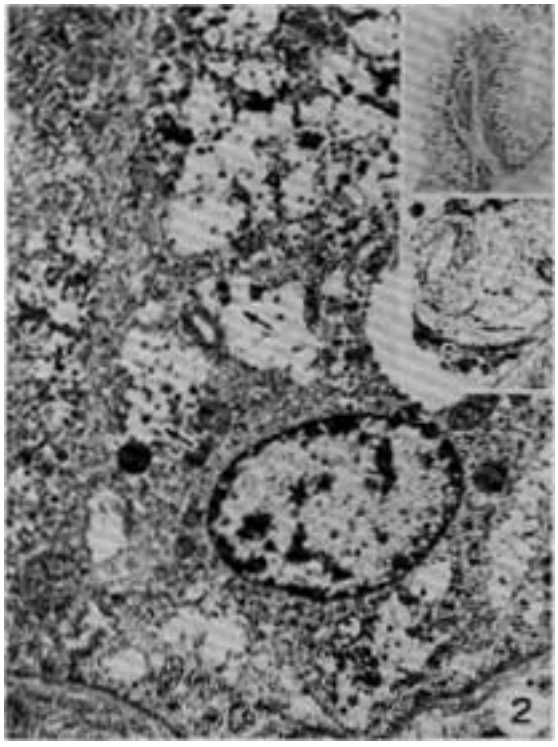


Fig. 1. An electron micrograph of duodenal gland cells in the camel. The supranuclear area has several Golgi complex (arrow) associated with secretory granules. The secretory granules were electron-lucent. × 7,000. Inset: A light micrograph of the duodenum stained with HE. The duodenal glands were located in the submucosal layer in the duodenum. × 10.

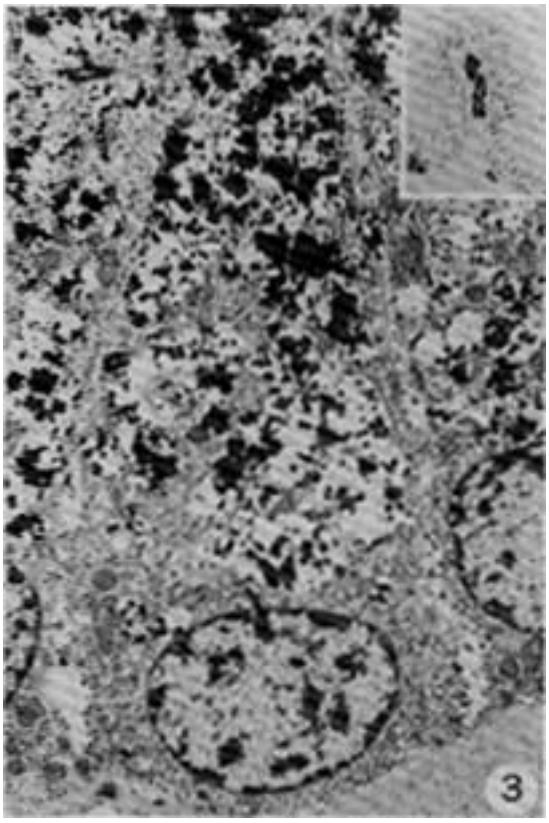
Fig. 2. An electron micrograph of duodenal gland cells stained with PA-TCH-SP-PD. There are a small number of silver granules (arrow) located on the secretary granules. × 7,000. Upper inset: A light micrograph of the duodenum stained with PAS. Gland cells showed a weak positive reaction to neutral carbohydrates. × 10. Lower inset: An electron micrograph of duodenal gland cells stained with PA-TCH-SP-PD. Golgi complex (G) was strongly positive and associated granules having numerous number of tiny silver granules (arrows). × 10,000.

were sectioned and reacted with periodic acid (PA)-thiocarbohydrazide (TCH)-silver protein (SP)-physical development (PD) and HID or LID-TCH-SP-PD according to the method of Kitamura *et al.* [4].

The camel duodenal glands were found to be distributed in the submucosa of the upper part of the small intestine for approximately 2 m caudal from the pylorus. They were separated into lobules by relatively well-developed interlobular connective tissues that can be classified morphologically as branched tubuloalveolar glands. The terminal portion was formed by typical mucous cells. The glandular cells were cuboidal, about 9×18 μm in diameter, and had round nuclei located in the basal cytoplasm. The Golgi complex was well-developed and occupied an extensive region of the cytoplasm. Another prominent feature noted in the supranuclear cytoplasm was the varying number of secretory granules, ranging from 0.6 to 2.3 μm in diameter (Fig. 1). Histochemically, the secretory granules were weakly positive for PAS, strongly positive for HID and LID, and moderately positive for AB (pH 1.0) and AB (pH 2.5). In the reaction with AB (pH 2.5)/PAS, all of the glandular cells were strongly stained bluish purple. When the duodenal glands were treated with lectins, they showed different staining patterns. All secretory granules weakly reacted with Con A and GS-II, which bind specifically to D-mannose, D-glucose and N-Acetyl glucosamine. When treated with GS-I and SBA, which bind specifically to galactose, the glandular cells showed various degrees of weak positivity to these lectins. PAN, which binds specifically to sialic acid, moderately stained all granules. The light microscopic histochemical results are summarized in Table 2.

In electron microscopic histochemistry, the glandular cells stained with the PA-TCH-SP-PD procedure, all secretary granules, glycogen particles, Golgi complex and plasma membrane exhibited positive results (Fig. 2). With the HID and LID-TCH-SP-PD procedures, only secretory granules were found to yield strong positivity (Figs. 3 and 4). These results indicated that the camel duodenal gland cells contain mainly acidic carbohydrates.

Since the discovery of the duodenal glands by Wepfer in 1697 [3], the secretion of the duodenal glands has been



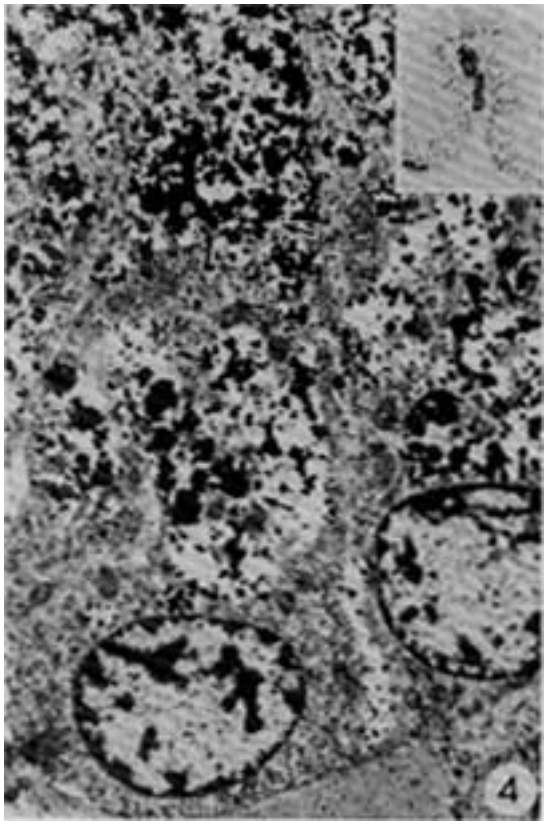


Fig. 3. Duodenal gland cells stained with HID-TCH-SP-PD. There are numerous silver granules located on the secretory granules (arrows). × 7,000. Inset: A light micrograph of the duodenum stained with AB (pH 2.5). Gland cell showed a positive reaction to acidic carbohydrates. × 10.

Fig. 4. Duodenal gland cells stained with LID-TCH-SP-PD. There are numerous silver granules located on the secretory granules (arrows). × 7,000. Inset: A light micrograph of the duodenum stained with AB (pH 1.0). Gland cells showed a positive reaction to acidic carbohydrates. × 10.

Table 2. Results of light microscopic histochemical stainings of the duodenal glands

PAS	+/Br
HID	+++/Br
LID	+++/Br
AB(pH1.0)	++/B
AB(pH2.5)	++/B
AB(pH2.5)-PAS	++/BP
Con A	+/Br
GS-I	+/Br
GS-II	+/Br
SBA	+/Br
PNA	++/Br

Intensity of staining reaction: +++, strong; ++, moderate; +, weak; Color of reaction: R, red; Br, brown; B, blue; BP, bluish purple.

presumed to play an important role in the physiology of digestion. They have been studied by light and electron microscopy in many species to determine the extent of their distribution, density and their cell constituents. In the bovine, Takehana *et al.* [10] reported that there are two types of mucous cells in the duodenal gland lobules. In the present study, it was found that the duodenal gland in the camel is composed of only one type of mucous cells containing acidic carbohydrates. Previous studies have shown that the secretion of the duodenal gland neutralizes gastric hydrochloric acid in cooperation with pancreatic juice, bile, and intestinal juice [1, 3]. In the camel, the secretary granules of the duodenal glands contained mainly acidic carbohydrates, although the duodenal secretion is mainly neutral carbohydrates and has a neutralizing role. The present results are in accordance with the statement by Maloiy [6] concerning the decreased pH of the digest in the camel abomasum.

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