The Fine Structure of Endocrine Cells in the Sheep Pancreas

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The Fine Structure of Endocrine Cells in the Sheep Pancreas

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Summary

The fine structure of endocrine cells of the sheep pancreas was studied. The five cell types were identified mainly by the character of their specific granules. A cells had an indented nucleus. Mitochondria of ring-form were observed, though rarely. B cells had a spherical nucleus and in rare cases their secretion granules had a cristalline-shaped core. D cells had abundant secretion granules, and more over the morphometry of the granule density was high. D_1-like cells were observed among the exocrine cells. Their granules were more uniform and round than those of D cells. EC cells were also observed among the exocrine cells, and their abundant granules varied in size and form. By morphometry of Penel and Simon's transformation equations, the density of the granules per cube micrometer of A, B and D cells was calculated as 8.93, 9.42 and 157.56, respectively.

The fine structure of the endocrine cells of the pancreas has been reported in various mammals, such as mouse (1), rat (2), hamster (3), rabbit (4) and cat (5). In domestic ruminant, however, the fine structure of their pancreas endocrine cells has not been fully reported, except for that of cattle (6). In primates (7), cell types reported in their pancreas endocrine cells are A, B, D, D_1 and EC cells. In the sheep, there is no report on the cell types and their fine structure.

On the other hand, it is known that the insulin secretion in the sheep is somewhat different from other monogastric mammals (8). The insulin secretion from B cells of sheep islet was greater than that of monogastric mammals when stimulated by propionate and butyrate, known products of rumen fermentation in the ruminant.

The purpose of this study is to investigate the cell types and their fine structure of the sheep pancreas endocrine cells by electron microscopy, and morphometry of the secretion granules of A, B and D cells. Special attention was paid to the morphology of A and B cells which are mainly concerned with insulin secretion in the pancreas.

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Materials and Methods

Two castrated male sheep of 35 Kg body weight were used in this study. Blood samples were obtained from them by jugular vein 5 min. before slaughter. Plasma glucose was determined by the glucose oxidase method of Huggett and Nixon (9). Plasma insulin level was measured by the dextran-coated charcoal method of Herbert *et al.* (10).

Small pieces of the pancreas were removed immediately after slaughter, fixed in 2.5% solution of glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4), and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4). They were then dehydrated in a graded series of ethanol and embedded in Epon resin through propyren oxide. For electron microscopy, ultrathin sections were double-stained with uranyl acetate and Reynold's lead citrate, and examined by an electron microscope (JEM 100B).

For morphometry, electron micrographs were printed with a magnification of 33,600, and the diameter of the secretion granules in A, B and D cells were calculated by measuring the profile diameters. Using the Penel and Simon's transformation equations (11), the distribution of measured diameters was converted into that of real granule diameters and the density of the granules per cube micrometer of each cell cytoplasm.

Results

Plasma glucose level was 67.0 mg/100 ml and immunoreactive insulin (IRI) was 16.99 μU/ml in average, respectively.

The endocrine cells of the sheep pancreas had at least five cell types; A, B, D, EC and D_1-like cells which could be differentiated from one another mainly by the character of their specific granules. The islets of Langerhans were observed in three types; A, B and D cells (Fig. 1). EC and D_1-like cells were observed among the exocrine cells.

A cells

The nucleus of the A cells was generally indented or reniform (Fig. 1). The mitochondria were elongated and sometimes branched (Fig. 2). The mitochondria of ring-form were present, but rare (Fig. 3). The Golgi apparatus and the endoplasmic reticulum were slightly developed, with free ribosomes in the cytoplasm. The size of A-granules was approximately 200–300 nm in diameter. They were round in profile and had a narrow halo between the limiting membrane and the core. The core was composed of electron-dense materials (Figs. 2, 3).

B cells

The nucleus of the B cells was generally spherical (Fig. 1). The endoplasmic reticulum was well developed. The mitochondria were oval, round and sometimes

elongated. The Golgi apparatus was well developed and situated close to the nucleus (Fig. 4). The B-granules varied in size and were approximately 250–400 nm in diameter. The halo between the limiting membrane and the core varied in width. The electron density of the core varied from dense to pale. Crystalline-shaped cores were rarely observed (Fig. 5).

D cells

D cells had an oval nucleus (Figs. 1, 6). The endoplasmic reticulum, the mitochondria and the Golgi apparatus were relatively undeveloped (Fig. 6). The D-granules were mostly oval but some were spherical or elongated. Their size were approximately 150–200 nm in diameter. The core of the D-granules had a moderate electron density. No halo between the limiting membrane and the core was observed (Fig. 7).

D_1-like cells

D_1-like cells were observed among the exocrine cells. They had an indented nucleus, relatively scant organellae such as endoplasmic reticulum, mitochondria and the Golgi apparatus. The D_1-like-granules were more uniform and round than the D-granules (Fig. 8).

EC cells

EC cells were observed among the exocrine cells. EC-granules were abundant and varied in size and shape. The core of the granules had a moderate electron density (Fig. 9).

TABLE 1. *The Density Distribution of A-, B- and D-granules per μm^3 of Each Cells*

Diameter (nm)	The density of granules/μm^3		
	A-granules	B-granules	D-granules
0- 29.7	0	0	0
- 59.4	0.29	0.07	12.22
- 89.1	0.39	0.30	49.28
-118.8	0.65	0.48	58.10
-148.5	1.19	0.58	33.33
-178.2	1.92	1.54	4.16
-207.9	2.00	2.21	0.47
-237.6	1.87	1.82	—
-267.3	0.53	1.31	—
-297.0	0.08	0.77	—
-326.7	—	0.23	—
-356.4	—	0.07	—
-386.1	—	0.04	—
Total	8.93	9.42	157.56

Morphometry

The calculated distribution of the density of the secretion granules of varying diameter is shown in Fig. 10. The total density of the granules is shown in Table 1. The granules of A cells were shown as spheres of about 60–300 nm in diameter. Their total density was 8.93 per cube micrometer of A cell cytoplasm. The granules of B cells were shown as spheres of about 60–390 nm in diameter. Their total density was 9.42 per cube micrometer of B cell cytoplasm. The granules of D cells were shown as spheres of about 60–210 nm in diameter. Their total density was 157.56 per cube micrometer of D cell cytoplasm.

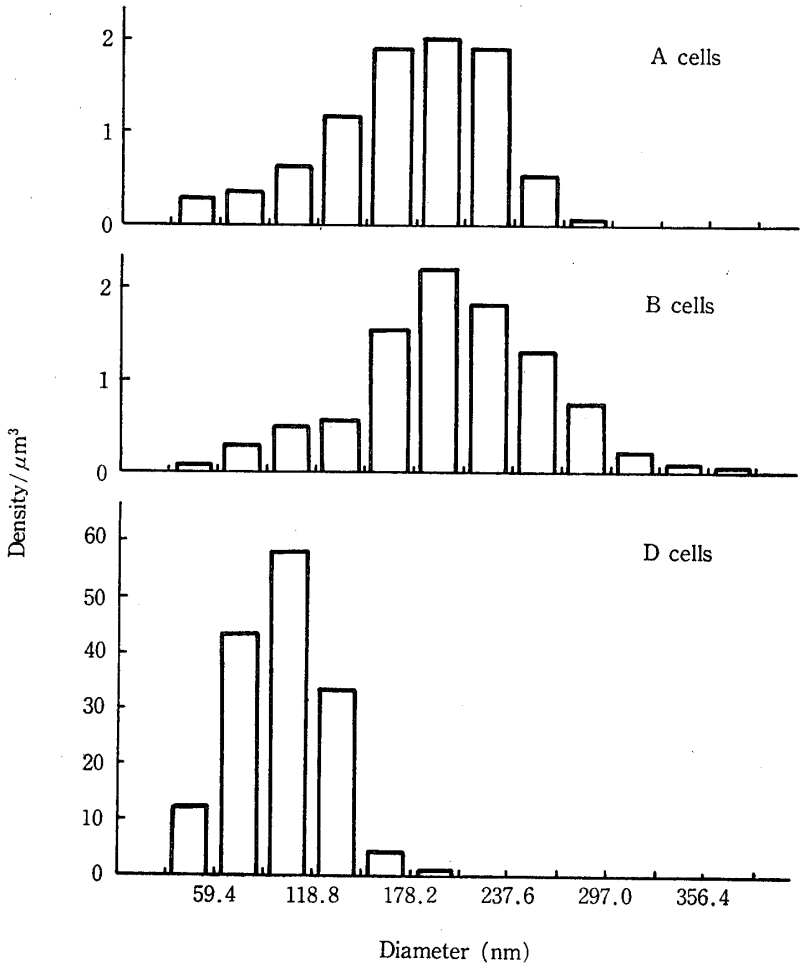

FIG. 10. The density distribution of A-, B- and D-granules per cube micrometer of each cells.

Discussion

The result of this study showed that the fine structure of the endocrine cells of the sheep pancreas was generally similar to that of cattle (6), rodent (1–3), and other mammal (4, 5).

The shape of mitochondria of the sheep A cell was slightly different from that of the cattle (6). Namely, the former were elongated and sometimes branched. Mitochondria of ring- or half-moon-shape were frequently observed in the cattle (6), and in sheep but rarely. In lambs, such mitochondria were observed frequently (*). The sheep A cells had generally indented or reniform nuclei, contrary to those of B and D cells. This type of nuclei seems to be characteristic of the A cells of the sheep. This helps in the identification of A cells in sheep.

The plasma glucose level and the IRI level were within the basal levels known for sheep (12). The sheep were judged, therefore, as "normal" as far as insulin secretion was concerned.

The cristalline-shaped cores of the B-granules were absent in cattle (6). In sheep, however, such cores were observed, though rarely. The morphology of D_1 cells is similar to that of D cells in many respects. The D_1-granules were, however, more regularly round and more uniform in size (7). D_1 cells also had abundant cytoplasmic filaments (13). In this study, even enough D_1-like cells among the exocrine cells had uniform round granules, abundant cytoplasmic filaments were not observed. Thus it remained in question whether D_1-like cells in this study are true D_1 cells.

The number of D-granules was highest among all granule types of islet cells in the cattle (6). In morphometry, the density of D-granules was highest, being 157.56 per cube micrometer, which was in good agreement with the result just stated.

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Plate 1

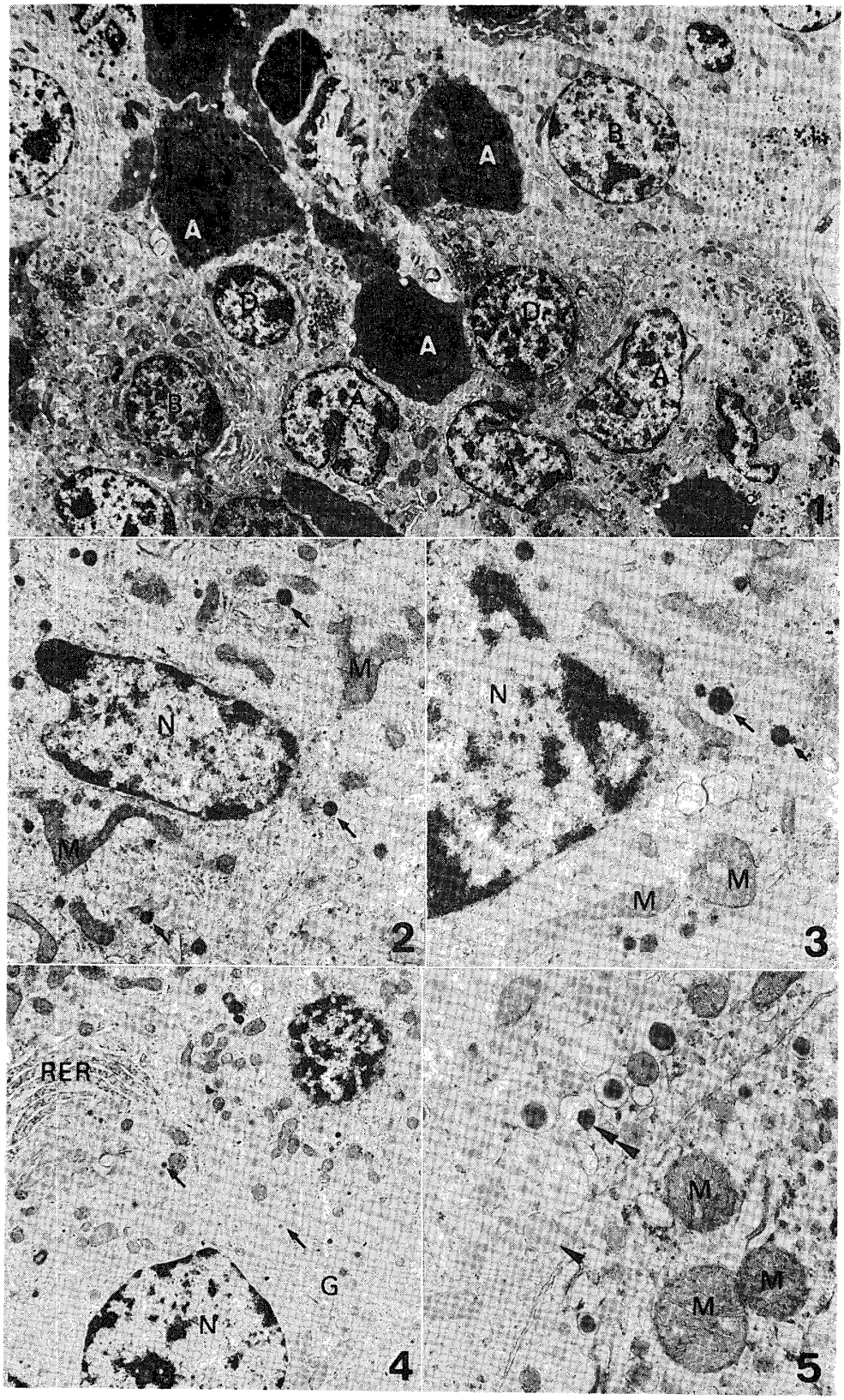
Fig. 1. Islet of Langerhans in the sheep pancreas, showing A cells with indented nucleus (A), B cells (B) and D cells (D) with spherical nucleus. × 2,900

Fig. 2. A cell with branched or elongated mitochondria (M) and secretion granules (↑). × 9,000

Fig. 3. A cell with ring form or elongated mitochondria (M), nucleus (N) and secretion granules (↑). × 12,000

Fig. 4. B cell with developed Golgi apparatus (G) and rough endoplasmic reticulum (RER). Nucleus (N). × 6,300

Fig. 5. Secretion granules and mitochondria of B cell. Crystalline-shaped core (▲) and pale core (▲) of the granules, and spherical mitochondria (M) were observed. × 16,300


A
A
A
A
A
B
B
D
D
D
1
N
M
M
2
N
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3
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G
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5

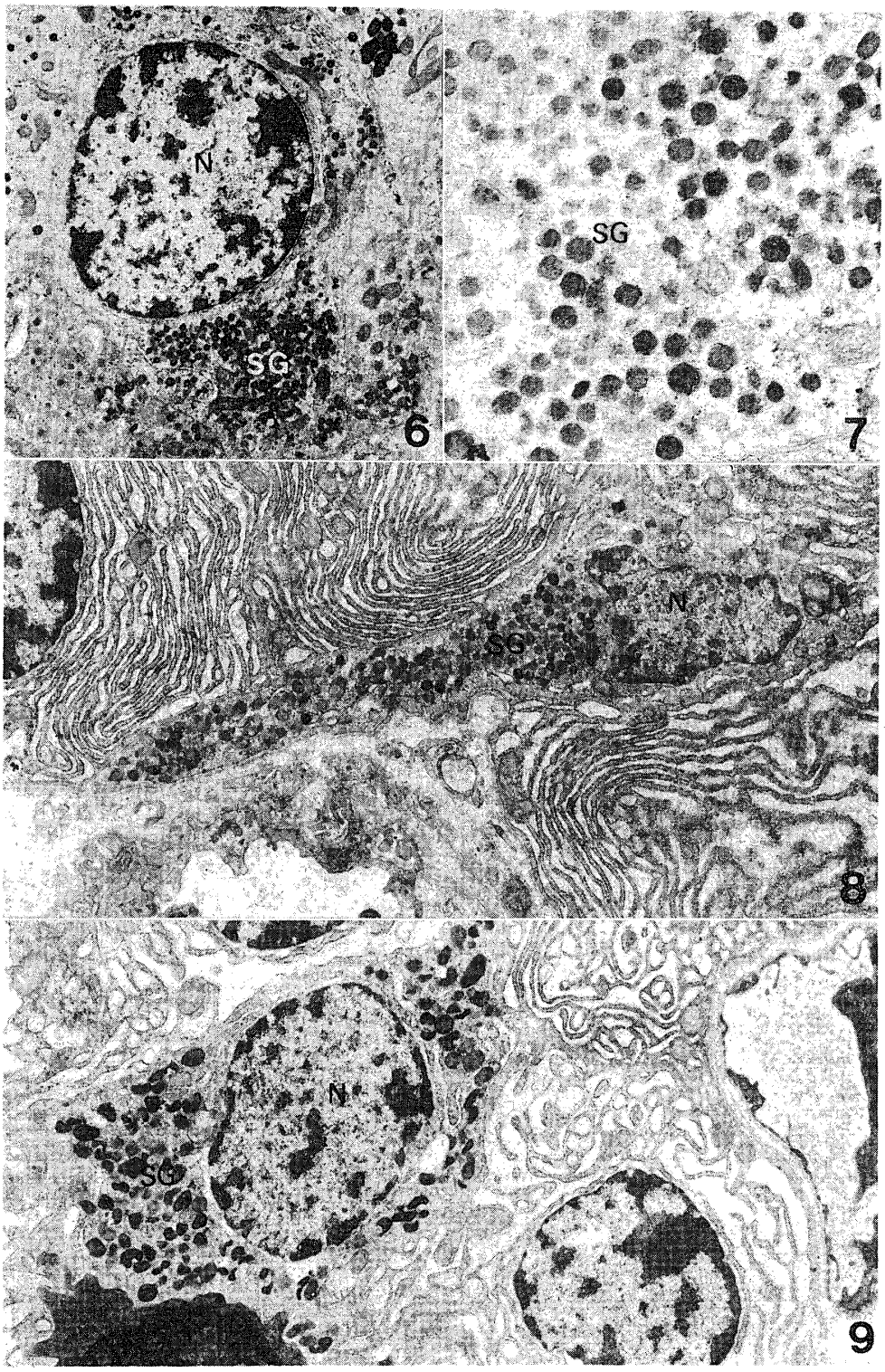
N
SG
6
SG
7
N
SG
8
N
SG
9

PLATE 2

FIG. 6. D cell with abundant secretion granules (SG). Nucleus (N). × 6,700

FIG. 7. The secretion granules of D cells (SG). No halo between the limiting membrane and the core is observed. × 17,400

FIG. 8. D_1-like cell among exocrine cells. Indented nucleus (N) and abundant secretion granules (SG). × 7,500

FIG. 9. EC cell among exocrine cells. Abundant secretion granules varied in size and form (SG). Nucleus (N). × 7,500