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Morphological Studies in Dogs with Chronic Pancreatic Insufficiency

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Summary. The pancreas of dogs with the clinical syndrome of chronic pancreatic insufficiency was examined macroscopically, by light- and electron microscopy and immunohistochemically. The pancreatic tissue was reduced in volume, the lobular architecture was disturbed and the ductal system prominent. Many acini were shrunken and contained cells with pyknotic nuclei. The islets of Langerhans were highly irregular, often difficult to identify. Many B cells occurred isolated within the exocrine tissue. Ultrastructurally, changes in the acinar cells included alterations in the chromatin pattern and dilatation of the cisternae of the rough endoplasmic reticulum. In endocrine cells nuclear deformations, chromatin condensation and progressive loss of secretory granules were seen, accompanied by vacuolization of the cytoplasm. The changes included all endocrine cell types. This complex of pancreatic lesions is considered to be of degenerative origin. The aetiology of the disease is unknown.

Key words: Dog – Pancreas – Exocrine – Endocrine – Insufficiency – Degeneration – Atrophy.

Introduction

The clinical syndrome of chronic pancreatic insufficiency in dogs is described as a complex, slowly evolving disease, which primarily affects the exocrine portion of the organ (Freudiger, 1976). A triad of symptoms, i.e. (1) wasting, (2) polyphagy, and (3) color and texture of feces directly determined in composition of diet, is characteristic of the disturbance. The pathological changes consist mainly of atrophy of the pancreas (especially in German shepherds) and, less frequently, chronic pancreatitis (in other breeds) (Eikmeier, 1964; Jubb and Kennedy, 1970; Freudiger, 1972). Chronic pancreatic insufficiency of atrophic

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origin occurs most frequently in adolescent or young adults, seldom in very young or mature animals. Chronic pancreatic insufficiency of inflammatory origin occurs mainly in adult or old dogs. The aetiology is still largely unknown. Genetic studies of pancreatic atrophy in German shepherds suggest the possibility of an autosomal recessive inheritance (Weber and Freudiger, 1977). The pathogenesis of the atrophy is unknown.

The question of whether we are dealing with atrophy or hypoplasia of the exocrine tissue in this pathological picture has been much debated in the literature. Holroyd (1968) and Jubb and Kennedy (1970) considered the disease to be a consequence of pancreatic hypoplasia; other authors (Archibald and Whiteford, 1953; Thordal-Christensen and Coffin, 1956; Köhler and Stavrou, 1967; Freudiger, 1971; Hill et al., 1971; Hashimoto et al., 1979) feel that it follows progressive degenerative change.

The occurrence of simultaneous endocrine insufficiency is questionable. Hill et al. (1971) noted a reduction of endocrine function in all dogs affected with chronic pancreatic insufficiency, but others have noted only occasional cases with impaired islet activity (Archibald and Whiteford, 1953; Eikmeier, 1964; Freudiger, 1971; Hashimoto et al., 1979). A similar divergence of opinions exists as to the function of the affected islet cells in chronic pancreatitis in man (Hess, 1969; Ritter, 1971; Raptis, 1974; Klöppel et al., 1978).

The results of glucose tolerance tests performed at our small animal clinic on a group of dogs with chronic pancreatic insufficiency, correlated with histopathological findings, convinced us that these changes are of a degenerative nature and involve both exocrine and endocrine tissue. The discrepancies in published descriptions of this condition induced us to expand the study with immunohistochemical and ultrastructural investigations.

Material and Methods

1. Clinical. The study was undertaken on 14 dogs with clinical chronic pancreatic insufficiency (Freudiger, 1972). The oral glucose tolerance test was performed as follows: 1 g glucose/kg body weight was given orally followed by glucose determinations on venous blood taken at hourly intervals, using the glucose-oxidase method (Boehringer).

2. Conventional Histology. Multiple blocks of Bouin fixed pancreatic tissue were embedded in paraffin and in Spurr low viscosity medium. Four-6 μ paraffin sections were stained with haematoxylin-eosin, semithin (1–2 μ) Spurr sections were stained with toluidine-blue.

3. Immunohistochemistry and Electron Microscopy. For these investigations we selected 4 dogs with clinical chronic pancreatic insufficiency and 2 matched controls as seen in Table 1.

The tissue for immunohistochemistry was fixed in formalin and embedded in paraffin. For immunoperoxidase staining, 6 μ sections were deparaffinized, washed and incubated in the following: Coon's buffer, a 1:30 dilution of rabbit serum, guinea pig antiporcine insulin IgG 1:100[1], rabbit anti-guinea pig peroxidase complex 1:50, diaminobenzidintetrahydrochloride. Sections were dehydrated and mounted in Eukitt.

Tissue for electron microscopy was fixed in S-collidin-buffered (pH 7.3) 2% glutaraldehyde, post-fixed in 1% OsO_4, dehydrated with acetone and embedded in Spurr's resin. Islets of Langerhans

[1] Guinea pig anti-porcine insulin IgG was prepared by Dr. P. Donatsch, Sandoz, Basel

Table 1. Characteristics of dogs (male) used for immunohistochemical and ultrastructural studies

Dog No.	Breed	Age (months)	Remarks
1	German shepherd	36	–
2	German shepherd	18	Descendant of parents with chronic pancreatic insufficiency (Weber and Freudiger, 1977)
3	German boxer	17	–
4	Abruzzian shepherd	5	–
Control 1	German shepherd	96	Euthanized because of hip joint dysplasia
Control 2	German shepherd	36	Euthanized because of hip joint dysplasia

were selected from semithin sections. The ultrathin sections were contrasted with uranyl acetate and lead citrate and cut with an Om U2 (Reichert) microtome. Observations and photographs were made with a Philips EM 300 microscope.

Results

1. Clinical Observations and Gross Pathology

All animals with chronic pancreatic insufficiency presented the classical triad of clinical signs consisting of polyphagy, loss of weight and typical steatorrheic faeces depending on diet composition. The oral glucose tolerance test was typically at the higher limits of normality (Fig. 1).

Each of these animals exhibited a marked reduction of pancreatic volume at gross examination.

2. Observations by Light Microscopy

In the *exocrine pancreas* of control dogs the compact glandular structures are predominant. Acini are closely packed, arranged in lobules and have a consistent affinity for stains. The number of zymogen granules in acinar cells is generally large with some degree of variation depending on physiological conditions (Fig. 2).

The structure of the exocrine glandular tissue in affected dogs is extensively altered (Fig. 4). Most acini are reduced in volume and contain cells whose stainability varies greatly. Markedly changed lobules can be found isolated in fat and loose connective tissue (Fig. 6). Zymogen granules are reduced in number or are completely absent (Fig. 5). Some cell nuclei are apparently unchanged, others are clearly pyknotic (Fig. 7). Isolated round cell infiltrates can also be observed.

Langerhans' islets of control dogs are generally compact and are clearly demarcated from exocrine tissue (Fig. 3).

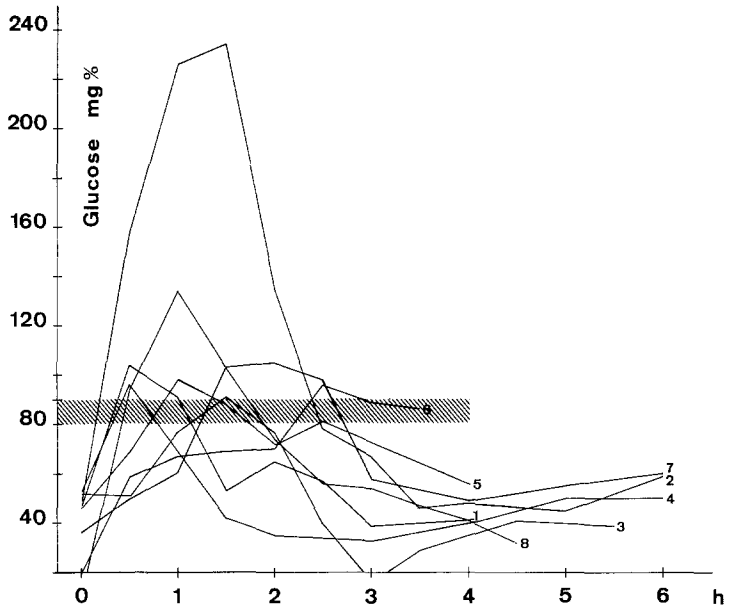


Fig. 1. Glucose tolerance test in 8 dogs with chronic pancreatic insufficiency. Shaded area gives limits of normality

In affected dogs, as a consequence of the disturbed architecture, recognition of islets is difficult. Frequently B cells are isolated, dispersed in the pancreatic tissue and difficult to distinguish from modified acinar cells. A better demonstration of the distribution pattern of endocrine cells was obtained by immunohistochemistry.

The islets of Langerhans of control animals are seen as more or less compact structures (Fig. 8). The B cells appear dark and richly granulated.

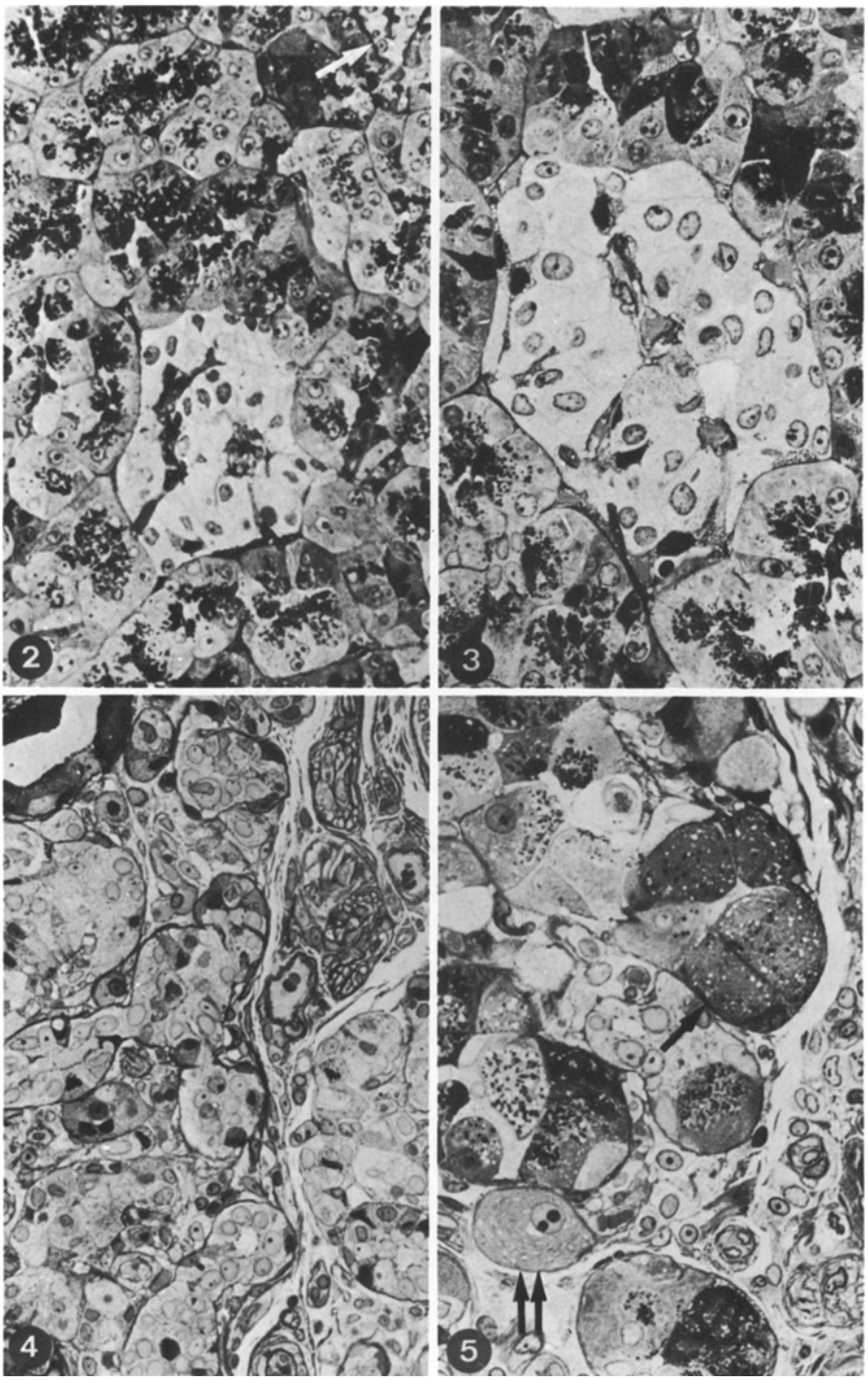
The appearance of the pancreas of dogs with chronic pancreatic insufficiency is clearly different (Fig. 9). In addition to a few isolated, more or less preserved islets, irregularly arranged clusters of endocrine cells are present with partial

Fig. 2. Control dog pancreas. Exocrine cells appear richly granulated and regularly arranged to form typical acini. Islets are distinct. Ducts (⇧) unconspicuous. Semi-thin section (*SF*), toluidine-blue stain (*TB*). 330×

Fig. 3. Appearance of a control dog pancreatic islet. Cell nuclei are round or ovoid and of regular appearance. SF, TB. 528×

Fig. 4. Pancreas from dog with chronic pancreatic insufficiency. Exocrine cells are mostly degranulated, acinar structure is generally lost and typical islets are absent. SF, TB. 528×

Fig. 5. Detail of affected pancreas. Structure of acini is irregular, intensity of cytoplasmic staining highly variable from cell to cell. Zymogen granules are reduced in amount (⇧), sometimes totally absent (⇧⇧). Dispersed endocrine cells, connective tissue elements and cells from ducti are recognizable in the widened interstitium. SF, TB. 528×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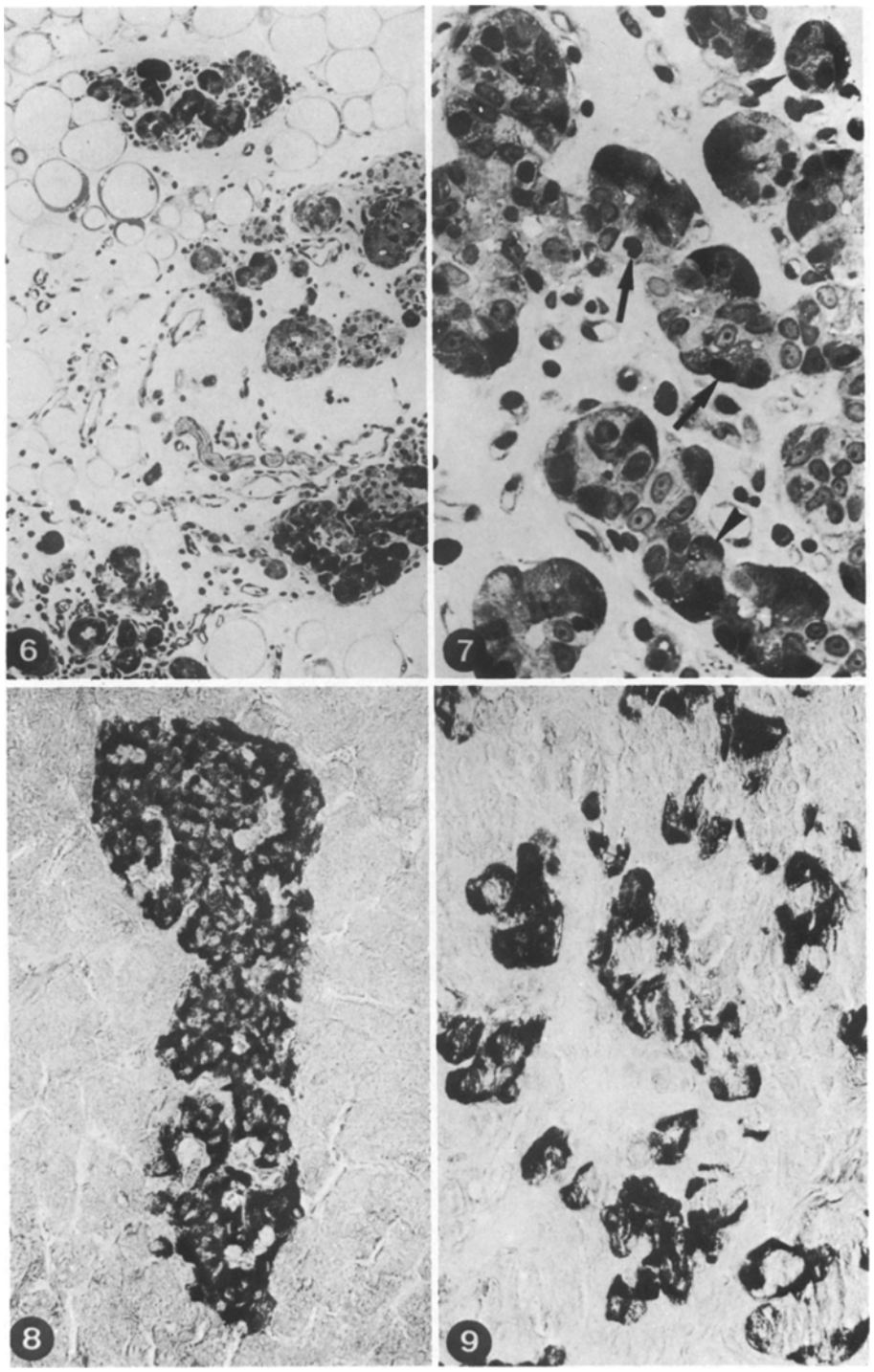

Fig. 6. Area of affected pancreas with high degree of change. Small clusters of abnormal acini are left within a loose matrix consisting largely of fibrous and fatty tissue. SF, TB. 132×

Fig. 7. Detail from Fig. 6 Acini are reduced in size (↑), cells show great differences in cytoplasmic staining and pyknotic nuclei (▲). SF, TB. 528×

Fig. 8. Immunohistochemical demonstration of insulin in B cells of a normal islet (peroxidase reaction). 300×

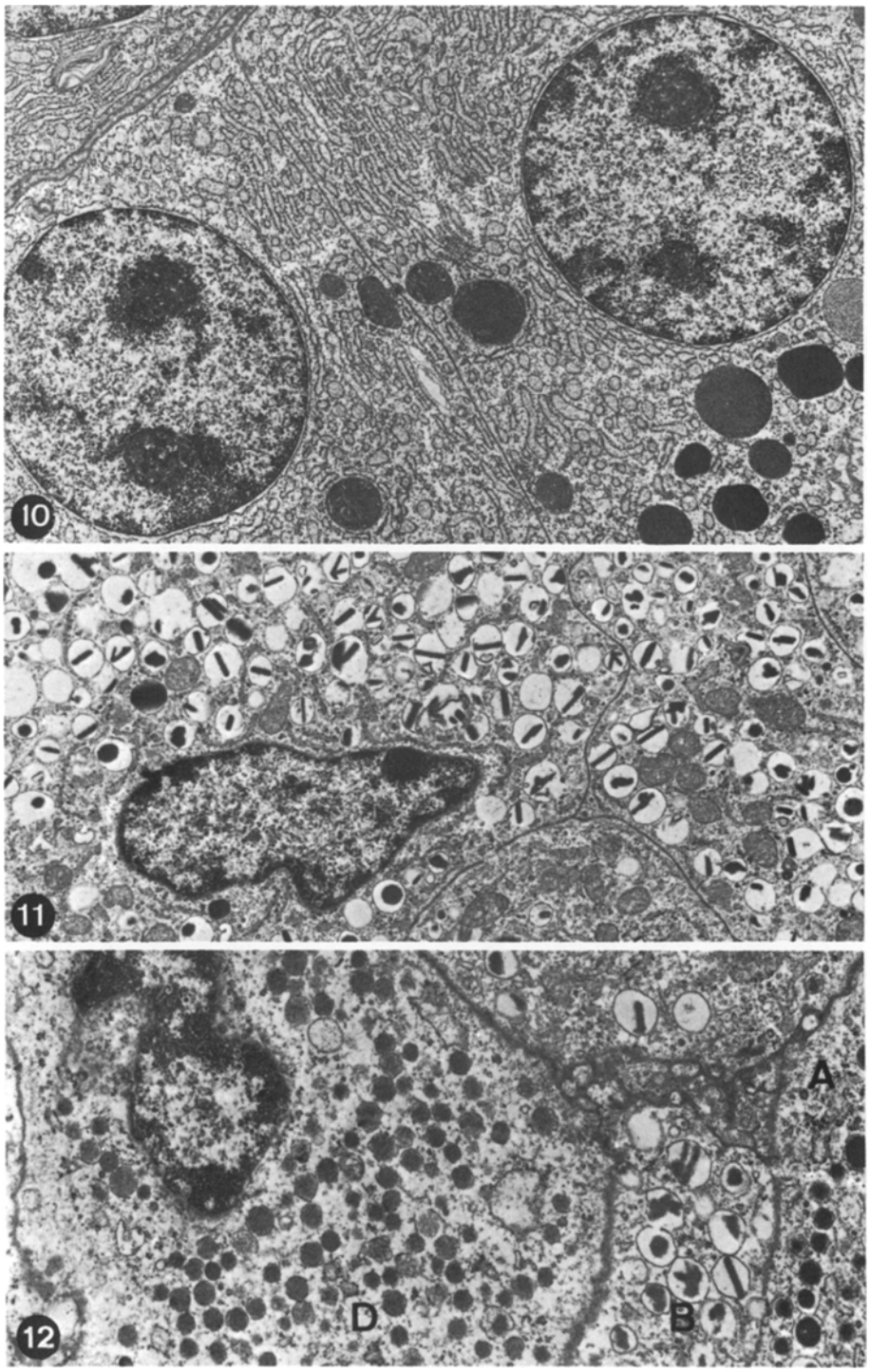

Fig. 10. Exocrine cells from control dog pancreas. Nuclei are round with a distinct chromatin pattern. Zymogen granules of various electron density can be seen in the cytoplasm. 7,568×

Fig. 11. B cells from a control dog. Insulin granules are different in size, rod-shaped or round, show a large halo and are regularly distributed throughout the cytoplasm. 7,568×

Fig. 12. D cell (*D*) from a control dog. Compared with B (*B*) and A (*A*) cells, secretion granules are of varying electron density, mainly round and ragged in appearance, and only sometimes with a halo. 13,266×

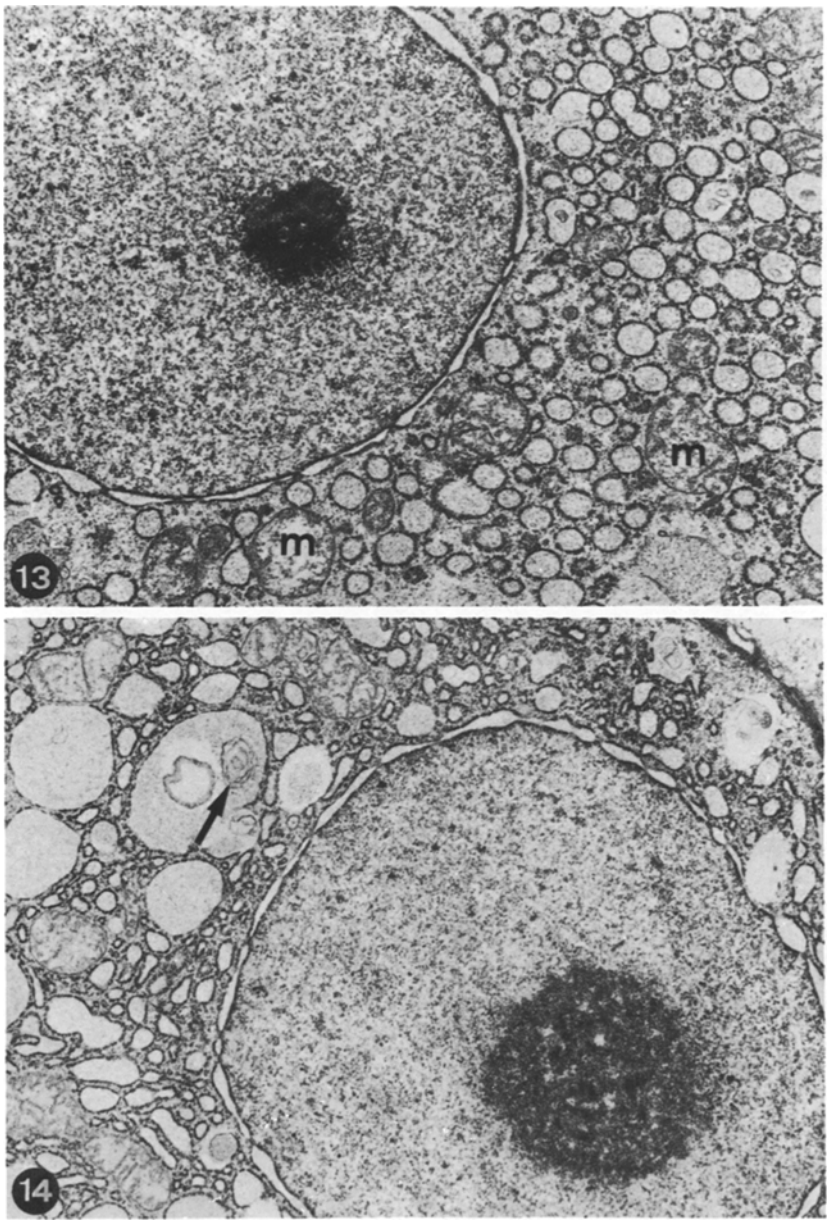

Fig. 13. Exocrine cell from affected pancreas. Nuclear chromatin is homogeneously dispersed, perinuclear space and cisternae of rough endoplasmic reticulum are irregularly dilated, mitochondria are swollen (*m*). 12,060 ×

Fig. 14. Further evolution of change shown in Fig. 13. Dilatation of reticulum cisternae has progressed. Myelin figures appear in the cisternae (⬆). 12,060 ×

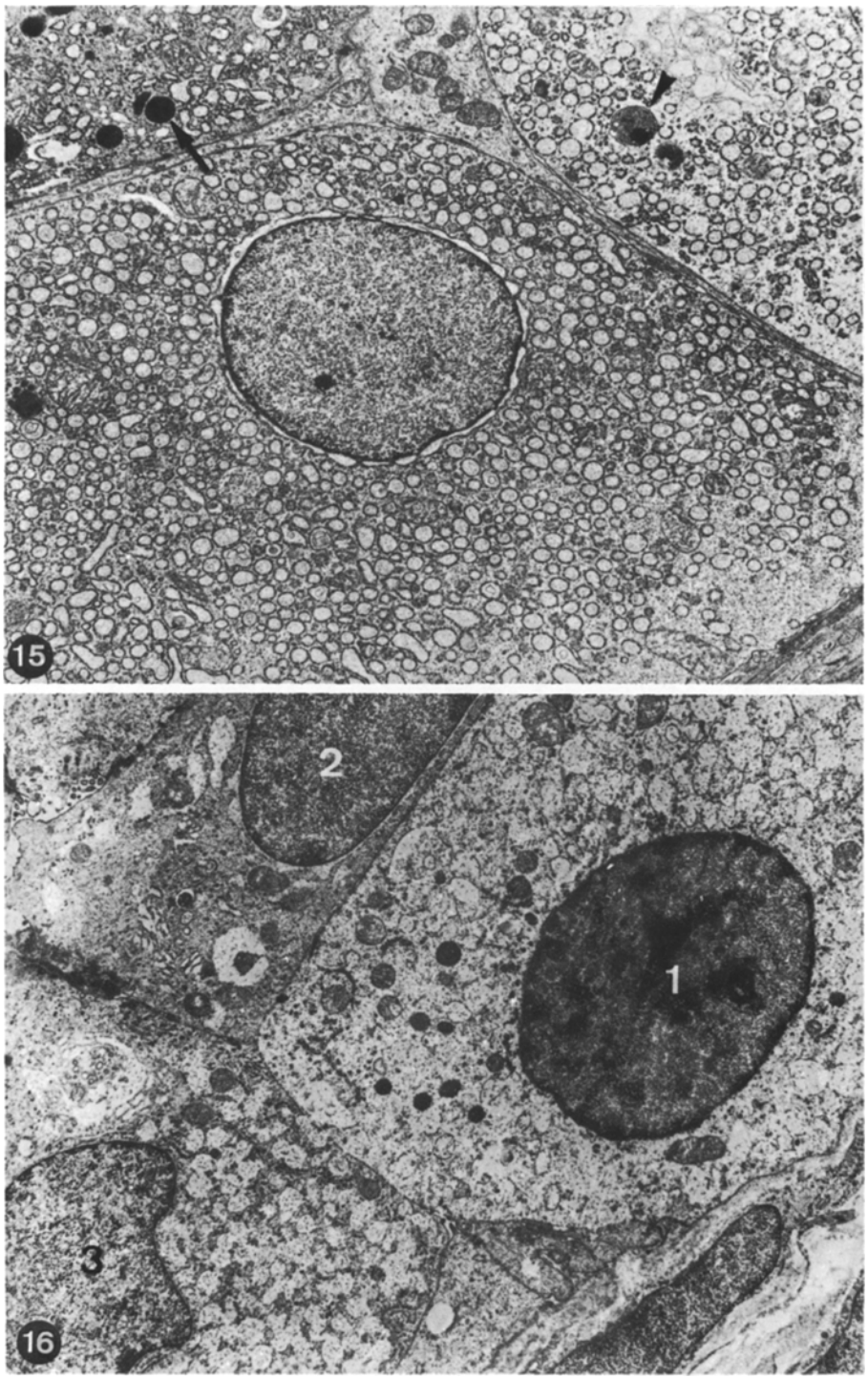

Fig. 15. As consequence of cisternal dilatation the cytoplasm of the exocrine cells in dog with chronic pancreatic insufficiency is increased in volume. Secretion granules are rare (▲) or absent. Lysosomes (▲) occur with increased frequency. 7,568 ×

Fig. 16. Exocrine cells (*1–2*) with advanced changes. Nuclei become pyknotic (*1*), swelling of organelles pogresses and cytoplasm loses detail. 7,568 ×

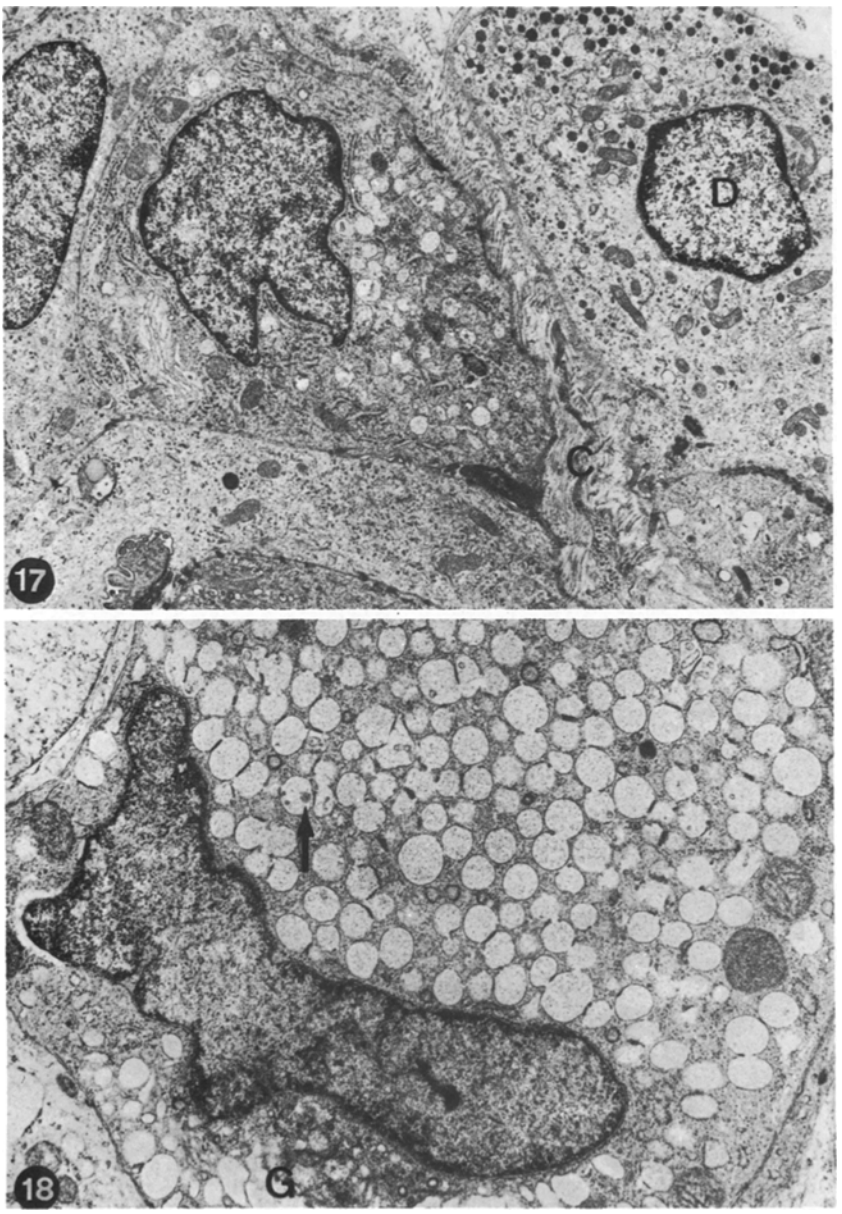

Fig. 17. Islet cells from an affected dog. While a D cell (*D*) is still recognizable, other endocrine cells have mainly lost their characteristics. Adjacent to D cells some collagen fibers (*C*) can be recognized. 6,880 ×

Fig. 18. Detail of abnormal islet cell. Secretion granules (▲) have almost disappeared. Cytoplasm is filled by empty vacuoles. Golgi apparatus (*G*) dilated. 12,060 ×

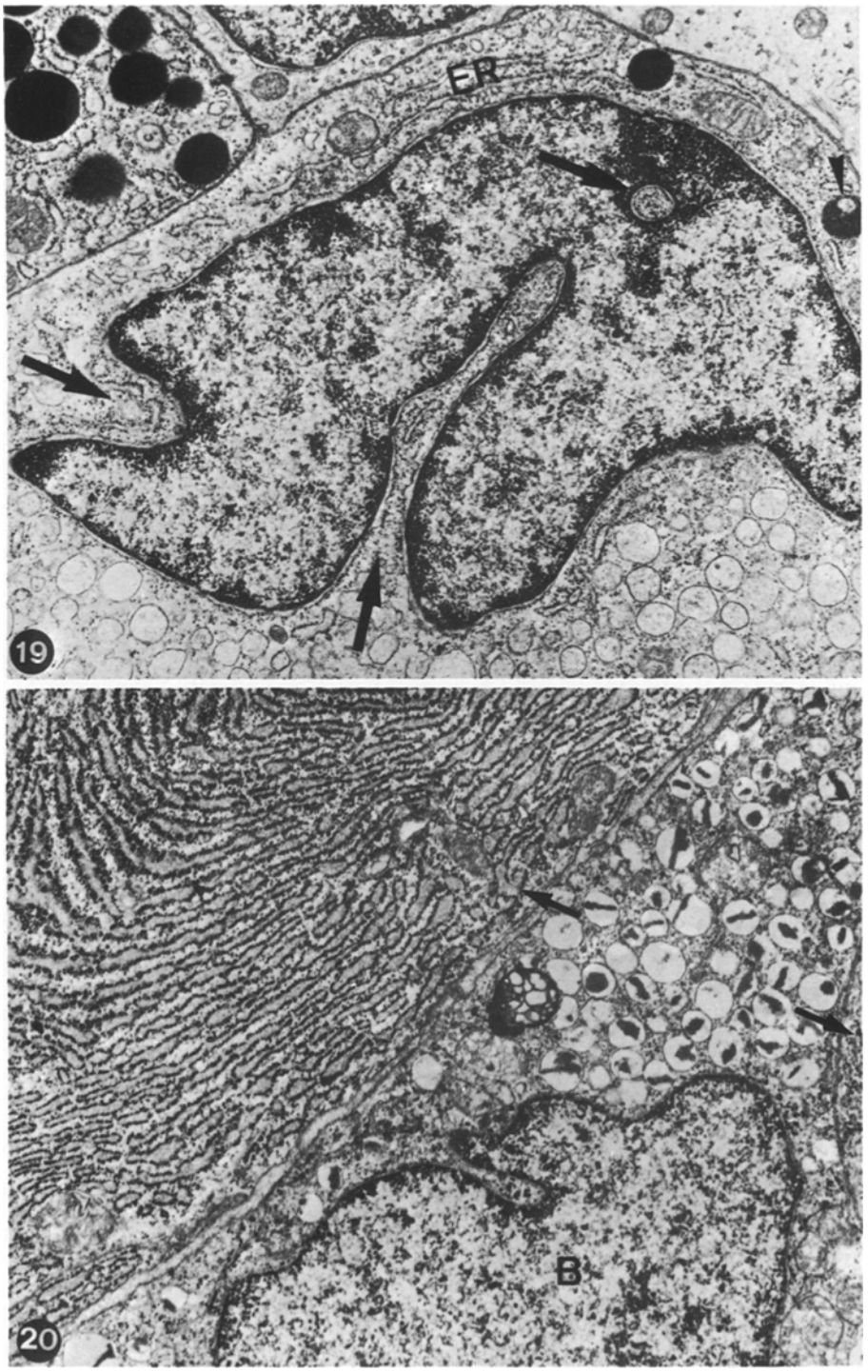

Fig. 19. Abnormal islet cell. Nucleus shows deep invaginations (▲), empty vacuoles fill the cytoplasm. Lysosomes (⟩) are frequent. The rough endoplasmic reticulum (*ER*), contrary to what observed in exocrine cells, appears unchanged. 13,266×

Fig. 20. B cell (*B*) from an affected dog. The endocrine cell occurred isolated and is surrounded by well preserved exocrine cells (▲). 13,266×

or total disorganization. The B cells appear in small groups or as solitary cells within the exocrine tissue. Some B cells contain large amounts of immunoreactive insulin, perhaps a sign of a compensatory hyperactivity.

3. Electron Microscopy

In control animals (Fig. 10), the nuclei of *acinar cells* are round and show a typical chromatin distribution. The perinuclear space is regular and the cytoplasm is packed with parallel oriented cisternae of endoplasmic reticulum. Mitochondria are round or ovoid with an electron dense matrix and tightly packed cristae. Secretory granules are generally abundant although there are variations in amount, size and electron density.

In affected dogs (Figs. 13–16), the nuclei of exocrine cells contain finely granulated, cloudy, homogeneous chromatin. The perinuclear space is irregularly dilated. In the cytoplasm the cisternae of rough endoplasmic reticulum are swollen with formation of vacuoles, which sometimes contain myelin bodies. The cells are generally degranulated. In cells with a greater degree of change, identification of organelles is difficult.

In control dogs (Figs. 11, 12) *islet cells* have ovoid nuclei with clearly recognizable nucleoli. Sometimes slight invaginations of the nuclear envelope can be found. Cell organelles appear normal and secretory granules are generally abundant.

In affected animals (Figs. 17–20), the polymorphic nuclear changes are impressive. While in some cells invaginations of the nuclear envelope may be slight (Fig. 17) in others they are very deep (Fig. 19) giving the nuclei an odd appearance. The chromatin is finely granulated and often accumulated at the periphery of the nucleus (Fig. 18). Nucleoli are loose textured and rarely found. Secretory granules are reduced in number, are contracted or collapsed and often only empty vacuoles remain (Figs. 18, 19). The Golgi apparatus appears dilated (Fig. 18); sometimes lysosomes are found (Fig. 19). From the appearance of the secretory granules, B cells are mostly affected, yet all islet cell types can be involved (Fig. 17). Relatively unchanged B cells, apparently isolated from other endocrine cells and completely surrounded by exocrine tissue, are of common occurrence (Fig. 20).

Discussion

Clinical and anatomical findings in the affected dogs corresponded to those reported in the literature for the syndrome of chronic exocrine pancreatic insufficiency, as generally occurs in the German shepherd (Thordal-Christensen and Coffin, 1956; Eikmeier, 1964; Köhler and Stavrou, 1967; Holroyd, 1968; Freudiger, 1971; Hill et al., 1971; Hill, 1972; Hashimoto et al., 1979).

The changes observed by conventional light microscopy consisted in an alteration of the glandular architecture with occurrence of prominent ducts and a reduction in the number of secretion granules. While some authors (Archi-

bald and Whiteford, 1953; Thordal-Christensen and Coffin, 1956; Köhler and Stavrou, 1967; Freudiger, 1971; Hill et al., 1971; Freudiger, 1976; Hashimoto et al., 1979) consider these changes to be degenerative, others (Holroyd, 1968; Jubb and Kennedy, 1970) describe similar changes as pancreatic hypoplasia. Because of this controversy on the pathogenesis of chronic pancreatic insufficiency we tried to obtain additional information from semithin sections and ultrastructural studies.

With the aid of semithin sections we frequently observed the presence of pyknotic nuclei or complete disaggregation of acinar cells. These findings are suggestive of a degenerative process. A similar conclusion was reached by Hill et al. (1971), who by studying fetal pancreas and finding it totally different from the tissue of affected dogs, weakened the hypoplasia thesis.

The ultrastructural changes are characterized by an abnormal chromatin distribution in the nucleus and by a segmental dilatation of the perinuclear space. The rough endoplasmic reticulum is also dilated and the number of secretion granules is markedly reduced. These lesions, together with the changes we observed in other cell organelles are of a degenerative character. Loss of zymogen granules, whorl formation and dilatation of the rough endoplasmic reticulum were observed by Churg and Richter (1971) after ligation of the pancreatic duct and were interpreted as degenerative changes. Chenard and Auger (1968), Boquist (1969) and Bockman et al. (1973) induced analogous changes of the rough endoplasmic reticulum experimentally by means of different injuries. Hashimoto et al. (1979) observed a progressive degranulation and loss of acinar cells and described this as a mild form of degeneration. Because of the parallelism between the findings of previous reports and those of this study we believe that the observed acinar changes are degenerative in character.

Generally, identification of islet cells has been considered to be difficult, but Köhler and Stavrou (1967) described well preserved islets in their cases. The endocrine changes may have been missed because, as in man, there is only occasionally clinically manifest diabetes mellitus (Archibald and Whiteford, 1953; Eikmeier, 1964; Ammann, 1967; Köhler and Stavrou, 1967; Hess, 1969; Spiro, 1971). Anderson (1972) however, is convinced that a majority of cases of diabetes in dogs may be the result of chronic or acute pancreatitis. Thordal-Christensen and Coffin (1956) believe that damage to the islet apparatus occurs in chronic pancreatitis, but not in pancreatic atrophy. Hess (1969) remarks that the endocrine insufficiency appears relatively late during the course of chronic pancreatitis in man and rarely in severe form. Because of this it usually receives little attention. Klöppel et al. (1978) observed a shift in the islet cell population in favor of the A cells after chronic pancreatitis. As a pathogenetic factor they postulated a local circulatory insufficiency due to the scarring of the tissue.

Our initial assumption that the secretory capacity, especially of insulin, was reduced, is supported by the observed dispersion of the cells of the islets of Langerhans and the appearance of isolated B cells, probably regenerative forms, in the exocrine part. This observation corresponds to those of Gepts and De Mey (1978) in human juvenile diabetes. Orci and Unger (1975) suggested that the arrangement of A, B and D cells is important to the normal functioning

of the islet. Taborsky et al. (1978) and Schauder et al. (1978) mentioned possible topographical interactions between endocrine cells. On the basis of this information it is logical to assume that a so called abnormal distribution pattern of A and B cells in the islet (Suzuki et al., 1975; Klöppel et al., 1978; Schauder et al., 1978; Taborsky et al., 1978) resulting from the dispersion, gives an indication of functional injury.

The electron microscopic findings were characterized predominantly by degenerative changes. Deformations of the nucleus, shrinkage of the karyoplasm and condensation of chromatin on the inner aspect of the nuclear membrane were seen in B cells. Progressive degranulation was evident in the cytoplasm. The appearance of the other cytoplasmic organelles was suggestive of a regressive lesion. Menser et al. (1978) produced similar changes in B cells of rabbit fetuses by experimental infection with Rubella virus. Derenzini et al. (1978) observed similar ultrastructural changes in B cells of mice after poisoning with α-Amanitin.

We conclude that in our cases a progressively evolving disease is responsible for the lesions described and that both in the exocrine and endocrine pancreas the changes are of a degenerative, yet not specific, type. The causes may be various. Possibly, congenital enzymatic defects may be responsible for the spontaneous disease in the dog.

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References

Ammann, R.: Fortschritte in der Pankreasfunktionsdiagnostik. Berlin, Heidelberg, New York: Springer 1967

Anderson, N.V.: Pancreatitis in dogs. Vet. Clin. North Am. **2**, 79–97 (1972)

Archibald, J., Whiteford, R.D.: Canine atrophic pancreatitis. J. Am. Vet. Med. Ass. **122**, 119–125 (1953)

Bockman, D.E., Schiller, W.R., Suriyapa, Ch., Mutchler, J.H.W., Anderson, M.C.: Fine structure of early experimental acute pancreatitis in dogs. Lab. Invest. **28**, 584–592 (1973)

Boquist, L.: The effect of excess methionine on the pancreas. Lab. Invest. **21**, 96–104 (1969)

Chenard, J., Auger, C.: Cytoplasmic changes in pancreatic acinar cells of the rat caused by one-Aminocyclopentane carboxylic acid (ACPC). Am. J. Pathol. **52**, 825–840 (1968)

Churg, A., Richter, W.R.: Early changes in the exocrine pancreas of the dog and rat after ligation of the pancreatic duct. Am. J. Pathol. **63**, 521–534 (1971)

Derenzini, M., Betts, C.M., Busi, C., Fiume, L.: Ultrastructural changes in B-cells of pancreatic islets in α-Amanitin-poisoned mice. Virchows Arch. B Cell Path. **28**, 13–20 (1978)

Eikmeier, H.: Zur Klinik der Pankreaserkrankungen des Hundes. Berlin. München. tierärztl. Wochenschr. **77**, 425–427 (1964)

Freudiger, U.: Die Erkrankungen des exokrinen Pankreas des Hundes. Kleintierpraxis **16**, 201–211, 229–234 (1971)

Freudiger, U.: Die Diagnose der chronischen exokrinen Pankreasinsuffizienz. Schweiz. Arch. Tierheilk. **114**, 476–487 (1972)

Freudiger, U.: Epidemiologie, Ätiologie, Klinik und Diagnose der chronischen exokrinen Pankreasinsuffizienz. Prakt. Tierarzt **57**, 301–308 (1976)

Gepts, W., De Mey, J.: Islet cell survival determined by morphology. Diabetes **27**, Suppl. 1, 251–261 (1978)

Hashimoto, A., Kita, I., Okada, K., Fujimoto, Y.: Juvenile acinar atrophy of the pancreas of a dog. Vet. Pathol. **16**, 74–80 (1979)

Hess, W.: Die chronische Pankreatitis. Bern und Stuttgart: Hans Huber 1969

Hill, F.W.G.: Malabsorption syndrome in the dog: A study of thirty-eight cases. J. Small Anim. Pract. **13**, 575–594 (1972)

Hill, F.W.G., Osborne, A.D., Kidder, D.E.: Pancreatic degenerative atrophy in dogs. J. Comp. Pathol. **81**, 321–330 (1971)

Holroyd, J.B.: Canine exocrine pancreatic disease. J. Small Anim. Pract. **9**, 269–281 (1968)

Jubb, K.V.F., Kennedy, P.C.: Pathology of domestic animals, 2nd ed., vol. 2, 697 p. New York and London: Academic Press 1970

Klöppel, G., Bommer, G., Commandeur, G., Heitz, Ph.: The endocrine pancreas in chronic pancreatitis. Virchows Arch. A Path. Anat. and Histol. **377**, 157–174 (1978)

Köhler, H., Stavrou, D.: Ein Beitrag zur Pancreasatrophie beim Hund. Dtsch. tierärztl. Wochenschr. **74**, 150–153 (1967)

Menser, M.A., Forrest, J.M., Bransby, R.D.: Rubella infection and diabetes mellitus. Lancet **I**, 57–60 (1978)

Orci, L., Unger, R.H.: Functional subdivision of islets of Langerhans and possible role of D cells. Lancet **II**, 1243–1244 (1975)

Raptis, S.: Enterohormone und endokrines Pankreas. Stuttgart: Georg Thieme 1974

Ritter, U.: Erkrankungen des exkretorischen Pankreas. Stuttgart: Georg Thieme 1971

Schauder, P., Mc Intosh, Ch., Panten, U., Arends, J., Arnold, R., Frerichs, H., Creutzfeldt, W.: Dynamics of somatostatin and insulin release from isolated rat pancreatic islets: Evidence for intraislet interactions between B cells and D cells. Metabolism **27**, Suppl. 1, 1211–1214 (1978)

Spiro, H.M.: Some observations on chronic pancreatitis. In: The exocrine pancreas, Beck, I.T., Sinclair, D.G., (eds.), pp. 212–225. London: J. and A. Churchill 1971

Suzuki, T., Ito, S., Sasaki, H.: Comparative observation of the pancreatic A and B cells by the enzyme antibody method: Light microscopic findings in dog and mouse tissue. Endocrinol. Japon. **22**, 531–536 (1975)

Taborsky, G.J., Smith, Ph.H., Porte, D.: Interaction of somatostatin with the A and B cells of the endocrine pancreas. Metabolism **27**, Suppl. 1, 1299–1302 (1978)

Thordal-Christensen, A., Coffin, D.L.: Pancreatic diseases in the dog. Nord. Vet.-Med. **8**, 89–114 (1956)

Weber, W., Freudiger, U.: Erbanalytische Untersuchungen über die chronische exokrine Pankreasinsuffizienz beim Deutschen Schäferhund. Schweiz. Arch. Tierheilk. **119**, 257–263 (1977)

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