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A Case of Canine Insulinoma

J. KOLEVSKÁ[1], R. HUSNÍK[1], V. BRUNCLÍK[1], V. MANDYS[2], Z. VERNEROVÁ[2], P. SCHÁNILEC[1], L. BARTOŠOVÁ[3], M. SVOBODA[1]

[1]Clinic of Dog and Cat Diseases, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]Department of Pathology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic
[3]Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

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Abstract

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The paper reports on a ten-year-old German Shepherd presented with a history of episodic weakness and convulsions after exercise. The patient showed *status epilepticus* upon presentation. Glucose concentrations repeatedly determined by glucometer were below detection threshold. After intravenous administration of glucose the condition always temporarily improved, however after 1–2 hours the situation reverted. Insulinoma was diagnosed on the basis of parallel determination of hypoglycaemia (glucose 3.3 $mmol \cdot l^{-1}$) and hyperinsulinaemia (2.398 $pmol \cdot l^{-1}$). A poor prognosis was stated and the owner decided for euthanasia. Necropsy revealed a tumour of pancreas. Subsequent pathological, immunohistochemical and electron microscopic examinations confirmed the diagnosis of insulinoma.

Pancreatic islet cell tumour, clinical symptoms, immunocytochemical examination.

Insulinoma (pancreatic islet cell tumour) is a tumour related to insulin overproduction, which is rarely diagnosed in the dog, ad in humans, as well. This neoplasm occurs more frequently in dogs of medium sized and large breeds without any particular breed or sex predisposition (Kruth et al. 1982; Mehlhaff et al. 1985). Medium aged dogs around 8 years old are most frequently affected (Rijnberk 1996). While in healthy animals insulin concentration is regulated by serum glucose concentration (insulin secretion is increased postprandially), in animals affected by insulinoma this mechanism does not work. Insulin concentration is also continuously increased in cases of hypoglycaemia (Feldman and Nelson 1996). There are multiple symptoms of the disease, which are mostly based on hypoglycaemia. This condition results in neuroglycopaenia and stimulation of the sympathetic nervous system. Neurological and psychic symptoms are frequent, in particular ataxia, tremor, depression/lethargy, convulsions, loss of consciousness, disorders of sight, behavioural disorders (Steiner et al. 1996). The symptoms get worse after prolonged starvation and physical exercise (Zamrazil 1997). Some of the patients with insulinoma gain weight in response to hypoglycaemia (Rijnberk 1996; Zamrazil 1997). In humans, about half of insulinomas are multihormonal and produce glucagon, pancreatic polypeptide, somatostatin, or gastrin as well as insulin (Mukai et al. 1982). Usually no typical symptoms due to the overproduction of the hormones are detected (Zamrazil 1997).

The present paper describes a case report of this rare tumour that occurred in a dog in the Czech Republic.

Address for correspondence:
MVDr. Jana Kolevská, Ph.D.
Clinic of Dog and Cat Diseases
Faculty of Veterinary Medicine
University of Veterinary and
Pharmaceutical Sciences
Palackého 1–3, 612 42 Brno,
Czech Republic

Phone: + 420 602 771 872
E-mail: kolevskaj@email.cz
http://www.vfu.cz/acta-vet/actavet.htm

Materials and Methods

Physical examination

A ten-year-old male German Shepherd was presented at the Clinic of Dog and Cat Diseases, Faculty of Veterinary Medicine, Veterinary and Pharmaceutical University Brno. A complete physical examination of the patient was carried out.

Laboratory examinations

Blood samples were collected and preserved with EDTA prior to haematological examination with Beckman coulter ACT 8. Differential blood count was evaluated after staining with standard May-Grünwald and Giemsa-Romanowski stains. Biochemical examination was carried out in serum after standard centrifugation. Cobas Mira S analyser was used to determine biochemical parameters. Insulin was determined by chemiluminiscence immunoanalysis (Immulite Insulin). Sensitivity of insulin determination was 2 $mIU \cdot l^{-1}$ (14.36 $pmol \cdot l^{-1}$). Calibration range upper limit was 400 $mIU \cdot l^{-1}$ (2.872 $pmol \cdot l^{-1}$). Approximate glucose determination was carried out by glucometer (Glukokard II).

Necropsy

Necropsy of the pancreas was carried out after euthanasia with Thiopental (natrium-5-aethyl-5-/1-methyl-butyl/-2-thiobarbituricum, manufactured by SPOFA, Prague, Czech Republic).

Tissue specimens were fixed in formalin, routinely processed and paraffin embedded. The slides were stained with haematoxylin and eosin and with Masson's trichrome to characterise tumour morphology.

Formalin-fixed paraffin-embedded tissue samples were used for immunohistochemistry examinations. Sections 5 µm thick were deparaffinised, hydrated, and treated with citrate buffer (pH 6) three times for 5 min in a microwave oven. Endogenous peroxidase activity was quenched by pretreatment of the specimens with 2.73% peroxide in 0.1% sodium azide diluted in distilled water. Slides were incubated with primary antibodies listed in Table 1. Synaptophysin and NSE, the markers of neuroendocrine cells, and CD57, a marker of neurosecretory granules, and insulin were detected with mouse monoclonal primary antibodies. Chromogranin A (CgA), another marker of neurosecretory granules, was detected using rabbit polyclonal primary antibody. After incubation with the primary antibody, immunodetection was performed using either a LSAB+ Peroxidase Kit (DAKO, Glostrup, Denmark), or an EnVision Peroxidase Kit (DAKO, Glostrup, Denmark). Chromogen 3,3-diaminobenzidine (FLUKA, Buchs, Switzerland) was used in all specimens, and counterstaining was performed with Harris haematoxylin. Appropriate positive controls were processed simultaneously. Specimens incubated without primary antibody were used as negative controls.

Stained slides were evaluated semi-quantitatively, according to the quantity and distribution of positive neoplasm cells, using optical microscope Nikon ECLIPSE E 400.

Preparation for electron microscopy was carried out as follows: Formalin-fixed tissue samples were postfixed in osmium tetroxide and embedded into Durcupan-Epon mixture. Thin sections were stained with uranyl acetete and lead citrate and examined under transmission electron microscope JEOL 1200.

Case description and results

The patient was presented with a history of episodic weakness and convulsions after exercise. Symptoms occurred three months before presentation for the first time. At the beginning the owner observed reduction of problems when the dog was fed after the manifestation of weakness. Over the time the symptoms were becoming more severe. Weakness was accompanied with convulsions, symptomatic episodes became longer and intervals between them were shorter. Upon first presentation the patient already showed *status epilepticus*. The following symptoms were detected by physical examination: clonic convulsions, polypnea, dehydration, hyperthermia (40.7 °C), tachycardia (heart rate of 160). Indicative determination of blood glucose with glucometer determined a value below the detection limit of the instrument (1.6 $mmol \cdot l^{-1}$). Intravenous infusion and continuous glucose administration temporarily improved glycaemia, however after 1-2 h the concentration of glucose again decreased below detection limit of the glucometer.

Table 1
Panel of primary antibodies used in immunohistochemical techniques

Antibody	Source	Clone	Dilution	Results
Insulin	Sigma-Aldrich	K36aC10	1 : 1 000	+ diffuse
Synaptophysin	DAKO	SY38	1 : 20	+ diffuse
Chromogranin A	DAKO	A 430	1 : 200	+ irregular
NSE	DAKO	BBS/NC/VI-H14	1 : 200	+ scattered
CD57	DAKO	NK-1	1 : 50	+ scattered

Haematological examination detected haemoconcentration, leucocytosis with left shift, and lymphopaenia. Biochemical examination revealed slightly elevated concentrations of urea, creatinine and activity of alanine aminotransferase. Acid-base balance was within reference range. Insulin concentration was significantly increased (2.398 pmol·l^{-1}). Glucose concentration (3.3 mmol·l^{-1}) was repeatedly determined together with insulin concentration (Table 2).

Table 2
Haematological and biochemical values in the dog presented for examination

Parameter (unit)	Presented patient	Reference range
Haemoglobin (g·l^{-1})	209	120–180
Haematocrit (l·l^{-1})	0.61	0.37–0.55
Erythrocytes (10^{12}·l^{-1})	8.86	5.4–8
Leukocytes (10^{9}·l^{-1}))	27.2	6–18
Neutrophils bands (10^{9}·l^{-1})	4.624	0–1
Neutrophils mature (10^{9}·l^{-1})	21.256	3–11.4
Lymphocytes (10^{9}·l^{-1})	0.504	0.8–3.8
Monocytes (10^{9}·l^{-1})	0.816	0–1.8
Eosinophils (10^{9}·l^{-1})	0	0–1.9
Reticulocytes (10^{9}·l^{-1})	0	20–60
Total protein (g·l^{-1})	60.5	55–75
Albumin (g·l^{-1})	27.3	23–34
Glucose (mmol·l^{-1})	3.3	3.3–7
Creatinine (μmol·l^{-1})	163.5	20–110
Urea (mmol·l^{-1})	10.24	3–9
ALP (μkat·l^{-1})	0.93	0.1–1.5
ALT (μkat·l^{-1})	6.18	0–1
AST (μkat·l^{-1})	0.48	0–1
Triglycerides (mmol·l^{-1})	0.68	0.5–1.7
Cholesterol (mmol·l^{-1})	5.16	2.7–7.0
Sodium (mmol·l^{-1})	155	140–158
Calcium (mmol·l^{-1})	2.35	2.2–2.9
Potassium (mmol·l^{-1})	3.88	3.8–5.8
Phosphorus (mmol·l^{-1})	1.91	0.5–2.6
Insulin (pmol·l^{-1})	2.398	14.4–70

ALP Alkaline phosphatase, ALT Alanine aminotransferase, AST Aspartate aminotransferase

With regard to history (episodic weakness and convulsions after exercise), clinical symptoms (*status epilepticus*) and laboratory findings of concurrent hypoglycaemia and hyperinsulinaemia, insulinoma was diagnosed. After having been informed about the diagnosis and poor prognosis, the owner was not interested either in treatment or in additional examinations which might confirm or exclude possible metastases, and decided for euthanasia of the patient. The owner did not agree with total autopsy but permitted a necropsy of the pancreas to confirm the diagnosis.

Necropsy of the pancreas revealed a grey-white tumour of the size of 2 × 3 × 1.5 cm (Plate I, Fig. 1). The tumour consisted of relatively small uniform cells containing predominantly round or oval nuclei with small inconspicuous nucleoli. The tumour cells

were arranged in the solid nests or cords, separated by fine connective tissue septa (Plate I, Fig. 2, Plate II, Fig. 3). The tumour was only partially encapsulated and extended into the adjacent pancreatic tissue.

The results of immunohistochemical study are summarised in Table 1. The tumour showed strong diffuse cytoplasmic staining for insulin and for synaptophysin. Positive staining for CgA was observed in irregular pattern. NSE and CD57 showed only rare, scattered immunoreactivity (Plate II, Fig. 4, Plate III, Fig. 5).

The results of electron microscopic examination were as follows: The tumour cells contained regular nuclei with fine chromatin granules. Cisternae of rough endoplasmic reticulum were presented within the cytoplasm. Round to oval membrane-bound, dense-core granules with distinct halo were observed in a vast majority of the cells (Plate III, Fig. 6). Other organelles such as mitochondria, ribosomes, lysosomes, and lipid droplets were rarely seen.

The above-mentioned examinations confirmed the diagnosis of a pancreatic islet cell tumour – insulinoma.

Discussion

In contrast to many other neoplastic diseases, the clinical presentation of insulinoma is rarely a result of destructive growth of the tumor. It is rather the symptoms of hypoglycemia, caused by the uncontrolled secretion of insulin, that make the patient/client seek medical advice (Feldman and Nelson 1996).

Diagnosis of insulinoma includes the proof of increased insulin secretion, which is not appropriate to current needs of the organism. Values above 144 $pmol \cdot l^{-1}$ in a patient with typical symptoms and concurrent hypoglycaemia (glucose below 3.3 $mmol \cdot l^{-1}$) confirm the diagnosis of insulinoma (Feldman and Nelson 1996). Rijnberk (1996) proposes the values of insulin of 70 $pmol \cdot l^{-1}$ and of glucose below 3.5 $mmol \cdot l^{-1}$ as the diagnostic threshold. The values detected in our case corresponded to this scheme.

In the patient presented in the paper, final diagnosis was confirmed by pathological, immunohistochemical and electron microscopical examinations as a pancreatic islet cell tumor (insulinoma).

Canine insulinomas are characterized by their firm-to-hard consistency and distinctive pale grayish-purple color (Jubb 1993). They are either discrete, focal, sharply delineated, expansile masses surrounded by a thin connective tissue capsule, or multilobular, poorly encapsulated masses infiltrating the adjacent exocrine parenchyma. Invasion of the capsule and entrapment of exocrine ducts and the parenchyma is common (Hawkins et al. 1987). Tumor cells are often tightly packed and divided into small islands by a fine fibrovascular stroma (Hawkins et al. 1987). Besides this predominantly solid medullary pattern, trabecular patterns also can be found. However, there is no apparent correlation between cytoarchitecture predominant cell type, and tendency to metastasize (O´Brien et al. 1987). The neoplastic cells are mildly pleomorphic, oval to polyhedral, with prominent round pale basophilic nuclei with a stippled chromatin pattern and abundant pale eosinophilic granular cytoplasm. The mitotic rate is low. On immunocytochemical examination, the majority of cells contain insulin but in smaller amounts than in non-tumorous islet cells (Hawkins et al. 1987). The malignant nature of the tumor may become evident only after metastases have been discovered (Lam and Lo 1998). In the case described in this paper the results of pathological examination were in accordance with the informations mentioned above. Electron microscopic findings and the results of immunocytochemical examination were in accordance with the information provided by Wiedemann and Franke (1985), Wieczorek et al. (1998), Cox (1999) and Portel-Gomes et al. (2001).

Surgical excision is considered the optimal therapy for insulinoma because when successful, it leads to long survival times. In malignant inoperable forms a conservative treatment is recommended. Glucocorticoids show a limited, short-term effect. Diazoxid is usually effective in blocking insulin secretion. Treatment with beta-blockers and calcium blockers is sometimes also effective. Somatostatin analogues (Sandostatin) not only reduce insulin secretion but may also slow down or terminate the growth of tumours. Their effect is however not stable in all cases. Cytostatic drugs (5-fluorouracil and streptozocin) are used in particular in cases of metastases in the liver (Zamrazil 1997).

The prognosis of the disease is rather dubious to poor and depends on the degree of malignancy and stage of the tumour (Feldman and Nelson 1996). Average survival period in dogs with conservative treatment is 12 months after the first symptoms of hypoglycaemia occur. Survival period after surgery depends on the presence of metastases. Nevertheless, Feldman and Nelson (1996) reported that 1/3 of all dogs treated by surgery died during the intervention or within one month after it due to uncontrollable hypoglycaemia or complications after surgery.

No treatment was started in the case described in this paper. The reason was poor prognosis and owner's decision for euthanasia.

Inzulinom u psa

V příspěvku je popsán případ desetiletého německého ovčáka, který byl předveden s anamnézou epizodické slabosti a křečí po zátěži. V době příjmu se nacházel ve *status epilepticus*. Koncentrace glukózy opakovaně stanovené glukometrem v době prezentace se nacházely pod mezí detekce. Po intravenózní aplikaci glukózy se stav vždy přechodně upravil, ale v 1-2 hod. intervalech se opakoval. Na základě paralelního a opakovaného zjištění hypoglykémie (glukóza 3,3 $mmol \cdot l^{-1}$) a hyperinzulinémie (2,398 $pmol \cdot l^{-1}$) byla stanovena diagnóza inzulinom. Po vyslovení nepříznivé prognózy byla na přání majitele provedena eutanázie. Nekropsií byl následně zjištěn nádor pankreatu, mikroskopické, imunohistochemické a ultrastrukturální vyšetření potvrdilo diagnózu inzulinomu.

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References

COX, D 1999: Pancreatic insulin-secreting neoplasm (insulinoma) in a West Highland white terrier. Can Vet J **40**: 343-345

FELDMAN, EC, NELSON, RW 1996: Beta-cell neoplasia: insulinoma. In: FELDMAN, EC, NELSON, RW: Canine and Feline Endocrinology and Reproduction. WB Saunders Comp., Philadelphia, pp. 422-441

HAWKINS, KL, SUMMERS, BA, KUHAJDA, FP, SMITH, CA 1987: Immunohistochemistry of normal pancreatic islets and spontaneous islet cell tumors in dogs. Vet Pathol **24**: 170-179

JUBB, KVF 1993: The pancreas. In: JUBB, KVF, KENNEDY, PC, PALMER, N (Eds): Pathology of Domestic Animals. Academic Press Inc., San Diego, pp. 407-424

KRUTH, SA, FELDMAN, EC, KENNEDY, PC 1982: Insulin-producing islet cell tumors: establishing a diagnosis and the clinical course for 25 dogs. J Am Vet Med Assoc **181**: 54-58

LAM, KY, LO, CY 1998: Pancreatic endocrine tumors: classical features and current insights. Cancer J **11**: 18-26

MEHLHAFF, CJ, PETERSON, ME, PATNAIK, AK, CARILLO, JM 1985: Insulin-producing islet cell neoplasm: surgical considerations and general management in 35 dogs. J Am Anim Hosp Assoc **21**: 607-612

MUKAI, K, GREIDER, MH, GROTTING, JC, ROSAI, J 1982: Retrospective study of 77 pancreatic endocrine tumors using the immunoperoxidase method. Am J Surg Pathol **6**: 387-399

O´BRIEN, TD, HAYDEN, DW, O´LEARY, TP, CAYWOOD, DD, JOHNSON, KH 1987: Canine pancreatic endocrine tumors: immunohistochemical analysis of hormone content and amyloid. Vet Pathol **24**: 308-314

PORTEL-GOMES, GM, GRIMELIUS, L, JOHANSSON, H, WILANDER, E, STRIDSBERG, M 2001: Chromogranin A in human neuroendocrine tumors: an immunohistochemical study with region-specific antibodies. Am J Surg Pathol **25**: 1261-1267

RIJNBERK, A 1996: Hypoglycemia due to islet-cell tumor. In: RIJNBERK, A: Clinical Endocrinology of Dogs and Cats. Kluwer Academic Publishers, Dordrecht, pp. 112-115

STEINER, JM, BRUYETTE, DS 1996: Canine insulinoma. Comp Cont Educ Pract Vet **18**: 13-23

WIECZOREK, G, POSPISCHIL, A, PERENTES, E 1998: A comparative immunohistochemical study of pancreatic islets in laboratory animals (rats, dogs, minipigs, nonhuman primates). Exp. Toxic. Pathol. **50**: 151-172

WIEDEMANN, B, FRANKE, WW 1985: Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38.000 characteristic of presynaptic vesicles. Cell **41**: 1017-1028

ZAMRAZIL, V. 1997: Hypoglykémie vznikající nalačno. In: STÁRKA, L. Endokrinologie. MAXDORF, Praha, pp. 267-270

Plate I
Kolevská J. et al.: Insulinoma ... pp. 353-358

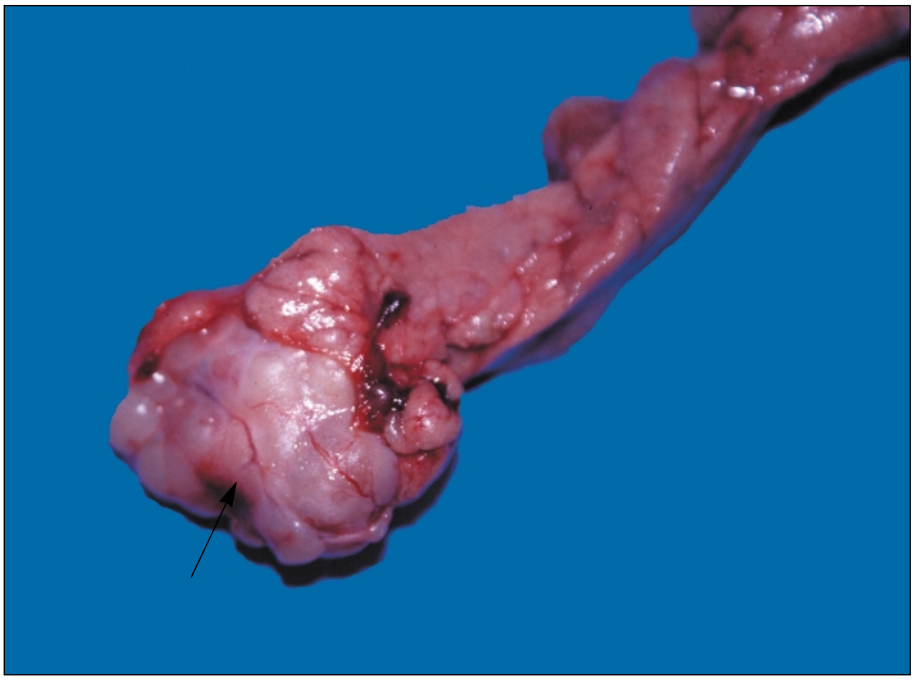

Fig. 1. Photograph of pancreatic insulin-secreting islet-cell tumor (presented case).

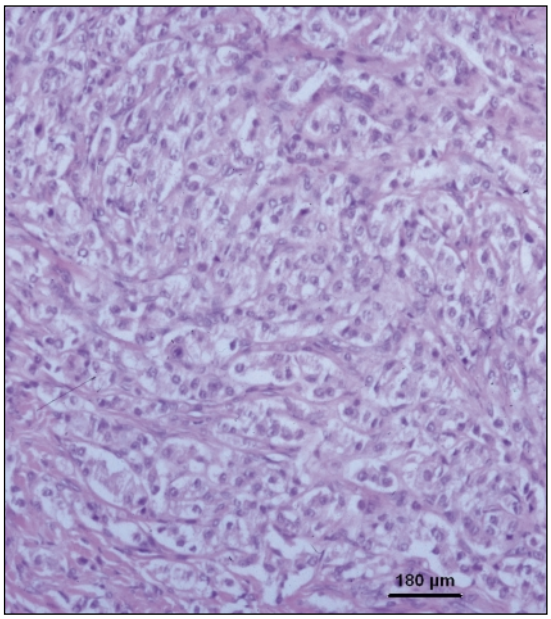

Fig. 2. At low power, the tumour is arranged in solid nests or cords (H&E, original magnification, 40).

Plate II

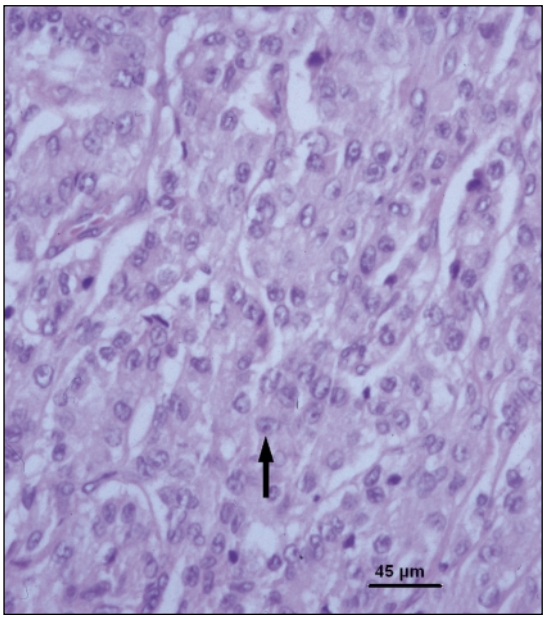

Fig. 3. High-power view of polygonal tumour cells containing round or oval nuclei (arrow) with small inconspicuous nucleoli (H&E, original magnification, × 200).

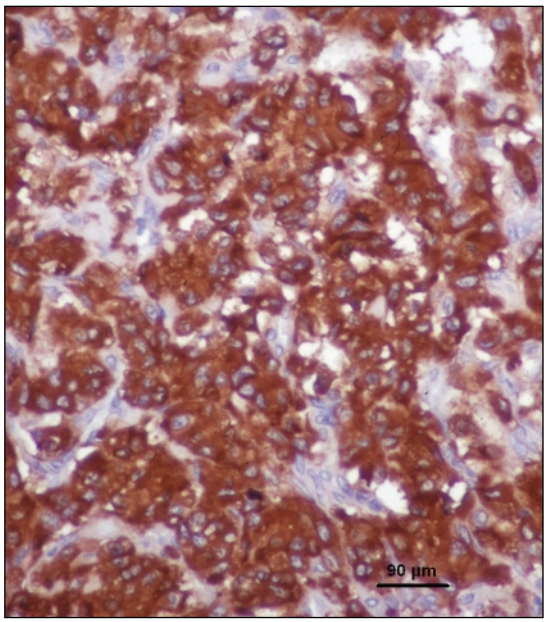

Fig. 4. Positive immunohistochemical staining of tumour cells (brown colour). Strong diffuse immunoreactivity for insulin (original magnification, ×100).

Plate III

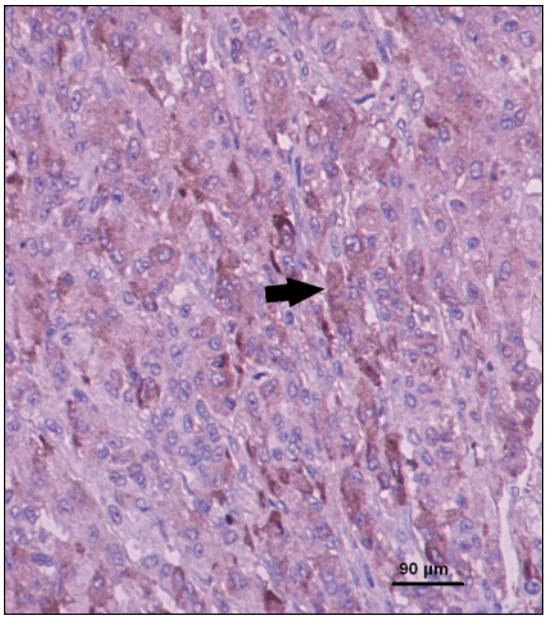

Fig. 5. Irregular patchy immunostaining for CgA in tumour cells (arrow) (original magnification, × 100).

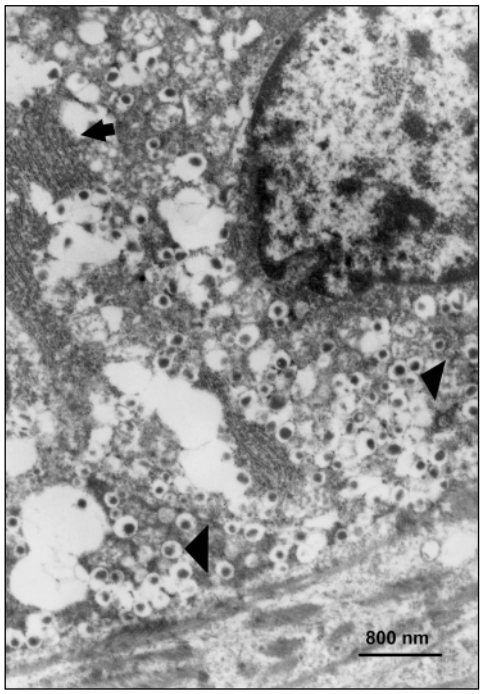

Fig. 6. Electron micrography shows the tumour cell containing cisternae of rough endoplasmic reticulum (arrow) and numerous granules (arrowheads) within the cytoplasm (× 20.000).