Detection of Chromogranin in Neuroendocrine Cells With a Monoclonal Antibody

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A monoclonal antibody (LK2H10) produced against a human pheochromocytoma reacted immunohistochemically with 126 normal and neoplastic endocrine tissues with secretory granules which were formalin-fixed and paraffin-embedded. Antibody LK2H10 did not react with 46 other endocrine tissues or tumors without secretory granules nor with 113 normal and neoplastic nonendocrine cells and tumors. Tumors with abundant secretory granules showed intense and diffuse staining, and tumors with few granules, such as Merkel cell carcinomas, neuroblastomas, and small cell carcinomas of lung, showed focal staining. Antibody LK2H10 did not react with melanomas, nevi, posterior pituitary, peripheral nerve tissues, or neurons. The target structure of LK2H10 was identified as human chromogranin, of which the major fraction was chromogranin A (mol wt 68,000 daltons). Preabsorption with purified chromogranin A blocked immunoperoxidase staining by LK2H10 in normal adrenal medulla, in the anterior pituitary, and in a pheochromocytoma. Ultrastructural immunohistochemistry with LK2H10 showed that chromogranin was present in cytoplasmic secretory granules. These results indicate that chromogranin is widely distributed in the secretory granules of most polypeptide-producing endocrine tissues, and it is readily detected with the use of monoclonal antibody LK2H10. The detection of this marker can be very helpful as a diagnostic aid for neuroendocrine cells and tumors. (Am J Pathol 1984, 115:458–468)

THE GROUP of cells which are widely dispersed throughout the body and share some common characteristics, including dense-core secretory granules and the ability to take up amine precursors and modify them by decarboxylation, are known as APUD cells. These cells and related tissues comprise the diffuse neuroendocrine system (DNES).[1] Although many of the cells of the DNES are not derived directly from neural crest precursors,[2,3] the relationship of the cells in this widely diffuse group of cells has been reconfirmed by the immunologic detection of neuron-specific enolase (NSE) in the cytoplasm of all cells of the DNES.[4,5] NSE is an isozyme of the glycolytic enzyme enolase, which is also in neurons but not in endocrine cells which do not form part of the DNES, such as thyroid follicular and adrenal cortical cells.[6] Because of the recent work demonstrating the specificity of NSE for neuroendocrine cells and neurons, it is possible that other markers for endocrine cells will also be developed. Several reports have indicated that with the use of hybridoma technology,[7] it is possible to develop monoclonal antibodies to previously undetected hormones and other substances associated with a broad spectrum of endocrine and neural cells.[8–10]

Recent evidence indicates that chromogranin, which was originally described in the catecholamine-containing granules of bovine adrenal medulla,[11,12] may be widely distributed in endocrine tissues.[13–15] Chromogranin comprises a group of acidic polypeptides of various sizes which forms a major part of the soluble proteins in the secretory granule of the adrenal medulla.[12] Chromogranin A is the largest of the polypeptides (68,000 daltons) and makes up about 40–50% of the total soluble granule proteins of the adrenal medulla. A recent report indicated that bovine parathyroid glands contained a structural protein similar in amino acid composition to chromogranin A,[13] while in another study, a polyclonal antiserum to human chromogranin A was found to react

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immunohistochemically with various endocrine tissues.[14] We have recently developed a monoclonal antibody (LK2H10) against a chromograninlike substance and in a preliminary report[15] indicated that antibody LK2H10 reacts specifically with a wide range of tissues and tumors of the DNES. In this report we show that antibody LK2H10 is directed against chromogranin A and other related chromogranins. A detailed description of LK2H10 reactivity with endocrine tissues and ultrastructural studies demonstrating that chromogranin is present in secretory granules is also included.

Materials and Methods

Production of Monoclonal LK2H10 Specific for Human Chromogranin

A Balb/c mouse was immunized with 1-sq mm pieces of a human pheochromocytoma that had been minced with a razor blade and mixed with Freund's adjuvant prior to intraperitoneal injection. After 5 biweekly injections, the mouse was sacrificed and the spleen cells were fused with NS-1 mouse myeloma cells essentially as described by Galfre et al.[16] After 14 days in medium containing hypoxanthine, aminopterin, and thymidine, culture supernatant fluids from growing clones were screened for antibody activity against cryostat sections of the original tumor by indirect immunofluorescence. After this step, 50 clones were selected and 2 weeks later were retested for reactivity with cryostat and formalin-paraffin-fixed tissue sections of the same tumor by avidin-biotin complex (ABC) peroxidase staining. Hybridoma LK2H10 was selected for additional testing because it reacted with tumor cells but not with any connective tissue elements in both cryostat and paraffin tissue sections. The hybridoma secreting antibody LK2H10 was successfully subcloned by limiting dilution over mouse spleen feeder cells. A single large batch of hybridoma culture fluid containing LK2H10 antibody was collected and used throughout this study at a 1:10 dilution because strong staining was seen even up to a 100-fold dilution.

Purification of Human Chromogranin

A human autopsy adrenal gland dissected for removal of most of the cortical region was extracted by high-speed blending in a Waring blender with about 10 volumes of ice-cold phosphate-buffered saline (PBS). The extract was then cleared of debris by centrifugation at 10,000 rpm in a Beckman centrifuge. Antibody LK2H10 was purified from mouse ascites fluid by protein-A/Sepharose chromatography[17] and then coupled to Sepharose 4B by the cyanogen bromide method.[18] The soluble adrenal extract was applied to the LK2H10 antibody-Sepharose column, and the column was washed with PBS until the effluent O.D. 280 mm reading was <0.01. The bound material was then eluted with 2N acetic acid; and after pH neutralization with Tris base, the affinity purified chromogranins were dialyzed against H_2O and concentrated by vacuum evaporation.

Purified human chromogranin A was kindly provided by Dr. Dan O'Connor at the Veterans Administration Hospital, San Diego, California. The molecule was purified to electrophoretic homogeneity from isolated catecholamine storage vesicles of a human pheochromocytoma as detailed elsewhere.[19]

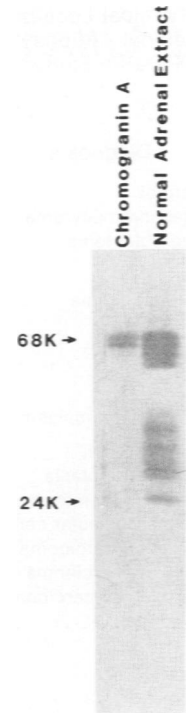


Figure 1 – Molecular weight analysis of endocrine granule protein detected by antibody LK2H10. An electrophoretically purified preparation of human chromogranin A from pheochromocytoma catacholamine storage vesicles and LK2H10 affinity purified proteins from a human adrenal gland extract (see methods for details) were first separated by molecular weight in a SDS-polyacrylamide slab gel and then transferred to nitrocellulose paper for immunoblotting. Treatment with antibody LK2H10 followed by peroxidase conjugated anti-mouse Ig antibodies showed that LK2H10 antibody reacts with the chromogranin A molecule as well as with a complex array of polypeptides (chromogranin) from the adrenal medullary extract.

Immunoelectroblot Staining

About 10 μg of human chromogranin A or LK2H10 affinity-purified endocrine protein were separated by molecular weight into individual polypeptides in a one-dimensional polyacrylamide slab gel (1.5 mm ×

Table 1 – Immunohistochemical Localization of Chromogranin by Monoclonal Antibody LK2H10 in Normal and Neoplastic Paraffin-Embedded Endocrine Tissues

Organ/Tissue	Diagnosis	Staining with LK2H10*
Adrenal medulla	Normal	10/10
	Pheochromocytoma	25/25
	Neuroblastoma	4/10
Paraganglia	Normal	1/1
	Paraganglioma	5/5
Adrenal cortex	Normal	0/10
	Carcinoma	0/2
Pancreas	Normal islet	5/5
	Endocrine neoplasm	15/20
Thyroid	Normal C-cell	5/5
	C-cell hyperplasia	5/5
	Medullary carcinoma	6/6
	Normal follicular cells	0/10
	Follicular carcinoma	0/3
	Papillary carcinoma	0/3
	Hürthle cell carcinoma	0/3
Parathyroid	Normal	2/2
	Adenoma	2/3
Anterior pituitary	Normal	5/5
	Adenoma	6/10
Posterior pituitary	Normal	0/5
Stomach	Normal endocrine cells	3/3
	Carcinoid	3/3
Small bowel	Normal endocrine cells	3/3
	Carcinoid	2/2
Colon	Normal endocrine cells	4/4
Lungs	Normal endocrine cells	1/3
	Small cell carcinoma	4/10
Skin	Merkel cell carcinoma	2/3
Fetal adrenal medulla	Normal	4/4
Fetal pancreatic islet	Normal	4/4
Fetal adrenal cortex	Normal	0/4
Placenta	Normal	0/4
	Choriocarcinoma	0/2

* Positive cases/total number of cases.

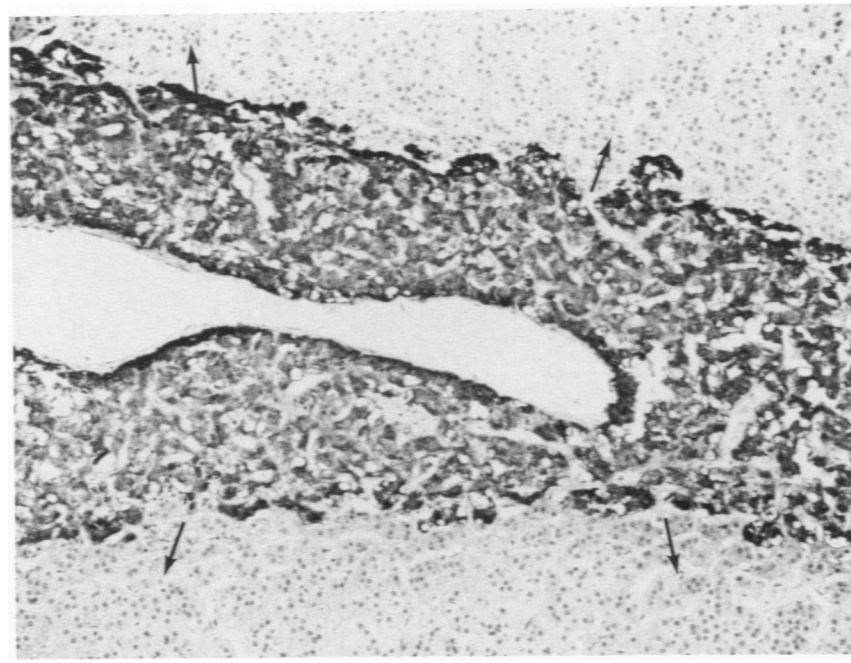
160 mm) containing sodium dodecyl sulfate and the buffer system of Laemmli.[20] After electrophoresis was completed, the proteins in the gel were electrophoretically transferred at right angles (60 V for 4 hours) to nitrocellulose paper as described by Towbin et al.[21] To visualize proteins reactive with antibody LK2H10, we first incubated the nitrocellulose paper for 1 hour with 4% bovine serum albumin–PBS to block nonspecific binding. Subsequent treatments included 1 hour with the monoclonal antibody LK2H10 or a control spent culture medium (diluted fourfold in PBS) and 1 hour with peroxidase-conjugated anti-mouse IgG antibody (Cappel Laboratories) (diluted 1:400 in PBS–10% fetal calf serum) and 10 minutes in diaminobenzidine-HCl (20 mg/dl) and H_2O_2 (0.05%). All of the above steps were separated by three washes in PBS (2 minutes each). The level of nonspecific staining was determined by the use of a culture medium containing a monoclonal antibody to human Ia antigens.

Immunohistochemical Staining of Tissues

The avidin–biotin complex (ABC)/immunoperoxidase method of Hsu[22] was used as previously described.[23] Formalin-fixed paraffin embedded sections cut at 4 μ were dewaxed, then treated with H_2O_2–methanol (1%) for 15 minutes. After washing in PBS, pH 7.2, and treatment with suppressor serum for 10 minutes, the tissues were incubated with a 1:10 dilution of LK2H10 antibody for 60 minutes, followed by washes in PBS, and then incubated with biotin IgG (Vector Lab, Burlingame, Calif) for 30 minutes. After PBS washes and incubation in avidin–biotin/peroxidase complex (Vector Labs, Calif) for 30 minutes, the tissues were treated with diaminobenzidine-HCl (20 mg/dl) with 0.05% H_2O_2, washed with distilled water, and counterstained with hematoxylin.

Ultrastructural immunohistochemical studies were done with the ABC and colloidal gold techniques.[24] Tissues were fixed in 4% paraformaldehyde, 1% glutaraldehyde in phosphate buffer, pH 7.2, for 1 hour. Sections were embedded in Polybed/Araldite. Ultrathin sections were placed on nickel grids for immunostaining. After etching in 5% H_2O_2 for 10 minutes and washing in PBS, the sections were treated with suppressor serum (5% normal horse serum) for 15 minutes, then incubated with the primary antibody at 1:10 and 1:50 dilutions for 60 minutes at room temperature. After PBS, biotin IgG, and avidin–biotin complex treatments for 30-minute periods, the sections were stained with DAB for 5 minutes, then counterstained with 2% uranyl acetate for 30 minutes. For colloidal gold staining, the grids were treated with methanol–H_2O_2, followed by 1% BSA, for 10 minutes. After washing in PBS they were incubated with the monoclonal antibody for 60 minutes at 1:10 and 1:50 dilutions. Sections were washed in PBS, then treated with protein A linked to 20–25 nm colloidal gold particles (EY Labs, San Mateo, Calif) for 60 minutes. Colloidal gold was diluted 1:8 before using. After washes in PBS and H_2O, the grids were counterstained with 2% uranyl acetate. The sections were viewed with a Zeiss 109 electron microscope.

Controls for immunohistochemical study consisted of 1) omission of the primary and secondary anti-



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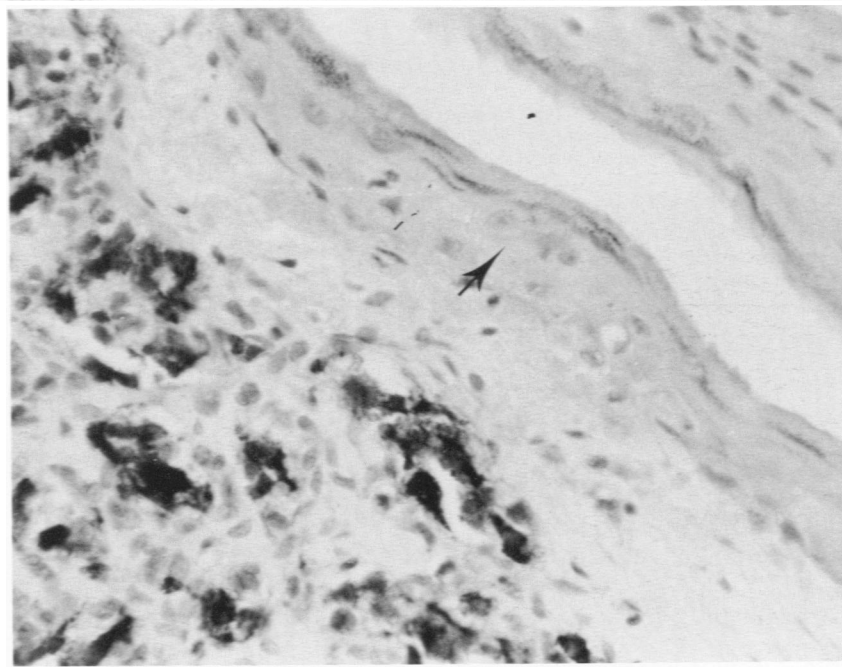

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Figure 2—Normal adrenal medulla stained with antibody LK2H10. The dark brown medullary cells are positive, whereas the adrenal cortical cells (*arrows*) are negative for chromogranin. (Immunoperoxidase, x 132) **Figure 3**—Paraganglioma from the middle ear (glomus jugulare) stains positively with antibody LK2H10, whereas the squamous epithelium (*arrow*) is negative. (Immunoperoxidase, x 208)

bodies and 2) substitution of antibody LK2H10 absorbed with chromogranin A in place of the primary antibody. Absorption was done by incubating 0.5 μg/ml of chromogranin A with 1 ml of a 1:10 dilution of the antibody LK2H10 at room temperature for 2 hours, then at 4 C for 18 hours. After centrifugation at 10,000 rpm for 60 minutes, the supernatant was used in place of the primary antibody.

Results

Evidence for Chromogranin Specificity of Monoclonal Antibody LK2H10

To assess the nature of the molecule detected by LK2H10, we used an affinity chromatography column containing this antibody to purify reactive proteins from a soluble extract of a human autopsy adrenal

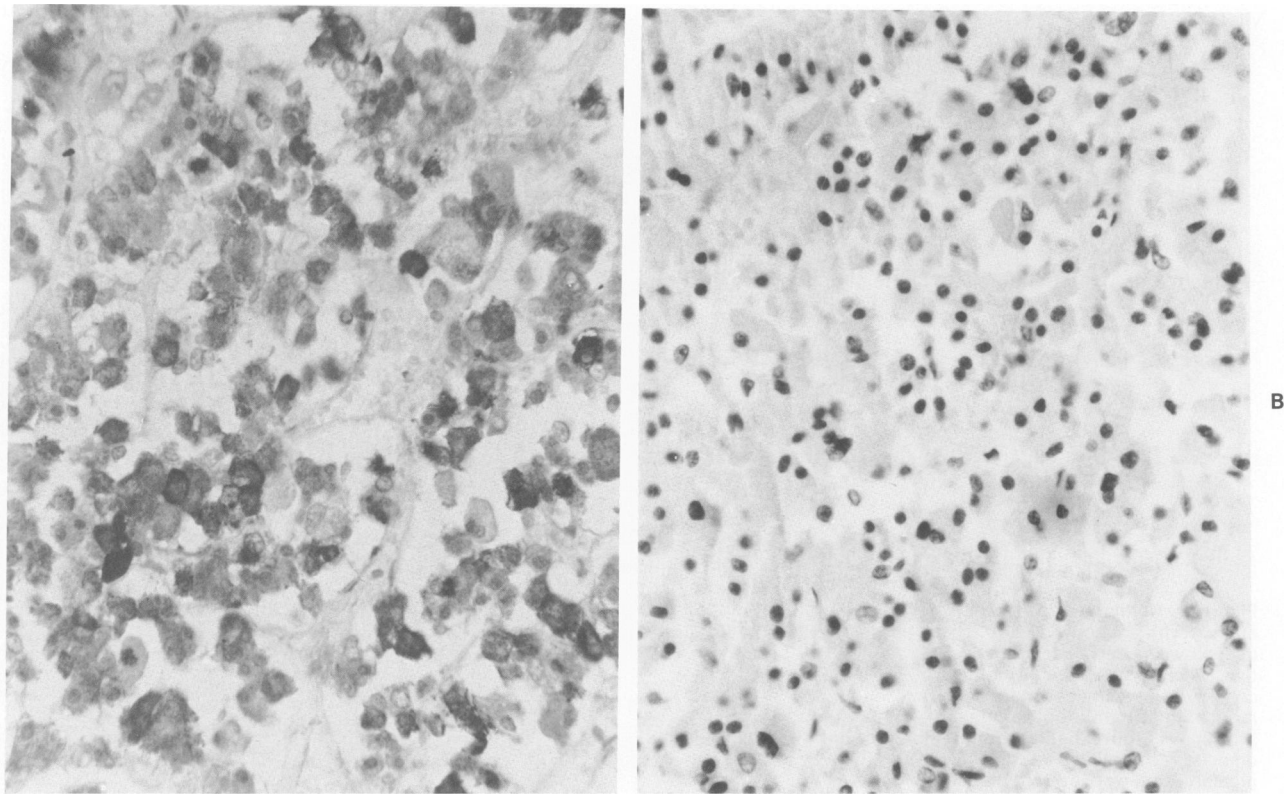


Figure 4A – Normal anterior pituitary showing variable staining of cells with antibody LK2H10. **B** – Absorption of antibody LK2H10 with chromogranin A before immunostaining of the anterior pituitary resulted in complete blocking of immunoreactivity. (Immunoperoxidase, × 330) (With a photographic reduction of 6%)

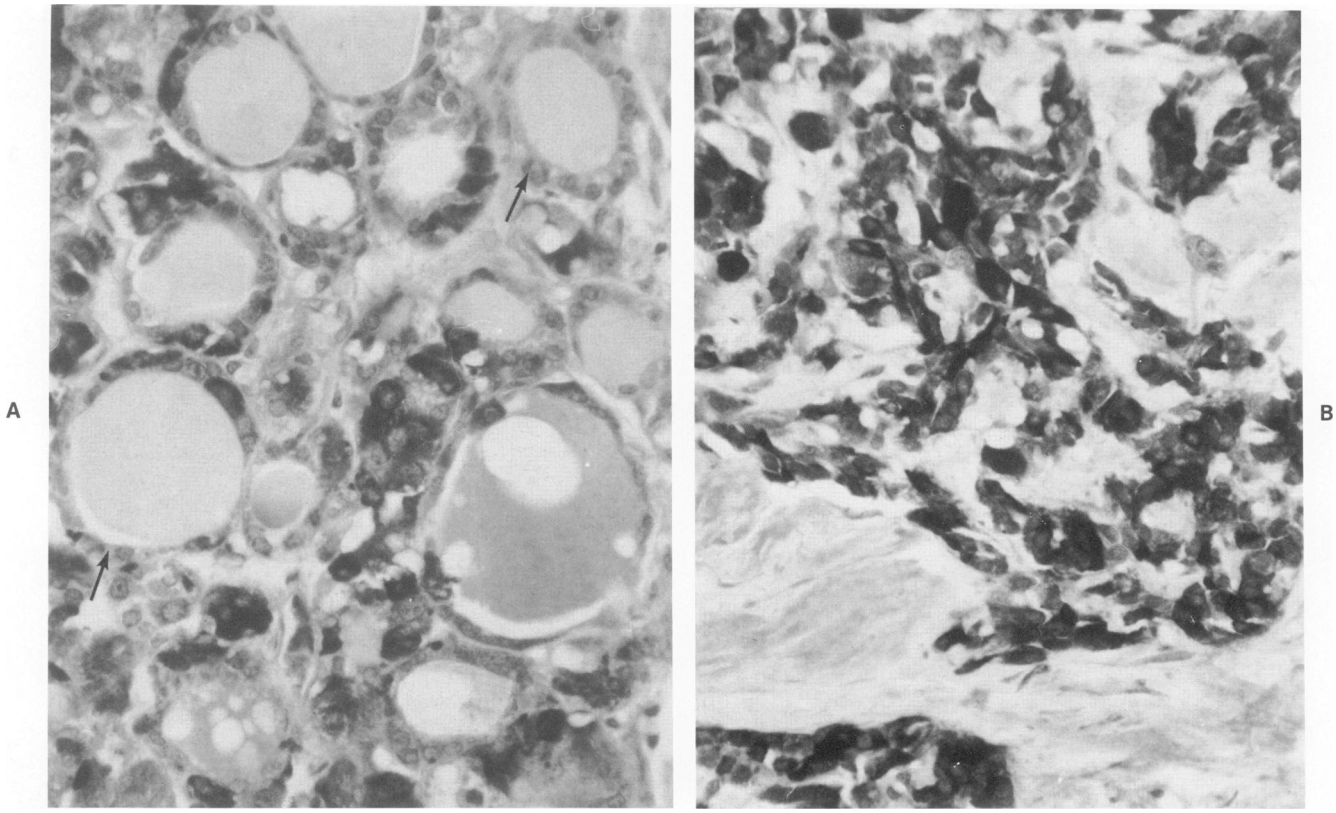
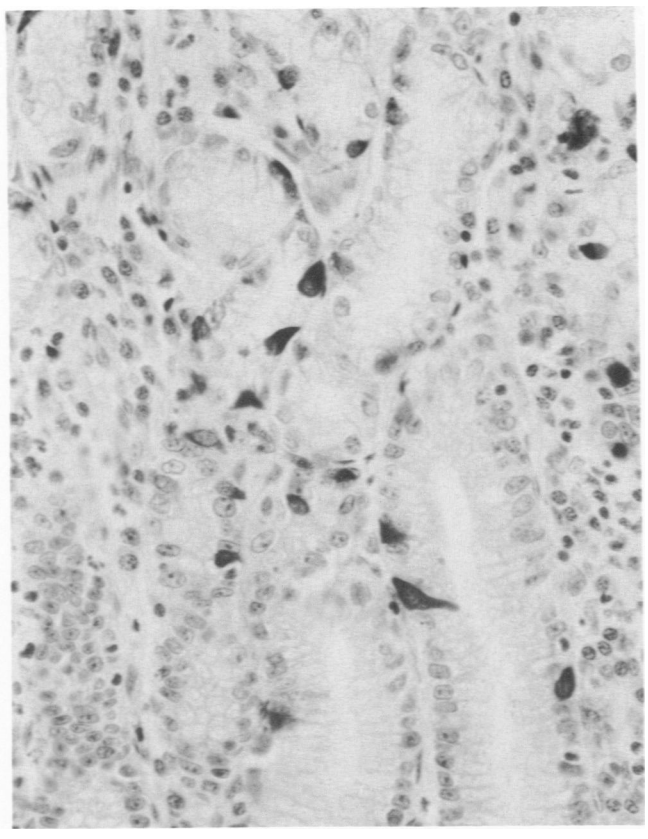
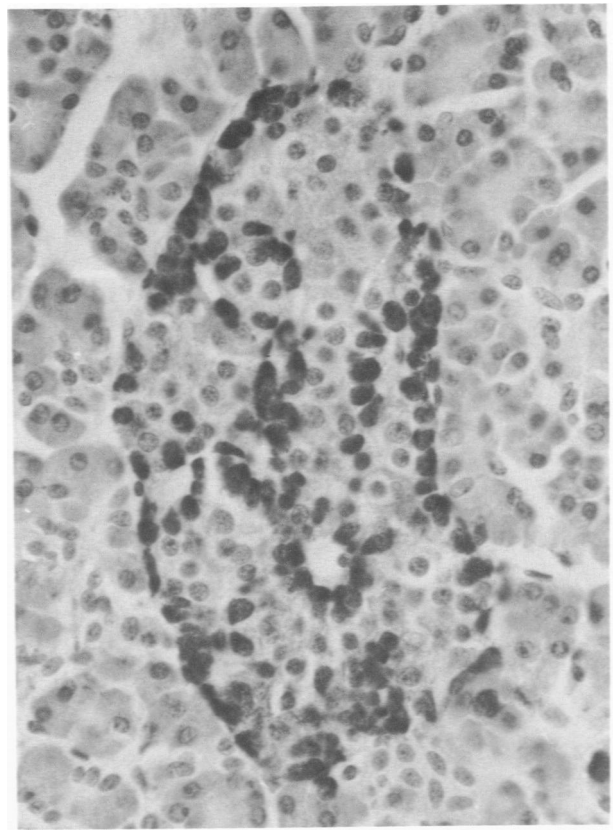
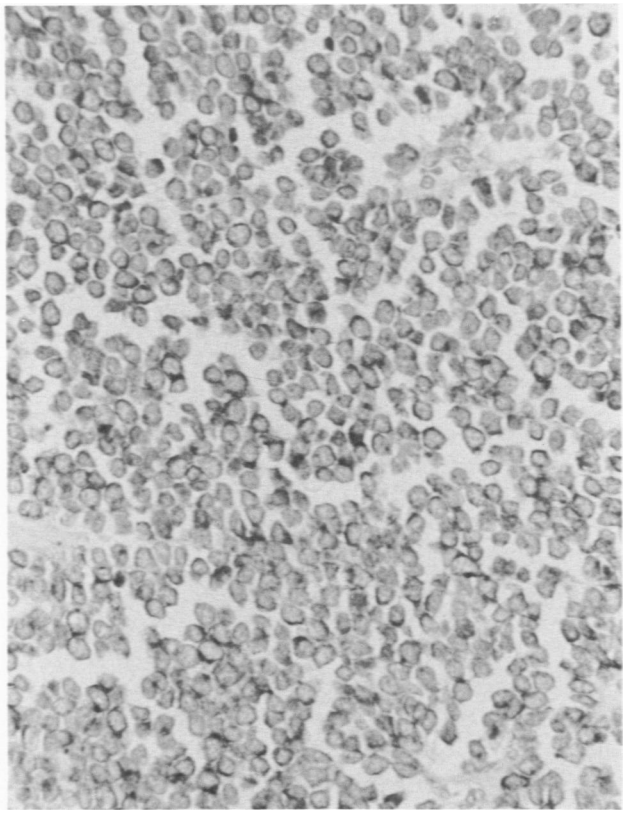


Figure 5A – Positive immunoreactivity of antibody LK2H10 in normal and hyperplastic thyroid C-cells. The follicular epithelial cells (*arrows*) are negative. (Immunoperoxidase, × 330) **B** – Infiltrating medullary thyroid carcinoma showing strong immunoreactivity with antibody LK2H10. (Immunoperoxidase, × 330) (With a photographic reduction of 6%)







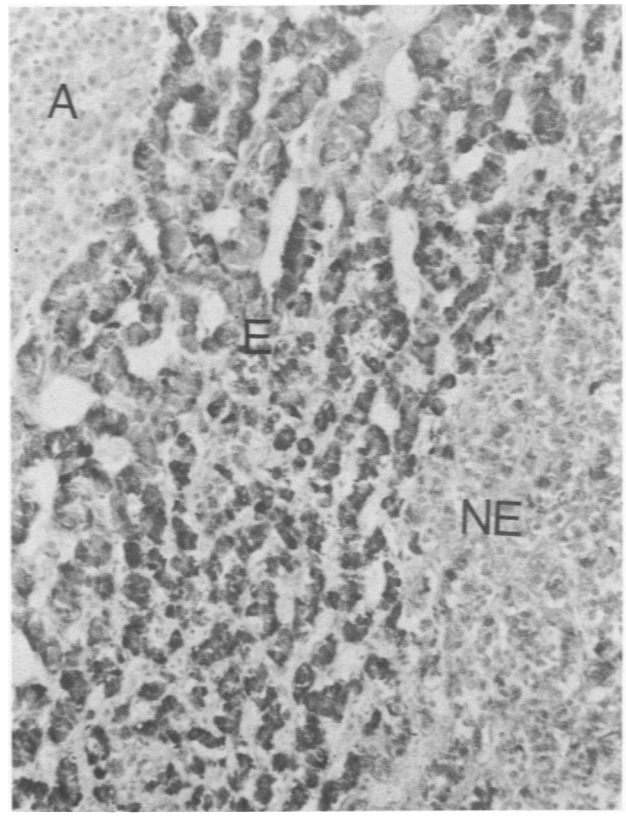


Figure 6 – Gastric endocrine cells show positive reactivity with antibody LK2H10, whereas the rest of the glands are negative. (Immunoperoxidase, ×330) **Figure 7** – Pancreatic Islet of Langerhans stained with antibody LK2H10. The glucagon cells, which are predominantly around the periphery of the islet, show strong staining, whereas the other endocrine cells show weak to absent staining. The pancreatic exocrine cells show no staining. (Immunoperoxidase, ×330) **Figure 8** – Merkel cell carcinoma of skin showing staining in the thin rim of the cytoplasm of the tumor cells with antibody LK2H10. (Immunoperoxidase, ×330) **Figure 9** – Porcine adrenal medulla stained with antibody LK2H10. The cordlike pattern of epinephrine-producing cells (*E*) shows strong reactivity, whereas the compact clusters of norepinephrine-producing (*NE*) cells show less intense staining. The adrenal cortical cells (*A*) are negative. (Immunoperoxidase, ×132) (All with a photographic reduction of 5%)

Table 2—Immunohistochemical Localization of Chromogranin by Monoclonal Antibody LK2H10 in Paraffin-Embedded Nonendocrine Tissues and Tumors

Organ/Tissue	Diagnosis	Staining with LK2H10*
Central nervous system	Normal neurons	0/4
Brain	Normal astrocytes	0/4
	Astrocytomas	0/5
	Normal pineal	0/1
Peripheral nervous system	Normal nerves	0/11
	Neurofibroma	0/4
	Neurilemoma	0/4
Alimentary system		
Stomach	Carcinoma	0/1
Colon	Carcinoma	0/4
Liver	Normal	0/2
Pancreas	Normal exocrine	0/5
Reproductive system		
Breast	Normal	0/2
	Carcinoma	0/3
Prostate	Normal	0/2
	Carcinoma	0/1
Fallopian tube	Normal	0/4
Ovary	Endodermal sinus tumor	0/1
Lymphatic system		
Lymph Node	Normal	0/1
	Lymphoma	0/3
Spleen	Normal	0/1
Skin		
Epidermis	Normal	0/4
	Nevi	0/10
	Melanoma	0/5
Urinary System		
Kidney	Normal	0/2
	Carcinoma	0/3
Ureter	Normal	0/2
Muskuloskeletal system	Normal skeletal muscle	0/3
	Normal smooth muscle	0/2
Soft Tissues	Rhabdomyosarcoma	0/3
	Synovial Sarcoma	0/1
	Malignant fibrous histiocytoma	0/2
	Hemangioma	0/1
Respiratory system		
Lungs	Squamous cell carcinoma	0/2
Larynx	Squamous cell carcinoma	0/1

* Positive cases/Total number of cases.

gland. These isolated proteins, together with a reference preparation of human chromogranin A, were separated into individual polypeptides by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then transferred to nitrocellulose paper for immunoblotting. Immunoperoxidase staining with antibody LK2H10 showed reactivity with two polypeptides (about 68,000 daltons) of the chromogranin A preparation of virtually equivalent size. These same two polypeptides were also identified in the purified adrenal gland extract in addition to a large number of lower molecular weight polypeptides (Figure 1). The same polypeptides seen in Figure 1 were also visualized by Coomassie blue staining of the polyacrylamide gel prior to nitrocellulose transfer.

Immunohistochemical Staining

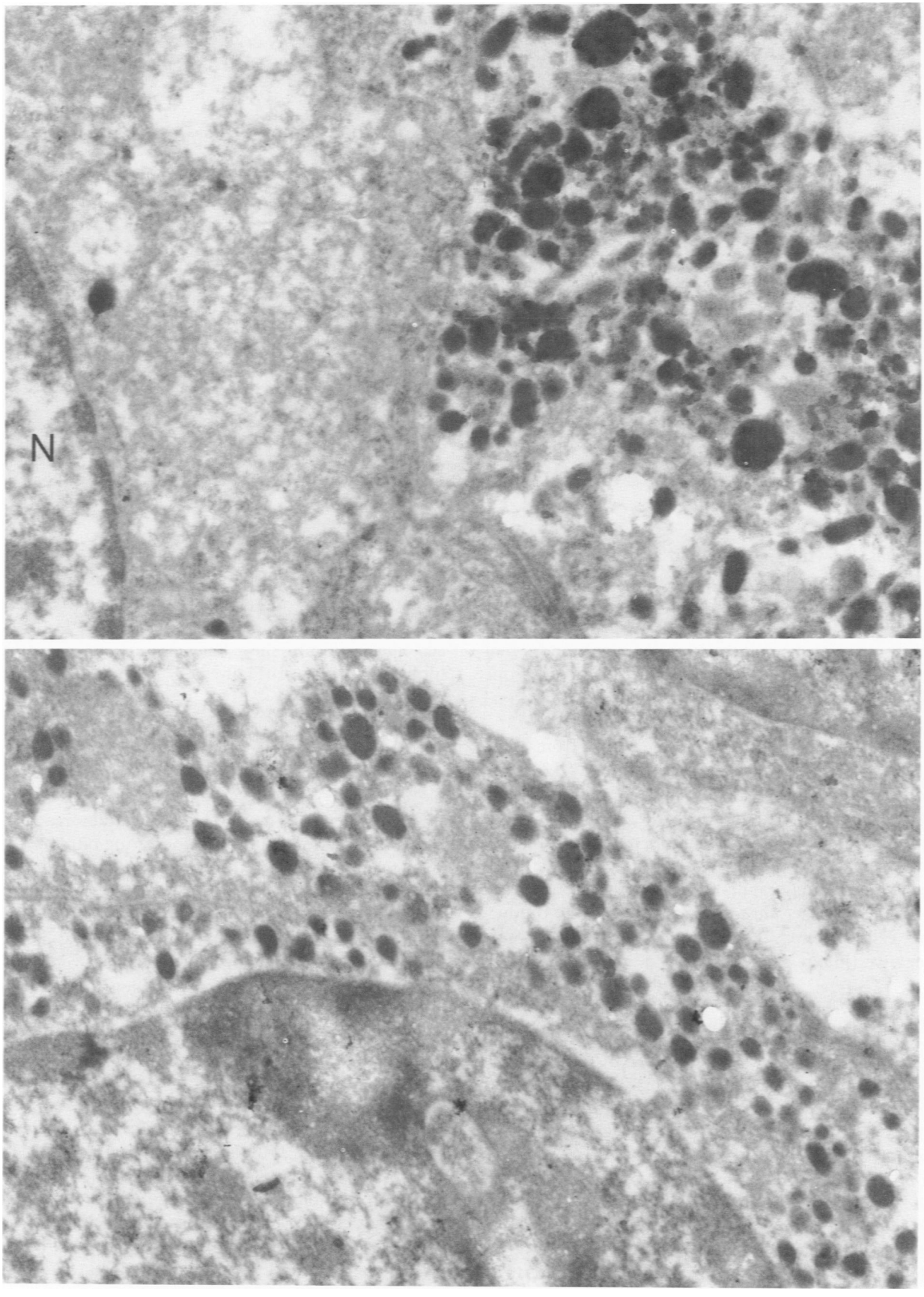
The binding of antibody LK2H10 to normal endocrine tissues and tumors is summarized in Table 1. Most endocrine tissues known to contain secretory granules reacted with antibody LK2H10 (Figures 2–8). Fetal endocrine tissues from 11–18-week fetuses reacted as well as adult tissues (Table 1). Some tumors known to contain few endocrine granules reacted weakly or not at all with LK2H10 antibody. These included neuroblastomas, Merkel cell tumors of the skin, and small cell carcinomas of the lung. Endocrine cells without secretory granules failed to react with antibody LK2H10. Nonendocrine tissues and tumors showed no reactivity (Table 2). Neurons from the hypothalamus and the nerve terminals of the posterior pituitary along with melanomas and nevi did not react with LK2H10 (Table 2). Although most normal anterior pituitary cells were positive with antibody LK2H10, some cells were negative. The four pituitary adenomas that were negative were all prolac-

Table 3—Immunohistochemical Cross-reactivity of LK2H10 Monoclonal Antibody With Paraffin-Embedded Normal Tissues from Various Animals

Species	No. positive/No. tested			
	Adrenal medulla	Pancreatic islet	Anterior pituitary	Thyroid C-cells
Monkey	3/3	0/2	ND	ND
Pig	1/1	1/1	ND	1/1
Cow*	0/1	ND	ND	ND
Sheep	0/1	0/1	0/1	0/1
Dog	0/3	0/2	0/1	0/2
Guinea pig	0/1	ND	0/1	ND
Rabbit	0/1	ND	ND	ND
Rat	0/1	0/1	ND	ND
Mouse	0/1	0/1	ND	ND

ND, not done.
* Acetone-fixed cryostat section.

Figure 10A—Ultrastructural immunohistochemical localization of antibody LK2H10 in the secretory granules of a mediastinal paraganglioma by the avidin-biotin complex method and diaminobenzidine-HCl. The nucleus (*N*) and cytoplasm of a cell without granules are negative, whereas most of the secretory granules in an adjacent cell show positive reactivity in the granule matrix. (×33,000) • **B**—Absorption of antibody LK2H10 with chromogranin A before immunostaining of the paraganglioma resulted in complete blocking of immunoreactivity in the granules. (×35,400)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A
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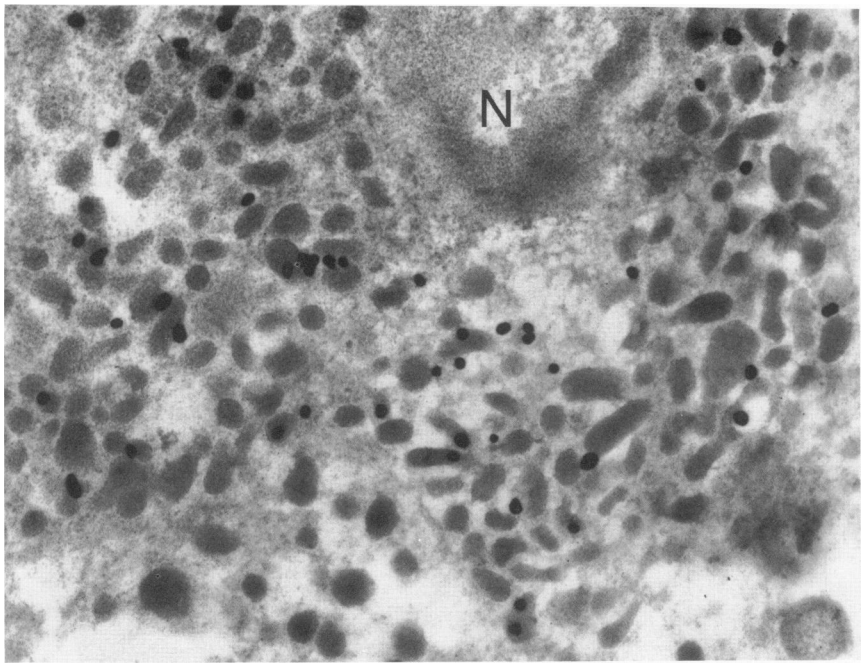

Figure 11 — Ultrastructural localization of antibody LK2H10 in the secretory granules of a paraganglioma using the Protein A-colloidal gold technique. The 20–25-mm colloidal gold particles are localized primarily over the granule matrix, whereas the nucleus (*N*) and the remainder of the cytoplasm are negative. (×35,400)

tinomas; growth hormone[5] and an ACTH-producing adenomas were positive. Glucagon-producing cells in the normal pancreatic islets stained intensely, and the insulin-producing cells had the weakest immunoreactivity. The five pancreatic endocrine tumors that were negative were insulinomas; gastrinomas,[10] one glucagonoma, and four tumors with mixed hormonal profiles were all positive with antibody LK2H10.

Chromogranin in the adrenal medulla from monkeys and pigs cross-reacted with antibody LK2H10; adrenal medullary tissues from cows, rabbits, sheep, guinea pig, dogs, rat, and mice did not (Table 3). The epinephrine-producing cells in the pig adrenal, which showed a cordlike pattern separated by wide thin-walled sinusoidal capillaries, stained more intensely than the compact clusters of norepinephrine-producing cells; whereas the adrenal cortical cells were negative (Figure 9).

Ultrastructural Localization

Ultrastructural studies with the ABC and the colloidal gold techniques in a paraganglioma from the anterior mediastinum showed that the target structure of LK2H10 was present within cytoplasmic secretory granules (Figures 10 and 11). Similar results were found with a growth-hormone–producing pituitary adenoma (data not shown). Absorption with chromogranin A abolished staining in the secretory granules in both light-microscopic and ultrastructural studies. (Figures 4B and 10B). The deposition of electron-dense immunoreactive antigen antibody complexes was seen predominantly in the granule matrix in approximately 30% of the granules with the colloidal gold technique. The remainder of the cytoplasm and cell nuclei did not show any immunostaining at the ultrastructural level (Figure 11).

Discussion

These studies indicate that human chromogranin is present in the secretory granules of most cells of the DNES. The reason for the lack of immunoreactivity in hypothalamic neurons and posterior pituitary tissues, which are known to contain secretory granules, is not known. Possible reasons include the following: 1) chromogranin of a similar structure is present in these tissues, but in lower concentrations that are not detectable by our immunohistochemical assays; 2) chromogranin with a different antigenic structure is present in these tissues and is thus not detectable by antibody LK2H10. The widespread distribution of chromogranin in bovine endocrine tissues was recently reported by O'Connor.[25] Using a sensitive radioimmunoassay, he found immunoreactive chromogranin in bovine anterior pituitary and posterior pituitary; however, the level of chromogranin in the anterior pituitary was three times greater than in the posterior pituitary; whereas the levels in the adrenal medulla were 30 times greater than in the anterior pituitary. In addition, chromogranin levels in bovine hypothalamus was 1/16 as much as in the posterior

pituitary.[25] These findings indicate that significantly less chromogranin is present in the posterior pituitary and hypothalamus, which is probably why these tissues are negative in our immunohistochemical assays.

The presence of immunoreactive chromogranin in the adrenal medulla of pigs and monkeys indicates that the distribution of this molecule in endocrine tissues of these animals may be similar to that of human chromogranin. Chromogranin in bovine, canine, and other species that did not react with antibody LK2H10 is probably structurally different from human chromogranin. The differences between the staining intensity of epinephrine and norepinephrine-producing cells in the porcine adrenal medulla, although not quantitative, indicate that higher concentrations of chromogranin are associated with granules of epinephrine-producing cells. The distinct histologic separation of epinephrine and norepinephrine-producing cells in porcine adrenal medulla has been noted by previous investigators.[26]

The immunoreactivity of pituitary gland cells with LK2H10 indicates that some normal pituitary cells and tumors were not reactive to this antibody. The four pituitary adenomas that were negative were all prolactin-producing tumors. Likewise, in the normal pancreatic islets, glucagon-producing cells had the strongest immunoreactivity; whereas insulin-producing cells had weak to absent immunoreactivity. These findings suggest that these subsets of pituitary and pancreatic endocrine cells have undetectable levels of immunoreactive chromogranin or have chromogranin of a different form, which is not detected by antibody LK2H10. Additional studies will be needed to clarify these observations. Similar studies will also be required for us to determine whether chromogranin is present in granule-containing cells of nonendocrine tissues such as the pancreatic exocrine cells, which were not reactive to antibody LK2H10.

Normal parathyroid tissues and parathyroid adenomas both showed positive immunoreactivity with LK2H10. Cohn et al[13] found that secretory protein I from the bovine parathyroid was structurally similar to bovine chromogranin A and suggested that this protein was stored and released with parathyroid hormone. Our ultrastructural studies provide direct evidence showing that chromogranin is present in the secretory granules of endocrine tissues. This observation is consistent with the less intense staining seen in neuroblastomas and Merkel cell tumors, which are known to contain relatively few secretory granules. The observation that chromogranin is localized ultrastructurally in the matrix of the granule in a paraganglioma and in a growth-hormone–producing pituitary adenoma is in agreement with the ultrastructural localization of chromogranin in the granule matrix of bovine adrenal medulla by Aunis et al,[27] who used polyclonal antisera against bovine chromogranin A.

The presence of chromogranin in most cells of the DNES is consistent with observations that these cells share many unique properties, eg, neuron-specific enolase,[4-6] despite the fact that not all DNES cells are derived from the neural crest.[1-3] This ubiquitous distribution suggests that chromogranin may have a general role in storage of and/or release of biologically active substances from neurosecretory granules. Previous studies of bovine adrenal glands showed that chromogranin helps to stabilize the soluble portion of the secretory granule through interaction with ATP and catecholamines.[28-29] Whether chromogranin functions similarly in DNES cells outside the adrenal glands is presently unknown but should be given serious consideration in light of the following observations: 1) pancreatic glucagon-secreting cells, which produce chromogranin, can synthesize catecholamines during fetal development[30]; 2) higher chromogranin levels seem to be linked to epinephrine storage granules, rather than norepinephrine storage granules, in the pig adrenal gland (this article); and 3) the adrenal gland, which contains the highest levels of catecholamines, also contains the highest levels of chromogranins.[25]

The observation that chromogranin is released into the serum after splanchnic stimulation[11] suggests that this may be a useful marker in patients who have neoplasms of the adrenal medulla and other neuroendocrine organs. Antibody LK2H10 may be used as a diagnostic marker in the immunohistochemical evaluation of endocrine tumors with secretory granules in both the serum and tissues of patients with neuroendocrine tumors.

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