The Variable Distribution of Chromaffin Paraganglia Around the Inferior Mesenteric Ganglion in Young Cats, With Corroborative Morphological Observations

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ABSTRACT

The anatomical distribution of chromaffin paraganglia (PG) associated with the inferior mesenteric ganglion (IMG) in cats was defined initially by perfusing animals with a combined staining/fixing solution containing potassium dichromate and glutaraldehyde. The dichromate component stained and distinctly mapped 3 to 6 chromaffin paraganglia that that were distributed randomly around the surface of each IMG. On the other hand, glutaraldehyde preserved the morphological integrity of the chromaffin organs for subsequent histological and ultrastructural evaluation. The IMG always appeared as a paired structure surrounding the inferior mesenteric artery (IMA). Elongated paraaortic chromaffin organs occurred ventral to the abdominal aorta in the midretroperitoneum. Histological evaluation showed that a paranglion was separated from the adjacent IMG by a vascular and connective tissue capsule. Large chromaffin cell clusters occured within the IMG proper, but a continuity between intraganglion and paraganglion chromaffin tissue was not evident. All chromaffin cells contained granules with dense cores and otherwise displayed features consistent with chromaffin cells in general. Nerve endings containing clear vesicles established contact with the intraganglion chromaffin cells; however, terminals were never observed contacting the paraganglion or paraaortic chromaffin cells. This study shows clearly that a variable number of chromaffin paraganglia are juxtapositioned to the IMG in cats, and that similar cells assimilate in clusters of considerable size within the IMG and alongside the abdominal aorta.

INTRODUCTION

Stilling (1890) originally described collections of cells that displayed a characteristic chromaffin reaction following fixation with potassium dichromate and were associated with the sympathetic plexuses in various animals. In addition, this pioneer investigator reported that the cells were morphologically similar to their adrenal medullary counterparts. Köhn (1902) later assigned the name paraganglia to those cell collections and introduced the term "chromaffin" to designate a specific collection of extramedullary cells in the midretroperitoneum. The term paraganglia quickly gained acceptance and now is used routinely to describe extramedullary chromaffin tissue in general. However, it does not define the total expanse of this tissue type because paraganglia refers only to minute structures that are directly apposed to sympathetic ganglia. But studies based upon potassium dichromate mapping have shown, without question, that very large deposits of chromaffin tissue occur in non-paraganglion, paraaortic locations [3, 4, 5]. It is now known that paraaortic chromaffin bodies represent the largest deposit of extra-medullary chromaffin tissue in dogs [6], and that the largest paraganglion observed in the human embryo is a paired mass around the origin of the inferior mesenteric artery [7].

Chromaffin cells that are located away from the adrenal medulla have attracted much interest in recent years and several investigators have observed small granule-containing cells within various sympathetic ganglia [8, 9, 10]. These unique intraganglion cells are further categorized as small intensely fluorescent (SIF) cell capable of functioning as interneurons in inhibiting or modulating ganglion transmission via their interposition between pre-and postganglionic neurons [11, 12]. Other chromaffin cells are located within various of the sympathetic ganglia and specifically exist in this location as clusters of varying size. These particular chromaffin cells, however, lack the

synaptic contacts that would be necessary for direct interneuronal action. Clustered intraganglion chromaffin cells are highly vascular, posses many catecholamine-containing granules and may represent a localized neuroendocrine system capable of releasing products into regional blood vessels [13, 14, 15]. This arrangement could represent a neurosecretory rather than a synaptic action by the ganglion chromaffin elements. Whatever the case, the physiological significanse of such a voluminous extraadrenal chromaffin system is vague, since much of it appears to lack a function innervation [16].

In view of the voluminous distribution of paraganglion and intraganglion chromaffin cells, it seems important now to examine closely the relationship between sympathetic ganglia and their adjacent paraganglia, and particularly between the principal autonomic neurons and the chromaffin cells which reside within the IMG and closely border the postganglionic neurons. The actual number of paraganglia that may appose the inferior mesenteric ganglion also is an important issue that deserves to be investigated.

This work utilized a method [5, 6] to stain and concurrently preserve the morphology of chromaffin organs by combining potassium dichromate salts with glutaraldehyde at a near-neutral pH level. Potassium dichromate produces a gross chromaffin reaction that is easily visible and thus "maps" chromaffin tissue, while glutaraldehyde maintains the morphological integrity of the stained tissues for ultrastructural examination and unequivocable identification as chromaffin, granule-containing organs.

MATERIALS AND METHODS

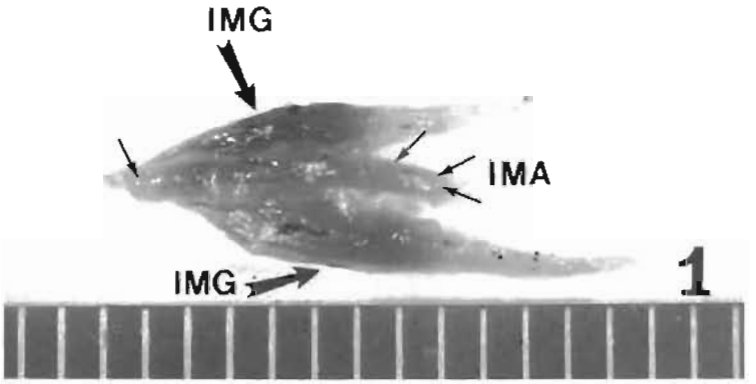
Nine young cats, irrespective of sex and weighing between 3.0 and 5.0 kg, were housed individually in spacious metal cages. The animals received continuous food and water and were kept in 12:12 hour periods of light/darkness. Prior to use, all animals were anesthetized with 50 mg/kg, IP, Nembutal (sodium pentobarbital) and ultimately sacrificed by intracardiac perfusion with cold 3% glutaraldehyde adjusted to pH 7.2-7.4 range with 0.1M sodium phosphate buffer. The vascular perfusion was preceded by a phosphate-buffered saline flush in order to empty the vascular tree of blood. The retroperitoneal tissue block (RTB) from the adrenal glands to the abdominal aortic bifurcation was removed and fixed for an additional 60 minutes in the original perfusion fluid. This RTB then was transferred to a solution of 3% glutaraldehyde/2.5% potassium dichromate (pH 6.8-7.0) to accomplish anatomical staining of extramedullary chromaffin tissue and concurrently preserve its morphological character. The RTB was washed overnight in 0.1M phosphate buffer containing 8% sucrose in order to clear excess dichromate ions and make the stained chromaffin tissues clearly visible. The IMG and attached PG were dissected from the RTB, cleansed of connective and fatty tissues and photographed with Kodak Kodachrome 200 slide film. Paraaortic chromaffin organs also mapped by this method were removed and similarly photographed. The ganglion/paraganglion tissue blocks then were bisected so that each block contained a portion of the ganglion and its attached paraganglion. These blocks were secondarily fixed in osmic acid, also buffered with sodium phosphate, and processed routinely for light and ultrastructural study.

RESULTS

Anatomical Mapping:

The inferior mesenteric ganglion (IMG) was present in all animals, usually as a paired structure on either side of the inferior mesenteric artery (IMA) to which it was firmly bound by a capsular meshwork of fibrous and fatty connective tissue (Fig. 1). The capsular material was dissected away in order to illustrate to best advantage the ganglion as well as the adjoined paraganglia (PG) (Figs. 2-5). The individual inferior mesenteric ganglia were flattened, elongated structures and varied from 3-5 mm in length by 1-2 mm in width. Nerve fibers associated with the ganglia were particularly evident at their caudal aspects (Figs. 1, 3, 5).

The dichromate-stained PG were outlined clearly upon the ventral or dorsal aspect of all inferior mesenteric ganglia examined (Figs. 1-5). One particualr IMG showed 6 chromaffin bodies attached to its surface (Table #1). The PG usually measured 1 mm in length by 0.5 mm in diameter. Some of the chromaffin PG were associated primarily with nerves and not actually attached to the IMG proper (Figs. 3, 4). Other similarly stained chromaffin bodies occurred throughout the retroperitoneum in a ventral paraaortic position. One such organ measured 13 mm in length by 1mm in width and gradually tapered into slender strands toward its rostral and caudal aspects (Fig. 6).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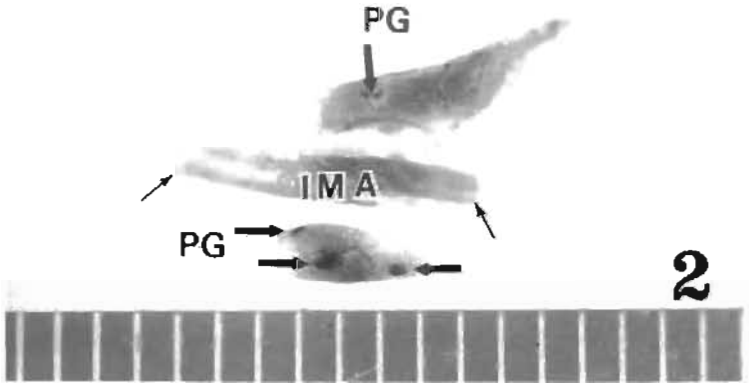
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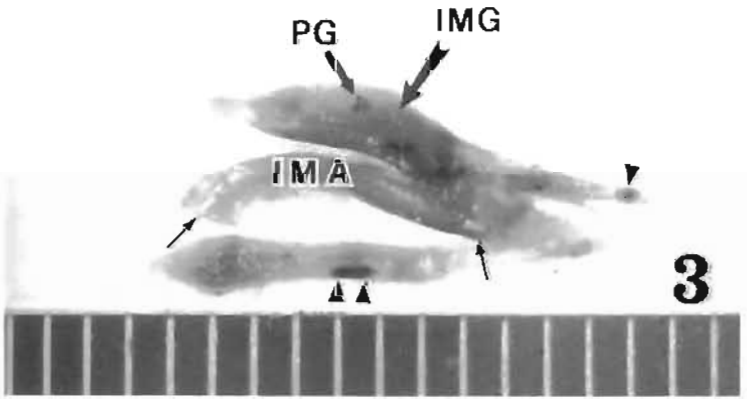
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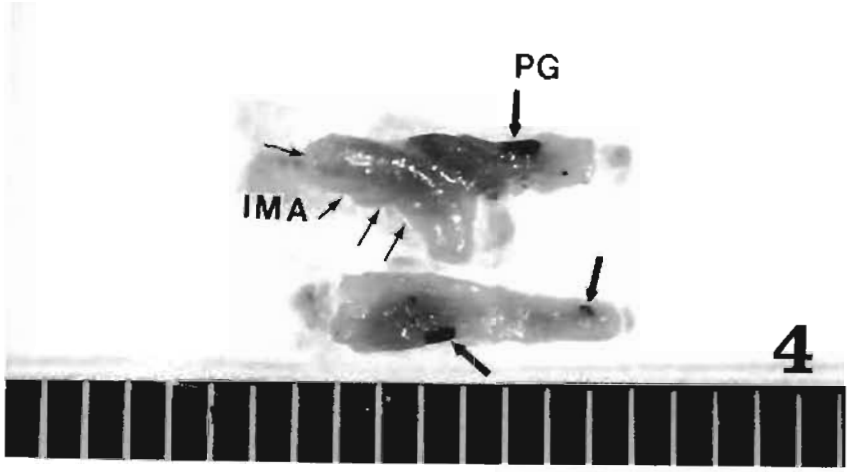
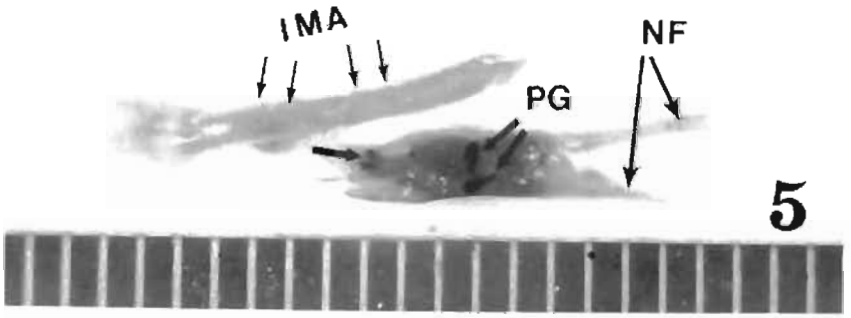


Figures 1-5. Photomicrographs of potassium dichromate-stained gross specimens showing the inferior mesenteric artery (IMA; small arrows), inferior mesenteric ganglion (IMG; large arrows) as well as associated paraganglia (PG; medium arrows). A connective tissue sheath firmly bound the IMG complex to the IMA (Fig. 1). This material was dissected away in order to better expose the IMG and PG, thus the true relationship between ganglion and artery is disturbed in Figs. 2-5. PG could be seen easily on the surface of the IMG following potassium dichromate staining. Other PG also were localized along the inferior mesenteric nerves entering or leaving the ganglion complex (arrowheads, Fig. 3). NF = Nerve Fibers.
Scale = millimeters (mm).



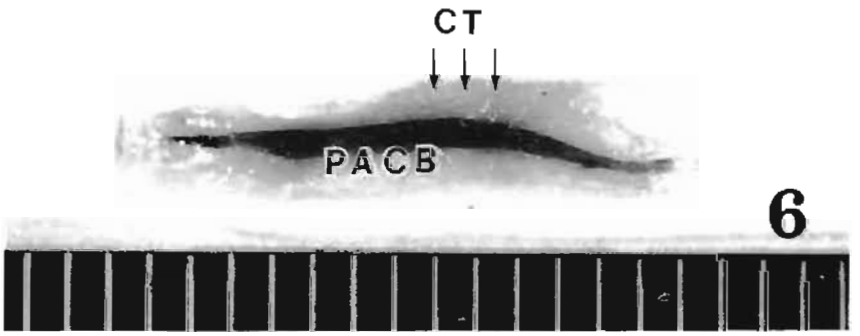


Figure 6. A paraaortic chromaffin body (PACB) that stained intensely following potassium dichromate mapping. The organ was situated alongside the abdominal aorta, surrounded by connective tissue (CT), and measured 13 mm in length by 1 mm in width.
Scale = millimeters (mm).

Histological Structure:

Connective tissue and blood vessels formed a well-vascularized capsule that separated and surrounded the IMG/PG complex (Fig. 7). The paraganglion was always separated from the adjacent ganglion by this ever-present connective tissue partition, thus a continuity between paraganglion and ganglion tissues was never observed (Fig. 7).

Paraganglia were comprised of small, deeply basophilic epithelial cells with pale central nuclei and prominent nucleoli (Fig. 7). This striking basophilia was a consequence of potassium dichromate staining and accounted for the darkened organs that were mapped at the anatomical level (Figs. 1-6). Chromaffin cells were grouped closely around thin-walled blood vessels that branched and anastomosed freely throughout the gland. Neurons were never observed within a paraganglion.

Occasionally, clusters of darkly stained chromaffin cells appeared within the IMG parenchyma (Fig. 8). The clusters varied considerably in size, with some showing over 60 nuclear profiles in cross section, while others were only a few cells across. These intraganglion chromaffin cells were grouped around blood vessels and separated from neurons by nerve bundles as well as connective tissue fibers and cells. These chromaffin cells were distinguished easily from neurons as the nerve cells were noticeably larger and possessed nuclei which often were as large as one entire chromaffin cell. In addition, neurons lacked cytoplasmic vesicles and, therefore, the basophilia noted in the small chromaffin cells was absent.

Paraganglion (Fig. 7) and intraganglion (Fig. 8) chromaffin cells generally appeared identical in size and basophilic character. A continuity between paraganglion and intraganglion chromaffin cells was never seen, again because of the presence of capsular material between the two. The inferior mesenteric ganglion was a composite of large neurons, connective tissue, myelinated and unmyelinated fibers, and numerous blood vessels (Figs. 7, 9).

In addition to whole chromaffin cells which were easily identifiable, the ganglion also contained dark, rounded, and deeply basophilic processes that appeared to contact the individual neurons (Fig. 9). The exact association between these dark structures and the ganglion neurons could not be ascertained solely by light microscopic study, and further ultrastructural evaluation was necessary.

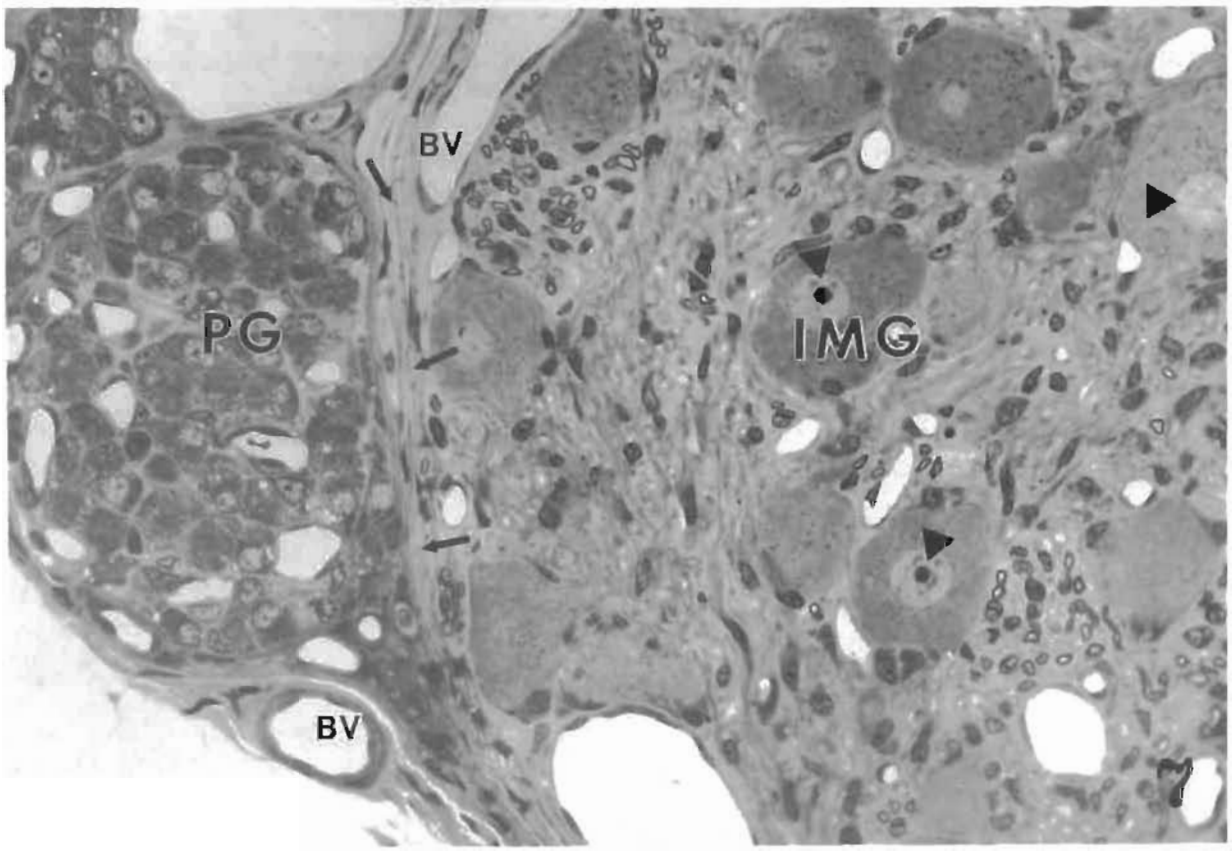
Innervation:

Granule-containing processes, possibly originating from the clustered ganglion chromaffin cells, seemed to terminate directly upon the neuronal soma, while others were scattered throughout the ganglion and not necessarily associated with neurons (Fig. 10). Study of adjacent sections showed that the processes were widely separated from the neuronal soma by satellite cell cytoplasm which always surrounded the nerve cells (Fig. 11). The satellite cells were easily recognized as non-neuronal or non-chromaffin since they did not show the obvious granularity of chromaffin cells or the Nissl material characteristic of IMG neurons.

Nerve endings were never observed upon the paraganglion cells. Conversely, several of the ganglion chromaffin cells did receive nerve terminations. The synaptic terminals abutting upon the chromaffin cells contained a multitude of vesicles with clear cores in addition to a few larger ones with electron dense cores (Fig. 12). The region of apposition included an area of thickened pre-and postsynaptic membranes and also showed a density on the postsynaptic side of the complex. Most nerve endings contained one such area of specialization, but a few others showed two similar areas. Only the presynaptic vesicles with the clear cores were associated with the synaptic areas. On the postsynaptic side, the dark chromaffin granules were distributed randomly and did not show a preferential orientation with the synaptic region (Fig. 12).

Ultrastructural Observations:

Favorable sections that included both paraganglion and ganglion chromaffin cells showed clearly that all chromaffin cells displayed a conspicuous cytoplasmic granularity (Fig. 13). This feature was a consequence of the numerous, dense granules that completely filled the confines of the cell, usually in such large volume that they obscured the usual organelles. The individual granules were small and displayed very dense central cores (Figs. 13, 14). Many blood vessels were evident amongst the chromaffin cells. However, the individual cells did not abut on the blood vessels directly, as satellite cell processes and intercellular space occurred between the chromaffin cell and endothelial lining (Fig. 14). The capsule originally noted by light microscopy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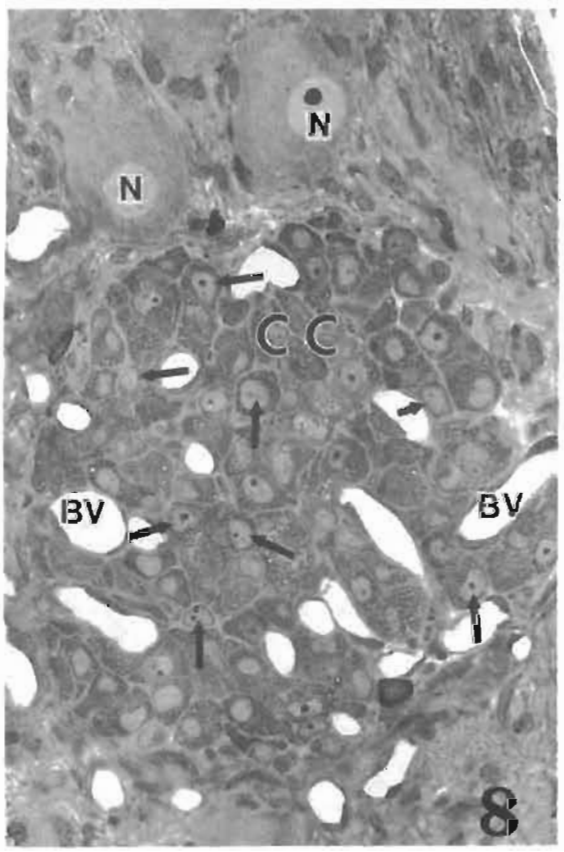
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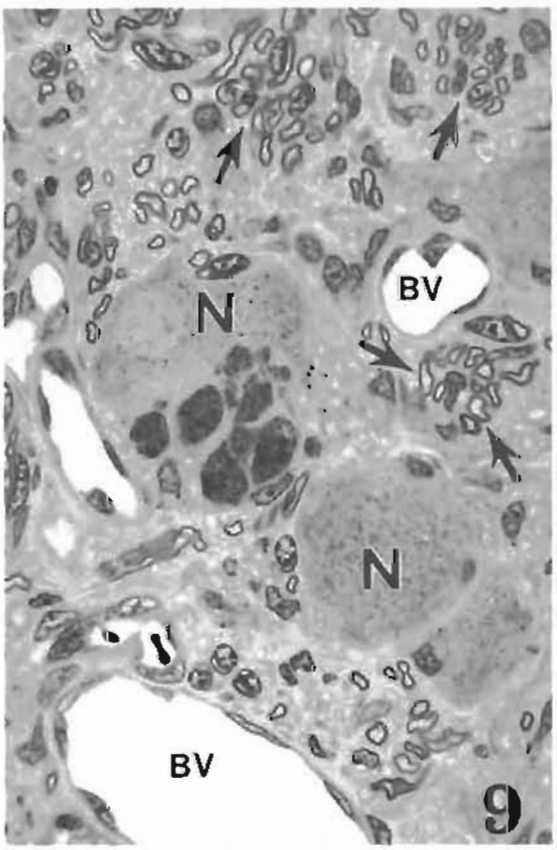
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was composed of fine collagen fibers elaborated by many fibroblasts (Fig. 14).

DISCUSSION

Catecholamine-containing, chromaffin-positive cells morphologically similar or identical to the cells of the adrenal medulla are widely distributed throughout the entire abdominal cavity. Very early studies (1, 2) initially described extraadrenal collections of cells that reacted with potassium dichromate salts and were morphologically analogous to the cells of the adrenal medulla. Köhn (1902) then applied the name of "paraganglion aorticum abdominale" to a particular collection of chromaffin cells located in the midretroperitoneum. The term paraganglia since has been used universally to describe chromaffin-positive tissue located at sites away from the adrenal medulla. Histochemical studies by Eränkö and Härkonen (1965) also described chromaffin cells in the superior cervical ganglion of rats, and soon afterwards Elfvin (1968) discovered chromaffin bodies adjacent to the same ganglion in the rabbit.

Several works (8, 9, 10, 14, 15), have described the presence of "small neurons", "small granule-containing cells", or "small intensely-fluorescent cells" within different ganglia in various species. The names attributed to the cells are different because they are predicated upon the particular method (i.e., histochemical or ultrastructural) used to identify them; however, all of the nomenclature referes to a common chromaffin cell type that exists within sympathetic ganglia. On the other hand, the term "paraganglia" refers to groups of chromaffin cells that are very closely associated with the surface of a sympathetic ganglion. The exact number and distribution of paraganglia associated with the inferior cervical ganglion, in this case, has not been explored.

The present work clearly indicates that the inferior mesenteric ganglion in young cats may have as many as 6, or as few as 3, chromaffin paraganglia associated with its dorsal or ventral surfaces. The fact that the organs, eventhough they are microscopic in size and difficult to visualize with the unaided eye, can be identified as paraganglia and chromaffin in nature is made possible by the positive staining action of potassium dichromate as used in the present mapping technique. The important revelation by Henle (1865) that chrome salts, such as potassium dichromate, oxidized the catecholamine products in adrenomedullary cells to yield brown oxidation products (i.e., the "chromaffin" reaction) is of utmost value when tracing adrenomedullary-like chromaffin tissue. The present method utilizes potassium dichromate in combination with glutaraldehyde to concomitantly stain and preserve chromaffin organs. The staining capability of the mixture produces, in effect, a chromaffin reaction at the anatomical level that effectively and accurately maps chromaffin paraganglia. The action of glutaraldehyde, on the other hand, preserves the morphology of the organs for unequivocable

Figure 7. A survey light photomicrograph illustrating a large group of chromaffin cells (PG) immediately adjacent to a Inferior Mesenteric Ganglion (IMG). The paraganglion and ganglion are distinctly separated by a thin capsule containing Blood Vessels (BV) and connective tissue elements (arrows). The ganglion shows many neurons with prominent nuclei and nucleoli (arrowheads) as well as blood vessels and neuropile rich in fibers and glial elements. The adjacent paraganglion is a composite of cells that are considerably smaller than ganglion neurons. Each cell exhibits a profound cytoplasmic basophilia, a consequence of catecholamine storage, and many small blood vessels throughout the parenchyma.
Magnification: X 850 approx.

Figure 8. A light photomicrograph showing a group of chromaffin cells (CC) nestled within the IMG. This rounded cluster contained more than 50 nuclear profiles (arrows) and many Blood Vessels (BV). Neighboring neurons with their distinctive nuclei and nucleoli are easily visible (N). These intraganglion chromaffin cells also show the cytoplasmic basophilia originally seen in the paraganglion cells.
Magnification: X 1,000 approx.

Figure 9. A photomicrograph showing several Neurons (N) and many darkly stained structures in close apposition that presumably represent processes from chromaffin cells. However, they are not easily identified at this level of resolution. Blood Vessels (BV) and many groups of myelinated axons (arrows) are seen throughout the ganglion.
Magnification: X 1,000 approx.

identification at the histological and ultimately the ultrastructural level. The granule-containing nature of the individual cells is verified when the glutaraldehyde-fixed tissues are studied with the electron microscope, and the presence of numerous dark-staining catecholamine granules becomes clear.

In addition to localizing chromaffin paraganglia, the present method of study also demonstrated voluminous chromaffin organs that were situated along the abdominal aorta. Also, detailed study of the adjacent inferior mesenteric ganglion revealed the occurrence of large islands of chromaffin cells within the ganglion proper. Chromaffin cells thus were effectively mapped in various extraadrenal positions.

The nerve supply to paraaortic, paraganglion, or intraganglion chromaffin cells is not thoroughly understood. The early studies of Hollinshead (1937) proposed that the main abdominal paraganglion in cats and dogs received preganglionic cholinergic impulses mediated via thoracic splanchnic nerves. Thompson and Gosling (1976), however, did not observe appreciable amounts of acetylcholinesterase (AChe) activity within the human pelvic paraganglia and thus disagreed with the concept that paraganglia represented cholinergic end organs. On the other hand, Jacobowitz (1970) and Hervonen (1971) noted ample AChe fibers in paraaortic chromaffin organs in cats and human fetuses.

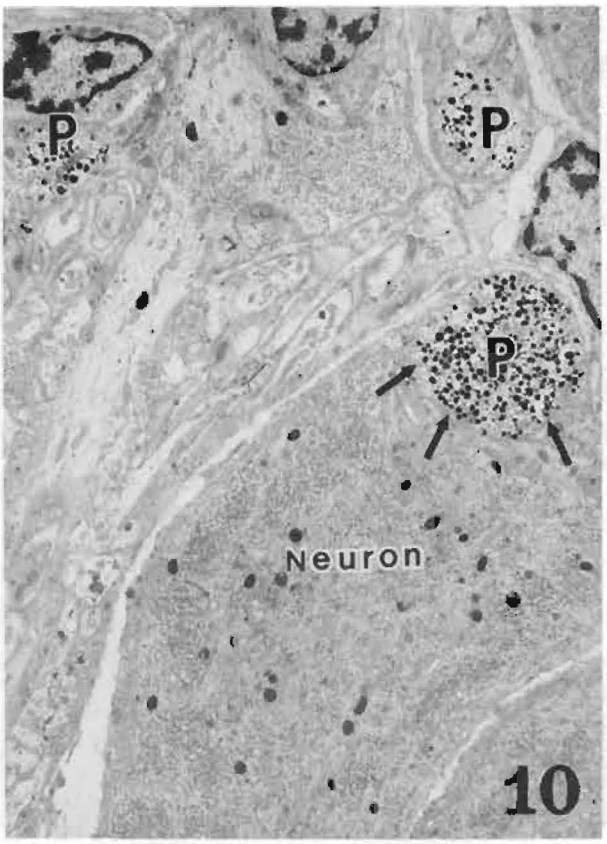
Several ultrastructural studies have been somewhat inconclusive in clearly elucidating the innervation of extraadrenal chromaffin cells. Hervonen and co-workers (1978), and also Coupland and Weakley (1970), did not locate terminals at the submicroscopic level in paraaortic bodies of humans and rabbits. It is perplexing that paraaortic chromaffin cells in certain species appear to lack a functional innervation, considering that similar paraganglion cells in guinea pigs (14) as well as Syrian hamsters and squirrel monkeys (26) show distinct ultrastructural innervation patterns. Indeed, chromaffin cells in the guinea pig inferior mesenteric paraganglia (14) possess a dual cholinergic/noradrenergic nerve supply, and it well known that synapses occur with regularity in the adrenal medulla (27) and vagal paraganglia (28). Coupland et al. (1982) simply wondered why the voluminous but noninnervated abdominal paraaortic chromaffin bodies would persist throughout life in rabbits, unless their amine pool was of physiological importance.

This study did not produce evidence supporting an innervation pattern of any sort to the paraganglion cells associated with the inferior mesenteric ganglion in cats. This finding would agree with the work of Autillo-Touati and Seite (1980) that the paraganglia adjacent to the celiac ganglion in the cat also were not innervated. However, the intraganglion chromaffin cells presently studied received synaptic endings appearing to be cholinergic judging by the preponderance of small clear core vesicles; nevertheless, the dual presence of peptide-like components in such endings (29, 30) cannot be discounted solely on the basis of structure.

Figure 10. Low power electron micrograph defining the ultrastructural appearance of the dark structures first observed approximating ganglion neurons at the light microscopic level (see Fig. 9). These structures actually represent cytoplasmic Processes (P) emanating from the ganglion chromaffin cells. One such process appears to contact the neuron cell body (arrows). Other smaller processes, also containing granules, are scattered throughout the field.
Magnification: X 4,800 approx.

Figure 11. A higher power electron micrograph that clearly defines the junction between chromaffin cell Processes (P) and ganglion neurons. A distinct barrier of Satellite Cell cytoplasm (SC) and collagen-containing intercellular spaces (arrows) exists between the chromaffin cell and ganglion neuron.
Magnification: X 15,600 approx.

Figure 12. A high power electron micrograph showing a granule-rich Chromaffin Cell (CC) receiving a Synaptic contact (SYN). The synaptic ending contains predominantly clearcore vesicles, but a number of larger vesicles with darker cores also are seen (arrows). The area of contact shows membrane density on the pre-and post-synaptic membranes.
Magnification: X 27,600 approx.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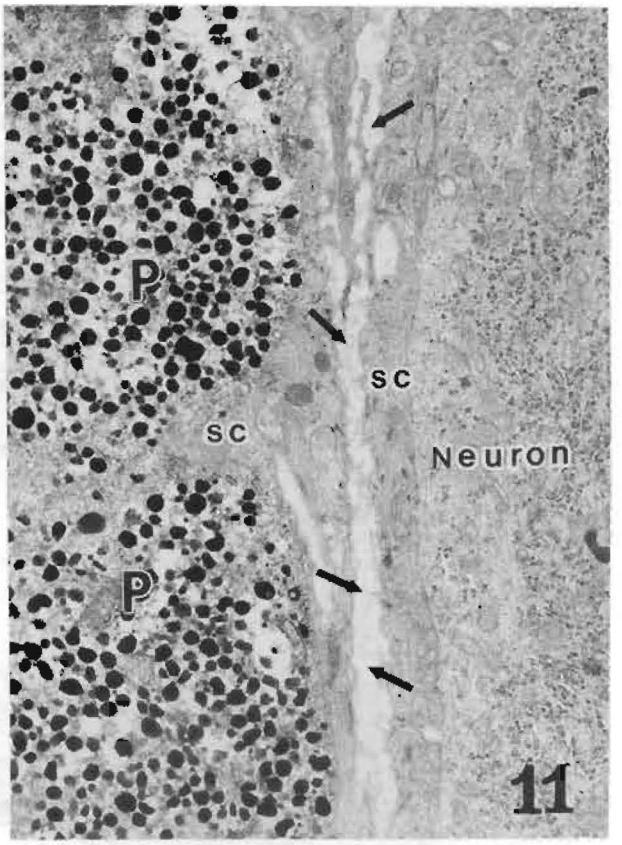
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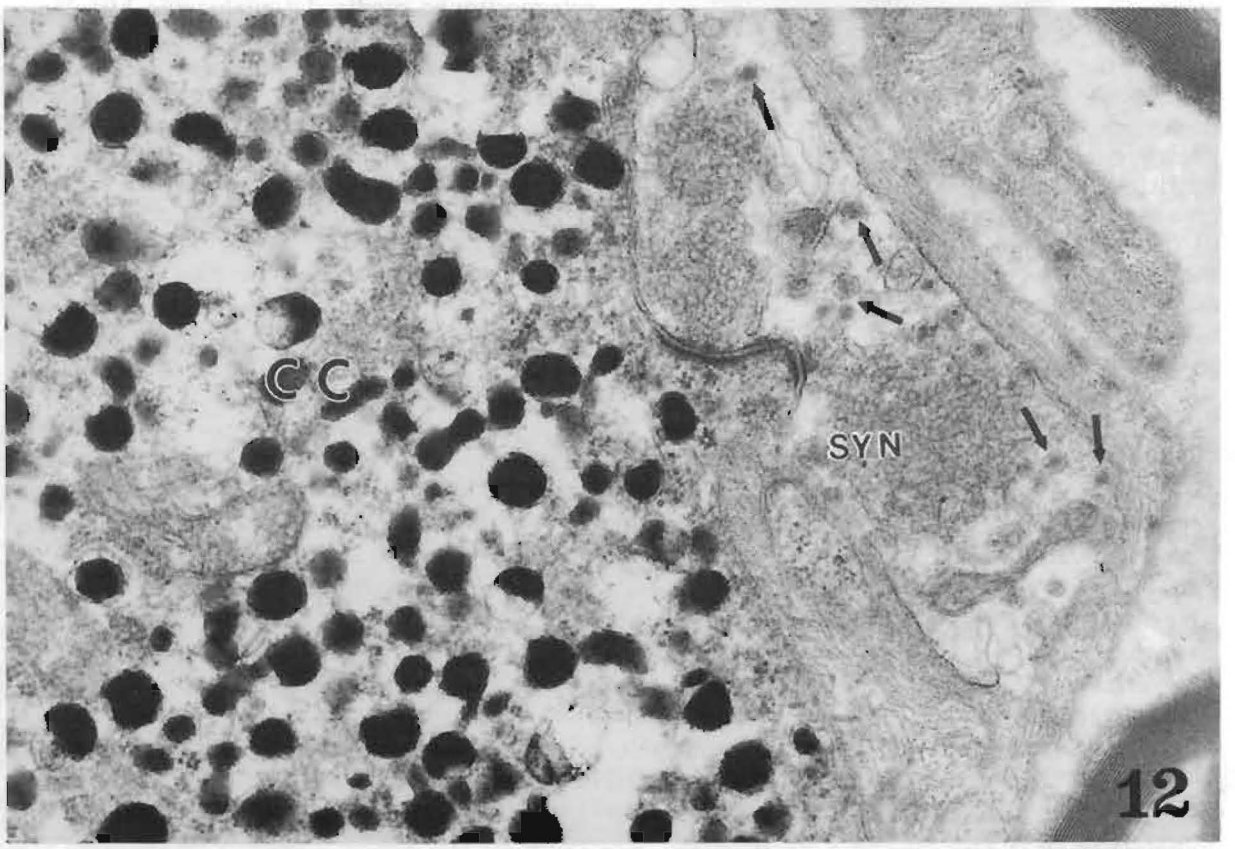
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Large granule-filled processes neared the ganglion neurons, but a distinct buffer of satellite cell cytoplasm prevented the axonal-like chromaffin processes from synapsing directly upon the principal neurons. Thus, the absence of efferent contacts from the ganglion chromaffin cells to the neurons would appear to negate a direct interneuronal role for the clustered type of chromaffin cells, as is likely the case when singular chromaffin elements establish inhibitory synaptic contacts upon nerve cells in the superior cervical ganglion (8, 9).

The overall, general pattern of chromaffin cells innervation that seems to emerge from studying the cat inferior mesenteric ganglion is that probably intraganglion chromaffin cells in this species are innervated by terminals not unlike the cholinergic endings often seen in the adrenal medulla. By contrast, the large and persisting paraaortic chromaffin organs as well as paraganglion chromaffin tissue appears to exist without innervation. It seems that synaptic contacts on these cells, if indeed they do exist, surely would have been discovered easily by the investigators actively studying these important organs.

In summary, this work localized and enumerated paraganglion tissue in young cats by combining glutaraldehyde and potassium dichromate in a manner that produced well preserved tissues for morphological study. In addition, the gross chromaffin reaction consistently mapped the extraadrenal distribution of chromaffin tissue with great accuracy. This mapping clearly indicated that several paraganglia were randomly distributed upon the surface of the inferior mesenteric ganglion, and that large collections of similar cells resided in paraaortic and intraganglion regions. The close apposition between paraganglia and the inferior mesenteric ganglion, as well as between the intraganglion chromaffin cells and nearby neurons, is an anatomical reality. The significance, if any, of such junctions is a matter for further study.

RESUMEN

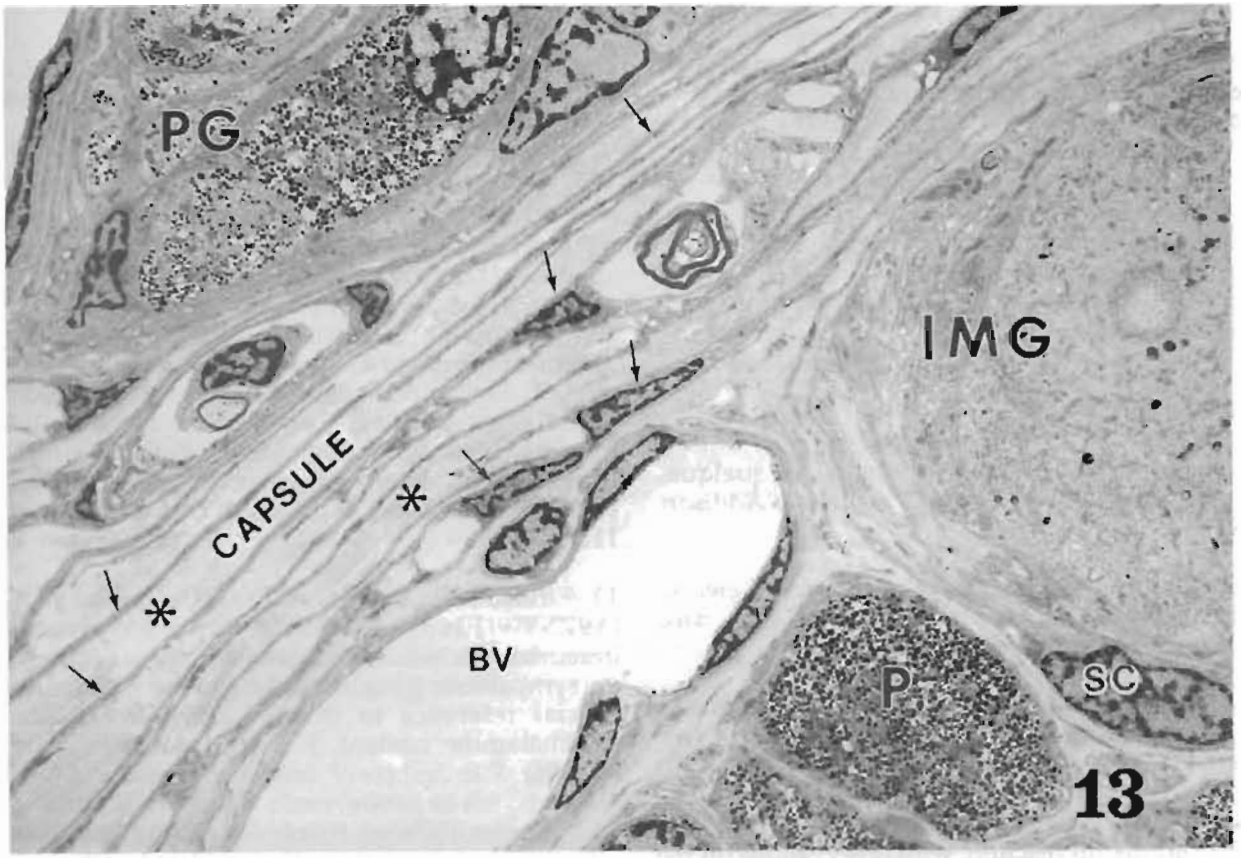
La distribución anatómica de los paraganglios cromafinos (PG), asociados al ganglio mesentérico inferior (IMG) de los gatos fue definida inicialmente con una solución combinada colorante/fijadora de dicromato de potasio y glutaraldehido. El dicromato coloró y mapeó distintivamente 3 a 6 paraganglios cromafinos distribuidos al azar, alrededor de la superficie de cada IMG. Por otra parte, el glutaraldehido preservó la integridad morfológica de los órganos cromafinos para subsucuentes evaluaciones histológicas y ultraestructurales. El IMG siempre se observó como una estructura pareada rodeando la arteria mesentérica inferior (IMA). Los órganos cromafinos paraaorticos alargados se hallan ventrales a la aorta abdominal en el retroperitoneo medio. La evaluación histológica demostró que un paraganglio está separado del IMG adayacente por una cápsula de tejido vascular y conectivo. Grandes acúmulos de células cromafinas están presentes dentro del propio IMG, pero no fue evidente una continuidad entre el tejido cromafino intraganglionar y paraganglionar. Todas las células cromafinas contienen gránulos con núcleos densos y además muestran características que son consistentes con las de las células cromafinas en general. Terminales nerviosos conteniendo vesículas claras mostraron contacto

Figure 13. A low power electron micrograph that defines the ultrastructural relationship between one Inferior Mesenteric Ganglion (IMG) and the companion Paraganglion (PG). The two bodies are separated by a capsule comprised of several layers of collagen fibers (*) as well as many fibroblast cells (arrows). Blood Vessels (BV) also are a component of the capsule material. In this case, the IMG shows Processes (P) from chromaffin cells that are filled with dark granules and appear identical to the chromaffin cells that make up the PG. A Satellite Cell (SC) surrunds one IMG neuron.

Magnification: X 4,200 approx.

Figure 14. A higher power electron micrograph showing paraganglion Chromaffin Cells (CC) in greater detail. The cells contain prominent nuclei with clumpled chromatin material (arrows). The most conspicuous characteristic of all chromaffin cells is the presence on dense cytoplasmic vesicles that represent individual catecholamine granules and completely fill the cell interior. Blood Vessel = (BV).

Magnification: X 7,200 approx.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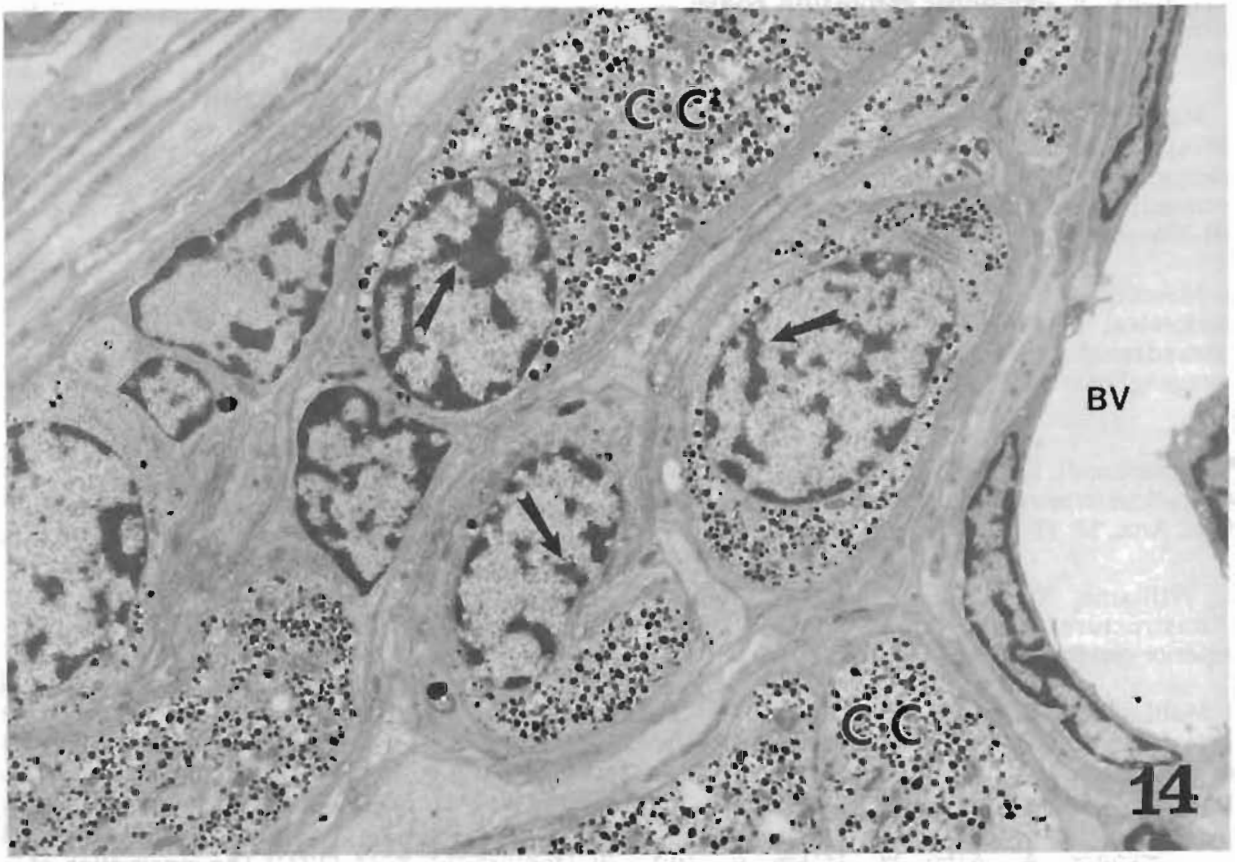
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con las células cromafinas intraganglionares, sin embargo, los terminales nunca se observaron en contacto con células cromafinas paraganglionares o paraaorticas. Este estudio demuestra claramente que un número variable de paraganglios cromafinos están yuxtapuestos a el IMG en los gatos, también que células similares están agrupadas en racimos de tamaño considerable dentro del IMG y a lo largo de la aorta abdominal.

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