HISTOCHEMICAL STUDY OF THE PANCREAS WITH REFERENCE TO 'NEURO-INSULAR COMPLEX' IN *Lepus europaeus* – Bulgarian rabbit, AS VISUALISED BY CHOLINESTERASE TECHNIQUE

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ABSTRACT
An investigation was undertaken for the study of distribution, association and relation of islet cells with neural elements such as ganglia (large, medium and small in size), nerves of nerve cells, nerves of neural networks, coarse nerves, fine nerves, nerve bundles, nerves of blood vessels and pancreatic duct, with reference to 'Neuro-Insular Complex', in the pancreas of *Lepus europaeus*-Bulgarian Rabbit, as visualized by cholinesterase technique.
Formations of the 'Neuro-Insular Complex' in the vicinity of the ganglia (either lying on the periphery of islet cell or in the vicinity of islet cell- this is one type of complex) and in the vicinity of nerve cell, lying on the periphery of the islet cell-this is another type of complex, were recorded. In addition to this, so many other types of the neuro-insular complexes, were recorded which have been described and discussed.
Islet cells of different shapes (rounded, oval and irregular) and sizes (small, medium and large),were recorded in the vicinity of the blood vessels, pancreatic duct and their ductules and in the endocrine region of the pancreas. The nerves of the islet cells formed the envelope-like structure on each islet cell, in different forms. At times, the nerves of these islet cells were in close relation and associations with the nerves of the neural networks and plexuses either of the blood vessels or the pancreatic duct. These associations and relations among the islet cells and neural elements may also be termed as 'Neuro-Insular complexes'.
Keywords: Islet cells, Neuro-Insular Complex, Mammalian Pancreas

INTRODUCTION

Earlier, researcher [1] described a peculiar structure which consisted of autonomic nerve cells and islet cells and named 'complexe sympathico insulaire'. He [2] reported the complex in contiguity with nerve cells and vascular supply and came to conclusion that the neuro-insular complex is a constant and normal structure of the mammalian pancreas. He characterized the neuro-insular complex as a non-chromaffin paraganglion and described three types of neuro-insular complexes, according to the relative number of the islet cells and associated neurons. Other investigator [3] regards the complex as a rare and accidental phenomenon seltenes zufälliges zusammentreffen" with no particular function. Previous investigator [4] observed no special nerve fiber connection between the nervous and insular elements of complex. Earlier worker [5] stated that the complexes are formed by only a part of the primary islets and persists postnatally. Another researcher [6] recorded two types of complexes in the pancreas. However, other investigators [7, 8] have given contradictory and confusing results for the staining of the islet cells. However, He [7] recorded positive reaction to AChE substance for islet cells and on the contrary other investigator [8] reported that the islet cells yielded no positive reaction to AChE activity and ganglion cells were absent. Further in the year, 1987 other researcher [9] recorded rarely nerve cells around or within the islet tissue and a few ganglia were seen in the vicinity of the acini, islet tissue and the ducts. A few acetylcholinesterase (AChE)- positive nerve fibers could be traced within and around the islet tissue [9]. However, other group of investigators, [10-12] have given a clear picture and have established untouched facts that islet cells, blood vessels, pancreatic duct and their ductules, ganglionic nerves, nerve cells, nerve endings, nerves and nerve bundles, showed AChE-positive reaction. On the

contrary, the acinar cells and the connective tissues gave negative reaction to AChE substance.

From the records, it is clear that very less attention has been paid towards the associations of the islet cells alongwith neural elements and other structures and formations of various types of neuro-insular complexes by using cholinesterase technique by earlier many investigators [7-9, 13, 14], and either have failed or ignored them.

The present study, analyzed the neuro-insular complexes in the pancreas of rabbit, under the following items: 1) Distribution, shapes and sizes of the islet cells. 2) Formation of the neuro-insular complexes on various islet cells, relation and associations of islet cells with neural elements, such as ganglia, nerve cells, nerve fibers, nerve bundles and the formation of neural networks and plexuses in the acini (exocrine pancreatic region), blood vessels and pancreatic ducts.

MATERIALS AND METHODS

The anesthesia (ether, as anesthetic agent was used) was given to healthy, laboratory, male adult rabbits. After opening the diaphragm, each pancreas was removed and fixed in 10% cold neutral formalin. Before fixing, pancreas was treated with carbon dioxide gas for 15-20 min and 35 micron thick tissue sections were cut by freezing microtome in the pathology laboratory of Higher Medical Institute, Sofia-31(Bulgaria). The staining solutions and procedures to histochemical AChE activity demonstration were followed as it has been described by previous investigators [10-12, 15].

RESULTS

Distribution, shapes, sizes and innervation of islet cells.

Frequently distributed islet cells were recorded to be AChE-positive which were small, medium and large in size and rounded oval and irregular in shape. Each islet cell was profusely and richly innervated in different pattern and forms by fine branches of the nerves which formed the envelope on each islet cell. The nerves of the envelopes, formed on islet cell were either related with the nerves of the neural networks, with the nerves of the insular plexus and their branches and the nerves of the pancreatic duct or with the ganglionic nerve fibers of the perivascular ganglia and at times with the nerves of the periductular ganglia.

Formation of the neuro-insular complexes, and their relation and associations with neural elements.

Each islet cell was closely related and associated with the ganglia of different shapes and sizes, nerve cells, nerves of the neural networks of exocrine part, coarse nerve fibers, nerve bundles, nerves of blood vessels and also the nerves of the pancreatic duct. These associations took the shape of the envelopes which termed as the neuro-insular complex.

Neuro-insular complexes were recorded of **sixteen types**, on the basis of relation and associations with neural elements.

Type I complex: The medium and small sized ganglia, arranged in chain-like fashion, lying either on the islet cell or in the vicinity of islet cells, were observed. The nerves of these ganglia were closely related and associated with the nerve bundle, coarse nerves and nerves of inter-lobular networks. The nerves of the complex formed the continuity in the entire region (Fig.1).

Type II complex: The medium-sized, irregular-shaped and AChE-positive ganglia, lying on the periphery of an oval-shaped islet cell, was recorded. The nerves of this ganglion were related with the chain of ganglia, nerves of other vascular network, nerves of acinar network and nerves of the peri-insular plexus, through the nerves of other islet cells (Fig.2). At times, AChE-positive, oval-shaped (bipolar) and irregular-shaped (tripolar) ganglia were observed, lying on the periphery of small and oval-shaped islet cell. Each ganglia, sends their ganglionic nerve fibers to other islet cells which formed the envelope-like structure, in association with the nerves of acini, blood vessels and coarse nerves.

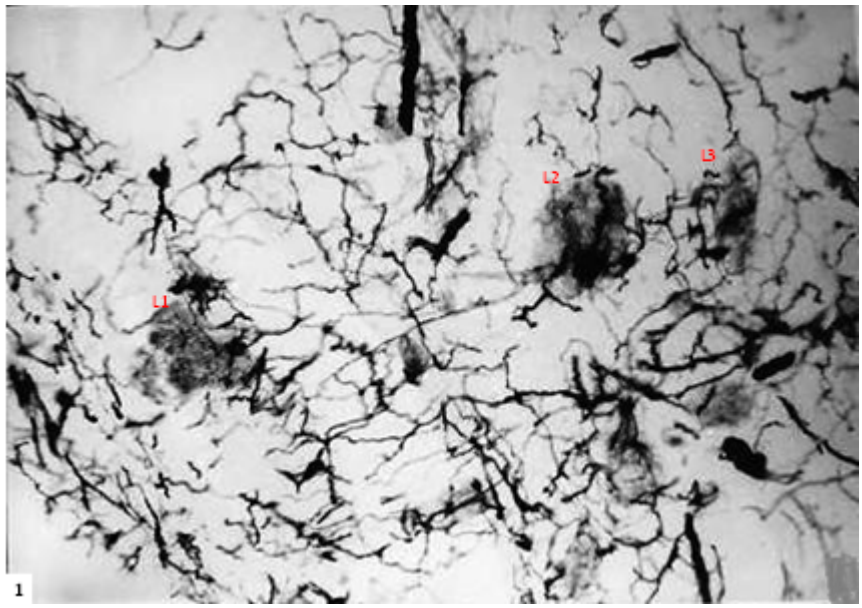

Fig. 1. Shows the formation of neuro-insular complexes on islet cells (L1, L2, L3).These complexes were formed by the ganglionic nerves, ganglia, nerve cells and nerves of the perinisular plexuses. X.150.

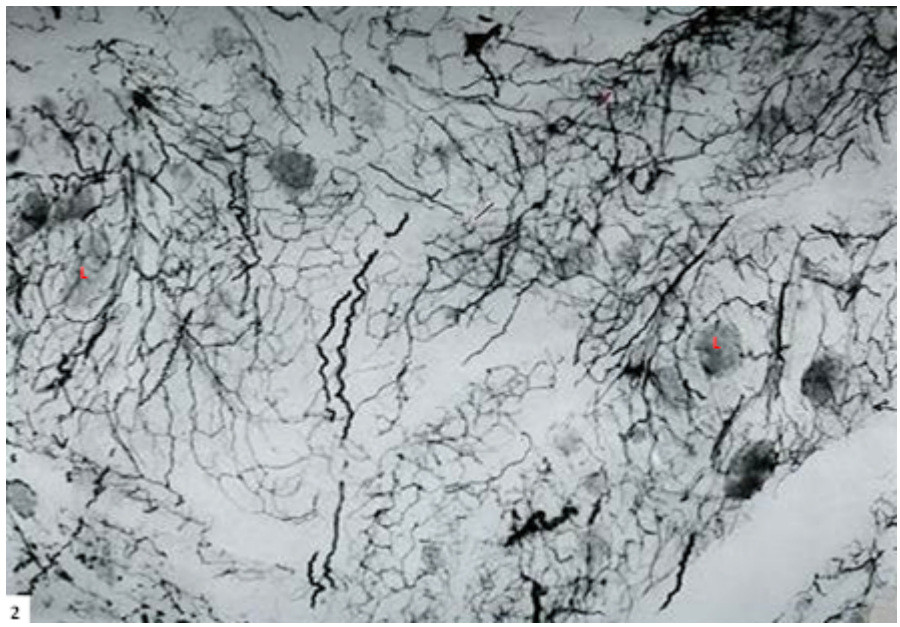

Fig. 2. Shows the distribution of coarse nerves, chain of nerve cells which were commonly related with the nerves of the neural networks. These nerves formed the neuro-insular chain of nerve cells, participated in such formations. X.150

Type III complex: The very small sized and AChE-positive ganglia, were observed in the vicinity of islet cells.These ganglia were arranged in chain-like fashion and send their nerves to islet cells and on the other end were related with tripolar and medium-sized ganglia. These nerves were related to the other chain of very small ganglia, nerves of networks and coarse nerves. At times, the smaller ganglia, along-with their ganglionic nerve fibers, formed the peri-insular plexus, in association with coarse nerves. The nerves of the plexus, encircled the which called as neuro-insular complex. **Type III** complex was very rare (Fig.3).

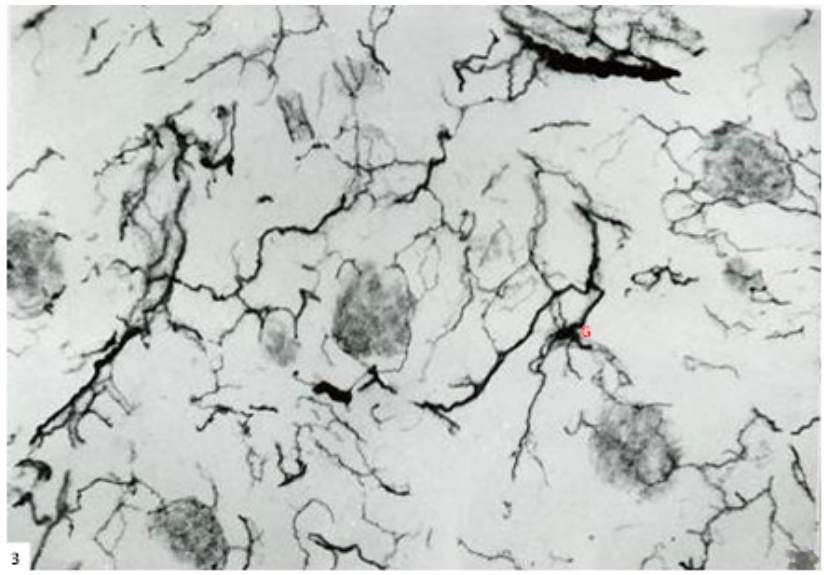

Fig. 3. Shows the formation of complexes by the nerves of vascular ganglia (G), coarse nerves and nerves of chain of nerve cells. X.150

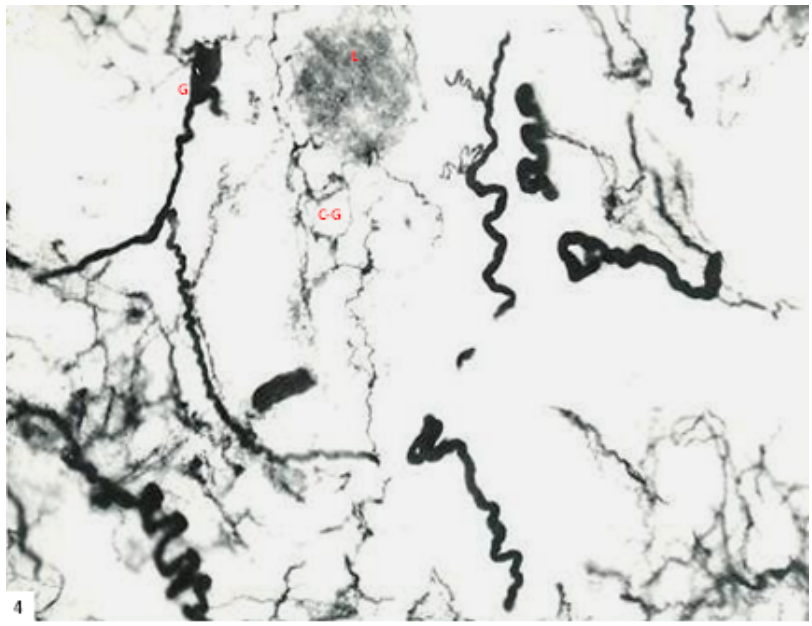

Fig. 4. Shows the formation of complex on islet cell (L) by the nerves of chain of very small ganglia (C-G) and nerves of AChE-positive ganglia, lying in the vicinity. X.150.

Type IV complex: Irregular -shaped, small sized, peri-vascular and AChE-positive ganglia, sends their nerves to islet cells. These nerves took part in the formation of peri-insular plexus at one end and on the other end reached to other islet cells. The nerves of the plexuses formed the envelope on each rounded and medium sized islet cell. Vascular nerves also participated in the formation of the network (in the acini region) and plexuses near islet cells (Fig.4). **Type IV** complex was very common.

Type V complex: The medium sized irregular-shaped ganglia were observed in the vicinity of islet cell and their nerves joined the nerves of the chain of very small ganglia. The ganglionic nerve fibers, in association with

the nerves of lobular cells, formed the envelope on islet cell (rounded and large in size) and on the other end, the nerves of the chain of ganglia were closely related and associated with the coarse nerves (Fig. 5).

Type VI complex: The nerves of tripolar (medium and vascular), irregular-shaped and AChE-positive ganglia with their ganglionic nerves, formed the envelope on the numerous islet cells (rounded, medium and large and oblong in shape), lying in the vicinity of islet cells. Usually, the ganglionic nerves participated in the formation of neural network in the lobular region and on the other end, formed the peri-insular plexuses. The nerves of these plexuses, in association with the nerves of the neural networks, formed the composite structure, named as the neuro-insular complex in the endocrine region. **Type VI** complex was rare (Fig.6).

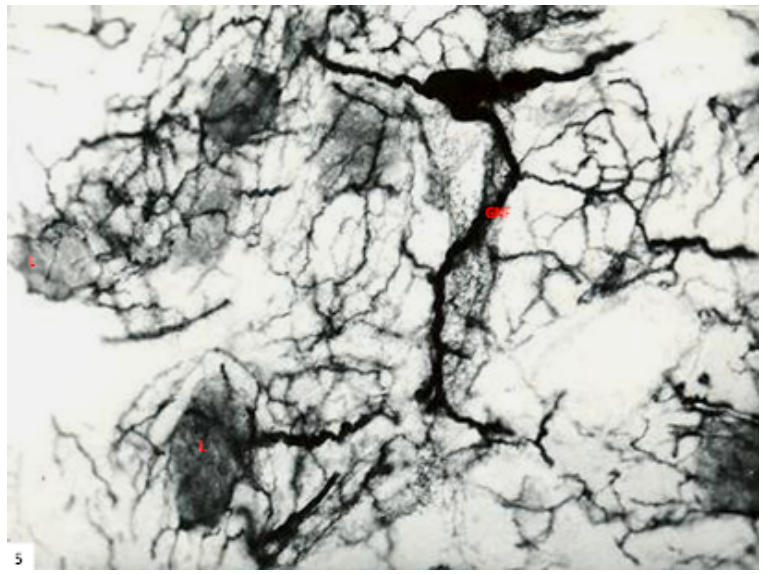

Fig. 5. Shows the formation of complexes on islet Cells (L) by ganglionic nerve fibres (GNF), in association with the common nerves of peri-insular plexuses and nerves of neural networks. X.150

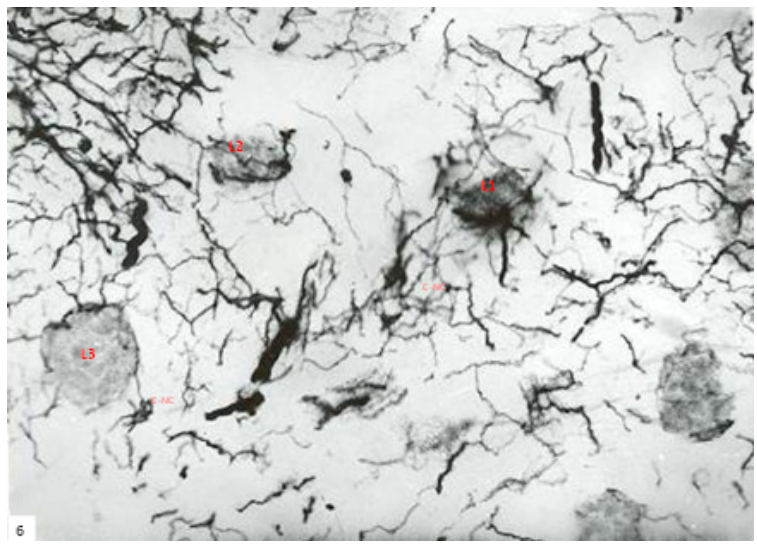

Fig. 6. Shows the formation of neuro-insular complexes on islet cells (L1, L2, L3), in different pattern. Ganglionic mass was recorded lying on islet (L1) and formed envelope, in association with the nerves of chain of nerve cells (C-NC) and vascular nerves. On the other end, nerves of chain of nerve cells and vascular nerves formed the envelope on islet (L2) and on other islet cell (L3), formed the envelope, in association with the nerves of nerve cells and nerves of chain of nerve cells (C-NC). X.150.

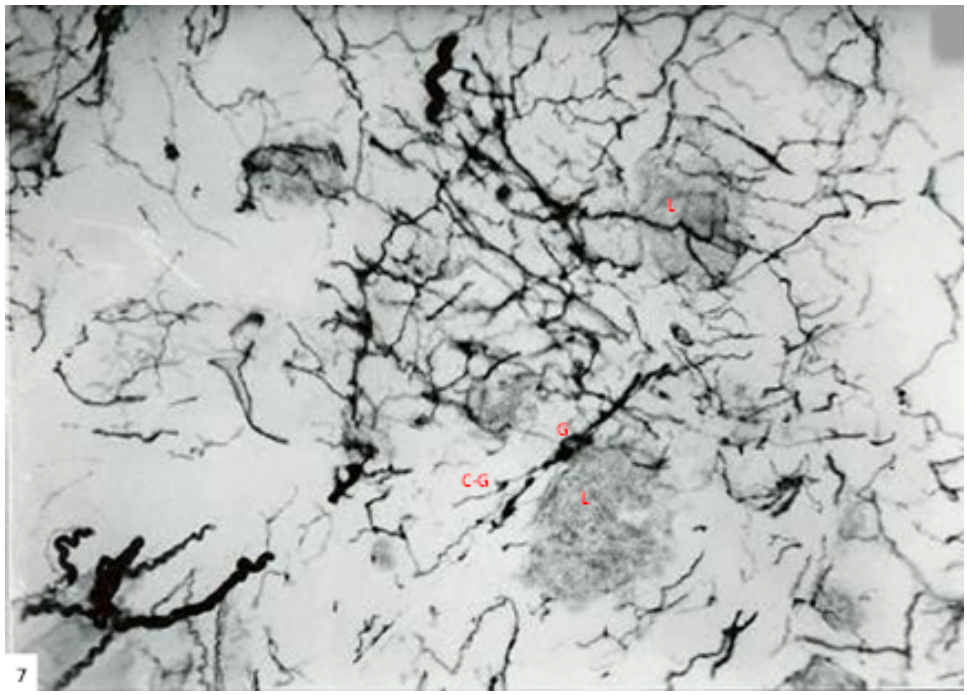

Fig. 7. Shows the formation of neuro-insular complexes. These complexes formed by common nerves of chain of small ganglia (C-G) and nerve cells.Periinsular ganglia (G) can be marked. X.150.

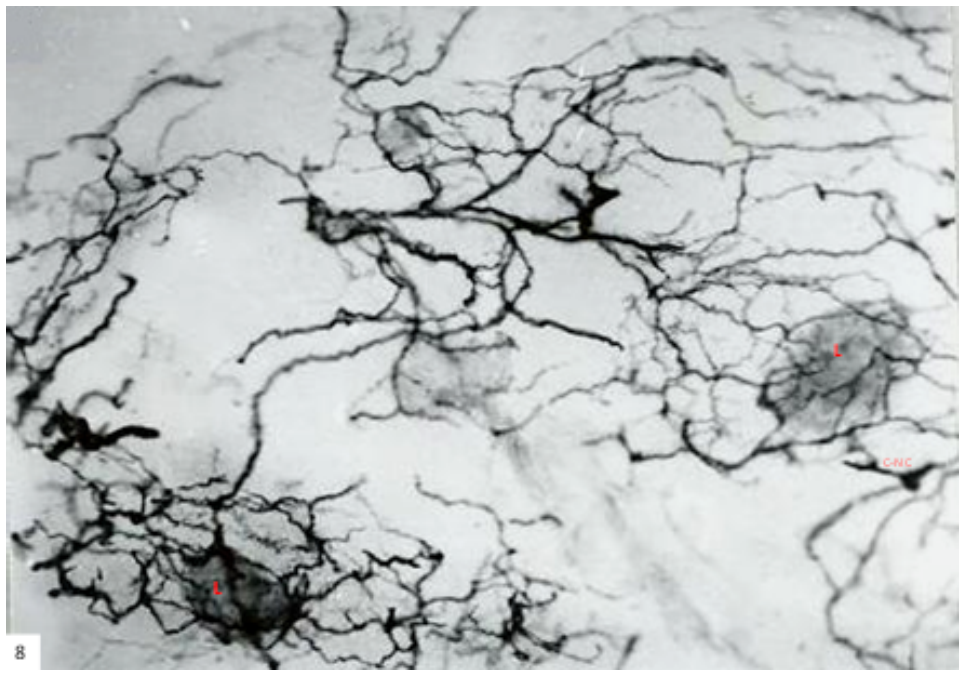

Fig. 8. Shows the formation of neuro-insular complexes on islet cells (L) by the nerves of chain of small ganglia (C-G), in ssociation with the nerves of the periinsular plexuses at one end and on the other end with the nerves of nerve cell (NC), nerves of acini and vascular nerves. X.150.

Type VII complex: The close relation and association of islet cells with the fine nerves of the neural networks and coarse nerves, was recorded. These nerves formed the envelope–like structure on islet cell (round and medium in size). On the other end, the ganglionic mass was observed on the small islet cell. The nerves of these

ganglia were closely related to the nerves of the peri-insular plexus. **Type VII** complex was also rare (Fig. 7).

Type VIII complex: The nerves of the vascular plexus, in association with the nerves of the neural network, formed the complex on each islet cell, lying in the vicinity. The nerves of the complex were either related or associated with the nerves of the chain of nerve cells at one end and on the other end with the nerve cells. **Type VIII** complex was also rare (Fig. 8).

Type IX complex: Medium, large-sized, elongated, rounded, oval-shaped and AChE-positive ganglia, along with their ganglionic nerves participated in the formation of envelopes on islet cells. The nerves of envelopes of islet cells were closely related with the nerves of peri-insular plexus and ganglia (oval-shaped), through the nerves of the acini and the nerves of the blood vessels (Fig. 9).

Type X complex: A rounded, small and AChE-positive ganglion was recorded, on the periphery of a small and rounded islet cell. Fine divisions of the periinsular plexus, formed the envelope on the islet cell in association with the nerves of the large, elongated ganglia, lying in the vicinity. **Type X** complex was rare but unique (Fig.10).

Type XI complex: The AChE-positive nerve bundles, coarse nerves and ganglionic nerve fibres, formed the envelope-like structure on the islet cell (rounded and large in size).The nerves of the envelope, were closely related with the vascular nerves and the nerves of the acini (Fig.11). **Type XI** complex was also rare and unique.

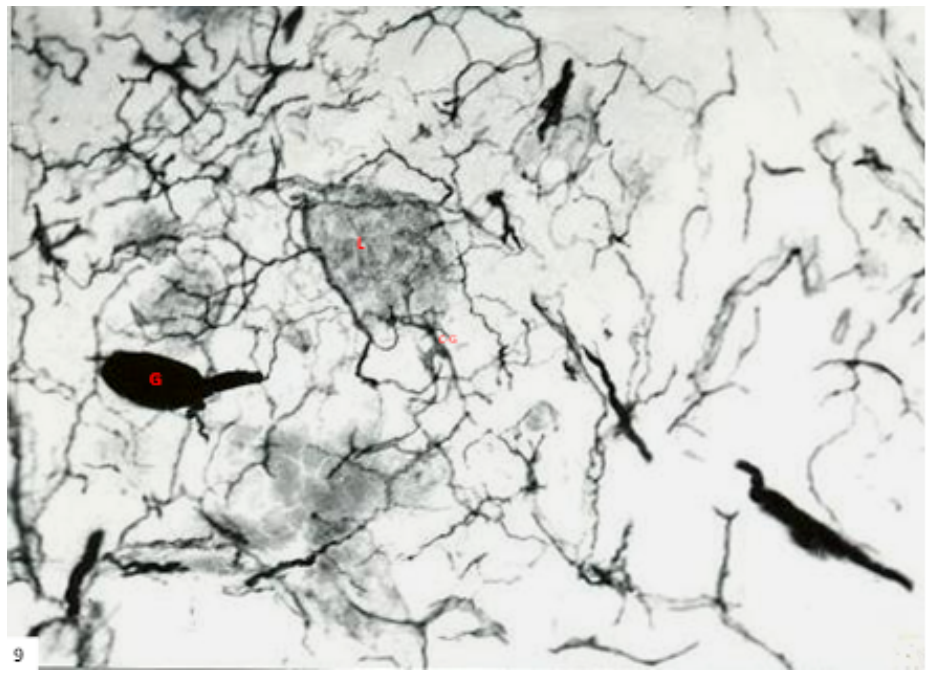

Fig. 9. Shows the formation of neuro-insular complexes on islet cells (L) by the nerves of chain of small ganglia (C-G), vascular nerves (VN), nerves of acini and ganglia.AChE-positive ganglia of irregular-shaped, also shared in the formations. X.200.

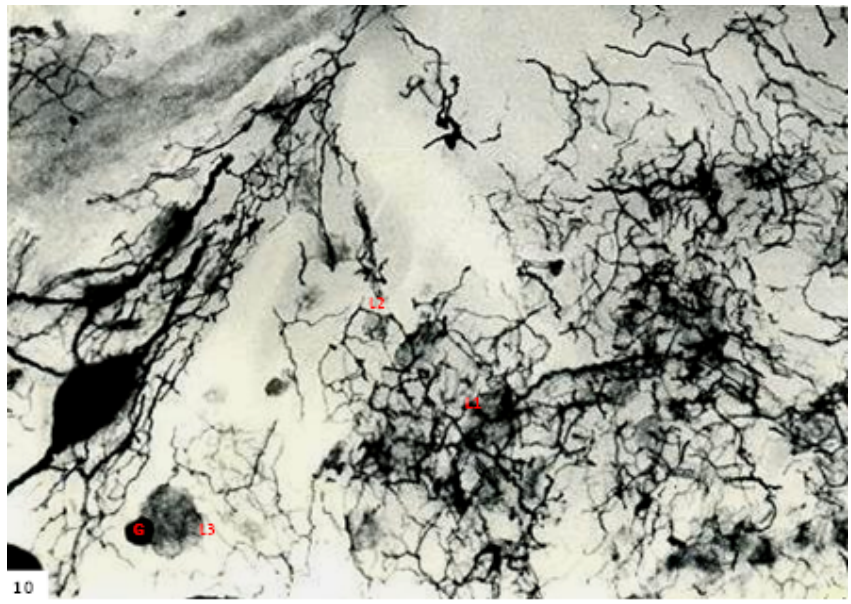

Fig. 10. Shows the formation of neuro-insular complex on islet cells (L).The complex was formed by fine nerves of chain of small ganglia on islet (L1) and nerves of periinsular plexus, and on other islet (L2) cell, the coarse nerves and nerves of periinsular plexus , formed the envelope. In other place, the rounded and AChE-positive ganglia covered the small islet cell (L3). X150.

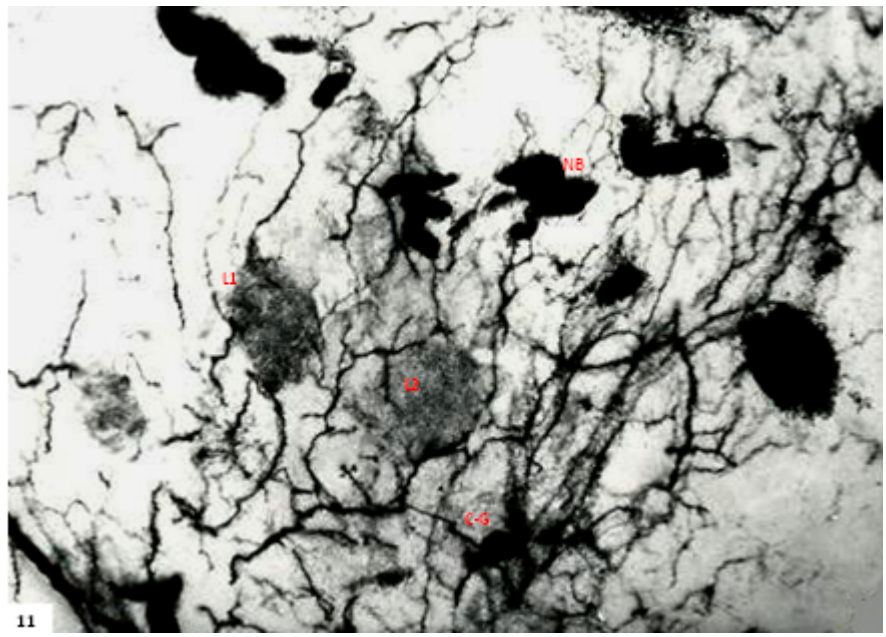

Fig. 11. Shows the formation of neuro-insular complexes on islet cells (L1, L2) by the common vascular nerves, nerves of chain of ganglia (C-G) and nerve bundles (NB). X.150.

Type XII complex: The AChE-positive ganglia arranged in chain–like fashion were recorded on the periphery of small and rounded islet cell. The ganglionic nerves, formed the peri-insular plexuses, in association with the nerves of the acini. These nerves later on, formed the envelope-like structure on islet cells, at one end and on the other end were related with the nerves of interlobular nerve cell. **Type XII** complex was a peculiar and rare (Fig.12).

Type XIII complex: The nerve cells were recorded, lying on the periphery of small and rounded islet cell. The nerves of the nerve cells were in close relation with

the nerves of the chain of ganglia, at one end and on the other end with the nerves of the peri-insular plexus and neural network. **Type XIII** complex was occasionally seen (Fig. 13).

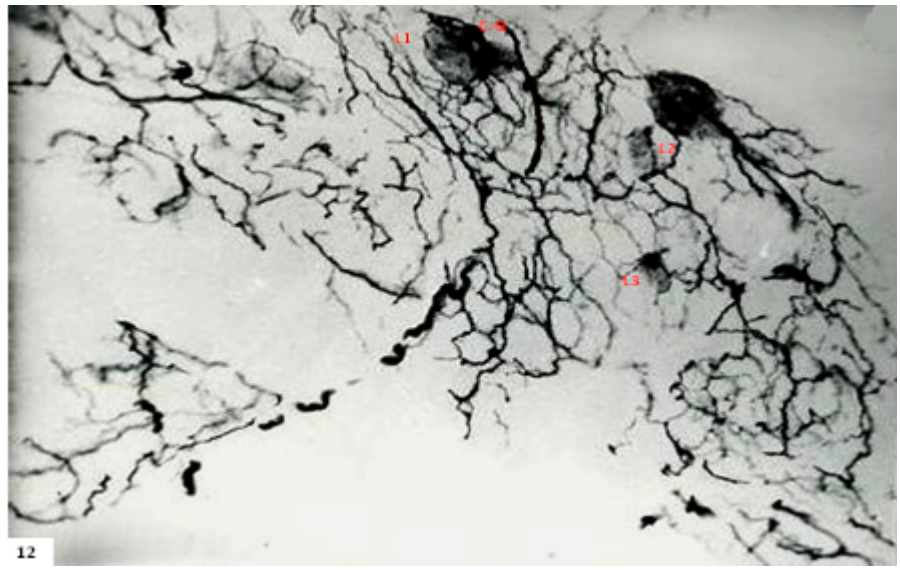

Fig. 12. Shows the formation of neuro-insular complexes on medium-sized and rounded islet cells (L1, L2, L3). On islet (L1), the chain of ganglia (C-G) covered entirely and formed the complex by the nerves of the chain of small ganglia and nerves of periinsular plexus. On other islet (L2), the plexus was formed by the nerves of periinsular plexus and nerves of the chain of ganglia and on the third islet cell (L3), the tetrapolar, and medium sized and AChE-positive ganglia covered a part of it and formed network of fine divisions of the nerves. X.150.

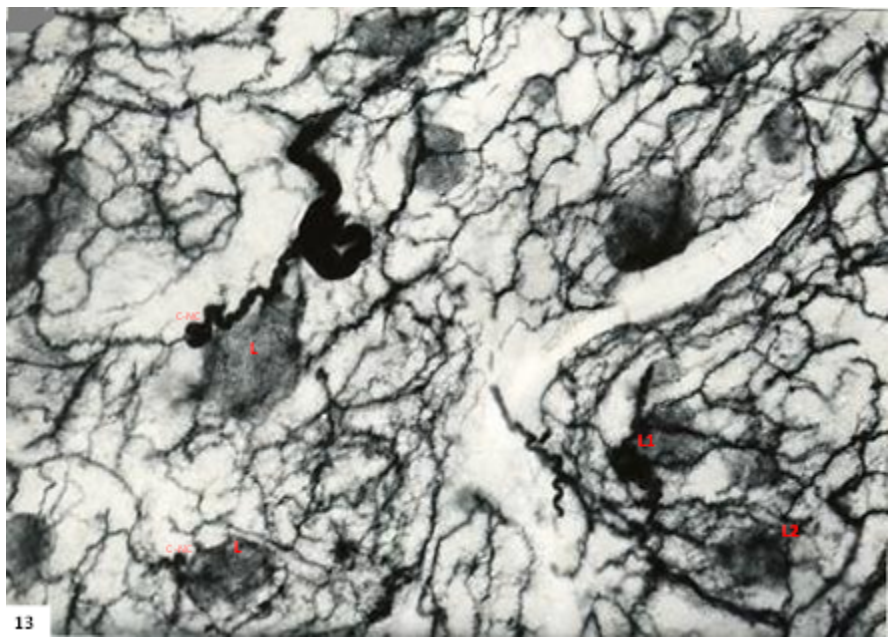

Fig. 13. Shows the formation of the neuro-insular complexes on islet cells (L1, L2) by common nerves of nerve cells and nerves of peiinsular plexuses at one end and on the other end, the complexes on other islet cells, were formed by the nerves of chain of nerve cells (C-NC) and nerves of neural networks. The nerve cells were recorded lying on the periphery of the islet cells. X.200.

Type XIV complex: Chain of nerve cells was observed on islet cell (rounded and medium in size).The nerves of the nerve cells, were closely related with the nerves of the large-sized, irregular-shaped and AChE-positive ganglia. **Type XVI** complex was very rare (Fig.14).

Type XV complex: The peripheral coarse nerves of pancreatic duct, nerves of chain of nerve cells, in association with other coarse nerves, formed the envelope over rounded and large islet cell. The fine branches of nerves cells were closely related and associated with the nerves of the peri-insular plexus, around the islet cell. **Type XV** complex is unique and rare (Fig.15).

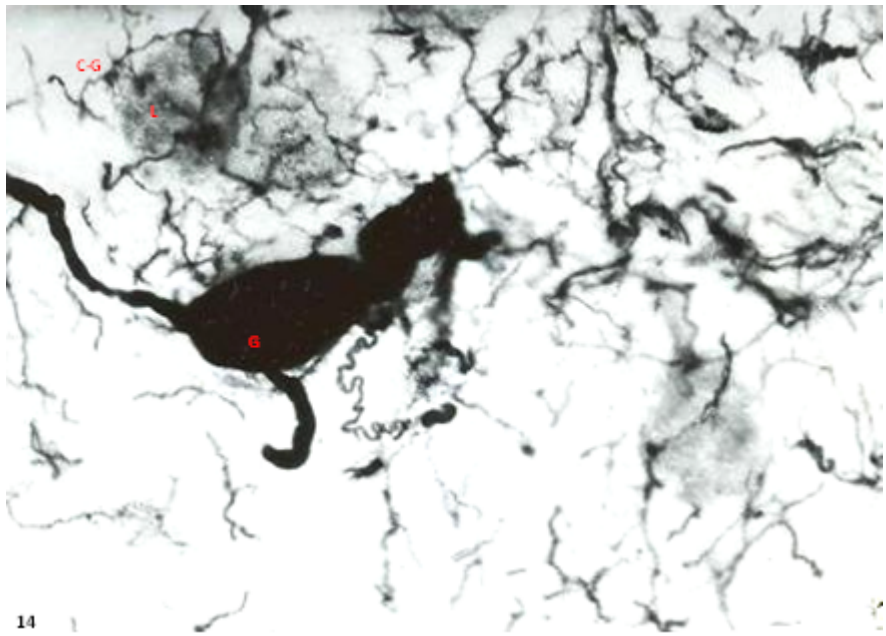

Fig. 14. Shows the formation of the neuro-insular complex on islet cell (L), by the chain of ganglia (C-G) and their nerves which covered the islet cell.The ganglionic nerves were related and associated with the nerves of the large sized, irregular-shaped and AChE-positive ganglia (G) and chain of nerve cells (C-NC). X.250.

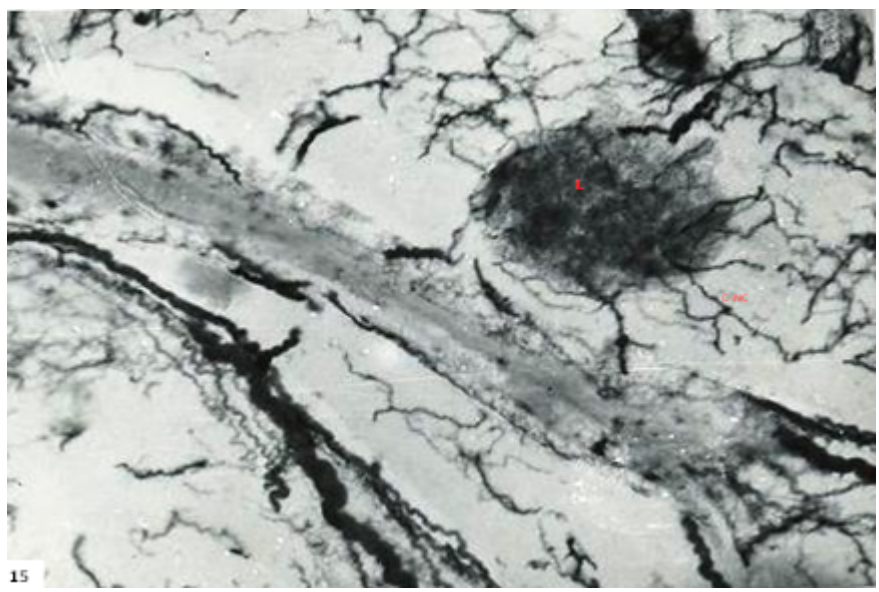

Fig. 15. Shows the formation of neuro-insular complex, on large, rounded and AChE-positive islet cell (L), by the nerves of the chain of nerve cells (C-NC), nerves of the periinsular plexuses and peripheral nerves of the pancreatic duct. X.200.

Type XVI complex: was formed by the ganglionic nerves of bipolar, rounded, oval-shaped and AChE-positive ganglia, nerves of the insular plexuses, nerves of the neural networks of acini, coarse nerves and vascular nerves. AChE-positive, bipolar and rounded ganglia (medium-sized) was also recorded on the periphery of small sized islet cell. The envelope on this islet cell was formed by the nerves of chain of intra-lobular nerve cells, coarse nerves and nerves of the acini. On the other side, the complex was formed by the nerves of the chain of nerve cells, nerves of neural network of acini and nerves of intra-lobular irregular-shaped and medium sized ganglia (Fig.16).

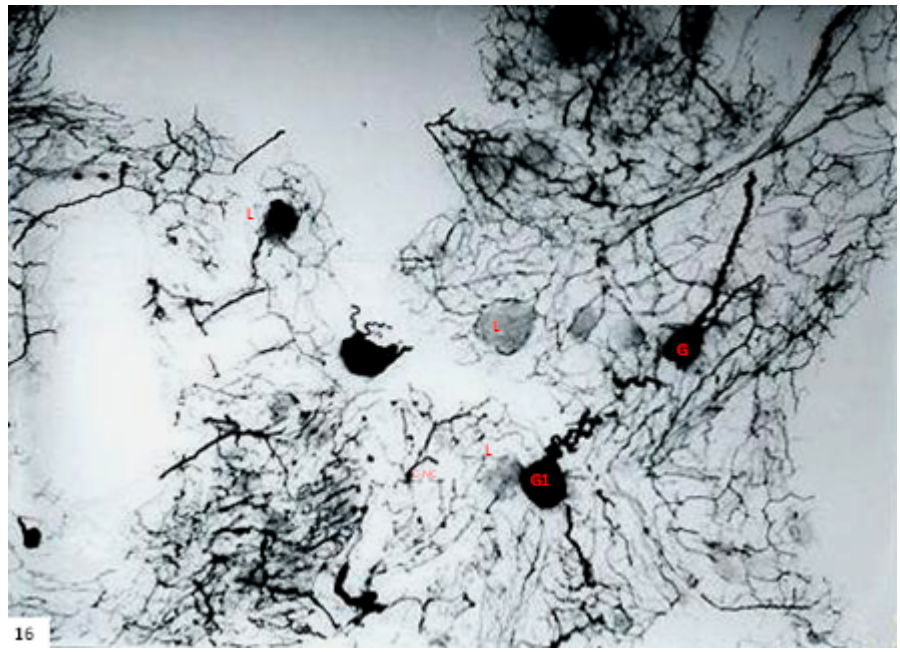

Fig. 16. Shows the formation of the neuro-insular complexes by the nerves of chain of nerve cells, nerves of the acini, ganglionic nerves of the AChE-positive ganglia (G) and vascular nerves. A Periinsular ganglion (G1) was also recorded lying on the periphery of the islet cell. This pattern of complex formation was commonly observed in many islet cells. X.150.

DISCUSSIONS

Early researches of the investigators [7-9, 13, 14, 16, 17] have either ignored or left many details untouched and undecided, although they have applied the histochemical cholinesterase method, even then they have recorded confusing and conflicting results regarding the reaction for the AChE activity. Other investigators [1, 3, 5, 18, 19] had attracted the attention regarding the presence of the neuro-insular complex, formation, and their relation and association with the neural components and other structures of the pancreas of mammals.

In the light of recent researches on the pancreas of rabbit, as visualized by the modified cholinesterase technique [11] where it was recorded that the pancreas was richly and profusely innervated by AChE-positive nerves [9], i.e., myelinated (thick and coarse), non-myelinated (thin), nerve bundles, ganglia (of different shapes and sizes), nerve cells (generally in chain form), the nerves and ganglia of blood vessels and pancreatic duct have participated in the formation of neural networks in the exocrine part and insular plexuses,around the islet cells of different shapes (rounded, oval and irregular) and sizes (small, medium and large). Some islet cells showed strong positive reaction to AChE-activity.

In the formation of the neuro-insular complexes, the nerve cell, chain of nerve cells, ganglia and chain of

ganglia and nerves of different nature were recorded in association with the islet cells, at different levels.

At times, the ganglia, nerve cells and chain of nerve cells were observed on the periphery of the islet cells. On the basis of relation and associations of islet cells with the neural elements, the different types of neuro-insular complexes were recorded in the endocrine part which have not been reported so far by the previous investigators [7-9, 13, 14, 17] except a few earlier investigators who reported the existence of the complex [1, 2, 4, 6, 18, 20]. A few investigators could only described two types of the neuro-insular complexes [6]. At several places the islet cells were recorded to be closely related with AChE-positive ganglia and nerve cells.These associations have some functional importance and they might be playing the important role in relay of senses from one place to another.

Previous investigators could not provide the detailed information regarding numerous formations of the neuro-insular complexes and their associations and relations with neural components of the exocrine and endocrine components, as recorded in the rabbit's pancreas, i.e., **sixteen types** and their relations and associations with various neural elements at different levels while earlier researchers [2, 7, 9, 13, 19, 20] have not mentioned the above details of the neuro-insular complexes, as described at the present work. However, it may be due to faulty use of the method which they applied, either old histological staining technique or cholinesterase technique [2, 3, 5-9, 13, 14, 18, 20, 21].

Although controversial and confusing results were recorded for the reaction of AChE activity while applying the cholinesterase method [7] recorded AChE-positive reaction for islet cells and other researcher [8] recorded no positive reaction for islet cells and absence of ganglion cells.

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