Microscopical Studies on the Pineal Gland (Epiphysis Cerebri) of Balady Goat

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Abstract

Thirty pineal glands of Balady goats of 2-3 years of age were used in this investigation. Immediately after sacrifice, they were fixed or put in liquid nitrogen for histochemical enzymatic reactions. Different stains were applied after routine processing. The glands were situated anterior to superior colliculi as evagination of the roof of 3rd ventricle. The glands were of 5-7 millimeter in length, 4 millimeter in breadth and 3 millimeter in thickness. Glands were invested in thin capsule with subcapsular spaces in some areas. Septae extending from the capsule divided the glands into small lobules containing groups of parenchymal cells. Stroma revealed conspicuous capillary network, septae were of dense reticular and collagenic but no elastic fibres. Nerve fibres were very few of thin and thick axons coming from brain stem to the gland. The parenchymal cells of pineal gland of goat were: very few neurons, dark pinealocytes of basophilic cytoplasm and eccentric spherical vesicular nuclei, light pinealocytes of clear or slightly basophilic cytoplasm with spherical vesicular centrally located nuclei, most of pinealocytes tend to be in close contact with each other, binucleated light cells were present. As well, glial cells were present represented by astrocytes in close relation to the pinealocytes as well as microglial cells either present singly or in groups. There were no pigments could be observed in any of all cells. Cavities as a result of apoptosis were common at this age in this animal especially in the middle (Centre) of the gland, not present superficially. Short recess could be observed as continuation of the ependymal cells lined extensions of third ventricle was discerned. Regarding the enzymatic reactions, strong reaction in the pinealocytes was for SDH-ase but moderate for non-specific esterase, slight reaction for ATP-ase and 5-Nucleotidase but traces of lipids and few stromal cells were alcianophilic through the pinealocytes, while PAS reaction was strong in septae and some of pinealocytes. Other results were mentioned and discussed,

Introduction

The pineal gland is a small pyriform organ occupying a depression between the superior colliculi. The primary role of it is to mediate the influence of the photoperiod on the neuroendocrine reproductive axis (1). Assumption may be premature and may apply only to some species. The gland serves as an intermediary between the external environments especially the photoperiod and the organism as a whole. In effect, the gland keeps the organism in proper synchrony with the prevailing environmental conditions. It is an active endocrine gland secreting melatonin that is be synthesized from the neuro-transmitter serotonin (2). The production of this hormone is rhythmic, where it is lowest during daylight and highest at night (3), It is a very effective antioxidant and is considered as a free radical scavenger (4). It has a role in the maturation of the reproductive organs (5) and affects other endocrine glands such as the adrenal gland (6). Its histological and histochemical structures as well as some ulltrastructures were investigated in many species, e.g. fox (7), dog (8), squirrel (9), human (10), deer (11), cat (12), sheep (13), bovine (14), rat(15) and other mammals (16).

Hence, the aim of this work was to throw some light on the structure under the microscope for the pineal gland of goat due to few if any investigated it.

Material and Methods

Thirty pineal glands of Balady goat aged 2-3 years were used in this investigation, their length, width and thickness were measured after recording the position of the glands. Every gland was extirpated at its connection with the brain stem and immediately fixed either in buffered neutral formalin, Bouin's fluid , Susa or Carqnoy fixatives. Other glands were freezed in liquid nitrogen (-196 ^{o}C) and transferred to cryostat (-20 ^{o}C) to be cut at thickness of 10 micrometer and subjected to photochemical methods for lipids, glycogen and some phosphorylases and dehydrogenase enzymes. The specimens other than those put in

liquid nitrogen were dehydrated, cleared and paraffin impregnated till 6 micrometer thick sections were prepared and stained with different stains. The methods used in this investigation were adopted from (17; 18; 19).

N.B. All specimens were taken at 11 O'clock A. M.

Results

The pineal gland was situated rostral to the superior colliculi in the midline. So overlying a depression at the roof of the 3rd ventricle, anterior to the colliculi. The gland is situated obliquely caudally having antero-posterior compressed spherical form. Its rostral surface was smooth without any projections while its caudal surface revealed an irregular configuration with distinct projections with fissures in between (Fig. 1).The pineal gland of goat had an-average length of 5-7 millimeters an average breadth of 4 millimeters and an average thickness of 3millimeters.As it is developed as an evagination of the roof of the third ventricle, hence the gland is separated from the third ventricle by nerve fascicles containing nerve axons that extended to the interior of the pineal parenchyma then followed by an ependymal cell layer of long ciliated simple columnar cells with oval nuclei (Fig. 2).The pineal gland is invested in thin capsule of connective tissue having collagenic, a lot of reticular (Fig. 3) but no elastic fibres, The capsule as a continuation of pia mater, it is in some areas appeared very thin or revealed subcapsular spaces (Fig. 1).In some regions, the capsular pia mater exhibited wide blood vessels to which tributaries were seen leaving the gland (Fig. 4).From the capsule many thin septae were seen directed to the interior of the gland forming the main part of the stroma (Figs. 1 & 3).The septae were rich in collagenic fibers (Figs. 5 & 6) as also the reticular fibers (Fig. 3).The septae carried the blood vessels where the reticular fibers were condensed around these blood vessels (Fig. 7), most of septae revealed periseptal narrow spaces (Figs. 5 & 6).Throughout the stroma there is conspicuous capillary network in the stroma of the pineal gland of goat (Figs. 8 & 4).

In the center of the gland there were irregular variable shaped cavities free of intact cells except remnants of them or lined with small cells with small compact and dense nuclei (Fig. 9).The septae were strongly reacting with PAS (Figs. 10 & 11). but no reaction for alcian blue (Fig. 12). Some few glial cells were alcianophilic. There were no any lymphocytic aggregations either in the stroma or in the parenchyma. Unmyelinated nerve fibres were very few, thin but rarely thick, associated to some pinealocytes or accompanying blood vessels and passed either singly or in groups (Fig. 13). Short recess was discerned extending from 3rd ventricle with lining ependymal cells.

Parenchyma:

The highly branched septae enclosed in between small cellular groups of parenchymal cells in small lobules. Those cells could be differentiated into the following:

1. Neurons (Figs. 10 & 14), were very few in number, of polyhedral large perikaryonic region, basophilic cytoplasm, large vesicular oval or spherical nuclei with distinct nucleoli.
2. Dark pinealocytes (Fig. 14), numerous in number of oval contours having basophilic cytoplasm, eccentric smaller vesicular nuclei which appeared spherical, the cells appeared as doublets or in groups especially near the septae or blood vessels.
3. Light pinealocytes (Fig. 14), were scattered between the dark ones, those cells were smaller, with less vesicular nuclei that were centrally located, and mostly their cytoplasm was lightly stained or appeared as clear cells.
4. Glia cells (Fig. 15), those cells are situated in relation to pinealocytes with oval dense nuclei as astrocytes (Fig. 10). As well, fewer in number microglia were having elongated dense nuclei and passed in groups or singly in patchy areas (unevenly distributed).

There were no pigments could be observed in any of all pinealocytes of goat pineal gland.

Histochemical Reactions:

Although most of pinealocyes gave PAS-ve reaction some of the light pinealocytes revealed PAS+ve reaction (Fig. 10). Also, those cells exhibited alcianophilia, but most of pinealocytes were alcianophobic (Fig. 12). As for glycogen, the parenchymal cells were negative for best's carmine.

Succinate Dehydrogenase:

As general, the parenchymal cells in low power revealed a strong reaction (Fig. 17), while by high power (Fig. 18), the large dark pinealocytes were intensely reactive as numerous diformazan granules having blackish blue colour.

Although ATP-ase reaction was slight in parenchymal cells, negative in trabeculae (Fig. 19), the reaction in parenchymal cells was moderate in reaction for 5-nucleotidase (Fig. 20).

About lipids, traces were present in parenchymal cells (Fig. 21). The reaction for non-specific esterase was moderate especially in pinealocytes, but negative in other cells (Fig. 22).

Discussion

Although pineal gland is present in all mammals and birds, it was present only in Indian spotted owlets and absent in adults of them (20).Regarding the dimensions given for pineal gland of Balady goats, a condition which is denied in all available literature for all pineals of different mammals and brids.The results on goat pineal recorded by (21) is in a complete agreement with this investigation especially for the histochemical reactions, but differed in reticular fibres distribution where the author described the gland to be rich in reticular fibres without pointing to their concentration in capsule and septulae. Also, the author did not describe the cavities in the rniddle or center of the gland or microglia, but only pointed to interstitial glial cells as well as high vascularity of capillaries did not mention by the author.

Respecting the presence of few neurons in the pineal gland of goat, it is going in hand with the findings in chicken pineal by (22), while binucleated light pinealocytes in goat pineal have not mentioned by any of the reviewed articles.As for pigments and glycogen which could not be seen in goat pineal, those were detected as common deposits in fox pinealocytes (7), but the aforementioned authors agreed with this investigation in detection of astrocytes, capillaries of non-fenestrated type and adrenergic nerve fibres. The results of this investigation detected periseptal spaces which are considered as perivasular spaces due to blood vessels passing through these septae, hence, these perivascular spaces could be observed in domestic fowl pineals (23).

Regarding the parenchymal cells of pineal gland of goat, they were dark and light pinealocytes as well as astrocytes and microglia. Those were described in dog except the microglia (8). In rat (15) described dark and light pinealocytes and neuroglial cells. In Indian palm squirrel (9). Large and small pinealocytes could be observed and moderately stained with PAS and Sudan black. The latter reactions in goat pineal were positive for PAS only in light pinealocytes, but traces of lipids were present in the parenchymal cells. As well, (20) described intrapinealocyte lipid droplets. In avian pineal glands, there were 3 types of cells; photosensory cells, neuronal cells and secretoty cells (24). Perivascular phagocytes having antigenic properties, microglia and antigen presenting cells were revealed in avian pineal gland (16). From the latter only microglia could de described in goat pineal. As well, the latter described neurons which were very few in goat pineal under this work. The neurons in most animal pineals were mentioned by (25).

The cellular lined cavities recorded in goat pineal did not simulate those of Indian palm squirrel (9) where in this animal the lining cells were pinealocytes and glial cells, but in goat, the lining cells were very small cells did not simulate either pinealocytes or glia cells, also the cavities revealed PAS+ve materials which were absent in goat pineal. In this regard, the brain sands or corpora arebasie present in human pineal (26) were not seen in the goat pineals.

Although the stroma of goat pineal contained no lymphocytic groups or aggregations of them, those were encountered in Indian palm squirrel (9), as also, in chicken pineals lymphopoiesis was occurring till 4 months post hatching (27) or till 6 months (28) in capsule and trabeculae or even insular Lymphoid tissue was maintained in pineal stroma of chickens even at 3 years of age (29). Moreover, accumulations of lymphatic tissue continued in adult house sparrow (30).

According to this work, to distinguish between the 2 types of pinealocytes, it is based on cytoplasmic affinity for staining, where the dark ones revealed more basophilic cytoplasm and eccentric nuclei, while the light cells revealed mostly few or no basophiles and centrally located nuclei. The light pinealocytes though revealed no stainable granules but doubtedly contained flocculent martial in the cisternae of the endoplasmic reticulum (1).The presence of two varieties of pinealocytes may be due to different substances secreted by the pineal gland as melatonin and serotonin (31). Thyrotrophic releasing hormone and luteinizing releasing hormone (32), melanocyte stimulating hormone (33) and angiotensin II (34). The gland also secretes other pineal peptides (35). It was reported that dark pinealocytes contain more calcium than light cells (36). Many authors suggested different classifications for pineal parenchymal cells in different animals (31&37).Through this investigation the neuroglial cells were smaller in size and less numerous than pinealocytes, exhibited deeply stained nuclei, the glial cells provide a netowrk surrounding the pinealocytes, as astrocytes which are responsible for transport of energy, neurotransmitters and metabolic products to the pinealocytes (38).The gradual progression of pinealocytes degeneration with age by(15), may be explained by the gradual decrease of melatonin secretion causing more degeneration of pinealocytes. This may be confirmed by the results of (39) that proved the melatonin having a role in maintenance of pinealocyte viability. This is also confirmed by the cavities revealed in this investigation in 2-3 year aged goat pineal.

Regarding the multiple intercellular contact between the pinealocytes of goat pineal, this may occur in an attempt to compensate for the decrease in the adrenergic innervations of pinealocytes as reported

by (40) providing links between the adjacent cells, as also was described by (15).In goat pineal, a short recess could be observed as extension from the third ventricle and lined by ependymal cells, hence simulating their presence in indian palm squirrel (9), as also were present in red deer male pineal as 2-3 branches that reached the distal part of the gland and appeard to be lined by continuous layer of ependymal cells (11). In developing sheep pineal gland,(41) suggested that the gland has a secretory function in uterine life, also they described 3 types of cells in sheep pineal gland (pinealocytes, interstitial cells and pigment cells) of these cells, the pigment cells could not be detected in goat pineal here, though they did not point to neurons in sheep pineal which were detected in goat pineal.

Respecting the nerve fibres detected in the pineal gland of goat, these are originated from perikarya located in sympathetic superior cervical ganglia and parasympathetic sphenopalatine and otic ganglia (16), the sympathetic ganglia contain norepinephrine and neuropeptide as neurotransmitters, while the parasympathetic ganglia contain VIP; vasocative intestinal peptide and peptide histidine isoleucine. Recently, nerve fibres originating from perikarya located in the brain containing GABA, orexin, serotonin, histamine, oxytocin and vasopressin innervate the pineal gland directly via the pineal stalk. So, mammalian pineal gland pinealocytes can be influenced by a plethora of neurotransmitters (16). The strong succinate dehydrogenase activity in goat pineal gland is dued to enrichment in mitochondria that accumulate respiratory enzymes (42). So, indicating high respiratory activity of the pinealocytes. As it was resorded in this work that slight ATP-ase reaction could be seen in the parenchymal cells of pineal of goat, so, ATP-ase acts as the energy currency of the cell, transferring free energy derived from substances of higher energy potential to those of lower energy potential (43), thence the slight reaction is indicating low activity of cells may be due aged goats (2-3 years) of this work which is confirmed by areas of cavities revealing an apoptotic condition at that age in goat pineal.

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Legends of Figures

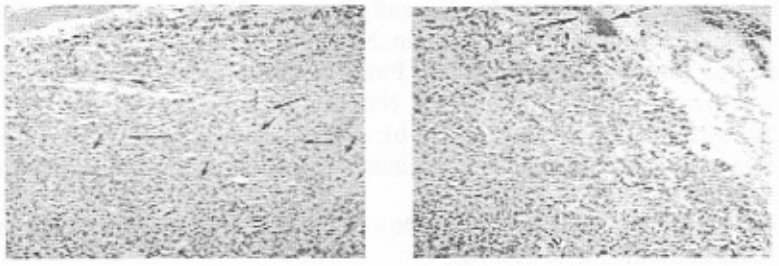

Fig.1: Photomicrograph of pineal gland of balady goat, showing the capsule (c), subcapsular spaces(s), caudal surface of the gland having projections and fissures in between (arrows). Crossmon's trichrome, X 100.

Fig.2: Photomicrograph of pineal gland of balady goat, revealing its relation to third ventricle, pineal gland (p), roof of third ventricle (R), ependymal cells (e) lining the venricle.(V) Van Gieson's st., x 100.

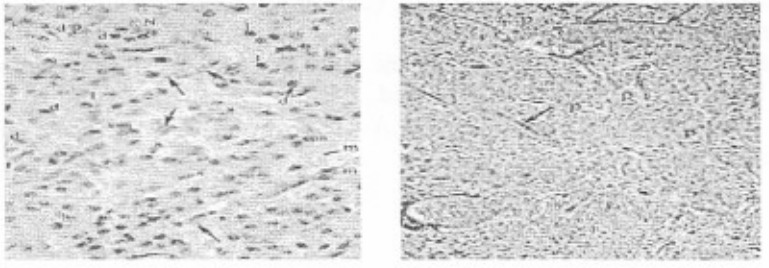

Fig.3: Photomicrograph of pineal gland of balady goat, revealing capsule (c) rich in reticular fibres, stroma has septae of dense reticular fibres in all septae and around blood vessles (arrows) with apparent periseptal spaces (s). Silver impregnation, x 100.

Fig.4: Photomicrograph of pineal gland of balady goat, revelaing subcapsular sinuses (s), periseptal and perivascular spaces (v), wide venules in subcapsular region (arrows), sroma rich in branched septae. H & E, X100.

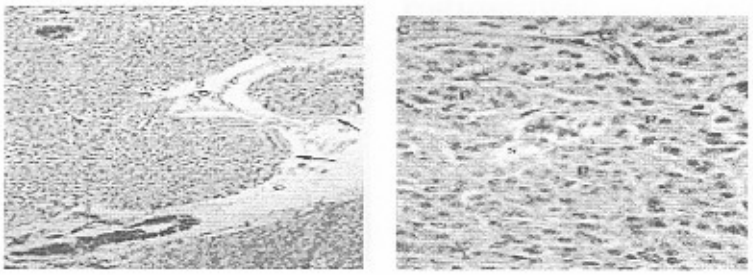

Fig.5: Photomicrograph of pineal gland of balady goat, revealing septae rich in collagenic fibres (arrow), small groups of parenchymal cells (p), periseptal spaces (s). Crossmon's trichrome, x 100.

Fig.6: Photomicrograph of pineal gland of balady goat, revealing dense collagenic fibres in septae (c), Parenchymal cell groups (p), periseptal spaces (s). Crossmon's trichrome, x400.

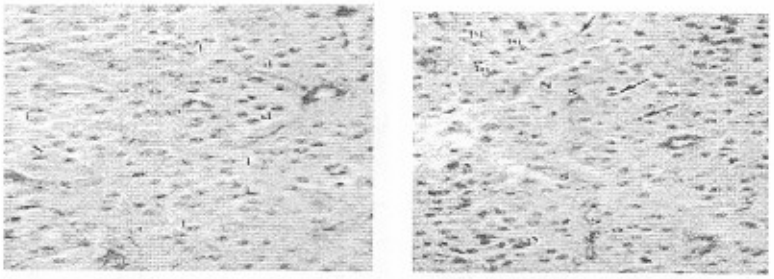

Fig.7: Photomicrograph of pineal gland of balady goat, revealing condensed reticular fibres to the septae (arrow and around blood vessels in septae. Silver impregnation, x. 400.

Fig.8: Photomicrograph of pineal gland of balady goat, revealing conspicuous blood capillaries network in the stroma (arrow). H & E. x 100.

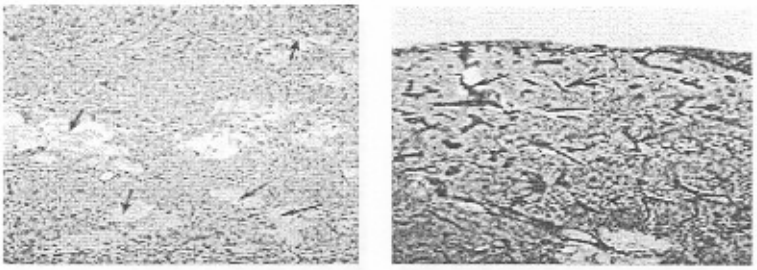

Fig.9: Photomicrograph of pineal gland of balady goat, revealing irregular cavities (c) especially in the middle (centre) of the gland except remnants of apoptotic cell debris. PAS tech, X 100.

Fig.10: Photomicrograph of pineal gland of balady goat, revealing PAS+ve septae (s), astrocytes (arrows) in relation to light pinealocytes (bi). Alc. blue-PAS tech., X 400.

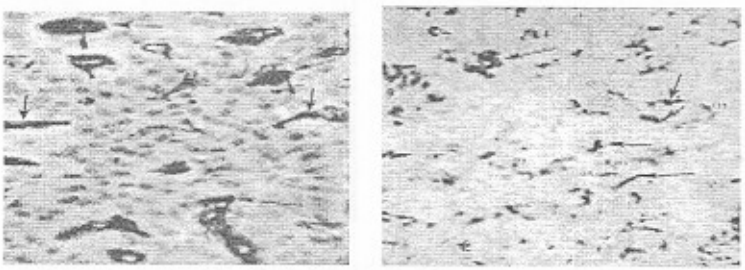

Fig.11: Photomicrograph of pineal gland of balady goat, revealing dark pinealocytes (d), ligh pinealocytes (l), Neuron (N), alcianophobic, septae were strongly reacted for PAS. Alc. Blue-PAS tech. X. 400.

Fig.12: Photomicrograph of pineal gland of balady goat, revealing alcianophobic spetae, some few glial cells were alcianophilic (arrows), pinealocytes are alcianophobic. Alcian blue, x 100.

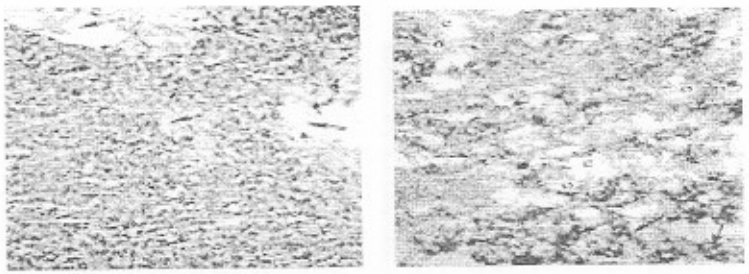

Fig.13: Photomicrograph of pineal gland of balady goat, revealing nerve fibres (f) deep to pineal gland (p) in the roof of the third ventricle, some of nerve fibres are in groups directed from brain stem to the gland (arrows). Holme's method, X 100.

Fig.14: Photomicrograph of pineal gland of balady goat, revealing neuron (N), dark pinealocytes (d), light pinealocytes (L) astrocytes (s) enrichment of blood capillary network (arrows), microglia (m) singly or in groups, close contact of pinealocytes (con). H & E. X 400.

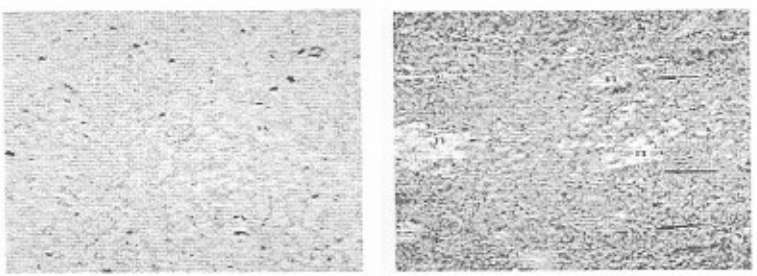

Fig.15: Photomicrograph of pineal gland of balady goat, revealing glial cells either microglia (m) or astrocytes (as), reticular fibres (arrows). Anderson method, X 400.

Fig.16: Photomicrograph of pineal gland of balady goat, revealing negative raction for glycogen in all parenchymal cells, as also - ve reaction in the capsule. Best's carmine method, X 100.

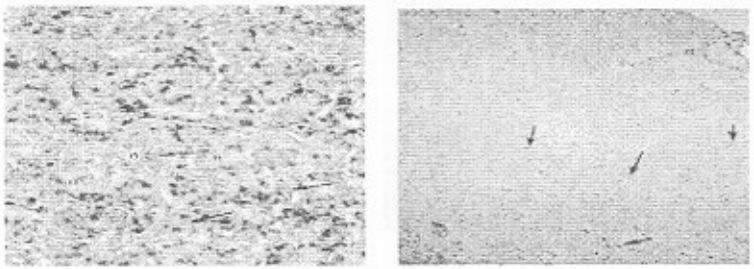

Fig.17: Photomicrograph of pineal gland of balady goat revealing strong succinate dehydrogenase SHD) in parenchymal cells, mainly the pinealocytes, but negative in septae, and cavities of apoptotic regions (arrows). NBT method, X 100.

Fig.18: Photomicrograph of pineal gland of balady goat revealing an intense reaction of SDH in some pinealocytes (arrows) but strong in others (o) and negative in apoptotic cavites (c). NBT method, X 400.

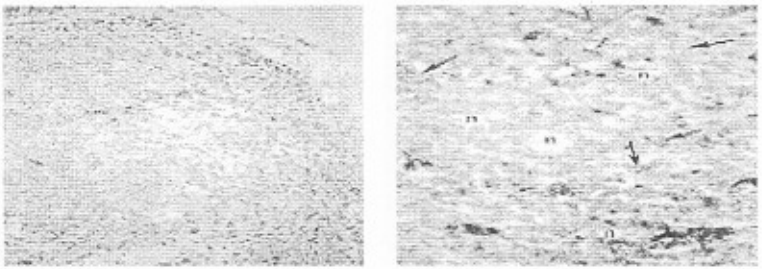

Fig.19: Photomicrograph of pineal gland of balady goat revealing slight reaction of ATP-ase in pinealocytes and negative reaction in septae and blood vessels. Lead method, X 100.

Fig.20: Photomicrograph of pineal gland of balady goat revealing moderate reaction for 5-Nucleotidase in pinealocytes Arrows), negative in blood vessels and septae (n). Lead method, X. 100.

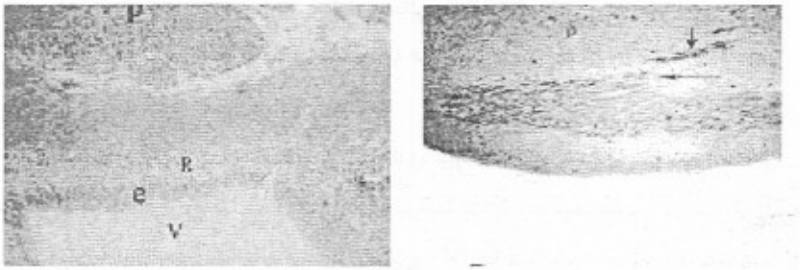

Fig.21: Photomicrograph of pineal gland of balady goat revealing traces of lipids in pinealocytes (arrows), no reaction in the apoptotic cavities of the central region of the gland (n). Sudan black B method, X 100.

Fig.22: Photomicrograph of pineal gland of balady goat revealing moderate reaction of non-specific esterase (arrows), negative in other cells (n). alpha-naphthyl acetate method, X. 100.

دراسات نسيجية ونسجوكيميائية للغدة الصنوبرية في الماعز البلدي

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تم استخدام ثلاثين غدة صنوبرية من ماعز بلدي تراوحت أعمارها بين ٢-٣ سنوات, حيث تم تثبيت العينات في مثبتات خاصة أو وضعها في الأزوت السائل لدراسة الانزيمات المختلفة وتم تحضير قطاعات بسمك ٦ ميكرون لغير الانزيمات والدهون وقطاعات بسمك ١٠ ميكرون للانزيمات والدهون وقد أظهرت النتائج ما يلي :

١- كانت الغده الصنوبرية موجودة أعلي البطين الثالث للمخ أمام الأكيمات العلوية بطول ٥-٧ ملليمترات وعرض ٤ ملليمترات وسمك ٣ ملليمترات, وقد أحيطت الغدة بحافظة بها فراغات تحتية في بعض المناطق وامتدت من المحفظة حواجز قسمت الغدة الي قصيصات تحتوي علي مجموعات من الخلايا المتنية أما سدي الغدة فكان غنيا بشبكة من الشعيرات الدموية , وقد احتوت الحواجز علي ألياف كلاجينية وشبكية , ولكن اختفت الألياف المرنة كانت الألياف العصبية قليلة ما عدا قرب البطين الثالث , حيث توجهت حزم عصبية من هذه المنطقة للدخول في الغدة من أسفل .

الخلايا المتنية كانت كما يلي :

القليل من الخلايا العصبية , خلايا صنوبرية داكنة ذات أنوية غير مركزية , خلايا صنوبرية باهتة ذات أنوية مركزية , وكانت بعض الخلايا مزدوجة الأنوية ثم خلايا داعمة تمثلت في خلايا نجمية كانت متلازمة مع الخلايا الصنوبرية الباهتة ثم أخيرا خلايا دبقية دقيقة .

أمكن توضيح بعض المناطق كفجوات نتيجة موت مبرمج لبعض الخلايا من الغدة .

كان التفاعل لحمض البيرأيودك شف قويا في الحواجز وبعض الخلايا الصنوبرية . بالنسبة للدهون والانزيمات في الخلايا الصنوبرية فقد أظهرت الخلايا أثارقليلة لتفاعل للدهون وكان التفاعل لانزيم السكسنك ديهيدروجينيز قويا أما التفاعل بالنسبة لانزيم ثلاثي الفوسفات الأدينوزيني بسيطا , وكذلك بالنسبة لانزيم ٥- نيوكليوتيداز , بعض خلايا السدي أظهرت تفاعلا بسيطا للألشيان الأزرق.

تم تسجيل النتائج الأخري ومناقشتها .