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PHYSIOLOGICAL STUDY OF THE FIBRE COMPONENT OF ELN INNERVATING THYROID GLAND

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ABSTRACT

Many studies in anatomical literatures reported that the external laryngeal nerve (ELN), one of the superior laryngeal branches of the superior laryngeal nerve (SLN) is not a purely motor nerve as was thought earlier. Some anatomists have established that the ELN innervates the thyroid gland through a branch. Sun and Chang, 1991 reported thatthe thyroid gland receives branches from the ELN which may be sympathetic in nature. In a recent study, Vijesh and Sun, 2012 proved that ELN is a mixed nerve and prove the thyroid glandular branch is postganglionic sympathetic in nature, which may play an important function in vasomotor and secretory functions of thyroid gland. This present study aims to understand the function of the branch of ELN innervating the thyroid gland. This study is unique in itself since no such study has been reported till now .Surgical procedures have been conducted by making a cut between the thyroid gland and the branch of the ELN that innervates the upper pole of the thyroid gland in dogs. Three experimental groups A, B and C were formed. Every group had a dog used as sham. Thyroid glands were collected from the dogs after 4, 6 and 8 days post surgeries. Blood samples were collected both before and after surgery from all groups. Some thyroid glands showed the presence of colloid goiter with single cuboidal or flattened epithelial cells surrounding the follicles. Follicles of variable sizes and of colloid material in the interfollicullar spaces were seen in group A. Group B and C showed similar kind of follicles along with different kinds of colloids. Group B and C showed significant increase in the TSH and T4. This result suggests that the branch of ELN innervating the thyroid gland may be sympathetic nature.

KEYWORDS : External laryngeal nerve, thyroid gland, gelatinous colloid, TSH, T4

The ELN is described as a small branch of the superior laryngeal nerve (SLN) that innervates the cricothyroid muscle, the inferior constrictor, and pharyngeal plexus and has connections with the superior cardiac nerve, cervical sympathetic ganglion and the Thyroid gland. The ELN supplies the thyroarytenoid muscle was reported by Kochilas et al., 2008 and Nasri et al., 1997 to the subglottis mucosa by Kochilas et al., 2008, Adazu and Wyke, 1979 and Maranillo et al., 2003, to the pharyngeal plexus by Rueger, 1972, to the cricothyroid joint by Kochilas et al., 2008, Maranillo et al., 2003 and to membrane by Durham and Harrison, 1964 and Maranillo et al., 2003. Nonidez, 1931 in one of his studies concluded that the thyroid gland of dog received innervations from the superior cervical, sympathetic ganglion (SCSG) and SLN. Presence of small twigs emanating from the ELN which coursed downwards towards the thyroid gland along with branches of the thyroid artery to reach the thyroid gland was reported by Moosman and Weese, 1968. In fluorescence histochemical study conducted by Hisa, 1982 presence of noradrenergic nerve fibers carried by the superior and external laryngeal nerves was demonstrated. Sasaki et al., 1999 reported absence of a sympathetic or external superior laryngeal nerve contribution in cricopharyngeus muscle. A recent case report by Kochilas et al., 2008, mentioned that the ELN was found to give off branches that pierced the upper pole of the thyroid gland. Thyroid gland, one of the largestendocrine gland is located on the anterior side of the neck. Sun and dong, 1997 in their study described SLN as a variety of anatomical configurations of its ELN branch which may need to be considered during surgery. Furlan, 2002 in his research paper confirmed Sun and Chang findings Durham and Harrison, 1964 and Maranillo et al., 2003 reported that the changes in the quality of voice resulting from the ELN injury may be transient or persistent. The principal function of thyroid gland is production of triiodothyronine (T3), thyroxin (T4), and calcitonin hormones. The production of thesehormones is regulated by TSH (thyroid stimulating hormone) which is released by anterior pituitary gland (gray's).

Our current study aims to establish the function of ELN and its role in the functioning of thyroid gland.

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MATERIALS AND METHODS

Surgery of Thyroid gland in dogs: 15 live healthy dogs were obtained from the animal department of Chongqing medical university and were divided into 3 groups named as A, B and C. Each groups had 5 dogs. One dog in every group was kept as sham dog and other 4 as experimental dogs. Experiment was conducted on all three groups on the same day. All the 15 dogs of were injected with 2.5% sodium pentobarbital at a dose of 1.2 ml/kg body weight. 5ml of fresh blood was drawn from the long saphenous vein of the dogs and was marked as d1 (day 1). The thyroid glands were exposed along with SLN, ELN and the corresponding loops by careful dissection under sterile conditions. The Laryngeal nerves along with their branches, the Superior cervical ganglion and the ganglion nodosum were exposed. The communications between the cervical sympathetic trunk and the SLN were identified as shown in figure 1 (a) & (b.). The connection between the thyroid gland and the branch of the ELN that supplies the upper pole of the thyroid gland was identified and cut in the experimental dogs and was kept intact in thesham dog. The neck of the dogs was sutured back. Four days later, a blood sample was collected from all the 5 dogs in group A and was marked as d5 (day 5). This time the dogs were sacrificed and once again the neck of the dogs was opened to collect the thyroid gland of the dogs for histological studies. The blood samples collected before and after surgery were subject to hormonal assay. The same protocol was carried out on group B and group C dogs. Blood samples and thyroid glands of group B dogs were collected after 6 days and marked as d7 (day 7) and for group C dogs the same was done after 8 days and marked as d9(day 9) after surgeries respectively.

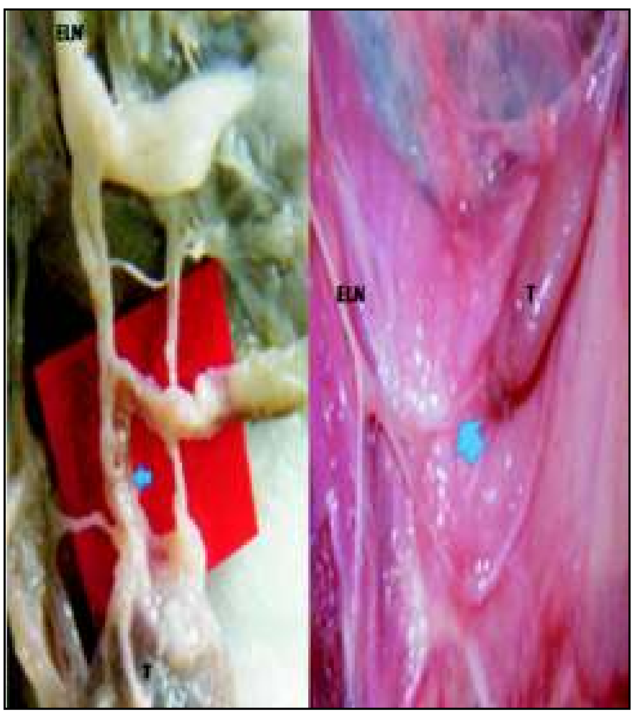


Figure 1 (a) **Figure 1 (b)**

⇦ Points the glandular branch of ELN supplying the upper pole of the thyroid gland & T indicates Thyroid gland

Figure 1 (a & b) : Branch of ELN Innervating the Upper Pole of the Thyroid Gland Exposed in Live Dog During the Surgery

Histological Analysis

HE staining was performed to study the histopathological changes in the thyroid glands of all groups after surgery.

Physiological Analysis

Elisa was performed to assess the T4 level before and after the surgery of all groups. The Canine Elisa kit for T4 was purchased from R&D (shanghai), China.

Manufacturer's procedure was followed to perform the assay. TSH assay was done by performing RIA. The kit was purchased from cisbiobioassays (France) and manufacturer's protocol was followed to conduct the study

Statistical Analysis

All data were presented as the mean±SEM. Statistical analysis was performed with repeated measures analysis of variance, paired T test and independent sample t test by using SAS8.0 for Windows. A P-value <0.05 was considered statistically significant.

Dogs from all groups were fed with food with adequate amount of iodine salt both before and after the surgery.

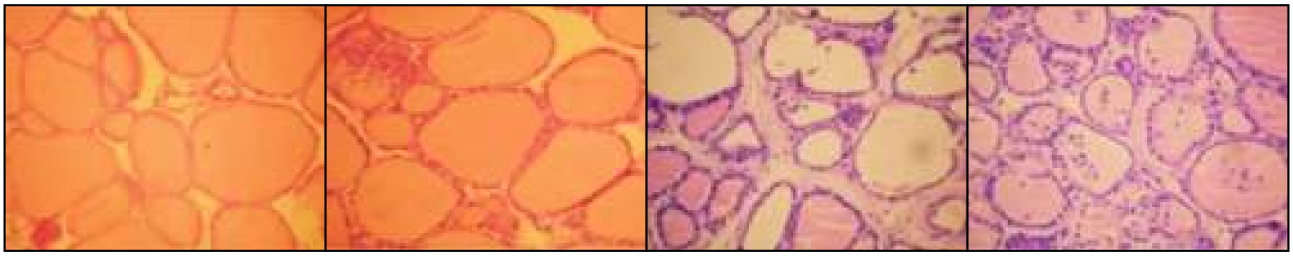
All the experimental protocol was approved by the Animal Ethical Committee of Chongqing Medical University.

RESULTS

Microscopic examination of the thyroid glands of three different groups showed the following observations:

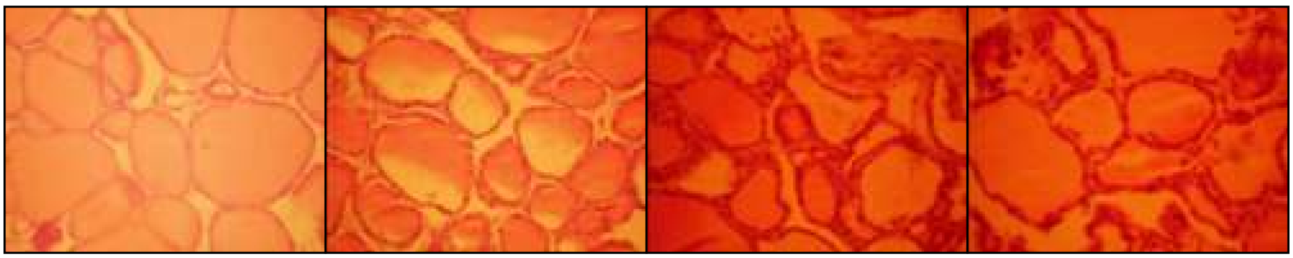
Group A

Unlike normal and sham group thyroid gland, the



2(a) 2(b) 2(c) 2(d)

Figure 2 : (a) & (b) Showing Normal Thyroid Gland and Thyroid Gland of Sham Dog of Group A Respectively. (c) & (d) Showing Thyroid Gland of Experimental Dogs of Group A. (Day 5, Four Days After Surgery) Presence of Large, Some Medium and Small Sized Follicles are Seen. Epithelial Cells are Detached From the Folliclesand are Lined With Single Layer of Either Cuboidal or Flattened Epithelial Cells. Colloid Materials in Some of the Interfollicular Spaces are Observed. Magnification100x



3(a) 3(b) 3(c) 3(d)

Figure 3 : (a) & (b) Showing Normal Thyroid Gland and Thyroid Gland of Sham Dog of Group B respectively. (c) & (d) Showing Thyroid Gland of Experimental Dogs of Group B. (Day 7, Six Days After Surgery) Colloid Goiter Present. Peripheral Vacuolation and Shred Type With Clear Areas in the Follicle is Seen. Cuboidal or Flattened Follicles With Widened Intercellular Space in the Epithelial Cells is Observed. The Gelatinous Colloid is Absorbed by the Epithelium in Most of the Follicles. Enlargement of Some Follicles Filled With Gelatinous Colloid While Decreases in Size of a Few Follicular Cells are Noticed. Magnification100x

thyroid gland of group A showed presence of variable sized follicles. Some of which were large, some medium and some were small in size. Detachment of the epithelial cells from the follicles was distinct. Follicles lined with single layer of either cuboidal or flattened epithelial cells were present. In some, presence of colloid materials in the interfollicular spaces was observed figure 2, (a), (b), (c) & (d).

Group B

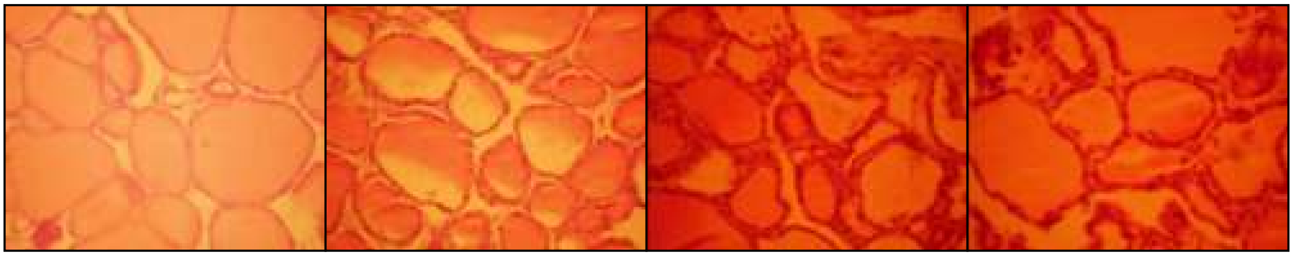
Presence of colloid goiter was evident. Some of the colloids were empty and some reabsorbing type epithelial cells were also present. Some of which had peripheral vacuolation and some were shred type with clear areas in the follicle are mostly cuboidal or flattened with widened intercellular space in the epithelial cells. The gelatinous colloid is absorbed by the epithelium by most of the follicles. Enlargement of some follicles filled with gelatinous colloid and decreased in size of a few follicular cell were noticed. These findings were very different from that of normal and sham group thyroid glands.figure 3(a), 3(b), 3(c) & (d).

Group C

Distinct shrinkage of the follicular cells and increase in interfollicular space was evident. Presence of follicles filled with gelatinous colloid was observed. These features were distinctly different from that of normal and sham group thyroid gland. figure 4(a), 4(b), 4(c) & 4(d).

Assay of Endocrine Function

A significant increase in the level of TSH and T4 was observed between d1 and d7 in groupB and between d1



4(a) 4(b) 4(c) 4(d)

Fig 4- (a) & (b) Showing Normal Thyroid Gland and Thyroid Gland of Sham Dog of Group C Respectively. (c) & (d) Showing Thyroid Gland of Experimental Dogs of Group C. (Day 9, Eight Days After Surgery). Shrinkage of the Follicular Cells With Increase in Interfollicular Space is Noticed. Numerous Follicles Filled With Gelatinous Colloid are Observed. Magnification100x

Table1: Value of T4 (µg/L) in All Three Groups Before and After Surgery

Days	Sham Group	Experimental Group
d1 (all groups)	0.66	0.60
d5 (Group A)	0.63	0.53
d7 (Group B)	0.62	0.49
d9 (Group C)	0.67	0.45

Table 2: Value of TSH (µg/L) in All Three Groups Before and After Surgery

Days	Sham Group	Experimental Group
d1 (all groups)	0.12	0.14
d5 (Group A)	0.14	0.22
d7 (Group B)	0.11	0.25
d9 (Group C)	0.11	0.26

and d9 in group C, whereas no significant difference was observed in the TSH and T4 level between d1 and d5 in group A. The values are presented in table 1 and table 2.

DISCUSSION

Gray's Anatomy states that the nerves to the thyroid gland derive from the superior, middle and inferior cervical ganglia of sympathetic innervations. In Morris Human Anatomy, the vasomotor and secretory nerve supply of the thyroid gland is said to be consisted of unmyelinated fibres from the middle and inferior cervical ganglia of the sympathetic system. Nonidez (1931) in his study reported that thyroid gland comes from the superior cervical ganglion and the ELN, the ELN branches being called thyroid nerve and most of which are sympathetic. Shornicki et al. (1968), in a study observed that the ELN gave off branches reaching the thyroid in 25 dogs and 12 human bodies. Moosam (1968) reported of some small twigs from the ELN appearing parasympathetic in function reaching the thyroid gland along with the branches of thyroid artery. However in Sun and Chang's (1991) study, it has been reported that the ELN was not purely a motor nerve, but also carried sympathetic fibers that it derived from a communicating branch from the superior cervical sympathetic ganglion/ cervical sympathetic chain. Vijesh and Sun, 2012 proved that e ELN is a mixed nerve and also prove that the thyroid glandular branch is postganglionic sympathetic in nature, which may play an important function in vasomotor and secretory functions of thyroid gland.

Our present study targets and reports of the physiological and histological proof of the finding that ELN branches off to innervate the thyroid gland, which may be sympathetic in nature. Thyroid gland is the production of triiodothyronine (T3) and Thyroxin (T4).The thyroid gland is controlled by the thyroid stimulating hormone (TSH) produced by pituitary gland and thyrotropin releasing hormone (TRH) produced by hypothalamus (gray's).In this present study the histopathological changes observed in the thyroid gland after surgery are similar to that observed by shelke et al., 2009 in thyroid gland of buffaloes, in parenchymatous goiter by Vashistha and Dwivedi, 1975 and also by Verlekar, 1999 in his own communication on buffaloes. Sastry (1983) reported that colloid goiter may

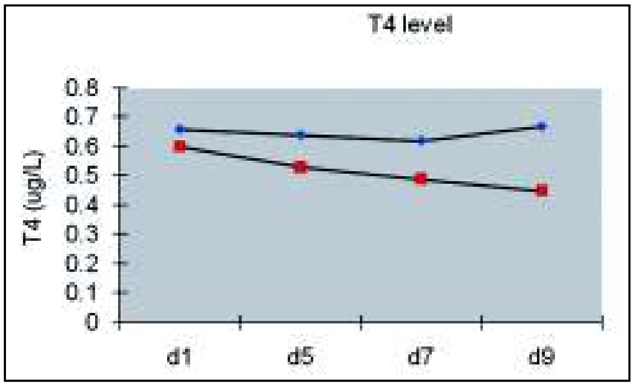


Figure 5 (a): Graphical Representation of assay of T4 Level Before and After Surgery

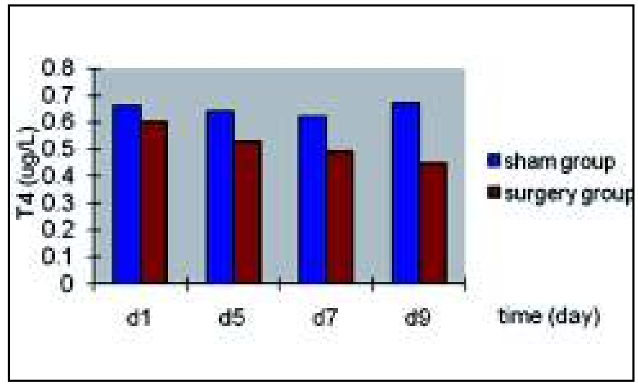


Figure 5 (b): Assay of T4 Level Before and After Surgery Depicted in Bar Diagram

d1 showing samples from all groups on day one, d5 = Group A, d7 = Group B & d9 = group C. Multiple comparisons between groups were done by HSD method. For d1 and Group A, the between the sham and experimental dogs was not significant with p = 0.6019> 0.05 and p = 0.4003> 0.05 respectively, whereas, for Group B and group C a significant difference between the sham and experimental dog were found at p = 0.0336 <0.05 and p = 0.01071 <0.05 respectively

Within groups: using paired T test, within sham dogs: t=-0.47 p=0.6501>0.05,no difference among d1,Group A, Group B and Group C was noticed
Within experimental dogs: t=-1.72 p=0.0473<0.05,there are significant differences between day1 and group A; t=-1.02 p=0.0221<0.05,there were significant differences between day1 and
Group B; t=-3.64 p=0.0105<0.05, there are significant differences between day1 and Group C

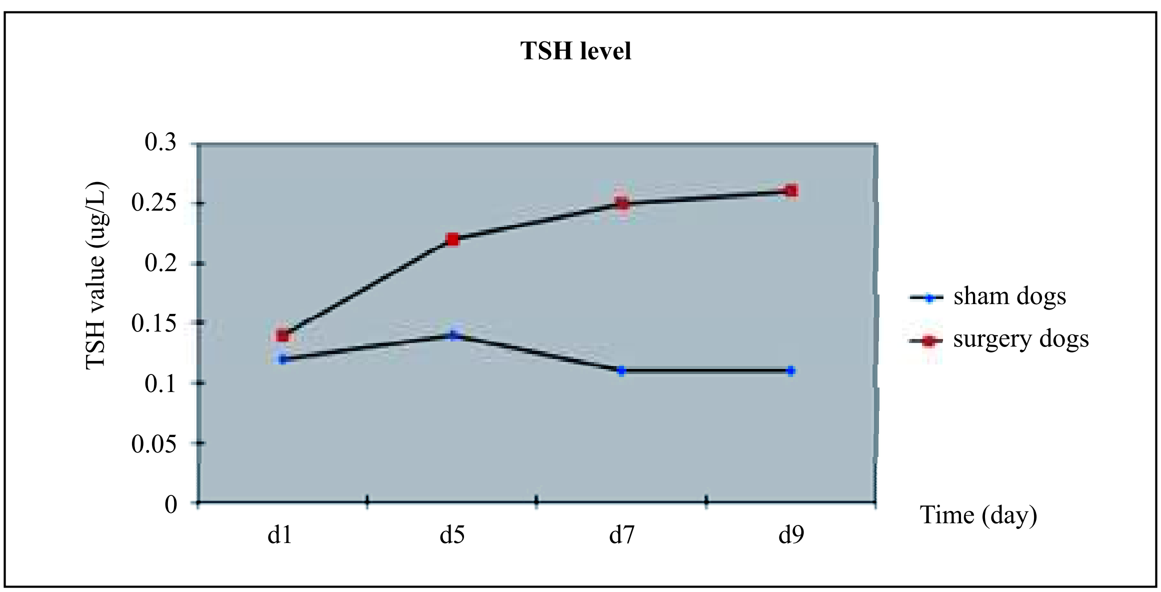


Figure 6 (a) : Graphical Representation of Assay of TSH Level Before and After Surgery

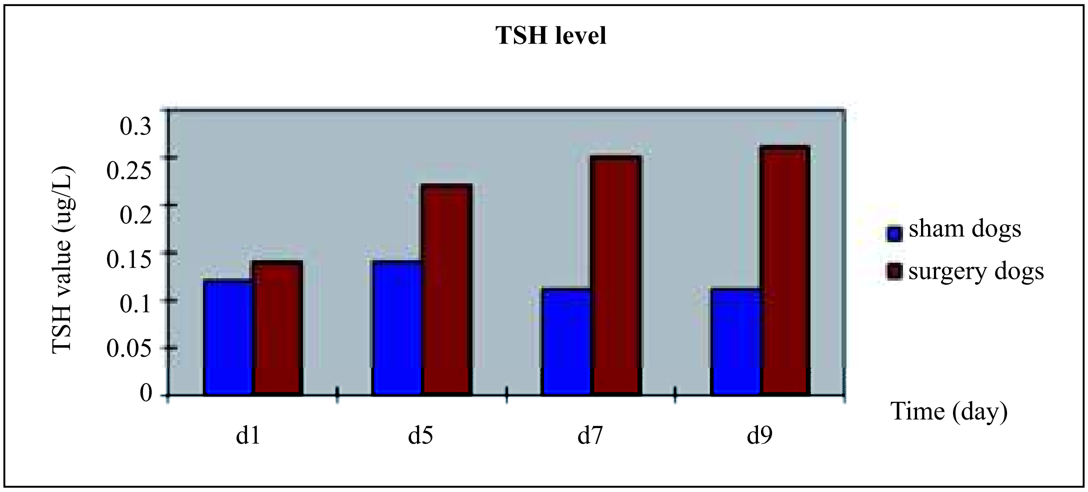


Figure 6 (b): Analysis of TSH Level After Surgery by a Bar Diagram

d1 showing samples from all groups on day one,d5 = Group A, d7 = Group B & d9 = group C.Multiple comparisons between groups were done by HSD method. For d1 and Group A, the between the sham and experimental dogs was not significant with p = 0.7462> 0.05 and p = 0.0201 <0.05 respectively, whereas, for Group B and group C a significant difference between the sham an experimental dog were found at p = 0.0093 <0.05 and p = 0.0017 <0.05 respectively.

within groups: using paired T test,
within sham dogs: t=-0.52 p=0.5977>0.05,no differences between surgery time
within experimental dogs: t=-6.31 p=0.0031<0.05,there are significant differences between day1 and Group A; t=-7.42 p=0.0026<0.05,there are significant differences between day1 and Group B ;t=-7.97 p=0.0013<0.05,there are significant differences between day1 and Group C

occurs due to low levels of iodine in soil and water, increased demand during pregnancy, diseased conditions and ingestion of goitrogenous substances like thiouracil, soybean, cabbage etc. Since in this present study all groups were provided with iodinated food, the histopathological and pathophysiological changes observed which are consistent with hypothyroidism is due to the cut made in the connection between the thyroid gland and the branch of the ELN that supplies the upper pole of the thyroid gland in the dogs. Probably the surgery inhibited the sympathetic control of ELN on thyroid gland. Owing to low iodine level an increase in TSH secretion from pituitary gland took place thus resulting in increase in T4 level. This compensatory increase led to the hypertrophy followed by hyperplasia of thyroid epithelium. This finding is similar to Jubb & Kennedy, 1985 where they opined that inadequate amount of iodine available for organic synthesis of thyroid gland was reported to be the major cause of such condition. They further added that due to this output of thyroid hormone is reduced and compensatory increase in amount of TSH released from pituitary gland hypertrophy resulting in the hypertrophy followed by hyperplasia of thyroid epithelium. In addition to this increase in amount of tissue capable of thyroxin synthesis, the thyrotrophic hormone increase the efficiency of thyroid in trapping iodine and in the absence of inhibitory influence of synthesizing thyroxin leads to parenchymatous goiter occurs. All the above observations clearly indicate that the innervations to the thyroid gland from the ELN may be sympathetic in nature.

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