Histological changes of the submandibular Salirary gland of mice maintained on a liquid diet

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Abstract
The purpose of this study was to examine the histological alterations that occurred in the submandibular glands of mice fed a liquid diet compared to a solid diet. Thirty-six mice were randomly divided into two groups. The control group received a solid diet, and the experimental group received a liquid diet. The mice were killed after 21 days. The glands were prepared in paraffin and analyzed with a light microscope. The results showed a statistically significant reduction of the submandibular gland acini of the mice from the experimental group compared to the control group, in which the diameter of serous acini in experimental group was (25.2), mucous acini (30.6), while for the control group the diameter of serous acini was (35.24), mucous acini (39.55). There was no significant reduction in the diameter and volume of both the straited ducts and the interstitial connective tissue.
Key Words: submandibular gland, salivary gland, diet, light microscopy.

Introduction
The term salivary glands should be taken to include any tissue that normally discharges a secretory product into the oral cavity, the functions of such secretion which is called saliva, are to moisten the mucous membrane of the upper digestive tract, to facilitate speech, to control the bacterial flora of the mouth, and to prepare food for digestion, the only mammalian saliva known to be toxic is that of the American short tailed shrew [1, 2].

Saliva is a complex fluid produced by a number of specialized glands, most of which is produced by the major salivary glands: parotid, sublingual and submandibular, but small contribution is made by the numerous small labial, buccal and palatal glands located in the mouth, in general saliva is produced in amount of 1000-1500ml per day [3, 4], the relative contribution of the major salivary glands is as follows:

- Submandibular gland 69%
- Parotid gland 26%
- Sublingual gland 5% or less.[5]

Many factors must be considered in the diet such as its texture, taste and consistence [6, 7]. According with Edgar and Jenkins [8] administration of diets requiring reduced or increased mastication of rats leads to atrophy or hypertrophy, respectively, of their salivary glands. Johnson and Sreebny [9] observed that when rats were fed with hard chow the weight, enzymatic content and protein synthesis of the parotid glands increased.

Scott and Gunn found atrophy of acini in the major salivary glands of rats fed a liquid diet [10]. On the other hand, Soraya and Orlando [11] analyzed the alterations caused by liquid diet on rat parotid glands and concluded that gland weight was reduced approximately 35% in rats on a liquid diet compared to control mice.

As far as we know, there are limited studies in the literature conducted in humans. The effects of nutrition and diet should be assessed in terms of flow secretion and saliva composition. Nevertheless, the findings in humans are markedly similar to the results of animal studies. Therefore, knowledge from the animal model is helpful for the understanding of the cellular gland alterations, as well as their influence on saliva composition [12].

The purpose of this study was to examine the histological alterations that occurred in the submandibular gland of the mice maintained on a liquid diet.

Materials and Methods
Thirty-six male albino mice with a mean weight of (26) gram were randomly divided into two groups. The control group was fed a solid diet and water. The mice of the experimental group were subjugated on a liquid diet. The liquid diet was prepared daily by mixing one part of solid bread (20 gm) and 5 parts of distilled water (100 ml). The mixture was blended for 3 minutes in the blender and offered to the animals.

After 21 days, control and experimental mice were killed by ether application, the submandibular gland from each side was carefully dissected intact, for histological studies; the glands were placed in 10% buffered formalin and processed by conventional methods for embedding in paraffin. Six-micrometer sections were obtained and stained with hematoxylin and eosin (H&E) for light microscopy evaluation.

The diameter of acini and other structures of both control and experimental groups were measured using calibrated ocular lens, fifteen observations were made for measuring the diameter of acini, each observation includes two measurements of the diameter of each acini then the mean of these two measurements is brought to the diameter of the acini.

Another fifteen observations were made to count the number of serous, and mucous acini per microscopic field at (40x) power, in both control and experimental groups. Statistical analysis significance was evaluated by *t*-test.

Results
The mice of both groups appeared to be healthy during the experimental period, the control group had a histological structure described as normal for the submandibular gland, sections stained with H&E revealed a mixed appearance of both mucous acini and serous acini, these two types of acini appear as separated groups of its own type as shown in Figures (1a, & 1b), in which the serous acini are rounded, their serous cells tend to be pyramidal in shape the nucleus is prominent, rounded and located in the basal third of the cell, the cytoplasm is granular appearance.

The mucous acini are rounded, in which the mucous cell is pyramidal in shape of flattened basal nucleus; mucous cells are larger than serous cells, the apical portion of the cell stains weakly with H&E stain, which indicates the mucous carbohydrate content of the cell as shown in figures (1a, &1b).

The histological appearance for the submandibular gland from mice on a liquid diet was similar to the submandibular gland of the control group, but serous acini and mucous acini suffer from atrophy as shown in figure (2a, & 2b).

Table (1) shows the mean of diameter of serous acini in both control and experimental groups, in which the diameter of acini of control group was(35.24), and the diameter of acini of experimental group was(25.2), which was a significant reduction in diameter of acini between the two groups.

Table(2) reveals the difference in the diameter of mucous acini in the two groups, which was in the control group (39.55), and in the experimental group(30.6) that was a significant decrease in the diameter of mucous acini.

Table(3)describes the number of serous and mucous acini per microscopic field, in which number of serous acini in the control group was (42.13), and in the experimental group was (39.9±10.2), while the number of mucous acini in the control group was(33.4±6.2), and in the experimental group was (30.46).

The striated ducts appeared normal in both control and experimental groups however there is a slight reduction in its diameter in the experimental group which was (28), while it was in the control group was (30.12), this reduction was not significant, as shown in Table (4).

Table(5)clarifies the alterations in the thickness of interstitial connective tissue between the two groups, the thickness in the control group was (6.11), and the thickness in the experimental group was (5), although this difference was not significant.

The experimental group shows little amount of adipose tissue infiltration in comparing with very little infiltration of adipose tissue in control group, as revealed in figure (3), also there was no vaculation in the cytoplasm of serous cells of the experimental group .

Discussion

The liquid diet induces acinar atrophy but other parenchymal components were apparently unaffected. The reductions in acinar diameters suggest that most of glandular atrophy after liquid deit feeding is due to acinar cell shrinkage rather than to losses of acinar cell number in the salivary gland, as reported in a study done by Scott and Gunn [13].

The cause of acinar atrophy is greatly linked to the loss of masticatory reflex stimulation[14]since submandibular gland is situated and surrounded by muscular coats by the platysma muscle superficially, rests on mylohyoid muscle and the deep lobe of the gland is located between the mylohyoid muscle laterally and hyoglossus muscle medially[15], all these muscles are included and assisted in the process of mastication and tongue movement.

In addition, it was reported that chewing stimuli and jaw movement during mastication evoke up the reflex of salivary flow rate to ten times than in resting state, which simultaneously induces masticatory muscles contractions[14].

This type of atrophy is termed as (physiologic or functional atrophy) which is reversible after removal of the causative agent [16].

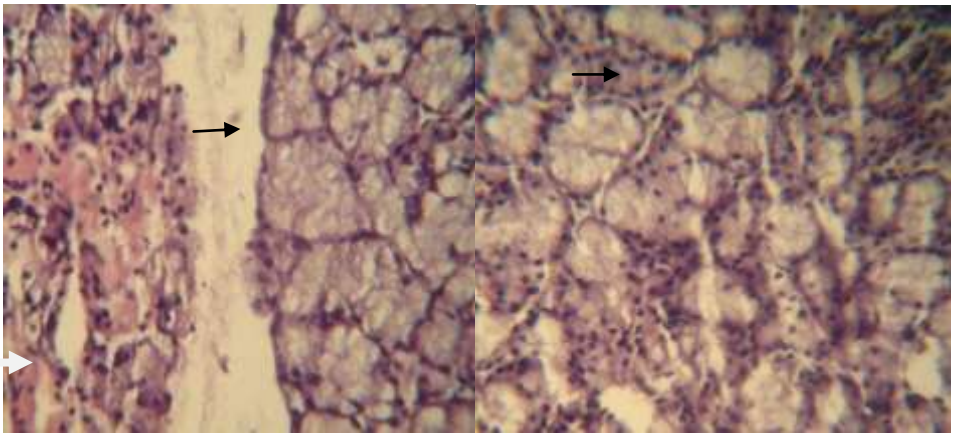
The atrophy of serous acini in this study is agreed with the study done by Scott and Gunn [10], the reduction in the size of serous acini, striated ducts, and interstitial tissue may be supported by other studies that reported reduction in weight of parotid gland in rats fed liquid deit after a period of fifteen days [11].

There were no vacuoles in the cytoplasm; this finding is not coordinated with study worked by Soraya and Orlando [11], which may be related to the high fluid consistency given to the mice in comparing to that given in reported study.

Hand and Ho[17] reported the presence of lipids in atrophic acinar cells; however this hypothesis was found to a slight degree in this study, and agreed with Walter at al study [16].

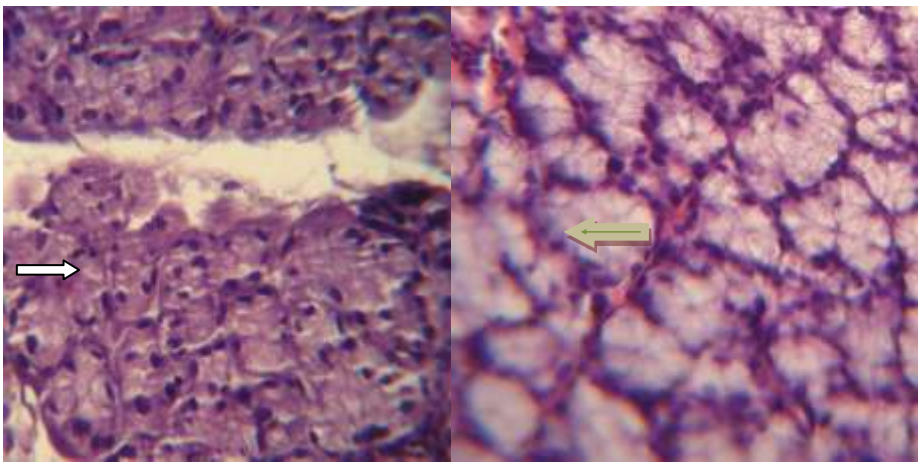
Conclusion

From the results of the present study, it can be concluded that; the diameter of serous acini, and mucous acini of submandibular salivary gland is affected by the consistency of diet when it contains a high liquid contents by decreasing its diameter.



(a) (b)

Figure (1): Shows submandibular gland of the control group (black arrow points on mucous acini, and white arrow points on serous acini).(40X)



(a) (b)

Figure (2): Reveals tissue of submandibular tissue of experimental group (green arrow points on mucous acini, white arrow points on serous acini) (40X).

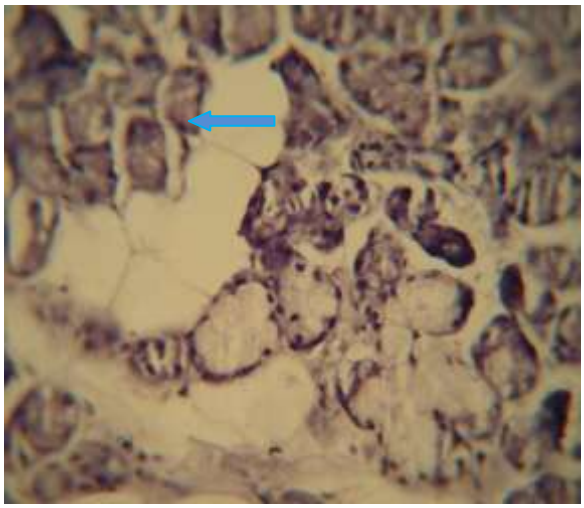


Figure (3): Shows serous and mucous acini with the infilteration of adipose cells in the experimental group (blue arrow points on adipose cell) (4ox).

Table (1): Shows diameter of serous acini in control, and experimental group expressed in Micrometers (µm).

	Control group	Experimental group
Diameter of acini	35.24±5.5	25.2±6.72

Table (2): Shows diameter of mucous acini in control, and experimental group expressed in Micrometers (µm).

	Control group	Experimental group
Diameter of acini	39.55±4.32	30.6±7.36

Table (3): describes the number of serous and mucous acini in both control and experimental groups.

	Control group	Experimental group
Number of srous acini	42.13±10.8	39.9±10.2
Number of mucous acini	33.4±6.2	30.46±8.7

Table (3): Reveals the diameter of striated ducts in control & experimental groups, expressed in micrometers (µm).

	Control group	Experimental group
Diameter of straited duct	30.12±4.4	28±5.02

Table (4): Clarifies thickness of interstitial connective tissue, in control and experimental groups expressed in Micrometers (µm).

	Control group	Experimental group
Thickness of interstitial C.T	6.11±1.45	5±2.69

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التغيرات النسيجية للغدد اللعابية تحت الفكية للفئران المتغذية على السوائل

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الملخص

الهدف من هذه الدراسة هو ملاحظة التغيرات التي تحدث على الغدد اللعابية تحت الفكية للفئران التي غذيت بالسوائل مقارنة بالفئران التي غذيت بالغذاء الصلب. ست وثلاثين فأراً تمّ تقسيمهم عشوائيا الى مجموعتين، مجموعة السيطرة غذيت بالطعام الصلب ومجموعة التجربة غذيت بالسوائل، تمّ قتل الفئران بعد مرور واحد وعشرون يوما واستصلت الغدد تحت الفكية وقطعت نسيجيا لفحصها بالمجهر الضوئي. وأظهرت النتائج نقص اقطار العنيبات المصلية والمخاطية نقصانا معنويا في مجموعة التجربة بالمقارنة مع مجموعة السيطرة . حيث كانت أقطار العنيبات المصلية لمجموعة التجربة(٢٥,٢) وأقطار العنيبات المخاطية لمجموعة التجربة (٣٠,٦) بينما كانت أقطار العنيبات المصلية لمجموعة السيطرة (٣٥,٢٤) و أقطار العنيبات المخاطية (٣٩,٥٥). ولم يكن هنالك نقصانا معنويا بالنسبة لأقطار وأحجام القنوات المخططة والنسيج الرابط البيني.