Histological changes of the parotid gland of mice maintained on a liquid diet

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Abstract
The purpose of this study was to recognize the histological alterations that occurred in the parotid 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 for histological technique in paraffin wax and examined by a light microscope. The results showed a statistically significant reduction of the parotid gland acini diameter of the mice from the experimental group compared to the control group, in which the diameter of serous acini in experimental group was (22.2) and in the control group was (29.13), there was no significant reduction in the diameter of striated ducts, and no significant reduction in interstitial connective tissue. The conclusion was the reduction in diameter of serous acini is greatly related to the decrease in the need of masticatory function.

Key Words: parotid 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 60%
- Parotid gland 30%
- 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.

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 parotid gland of the mice maintained on a liquid diet.

Materials And Methods
Thirty-six male albino mice with a mean weight of (25) gram were randomly divided into two groups. The control group was fed a solid diet and water; the mice of the experimental group were fed 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 mice daily.

After 21 days, control and experimental mice were killed by decapitation. The parotid 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 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 acini per microscopic field at (40x) power, in both the control and experimental group. Statistical analysis significance was evaluated by the Student *t*-test.

Results
The mice of both groups appeared to be healthy during the experimental period, along the experimental time the control group had a histological structure described as normal for the parotid gland, sections stained with H&E revealed serous acini, each serous acini consists of number of serous cells, in which the serous cells tend to be pyramidal in shape, with rounded prominent spherical nucleus, located either in the center or basal part of the cell, some sections show very small quantities of adipose cells as shown in figures (1) & (2).

The histological appearance for the parotid gland from mice on a liquid diet was similar to the parotid gland of the control group, but serous acini were atrophied, table

(1) shows the mean of diameter of acini in both control and experimental groups, in which the diameter of acini of control group was (29.13µm), and the diameter of acini of experimental group was (22.2µm), which was a statistically significant reduction in diameter of acini between the two groups.

Table (2) reveals the number of serous acini in both control and experimental groups, in which in control group was (46.31±11.4) while in experimental group was (40.26±10.3), however the difference between the two values was not significant.

The striated ducts appeared to be normal in both control and experimental groups however there is a slight reduction in its diameter in the experimental group which was (32.44µm), while it was in the control group was (33.3µm), this reduction was not significant, as shown in Table (3).

Table (4) reveals the alterations in the thickness of interstitial connective tissue between the two groups, the thickness in the control group was (11.4µ), and the thickness in the experimental group was (9.46), although this difference was not significant.

The experimental group shows small quantities of adipose tissue infiltration in the parenchyma as shown in figure (3), comparing with very little infiltration of adipose tissue in control group as previously shown in figure (1)& (2). Also there was no vaculation in the cytoplasm of serous cells of the experimental group.

Discussion

The liquid deit induced 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 parotid gland [(13)].

The cause of this acinar atrophy is greatly linked to the loss of masticatory reflex stimulation [(14)]since parotid gland is situated behind the ramus of the mandible in close relation to the lateral, medial pterygoid and masseter muscles (masticatory muscles) [(15)], 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 [(14)].

The present study did not evaluate the relation of saliva and caries, the importance of saliva in the caries process is well known [(4)]. Muñiz et al [(7)] reported that foods that require considerable mastication might induce a higher salivary flow rate via local reflex. Thus, the increased salivary flow rate, the increase in saliva and consequently in pH of dental plaque and the components of saliva may be major factors in the prevention of caries [(4)].

Conclusion

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

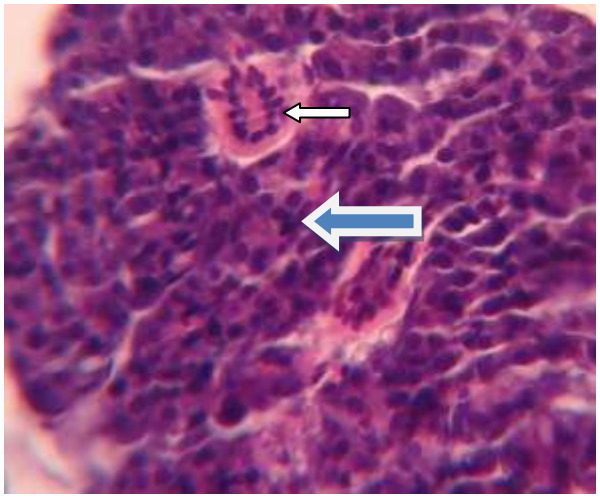

Figure (1): Shows parotid gland structure, serous acini (blue arrow), striated ducts (white arrow), interstitial connective tissue (white arrow head) (40x)

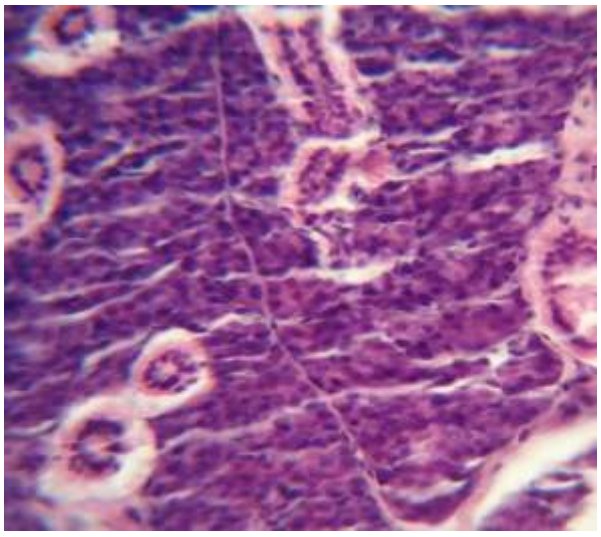

Figure (2): Reveals parotid tissue of experimental group. (40x)

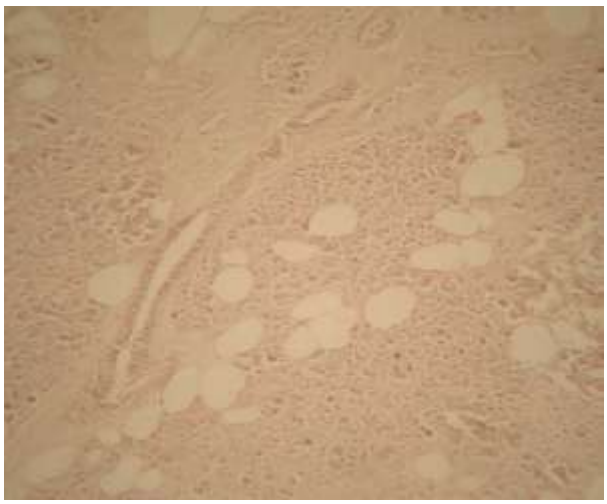

Figure (3): Infilteration of adipose cells in the parenchyma of parotid gland in the experimental group (10 x).

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

	Control group	Experimental group
Diameter of acini	29.13±5.5	22.2±5.62

Table (2): clarify the number of serous acini in control and experimental groups per microscopic field at (40x) power.

	Control group	Experimental group
Number of serous acini	46.31±11.4	40.26±10.3

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

	Control group	Experimental group
Diameter of straited duct	33.3±10.62	32.44±9.2

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

	Control group	Experimental group
Thickness of interstitial C.T	11.4±2.24	9.46±4.79

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التغيرات النسيجية للغدد اللعابية للفئران المعرضة للتغذية السائلة

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الملخص

الهدف من هذه الدراسة هو ملاحظة التغيرات النسيجية التي تحدث على الغدد اللعابية النكفية للفئران التي غذيت على السوائل مقارنة بالفئران التي غذيت بالغذاء الصلب. ست وثلاثين حيوان تمّ تقسيمهم عشوائيا الى مجموعتين، مجموعة السيطرة غذيت بالطعام الصلب ومجموعة التجربة غذيت بالسوائل، تمّ قتل الفئران بعد مرور واحد وعشرون يوما واسئصلت الغدد النكفية وقطعت نسيجيا في شمع البارافين لفحصها بالمجهر الضوئي. وأظهرت النتائج بنقص اقطار العنيبات المصلية نقصانا معنويا في مجموعة التجربة بالمقارنة بمجموعة السيطرة . حيث كانت أقطار العنيبات المصلية لمجموعة التجربة (22.2) وأقطار العنيبات المصلية لمجموعة السيطرة (٢٩,١٣) ولم يكن هنالك نقصاناً معنوياً لأقطار وأحجام القنوات المخططة والنسيج الرابط البيني. وأستنتج من الدراسة أن سبب هذا النقصان في أقطار العنيبات المصلية هو بسبب قلة الحاجة في مضغ الطعام.