

Morphological Alterations of the Parotid Gland of Rats Maintained on a Liquid Diet

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The purpose of this study was to analyze the morphological alterations that occurred in the parotid glands of rats maintained on a liquid diet compared to a solid diet. Thirty-six animals were randomly divided into two groups. The control group received a solid diet, and the experimental group received a liquid diet. The animals were killed after 8, 15 and 30 days. The glands were prepared for inclusion in paraffin and analyzed with a light microscope. The results showed a statistically significant reduction of the parotid gland weight of the animals from the experimental group compared to the control group at 15 and 30 days. The strongest morphological alteration displayed was the presence of cytoplasm vacuoles on the parotid glands of the animals maintained on the liquid diet. Specific stain techniques for glycoproteins and mucopolysaccharides could not identify the substances inside the vacuoles observed in the experimental animals. We conclude that a liquid diet caused atrophy of the parotid gland after 15 and 30 days.

Key Words: parotid gland, salivary gland, diet, light microscopy.

INTRODUCTION

Salivary gland secretions are rich in fluids, ions and proteins important for oral health and integrity of the teeth. Flow rate is strongly protective against caries, and individuals with reduced salivary output should be identified to modify treatment and prevention programs in ways that diminish caries risk (1). Patients displaying hypo-salivary flow or xerostomia usually show high caries activity, if other epidemiological variables are maintained (2,3).

The saliva exerts a major influence on plaque initiation, maturation and metabolism. During caries process, saliva interferes in several ways. When stimulated, it can increase the amount of protective ions such as calcium, phosphate and fluoride, that are related to the demineralization and remineralization process of enamel. The saliva also produces antimicrobial substances such as immunoglobulins, lysozyme, lactoferrin and salivary peroxidase, which can have an immediate effect on oral bacteria interfering with their metabo-

lism, producing acid and activating multiplication, or killing them directly (4-6). In other words, saliva's role in maintaining tooth integrity is a reflection of mechanical cleaning and carbohydrate clearance, post-eruptive maturation of enamel, regulation of the ionic environment in plaque fluid to provide a remineralizing potential and limitation of acid diffusion.

These protective properties are related to salivary flow. Therefore, any alteration of the integrity and activity of salivary glands can change saliva flow and composition. Many factors must be considered in the diet such as its texture, taste and consistence (7-9). According with Edgar and Jenkins (10), administration of diets requiring reduced or increased mastication of rats leads to atrophy or hypertrophy, respectively, of their salivary glands. Johnson and Sreebny (11) observed that when rats were fed with hard chow the weight, enzymatic content and protein synthesis of the parotid glands increased. On the other hand, Scott et al. (12) 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 animals.

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 (13).

The purpose of this study was to analyze the morphological alterations that occurred in the parotid gland of the rats maintained on a liquid diet.

MATERIAL AND METHODS

Thirty-six 90-day-old male Wistar rats with a mean weight of 210 g (Laboratory Animal Center of the University of Brasilia) were randomly divided into two groups. The control group was fed a solid diet (Purina) and water *ad libitum*. The animals of the experimental group were fed a liquid diet and water *ad libitum*. The liquid diet was prepared daily by mixing one part of solid chow (20 g) and 5 parts of distilled water (100 ml). The mixture was blended for 3 min and offered to the animals (14).

After 8, 15 and 30 days, control and experimental rats were weighed and killed by ether overdose. The parotid gland from each side was carefully dissected intact and weighed. 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), periodic acid-Schiff (PAS) and Alcian blue for light microscopy evaluation. Statistical significance was evaluated by the Student *t*-test.

Table 1. Wet weights (mg) of parotid glands from rats after 8, 15 and 30 days on a solid or liquid diet.

Observation time	Solid diet	Liquid diet
8 days	331.5 ± 130.2	224.4 ± 27.3
15 days	288.2 ± 41.9	193.0 ± 18.6*
30 days	271.4 ± 27.9	223.3 ± 25.8*

**p*<0.05 compared to solid diet

RESULTS

The animals of both groups gained weight in the same range, and appeared to be healthy during the experimental period.

The weight of the parotid glands for the three experimental periods are reported in Table 1. There was a decrease in gland weight of the experimental animals compared to the control animals at 8, 15 and 30 days. Nevertheless, this difference was not statistically significant for the period of 8 days.

During the experimental periods, the control group had a structure usually described as normal for the parotid gland. Sections stained with H&E revealed serous acini with pyramidal cells surrounding a small central lumen with a spherical, basal nucleus (Figure 1).

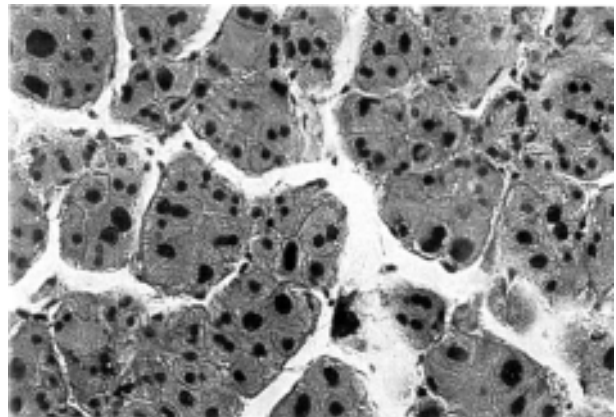


Figure 1. Control group. Histological aspect of the parotid gland from rats fed a solid diet for 8 days. H&E (40X)

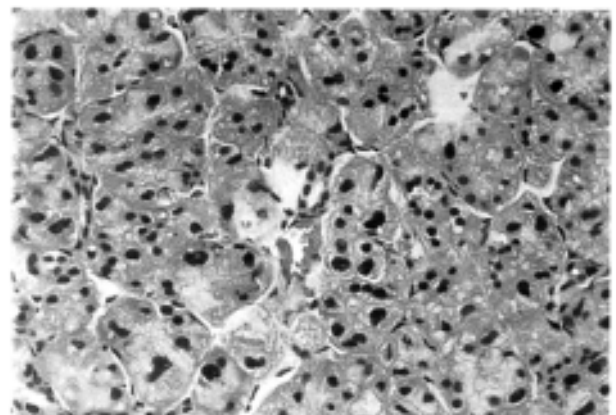


Figure 2. Experimental group. Histological aspect of the parotid gland from rats fed a liquid diet for 8 days. Some vacuoles can be observed. H&E (40X)

The histological appearance of the parotid glands from rats on a liquid diet was very similar to the parotid glands of the control group. The glandular parenchyma, intercellular spaces and nucleus did not suffer any important alteration. However, the serous acinus showed slight atrophy, and some acinar cells were degranulated. The salivary ducts were considered normal. Vacuoles in the cell cytoplasm of the parotid glands from the experimental group could be seen at all periods of experimentation. These vacuoles were more frequent at 15 and 30 days (Figures 2-4). PAS was used to identify glycoproteins and Alcian blue to identify mucopolysaccharides inside the vacuoles; however, the content of the vacuoles reacted negatively for both techniques (Figures 5 and 6).

DISCUSSION

The results showed a decrease of the parotid gland weight when rats were fed a liquid diet at all time periods; however, this difference was statistically significant only at 15 and 30 days. Although at 8 days, there was a decrease in the parotid gland weight in the treated group, the reduction was not statistically significant. This can be explained by the standard deviation figures, in which a higher SD (± 130.16) can be seen for the control group caused by one gland that showed an unexpected increase in its weight (Table 1).

The atrophy of animal glands fed a liquid diet has been observed in many different studies (15-19). There exists a consensus in the literature concerning the

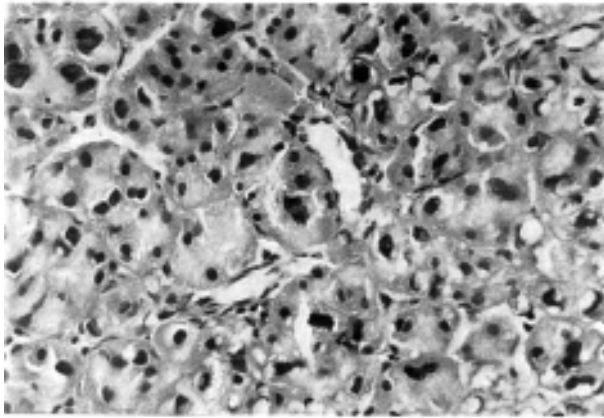


Figure 3. Experimental group. Histological aspect of the parotid gland from rats fed a liquid diet for 15 days. H&E (40X)

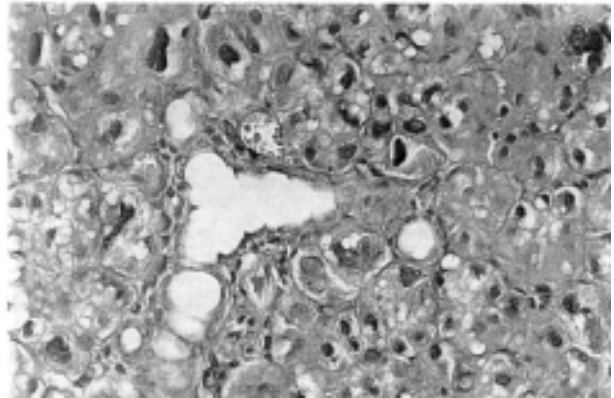


Figure 5. Experimental group. Histological aspect of the parotid gland from rats fed a liquid diet for 30 days. PAS reacted negatively (40X).

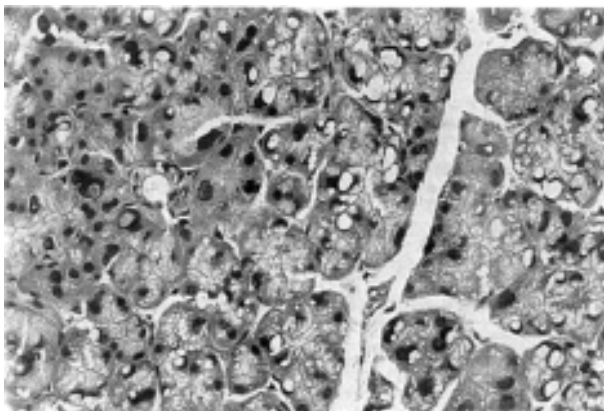


Figure 4. Experimental group. Histological aspect of the parotid gland from rats fed a liquid diet for 30 days. The vacuolization of the acinar cytoplasm is more evident. H&E (40X)

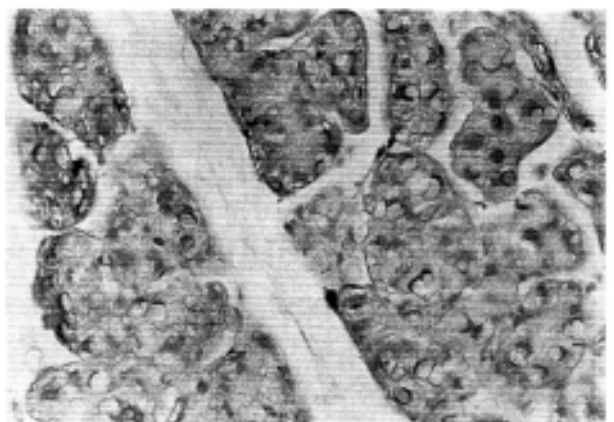


Figure 6. Experimental group. Histological aspect of the parotid gland from rats fed a liquid diet for 30 days. Alcian-blue reacted negatively (40X).

decrease of the size and weight of salivary glands when function is reduced by eliminating the need for mastication.

Generally speaking, morphological alterations were not seen when the parotid glands from the treated group were examined microscopically. The only important alteration was related to the presence of vacuoles in the cytoplasm of the acinar cells, greater at 15 and 30 days (Table 1).

Hand and Ho (17) report the presence of lipids in atrophic acinar cells. However, this hypothesis could not be confirmed in this study because the method used to fix the tissue was not appropriate for lipid studies.

Other studies have reported histological alterations in salivary glands from rats fed a liquid diet, with a reduction in size of acinar and duct cells (16,18,19). The results of the present experiment did not confirm these alterations, although the acinar cells apparently showed slight atrophy when compared to the control group. Nevertheless, these findings cannot be considered conclusive, since no morphometric study was conducted.

It could be concluded that the parotid gland weight of rats maintained on a liquid diet was smaller than the weight of rats fed with hard chow. This difference was statistically significant at 15 and 30 days. The cytoplasm of the acinar cells from the treated group displayed marked vacuolization, greater at 15 and 30 days, and specific stain techniques for glycoproteins and mucopolysaccharides were not able to identify these organic compounds inside the vacuoles.

Even though the present research did not evaluate the relation of saliva and caries, the importance of saliva in the caries process is well known (5). Muñiz et al (20) 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.

Thus, it is very important to understand the possible mechanisms that can modify the function and activity of salivary glands. There are some questions that were not answered and some associations that should be done. Could children that are maintained on a liquid diet for long periods suffer any kind of alteration in their salivary gland? If so, could this change affect saliva flow rate and composition?

RESUMO

Esse trabalho teve por objetivo analisar as alterações morfológicas das glândulas parótidas de ratos submetidos a uma dieta líquida. Trinta e seis animais foram divididos aleatoriamente em dois grupos. O grupo controle recebeu dieta sólida, e o grupo experimental recebeu dieta líquida. Os animais foram sacrificados 8, 15 e 30 dias após o início da experimentação. As glândulas foram incluídas em parafina e analisadas no microscópio de luz. Os resultados mostraram uma redução estatisticamente significativa no peso das glândulas parótidas dos animais do grupo experimental quando comparado aos dos animais do grupo controle nos períodos de 15 e 30 dias. A alteração morfológica mais importante foi a evidência de vacúolos no citoplasma das glândulas parótidas dos animais alimentados com dieta líquida. Os vacúolos citoplasmáticos reagiram negativamente às técnicas de coloração específicas para glicoproteínas e mucopolissacarídeos (PAS e Alcian blue). Concluiu-se que a dieta líquida causou atrofia das glândulas parótidas nos períodos experimentais de 15 e 30 dias.

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