Effects of protein deficiency (malnutrition) on the Submandibular gland on mice

Hashim M. abdul-kareem

College of sciences

Qadisiya university

Abstract:

Five weeks old male mice ware given a diet containing 2.5% protein for period of 5 weeks and its effects on submadibular salivary gland were compared with those in similar animals which were given a 20% protein diet. the submandibular salivary gland was studied using light microscopy protein - deficient animals F The were found to have a markedly reduced body weight compared to the controls this accompanied by a significant reduction in the cell volume of the granulated convoluted tubule cells of the submandibular gland, the granules of the cells were smaller and fewer than those in the controls the acinar cells also showed some reduction in size 1 because GCT cell granules contain a number of growth factors their diminished production should be an additional factor producing growth retardation of various tissues in protein deficiency.

Introduction:

Malnutrition due to a protein deficient diet is known to produce abnormal changes in a number of organs in man as well as in experimental animals. it produces growth retardation and results in the atrophy of small intestinal villi, damage and eventual atrophy of pancreatic acinar cells as well as changes in the central nervous system, delaying is maturation, Protein deficiency cause a distinct decrease in the size and weight of the rat parotid gland; the cell number decreases without alteration in the cell size (14 & 20).

The mouse submandibular gland has special biological interest as it is site of production of epidermal growth factor (EGF), never growth factor (NGF), the mesodermal growth factor and many other biologically active peptide (1). A particular interest is direct to the cells of its granulated convoluted tubules(GCT), the granules of which contain these growth factors. These tubules are situated between the intercalated dusts and the striated ducts. There is sexual dimorphism in the cells of the GCT (2), these being more abundant, larger and containing more granules in males tan in females. These granules develop in the postnatal period in mice, their development from striated duct precursors beginning in the second to third week.

Sexual dimorphism of these cells is established early. The size of the tubules and the number of granules increase more rabidly in males than in females(9).

The percent study was undertaken to observe the effect of a protein- deficient diet in the post- weaning period on the morphology of GCT cells of mouse submandibular gland. this was thought to be interest as the amount of granules in these cells is an indirect measure of the growth factors the animals produce.Up to now there has been no study on the effects of protein deficiency on the mouse submandibular gland.

Materials and Methods:

Fourteen male white Balb/C mice 5 weeks old were used for this study. They were divided into 2 groups each group containing 7 animals.

Group 1 was fed a diet containing 2.5% protein.

The animals of group 2 were used controls and were fed a diet containing 20% protein Feeding of the respective diets was maintained for a period of 5 weeks. the animals had free access to food and water throughout this period. The composition of the diet is given in table 1.

The weight of the animals and the amount of food eaten were measured at weekly intervals.

After 5 weeks of feeding, when the animals were 10 weeks old, the experiments were ended. Under ether anesthesia the hearts was exposed and transcardiac perfusion was carried out using 2.5% gluteraldehyde in 0.1M phosphate buffer at PH 7.2 perfusion was continued till the whole animals was fixed. After perfusion the submandibular glands were dissected free. One gland from each animal was processed for light microscopy (17), specimens were further fixed in 2.5% gluteraldehyde in 0.1 м phosphate buffer overnight they were cut by microtome LKB, and they were stained with haematoxylin and eosin.

The following morphometric measurements were made.

- volume fractions (Vv)

The volume fractions (Vv) of the acinar and the GCT cells of the gland were estimated by point counting. Using an Olympus light microscope with a drawing tube attachment the image of a 25 point lattice was superimposed on the section. Ten sections covering the whole extent of the gland were examined from each gland (16; 15).

- Mean nuclear diameter(D)

The mean diameter of the nuclei of the GCT cells and acinar cells were estimated at least 500 nuclei of each category was measured from each gland. it was assumed that the nuclei were almost spherical and the major minor diameters of each nucleus were measured (23; 25).

- Numerical density (Nv) and cell volume

Numbers of nuciei per unit area (Na) of acinar and

GCT components of the gland.

Numerical density (Nv) was calculated using the

formula:

$$Nv=Na/D+t \quad (23)$$

Where Na equals the number of nuclear profiles per unit area of the component concerned and (t) equals section thickness assuming that each cell contained using the formula:

Vcell1/Nv (18)

Measurements were made for each animal and these were then pooled to estimate the mean + S.E. for the control and experimental groups differences between the groups were tested using students tests.

Results:

Control animals gained weight throughout the course of the experiment. After 5 weeks of feeding. the body weights of the experimental group, in spite of the weight loss and the small size, the experimental animals were more active than the controls.

The submandibular gland contained mucous acini intercalated dusts, GCT, striated ducts and excretory ducts the bulk of the gland was made up of acinar cells and GCT cells.

In the control animals the GCT were more abundent than the mucous acini where as in the experimental group this relationship was reversed. the size of the tubules and of each constituent cell wes smaller in the experimental animals (figs.1,2) the GCT cell in controls had a basal nucleus and almost all of the supernuclear region was filled with secretion granules.

In the experimental animals the GCT cells besides their smaller size slowed other structural difference. Their nuclei were more central, and the secretory granules were smaller and far fewer.

Each acinus was formed by 3-4 pyramidal cells bounding a lumen smaller than that of the granulated tubules the cytoplasm contained a large number of mucous granules.

The nucles was small and pushed to the base of the cell. No obvious structural difference was noted between the cells of control and experimental groups.

The results of the morphmetrics measurements are given in table 2.

There was a significant reduction in the volume proportion of the GCT cells in the experimental animals the GCT acinar ratio was almost reversed in the experimental group compared to that in the controls.

The mean volume of the GCT cell was also markedly reduced in the protein-deficient animals and the number or granules in these cells were so few that they could not be quantitated.

Discussion:

The results show that the relative volume of the GCT cells and their granules in the protein deficient animals were significantly less than those in the controls.

The effect of the diet was not so pronounced in the acinar tissue besides the direct effect of protein depletion the changes in the GCT cells might have also been due to the lack of hormones which promote and maintain the size and activity of these cells. The postnatal development and growth of the GCT cells depend on the serum concentration of the testosterone (10;3;5) and thyroxine (22; 24; 21).

A review of the endocrine changes in protein deficiency shows a general agreement on thyroid and gonadal hypofunction in many species (13). Serum are lowered in the children affected a 13 and T4 protein malnutrition (12; 8).

A delay in maturation of gonads in young rats and the changes in adult rats and man have also been reported (13).

The effects of malnutrition are well understood.

In in vitro studies EGF (Epidermal Growth Factor) has been shown to produce a cascade of events which leads to an increase in the mitogenic of activity in a variety of cell types. These events include the simulation of active transport the activation of glycolysis activation of RNA and protein synthesis and initiation of DNA synthesis (4).

Epidermal growth factors (EGF) accelerates eyelid opening and tooth eruption when administrated to neonatal mice, especially during the first three days of life (6;11).

The decrease in GCT cell and its granule volumes as seen in the protein deficient animals will presumably result in a lowering of the concentration of EGF, NGF and other growth factors. Lack of these biologically active peptides may be an added factor contributing to body growth retardation soen in malnutrition.

References:

1-Barka, T.(1980). Biologically active polypeptides in submandibular glands journal of Histochemistry and Cytochemistry 28, 836-859.

2-Caramia, F. (1966a). Ultrastructure of the mouse submaxillary gland. 1 sexual differences Journal of Ultrastructure Research 16, 505-523.

mouse 3-Caramia, F.(1966b). Ultrastructure of the submaxillary gland.11.effect of castration on the male . Journal Ultrastructure Research 16,524-536.

4-Carpenter, G.& Cohen S. (1979). Epidermal growth factor Annual Review of biochemistry 48, 193-216.

5-Chretein, M. (1977), the action of testosterone on the differentiation of secretory activity of a target organ: the submaxillary gland of the mouse. International review of Cytology 50, 333-396.

6-Cohen, S. (1962). Isolation of a mouse submaxillary gland protein acceleration incisor eruption and eyelid opening in the newborn animal. Journal of biological chemistry 237, 1555-1562.

7-Cope, G.H. (1982). Stereological techniques and their application to the gastrointestinal tract and its glands. In techniques in life Sciences, volume p2 (ed.D.A.Titchem). New York: Elsevier biomedical.

8-Fhatun, F.U.H.(1982). Study of the thyroid function in protein energy malnutrition. Bangladish Medical Research Concil Bulletin 8, 68-71.

9-Gresik E.W.(1980) Postnatal development changes in submandibular glands of rats and mice journal of Histochemistry and Cytochemistry 28, 860-870.

10-Harvey H. (1952). Sexual dimorphism of submaxillary glands in mice in relation to reproductive maturity and sex hormones Physiological zoology 25, 205-222.

11-Hoath, S.B.(1986). Treatment of neonatal rat with epidermal growth factor: differences in time and organ response. Paediatric Research 20, 468-427.

12-Ingenbleek, Y. & Beckers, C. (1975). T3 and TSH levels in protein - calorie malnutrition in infants. lancet ii, 845-847.

13-Jaya Rao, K.S. (1982) the endocrines in protein energy malnutrition World review of nutrition and dietetics 39, 53-84.

14-Johnson, D.A., Sreebny, LM. & Enwonwu, C.O. (1977). Effect of protein energy malnutrition and or a powdered diet on the parotid gland and pancreas of young rats. Journal of Nutrition 107, 1235-1243.

15-Kareem, H.M.(1995). Changes in skeletal muscle fiber types, diameter and number during growth in hind limps of mice. journal of Basrah Research 2, 86-95.

16-Kareem, H.M. (1987). Structure and development of muscle in the Rainbow trout, Salmo gairdnrei and the brown trout, Salmo. Ph.D. thesis, University of Salford - Manchester, U.K..

17-Karnovesky M.J. (1995) dehydegluteraldehyde fixative of high A formal osmabality for used in light and election microscope J. cell bio. 69, 139B.

18-Loud, A.V. (1968). A quantitative stereological description of the structure of normal rat liver parenchymal cells. Journal of cell Biology 37, 27-46.

19-Micheal, H.R.; Gordon, I.K.; (2002). Histology, A text and Atlas with cell and Molecular biology 4th.Ed..

20-Presnell,J.K. and Schreibman 1 M.P.(1997) Humansons Animal tissue Techniques The Johns Hopkins Uni. Press, Baltimore, London.pp. 541.

21- Punkt, K.; Krug, H.; Huse, J.; and punkt, J. (1990) the correlation of cytophotometrically and biochemically measured enzyme activities. Acta Histochem. 99: 261-269.

22-Raynaud, J. (1984). The action of thyroid and adrenal glands on the submaxillary glands of mice. in Salivary gland and their Secretions (ed. L.M. Sreebny XJ.Meyer). London: Pregamon press.

23-Underwood. E.E. (1970). Quantitative Stereology, Massachusetts: Addison-Wesley.

24-Watson A.Y., Larson P.R. & Murphy R.A.(1982). thyroxine reveres deficits of never growth factor and epidermal growth factor in submandibular glands of mice with muscular dystrophy Endocrinology. 140, 893-905.

25-Yan, L.J; Levine, R.L.; and Safial, R.S. (1997) oxidative damage during ageing targets mitochordrial aconitase. Proc. Acad. Sci. USA 94: 11168-11172.

Nutrients	Control%	Experiment%
Protein	20	2.5
Strach	65	82.5
Cellulose	4	4
Cornoil	4.5	4.5
Salt mix	4.5	4.5
Vitamin mix	2.0	2.0

Table 1. The percentage composition of the diet

Item	Controls	Experimentals
GCT cell Vv	84.6 ± 2.5	42.5 ± 1.8***
GCT nucl. Diameter(nm)	4.9 ± 0.2	4.2 ± 0.3**
GCT cell volume (nm^2)	5125.4± 211.3	2085 ± 121.8***
Acinar cell Vv	28.6 ± 1.9	58.5 ± 2.8***
Acinar nuclei diameter(nm)	7.8 ± 0.2	6.1 ± 0.3 **
Acinar cell Volume (nm^2)	4092.8 ± 194.1	3420.3 ± 302.2*

Table 2. The effect of protein deficiency on the nuclear diameters, and the absolute cell volume for the experimental and the control group of animals $0.1^{*} > p > 0.05; p^{**} < 0.005; p^{***} < 0.0001$

Fig. 1. Acini and GCT from a control animal. the GCT cells contain a large number of secretory granules (ig); acini (A); granulated convoluted tubule (T). Toluidine blue. Mag. X400

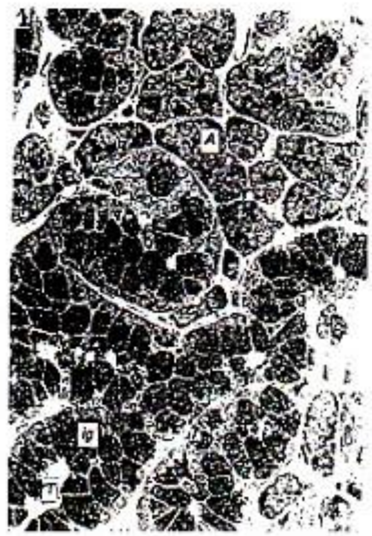
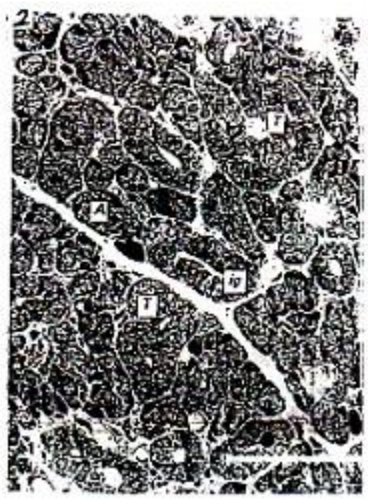


Fig.2. Acini and GCT from an experimental animal. the GCT cells and the tubules are smaller than these seen in fig. 1. the size and number of granules (ig) are also less. Acini (A); granulated convoluted tubules (T). Toluidine blue. Mag .X 400.



تأثير نقص البروتين على تركيب الغدة اللعابية تحت الفك

في الفئران

أ.م.د. هاشم محمد عبد الكريم

كلية العلوم – جامعة القادسية

الخلاصة :

في هذه الدراسة أعطيت الفئران بعمر خمسة أسابيع حمية غذائية حاويه على ٢,٥% بروتين لفترة خمسة أسابيع وقورن تأثيرها مع حيوانات أخرى أعطيت حمية غذائية تحتوي على ٪٢٠ بروتين. تم دراسة الغدة اللعابية تحت الفك مستخدما المجهر الضوئي والقياسات الشكلية .

وجدت الحيوانات ذات الحمية قليلة البروتين وبصوره واضحة تناقص وزن جسمها المقارنة إلى حيوانات السيطرة . وكان ذلك مترافقا باختزال معنوي في معدل حجم الخلايا للنبيبات الملتوية المحببة للغدد تحت الفكية . وكانت الحبيبات لتلك الخلايا صغيره وقليلة بالنسبة إلى حبيبات خلايا غدد حيوانات السيطرة . كذلك الخلايا الحبيبية أظهرت بعض الاختزال بحجمها بسبب إن الخلايا المحببة في النبيبات الملتوية تحتوي عدد من عوامل النمو وعدم إنتاجها سوف يؤدي إلى تراجع إنتاج عوامل لنمو النسج في حالة نقص البروتين .