

Light and Scanning Electron Microscopic Studies of Lingual Mucosa of Rat Pups of Phenylketonuria Mothers

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Abstract: The present study aimed to describe the morphology and structure of lingual mucosa of albino rat at 7 & 21 days pups. Specimens taken from tongue of pups of both control and experimental PKU mothers and examined by light and scanning electron microscopy. Filiform papillae appeared as small finger-like projections with rounded tips, its peripheral epithelial coat showed early keratinization. With the advancement of growth at 7 & 21 days postnatal, three types of filiform papillae were distinguished associated with much more keratinization of their free ends. At the same time, the gustatory fungiform papillae were more differentiated at 7 & 21 days, fungiform papillae is more differentiated form and became identical in morphology to that in the adult. The experimental rat pups of PKU mothers at 7 & 21 days, showed a considerable reduction of lingual keratinization. The stratification pattern of both filiform and fungiform papillae showed apparent degeneration of their cellular elements including vacuolar degeneration and necrotic cells with pyknotic nuclei. The outermost cells showed apparent cell loss. The lingual muscles were distorted and degenerated. Leukocytic infiltrations were abundant in between the lingual muscles. Fungiform papillae lacked differentiation.

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1. Introduction

The mucosa of the dorsal surface of the tongue differed markedly from that of the oral cavity. It is covered by functionally masticatory mucosa lined with different types of lingual papillae (Ten Cate, 1994). The gustatory system is unique in that its receptor cells develop from local epithelium rather than neurogenic ectoderm as in other sensory systems (Barlow and Northcutt 1995; Stone *et al.* 1995). In mammals, taste receptor cells are organized into taste buds which, on the tongue, are located within specialized papillae (Mistretta 1991). In rodents, taste papillae develop prenatally while taste buds subsequently appear within these papillae at about the time of parturition (Paulson *et al.* 1985). Thus, the formation of papillae is an important first step in development of the gustatory system in mammals.

Morphogenesis of fungiform papillae occurred in characteristic pattern during prenatal development in rodents (Mistretta 1991, El-Sharaby *et al.* 2001). Fungiform papillae formed in longitudinal rows on the anterior portion of the tongue with the medial rows forming prior to more lateral ones and the more anterior papillae developing first within each row. Although each type of papilla is morphologically distinct, the initial events in their development are histologically similar. Taste papillae begin as placodal thickenings in the lingual epithelium (Fujimoto *et al.* 1993). The placodal epithelium then

begins to grow into the underlying mesenchyme and evaginates into a raised structure. At the same time, nerve bundles begin to grow into the tongue and ultimately reach the lingual epithelium (Whitehead and Kachele 1994). Filiform papillae covered almost the entire part of the dorsum of the tongue and form a mechanical tough abrasive surface which is involved in mastication (Kullaa-Mikkonen *et al.* 1987).

Phenylketonuria is a disease resulting from insufficient phenylalanine hydroxylase expressed in the liver and converting phenylalanine to tyrosine. PAH is expressed only in the liver (Matalon *et al.*, 1989) and intern increase accumulation of phenylalanine metabolites including phenylacetate, phenyllactate, phenylpyruvate, and phenylethylamine in tissues causing adverse effects (Kaufman, 1999).

Maternal PKU is of particular concern, and it can lead to fetal malformations, including small head size (microcephaly), cardiac abnormalities, intrauterine growth retardation and mental retardation (Levy & Ghavami, 1996; Koch *et al.*, 2000). Thus, the present work aimed to investigate the effects of experimental PKU on the lingual mucosa during postnatal growth at 7 & 21 days pups.

2. Material and Methods

Thirty-two virgin female and fertile male albino rats 160-180g.b.wt (1 male /3 female) were used during experimentation. The animals supplied from

Hellwan Breeding Farm (Ministry of health, Cairo, Egypt) and used for experimentation. They were maintained under good ventilation and 12 hour light and dark cycles with free access of food and water ad libitum.

Induction of PKU:

Experimental induction of phenylketonuria (PKU) Beginning at the 6th day of gestation, each pregnant rat was intragastrically administered 30 mg DL-alpha methylphenylalanine/kg b.w. plus 60 mg/Kg b.w. L-phenylalanine at 12 h intervals throughout pregnancy till parturition as well as throughout lactation period till 21 days post-partum. The applied dose was chosen according to Huether et al. (1982) and Rech et al. (2002).

Experiments

Females were allowed to mate by keeping them with healthy fertile males for 12 hours overnight between 8 p.m. and 8 a.m. (at a ratio of 1 male per 2 females). In the next morning, the presumably pregnant rats were examined for the presence of vaginal plugs. Vaginal smears were carried out to give a precise determination of the onset of gestation. Twenty-eight pregnant animals were separated from the experimental animal colony and arranged into two main groups, with fourteen rats in each:

Gp1: Control and feed on standard diet and their pups were collected 7 & 21 days post-partum.

Gp2: PKU-treated pregnant. They were received PKU-treatment at the 6th day of gestation till the end of two weeks post-partum.

Delivered newly born and pup rats of both control and experimental mothers groups were scarified at 7 & 21 days post partum. Soft palate was separated at parturition, meanwhile tongue was separated at 7 and 21 days of pups. They were subjected for the following investigations:

1. Scanning electron microscope:

Fresh specimens of tongue were fixed immediately in 2.5% glutaraldehyde in 0.1M cacodylate buffer adjusted to pH 7.4 followed by washing in 5% sucrose in 0.1 M cacodylate buffer. The specimens were dehydrated using a graded series of 25%, 50%, 75%, 95%, and 100% acetone and critically drying in a carbon dioxide apparatus. The specimens were coated with gold in using and viewed using a joel 5300JSM (Musashino 3-chome Akishima Tokyo 196-8558, Japan).

2. Light microscopic investigations:

Both specimens of soft palate and tongue were fixed immediately in 10% formal saline for 24 hours, followed by dehydration in ascending grades of ethyl

alcohol, in xylol and mounted in molten paraplast (58-62 °C). Histological sections of five µm thick were made and stained with hematoxyline and eosin.

3. Results

a. Control animals:

At light microscopic levels both 7 & 21 days-old pups showed regular arrangement of 4 cell layers of the lingual mucosa including; stratum germinativum, spinosum, granulosum with distinct keratohyalin granules and stratum corneum which is more relatively thickened in 21 days-old pups. The filiform papillae covered the entire anterior part of the dorsal surface of the tongue, appearing as small finger-like projections with rounded or blunted tips; some of them had started to have a conical appearance. The projections of filiform papillae are slightly curved and pointed caudally towards the distal end of the tongue. The periphery of filiform papilla coated by a thin keratinized epithelium. Keratinization is clearly observed at the tips of each filiform papillae. The entire core of the papillae was formed of a fine collagen network & fibroblasts. The epithelium of the interpapillary regions is non-keratinized and appeared thicker than that covering the tips and lateral surfaces of the papillae. The width of filiform papillae at its base is much more wider than its free end. The epithelial cells at the periphery of the filiform papillae appeared flat with thin peripheral keratinized areas. The anterior and posterior cell columns of the filiform papillae and the interpapillary cell columns are clearly distinguishable. Fungiform papillae are far less numerous than the filiform type, and scattered among them. The fungiform papillae appeared developed with distinguished taste cells on its expanded upper surface. The musculature of the tongue consists of distinct skeletal muscle bundles that run in transverse, longitudinal and possibly oblique directions. The anterior portion of the tongue consist of interlacing fibers with a transverse bundle seen at the central portion of the tongue, however, the posterior portion consist of an outer transverse and inner double transverse bundles. In between the later is a longitudinal bundle that wraps around them separating them from each other and from the outer transverse bundle (Figs.1, A-A1).

In control, SEM revealed that the normal morphogenesis of filiform papillae proceeds during postnatal growth in parallel with keratinisation of the lingual epithelium. The filiform papillae cover the entire majority of the dorsal tongue surface. All the superficial surfaces of the filiform papillae show thin keratinisation coat early in 7 days old pups (Fig.2,A-D) and more keratinized at 21 days old (Fig.3,A-D). Three types of filiform papillae are

recognized. Fungiform papillae are identified as mushroom-shaped structures wider at the top than at the base and projected above adjacent smaller filiform papillae. They are broad and rounded and with slightly cornified surfaces that showed taste pore in its center at 21 days old pups.

b. Pups of PKU-treated mothers

Examination of light microscope sections of tongue lingual mucosa of 7 & 21 days showed a considerable reduction of lingual keratinization. The stratification pattern of both filiform and fungiform papillae showed apparent degeneration of their

cellular elements including vacuolar degeneration and necrotic cells with pyknotic nuclei. The outermost cells showed apparent cell loss. The lingual muscles were distorted and degenerated. Leukocytic infiltrations were abundant in between the lingual muscles. Fungiform papillae lacked differentiation (Fig.4, A1 & B1).

Under SEM, 7 & 21 days old pups of PKU mothers appeared with a considerable deformations of fungiform and filiform papillae. The size of the fungiform papillae is comparatively reduced. Keratinization was markedly diminished (Fig.5, A1-D1, Fig.6, A1-D1).

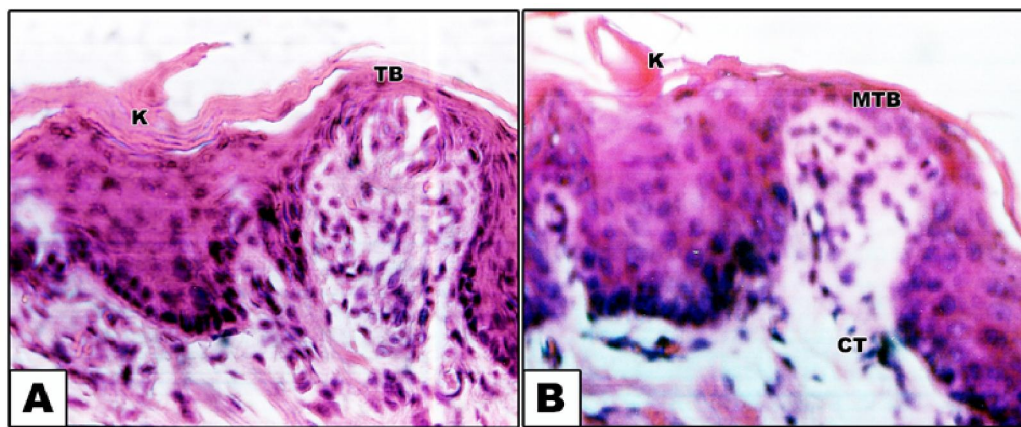


Fig. 1(A): photomicrographs of histological sections of 21 days old pups.

Control showing much more cornification, keratinization and differentiation of apical taste buds in fungiform papillae (B): PKU -treatment showing fungiform papilla with apical degenerated taste buds.

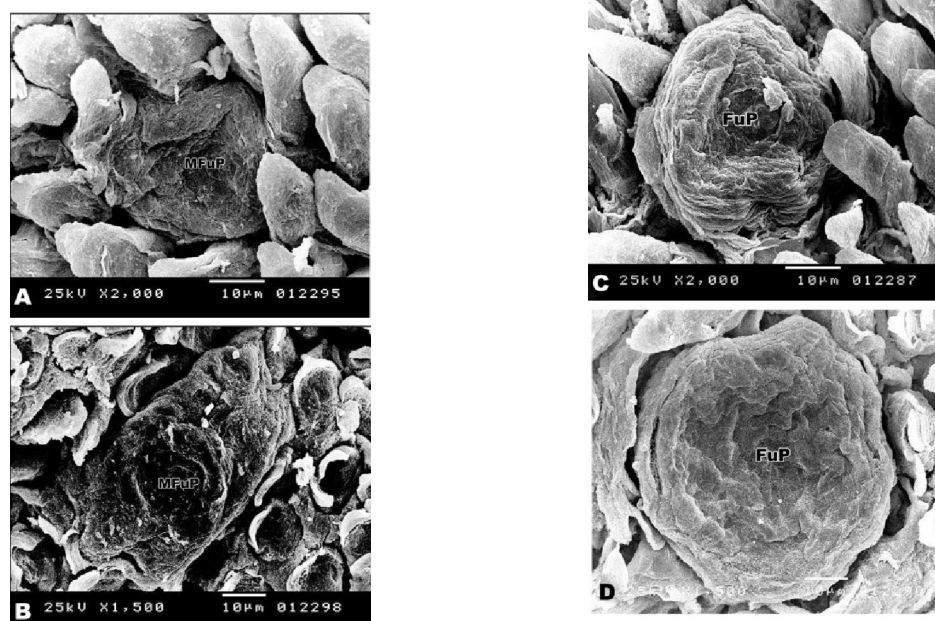


Fig.2 (A-D) :SEMs of lingual mucosa of 7 days old pups of control animals showing dense cornification of filiform papillae and more differentiation of fungiform papillae .

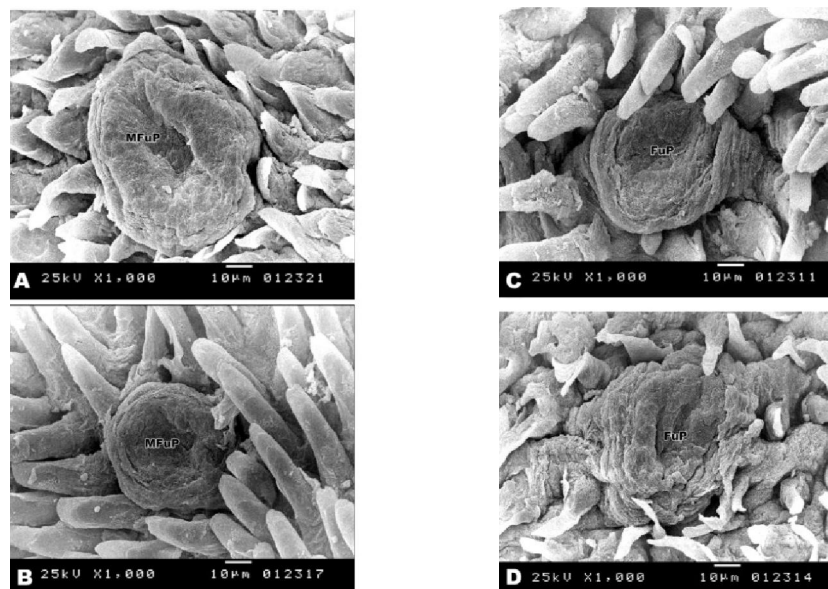


Fig.3 (A-D). SEMs of lingual mucosa of 21 days old pup. A-D. Control showing more organized filiform and fungiform papillae with distinguished taste pore.

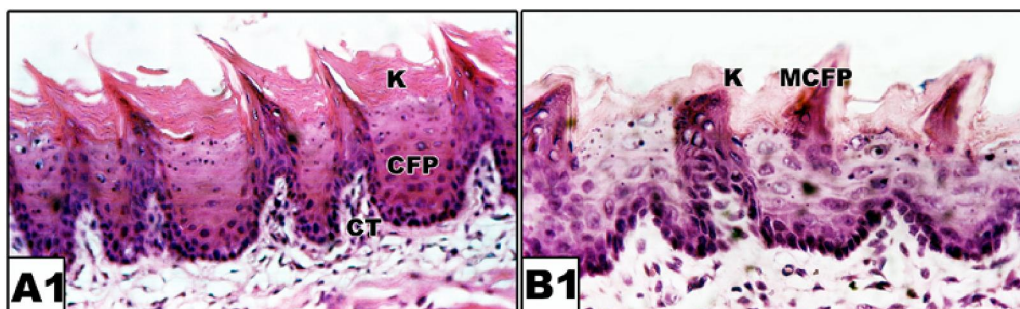


Fig. (4): photomicrographs of histological section of 21-days old pups.

A1-showing apparent keratinization and abundant connective tissue core in filiform papillae.

B1-showing atrophic filiform papillae with apparent degeneration of the mucosa layer and increase leukocytic infiltration in the connective tissue core.

4. Discussion

The normal morphogenesis of filiform papillae proceeds during postnatal growth in parallel with keratinisation of the lingual epithelium. The filiform papillae cover the entire majority of the dorsal tongue surface. All the superficial surfaces of the filiform papillae show thin keratinisation coat early in newly delivered pups and proceed during the postnatal life reaching highest keratinization at 4 weeks of growth. Three types of filiform papillae were recognized. The mechanical function of filiform papillae appeared to be very primitive during early postnatal life which involved only suckling of soft diet composed mainly of milk that need no friction and do not affect protective layer of dorsal tongue surface. As growth advanced at 4 week of age keratinization of lingual mucosa and filiform papillae attained much more growth and served its mechanical and protective function. The present results concurred with those of Dhoulily et al (1989) & Sawaf *et al.* (1990). Iwasaki *et al.* (1999) indicated that in rats the morphogenesis of the filiform papillae advances in parallel with keratinization of the lingual epithelium from just before birth to a few weeks after birth.

Baratz and Farbman (1975) reported that the epithelial mesenchymal interactions play an important role in the development of many organs such as teeth, salivary glands, hair and feathers, but do not occur in the differentiation of filiform papillae. This may simply be due to the relatively small size and close spacing of filiform papillae compared with other epidermal derivatives.

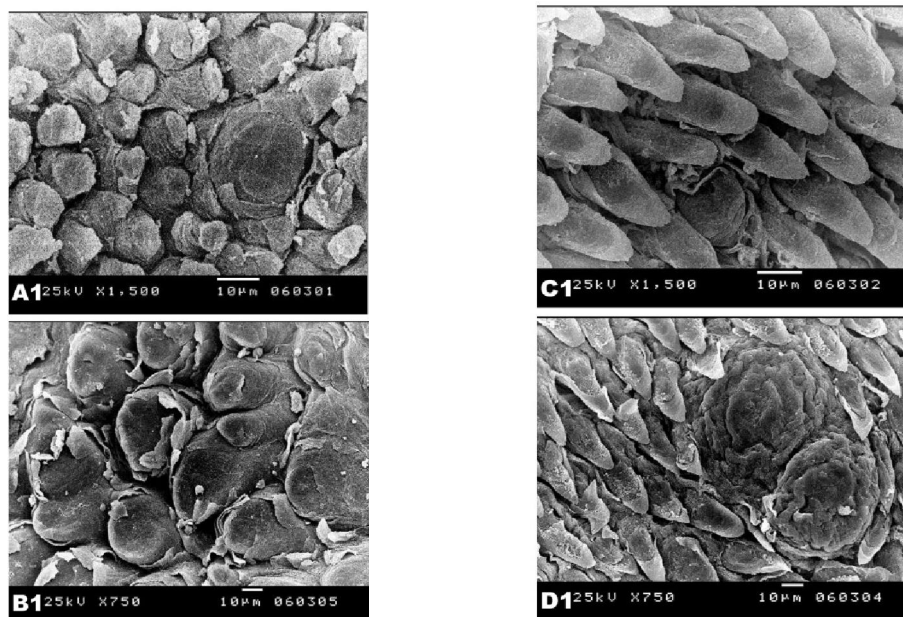


Fig.5 (A1-D1) :SEMs of lingual mucosa of 7 days old pup of PKU- treated animals showing developmental retardation of both filiform and fungiform papillae (shorted and thinning filiform papillae).

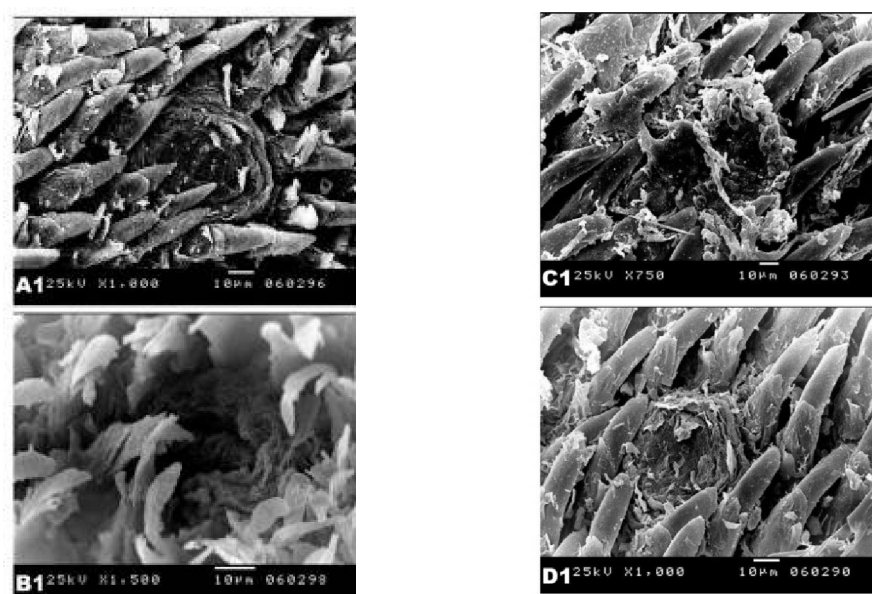


Fig.6 (A1-D1).PKU-treatment showing deformation of both filiform and fungiform papillae and retarded cornification.

Furthermore, keratinization and cleft formation occurred which began at the tips of the papillae and progressed downwards until the fused papillae separated from each other. The present observations confirmed the work of Baratz and Farbman (1975) during the development of rat filiform papillae.

Besides, the present finding of light and scanning electron microscopic observations are in accordance with Nagato *et al.* (1989) who reported

that the conical forms of filiform papillae served for taking food efficiently into the oral cavity, grinding it after has been crushed with the teeth in coordination with the palate. The thready filiform papillae might function as a heat-releasing organ and be involved in the control of body temperature.

On the other hand, fungiform papillae were firstly appear in newly born in primitive forms and preceded the circum vallate papillae. Although the

taste cells lacked their differentiation, the fungiform papillae may possess some important properties for tasting. With the advancement of growth both of the fungiform and circumvallate papillae reached much more development. The convex surfaced fungiform papillae were raised above the lingual mucosa. The vallate papilla was characterized by a papillary groove and an annular pad. Both types exhibited marked differentiation of taste cells either in the apical margin in case of fungiform papillae or in the lateral margins in circumvallate papillae. Taste buds and taste pores were clearly detected in fungiform papillae of 3 & 4 week old suckling neonates.

Similar findings of the distribution pattern of filiform, fungiform and vallate papillae were reported by Silva *et al.* (2002) after studying the surface structures of the lingual papillae in the rabbit.

From the present findings, dense keratinized surface of filiform papillae as well as the preceding eruption of deciduous teeth and the addition of some types of food to the diet, friction of the tongue occur in contact with food, teeth and other mucosal surfaces. The parakeratinized epithelium covering the papillae is transformed, orthokeratinized, and the papillae become conical in shape. Thus, differences in environment, diet and mastication may modify the morphology and structure of the tongue.

Pumplin *et al.* (1997) distinguished two types of cells in taste buds of circumvallate papillae of rats including dark (Type I) and light (Type II) cells. The cells were easily distinguished by their relative electron density, shape and topological relationships. Cells with electron-lucent cytoplasm (light cells) were circular or oval in outline, while those with electron-dense cytoplasm (dark cells) had an irregular outline with sheet-like cytoplasmic projections that separated adjacent light cells.

The gustatory papillae which depend on neuronal sensation of taste is mediated by three cranial nerves: facial (VII), glossopharyngeal (IX) and vagus (X). The trigeminal nerve (V) provides general sensory innervation to a region that overlaps the areas served by these other cranial nerves (Klasser *et al.*, 2009). Massive neuronal dysfunction may result in irreversible gustatory deficits and somatosensory dysfunction.

Phenylketonuria is an inherited metabolic disorder in the activity of hepatic phenylalanine hydroxylase characterized by the impaired conversion of phenylalanine to tyrosine. Thus phenylalanine accumulates in the blood to concentration sufficiently high to activate an alternatively pathway of degeneration (Gazit *et al.*, 2003).

Neuronal cell damage was reported by Binek *et al.* (1981) in experimental PKU neonatal mice.

It is known that phenylalanine and tyrosine compete for the same transporter across the blood brain barrier. In addition, the phenylalanine has more affinity to these transporters and higher plasma phenylalanine appears to block tyrosine transport as shown by evidence of lower levels of neurotransmitter in experimental animals and in humans (Krause *et al.*, 1985).

Castillo *et al.* (1988) induced experimental hyperphenylalaninemia in 5 days chick embryo and reported a significant decrease in the enzyme activities of 3-hydroxy-3-methylglutaryl CoA reductase and evalonate-5-decarboxylase that directly implicated in the regulation of cholesterologenesis. Decreased cholesterol synthesis may explain the impaired myelination and consequently impair differentiation and histogenesis of different body tissues.

Histogenesis of lingual mucosa need more supplements of cholesterol and amino acids for cell growth and differentiation. Binek-Singer and Johnson (1982) reported loss of several amino acids, especially large neutral amino acids, in the brain of newborn mice in experimental PKU.

Hyperphenylalaninemia were found to decrease the availability of tryptophan and tyrosine (Aragon *et al.*, 1982) and cause serotonin and catecholamine depletion in PKU (Herrero *et al.*, 1983) leading to drastically altered the synaptic transmission and influencing the brain function.

The observed deformation of lingual papillae and degeneration of taste buds in both fungiform papillae and soft palae seemed to be related to increased DNA damage as a result of reduced incorporation of amino acids into the protein molecules in experimental PKU (Binek *et al.*, 1981). Spero and Yu (1983) mentioned that hyperphenylalaninemia induced numerical increase of the necrotic cells of the brain of 21 day rat fetuses.

Decreased antioxidant activity may increase free radicals and enhanced massive cell damage in hyperphenylalaninemia (Halliwell and Chirico, 1993) and intern illustrate the retardation of oral defects including lingual and soft parts.

Hagen *et al.* (2002) indicated that the oxidative stress may be involved in the neuropathology of PKU through the inhibition of the antioxidant molecules including superoxide dismutase, catalase and glutathione peroxidase activities in the rat brain.

Gazit *et al.* (2003) attributed the neuronal damage to the hypoglycemic effect of phenylpyruvate. The increased hypoglycaemic manifestation of PKU led Himwich (1951) to investigate these criteria and reported that the reduction of oxygen consumption in the brain may be an important factor contributing to the neurological

defects.

Finally, the present author concluded that adequate nutrition is very important to pregnant women with PKU to prevent development of congenital malformation especially lingual mucosa and soft palate of their pups.

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