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Salivary amino acids and proteins in normal and malnourished Egyptian infants and young children*)

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With 3 tables

Exploration of derangement in different metabolic pathways in protein energy malnutrition (PEM) was usually assessed by investigating the changes in biological constituents as plasma, urine C.S.F. or liver biopsies. Among the biological fluids assessed in PEM cases, saliva received little attention. This biological fluid can be easily collected wihout pain or harm to the patients and at the same time, changes in its constituents might reflect changes in the function of the salivary glands themselves. It is stimulating that *Menaker* and *Miller* (10) who worked on rats found that the salivary glands in the body are affected by the dietary pattern.

Plasma proteins and amino acids were investigated and proved to be of value in understanding the metabolic derangement associating PEM and were helpful for diagnosis and prognosis of the disease (5, 6, 17).

The present study aims to investigate salivary amino acids and proteins in normal and protein-energy malnourished Egyptian infants and young children. Findings may throw light on any additional derangement in metabolic processes in PEM cases. The data might be also of diagnostic or prognostic value.

Material and methods

A number of 104 cases suffering from protein energy malnutrition, admitted to the Mounira children hospital, University of Cairo, form the material of this study. Their rage ranged from 6 to 36 months. Cases were selected free from infection or any associated complication other than protein-energy malnutrition. On clinical grounds and as shown previously (13) cases were categorized into:

Kwashiorkor (KWO)	(6 moderate, 13 severe)
Marasmus	(35 2nd grade, 13 3rd grade)
Marasmic kwashiorkor	(37)

22 healthy infants and young children of similar age range and socioeconomic standard were included to serve as controls.

A fasting morning whole saliva sample was collected from each subject during an optimum interval of 35 minutes. After cleansing the baccal cavity, sterilized cotton plugs were placed in the mouth using sterile forceps. Samples collected during the first 5 minutes were rejected. Cotton plugs newly ad-

Table 1. Essential free amino acid pattern in whole saliva of normal and PEM infants and young children (mg/100 ml).

		Leucine	Phenyl-alanine	Lysine	Valine	Threonine	Methionine	Trypto-phan	Total essential
Controls (22)	M.	1.00	2.16	2.07	0.92	2.51	1.04	0.35	10.05
	± S.E.	0.01	0.2	0.3	0.1	0.4	0.03	0.09	2.1
Moderate KWO (6)		2.25*	1.51*	1.68*	1.36*	3.25*	3.89*	0.88*	13.92*
		0.05	0.2	0.3	0.4	0.5	1.0	0.2	2.5
Severe KWO (13)		1.70*	2.20	2.07	1.20*	3.95*	1.82*	0.40	13.34
		0.06	0.3	0.4	0.2	1.0	0.3	0.05	3.1
2nd grade marasmus (35)		1.25	1.42*	1.66*	0.91	2.98	2.01*	0.27	10.50
		0.1	0.2	0.2	0.09	0.3	0.5	0.1	2.1
3rd grade marasmus (13)		1.03	0.89*	1.58*	1.07	3.08*	2.38*	0.29	10.32
		0.2	0.09	0.02	0.3	0.9	0.8	0.05	2.1
Marasmic KWO (37)		1.43*	1.94	1.94	1.21*	4.03*	2.70*	0.32	13.26*
		0.2	0.3	0.3	0.2	0.9	0.5	0.1	1.6

* Difference from controls is significant ($P < 0.05$)
( ) Number of cases

Table 2. Non-essential free amino acid pattern in whole saliva of normal and PEM infants and young children (mg/100 ml).

		Alanine	Glutamine	Aspartic acid	Serine + Glycine	Tyrosine	Aspara-gine	Cystine	Glutamic acid	Total non-essential
Controls	M.	1.40	1.35	1.81	2.49	3.66	1.79	2.33	1.03	15.86
	± S.E.	0.1	0.1	0.4	0.5	0.5	0.2	0.2	0.1	3.2
Moderate		1.63*	1.74*	2.43*	2.36	3.14*	1.80	2.60	0.94	18.54*
KWO		0.3	0.2	0.2	0.2	0.5	0.6	0.2	0.1	3.1
Severe		1.78*	1.39	1.67	3.18*	4.52*	1.63	2.07	0.44*	17.01*
KWO		0.2	0.3	0.4	0.9	1.0	0.3	0.5	0.05	4.5
2nd grade		1.46	1.37	1.92	2.6	4.53*	1.43*	1.80*	0.76*	15.95
marasmus		0.3	0.5	0.5	0.9	0.9	0.5	0.6	0.1	3.2
3rd grade		1.52	1.64*	2.03*	2.33	4.11*	0.84*	2.09	0.47	15.04
marasmus		0.08	0.3	0.3	0.6	0.9	0.1	0.3	0.1	3.5
Marasmic		1.92*	1.47	2.19*	3.61*	5.57*	1.30*	2.50	0.93*	19.49*
KWO		0.3	0.3	0.8	0.7	1.2	0.3	0.2	0.03	2.3

* Difference from controls is significant ($P < 0.05$)

Table 3. Total proteins and electrophoretically separated fractions in whole saliva of normal and PEM infants and young children (mg/100 ml).

	Total protein	Pre-albumin	Albumin	α-Anti-trypsin	α-Macro-globulin	Hapto-globulin	Trans-ferrin	Lipo-protein	Immuno-globu-lin A	Immuno-globu-lin M	Immuno-globu-lin G
Controls	66.23	–	19.04	4.21	–	6.63	7.64	1.25	20.96	1.18	5.32
	7.1		2.1	0.9		1.1	1.0	0.3	2.3	0.2	0.9
Moderate	131.69*	2.16*	36.15*	7.64*	1.19*	11.42*	12.91*	1.93*	23.22*	4.64*	30.43*
KWO	16.1	0.2	3.3	1.0	0.4	2.1	2.9	0.3	4.1	0.9	4.5
Severe	245.19*	3.41*	55.3*	10.62*	6.94*	19.70*	23.87*	4.42*	49.75*	8.56*	62.62*
KWO	23.1	0.9	3.7	1.8	2.1	2.6	3.3	0.9	6.5	0.9	7.2
2nd grade	58.42	1.03	16.88	3.86	0.16*	6.71	6.69	0.74*	13.59*	2.09*	6.67
marasmus	5.3	0.1	2.1	0.8	0.03	1.3	1.4	0.1	3.1	0.4	0.9
3rd grade	78.45*	1.19	22.48*	5.00	–	7.21	8.22	1.37	18.73	2.01*	12.24*
marasmus	9.4	0.1	4.1	0.9		1.4	1.5	0.3	3.4	0.09	2.8
Marasmic	124.28*	1.64*	32.36*	10.82*	0.56*	13.47*	9.13*	2.84*	22.74	3.87*	26.85*
KWO	17.5	0.9	2.3	1.3	0.09	2.6	2.4	0.9	1.9	0.9	5.3

* Difference from controls is significant ($P < 0.05$)

ministered after being soaked were placed in a plastic syringe, compressed, and the contents were received in a Wasserman tube.

Up to 4–5 ml of saliva were collected in this way. Samples were then centrifuged for 15 minutes at 3500 r.p.m. and the supernatant was separated in another tube. Saliva was then analysed for total proteins by the technique given by *Pesce* and *Strand* (12), electrophoretic separation of protein components was done according to the procedure of *Johanssen* (7) and the amino acid chromatography was carried out by the one-dimensional paper-chromatography technique as cited by *Whitehead* (18).

Results

The results showing the level of salivary amino acids in normal and protein-energy malnourished infants are shown in tables 1 and 2. Table 3 shows the values for salivary protein components.

Discussion

Although the level of most biological constituents as proteins, lipids, carbohytrates, minreals and vitamins in various fluids of malnourished patients is more or les standardized, yet little is know about the counstituents in saliva. As a result of the different studies previously done (2, 8, 9, 16), it is now accepted that 13 protein fractions occur in normal human saliva. These fractions are: prealbumin, albumin, α_1-antitrypsin, haptoglobin α_2-macroglobin, transferrin, lipoprotein, IgA, IgM and IgG, α_1-acid glycoprotein, α-lipoprotein and ceruloplasmin. In the the present work we could detect only the first 10 components mentioned before in saliva of either normal or malnourished infants. This may point to their absence or that they are present in quantities that are undetectable by the method applied. In concordance with similar findings (4, 15), albumin and IgA were the components most excreted in large amounts in normal whole saliva. In our cases this was followed by transferrin, haptoglobin, IgG, α_1-antitrypsin, B_2-lipoprotein and IgM.

No report could be traced in the literature showing the pattern of salivary amino acids in normal Egyptian infants. In this investigation, 16 amino acids could be identified, 7 of them are essential.

In our group of patients, the data obtained showed that most of the salivary amino acids and protein components are increased in oedematous form of the disease, relative to normal, in spite of being decreased in plasma (14). Such increase can be either due to increased permeability of gland cells to amino acids from circulation or as a result of tissue degeneration known to associate such condition (17). Such proposal may be supported from the finding of *Enwonwu* and *Glover* (3), who observed atrophic oedematous submandibular gland in experimental animals suffering from protein deficiency. The incapability of the submandibular salivary gland to synthesize protein under conditions of protein deficiency (10) can be another contributing factor for this process. The drastically lower volume of saliva excreted in protein-deficient cases (11) may cause concentration of such constituents in saliva and in turn elevation of their level. Disturbance in regulated transport of protein components between circulation and glandular cells and permeability due to protein deficiency

may also cause progressive release of these protein fractions from circulation to saliva via the salivary glands.

The increase of salivary amino acids and proteins in oedematous forms of the disease was not observed in non-oedematous cases. It seems that adaptability of marasmic children to the meager diet helps them to escape from such complication. However, in 3rd grade marasmus, most of the protein components showed higher values than normal, which may refer to decompensation of the severely malnourished subjects.

In general, amino acids that were markedly elevated in saliva of malnourished cases were the essential ones, particularly methionine. Such elevation in spite of dietary deficiency again may prove that these amino acids are derived from the degenerated gland cells.

It may be concluded that such elevation of amino acids, particularly the essential ones, and protein components in saliva of malnourished cases is the result of tissue degeneration which is more marked in oedematous than non-oedematous cases, although in 3rd grade marasmus it is partially manifested. Again, prospection of changes in a biological fluid as saliva could be safely used to reflect extent of tissue affection as such salivary glands cells.

Summary

Amino acids and proteins in whole saliva of normal and malnourished Egyptian infants and young children were investigated. 16 amino acids, 7 of them are essential, could be detected in saliva of normal infants. Electrophoretic analysis revealed the existence of 10 protein components in saliva of either normal or malnourished infants. In oedematous cases, salivary amino acids, particularly the essential ones, were increased as well as most of the protein components. In non-oedematous cases, the pattern was more or less normal, but in 3rd grade marasmus, amino acid and protein components of saliva were somewhat increased. The conclusion was drawn that such increase is mainly due to tissue degeneration which is more marked in oedematous than non-oedematous form of the disease.

Zusammenfassung

Der Gehalt an Aminosäuren und Proteinen im Speichel von normalen und unterernährten ägyptischen Kindern und Kleinkindern wurde untersucht. 16 Aminosäuren, davon 7 essentielle konnten im Speichel von normal ernährten Kindern nachgewiesen werden. Die elektrophoretische Analyse ergab den Nachweis von 10 Proteinen im Speichel von normalen und unterernährten Kindern. Bei ödematösen Kindern nimmt der Gehalt sowohl an Aminosäuren, besonders der essentiellen, als auch an Proteinen zu. Bei nicht ödematösen Fällen waren der Gehalt und die Zusammensetzung mehr oder weniger normal, aber bei Marasmus dritten Grades sind Aminosäuren und Proteine etwas vermehrt. Aus diesen Ergebnissen wird geschlossen, daß die Zunahme des Speichelgehaltes an Aminosäuren und Proteinen bei unterernährten ödematösen Kindern infolge einer Gewebsdegeneration ausgelöst wird, welche bei der nicht ödematösen Form der Krankheit weniger ausgeprägt ist.

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